DRUG MANAGEMENT OF EPILEPSY

Current problems and the possible role of calcium antagonists

A thesis by

John Gerarde Larkin MBChB MRCP (UK)

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Western Infirmary

Glasgow G11 6NT

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To my wife Louie; and to Peter, Anna and Catherine - without whom this thesis could not possibly have taken half the time.

DECLARATION

The work described in this thesis was carries out while I was employed as a registrar and as a research fellow in the Department of Medicine and Therapeutics (previously Department of Medicine), Western Infirmary, Glasgow. Some of the work has been published or presented to learned societies, and available reprints are submitted with the thesis. The work was greatly facilitated by the efforts of many friends and colleagues, who as far as possible are formally acknowledged. Unless otherwise indicated, the remainder of the work, and all of the statistical analysis, was carried out by myself. The writing of this thesis was entirely my own work.

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ACKNOWLEDGEMENTS

ABBREVIATIONS

6-beta OH-cortisol	6-beta hydroxycortisol
10-OH-CZ	10-hydroxy carbazepine (oxcarbazepine metabolite)
AED	antiepileptic drug
AND	androstenedione
AUC	area under (concentration-time) curve
BBB	blood-brain-barrier
bd	twice daily
Bmax	(maximum) receptor (or binding site) concentration
CA	calcium antagonist
CBZ	carbamazepine
CBZ-C	conventional carbamazepine tablets
CBZ-CR	controlled-release carbamazepine tablets
CBZ-E	carbamazepine-10,11-epoxide
CBZ trans-diol	10,11-dihydro-10,11-dihydroxycarbamazepine
CFFT	critical flicker fusion threshold (psychomotor test)
[^{3}H]CHA	tritiated cyclohexyladenosine
CI	confidence interval
CS	card sorting (psychomotor test)
CRT	choice reaction time (psychomotor test)
CRT1	recognition time (psychomotor test)
CRT2	movement time (psychomotor test)
CT	computerised tomography (tomogram)
DHAS	dehydroepiandrosterone sulphate
EEG	electroencephalogram
EMIT	enzyme-mediated immunoassay technique

EXPT.	experiment
FAI	free androgen index
FSH	follicle stimulating hormone
FT	free testosterone
FT4	free thyroxine
GTCS	generalised tonic-clonic seizure
h	hour
h.p.l.c.	high performance liquid chromatography
i.p.	intraperitoneal
IQ	intelligence quotient
I-V	intravenous
Kd	dissociation binding constant
kg(Kg)	kilogram
L (ml, ul/uL)	litre (millilitre, microlitre)
LH	luteinising hormone
LHRH	luteinising hormone releasing hormone
mg	milligram
mg/L*	milligrams per litre
min	minute
ml	millilitre
NIF	nifedipine
NIM	nimodipine
NSB	non-specific binding
OXC	oxcarbazepine

*   Style used for concentrations throughout text. Occasionally, in figures,

the convention mgL^{-1} or equivalent appears

P450	cytochrome P450 (monooxygenase/system)
PB	phenobarbitone
PHT	phenytoin
POLY	polypharmacy (with more than one AED)
PRIM	primidone
PRL	prolactin
PTZ	pentylenetetrazol
SD	standard deviation
SEM	standard error of the mean
SES	sexual experience scale
SES1	morality scale
SES2	psychosexual motivation scale
SES3	physical sexual motivation scale
SES4	attraction to marriage scale
SHBG	sex hormone binding globulin
T	testosterone
T3	triiodothyronine
T4	thyroxine
TDM	therapeutic drug monitoring
tds	three times daily
TLE	temporal lobe epilepsy
trans-diol	10,11-dihydro-10,11-dihydroxycarbamazepine
TSH	thyroid stimulating hormone (thyrotropin)
Tween 80	polyoxyethylene sorbitan mono-oleate preparation
ug, ul, uL	microgram, microlitre, microlitre
VPA	(sodium) valproate

SUMMARY

Prologue and Introduction

Epilepsy is a disease which has struck fear into the hearts of both sufferers and onlookers for many centuries. Only in the past hundred years or so has effective medication become available, and medical management still relies on a small group of antiepileptic drugs (AEDs). These can often abolish seizures, and very frequently diminish their frequency such that patients can enjoy a normal life-style. A significant minority of patients, however, do not respond satisfactorily. Combining different drugs is complicated by their sharing the same side-effect - sedation - which seems more additive than does any therapeutic effect. It is for the benefit of these patients with refractory epilepsy that research continues, to improve our use of the present AEDs, and to find new drugs which might, when used alone or in combination, improve their lot.

Therapeutic Drug Monitoring (TDM)

In Chapter 1, current use of TDM in the epilepsy clinic is analysed. By recording physicians' decisions both before and after serum anticonvulsant concentrations were made available at 488 clinic visits, we found that management decisions were affected at 23% of these consultations. However, physicians did not appear to follow a "target concentration strategy" as a high proportion of results (26%) in the "target range" were followed by a change in dosage. A drawback with the "target concentration strategy" was highlighted by the correlation between carbamazepine concentration and time since dosing ($P<0.005$). The possible benefits of an approach combining clinical and biochemical information are discussed.

Cognitive function

In Chapter 2, the effects of many AEDs on mental function are assessed. Deterioration in

"cognitive" or "psychomotor" abilities is a generally recognised side-effect of all current AEDs. However, most evidence compares patients taking AEDs with healthy volunteers, the effect on mental function of the disease itself thus being uncontrolled. Other studies show short term deterioration in volunteers given AEDs for a limited period, or in patients abruptly taking an increased dose. These respectively fail to allow for any beneficial effect which controlling seizures might have on mental function, and the effect of tolerance to the drugs' side-effects.

In EXPT. 2, 66 patients on AED therapy performed a battery of psychomotor function tests, and their results were compared with those of 14 untreated epileptic patients and 11 healthy controls. A clear "step-wise" deterioration in function was seen with reaction times, short-term memory, card-sorting, and finger-tapping speeds. Untreated epileptics fared worse than controls ($P<0.05$ - $P<0.001$) but better than treated patients ($P<0.05$ - $P<0.01$). This demonstrated the deliterious effect of epilepsy itself. The drugs may aggravate this, though clearly the treated patients had more severe epilepsy. No differences were found between the individual drugs.

In EXPT. 3, the effect of tolerance was demonstrated in a small group (n=13) of new patients commenced on carbamazepine. After an initial deterioration in reaction time ($P<0.05$) and finger-tapping ($P<0.001$) at one week, these abilities returned to normal by twelve weeks, while mean serum concentrations of carbamazepine only fell from 8.5 mg/L to 7.1 mg/L. The relevance of short-term cognitive deterioration demonstrated in many studies is thus brought into question.

Since diurnal variation in serum concentrations has been shown to correlate with carbamazepine neurotoxicity, EXPT. 4 tested the pharmacokinetics of a controlled-release preparation (Tegretol Retard, CBZ-CR). Eight healthy volunteers took this and

conventional carbamazepine 200mg bd for two weeks in a double-blind, crossover fashion. Serum concentrations "plateaued" for 56h after single dose CBZ-CR, while chronic dosing resulted in diurnal fluctuation of only 12% compared with 24% on conventional carbamazepine (P<0.025), and produced less rapid changes in concentration (P<0.02). Enzyme induction appeared similar with both preparations, but the bioavailability of CBZ-CR was possibly slightly lower. The "smoother" pharmacokinetic profile of CBZ-CR did not produce a detectable improvement in psychomotor function.

Enzyme induction

Many AEDs (carbamazepine, phenytoin, phenobarbitone) induce an increase in hepatic metabolising enzyme activity. This results in accelerated metabolism of the drugs themselves, of some other drugs which undergo oxidative metabolism, and of endogenous hormones. The clinical implications of this last aspect remain unclear.

In EXPTS. 5 and 6, thyroid and sex hormone concentrations were measured in 54 young epileptic men taking a variety of AEDs, and compared with the results in 14 untreated epileptics and 16 healthy controls. Falls in total and free thyroxine were confirmed in patients taking the inducing drugs carbamazepine and phenytoin. Nine such patients were in the "hypothyroid" range, but none had increased TSH concentrations or TSH response to TRH stimulation. Sodium valproate (a non-inducer) showed no effect, supporting the case for enzyme induction as the mechanism responsible for the phenomenon.

Concentrations of the sex hormone dehydroepiandrosterone sulphate were also reduced in induced patients (P<0.0001), while sex hormone binding globulin levels were increased (P<0.0001) causing a tendency for free testosterone to be lowered (P=0.06). These effects might help explain the reported hyposexuality in epilepsy. Again, sodium valproate showed no effect.

The keto-analogue of carbamazepine - oxcarbazepine - is reported to have similar efficacy to the parent compound, but not to cause enzyme induction. In EXPT. 7, this drug was given to eight healthy volunteers, in a dose of 300mg bd for two weeks, and various markers of enzyme induction were measured. Half-life of the drug's active metabolite - 10-hydroxy carbazepine - was unaltered. Similarly, antipyrine metabolism, 6-beta hydroxycortisol excretion, and endogenous hormone concentrations did not change during two weeks therapy. Oxcarbazepine may provide an improvement on carbamazepine in patients taking other drugs or those complaining of sexual difficulties.

It is, however, not proven that hormonal changes due to AED therapy affect sexual function. A long-term study of sexual function, hormone levels and AED concentrations in patients and controls is in progress in our unit. These and other possibly important factors, such as education and upbringing, will be correlated.

Calcium antagonists

Calcium influx across the neuronal cellular membrane is essential for intrinsic burst firing and the abnormal action potentials at the heart of initiating epileptogenic activity. Calcium antagonists inhibit these features *in vitro*, and are effective in many animal models of epilepsy. The dihydropyridines (e.g. nifedipine, nimodipine) have been particularly effective, promoting their appraisal as possible adjuvant AEDs.

EXPT. 8 was an open study of nifedipine in twelve epileptic patients given the drug as adjuvant therapy for three months. Eight patients completed the study, all of whom reported a decrease in seizure frequency compared with the previous three months ($P<0.01$). Two hitherto refractory patients remained seizure-free throughout the trial period. Since there was no placebo group, however, further investigation was required.

EXPTS. 9 and 10 were performed on mice, to supply pharmacokinetic data, and to assess the relative efficacies of nifedipine and nimodipine, the former drug having been largely ignored in previous work. Intraperitoneal injection of the dihydropyridines (6 mg/kg) followed by sacrifice of the animals at different time points allowed the estimation of half-lives in mouse blood and brain respectively for nifedipine (11.2min, 14.7min) and nimodipine (16.7min, 22.4min). Both drugs easily crossed the blood-brain-barrier, whether dissolved in alcohol/water (50/50) or suspended in Tween 80.

Using a pentylenetetrazol-induced seizure model, both drugs and carbamazepine were shown to be effective, orally and parenterally, in delaying seizure onset. However, nifedipine combined with carbamazepine was ineffective, suggesting a possible interaction.

EXPT. 11 was a placebo-controlled trial of nifedipine in 22 patients with refractory epilepsy. Only in the first of the two months active therapy did nifedipine show a significant improvement on placebo ($P<0.05$). EEG scores were also improved during active treatment ($P<0.05$), but, overall, the results were disappointing. A possible reason was the low circulating concentrations of nifedipine - less than half of those considered effective in angina. A significant correlation between seizure control and serum levels further suggested that the drug might be effective if taken in adequate dosage.

In EXPT. 12, nimodipine also disappointed in a placebo-controlled study in 22 patients. Again, circulating concentrations of the drug were low, and might explain its lack of efficacy.

An alternative explanation for the drugs' failure to impress in clinical trials was investigated in EXPT. 13. Possible mechanisms of action of established AEDs such as

carbamazepine include an effect on adenosine, one of "the brain's natural anticonvulsants". Carbamazepine has been shown to increase adenosine A_1 receptors in mouse brain. Using a binding assay involving [^{3}H]cyclohexyladenosine as radioligand at concentrations of 0.2nM - 29nM, we calculated adenosine receptor numbers and affinity in three areas of mouse brain. A slight increase in receptor numbers in the cerebellum of mice treated with carbamazepine was confirmed, but the dihydropyridines decreased the affinity of the same receptors.

This might be the basis of a deliterious interaction between carbamazepine and the dihydropyridines, reducing efficacy in clinical trials. It seems, however, more likely that enzyme induction due to concomitant AED therapy decreases the dihydropyridine's half-life and reduces concentrations. Further pharmacokinetic and dose-ranging studies are proposed - both in mice and man - for the novel dihydropyridine amlodipine, which has a longer half-life (35h) than nifedipine (6h) or nimodipine (2h). Meanwhile, we are continuing to assess the possibility that a more subtle interaction - perhaps involving the adenosine receptor, or even the dihydropyridine receptor (carbamazepine and other AEDs have calcium antagonist activity) - may underlie the relative failure of dihydropyridines in the clinical setting. It would be surprising if the calcium antagonists, with all their theoretical promise and success in seizure models, did not produce one useful antiepileptic drug.

PROLOGUE

"RECOVERING SOUL"

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Over the course of history the management of epilepsy has probably made use of a greater variety of remedies than that of any other disease. This reflects the long-standing bewilderment over its likely cause. In many societies, the aetiology of epilepsy has been attributed to the work of evil spirits. Indeed, the oldest written account of epilepsy so far known - that of a Babylonian treatise discovered on a stone tablet in South Turkey [Kinnier Wilson & Reynolds 1990] - is adamant: "If epilepsy falls once upon a person....it is the result of possession by a demon or departed spirit" and goes on to describe which demons cause which type of seizure. Other societies blamed friendly spirits. Hippocrates "et al" (c. 400 B.C.) was the first to ridicule this latter concept of "The Sacred Disease" as "a shelter for ignorant and fraudulent practices" [Temkin 1971a]. His suspicions fell upon the brain where, he felt, an excess of phlegm overspilled into the bloodstream. Widespread belief in the epileptogenic effect of attacks by spirits or demons, however, was slow to disappear. In Biblical times, it remained the conventional wisdom, as witness the Evangelist Mark's description of "the epileptic demoniac":

> there is a spirit of dumbness in him, and when it takes hold of him it
> throws him to the ground, and he foams at the mouth and grinds his teeth
> and goes rigid   [Mark 9: 18]

to which Luke adds:
> it is slow to leave him, but when it does it leaves the boy worn out
>
>   [Luke 9: 37]

The very word "epilepsy" comes from the Greek 'epilambanein' which means 'to seize'

or 'to attack' and such nomenclature continues to the present day, perhaps bringing with it an associated stigma.

From earliest days, however, we can also find precise clinical observations, such as the Hippocratic mention of convulsions on the contralateral side to skull injuries, or the superb description by Lucretius (? 2nd century A.D.) of a tonic-clonic seizure:

> As if by stroke of lightning, tumble down
>
> Before our eyes, and sputter forth and grunt,
>
> Blither, and twist about with sinews taut,
>
> Gasp up in starts, and weary out his limbs
>
> With tossing round......

Until eventually the patient

> ...Arises reeling, and gradually comes back
>
> To all his senses and recovers soul.   [Temkin 1971b]

Since the Ancients variously attributed the disease to demons, phlegms and the phases of the moon, it is hardly surprising that the remedies were equally disparate. Dietary measures were legion and ranged from abstention from meat to the avoidance of all food and drink. Phlebotomy or the drinking of vinegar were other simple measures, while more bizarre procedures included the provocation of sneezing last thing at night, or the rubbing of affected limbs with the genitals of a seal [Temkin 1971b]. The most direct approach was "trephining" - the production of a large hole in the skull, usually by cautery. Its versatility clearly suited all aetiologies as it could be used to allow the escape of phlegm or vapours - or indeed evil spirits - as required.

Progress in understanding the "Falling Sickness" [attrib. Paracelsus; cf. Esquirol 1845] was slow. Galen's idea (130-200 A.D.) of "animal spirits" in body tubes was still being quoted by Willis in the late 17th century [Stevens 1973]. Social attitudes were also slow to progress. Although Aristotle had long-before espoused an association between epilepsy and genius (still contemplated to this day cf. Caesar, Petrarch, Napoleon, Moliere, Van Gogh, Dostoyevsky), Albich in the 18th century warned "neither talk nor bathe with them, since by their mere breath they infect people" [Temkin 1971c].

During the more rational approach of the "Enlightenment" of the 18th and 19th centuries, attempts were made to classify epilepsy into idiopathic and secondary epilepsies, whose causes ranged from the respectably traumatic to the witnessing of a seizure while pregnant [Esquirol 1845]. The association with the moon's phases was scientifically laid to rest by Leuret [Temkin 1971d] notwithstanding today's interest in catamenial epilepsy, which was also noted in the early 18th century by Maisonneuve [Esquirol 1845].

"Modern epilepsy" may have begun with John Hughlings Jackson, who linked new electrophysiological evidence discovered by Fritz & Hitzig in 1870 with clinical observations in his "Investigation of Epilepsies" [1873] and defined epilepsy as "the name for occasional, sudden, excessive, rapid and local discharges of grey matter." Yet, Jackson still claimed "there can be no question that the ligature is a most valuable means of arresting such fits" [1873].

Some attempts at scientific evaluation of therapies, however, were proceeding. Around 1840, Esquirol twice-yearly submitted 30 female patients to a different traditional or novel remedy. A fine demonstration of the placebo response ensued:

"A new medicine invariably suspended the attacks with some,

for fifteen days; for a month, two months, and even three

months, with others."   [Esquirol 1845]

Nitrate of silver remained the thinking-man's drug of choice [Pereira 1839] until 1857,

when the first mention of potassium bromide was made during a paper at a meeting of

the Royal Medical and Chirurgical Society in London [Sieveking 1857]. Bromide,

probably the first genuinely effective therapy, was championed by Locock, and was used

by Hammond in 288 patients, producing an improvement in 243, with total seizure

control in 91 [Hammond 1871]. The related potassium iodide was used by others,

including Charcot [Charcot 1881].

Response to the availability of these drugs was perhaps over-enthusiastic. Overdosing

was common: "As you see, he is broken down in appearance, has large abscesses in his

neck, and is altogether in a bad condition. But this is better than to have epilepsy."

(Hammond) [Temkin 1971e] This, however, has to be viewed alongside contemporary

practices. A series of 72 cases of trephining was published as late as 1861, and in 1866

cliteroidectomy was still being practised by Baker [Duffy 1963]. By the 1880s, however,

surgery aimed at removing affected parts of the brain was meeting with more

conventional success. The foundations of modern-day therapy had been laid.

INTRODUCTION

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Classification of epilepsy

Drug treatment of epilepsy is dependent on some degree of classification of the disease. The official classification [CCT ILAE 1989] is a complicated hotch-potch of syndromes, but does acknowledge the major division of epilepsies into generalised seizures (generalised tonic-clonic seizures, absences, myoclonic, tonic, clonic) and epilepsies with partial or focal seizures (origins in frontal, temporal, parietal and occipital lobes). It is the management of generalised tonic-clonic (GTCS) and partial seizures with or without secondary generalisation which forms the basis of this thesis on adult epilepsy.

Partial seizures may be simple or complex (where there is loss of awareness) and may vary in character, depending on their site of origin in the brain. These are the epilepsies most often associated with a preceding "aura" and with other localising features such as *deja vu* phenomena and automatisms. Often, they are "symptomatic" epilepsies associated with a single anatomical or functional lesion, but "idiopathic" varieties do occur, where investigation may reveal more diffuse localisation of the origin, perhaps involving both hemispheres. Partial seizures may progress to secondary generalised seizures which can mimic entirely the presentation of a GTCS. Differentiation is best attempted by analysis of the initial clinical or encephalographic changes as it is early bilateral involvement which characterises the primary GTCS. Most patients presenting at the epilepsy clinic can be classified as suffering from generalised seizures alone, from partial seizures alone, or from both partial and secondary generalised seizures.

Clinical and pharmacokinetic data on the major anticonvulsants (or antiepileptic drugs - AEDs) which were available in 1986 are shown in Tables 1 and 2 [adapted from Brodie 1990]. It is generally accepted that the three "first-line" drugs from this group are

TABLE 1 - PRESCRIBING ANTICONVULSANTS IN ADULTS

DRUG	YEAR	INDICATIONS	STARTING DOSE	DAILY MAINTENANCE (INTERVAL)	TARGET RANGE
CARBAMAZEPINE	1963	Partial and GTCS	100-200mg bd	400-2000mg (bd*-qds)	4-10mg/L (17-42 umol/L)
CLOBAZAM	1979	Adjunctive therapy in refractory epilepsy	10mg nocte	10-40mg (od-bd)	None
CLONAZEPAM	1969	Myoclonic and GTCS Status epilepticus	0.5 - 1mg	2-8mg (od-bd)	None
ETHOSUXIMIDE	1958	Absence seizures tonic-clonic seizures	500mg	500-2000mg (od-bd)	40-100mg/L (283-708 umol/L)
PHENOBARBITONE	1912	Partial and GTCS clonic and tonic seizures Prophylaxis of febrile convulsions Resistant status epilepticus	30-60mg	60-240mg (od-bd)	10-40mg/L (40-172 umol/L)
PHENYTOIN	1936	Partial and GTCS Status epilepticus	100-200mg	100-700mg (od-bd)	10-20mg/L (40-80 umol/L)
PRIMIDONE	1952	Partial and GTCS	125-250mg	250-1500mg (od-bd)	5-12mg/L (23-35 umol/L)
SODIUM VALPROATE	1967	Primary generalised epilepsies Partial seizures Prophylaxis of febrile convulsions	200mg bd	400-3000mg (bd)	50-100mg/L (347-693 umol/L)

* bd only with controlled-release formulation   [adapted from Brodie, 1990]

TABLE 2 - PHARMACOKINETICS OF ANTICONVULSANT DRUGS

DRUG	ABSORPTION (BIOAVAILABILITY)	DISTRIBUTION VOLUME (L/Kg)	PROTEIN BINDING (% bound)	ELIMINATION HALF-LIFE (hours)	ROUTE(S) OF ELIMINATION	COMMENTS
CARBAMAZEPINE	Slow absorption (75-85%)	0.8 - 1.6	70-78	24-45 (single) 8-24 (chronic)	Hepatic metabolism CBZ-10,11-epoxide active metabolite	Enzyme inducer Autoinduction of metabolism
CLOBAZAM	Rapid absorption (90-100%)	0.7 - 1.6	87-90	10-30	Hepatic metabolism N-desmethylclobazam active metabolite	Tolerance Withdrawal exacerbation
CLONAZEPAM	Rapid absorption (80-90%)	2.1 - 4.3	80-90	30-40	Hepatic metabolism	Sedative Tolerance Withdrawal exacerbation
ETHOSUXIMIDE	Rapid absorption (90-95%)	0.6 - 0.9	0	20-60	Hepatic metabolism 25% excreted unchanged	More rapid clearance in children
PHENOBARBITONE	Slow absorption (95-100%)	0.51 - 0.57	48-54	72-144	Hepatic metabolism 25% excreted unchanged	Enzyme inducer Sedative
PHENYTOIN	Slow absorption (85-95%)	0.5 - 0.7	90-93	9-40	Saturable hepatic metabolism	Enzyme inducer Elimination half-life concentration-dependent
PRIMIDONE	Rapid absorption (90-100%)	0.4 - 0.8	20-30	4-12	Hepatic metabolism Active metabolites 40% excreted unchanged	Phenobarbitone a major metabolite
SODIUM VALPROATE	Rapid absorption (100%)	0.09 - 0.17	88-92	7-17	Hepatic metabolism	Minor enzyme inhibitor Concentration-dependent protein binding

[Adapted from Brodie 1990]

carbamazepine, phenytoin and sodium valproate. A case can be made for ethosuximide as the drug of choice in childhood absence seizures [Mikati & Browne 1988] (although its efficacy is at least equalled by sodium valproate [Sato et al. 1982]), but it has no place in the management of other epilepsies.

Primidone (PRIM) has undoubted anticonvulsant activity both in animals [Bogue & Carrington 1953] and in patients [Gruber et al. 1957, White et al. 1966] but suffers from important drawbacks. These include the presence of phenobarbitone as a major metabolite [Butler & Waddell 1956], which makes the anticonvulsant activity of primidone itself more difficult to assess. In a recent large controlled trial of carbamazepine, phenytoin, phenobarbitone and primidone [Mattson et al. 1985], the last was easily the least effective and least-well tolerated drug of the four. From the earliest animal studies [Barnes 1960], marked sedation has been recognised as a side-effect of primidone.This and other side-effects, such as gastrointestinal intolerance and psychosis, often lead to discontinuation of the drug [Mattson et al. 1985]. Physical dependence [Frey 1985] and withdrawal seizures [Norton 1970] are also major problems. There is thus no place for primidone as a first-line drug in the present-day management of epilepsy.

Phenobarbitone (PB), whose anticonvulsant properties were first discovered in 1912 by Loewe et al. [Gallagher & Freer 1985], suffers from many of the same drawbacks as primidone. It is highly sedative [Schmidt 1985] and can also cause hyperactivity and irritability [Consensus Statement 1980]. The long-term sequelae of its undoubted effects on higher cortical function [Guest et al. 1970, Consensus Statement 1980] are not yet clear. Tolerance occurs [Gallagher & Freer 1985] and withdrawal often provokes

seizures [Buchthal et al. 1968]. However, it lacks the added complication of a highly active metabolite, and was shown in the "Veterans' study" to be superior to primidone in treating secondary generalised seizures with respect to both efficacy and side-effects [Mattson et al. 1985]. While it is no longer a first-line drug, many patients continue to be well-controlled on phenobarbitone, and the problems of withdrawal often argue against stopping the drug entirely. It remains a possible second-line drug, to be considered in preference to primidone, and continues to play a major role in antiepileptic therapy in developing countries.

The **benzodiazepines** are the most recent entrants to Tables 1 and 2. Anticonvulsant effects were first demonstrated against electroshock and pentylenetetrazol-induced seizures in animals in 1960 by Randall and his colleagues. While diazepam remains arguably the drug of choice for status epilepticus [Simon 1985], the derivatives clobazam and clonazepam are most often used in epilepsy on a chronic basis. Both clonazepam [Shakir 1979, Mikkelsen et al. 1981] and clobazam [Allen JW et al. 1983] are effective adjuvant therapy in controlled studies, and clobazam in particular has markedly reduced sedative properties compared to typical benzodiazepines [Hindmarch & Gudgeon 1980, Trimble & Robertson 1986]. However, withdrawal seizures can be a problem [Fialip et al. 1987, Allen JW et al 1983] and loss of efficacy after variable periods of time on the drug has been widely reported [Trimble & Robertson 1986]. Clobazam is thus a useful member of the available group of anticonvulsants, and may have a particular place as adjunctive short-term therapy. In catamenial epilepsy, short courses of the drug around the time of menstruation have proven effective [Feely et al. 1982].

Phenytoin (PHT) was the first of the three first-line anticonvulsants to come into general use [Merrit & Putnam 1938]. Its main advantage at that time over phenobarbitone was its

activity against partial seizures, which had largely remained refractory to therapy [Jones & Wimbish 1985]. It was also obvious clinically that it was a less sedative drug. More sophisticated approaches have since suggested that it does cause some impairment of psychomotor and cognitive processing [Thompson et al 1981, Andrewes et al 1986]. These findings, however, have not been universal, and some studies have shown improved mental function in "retarded" epileptic patients [Goldberg & Kurland 1970], or "healthy" elderly subjects [Smith & Lowry 1975]. In toxic concentrations, phenytoin can cause ataxia, nystagmus, diplopia and vertigo [Reynolds 1989]. Its chronic side-effects include hirsutism and gum hyperplasia. It is thus often a cosmetically displeasing drug, but it is largely free from life-threatening side-effects. Interference with vitamin D metabolism may produce a degree of hypocalcaemia and osteomalacia [Ashworth & Horn 1977], while a mild macrocytic anaemia due to folate deficiency may also occur [Jones & Wimbish 1985]. Similar problems are found with phenobarbitone [Gallagher & Freer 1985]. The risk of the "foetal hydantoin syndrome" [Hanson et al 1976] has inhibited the use of phenytoin in pregnancy, but any gain from avoiding the drug has to be weighed against possible risks to the foetus from impaired seizure control. Phenytoin remains a very useful drug; perhaps less so in young women, but its once-daily dosage regimen is often an advantage.

Carbamazepine (CBZ), a drug chemically related to the tricyclic antidepressants, was introduced in 1963 [Theobald & Kunz 1963]. Its efficacy was found to be similar to that of primidone [Rodin et al 1976], and it has become a major anticonvulsant, particularly for partial seizures [Cereghino et al 1974, Troupin et al 1977]. It has generally low toxicity, although rashes occur in approximately 5% of patients [Pellock et al 1984] and hyponatraemia is a recognised side-effect [Gram & Jensen 1989]. The occasional

leucopenia and rare aplastic anaemia at one time prompted the use of haematological monitoring [Hart & Easton 1982] but this is now largely discarded [Loiseau & Duche 1989]. As with the other major anticonvulsants, CBZ does have neurological side-effects, mainly nausea, headache, dizziness and diplopia. Many of these may in part be associated with serum concentrations of the active metabolite carbamazepine-10,11-epoxide (CBZ-E) [Patsalos et al 1985, Schoemann et al 1984]. It is arguable that CBZ is one of the least sedative of the anticonvulsants [Thompson & Trimble 1982a, Andrewes et al 1986] although a definite effect on psychomotor function has been demonstrated in both healthy volunteers [Macphee et al 1986a] and patients [Macphee et al 1986b]. It may also produce some elevation of mood [Dalby 1975, Loiseau & Duche 1989] suggesting usefulness in patients with depression. The success of carbamazepine has led to the production of slow-release preparations and derived drugs such as oxcarbazepine, the place of which will be considered later.

The antiepileptic properties of **sodium valproate** (VPA) were first discovered accidentally in 1963 when valproic acid was being used in animal studies as a solvent for the drugs which were being formally tested [Meunier et al 1963]. As the most recently introduced of the major anticonvulsants, VPA has a wide background of successful trials in various animal models of epilepsy [Loscher 1985]. Clinical studies confirmed its efficacy in GTCS [Gram & Bentsen 1984, Shakir et al 1981] and partial epilepsy [Shakir et al 1981] as well as other epilepsies [Gram & Bentsen 1985]. It is a viable alternative to CBZ and PHT in both GTCS and partial seizures, while maintaining an advantage over them in myoclonic epilepsy and absence attacks [Sato et al 1982, Gram & Bentsen 1985, Fariello & Smith 1989]. Side-effects include hair loss, weight gain, and a tremor resembling benign essential tremor [Gram & Bentsen 1985]. These problems usually resolve with dose reduction or, if necessary, cessation of the drug. More worrying are the rare episodes of hepatitis, pancreatitis, thrombocytopenia or coma [Dreifuss 1989].

Fortunately, the incidence of these serious problems is low, even in children, where the overall incidence particularly of hepatitis is increased owing to the occasional patient with a metabolic defect [Gram & Bentsen 1985]. It is thus a close choice between VPA and ethosuximide for absence seizures, while VPA remains a useful drug of comparatively low toxicity in adult patients. It shares with carbamazepine a reputation for causing less sedation than some of the other anticonvulsants [Thompson & Trimble 1981, 1982a]. It is also notable for its lack of enzyme induction, and may be an enzyme inhibitor [Kapetanovic & Kupferberg 1980, Koch et al 1981] which could lead to toxicity if it is added to longstanding AED therapy [Levy & Koch 1982].

Scope of thesis

I have outlined above the background of the drugs currently used in epilepsy. This thesis will approach the problem of improving anticonvulsant therapy in two parts. In the first, an investigation will be made of the way in which these drugs are presently used, and of their drawbacks, with particular reference to psychomotor impairment and enzyme induction. A new formulation (controlled-release carbamazepine - "Tegretol Retard") and a new keto-analogue of carbamazepine (oxcarbazepine) will be assessed as we look for small but important advances in current therapeutic modalities. In the second part, I shall be investigating the potential of a new group of drugs for epilepsy - the calcium antagonists - in both clinical and animal studies.

Drug Assays

Assays of the common anticonvulsants CBZ, PHT, VPA and PB were all performed using an enzyme-mediated immunoassay technique (EMIT-SYVA, Palo Alto). A previously described [Macphee et al 1984] modification of the high performance liquid chromatography (h.p.l.c.) method of Meijer [1981] was utilised to measure CBZ-10,11-epoxide, and used 5-(p methyl-phenyl)-5-phenyl hydantoin as internal standard. Unbound CBZ concentrations were assayed by equilibrium dialysis using teflon dialysis cells separated by a presoaked semipermeable membrane (Dianorm). Plasma (0.9ml) and 0.13M (0.9ml) phosphate buffer were rotated at 4 r.p.m. for 24h in a water bath at 37 ^{O}C. Free CBZ concentration was calculated from the following equation:

$$f = Cl(F) / Cl(0) - Cl(F)$$

where f=free fraction; Cl(F)=final concentration of free ligand; and Cl(0)=starting concentration of ligand [Macphee et al 1986a]. Antipyrine was measured using a modification of the h.p.l.c. method of Shargel [1979] as outlined by Macphee et al [1984].

These assays were all performed in the laboratories of the Department of Medicine & Therapeutics (previously Department of Medicine), Western Infirmary, unless otherwise stated. Blood samples were centrifuged after allowing time for clotting, and the serum was stored at -20°C or lower until all of the appropriate samples had been obtained. Assays were then performed in batches to offset any slight time-to-time variation in the assays.

Pharmacokinetics

Antipyrine kinetics following oral ingestion of 600mg antipyrine were used as a measure of enzyme induction. Patients supplied 5ml samples of saliva for analysis after 0, 3, 5, 8, 12, 24 and 32 hours. Kinetic parameters were obtained using computer-assisted analysis. Antipyrine kinetics were routinely calculated in the laboratory using least-squares regression analysis assuming a one-compartmental model. The programme for Apple IIE microcomputer has been previously outlined [Birnie 1986a]. Kinetics of CBZ and other drugs were calculated by myself using a kinetics programme adapted for use on an Apricot F10 microcomputer [Birnie 1986b]. Later the statistical and pharmacokinetic package "STATIS" [CLYDESOFT] became available and was used for the analysis in EXPT 9.

Statistics

In general, statistical comparisons between groups were made using non-parametric tests. In some cases, the groups were reasonably large, and parametric testing might be deemed appropriate, but as few presumptions as possible have been made. The Wilcoxon rank test (or equivalent Mann-Whitney "U" test) has been used in many cases, with "matched-pairs" testing of related samples (usually the same patient on different regimens) where appropriate. Where more than two groups are being compared, an initial non-parametric test of analysis of variance (Kruskal-Wallis or Friedrich's analysis of variance) has been performed to decide whether there is a significant variation between all groups before going on to test specific pairs. This makes some allowance for the problems of multiple group vs. group analysis, as its failure to demonstrate a significant variance between all groups points against going any further with individual testing. This, however, results in the test being arguably too conservative, and an effort has been made to discuss the ramifications of this where appropriate e.g. EXPT. 8.

While non-parametric tests are employed, the presentation of data as medians and ranges has largely been avoided. Although more logical, such presentation is so rarely employed that the reader, in my opinion, does not develope any "feel" for data shown in this manner. Therefore, means with standard deviation (SD) or standard errors of mean (SEM) are shown for illustrative purposes in most figures where the full data set would be inappropriate. Correlations quoted are either Pearson's product moment correlations (r) or Spearmann rank correlations (r_s). All statistics were performed by myself either by hand or using MINITABS (release 5.1) on an Apricot F10 microcomputer. The methods for hand-performed tests, and the choice of test in each situation was determined from the monographs of Siegel [1956] and Howell [1985].

Consent

Patients and volunteers gave informed consent to their participation in all of the studies and trials; in particular, written consent was obtained for EXPTS. 3, 4, 7, 8, 10 and 11. All experiments received approval from the local ethics committee, and animal studies were approved by the Home Office.

PART 1

CURRENT PROBLEMS WITH ANTIEPILEPTIC DRUGS

This part will consist of three chapters. In the first, therapeutic drug monitoring (TDM) will be discussed and its influence in the epilepsy clinic in the Western Infirmary will be assessed. This will also give some demographic data on the patients attending the clinic, from whom subjects will be drawn for the later studies. Chapter 2 will discuss the ubiquitous side-effect of psychomotor and cognitive impairment with anticonvulsants and includes a cross-sectional study in the clinic. The efficiency of a controlled-release carbamazepine in "smoothing" diurnal variation of serum concentrations and thus possibly reducing sedation will be investigated. In chapter 3, the impact of enzyme induction, which is exhibited by many anticonvulsants, on exogenous hormones will be assessed, and the claimed lack of enzyme induction with oxcarbazepine will be tested.

CHAPTER 1

THERAPEUTIC DRUG MONITORING

CHAPTER 1

THERAPEUTIC DRUG MONITORING

INTRODUCTION

The correlation between clinical effects and serum drug concentrations was first investigated using antibiotics during the Second World War [Marshall 1940] and was later demonstrated with antimalarials [Shannon 1946]. From these beginnings, there has evolved a "target concentration strategy" of therapeutic drug monitoring (TDM) [Spector et al. 1988] whereby efforts are made to bring serum drug concentrations into a predetermined "therapeutic range" for that drug. Indeed, some would include "ensuring that plasma concentrations lie within a 'therapeutic' range" in their definition of TDM [Whiting et al 1984]. Vozeh [1987] more simply defines TDM as "(Routine) use of drug concentrations to individualise dosage."

The attributes of an appropriate drug are outlined in Table 3. [Spector et al 1988]. The "bottom line" is the belief that serum concentrations correlate better with clinical effect than does dosage. Of the anticonvulsants, phenytoin best fulfils these criteria, and its saturable kinetics, resulting in large concentration increases for relatively small changes in dosage [Richens & Warrington 1979], make it particularly suitable for TDM. However, extrapolation of phenytoin's suitability to other AEDs has not met with universal approval [Chadwick 1987].

Carbamazepine, like phenytoin, has a pharmacological effect generally proportional to serum concentration, but this is less true of VPA and the barbiturates [Chadwick 1985, Brodie & Feely 1988]. With VPA in particular, accumulation in the brain may be a relevant factor [Schobben et al 1980] and plasma concentrations may correlate only with

TABLE 3 APPROPRIATE DRUG FOR TDM

1. Efficient and relatively inexpensive assay available

2. Interindividual variation in drug absorption,
   elimination and distribution

3. Pharmacokinetic data available

4. Pharmacological effect proportional to serum concentration

5. Narrow therapeutic ratio

6. Constant pharmacological effect over extended time period

7. Therapeutic and toxic ranges well defined

[Adapted from Spector et al 1988]

toxicity and then only crudely [Turnbull et al 1983].

Most controversy, however, is aroused by the question of "well-defined therapeutic and toxic ranges" (No. 7). Many would argue that no drug fulfils this criterion, and that therapeutic ranges are an oversimplification. We can examine the range (10-20mg/L) of the "ideal" drug phenytoin as an example. Buchthal et al [1960] first looked at 12 patients new to PHT. On constant dosage for "three to four weeks" those with serum concentrations below 10 mg/L showed no clinical improvement, while those above did better. This rather arbitrary figure was then used as a cut-off point in assessing out-patients and showed - not surprisingly - that those with levels above this figure were better controlled than those below. Lund [1974] also showed that increased concentrations correlate with better control, but seizure count was already improving at concentrations around 6 mg/L. He also pointed out that an increase from 11.7 to 15 mg/L in the group - a change within the therapeutic range - produced an improvement in seizure control. Further work has shown complete control of seizures to occur at varying levels ranging from 3 to 50 mg/L [Schmidt & Haenel 1984]. It seems likely and logical that, with increasing PHT concentrations, more seizures will come under control, and that there is no magical figure above which this will occur.

The upper level of the range is even less well formulated. Buchthal et al [1960] gave 10-20 mg/L as a vague range where efficacy might be expected, while they found no serious neurotoxicity below 30 mg/L (mild symptoms began above 14 mg/L). Lund [1974] also found very little toxicity below 25 mg/L, while recently it has been suggested that these side-effects tend to appear only at much higher levels [Gannaway & Mawer 1981].

Despite such evidence, the range of 10-20 mg/L has been handed down almost as a *sine qua non* for PHT therapy. While the early studies were clearly useful in providing guidelines to the use of PHT, the further stage of treating blood levels as the most

important aspect of patient management - the "target concentration strategy" - is not justified by their observational results. It is doubtful whether the original authors would have envisaged such an extrapolation. The argument is further complicated by the suggestion of a "therapeutic window" whereby seizures recur above a certain concentration of PHT. The initial observation of seizure-like activity in animals given high-dose PHT [Gruber et al 1940] was later shown not to be accompanied by epileptogenic EEG activity [Bazemore & Zuckermann 1974]. Clinical studies have since postulated a "window" [Troupin & Ojemann 1975], but a careful study by Osorio and colleagues [1989] has virtually discounted such an occurence, although a small proconvulsant action of PHT could not be excluded. This would be in keeping with a previous appraisal of increased seizures in "phenytoin-toxic" patients [Stilman & Masdeu 1985] where one patient did not have epilepsy but suffered seizures following accidental ingestion of PHT.

There have been recent expressions of concern over clinicians' requests for, and application of serum drug concentrations in all specialties. Up to 74% of requests have been deemed "inappropriate" [Levine et al 1988] while the same epithet has been attached to clinicians' actions following 17-40% [Beardsley et al 1983, Bussey & Hoffman 1983] of results. One paper showed that seizure frequency only improved if "appropriate" action was taken [Beardsley et al 1983]. This is, in fact, one of the few instances where seizure control has been shown to be improved with TDM. However, confounding variables which may have resulted in "inappropriate" action were not analysed, and it shares with other papers the difficulty of defining "appropriateness". It may be necessary for a clinician to check drug concentrations not at steady-state or at an unusual time after ingestion. Allowances can be made for such problems clinically, but not in the protocol of many of these papers, which tend to be pharmacy-based [Pitterle et al 1985, Bussey & Hoffman 1983].

Nevertheless, there is a growing feeling in many quarters that the measurement of drug concentrations may be overdone [Brodie et al 1985, Chadwick 1987] and valuable resources misspent [Bussey & Hoffman 1983, Vozeh 1987]. The ready availability of drug concentrations may itself encourage clinicians to measure these more frequently [Ried et al 1989] and perhaps adopt the strategy of chasing the "therapeutic" range, thus treating the anticonvulsant concentration rather than the patient - a policy which can be shown to cause unnecessary toxicity [Woo et al 1988]. Unnecessary treatment failure may also ensue in inexperienced hands, if drugs are abandoned because they are "in the therapeutic range" yet ineffective. Training may be beneficial, and it has recently been shown that the introduction of a formal TDM programme can reduce the number of requests for drug concentrations [Wing & Duff 1989].

Vozeh [1987] has outlined the difficulties of assessing the cost-effectiveness of TDM. Part of the assessment must include an estimate of how often an available drug concentration will affect management. This has been investigated with regard to anticonvulsants by Marty et al [1981] in a small study which included only 42 patients taking either PHT or CBZ. To assess the effect on clinical management in our epilepsy clinic, an anticonvulsant assay service was set up on-site. A record was made of the physicians' clinical decisions before and immediately after the result became available. No specific instructions or protocol were followed for the adjustment of therapy, to allow analysis of the way clinicians would use such a service.

EXPT. 1   A YEAR OF THERAPEUTIC DRUG MONITORING IN THE
EPILEPSY CLINIC

AIM

To assess the impact of an on-site TDM service on patient management, and to investigate the way in which clinicians make use of such a service.

PATIENTS AND METHODS

The epilepsy service at the Western Infirmary is provided by Clinical Pharmacology. In 1986, the clinic was manned by a consultant clinical pharmacologist, myself as NHS registrar, and a "rotating" medical Senior House Officer. During the year for which data are presented (February 1986-87), an average of 22 patients attended the clinic each week, four of whom were new referrals. Patients for the study were preselected as requiring active management. Indications for monitoring included poor seizure control, unwelcome polypharmacy, clinical toxicity, suspect compliance and the recent introducion of a new drug.

Of the 1162 patient attendances, 488 (42%) were monitored. A total of 632 drug assays were performed (277 carbamazepine, 170 phenytoin, 113 valproate, 72 phenobarbitone) in 182 individual patients, most of whom were studied on more than one occasion (one visit - 52, two visits - 48, three visits - 33, four visits - 23, five visits - 15, six visits - 4, seven visits - 6, eight visits -1). Of these patients, 117 had partial seizures with or without secondary generalisation, while 65 reported only generalised tonic-clonic convulsions. 132 patients received one anticonvulsant, 47 took two, and 3 patients were treated with three antiepileptic drugs. These figures include monitored drugs only, and omit the

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benzodiazepines clobazam and clonazepam.

Age, type of epilepsy, dose of anticonvulsant(s), reason for assay, time of last dose and of blood sampling were entered for each study patient on a specially designed form prior to consultation (Figure 1). A venous blood sample was withdrawn from each patient and transferred into a lithium heparin tube for centrifugation and analysis. After the initial interview, the clinician recorded his or her management decisions on the same form. Anticonvulsant dose(s), addition or substitution of another drug, advice regarding poor compliance and time to next appointment were stipulated. The assay result(s) became available approximately 15 minutes after venepuncture. The doctor then noted whether the result was expected and reviewed his decisions while the patient waited.

Concentrations of CBZ, PHT, VPA and PB were measured using an enzyme immunoassay system (EMIT, Syva) and this service was kindly supplied by the Western Infirmary Biochemistry Department under the auspices of Prof. Percy-Robb. Phenobarbitone alone was assayed in the six patients taking primidone.

Statistics

Comparisons were made using non-parametric statistical tests. Chi-square test was employed for assessing the influence of: a) type of seizure on drug taken (PHT or CBZ, degrees of freedom = 1); b) concentration category (low, "therapeutic", high) on decision to change anticonvulsant dose (df = 2); and c) time-since-dosage on concentration category (df = 12). Drug doses and concentrations for different types of epilepsy were compared by the Mann Whitney 'U' test. Correlations quoted are Spearmann rank correlation coefficients.

HOSPITAL NUMBER _____   DATE _____

NAME _____   TIME OF SAMPLING _____

TYPE OF EPILEPSY _____   TIME OF DOSE _____

INDICATION

BASELINE [☐] DOSE TITRATION [☐] COMPLIANCE [☐] TOXICITY [☐] INTERACTION [☐]

DRUGS	DOSE	ANALYSIS	RESULT	EXPECTED	UNEXPECTED
CARBAMAZEPINE					
PHENOBARBITONE					
PHENYTOIN					
PRIMIDONE					
SODIUM VALPROATE					

UNEXPECTED

LOW [☐]   HIGH [☐]   UNDETECTABLE [☐]

ACTION

[PROPOSED]   [ACTUAL]

FOLLOW-UP

[PROPOSED]   [ACTUAL]

OTHER BIOCHEMICAL TESTS

FIGURE 1   Proforma for study of therapeutic drug monitoring

RESULTS

Details of anticonvulsant drugs, doses and concentrations are shown in Table 4. Carbamazepine was the most commonly prescribed drug and was used in a larger proportion of patients suffering from partial (70%) than from primary generalised epilepsy (36%, p<0.001). No other drug was associated with a particular seizure type. Patients with partial seizures received a higher dose of carbamazepine than those experiencing only generalised fits (means 860mg vs 600mg, p<0.005), although concentrations did not differ significantly between the groups (8.6 mg/L vs 7.7 mg/L). Patients experiencing partial seizures took similar doses of phenytoin (353mg vs 324mg) to those with GTCS but concentrations were higher in the former group (16.7 mg/L vs 13 mg/L, P<0.01). These results are consistent with the accepted wisdom that partial seizures are more difficult to control [Brodie & Porter 1990].

A wide range of concentrations at each daily dosage schedule was demonstrated for all drugs. This is illustrated for CBZ in Figure 2. The time elapsed since the previous dose correlated highly (P<0.001) with the classification of the results into low, "therapeutic" and high values (Figure 3). This effect was not seen with phenytoin or valproate. The distribution of assay results for each anticonvulsant is shown in Figure 4. Thirty-five per-cent of all concentrations were categorised as "unexpected" by the examining physician, and in 43% of these (19.2% of all results) a change in decision regarding management was thought appropriate. In 114 (23.3%) of the 488 patient visits, some alteration of management was brought about in response to the assay result (Table 5). The outcome of each patient visit, regardless of whether or not the assay result affected management, is summarised in Table 6. The proportion of results which were unexpected, or which altered management, did not vary significantly between the three clinicians (Table 7). The tendency for the registrar to anticipate results better than the others was, fortunately,

TABLE 4 Anticonvulsant doses and plasma concentrations (mean ± SD) in 182 actively managed epileptic outpatients

Drug	Number of Patients (Monotherapy)	Daily Dose (mg)	Commonest Daily Dose (mg) (mg/L)	Plasma Concentration (mg/L)
Carbamazepine	106 (69)	743 ± 415	800	8.2 ± 3.8
Phenytoin	59 (36)	336 ± 110	300	16.2 ± 8.2
Sodium Valproate	44 (22)	1220 ± 785	1000	80.9 ± 42
Phenobarbitone	20 (3)	111 ± 45	120	19.0 ± 8.9
Primidone	6 (2)	509 ± 220	50	17.8 ± 9.0*

* Phenobarbitone measured

TABLE 5 Changes in management following anticonvulsant assay at 488 patient visits

Increase in drug dosage	-	57	(11.7%)
Decrease in drug dosage	-	37	(7.5%)
Advice concerning compliance	-	8	(1.6%)
Addition/discontinuation of drug	-	3	(0.6%)
Earlier clinic appointment	-	45	(9.2%)
Later clinic appointment	-	13	(2.7%)
Total visits with one or more changes	-	114	(23%)

TABLE 6 Outcome of clinic visit regardless of whether decision changed

No alteration in management	- 271	(55%)
Increase in drug dosage	- 97	(20%)
Decrease in drug dosage	- 73	(15%)
Change in dose of two drug[s]	- 15	(3%)
Advice concerning compliance	- 12	(2.4%)
Addition of new drugs	- 8	(1.6%)
Admission to ward	- 8	(1.6%)
Stop current drug(s)	- 4	(0.8%)
Total patient visits	488	

TABLE 7 Proportion of results which were unexpected and which altered management - by grading of clinician

	SHO	REGISTRAR	CONSULTANT
PATIENTS SEEN	90	163	244
UNEXPECTED RESULTS	38 (42%)	58 (36%)	109 (45%)
MANAGEMENT CHANGES	24 (27%)	27 (16%)	56 (23%)

TABLE 8 Assay results below, within and above the 'therapeutic' ranges which were followed by a change in anticonvulsant dose

DRUG	BREAKDOWN	LOW	'THERAPEUTIC'	HIGH	TOTAL
CARBAMAZEPINE	All results	33 (12%)	140 (50%)	104 (38%)	277
	Dose changed	22 (67%)	32 (23%)	25 (24%)	79 (29%)
	Up/down	21/1	20/12	9/16	50/29
PHENYTOIN	All results	47 (28%)	76 (45%)	47 (28%)	170
	Dose changed	27 (57%)	23 (30%)	17 (36%)	67 (39%)
	Up/down	20/7	19/4	1/16	40/27
SODIUM VALPROATE	All results	32 (28%)	45 (40%)	36 (32%)	113
	Dose changed	15 (47%)	19 (42%)	10 (28%)	44 (39%)
	Up/down	9/6	10/9	1/9	20/24
PHENOBARBITONE	All results	10 (14%)	58 (81%)	4 (6%)	72
	Dose changed	2 (20%)	9 (16%)	3 (75%)	14 (19%)
	Up/down	0/2	3/6	0/3	3/11
ALL ASSAYS	All results	122 (19%)	319 (50%)	191 (30%)	632
	Dose changed	66 (54%)	83 (26%)	55 (29%)	204 (32%)
	Up/down	50/16	52/31	11/44	113/91

Carbamazepine 4-10mg/L (17-42 umol/L), Phenytoin 10-20mg/L (40-80 umol/L),
Sodium valproate 50-100mg/L (380-700 umol/L), Phenobarbitone 10-40mg/L (40-172 umol/L).
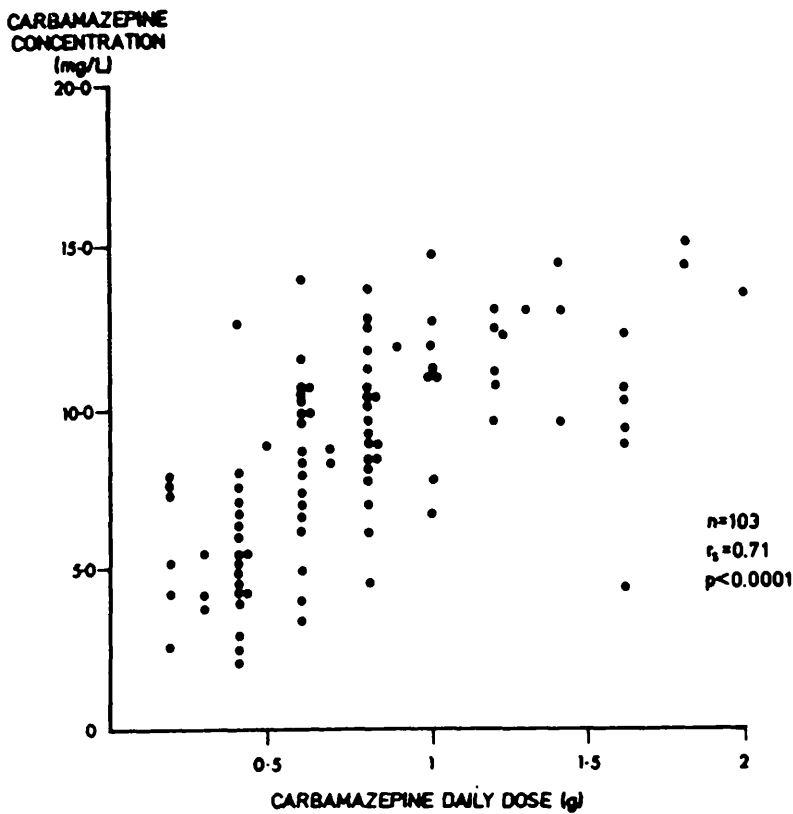
FIGURE 2   Carbamazepine doses and concentrations at the last outpatient attendance in 103 epileptic patients
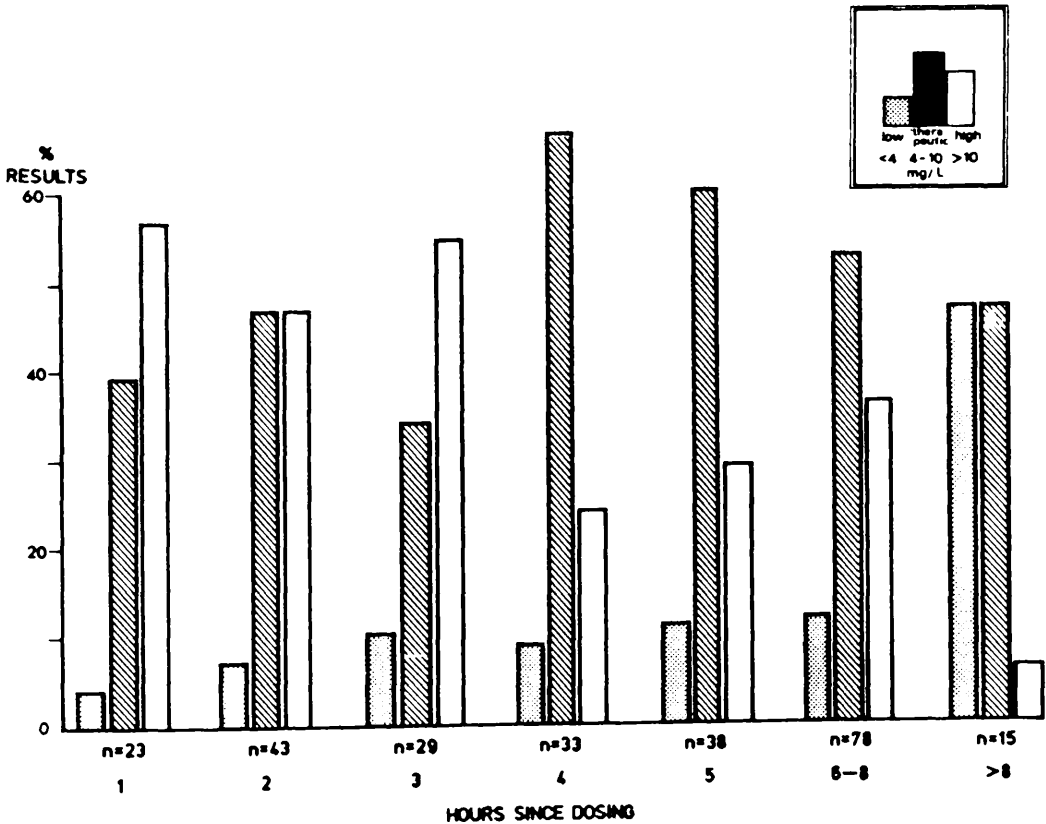
FIGURE 3   Proportion of low, "therapeutic" and high carbamazepine levels at varying times after the previous dose. Mean concentrations fell as the time since previous dose increased (Chi-square, P<0.001; Spearmann ranking, P<0.005)
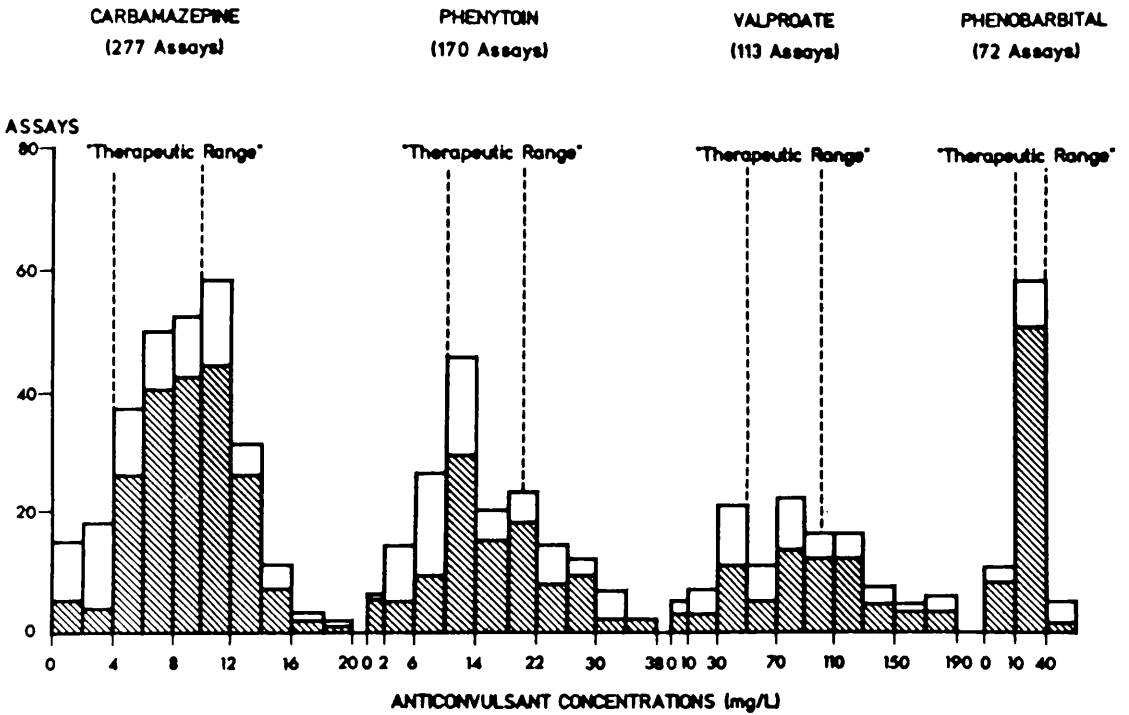
FIGURE 4   Distribution of 632 assays of 4 anticonvulsants. Unshaded areas represent results which were followed by a change in dosage. Putative "therapeutic" ranges are shown.
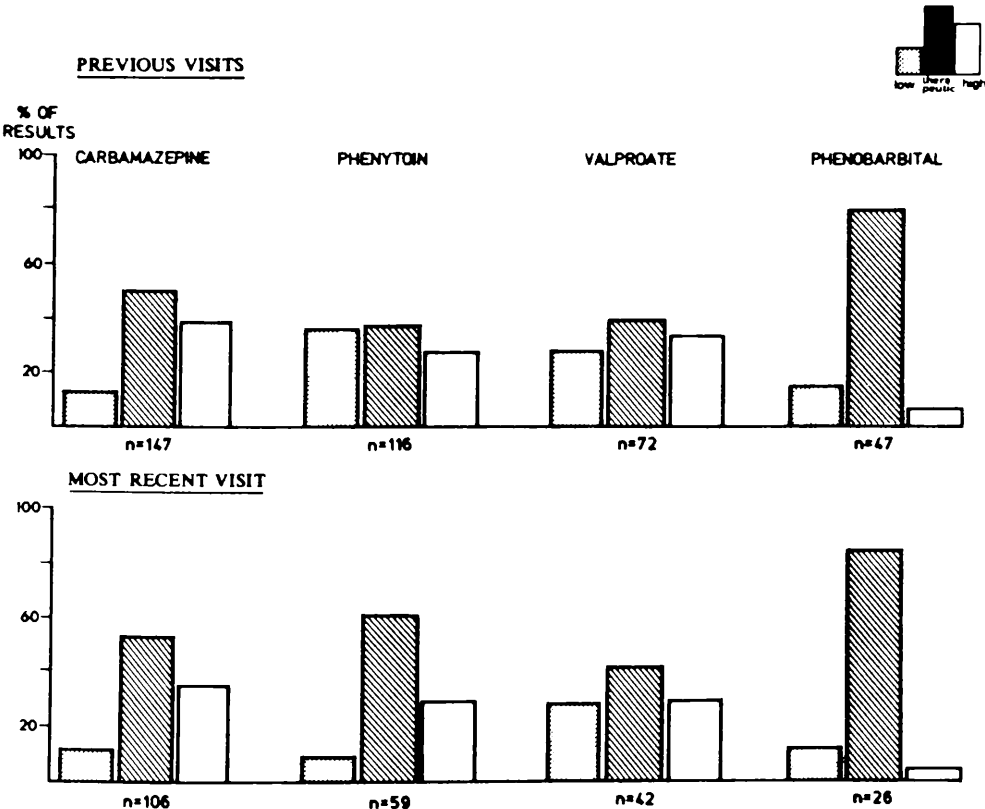
FIGURE 5   Proportion of results below, within and above the "therapeutic" range for carbamazepine (4-10 mg/L), phenytoin (10-20 mg/L), sodium valproate (50-100) mg/L and phenobarbitone (10-40 mg/L) following the patient's last clinic visit compared to previous visits.

not "statistically significant".

Table 8 displays the assay results according to whether they were below, within or above the "therapeutic" ranges for the four anticonvulsants i.e. CBZ 4-10 mg/L (17-42 umol/L), PHT 10-20 mg/L (40-80 umol/L), VPA 50-100 mg/L (350-700 umol/L) and PB 10-40 mg/L (40-172 umol/L) [Brodie & Feely 1988]. With both carbamazepine and phenytoin, a low result was more likely to be followed by a change in dosage than a "therapeutic" ($p<0.001$, $p<0.005$) or high ($p<0.001$, $p<0.05$) result, but there was no difference between the physicians' responses to "therapeutic" and high results. With valproate, the presence of a "therapeutic" range appeared to have even less influence on the likelihood of a change in dose which was recommended after 46% of low results, after 42% of "therapeutic" levels and after 29% of high concentrations.

Distribution of assay results obtained at the last clinic visit was compared with all previous analyses in Figure 5. Only with phenytoin was there a tendency for higher numbers of patients to enter the therapeutic range as the year progressed.

DISCUSSION

These data confirmed "interindividual variation in drug absorption, elimination and distribution" by showing the wide range of concentrations obtained in patients taking the same dose (Figure 2). This is a basic premise for the preferred use of serum concentrations over dosage in the monitoring of drug therapy. However, the striking relationship between CBZ concentration and time-since-dosing shows how a random result could cause confusion, and argues against any rigid adherence to a "therapeutic" range for this drug. In many cases, finding a concentration inside or outside the range

would entirely depend on when the blood sample was taken in relation to the previous dose. Sophisticated allowances for such complications are thus necessary if a target concentration strategy is to be adopted [Whiting et al 1984].

The receipt of an anticonvulsant concentration was followed by an alteration in patient management in 23% of monitored attendances. This was not an automatic consequence of the result, as more than half of the "unexpected" results were not followed by a change in decision. The "therapeutic" range had a smaller influence on decision-making than might be expected. A change in dosage was almost as common after a "therapeutic" level (26%) as after a high level (29%). From Figure 4, it would seem that only with phenytoin did the clinicians adopt, consciously or otherwise, a "target concentration strategy" [Spector et al 1988].

A low result was more likely to precipitate a change in dose (54%) than the other categories. A few of these patients had the dosage decreased, as it was decided to withdraw the drug. Those who were asymptomatic and seizure-free did not have their dose increased. An attempt to bring phenytoin and phenobarbitone from "subtherapeutic" to "therapeutic" levels in well patients has been shown to increase neurotoxicity without affecting seizure control [Woo et al 1988]. Many partially controlled patients in the present study, who would have continued as previously, had their dosage increased after a surprisingly low result. The number of patients (8) in whom poor compliance was identified appeared lower than might be expected. However, this includes only those individuals in whom poor compliance was previously unsuspected and not those known to be erratic in taking their medication.

The diagnosis of anticonvulsant neurotoxicity is a clinical one in the majority of affected individuals. There was little suggestion from these data that drug concentrations were being used to uncover latent sedation or ataxia. In 71% of the "high" concentrations, the

examining doctor considered the result satisfactory and the dose appropriate for the patient. Indeed, of the 55 patients with high results in whom a decision to alter the dose was made, an <u>increase</u> was advised at 11 consultations as the patient's seizure disorder was not fully controlled and there was no clinical evidence of toxicity.

Table 4 suggests a move towards earlier appointments as a result of the TDM. This may be misleading, however, as the physicians were choosing return appointment dates with the knowledge that a result would shortly be available. It is likely that, without TDM, clinicians would be requesting earlier appointments in order to see the patient with an anticonvulsant level to hand. The clinician's initial estimate of time to next appointment is therefore "assuming that I will soon be in possession of the result I expect". The shortened appointment times thus demonstrate the effect of occasional unexpected results, rather than of TDM itself.

This study of on-site anticonvulsant monitoring has demonstrated immediate impact on clinical management in more than 20% of patient visits - a similar figure to a previous small study [Marty et al 1981]. In our clinic, however, we did not seem to follow a "target concentration strategy". Clearly, other factors were taken into consideration before an alternative anticonvulsant dose was recommended. This more subtle effect of drug concentration results on management is incompatible with a "therapeutic range". Our unit has come to prefer the term "target range" though this also remains far from ideal. It does, however, lose some of the inflated "therapeutic" validity which a single anticonvulsant measurement cannot possess. Among epilepsy specialists, this might seem self-evident, however as recently as 1986, a group of experienced neurologists tackling computer-simulated case histories tended to miss ideal treatment regimens owing to a tendency to follow "therapeutic ranges" [Ward 1986]. Meanwhile, in general practice and

general medicine, patients frequently have their drug dosage tailored to achieve "therapeutic levels" rather than therapeutic effects. Hopefully, this is changing. A recent review states "One needs only to determine the 'therapeutic range' for the individual patient" [Dodson 1989]. The same writer continues "changing an epileptic drug dose based only on the drug level is like driving a car looking only at the speedometer". While this may be an overstatement, one would hope that the following quotation from a clinic letter will become a thing of the past:

"I saw E- for a follow-up visit....I am pleased to find that she is now well and symptom-free. I did check a phenytoin level and found this still to be in the toxic range. I telephoned and advised that E-'s phenytoin be cut down to 100mg b.d."

While on-site TDM clearly affects management, does it improve it? Preliminary evidence suggests that the number of clinic visits and assays required to optimise control can be decreased [Patsalos et al 1987, Wing & Duff 1989] and that seizure control might even be improved [Miller et al 1982, Wing & Duff 1989]. However, only small numbers of patients have been monitored, and the popular claim that "therapeutic drug monitoring, used intelligently, is of indisputable value to the individual epileptic" [Mucklow 1982] is not backed up by controlled studies. It can be shown that drug levels can be brought into predetermined ranges [Whiting et al 1984] but the further step that this necessarily improves the lot of the patient remains a presumption. The present study shows that we use TDM only as part of the over-all clinical picture, and this concurs with modern thinking [Chadwick 1987]. But perhaps even this needs testing. No-one as yet seems willing to face the problems of a proper, blinded, controlled study. Drug assays at our own clinic continue, and patients' future performance with regard to frequency of seizures and of attendance will be compared with these baseline data. Results will be partially helpful, but will not fully answer the question which began this paragraph.

CHAPTER 2

PSYCHOMOTOR FUNCTION AND ANTICONVULSANT DRUGS

CHAPTER 2

PSYCHOMOTOR FUNCTION AND ANTICONVULSANT DRUGS

INTRODUCTION

While an individual with epilepsy may have normal or above-average cognitive ability, epileptic patients as a whole tend to show impairment in memory, learning capacity [Hirtz & Nelson 1985] and academic achievement [Seidenberg et al 1986]. Intelligence may also be lowered, but that in "non-institutionalised" people with epilepsy usually approaches normal [Bourgeois et al 1983]. The relative importance of clinical and subclinical seizures [Kasteleijn-Nolst Trenite et al 1988, Siebelink et al 1988], underlying cerebral damage, and psychosocial problems is difficult to establish [Smith et al 1986, Dodrill 1986]. Anticonvulsant drugs are another complicating factor. Subtle changes in "psychometric" or "cognitive" function have been suggested to occur with all AEDs. In healthy subjects, limited treatment with phenytoin [Thompson et al 1981], carbamazepine [Macphee et al 1986a, Trimble 1987] and sodium valproate [Thompson & Trimble 1981] have been shown to impair memory, motor speed and mental processing at low dosing.

The relative psychomotor toxicity of the major AEDs when used in therapeutic doses is becoming clearer. Sedation with phenobarbitone and primidone was almost universal, so the introduction of phenytoin was an obvious improvement [Jones & Wimbish 1985], while CBZ was also clearly better than phenobarbitone in children [Schain et al 1977]. Recently, more sophisticated testing of psychomotor and cognitive function suggests that CBZ is superior to phenytoin [Dodrill & Troupin 1977, Andrewes et al 1986, Smith et al 1986, Gallassi et al 1988] and confirm both CBZ and phenytoin as improvements on barbiturates [Meador et al 1990]. These papers used a variety of tests of memory (digit

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recall, word list and prose memory), motor function (reaction time, finger tapping), decision making (choice reaction time) and tracking to investigate the cognitive side-effects of these drugs. These tests (of which the relevant ones will be further described later) are discussed in the individual papers and in the extensive review of Hindmarch [1980]. Using this battery of tests, some subtle differences have emerged, suggesting that the superiority of CBZ over PHT is more marked with regard to memory and learning [Evans & Gualtieri 1985, Andrewes et al 1986] than with purely motor function [Evans & Gualtieri 1985, Dodrill & Troupin 1977].

Studies with VPA have revealed only slight slowing of cognitive processing [Thompson & Trimble 1981] or reaction times [Sommerbeck et al 1977] and some workers have failed to show any deficit. [Harding et al 1985]. Its superiority to phenobarbitone has been confirmed [Vining et al 1987]. The assertion of Evans & Gualtieri [1985] that "CBZ appears to have the least harmful neuropsychological consequences of all the antiepileptic drugs, with the possible exception of valproic acid" still holds true, and these two drugs remain inseparable. One point of general agreement is that polypharmacy with more than one AED tends to produce a greater increase in sedative side-effects than in anticonvulsant efficacy [Morris et al 1987, Reynolds 1983]. This has led to a move towards monotherapy [Shorvon et al 1978, Thompson & Trimble 1982b] often producing less toxicity with no loss of effect or even increased efficacy [Shorvon & Reynolds 1979, Ludgate et al 1985]. A recent study in mice, however [Bourgeois 1988], suggested that this should not be assumed for all AED combinations, and showed that while VPA and PB had additive neurotoxic effects, VPA together with CBZ produced less problems. Some combinations may have a better therapeutic ratio than others.

Our unit has taken a particular interest in CBZ, as an anticonvulsant which is coming more and more into use world-wide. Although it is among the least sedative of the AEDs,

the evidence for a degree of psychomotor impairment is persuasive. A single dose of 600mg CBZ causes detrimental changes to many parameters in healthy subjects [Macphee et al 1986a], while an additional 400mg at night causes similar problems in patients stabilised on the drug [Macphee et al 1986b]. However, these studies only show acute effects, and it is possible that tolerance to psychomotor impairment developes. Studies have been performed which show impairment with chronic dosage, but here a common drawback is the use of normal healthy subjects as the only controls [e.g. Stores et al 1978, Tomson et al 1988, Tedeschi et al 1989] so the effect of epilepsy itself is not excluded. An attempt to delineate, if not overcome, this problem is made in EXPT 2.

Another aspect of psychomotor impairment with CBZ is the effect of diurnal fluctuation of blood concentrations. In our unit, daily fluctuation {(peak - trough concentration)/mean concentration x 100%} on twice-daily CBZ has been measured as 59% [Macphee et al 1987]. This occurs despite an initial half-life of CBZ which exceeds 24h, since autoinduction of metabolism lowers the half-life to approximately 12h with monotherapy, and further with polypharmacy [Eichelbaum et al 1985]. Hoppener and his colleagues [1980] found a similar degree of fluctuation and showed that neurological side-effects which did not relate to morning blood concentrations could be shown to correlate with fluctuating levels. The findings of Riva et al in 1984 were similar, while recent sophisticated analysis of saccadic eye movements [Tedeschi et al 1989] have confirmed the correlation. Hoppener suggested that thrice-daily dosing of CBZ should be mandatory to help reduce fluctuation, but this may reduce compliance. Riva thought his findings "should stimulate the pharmaceutical industry toward the development of CBZ preparations with more desirable pharmacokinetic properties." A controlled-release preparation might diminish side-effects, allow higher steady-state concentrations and improve compliance by allowing twice-daily dosage for all patients. The pharmacokinetics of such a preparation are assessed in this chapter.

EXPT. 2   PSYCHOMOTOR FUNCTION IN EPILEPSY PATIENTS AND CONTROLS

AIM

Using a battery of psychomotor and cognitive function tests, to compare the performance of epilepsy patients on therapy with that of untreated patients and healthy controls.

MATERIALS AND METHODS

This study was well under way before my arrival in the unit. Patients were recruited by Drs Brodie and Macphee. Psychomotor testing was performed by E. McPhail, and an alternative view of the data forms part of her MSc thesis.

Sixty-six patients with GTCS or complex partial epilepsy took part (Table 9). Patients exhibiting neurological deficit or poor control were excluded, and no patient was studied within 48 hours of a seizure. None had reported more than two seizures per month over the previous three months. Fifty-four patients were taking a single anticonvulsant (Table 10) and 12 took two or more AEDs. Drug dosage had remained unaltered for the previous three months in all patients, and none was taking any other drug known to impair psychomotor function. All of the polypharmacy group took CBZ (mean daily dose 920 mg, mean daily concentration 7.5 mg/L) together with one or more additional AED (3 each PHT and PB; 2 primidone, VPA and clobazam; 1 clonazepam). A 10 ml venous blood sample was taken into a heparinised tube at the time of testing for measurement of circulating anticonvulsant concentrations.

TABLE 9 Clinical details of control subjects and treated and untreated epileptic patients undergoing psychomotor function testing

Group	n	Male	Female	Age (years) (range)	Duration of disease (years) (range)
Controls	11	4	7	26.4 ± 5.1 SD (22-39)	-
Untreated	14	7	7	26.6 ± 3.2 SD (16-41)	3.7 ± 5 (0.25 - 14)
Treated	66	35	31	26.3 ± 7.5 SD (13-45)	9 ± 8.5 (0.5 - 36)

TABLE 10 Drugs taken as monotherapy by treated epileptic patients undergoing psychomotor testing

Drug	No. of patients	Mean dose (mg/day)(range)	Mean concentration (mg/L) (range)	Target range (mg/L)
Carbamazepine	30	720 (200-2000)	7.9 (3.1 - 14)	4 - 10
Phenytoin	15	360 (200-500)	18 (6.3 - 32.2)	10 - 20
Sodium valproate	9	1350 (400-3000)	56 (30 - 117)	50 - 100

TABLE 11 Distribution of centrencephalic and focal EEG findings

	Localised	Centrencephalic
Carbamazepine	17	6
Phenytoin	7	2
Valproate	2	6
Polypharmacy	7	3
Untreated	2	5

Fourteen patients who had been diagnosed as having epilepsy on the basis of more than one witnessed seizure, but who had been prescribed no anticonvulsant therapy for at least three months, were included as the untreated group. A further 11 age-matched healthy subjects were recruited from the staff of the Western Infirmary to act as controls. All but four epileptic patients had an electroencephalogram (EEG) result available. Thirty-five showed a lateral focus, 22 showed "centrencephalic" epilepsy, 7 were normal, 6 showed indeterminate "slowing" and 5 had some other abnormality. The distribution of localised and centrencephalic epilepsy among the treatment groups is shown in Table 11.

Psychomotor Testing

Psychomotor testing was performed at the same time (1400h-1600h) and in the same lighting conditions with all patients. They performed two initial complete runs to allow acclimatisation to the requirements of the series prior to completing the tests on a further three occasions. A mean value was obtained for each test. The tests employed are outlined below.

1. Critical Flicker Fusion Threshold (CFFT) was carried out using the Leeds Psychomotor Tester and involved the patients' appraisal of the frequency above which flickering diodes appeared as a constant light. The frequency at which the light "changed" was noted in three tests of increasing frequency (CFFTi) and of decreasing frquency (CFFTd). The mean of both tests was also calculated (CFFTm). This test assesses "integrative function", the aspect of coordination of sensory and motor function which is least affected by personality and motivation. CFFT is considered the best way of assessing drug-induced changes in this modality [Hindmarch 1980].

2. Choice Reaction Time (CRT), also performed with the Leeds Psychomotor Tester, measured the patient's reaction time in recognising a flash from one of six lights and touching one of six buttons equidistant from his index finger to extinguish it. A mean of thirty tests was recorded for recognition time (the time to lift the finger from its starting point - CRT1), movement time (CRT2) and total reaction time (CRT). This test assesses sensorimotor performance [Hindmarch 1980].

3. "Simple Simon" memory game involved the use of a commercially produced toy (M & B Games) which generates a randomised colour sequence of increasing length which the subject is asked to repeat. This tests short-term memory in a fashion similar to the digit-span technique [Wechsler 1972] but has no bias due to variable numeracy of patients and control subjects or the investigator's intonation of number sequences.

4. Finger tapping rate, using the index finger of the dominant hand, was determined over 60 seconds on an electronic calculator keyboard set in constant addition mode. This is a measure of pure motor function of the simple "ballistic" type [Hindmarch 1980].

5. Card sorting of a shuffled pack of conventional playing cards into aces, kings etc. on a table-top was employed as an over-all assessment of cognitive function - an "excellent example of a performance task which embraces sensory, motor and central components" [Hindmarch 1980].

Assays and statistics

All plasma concentrations of CBZ, PHT and VPA were measured immediately by enzyme immunoassay (Emit Syva). Comparisons between patient groups were made using the Mann-Whitney 'U' test. Correlations between age, duration of epilepsy and AED concentrations with psychomotor performance were obtained using the Spearmann

ranking procedure.

RESULTS

The mean and ranges for the performances of all tests in each group are shown, along with significance testing, in Table 12. Means ($\pm$ 2SEM) are shown graphically for the three components of choice reaction time in Figure 6, and of the other discriminatory tests in Figure 7. The untreated epileptic patients performed less well than control subjects in CRT1, CRT2, Simple Simon sequencing and card sorting. Their performance, however, was superior to treated patients in the same tests (except for CRT2 and finger tapping). There were highly significant differences between the healthy controls and treated patients for all tests with the exception of CFFT which did not distinguish between any of the groups.

No significant difference in psychomotor performance between the patients making up the four different treatment groups (CBZ, PHT, VPA, polypharmacy) was found for any test (Figures 8 & 9 show the results for CRT1 and finger tapping). There was no significant correlation between drug concentration and performance in any test for any of the monotherapy groups. Similarly, circulating CBZ (Fig 10; n=29, r_s=0.1), PHT (n=15, r_s=0.06) and VPA (n=9, r_s=0.04) correlated poorly with an "overall ability score" obtained by summing the ranks in the four discriminating tests (CRT, SS, CS, finger tapping) and listing the patients in order of decreasing performance. The 14 monotherapy patients above the "target" ranges performed no differently from the 39 within or below the range for each drug (Fig. 11). There was no overt association between overall ability score and age (r_s=0.08) or duration of epilepsy (r_s=0.15) in the treated patient group.

TABLE 12 Mean performance (and range) of subject groups in each psychomotor test

	Controls	Untreated epileptics	Treated epileptics
$CFFT_i$ (Hz)	28.5 (21-31.6)	28.4 (24.7-31.5)	27.5 (18.9-38.4)
$CFFT_d$ (Hz)	31 (26.6-38.6)	29 (24.3-34.5)	29.2 (20.8-40.9)
$CFFT_m$ (Hz)	29.8 (25.6-34.7)	28.7 (24.4-32.4)	28.4 (22.3-36.7)
CRT_1 (s)	0.36 (0.31-0.41)	0.44[c] (0.33-0.60)	0.52[de] (0.35-1.0)
CRT_2 (s)	0.18 (0.12-0.23)	0.28[a] (0.14-0.47)	0.27[d] (0.13-0.48)
CRT (s)	0.53 (0.44-0.64)	0.72[b] (0.46-1.1)	0.78[d] (0.54-1.5)
SS (sequence length)	10.4 (7-17)	7.6[a] (5-11)	6.9[de] (4-11)
CS (s)	50.1 (36-62)	63.1[b] (44-91)	75.1[df] (46-117)
FT taps/minute	370 (310-425)	344[a] (239-476)	302[de] (140-413)

CFFT = Critical flicker threshold (i = increasing, d = decreasing, m = mean)
CRT   = Choice reaction time (1 = recognition, 2 = movement)
SS   = 'Simple Simon' memory game
CS   = Card sorting
FT   = Finger tapping

Comparisons were made using the Mann-Whitney U test
Controls versus untreated patients : [a] = $P<0.05$, [b] = $P<0.01$, [c] = $P<0.001$
Controls versus treated patients : [d] = $P<0.001$
Untreated versus treated patients : [e] = $P<0.05$, [f] = $P<0.01$

RECOGNITION TIME   MOVEMENT TIME   CHOICE REACTION TIME
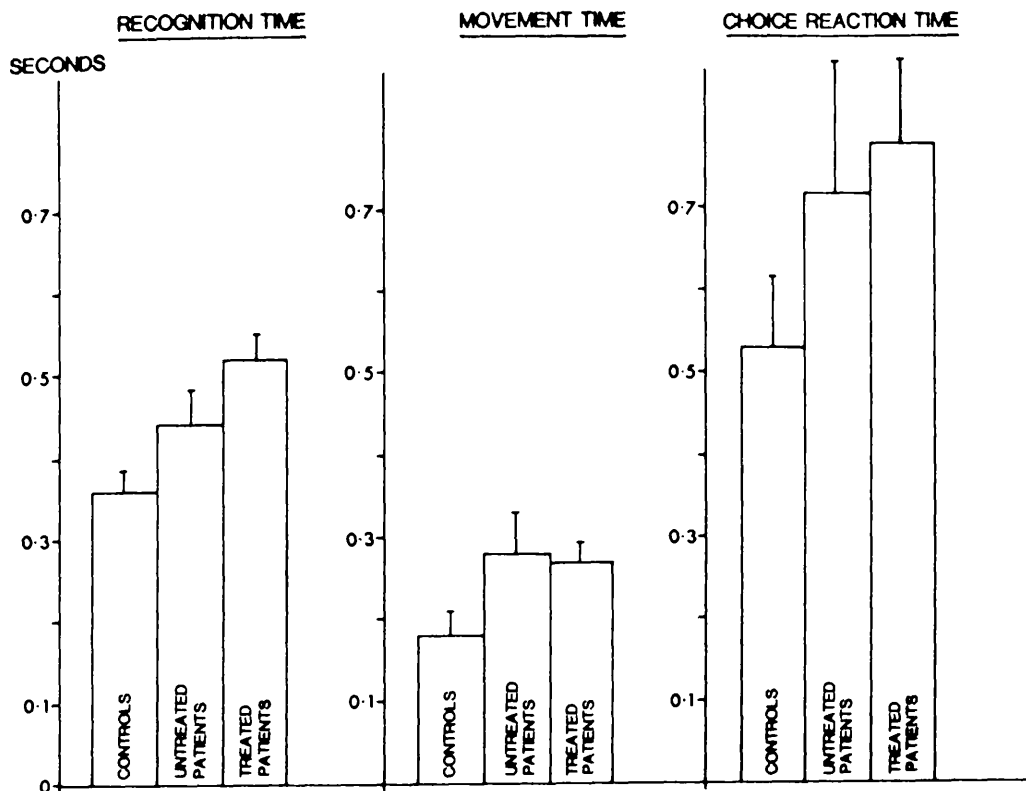
FIGURE 6   Choice reaction time and its two components in controls, untreated patients and treated patients. Values are mean ± 2 SEM. Significant differences were found between controls and untreated patients for all tests (P<0.05 - P<0.001), but only recognition time shows a difference between treated and untreated patients (P<0.05).

CARD SORTING   SIMPLE SIMON   FINGER TAPPING
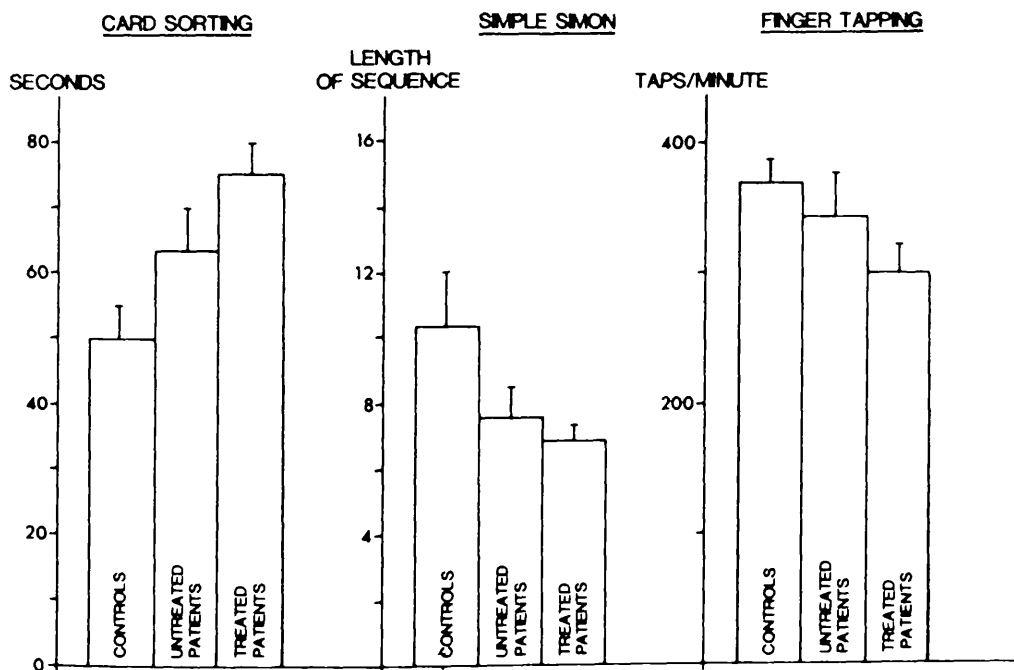
FIGURE 7   Card sorting, Simple Simon sequences and finger tapping rates in the three groups. Values are mean ± 2SEM. Controls differ from untreated patients in all tests (P<0.05 - P<0.01), while treated patients show further deterioration in performance in all tests (P<0.05-P<0.01).
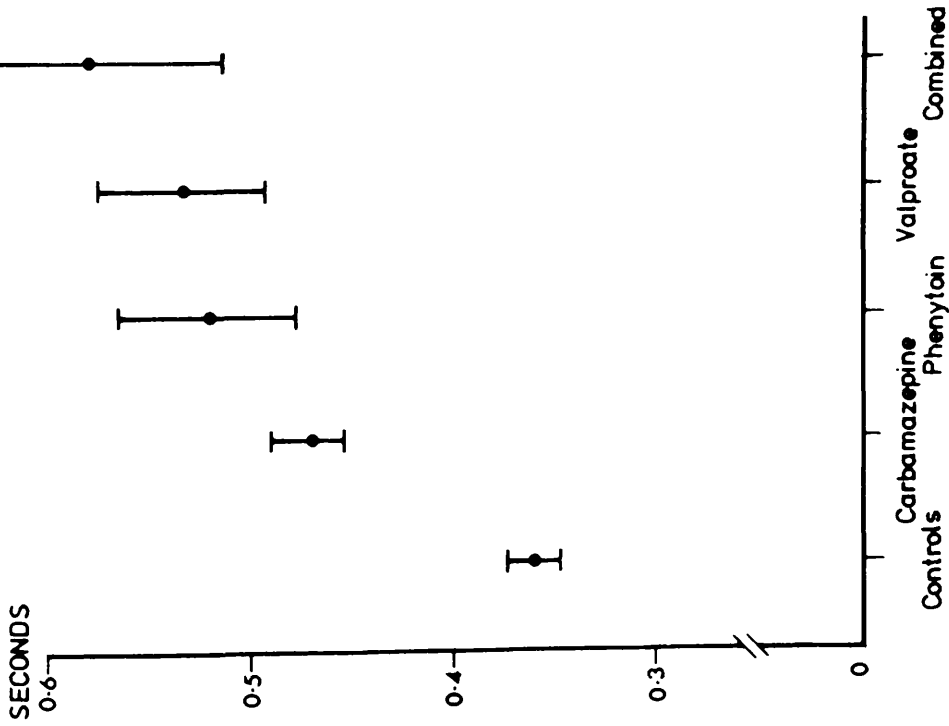
FIGURE 8   Recognition time in controls and patients on different AEDs. Values shown are mean ±SEM. Each drug group is significantly slower than controls.
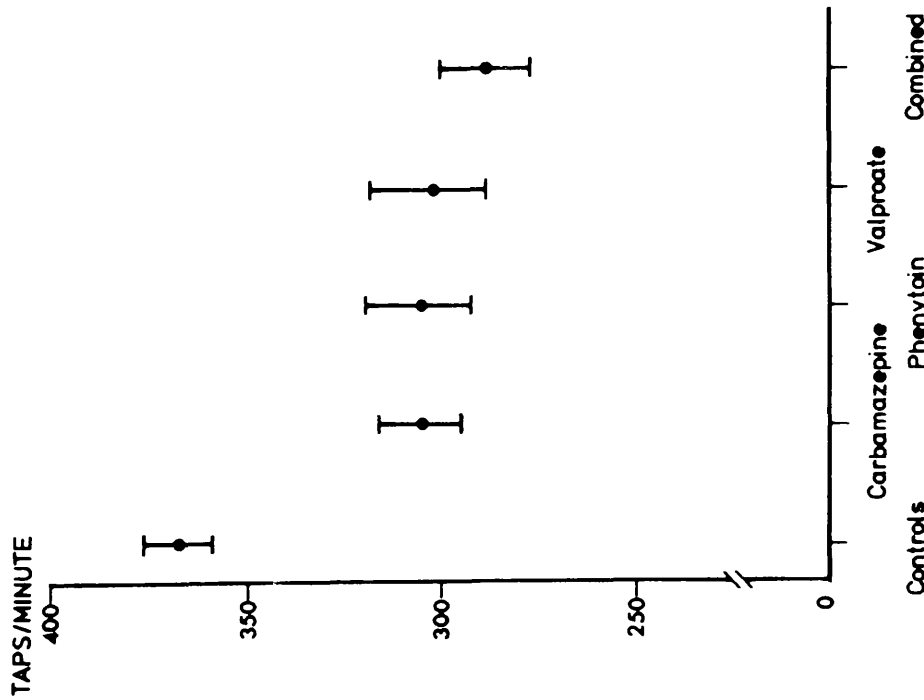
FIGURE 9   Finger tapping rate in controls and patients on different AEDs. Values shown are mean ±SEM. Each drug group is significantly slower than controls.

FIGURE 11 Lack of difference in overall ranking (CRT,SS,CS,FT) between patients with "toxic" serum levels of AED (i.e. above "therapeutic range") and those within the range.

FIGURE 10 Lack of correlation in 29 patients on monotherapy between carbamazepine concentration and overall ranked performance obtained by summating the four discriminating psychomotor tests.
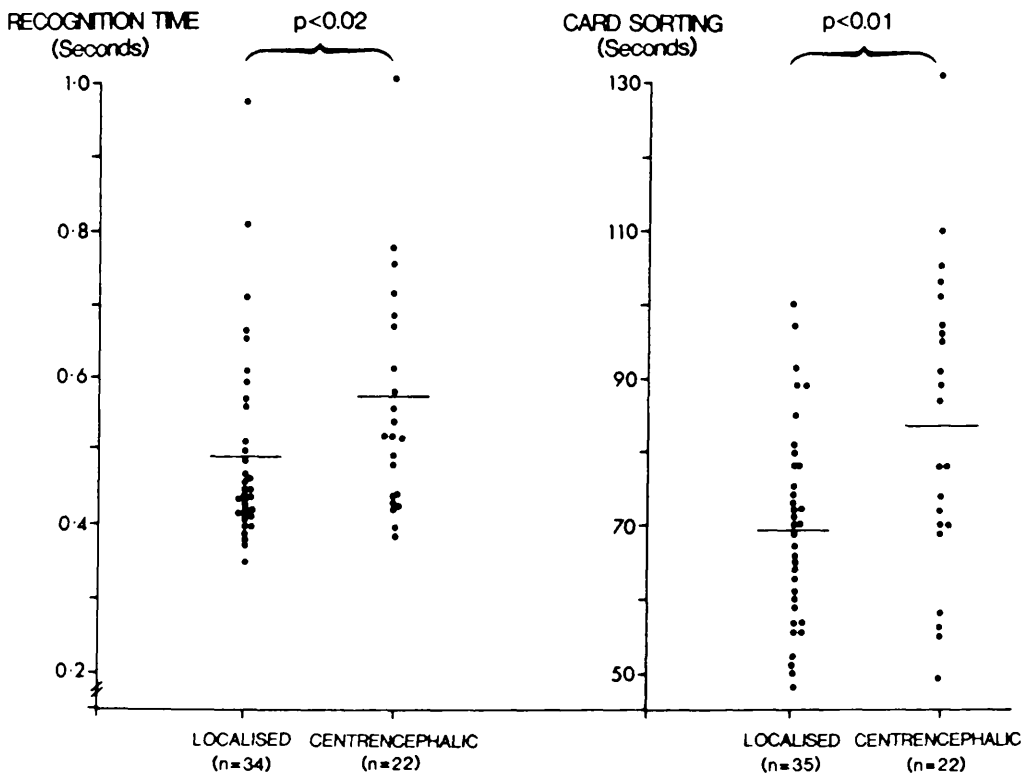
FIGURE 12   Recognition (CRT1) and card sorting times in patients with a localised EEG abnormality, and those with centrencephalic epilepsy.

Patients with centrencephalic epilepsy (n=22) demonstrated longer CRT1 (P<0.02) and card-sorting times (P<0.01) than those with discrete left- or right-sided EEG abnormalities (n=35; Figure 12). No significant differences in anticonvulsant concentrations were found between these two groups.

DISCUSSION

This study demonstrates impaired psychomotor/cognitive function as measured by three simple tests (CRT, "Simple Simon", card sorting) in epileptic patients on no medication compared with age-matched controls. A further reduction in function in these assessments (plus finger tapping) was apparent in a larger group of patients on chronic anticonvulsant medication. Although IQ, educational status and work experience were not considered, it is unlikely that these would have an important influence on the performance of these simple tests.

Young patients with good control were chosen to reduce the possible detrimental effect of age and of seizure activity on performance. These findings suggest that both epilepsy itself and AEDs contribute to the impairment of psychomotor/cognitive function. The failure of the CFFT to discriminate between the groups may be due to the lack of any "normal value" for this test, whose most useful function is to document serial changes in performance. A study published since this work was performed also failed to show any difference in CFFT between CBZ-treated patients and controls [Tomson et al 1988]. Meanwhile an American study performed about the same time did show a poorer performance at finger tapping, CRT1 and some other tests in untreated epileptic patients compared to controls [Smith et al 1986]. In the present study, the epilepsy in the untreated group was undoubtedly less severe than in the patients on drug therapy, so the study only goes some way towards separating the deleterious effects of AEDs and

epilepsy itself. What it does do is convincingly demonstrate the difference between untreated patients and the normal population, and promote caution in interpreting comparisons with healthy controls seen in other studies.

Another interesting feature is the lack of difference between untreated and treated patients with regard to the "movement phase" (CRT2) of the choice reaction time. Both groups are clearly worse than controls, but there is no further deterioration with treatment, in contrast to the effects on recognition time (CRT1). Perhaps we are seeing a degree of divergence between the neurotoxic effects of the drugs and of epilepsy itself.

The failure to demonstrate the "pecking-order" of psychomotor effects with the different AEDs is perhaps not surprising. Inter-subject variability may lead to the missing of effects in individual patients. These results also emphasise the subtlety of any differences between the drugs. Only since this work was completed have large studies with multiple testing [Vining et al 1987, Gallassi et al 1988, Gallassi et al 1990] or crossover designs [Meador et al 1990] shown convincing differences. The studies by Gallassi and his colleagues are probably the best evidence for psychomotor impairment with chronic carbamazepine and valproate treatment, as they show the detrimental effects gradually resolving following drug withdrawal.

The poorer performance in recognition and card sorting times in patients with centrencephalic epilepsy compared with those having focal EEG abnormalities was unexpected, particularly since a disproportionate number of the centrencephalic epilepsies were in what we would expect to be the "better" groups - VPA and untreated. It is generally accepted that patients with temporal lobe foci have poorer memories [Delaney et al 1980], reduced attention span [Stores et al 1978] and mental slowing [Halstead 1957]. Generalised spike-wave activity on the EEG has also been associated

with decreased intellectual performance [Goode et al 1970]. The present findings are at variance with this general trend.

The poor correlation between psychomotor impairment and anticonvulsant concentrations over a wide range of values was of interest. The suggestion that impairment is not confined to those patients at or above the upper limit of the target ranges is perfectly compatible with a previous finding that toxicity relates to concentration in the individual patient [Macphee et al 1986b]. The fact that this does not hold on a population basis supports our own view of TDM which came through in the previous chapter. Each individual has his or her own "therapeutic" and "toxic" ranges, and the population studies should be seen only as a guideline.

EXPT. 3   PSYCHOMOTOR FUNCTION IN NEW PATIENTS GIVEN

CARBAMAZEPINE PROSPECTIVELY - A BRIEF REPORT

AIM

To assess prospectively the effect on psychomotor function of starting and continuing

AEDs in patients recently diagnosed as having epilepsy.

METHODS

The previous experiment addressed the major problem with long-term studies - healthy

subjects as sole controls. Short-term studies with volunteers have shown psychomotor

impairment with CBZ [Macphee et al 1986a]. To ascertain the relevance of this to

chronic dosage I began seeing new patients before and after they commenced CBZ

200mg b.d. or VPA 200mg t.d.s. Psychomotor testing was performed as in the previous

study (except that a more conventional digit recall test [Weschler 1972] was substituted

for the 'Simple Simon' test) at 0, 1, 2, 4 and 12 weeks. A visual analogue scale 'alertness

score' was also included, where a patient marked a 10cm. line which ranged from "wide

awake" to "almost asleep". Tests were always performed between 1330h and 1500h, and

blood was obtained for measurement of serum anticonvulsant concentrations. Dose of

drug was increased if previous level was below "target range".

Recruitment was slow and a range of problems including variable compliance resulted in

a high drop-out rate. The study was discontinued when the Unit commenced a trial of a

novel anticonvulsant in new patients. At this stage, there were insufficient data on

patients taking VPA for meaningful analysis, but satisfactory longitudinal results from 13

patients taking CBZ were available for paired comparisons (Wilcoxon-rank).

RESULTS

Critical Flicker Fusion Threshold, digit recall and card sorting showed no significant changes from baseline at any time point. Total CRT (Fig. 13) increased after one week of CBZ (P<0.05) then returned to baseline levels. Finger-tapping similarly decreased after one week (P<0.001) before gradually recovering (Fig. 13). Figure 13 also shows the slight drop in serum CBZ concentrations over the twelve weeks.

DISCUSSION

It was impossible to study the planned number of patients for this study, and the information to be gained from it is therefore limited. It does seem to demonstrate, however, the development of tolerance to the detrimental effects of CBZ, as shown with finger tapping and CRT. A previous report of impaired CRT at one month with CBZ [Smith et al 1986] may have missed a subsequent improvement. Enzyme induction, with lowering of serum concentrations of CBZ, may play a part in the improvement, and the increased dosage in two patients, although almost maintaining "mean" concentrations, may not be adequate compensation. However, patients' subjective complaints suggested that maximum sedation took place in the first few days - before steady-state levels are reached. Unfortunately such alacrity of tolerance was not expected, and psychomotor tests were performed at the attainment of steady-state (one week), perhaps missing the initial effects. Such tolerance would explain the difference between the obvious psychomotor effects of CBZ in volunteer studies [Macphee et al 1986a] and the more
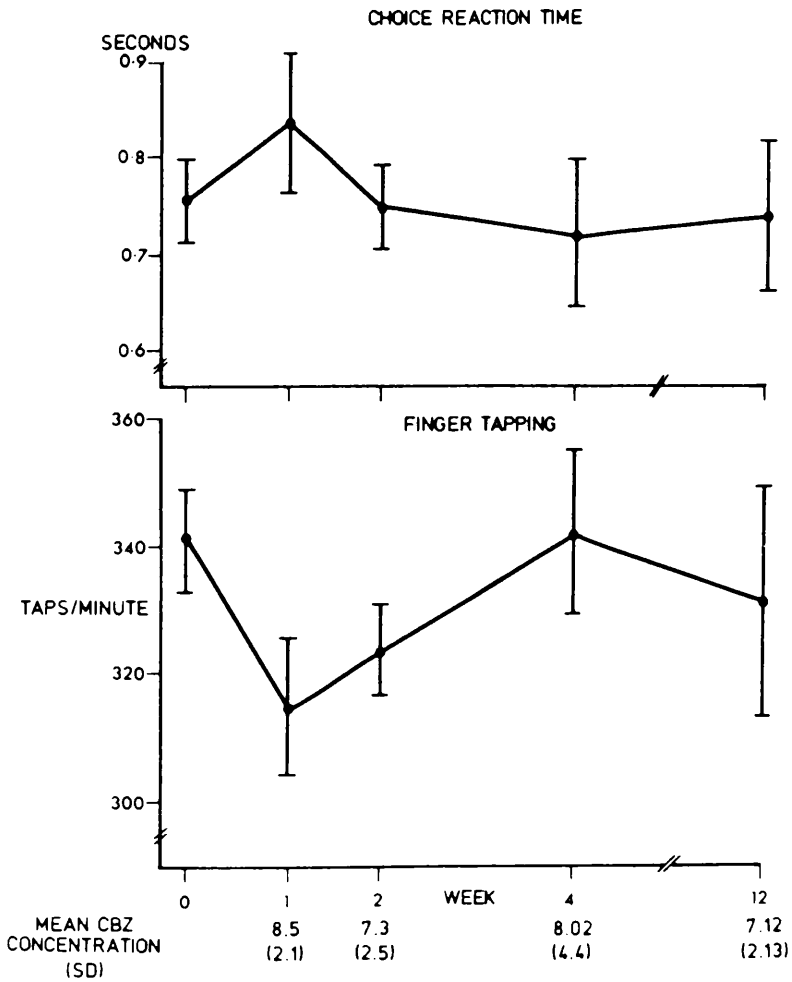
FIGURE 13   Changes in total CRT and finger tapping rate in 13 patients taking carbamazepine for 12 weeks. Values are mean ± SD. Both CRT (P<0.05) and finger tapping (P<0.001) deteriorate after one week, but recover to previous levels.

subtle impairment in patients taking the drugs long-term [Andrewes et al 1986, Aman et al 1990].

EXPT. 4   CONVENTIONAL AND CONTROLLED-RELEASE
          CARBAMAZEPINE IN HEALTHY SUBJECTS

AIM

To compare the pharmacokinetics of a controlled-release preparation of carbamezepine (Tegretol Retard, Ciba-Geigy, CBZ-CR) and conventional carbamazepine (Tegretol, CBZ-C).

METHODS

Eight healthy volunteers (seven male, one female; aged 25-47 years) took part in a double-blind, balanced cross-over study. No concurrent medication was taken during the study. A minimum wash-out period of one month was considered adequate between the two legs, each of which lasted three weeks, as enzyme induction has previously been found to abate two weeks after discontinuing CBZ [Rapeport et al 1983]

Single Dose

Following a single 400mg dose of CBZ-C or CBZ-CR, blood was drawn 0, 2, 4, 6, 8, 10, 14, 24, 32, 48 and 56h later for measurement of circulating CBZ, free CBZ and CBZ-10,11-epoxide (CBZ-E) concentrations. Psychomotor function tests were performed just prior to CBZ ingestion and 10h later, equivalent to the expected time to peak concentration [Evans & Gaultieri 1985]. Baseline antipyrine kinetics were also assessed 48h before CBZ ingestion.

Chronic dosing

Subjects took 200mg of the appropriate preparation at 0900h and 2100h daily for two weeks finishing with the 29th dose at 0900h on the 15th day. Tablet counting ensured compliance. Blood was withdrawn for drug analysis after the first and fifteenth (eighth day) doses at 0, 2, 4, 6, 8, 10 and 12h for "dosage interval" concentrations and again after the 29th dose (15th day) at times similar to those employed in the single-dose phase.

Psychomotor function tests were performed on days 1, 8 and 15 of each leg at 0, 3, 6 and 10h after the morning dose. The tests used were the same as those in EXPT. 3. Antipyrine kinetics were repeated on the eighth and fifteenth days of CBZ administration.

Assays, Kinetics and Statistics

Blood samples were centrifuged immediately after withdrawal and the serum was stored at $-20^\circ C$ for batch analysis. Total and free CBZ, CBZ-E and antipyrine were assayed as outlined in the Recurrent Methods section of the introduction to this thesis.

Antipyrine and CBZ kinetic parameters were also performed as outlined earlier. Diurnal fluctuation was defined as the difference between maximum and minimum concentrations expressed as a percentage of the mean [Riva et al 1984]. Comparative statistics were performed with the Wilcoxon matched-pairs signed-ranks test and confidence intervals were calculated using the Mann-Whitney confidence interval test.

RESULTS

Pharmacokinetics

Following a single dose of CBZ-CR, CBZ concentrations plateaued around 60% of the peak obtained with CBZ-C (Figure 14). Steady-state concentrations were slightly lower with CBZ-CR at 29 days (Figs. 15, 16) but this difference was not statistically significant with respect to either trough or average concentrations. Free CBZ levels followed a similar pattern to total CBZ (Fig. 17). The percentage protein binding was the same with both preparations, the mean (SD) values at each time point after the 29th dose ranging from 73.9 (4.5)% to 75.2 (2.4)% on CBZ-C and from 74.4 (2.7)% to 75.7 (2)% on CBZ-CR. CBZ-E levels on CBZ-CR were lower than on CBZ-C but not significantly so (Fig. 18).

Bioavailability

The area under the concentration-time curve for a dosage interval (AUC 0-12h) was calculated following the 15th and 29th doses (Fig. 19). After the 15th dose, mean results (SD) were CBZ-C 84 (17) mgh/L and CBZ-CR 85 (15) mgh/L (95% confidence interval {CI} for the difference +16 to -20). After the 29th dose, the AUC values were 80 (16) mgh/L and 75 (16) mgh/L respectively (95% CI for difference +23 to -12).

Fluctuation

Diurnal fluctuation of CBZ concentrations around the mean at steady-state (Fig. 20) was significantly less with CBZ-CR (12%) than with CBZ-C (24%, $P<0.025$, 95% CI difference +4% to +18%). The maximum increase in concentration in any 2h period is shown for each individual subject on the two preparations in Fig. 21. This was also lower with CBZ-CR ($P<0.02$). Decreased fluctuation of free CBZ and epoxide concentrations
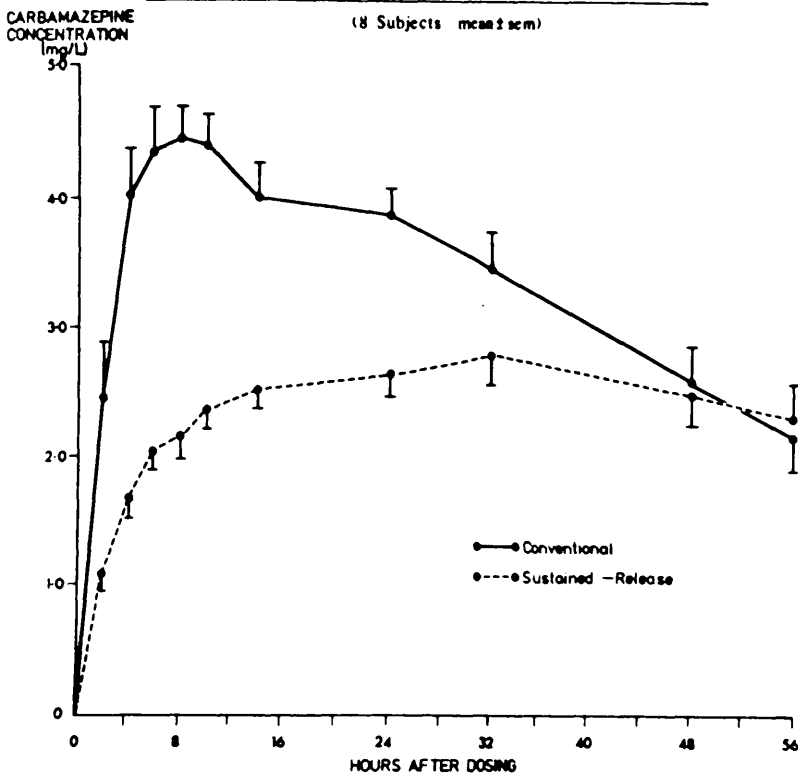
FIGURE 14   Mean serum drug concentration-time curves (± SEM) following a single 400mg dose of carbamazepine in eight subjects receiving conventional and controlled-release (sustained-release) preparations.
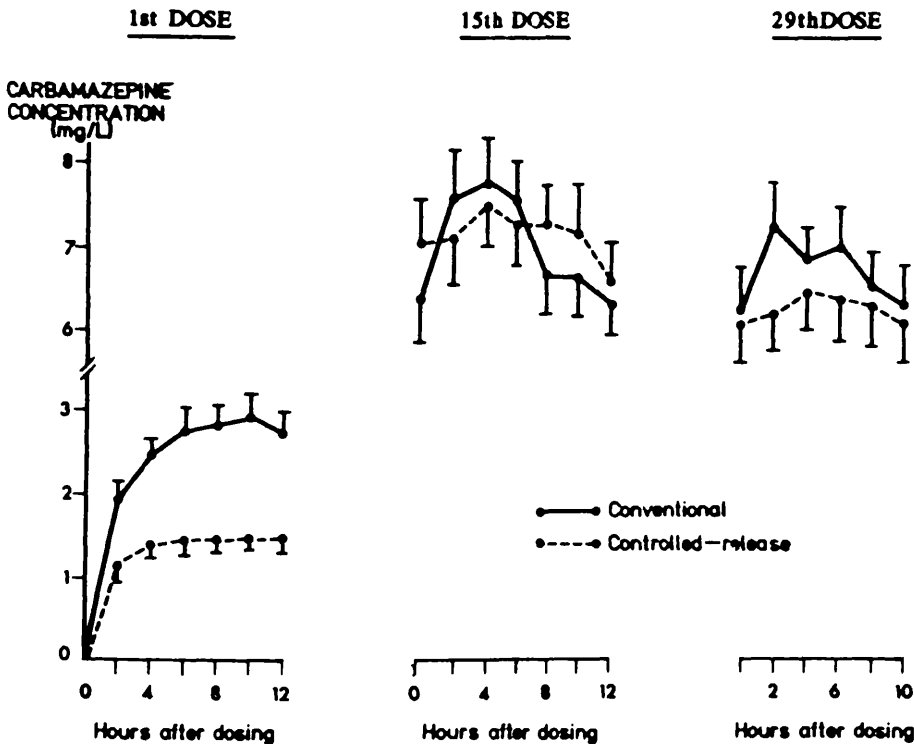
FIGURE 15   Mean diurnal serum drug concentration-time curves (± SEM) in eight subjects after 1st, 15th and 29th dose of conventional and controlled-release carbamazepine.
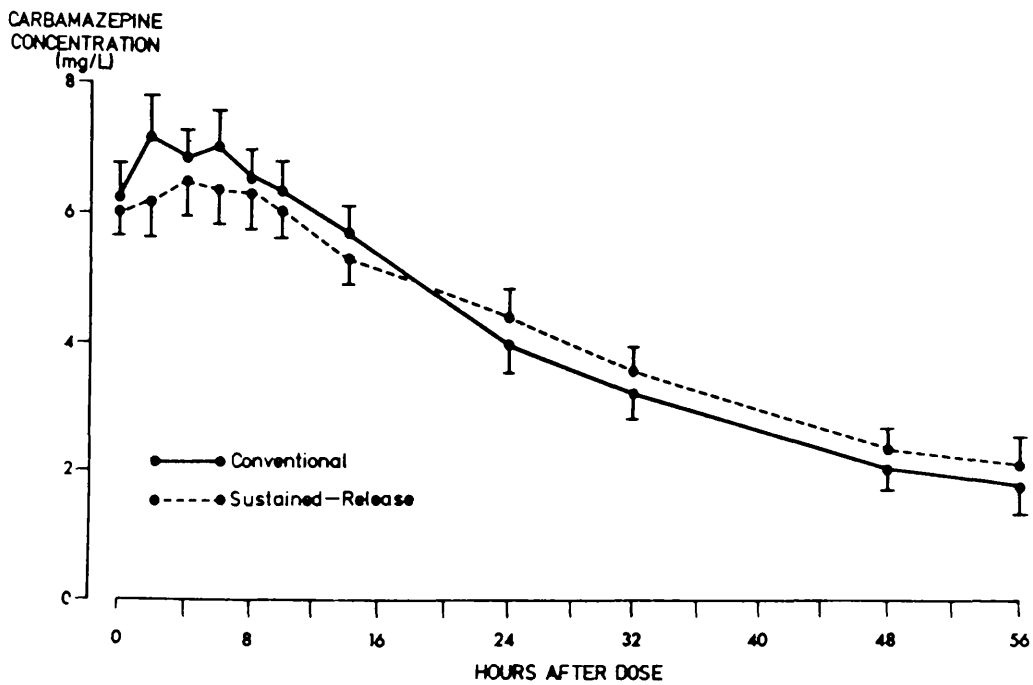
FIGURE 16   Mean serum drug concentration-time curves ($\pm$ SEM) in eight subjects
following the 29th dose of 200mg twice daily conventional or controlled-release
carbamazepine.
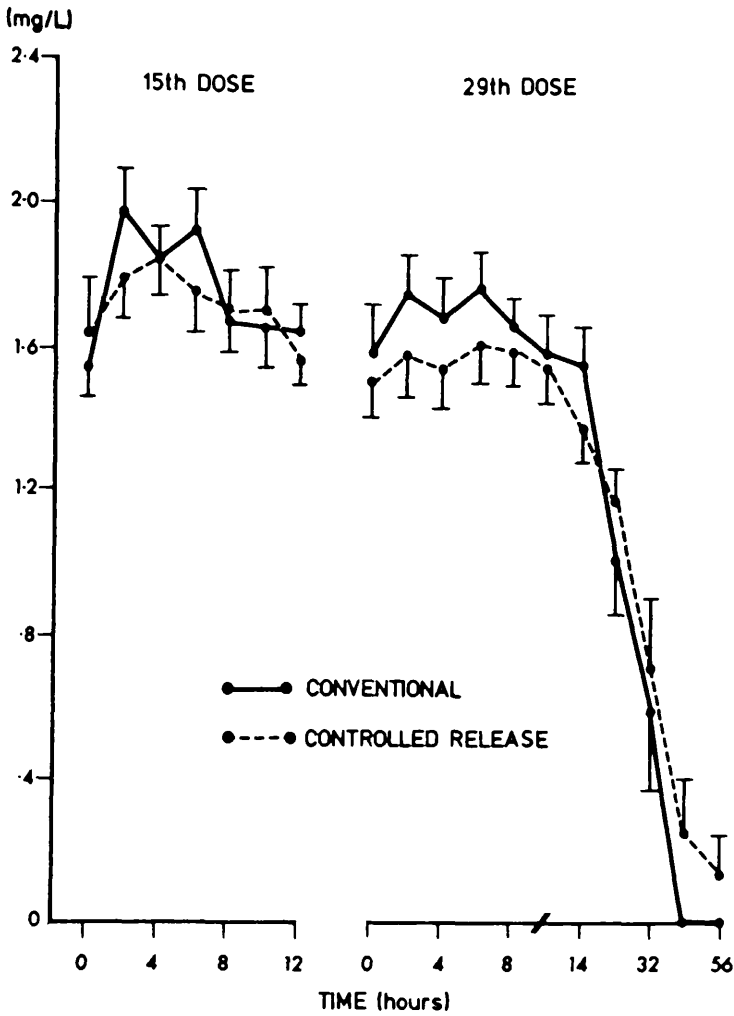
FIGURE 17   Mean diurnal serum drug concentration-time curves (± SEM) of free carbamazepine in eight subjects following 15th and 29th dose of conventional and controlled-release carbamazepine.
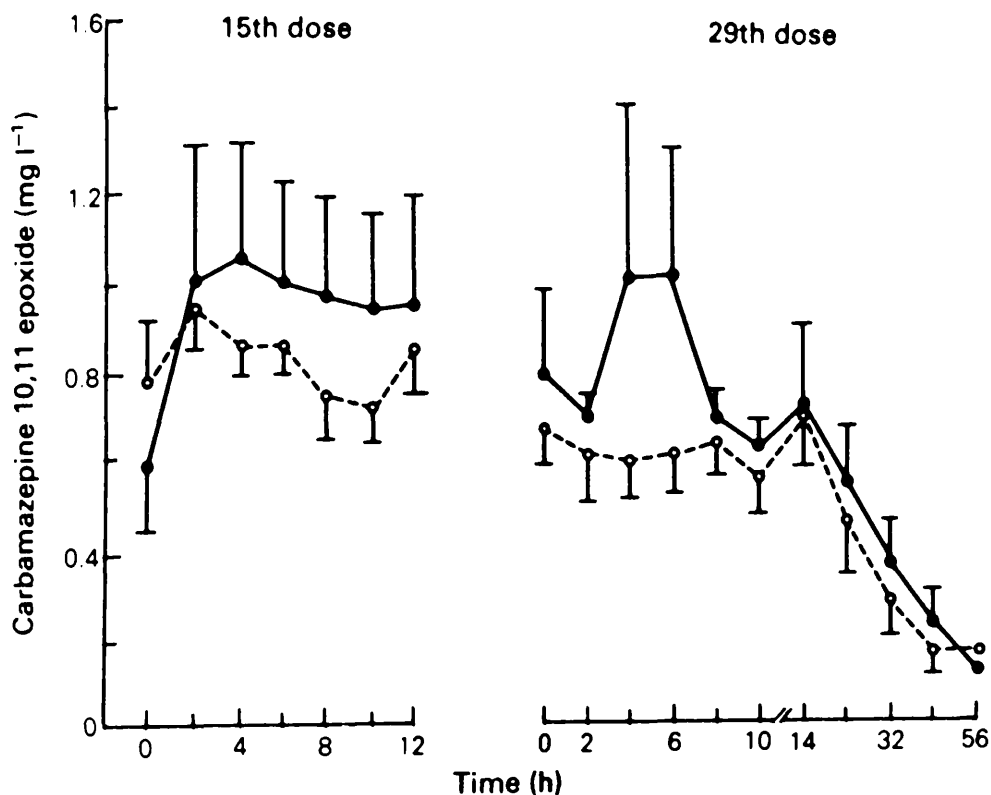
FIGURE 18   Mean diurnal concentration-time curves (± SEM) of serum carbamazepine-10,11-epoxide in eight subjects following 15th and 29th 200mg dose of conventional (●—●)and controlled-release (o--o) carbamazepine.
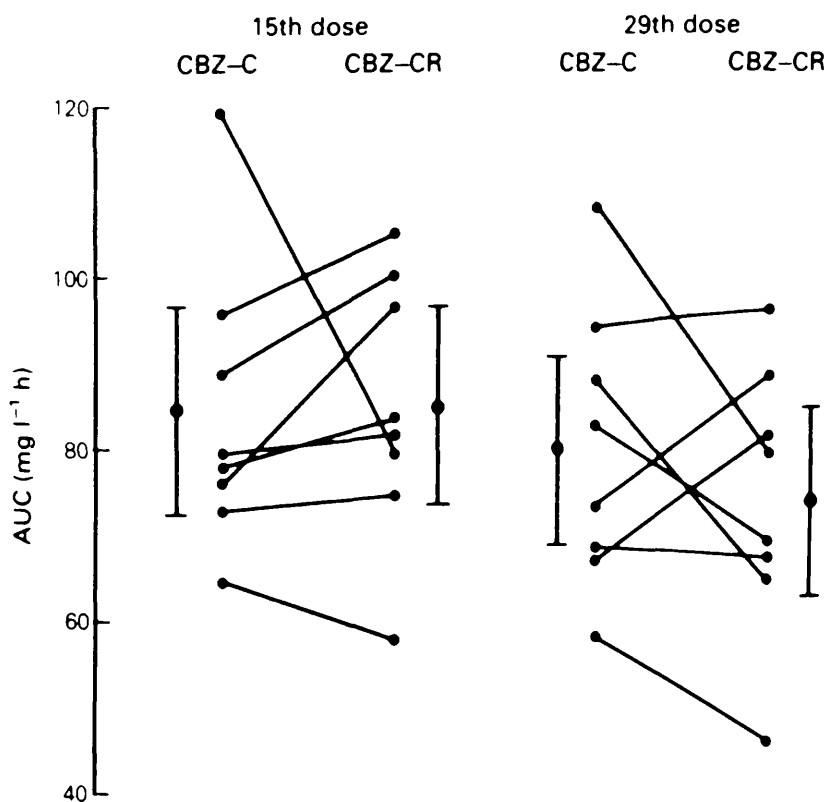
FIGURE 19   Areas under concentration-time curves (0-12h) following 15th and 29th dose of 200mg twice daily conventional (CBZ-C) and controlled-release (CBZ-CR) carbamazepine. Vertical bars represent mean ± SEM

FLUCTUATION OF [CBZ] DURING DAY
(┋ mean ± sem)
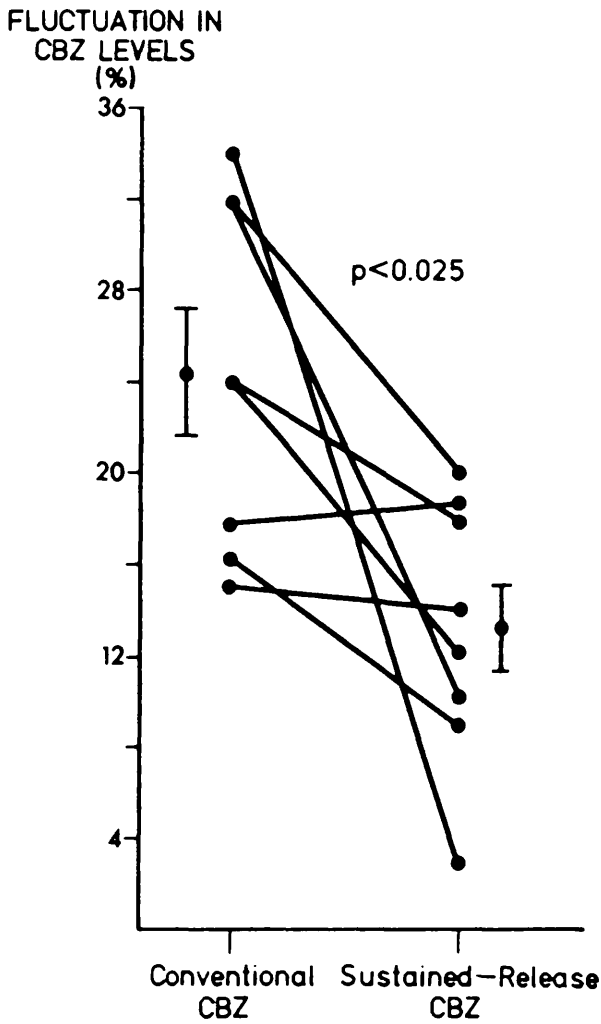
FIGURE 20   Diurnal fluctuation [(Cmax - Cmin)/mean x 100%] of carbamazepine
(CBZ) in eight subjects during dosing with 200mg twice daily of conventional and
controlled-release (sustained-release) preparations. Vertical bars represent mean ± SEM.
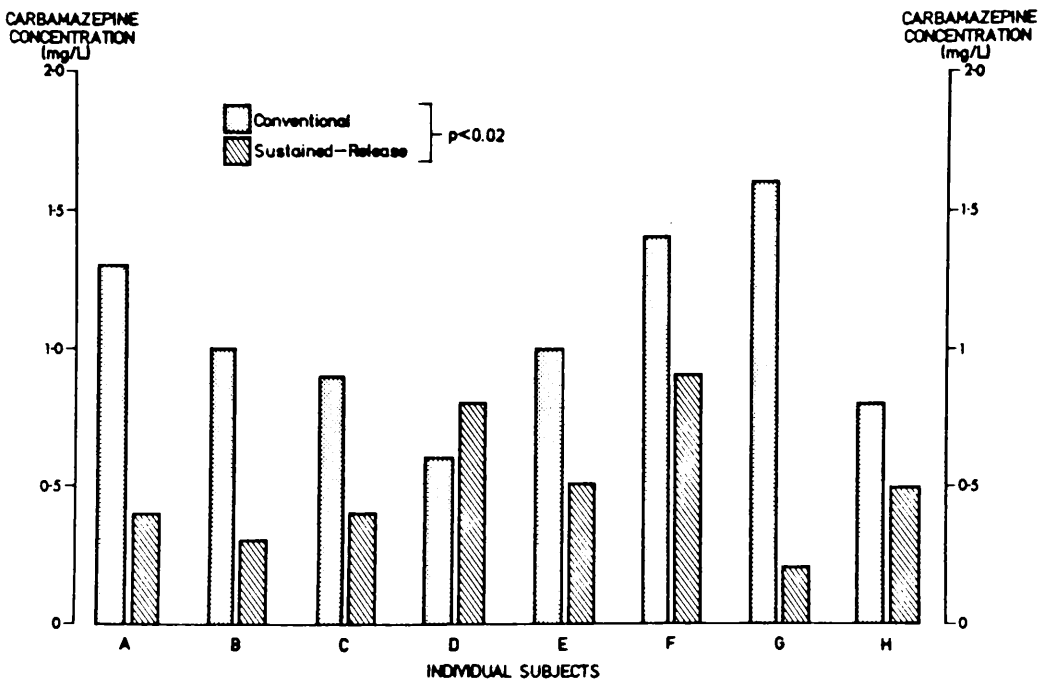
FIGURE 21   Maximum 2 hour increment in serum carbamazepine concentration in
eight subjects following the 29th dose of conventional and controlled-release (sustained-
release) preparations.
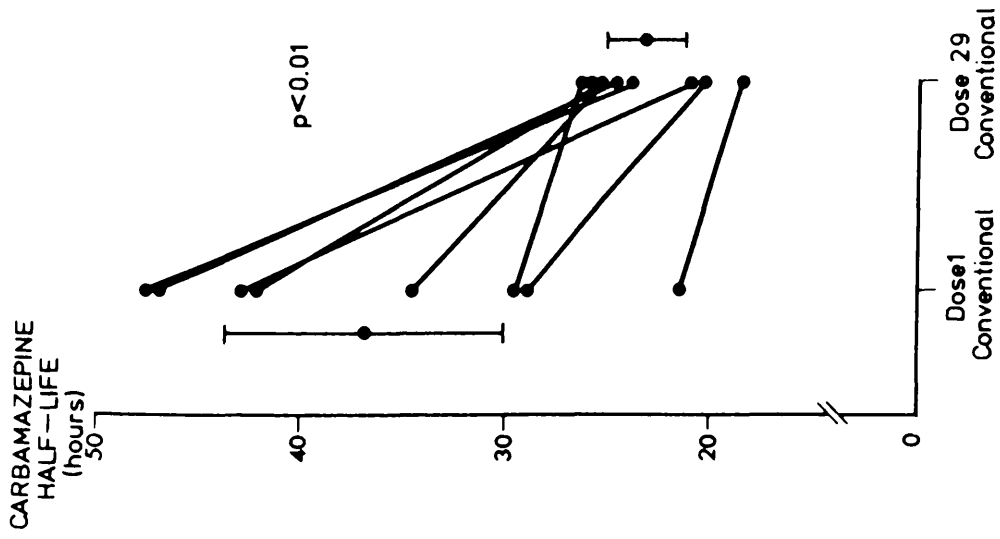
FIGURE 22   Half-life of carbamazepine in eight subjects before and after conventional carbamazepine 200mg b.d. for two weeks. Vertical bars represent mean ±2SEM.
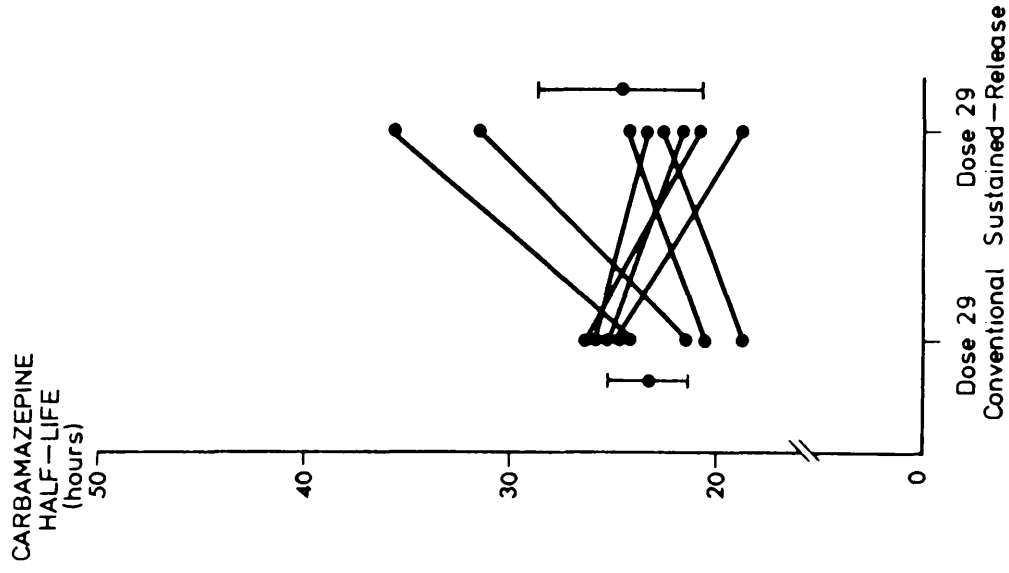
FIGURE 23   Half-life of carbamazepine in eight subjects after conventional or controlled-release (sustained-release) carbamazepine, 200mg b.d. for two weeks. Vertical bars represent mean ±2SEM.
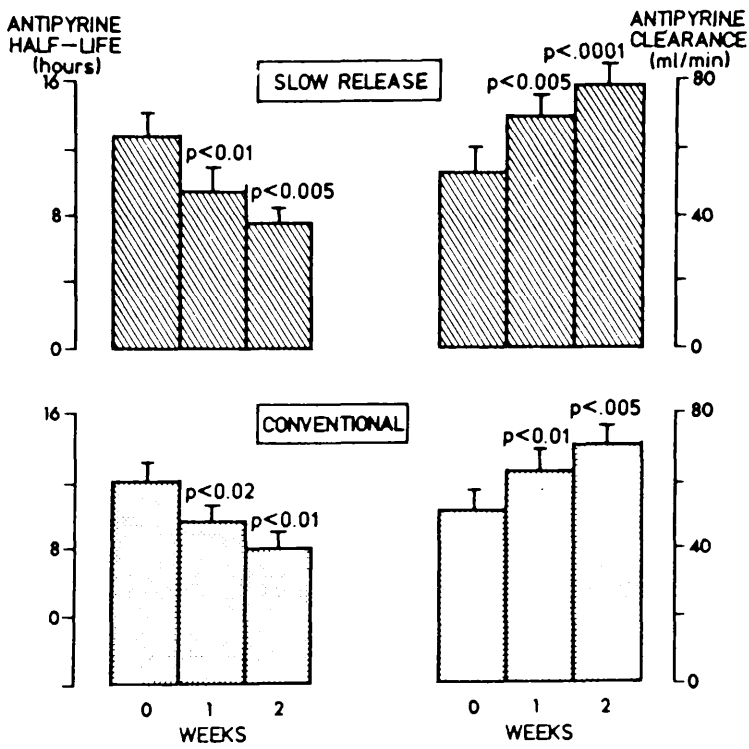
FIGURE 24   Effect of conventional and controlled-release (slow-release)
carbamazepine on antipyrine half-life and clearance following one and two weeks of
therapy.

TABLE 13 Mean performance of psychomotor function tests (SD) in eight subjects receiving conventional and controlled-release carbamazepine for 2 weeks

		Conventional carbamazepine				Controlled-release carbamazepine			
		1	2	3	4	5	6	7	8
CFFTi	(Hz)	26.9 (2.8)	26.2 (3.1)	26.9 (2.1)	27.6 (1.9)	27.5 (2.3)	27.1 (2.0)	27.0 (1.7)	27.3 (2.1)
CFFTd	(Hz)	28.4 (4.8)	27.5 (4.4)	28.6 (4.2)	28.9 (4.2)	28.8 (3.4)	28.4 (4.2)	28.3 (4.4)	28.5 (4.1)
CRT 1	(s)	0.342 (0.02)	0.338 (0.03)	0.338 (0.03)	0.331 (0.03)	0.342 (0.03)	0.342 (0.03)	0.334 (0.02)	0.334 (0.03)
CRT	(s)	0.484 (0.06)	0.481 (0.07)	0.486 (0.07)	0.475 (0.07)	0.490 (0.05)	0.480 (0.06)	0.480 (0.06)	0.471 (0.07)
Digit span	(n)	8.93 (2.2)	8.96 (1.6)	9.22 (1.4)	9.81 (2.3)	8.70 (1.8)	9.20 (1.9)	9.29 (1.7)	8.84 (3.2)
Card sorting	(s)	45.0 (5.0)	44.9 (4.1)	47.3 (6.3)	45.6 (7.3)	47.2 (6.9)	45.5 (6.2)	48.6 (6.7)	48.2 (6.6)
Finger rapping	(taps/min)	389 (31.2)	391 (31.6)	378 (62.2)	387 (30.5)	389 (20.5)	396 (28.2)	373 (59.3)	392 (25.6)
Alertness score	(mm)	75.0 (12.6)	75.6 (15.8)	72.0 (18.2)	78.8 (15.9)	82.6 (12.6)	80.7 (9.5)	76.4 (11.8)	83.5 (10.6)

Columns 1 and 5 are calculated from the pre-dosing measurements (n=16)
Columns 2 and 6 are from measurements following dosing on first day of chronic therapy (n=24)
Columns 3 and 7 include all measurements on days 8 of chronic therapy (n=32)
Columns 4 and 8 are obtained from all measurements on days 15 of chronic therapy (n=32)
CFFT = critical flicker fusion threshold (i - increasing frequency; d = decreasing frequency)
CRT = choice reaction time (1 = recognition time; CRT = complete manoeuvre)

36% [Hoppener et al 1980], 41% [Riva et al 1984] and 59% [Macphee et al 1987]. Our measure (24%) may be lower because of the strict adherence to a twice daily schedule by healthy volunteers. The controlled-release preparation showed an improvement (12%) on even this figure and this should have a beneficial effect on side-effects associated with large swings in blood drug concentration [Hoppener et al 1980, Riva et al 1984, Tomson 1984]. Anecdotally, many patients complain of maximum drowsiness and diplopia 30-120 minutes after ingestion of CBZ [Macphee et al 1986b] i.e. before peak drug concentrations are achieved. Our volunteers followed a similar pattern. This suggests that a rapid increase in concentration may be important in initiating the mild neurotoxic effects associated with the drug. If this is the case, the smaller increments shown with CBZ-CR (Fig. 21) may be as relevant as the reduction in peak concentration or fluctuation.

The contribution of CBZ-E to the psychotropic side-effects of CBZ is uncertain. The epoxide is at least as potent as CBZ in seizure models [Faigle et al 1977] and trigeminal neuralgia [Tomson & Bertilsson 1984]. Collaborative work we have carried out with RA Gillham (Psychology Department, Southern General Hospital, Glasgow) suggests a better correlation of psychomotor impairment with serum epoxide concentrations than with CBZ itself [Gillham et al 1988]. In that study, we found that 7 separate measures of mental performance (including performance IQ, CRT1, CRT2, finger tapping and backward digit span) showed deterioration which correlated with CBZ-E concentrations (range of $r = 0.299 - 0.610$), while only 4 correlated with CBZ concentrations.

The decreased half-life of CBZ following CBZ therapy confirms previous reports that CBZ induces its own metabolism [Eichelbaum et al 1975, Rapeport et al 1983]. Since it is not known, however, whether the extent of induction is a function of peak, trough or "average" drug concentrations, it is of interest to note that the mean half-life of CBZ following CBZ-CR (24.4h) was similar to that found with CBZ-C (23.2h). The effect of

CBZ-CR on antipyrine clearance was more impressive than that of conventional CBZ against baseline, but there were no significant differences when the two preparations were compared directly.

The AUC over a dosage interval at steady state was 7% lower with CBZ-CR. Although this was not a statistically significant decrease, we cannot exclude the possibility that the bioavailability of CBZ-CR may be slightly less than that of CBZ-C. A further study in our unit suggests that this may, in fact, be the case [McKee et al 1991]. This difference would not explain the reduced fluctuation, which was calculated as a percentage of the serum values. Increased induction with CBZ-CR is a further possible reason for decreased AUC, particularly since the AUCs after dose 15 were similar on the two preparations. No significant differences in CBZ half-life or antipyrine induction were found to support this suggestion.

The diurnal pattern of unbound CBZ resembled that of total CBZ, protein binding being similar with both formulations. Epoxide concentrations seemed "tighter" and at a lower level with CBZ-CR than CBZ-C. However, these differences did not reach conventional significance levels - possibly owing to the small numbers in the study. The smaller error bars with CBZ-CR in Figure 18 reflect the significantly reduced variation in CBZ-E concentration between individuals on this preparation, rather than reduced diurnal fluctuation.

Psychomotor testing failed to reveal differences between the two preparations. As with EXPT. 3, variability of these tests would work against disclosing differences in small numbers of healthy volunteers taking a relatively low dose. Subjective complaints suggested that symptoms were not experienced at times of peak concentration but much earlier. The timing of psychomotor testing at 10h post-dosing may thus have been less

than optimal. Tolerance to the psychomotor effects may be occurring before serum levels have peaked following the first dose!

This study demonstrated Tegretol Retard to be a true controlled-release preparation which reduced diurnal fluctuation of CBZ levels. Bioavailability is perhaps reduced, but enzyme induction is at least as marked as with conventional CBZ. Psychomotor testing again may have been mistimed, as the peak impairment did not seem to correspond with peak serum concentrations.

The problem of psychomotor impairment in epilepsy remains a complex one. As we saw in EXPT. 2, even relatively mild epilepsy, not requiring therapy, is associated with a significant degree of psychomotor impairment. A further deterioration in patients taking AEDs gave some evidence for a separate effect from the drugs themselves, but clearly these patients also have more severe epilepsy. The detrimental effects of AEDs in epileptic patients have largely been extrapolated from studies in healthy controls [e.g. Thompson et al 1980, Thompson & Trimble 1981, Macphee et al 1986a], but these are of necessity short-term studies. Other studies of patients themselves - where extra doses are given - [Macphee et al 1986b] also show a deterioration in performance, but these are also short-term, and as we see from EXPT 3, such changes may well return towards normal after some weeks.

Our collaborative study with psychologists [Gillham et al 1988] showed only minimal effects by CBZ on a wide range of psychomotor and cognitive tests, except when it was used in combination with another AED. All things considered, the evidence for marked cognitive impairment caused by the disease is much more convincing than that against the AEDs. Perhaps clinicians can take solace from the idea that the impairment caused by AEDs is outweighed by benefits in the same modalities due to a decrease in number of seizures - a known independent cause of psychomotor impairment [Seidenberg et al 1986]. A transient drowsiness 30-90 minutes following dosing with CBZ may be a small price to pay for an improvement in seizure frequency. Long-term damage is a genuine possibility however, though difficult to prove. Anecdotally, patients do very often complain in the clinic of memory deterioration. We have recently performed sophisticated psychological testing of 84 patients receiving monotherapy with CBZ (35), VPA (30), PHT (19) and 26 untreated patients [Gillham et al 1990]. Differences were subtle, but the phenytoin patients had poorer memory while the CBZ patients had slightly

impaired motor function. Interestingly, the VPA group were significantly better than those taking other drugs in these modalities. The results are in keeping with suggestions that different AEDs affect different modalities of mental function - PHT having more effect on memory and cognitive function, while CBZ may impair pure motor abilities [Andrewes et al 1986]. Whether phenytoin is more likely to cause long-term memory problems is unproven. The findings may also constitute early evidence that VPA may prove to be slightly less neurotoxic than CBZ.

The transient drowsiness following CBZ dosing may be open to amelioration using CBZ-CR. EXPT. 4 failed to prove this with regard to psychomotor testings, but did demonstrate pharmacokinetics which would appear much more favourable than those of CBZ-C. A recent study [Aldenkamp et al 1987] had the opposite emphasis, and while failing to show the pharmacokinetic effects in such detail, did demonstrate improved cognitive function with CBZ-CR compared with CBZ-C. A study of patients using a double-dummy technique with conventional CBZ is in progress in the unit to confirm this.

CHAPTER 3

ENZYME INDUCTION AND ANTICONVULSANT DRUGS

CHAPTER 3

ENZYME INDUCTION AND ANTICONVULSANT DRUGS

INTRODUCTION

Induction

The elimination of many anticonvulsants is largely dependent on metabolism by the family of monooxygenase enzymes in the liver. For example, carbamazepine is oxidised to carbamazepine-10,11-epoxide (CBZ-E), an active metabolite which is further enzymatically converted to 10,11-dihydro-10,11-dihydroxy-carbamazepine (CBZ trans-diol, cf Figure 25). As with many drugs, the mixed-function oxygenases, the terminal electron acceptor of which is cytochrome P-450, are of paramount importance in the biotransformation of anticonvulsants [Park 1982].

The activity of these enzymes can be assessed in various ways. The most common method is to measure the pharmacokinetics of the outmoded analgesic antipyrine. This drug is rapidly absorbed after oral ingestion, and is metabolised almost completely in the liver by the mixed function oxygenase system [Boobis et al 1981]. Pharmacokinetics can be calculated using either plasma or saliva concentrations. Increasing clearance and shortening elimination half-life can both give a good indication of enhanced mixed oxygenase activity due to induction [Park 1982]. Another method of detecting changes in an individual's monooxygenase enzyme activity is to measure the 24h urinary excretion of 6 beta-hydroxycortisol which is increased by inducing drugs [Park 1981]. This minor metabolite of cortisol is formed in the liver by enzymes associated specifically with cytochrome P450 [Park 1982]. This entirely non-invasive technique can be used in children, where it has been shown to correlate with antipyrine clearance in young epileptic patients given CBZ [Ohnhaus & Park 1979].
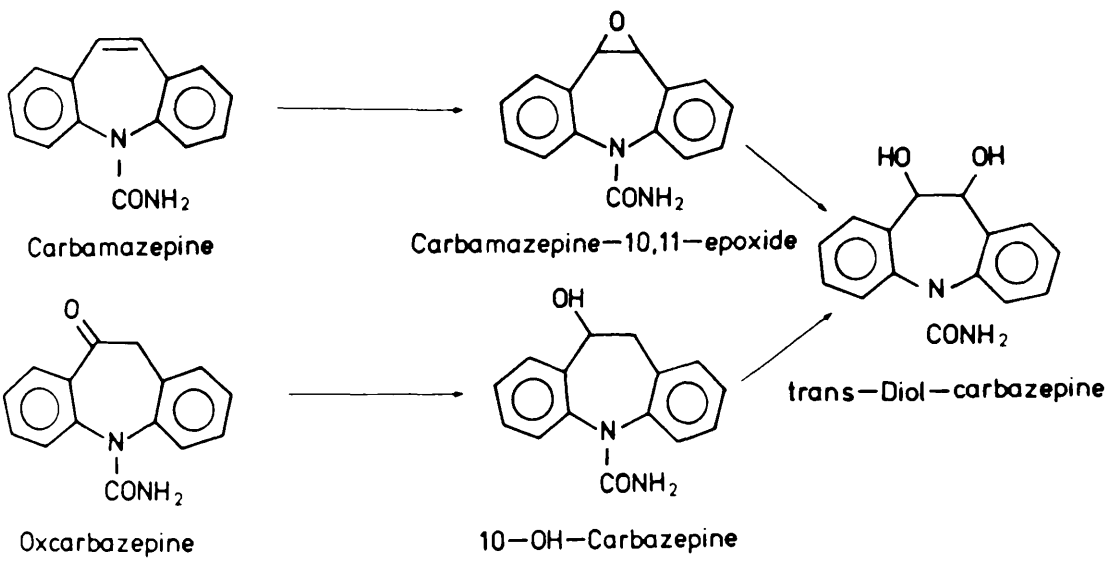
FIGURE 25 Metabolism of carbamazepine and oxcarbazepine.

Using such techniques, various workers have produced overwhelming evidence that PB [Burstein & Klaiber 1965, Ohnhaus & Park 1979, Vessell & Page 1969], PHT [Werk et al 1964, Petruch et al 1974] and CBZ [Moreland et al 1982, Rapeport et al 1983] can all induce hepatic enzymes, while this does not appear to be the case with VPA [Perucca et al 1984]. One possible consequence of this induction is accelerated metabolism of the drugs themselves ("autoinduction") with, for example, a resultant decrease in CBZ half-life [Eichelbaum et al 1975] reducing steady-state levels and necessitating an increase in dosage [Macphee & Brodie 1985]. Similarly, the dosage of concurrent medication also metabolised by the same enzyme system may require to be increased if an AED such as CBZ is added to the regimen. The most notable example is the oestrogen-containing contraceptive pill, where the introduction of an anticonvulsant has been shown to cause breakthrough bleeding and contraceptive failure [Orme 1982, Mattson & Cramer 1985]. On the other hand, the addition of an enzyme-inhibiting drug to a regimen containing e.g. CBZ can offset the effect of enzyme induction, thus causing an abrupt rise in CBZ levels and consequent toxicity. This may occur with VPA [Levy & Koch 1982] or with other commonly used enzyme inhibitors such as cimetidine [Macphee et al 1984] or verapamil [Macphee et al 1986c].

Hyposexuality

A controversial aspect of enzyme induction is the possible effect of AEDs on endogenous hormones, and the part they may play in the hyposexuality which has been recognised in epilepsy patients since 1954, when Gastaut & Collomb described a group of 36 patients with temporal lobe epilepsy (TLE), of whom 26 they considered as having absent or reduced sexual activity. The nature and prevalence of this hyposexuality has since been under investigation. Most studies find a decrease in sexual potency rather than

libido [Saunders & Rawson 1970, Pritchard 1980], though either may be present alone [Saunders & Rawson 1970]. A particular association with TLE was further championed by Hierons & Saunders [1966] and others [Kolarsky et al 1967, Pritchard 1980, Herzog et al 1986]. Many of these studies are presented in a way which makes it difficult to decide whether control groups with other epilepsy syndromes were considered. However, Saunders & Rawson [1970] simply interviewed 100 epileptic patients of whom 33 had TLE. Twelve patients had a "sexual disorder" and all of these were in the TLE group. It has since been argued that not only might TLE cause an endocrine disorder, but that the endocrine abnormalities themselves may predispose patients to epileptic seizures [Herzog 1989].

Many studies have also shown that earlier onset epilepsy is more likely to be associated with hyposexuality [Gastaut & Collomb 1954, Taylor 1969] with only one in disagreement [Pritchard 1980].

Hyposexuality and Induction

The role of antiepileptic drugs and induction in this hyposexuality is unclear. Circulating concentrations of sex-hormone binding globulin (SHBG) have been found to be markedly increased by AEDs [Victor et al 1977, Toone et al 1980]. Total testosterone is usually normal [Toone et al 1984, Rodin et al 1984] or increased [Toone et al 1982], but free testosterone is lowered [Dana-Haeri et al 1982, Toone et al 1983]. Luteinising hormone is raised [Toone et al 1980, Rodin et al 1984]. Toone [1985] has enumerated various possible sequences for these changes, favouring an increased metabolism of free testosterone as the first step, but also mentioning the possible roles of: a) increased SHBG; b) decreased testosterone synthesis due to a direct toxic effect on the testes; c) a failure of central mechanisms in response to reduced free testosterone. In this chapter, the effects of the drugs on young epileptic men will be investigated. EXPT 5. separates the

patients taking different AEDs, to investigate the possibility that the non-inducing drug VPA has different effects from the others. An untreated epilepsy group is also included and compared to healthy controls. A likely sequence for the hormonal changes is more fully discussed in the conclusion to this chapter.

Thyroid function

The effect of AEDs on thyroid function has also come under scrutiny. Depression of thyroid hormones was first reported with PHT in 1961 [Oppenheimer et al 1961]. Both PHT and CBZ have since been found to be associated with a reduction in total (T4) and free (FT4) thyroxine [Liewendahl et al 1978, Heyma et al 1977, Yeo et al 1978]. Triiodothyronine (T3) has been less consistently found to be decreased, and this may reflect changes of a smaller magnitude [Smith & Surks 1984]. Again, enzyme induction may be involved [Larsen et al 1970], although failures to reproduce the effect with the inducers primidone [Liewendahl et al 1978] and phenobarbitone [Yeo et al 1978] are perhaps surprising. Some support for the inductive mechanism is gained from the evidence that the inducing antibiotic rifampicin lowers T4 concentrations [Ohnhaus & Studer 1983]. Again, this chapter compares the non-inducing VPA with other AEDs to further assess the case for this mechanism.

Oxcarbazepine

Attempts to modify the structure of CBZ to reduce side-effects have produced oxcarbazepine (OXC), the 10-keto analogue (Figure 25). It has similar anticonvulsant efficacy to CBZ in animal models [Baltzer & Schmutz 1977] and in clinical trials [Houtkooper et al 1987, Reinikainen et al 1987, Dam et al 1989]. Psychomotor impairment was decreased in these clinical studies, and rashes were less frequent than

with CBZ in one [Dam et al 1989]. Hyponatremia may, however, be at least as common with OXC as with CBZ [Nielson et al 1988].

The metabolism of OXC differs markedly from that of CBZ. While CBZ is oxidised to CBZ-10,11-epoxide, OXC is immune to oxidative attack (Fig. 25) and is instead reduced to 10-hydroxy-carbazepine (10-OH-CZ). Indeed it is the anticonvulsant properties of this metabolite which account for the drug's pharmacological actions [Schutz et al. 1986], as OXC itself is only briefly present in the circulation [Theisohn & Heimann 1982, Kramer et al 1985]. As a consequence of this variation in metabolic profile, OXC may lack the enzyme-inducing properties of CBZ [Kramer et al 1985] and this postulation is tested later in this chapter (EXP 7).

EXPTS. 5 & 6 THYROID AND SEX HORMONES IN YOUNG EPILEPTIC MEN RECEIVING LONG-TERM ANTIEPILEPTIC MEDICATION

AIM

To determine sex hormone and thyroid hormone profiles in epileptic patients, and assess the effect of enzyme induction and epilepsy itself by including a group taking the non-inducing AED sodium valproate, and an "untreated epilepsy" control group.

PATIENTS AND METHODS

Fifty-four patients taking CBZ, PHT, VPA or a combination of drugs including either CBZ or PHT (POLY) were recruited (Table 14). All were compliant as judged by consistent AED concentrations at out-patient attendances. No other drugs were being prescribed, and no patient was known to abuse alcohol. Treatment had been unaltered for at least three months, and no patient had suffered a seizure in the previous 48h. For comparison, 14 untreated epileptics and 16 unmedicated members of laboratory and medical staff were included as controls.

Following a 30 minute supine rest, 40 ml blood was withdrawn through an indwelling cannula. Samples were centrifuged, and serum (hormone assays) and plasma (AED assays) were stored at 20^oC until batch analysis. "Sex hormones" assayed included testosterone (T), sex-hormone binding globulin (SHBG), dehydroepiandrosterone sulphate (DHAS), androstenedione (AND), luteinising hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL). Thyroid function tests consisted of serum thyroxine (T4), free thyroxine (FT4), triiodothyronine (T3) and thyrotropin (TSH).

TABLE 14 Mean and SD of age, seizure type, and AED concentration in all patient groups and in controls

Group	Age (yr)	Seizure type	AED concentration (mg/L)	
Healthy subjects (n = 26)	31 (7)	-	-	
Untreated epileptic patients (n = 14)	28 (8)	8 CPS + SG 6 GTCS	-	
CBZ (n = 18)	29 (7)	11 CPS + SG 7 GTCS	9.8 (4.1)	
PHT (n = 13)	32 (8)	5 CPS + SG 8 GTCS	17.4 (12.7)	
VPA (n = 10)	23 (5)	3 CPS + SG 7 GTCS	75.6 (34.7)	
Combination therapy (n = 12)	36 (7)	9 CPS + SG 3 GTCS	11.5 (3) 17.6 (6.9) 24.8 (15.8) 64, 55	CBZ, n = 8 PHT, n = 5 PB, n = 8 VPA, n = 2

AED, antiepileptic drug; CPS, complex partial seizures; SG, secondary generalisation; GTCS, generalised tonic-clonic seizures; CBZ, carbamazepine; PHT, phenytoin; VPA, valproate

A subgroup of 6 controls, 7 untreated patients and 33 treated patients (11 receiving CBZ, 9 PHT, 6 VPA and 7 POLY) underwent a combined pituitary stimulation/TRH test. After a supine rest and baseline measurements through an indwelling cannula, an intravenous injection of 100 ug. LHRH and 200 ug. TRH was administered. Further venous blood samples were obtained after 20 and 60 minutes for LH, FSH, PRL and TSH. These procedures were performed by Dr. GJA Macphee.

Assays

Hormone assays were performed by radioimmunoassay as previously described [Connell et al 1984a, Connell et al 1984b]. SHBG was determined by a modification of the method of Rosner [1972]. All had a mean interassay coefficient of variation of less than 10%. None of the AEDs or their metabolites was known to interfere with any of the assays. Free testosterone (FT) was derived from the formula: FT (pM) = total T x % free T x 1000/100, where % free T = -2.38 $\log_{10}$ (SHBG nM) + 6.11. This correlates very closely with free testosterone [Nanjee & Wheeler 1985].

Kinetics and Statistics

Areas under the concentration-time curve (AUC) for individual hormones following pituitary stimulation were calculated by the trapezoidal rule. Although means are given, all comparative statistics were non-parametric. Initial Kruskal-Wallis of the six groups was followed up by the Mann-Whitney 'U' test in positive cases. As well as analysing the differences between the six groups of subjects, the untreated epileptics and healthy controls were amalgamated for comparison with the VPA-treated group and those receiving one or more enzyme-inducing AED. Correlation coefficients were obtained by the Spearman ranking procedure.

RESULTS

Sex Hormones

The individual results of all subjects for each measurement are shown in Figure 26 (a)-(h). Three measurements (SHBG, H=30.4, P<0.001; DHAS, H=16.8, P<0.005; PRL, H=11.1, P<0.05) showed significant variation between the six subject groups. SHBG was increased in the CBZ (P<0.05), PHT (P<0.0001) and POLY (P<0.0001) groups, and DHAS was significantly decreased by the same drugs, relative both to controls (all P<0.001) and to the VPA group (P<0.02, P<0.01, P<0.05). PRL was increased only in the CBZ group (P<0.005). In no measurement did the VPA group differ from either set of controls, nor did the untreated patients differ from the healthy controls. The results are summarised {means(SD)} in Table 15. Some individual group results - such as the polypharmacy LH and FT, and the untreated patients' androstenedione - seemed altered, but the Kruskal-Wallis for all groups did not reach significant levels for these hormones. Amalgamation of the untreated patients and controls (n=30) and comparison with VPA (n=10) and induced patients (n=44) confirmed a marked increase in SHBG (P<0.0001) in induced patients vs. controls and a decrease in DHAS vs. both controls (P<0.0001) and VPA patients (P<0.005). A decrease in free testosterone in the induced group just failed to reach statistical significance (P=0.06).

Circulating AED concentrations were correlated with hormonal values for CBZ and PHT monotherapy patients. The only significant value obtained was an inverse relationship between CBZ and free testosterone (r_s = -0.54, P<0.05; Fig. 27)

Results for the LHRH/TRH stimulation test are shown in Table 16. The $AUCs_{0-60}$ were similar to controls in all patient groups for LH and FSH. For PRL, it was greater in the CBZ group only (P<0.05).

TABLE 15 Mean (SD) sex hormone and binding globulin concentrations in 93 subjects

	Norm n = (26)	Untreated (14)	CBZ (18)	PHT (13)	VPA (10)	POLYPHARM (12)
LH (U/L)	4.9 (2.8)	6.1 (2.6)	7.2 (3.6)	8.3 (7)	7.6 (7.8)	11.5 (10.3)
FSH (U/L)	4.2 (2.1)	5.3 (6.4)	4.2 (3.1)	7.8 (10.1)	3.1 (1.5)	7.5 (8.1)
* PROLACTIN (U/L)	136 (83)	207 (159)	380 (299)	199 (95)	227 (218)	190 (191)
TESTOSTERONE (nmol/L)	15.4 (4)	21.7 (20.3)	16.6 (8)	22.6 (11.1)	17.3 (5.8)	17.4 (3.6)
FREE TESTOSTERONE (pmol/L)	452 (121)	538 (256)	430 (229)	426 (184)	443 (174)	339 (96)
**SHBG (nmol/L)	22.9 (2)	28.3 (6.2)	31.1 (2.9)	61.2 (17.2)	32.9 (6.2)	55.8 (6.9)
***DHAS (umol/L)	7.4 (4.1)	7.7 (2.9)	3.1 (1.9)	1.9 (1.8)	5.9 (3.7)	2.6 (2.6)
A'ENEDIONE (nmol/L)	5.8 (2.4)	8.2 (1.7)	6.1 (1.8)	9.3 (9.3)	8.2 (3.3)	6.8 (2.6)

* $P < 0.00$ ** $P < 0.01$ *** $P < 0.005$

TABLE 16 Areas (mean ± SD) under concentration-time surve (0-60 min) for LH, FSH and PRL following combined gonadatrophin and TRH stimulation in 33 male epileptic patients receiving CBZ. PHT, VPA, or combination treatment and in 13 drug-free controls

	Area under the concentration-time curve (0-60 min)		
Group	LH (U/L/min)	FSH (U/L/min)	PRL (U/L/min)
Controls (n = 13)	1,771 ± 563	499 ± 290	42,100 ± 18,200
CBZ (n = 11)	1,449 ± 644	498 ± 430	62,900 [a] ± 22,700
PHT (n = 9)	1,682 ± 801	692 ± 672	46,400 ± 21,900
VPA (n = 6)	1,530 ± 688	372 ± 130	43,900 ± 18,400
Polypharmacy (n = 7)	1,829 ± 718	721 ± 914	60,928 ± 45,130

LH   - Luteinising hormone

FSH - Follicle Stimulating hormone

PRL - Prolactin

TRH - Thyrotropin-releasing hormone

[a] $P < 0.05$ vs. controls, statistics obtained by Student's t test for unpaired values.
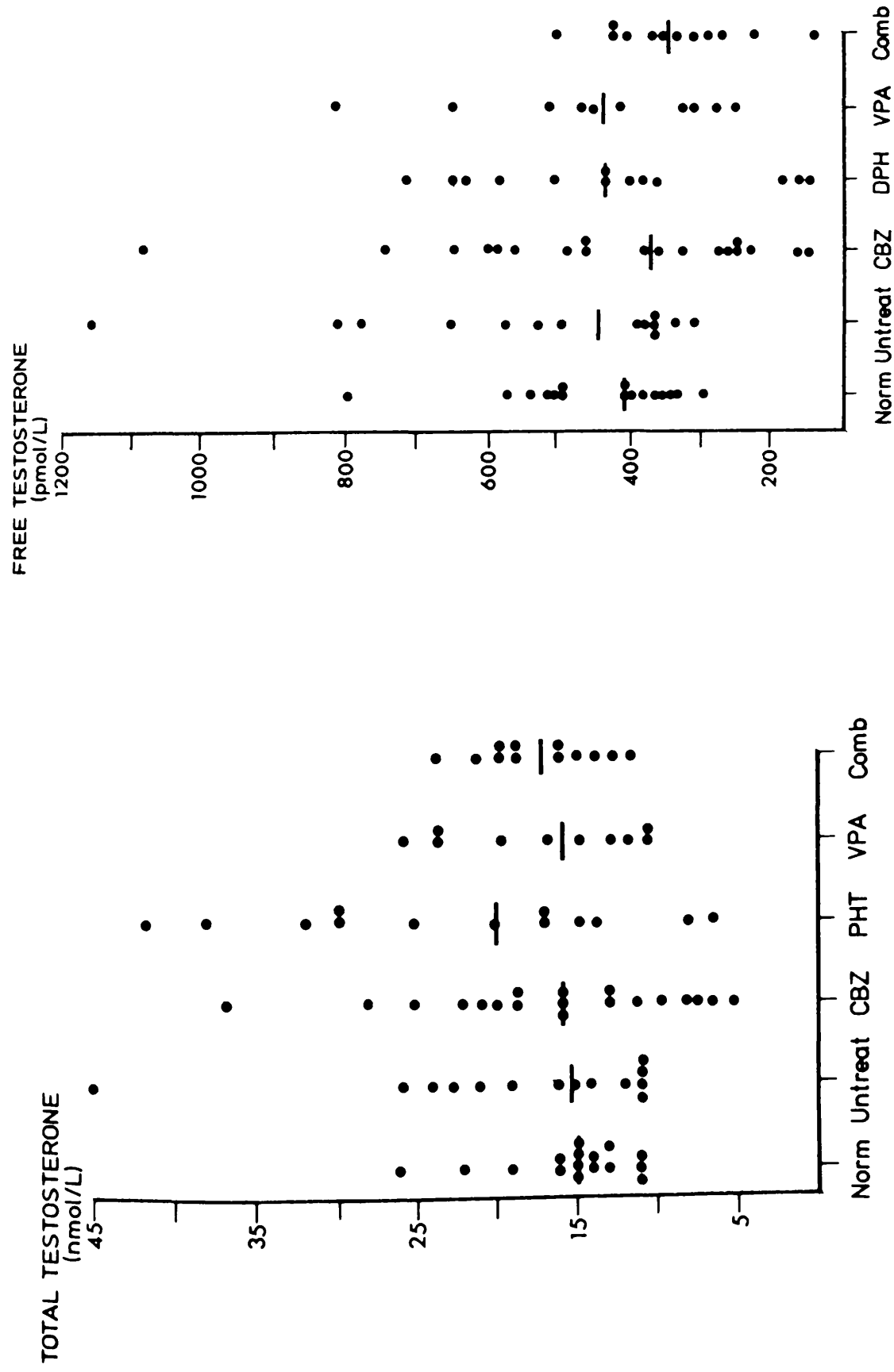
FIGURE 26(a)   Testosterone

FIGURE 26(b)   Free Testosterone

FIGURE 26(a-h)   Sex hormone and SHBG concentrations in normal controls (norm), untreated patients (untreat) and patients taking CBZ, PHT, VPA or combination therapy (comb).

FIGURE 26(d) Androstenedione.

FIGURE 26(c) Dehydroepiandrosterone sulphate.

PROLACTIN
(U/L)

●1200

Norm Untreat CBZ DPH VPA Comb

FIGURE 26(f)   Prolactin

SHBG
(nmol/L)

●260

Norm Untreat CBZ DPH VPA Comb
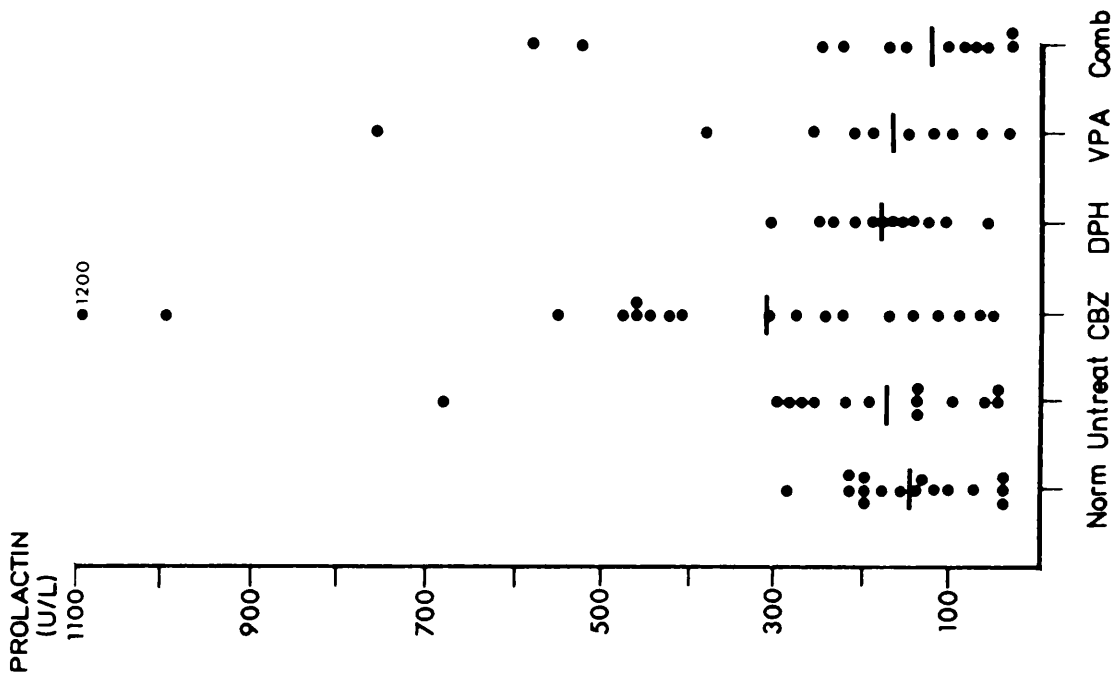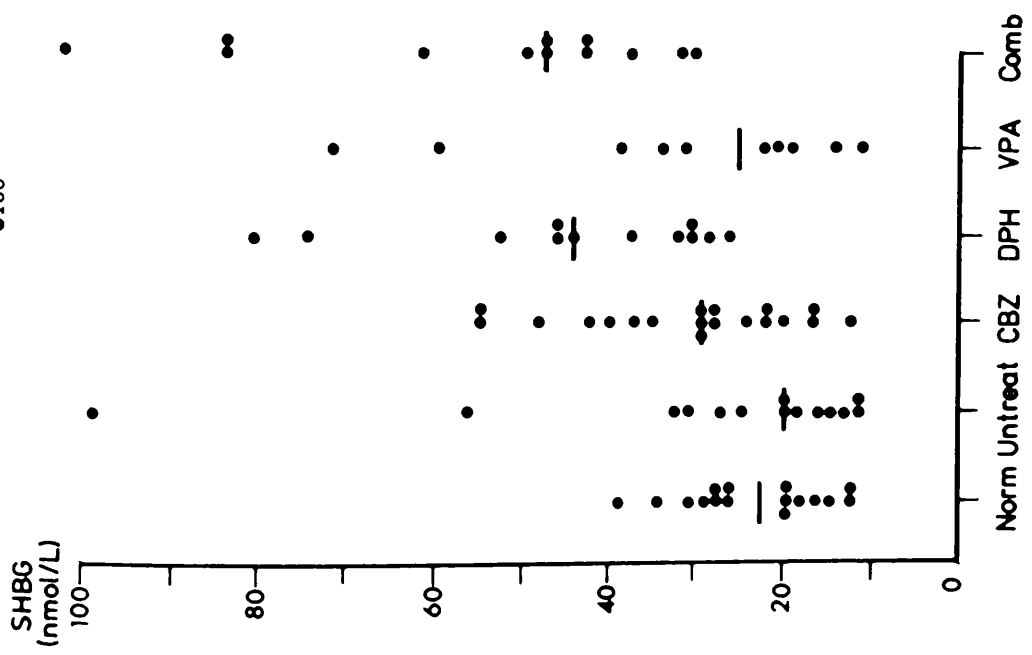
FIGURE 26(e)   Sex Hormone Binding Globulin.
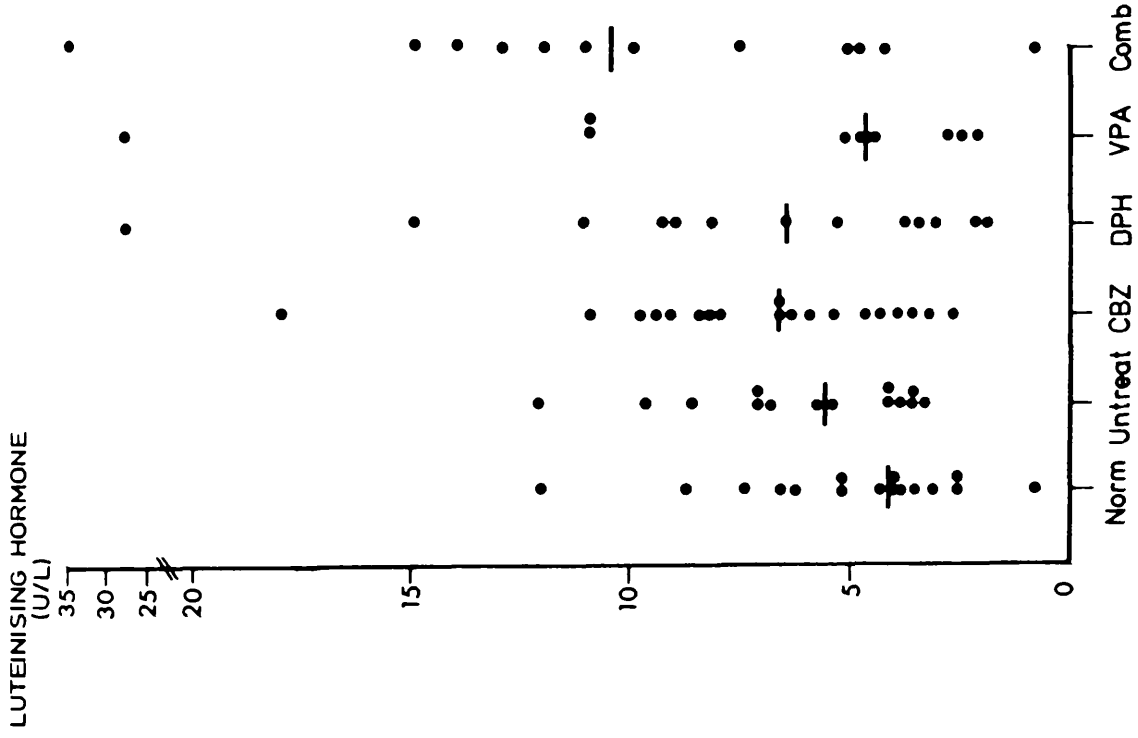
FIGURE 26(h)   Luteinising Hormone.
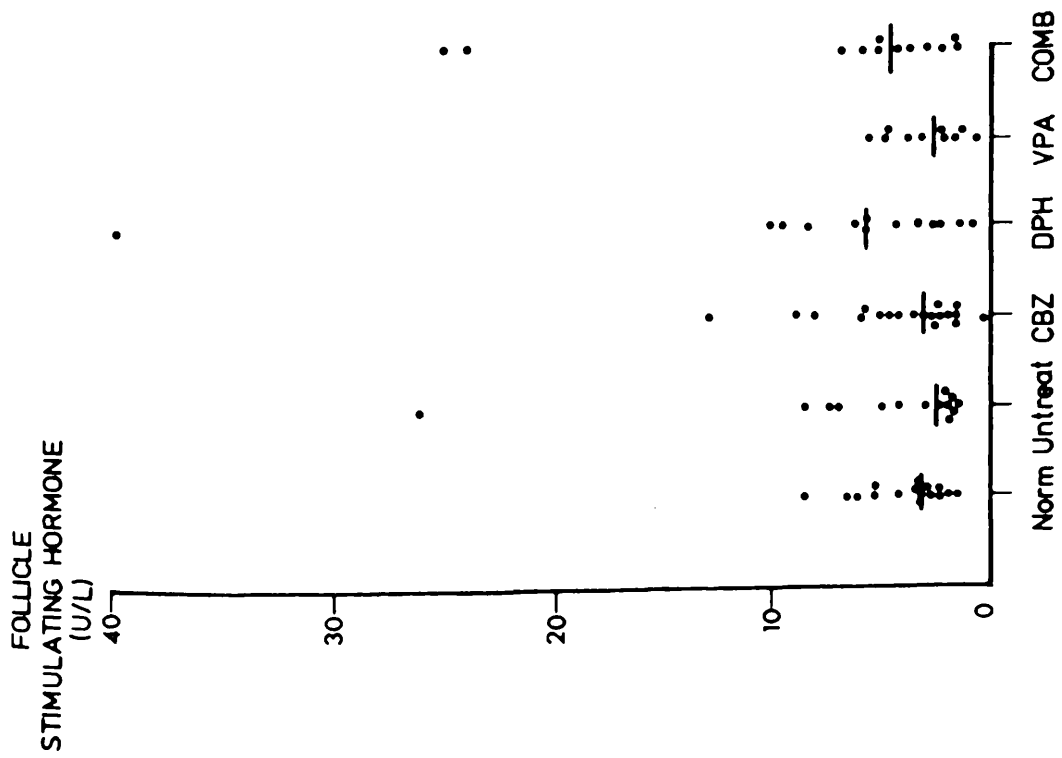
FIGURE 26(g)   Follicle Stimulating Hormone.

Free Testosterone
(pmol/L)

r = -0.54
p < 0.05

Total Carbamazepine (mg/L)
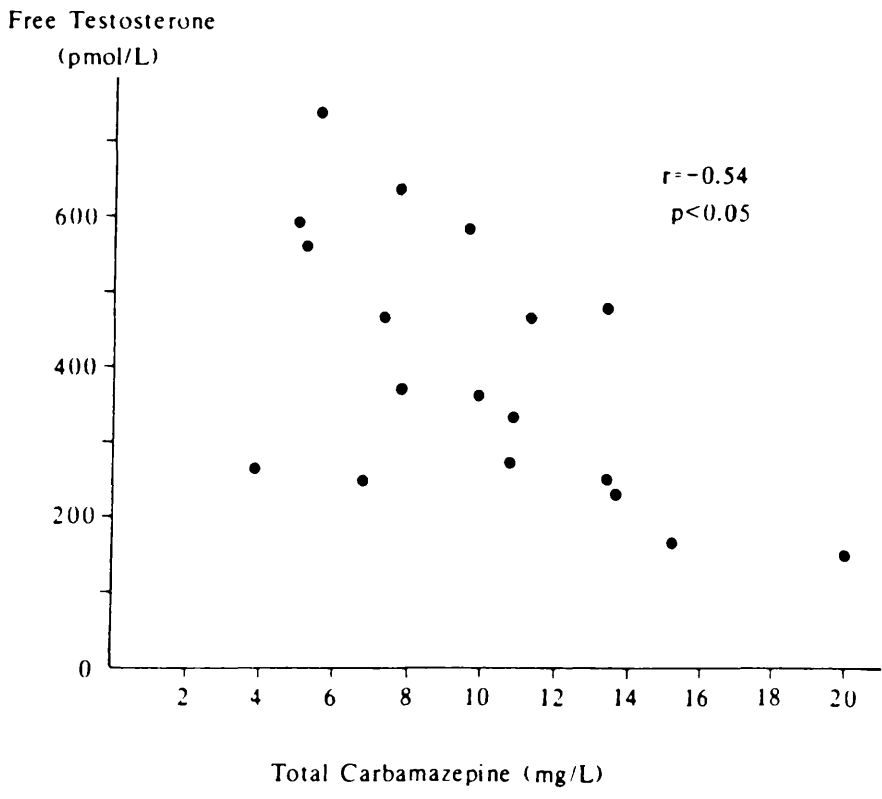
FIGURE 27   Correlation of serum carbamazepine concentrations and calculated free testosterone levels in 18 male epileptic patients receiving the drug as monotherapy.

Thyroid Hormones

Thyroid function test results were not available on five of the control group (leaving n=11). A summary of the data in the six groups is shown in Table 17. TRH test results are presented as the percentage rise of TSH to the peak from baseline. Individual data measurements for controls, patients receiving VPA monotherapy, and those on inducing drugs are shown in Fig. 28.

Nine of the T4 estimations in the patients receiving enzyme-inducing AEDs (2 CBZ, 4 PHT, 3 POLY) were below the reference range for the assay (55-144 nmol/L), the lowest being 28 nmol/L. None of the subjects who were not taking inducing drugs was below this range. Five subjects had free T4 concentrations below 10 pmol/L. Again, all were on inducing drugs (1 CBZ, 1 PHT, 3 POLY). No patient in the study had abnormal high basal or stimulated TSH concentrations.

Kruskal-Wallis analysis showed differences in T4 when the subjects were divided into either six groups (H=25.9, P<0.0005) or three groups (H=24.8, P<0.0005). Patients on inducing drugs had lower circulating T4 concentrations than both controls (P<0.001) and patients taking VPA (P<0.001). Significance testing for the individuals taking inducers against the three other groups is shown in Table 17. Valproate patients had slightly higher T4 concentrations than controls (95% CI +28 to -8 nmol/L), but the difference was not statistically significant.

Differences were also present in the FT4 results among the six groups (P<0.005) or the three groups (P<0.005). FT4 concentrations in induced patients were lower than in either controls (P<0.05) or VPA-treated patients (P<0.002). The latter again had slightly, but not "significantly", higher levels than controls (95% CI +5 to 0 pmol/L).

TABLE 17 Mean (SD) results of tests of thyroid function in healthy controls and in 5 groups of epileptic patients

	Controls (n = 11)	Untreated epileptics (n = 14)	Sodium Valproate (n = 10)	Carbamazepine (n = 19)	Phenytoin (n = 13)	Combination treatment (n = 12)
Total thyroxine (T_4) (nmol/L)	86.6 (21)	92.9 (22)	99.9 (20)	71.6[a,d,f] (18)	65.0[c,e] (21)	65.2[a,d,f] (15)
Free thyroxine (FT_4) (pmol/L)	16.4 (3.5)	16.0 (5.2)	18.3 (2.9)	14.3[e] (3.2)	14.1[e] (4.2)	12.2[b,f] (2.8)
Tri-iodothyronine (T_3) (nmol/L)	1.71 (0.25)	1.86 (0.28)	1.80 (0.39)	1.76 (0.2)	1.53 (0.34)	1.77 (0.37)
Thyrotropin (TSH) (mU/L)	1.26 (0.75)	1.48 (0.72)	1.53 (0.78)	1.50 (0.69)	1.03 (0.4)	1.51 (0.73)
TRH test (% TSH rise)	573 (376)	648 (214)	683 (327)	768 (340)	574 (336)	554 (240)
	(n=6)	(n=7)	(n=6)	(n=11)	(n=9)	(n=8)

Kruskal-Wallis showed significant variation in T_4 (P<0.005) and FT_4 (P<0.005). Mann-Whitney vs Controls [a] P<0.05, [b] P<0.01 ; vs Untreated epileptics [c] P<0.01, [d] P<0.005; vs Valproate [e] P<0.005, [f] P<0.001
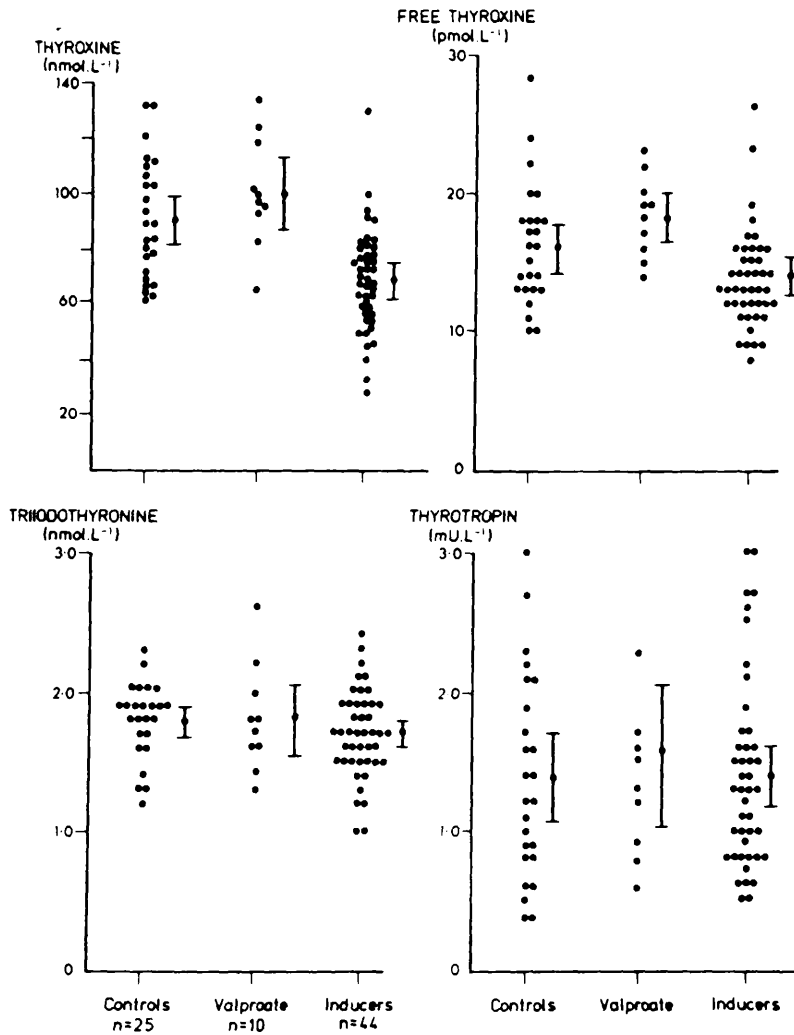
FIGURE 28   Thyroid hormone and thyrotropin (TSH) concentrations in controls, in patients receiving sodium valproate (VPA) monotherapy, and in those taking enzyme-inducing AEDs either singly or in combination. Vertical bars are mean (±SEM). Total and free thyroxine were lower in the inducer group than in controls (P<0.001, P<0.05) and VPA patients (P<0.001, P<0.002). Mann-Whitney 'U' test.

No significant correlation was found between circulating CBZ or PHT concentrations and T4 or FT4 concentrations in the monotherapy patients. There were no significant variations in the six or three groups for T3 or basal TSH. The PHT patients had lower concentrations of T3 and TSH than both controls and the other drug groups using simple Mann-Whitney testing, but the non-significant Kruskal-Wallis test suggests, as with some of the sex hormones, that this could be due to the compounding effect of multiple analyses. None of the TRH tests performed gave results suggestive of hypothyroidism. All of the tests gave normal rises of TSH (> 100%) and there were no important differences between the groups.

DISCUSSION

Sex Hormones

The similarity between untreated and healthy subjects suggests that epilepsy per se did not affect the hormone profile. Hypothalamic-pituitary function may be affected by ictal activity through limbic structures [Mattson & Cramer 1985], but all patients were studied following a 48h seizure-free period, and any central hormonal dysfunction provoked by complex partial seizures is known to reverse within hours [Pritchard et al 1983]. The "untreated epileptics" control group is not ideal, as they have less severe epilepsy, but the ideal group of patients with regular untreated seizures over a long period is, of course, unachievable.

Total T concentrations were unaltered in all drug groups, in keeping with many previous and subsequent reports [Dana-Haeri et al 1982, Toone et al 1984, Rodin et al 1984, Isojarvi et al 1989b], although others have shown an increase [Toone et al. 1982, Isojarvi et al 1990]. There was, however a trend towards a decrease in free T in induced patients

(P=0.06) and it is this active unbound fraction [Anderson 1974] which some workers have found to be decreased in patients with reduced sexual drive [Toone et al 1983]. This finding appears to be confirmed by later work [Toone et al 1984, Fenwick et al 1985], but these papers quote the same data. Other workers have found both free and total T to relate to the volunteered symptoms of low sexual drive [Rodin et al 1984] or decreased nocturnal erections [Fenwick et al 1986]. While any alteration in free T was marginal in the present study, definite changes occurred with SHBG, DHAS and PRL.

The increased SHBG would be expected to influence FT, but there was only a borderline decrease (P = 0.06) seen and only when all of the induced patients' results were amalgamated. This would suggest at least some normal functioning of the pituitary response to low FT levels, which would normally increase testosterone production by releasing LH. The increased LH concentrations in the induced groups just failed to reach significant levels (Mann-W P<0.02, but Kruskal-Wallis not significant) but are consistent with a response, while LHRH stimulation tests also suggested that the hypothalamic-pituitary axis was intact. However, the slight fall in FT may suggest that homeostatic mechanisms are not working to normal capacity. There may be an inability of the testicular Leydig cells to increase synthesis further. Healthy subjects treated with CBZ have shown an initial fall in free T which was fully compensated after three weeks of therapy [Connell et al 1984a]. A failure of homeostasis in epileptic patients could arise through a direct toxic effect of AEDs on the testes as suggested by Toone [1985]. Alternatively, inducing drugs may stress the individual's homeostatic responses by increasing SHBG and accelerating testosterone metabolism, while the eventual outcome is dependent on each person's homeostatic mechanisms - some of which may be relatively deficient as a normal variant, rather than due to epilepsy. This might explain why some patients complain of sexual problems while others do not, and why population studies such as this only find marginal changes in mean values of free T.

DHAS is a major androgenic steroid secreted principally by the adrenal gland [Bentley 1980a]. Its increased release in boys at the age of 9-10 has been described as a posiible "adrenarche" initiating puberty [Ducharme & Collu 1982]. Low levels have been associated with increased mortality in older men [Barrett-Connor et al 1986], although its precise function as a sex hormone is unknown. Our results show a marked reduction in DHAS in all groups receiving enzyme-inducing AEDs. These findings confirm earlier reports of reduced DHAS in healthy male subjects treated with CBZ [Connell et al 1984a] and in male and female epileptic patients receiving CBZ and PHT [Levesque et al 1986]. Why DHAS should be more obviously affected than other hormones is unclear, but it may simply be a manifestation of unequal effects on different enzymes. Pregnenolone and progesterone are metabolised to DHAS and androstenedione respectively via 17alpha-hydroxypregnenolone or 17alpha-hydroxyprogesterone. All steps are interchangeable and androstenedione may be further reversibly transposed to testosterone [Bentley 1980a]. It is easy to see how varying degrees of enzyme induction might result in differential effects on hormone levels. Alternatively, the negative feedback response may be more sensitive to changes in other hormones e.g. testosterone.

Prolactin concentrations may also be of some importance, since hyperprolactinaemia is associated with impotence [Cooper 1986] and hypogonadism [Bentley 1980b]. Elevation of PRL may occur in epilepsy for some hours following generalised [Trimble 1978] or partial seizures [Pritchard et al 1983, Rao et al 1989]. It has been suggested that baseline levels may be increased in patients taking multiple AEDs [Toone et al 1983] or CBZ alone [Franceschi et al 1984, Rodin et al 1984], but other papers have reported no difference [Isojarvi et al 1988] or even a decrease in baseline levels [Isojarvi et al 1989a]. LHRH/TSH stimulation has failed to show differences, except in female patients at 2 hours [Dana-Haeri et al 1984], although metoclopramide stimulation may demonstrate more convincingly an increased PRL response in CBZ-, PHT- and PB-treated patients [Franceschi et al 1984]. Since the present study, Isojarvi and his co-workers first found

no enhancement of PRL response to metoclopramide in patients taking CBZ [1989a], then found such an enhancement in similar studies [1989b, 1990]. This last report also showed a low baseline PRL in patients taking PHT. The changes appear worryingly inconsistent, but the present study conforms to the general trend for CBZ to be the AED most likely to affect PRL levels, which may suggest that this drug has a differential effect on dopaminergic function. An alternative mechanism, suggested by Isojarvi et al [1989a] is by way of the changes in thyroid hormones, which are also known to affect PRL responses [Yamaji 1974].

The effect of AEDs on central endocrine function is unclear. Impaired LH response to LHRH stimulation has been noted in treated epileptics already known to have sexual hypofunction [Spark et al. 1984] and Masala and his co-workers have shown impairment of LH response with PB [1980] but not with VPA [1981]. However, an exaggerated response to LHRH - consistent with primary gonadal dysfunction - has been shown with PHT but not CBZ [Dana-Haeri et al. 1984]. The present study showed adequate responses in all drug groups - even polypharmacy - and does not implicate a clinically relevant alteration in pituitary responsiveness being caused by AEDs.

Thyroid Hormones

While the incidence of clinical hypothyroidism due to anticonvulsants can be counted on anecdotal fingers [Aanderud & Standjord 1980, Blackshear et al 1983], depression of T4, FT4 and T3, in the absence of changes in TSH, is well documented [Liewendahl et al. 1978, Aanderud et al. 1981, Connell et al. 1984b]. Our results, with the inclusion of an untreated epilepsy group, confirm that this is an effect of CBZ and PHT and is not associated with the disease itself. Nine (20%) and five (11%) of the patients receiving enzyme-inducing drugs had "hypothyroid" total T4 and FT4 concentrations respectively,

while no subjects in the other groups were thus affected. T3 concentrations were not shown to be altered, but we may have failed to pick up an effect smaller than that on T4 and FT4. The statistical analysis of individual groups in Table 17 are shown as calculated without a correction factor, and demonstrate that the differences between inducers, VPA, and controls are not produced by any one drug.

The lack of response of TSH to the lowered T4 concentrations remains unexplained. The pituitary-mediated negative feedback system can be provoked by either T4 or T3. Although the initiating steps may be T3-dependent, T4 conversion within the pituitary may supply T3 for this mechanism [Larsen 1982]. None of the 9 patients with "hypothyroid" T4 concentrations had an inappropriate TSH response (range 0.8-2.7 mU/L). This may be a consequence of normal T3 concentrations, although an influence of CBZ and PHT on pituitary feedback cannot be excluded. Phenytoin has been shown to depress TSH response to TRH in both hypothyroid patients and animals [Surks et al. 1983]. However, if this effect is important, it is perhaps surprising that more patients do not become clinically hypothyroid.

The role of enzyme induction in the lowering of T4 and FT4 is unclear. Following the initial observation that PHT reduced serum protein bound iodine [Oppenheimer et al. 1961] it was shown that PHT displaced T4 from thyroxine binding globulin [Wolff et al. 1961, Oppenheimer et al. 1962,] and this was assumed to be the mechanism. However, methods developed for the estimation of FT4 showed that the free fraction was not increased and thus both total and free thyroxine were reduced [Chin & Schussler 1968], suggesting that the decrease in globulin binding could not be the whole story. Enhanced clearance of T4 was put forward as a possible mechanism [Larsen et al. 1970]. Although T4 is normally metabolised by mono-iodination to T3 or reverse T3 - processes not normally susceptible to induction by AEDs - it may also undergo deamination and decarboxylation [Chopra et al. 1978], of which the former at least can be catalysed by the

P450 system and will thus be inducible [Park 1982]. Induction of this "wastage pathway" may increase its relative importance to T4 breakdown. In support of this suggestion, it has been shown that isolated livers from PHT-treated rats exhibit accelerated metabolism of T4 [Mendoza et al. 1966]. An alternative theory put forward by Rao et al [1987], is of interest in view of the second part of this thesis concerning itself with calcium antagonists. Since one of these drugs, flunarizine, causes similar changes in T4 to those described above, they suggest that interference with calmodulin transport of thyroid hormones may be the basis of the effect. There is, however, little supportive evidence.

Recent studies have shown little [Haidukewych & Rodin 1987] or no effect [Connacher et al. 1987] with VPA on thyroid hormones. The first of these papers suggested a possible small decrease in hormone levels caused by VPA monotherapy, but comparisons were made with standard reference ranges rather than specific control groups. The present study included healthy and untreated controls, and the confidence intervals appear to exclude the possibility that VPA lowers T4 and FT4, and may even suggest that VPA increases T4 concentrations, which would be consistent with previous indications of a small inhibitory effect on oxidative metabolism [Levy & Koch 1982].

This study demonstrates a wide range of effects of AEDs on sex and thyroid hormones. The most striking overall finding is the disparity between the effects of VPA and those of the other AEDs, which supports the case for enzyme induction as the basis for all of the changes seen. A recent study [Isojarvi et al 1990] has suggested changes in serum oestradiol, LH and FSH in males taking VPA, but the patients were markedly younger than the controls, and this may have influenced the results. The cumulative evidence is against any significant effect with VPA. While the clinical relevance is uncertain, VPA may be preferrable to other AEDs in the occasional case of hypothyroidism [Aanderud & Strandjord 1980] or in male patients reporting sexual dysfunction. An analogue of CBZ

which does not induce enzymes could present another appealing option, so the reports of oxcarbazepine being such a drug required serious consideration and investigation.

EXPT. 7   OXCARBAZEPINE AND ENZYME INDUCTION

AIM

To investigate the enzyme-inducing properties of oxcarbazepine in healthy subjects by assessing the effect of two weeks' therapy on concentrations of the active metabolite 10-OH-CZ, and on other endogenous and exogenous substances metabolised by inducible pathways.

MATERIALS AND METHODS

Eight healthy male volunteers (aged 22-43 years, weight 70-89kg.) took part in a study of similar design to EXPT. 4. The study was performed in two stages.

Single dose

After an overnight fast, 300mg of OXC was ingested at 09.00h and blood drawn from an ante-cubital vein 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32, 48, 56, 72 and 96h later for measurement of serum OXC, its major metabolite 10-OH-CZ and a further metabolic product 10,11-dihydrotrans-10,11-dihydroxy-carbamazepine (trans-diol).

Chronic Dosing

After a washout period of 7 days, subjects commenced taking OXC 300mg. at 9.00h and 21.00h. daily for two weeks finishing with the 29th. dose at 9.00h. on the 15th. day. Blood was drawn for trough (pre-dosing) serum concentrations of the three chemical moieties before doses 1, 2, 3, 4, 5, 7, 15, 21, 23 and 29. After dose 29, serial blood

samples were taken as in the single dose stage.

Induction Markers

Antipyrine kinetics were assessed at least 48h before the single dose stage ("baseline"), on days 8 and 15 of chronic dosing, and two weeks after OXC was discontinued ("post-study"). Urinary excretion of 6 beta-hydroxycortisol in a 24h sample was measured at baseline, on days 8 and 15, and post-study as were serum concentrations of Testosterone (T), Androstenedione (AND), Dehydroepiandrosterone (DHAS), Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH) and Sex Hormone Binding Globulin (SHBG). Free androgen index (FAI) was calculated from the expression (Tx100/SHBG)%. Routine biochemical tests were performed at baseline and on day 15.

Assays

Blood samples were centrifuged after withdrawal and the serum stored at $-20^{\circ}C$ for batch analysis. The concentrations of OXC and its metabolites were determined using the h.p.l.c. method of Kumps [1984] as we found that antipyrine did not interfere with this assay. The limit of quantitation was 0.05ug/ml for all three compounds, and the % coefficients of intra-assay variation for OXC, 10-OH-CZ and trans-diol were 5.4%, 4% and 4.1% respectively. A sample chromatogram of two runs of "prepared standard" concentrations is shown in figure 29. This shows the measurement of trans-diol, 10-OH-CZ and OXC in concentrations of 2, 10 and 1 mg/L respectively, then 1.5, 7.5 and 0.75 mg/L.

Antipyrine concentrations in saliva were measured using h.p.l.c. as previously described [MacPhee et al. 1984]. Serum T, AND, DHAS, FSH, LH and PRL were determined by

```
 ·  4.45
·  5.27          5.29    ( / )    22638              3      7.856    +/ ) ?
   5.85          6.06             4477       R      1
   6.95          6.92             347               4      0.168
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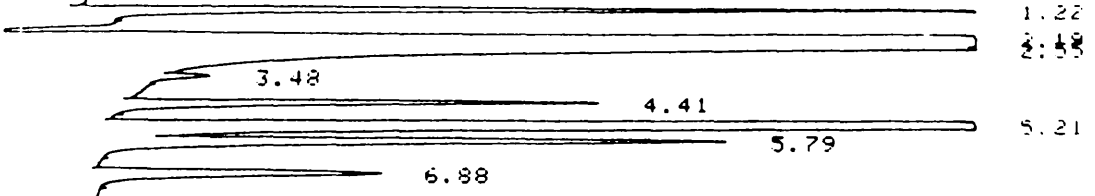
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       5.79   6.06   4875   R   1   1
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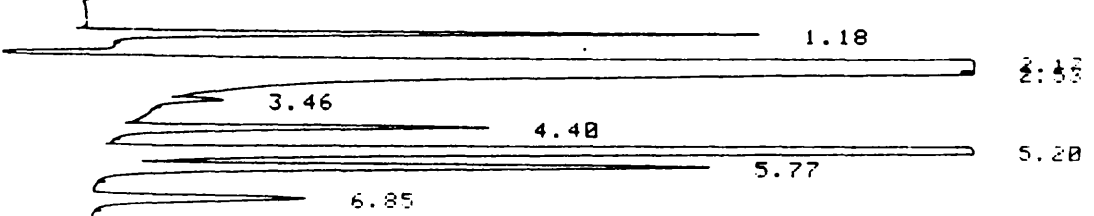
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       5.77   6.06   4729   R .75 1
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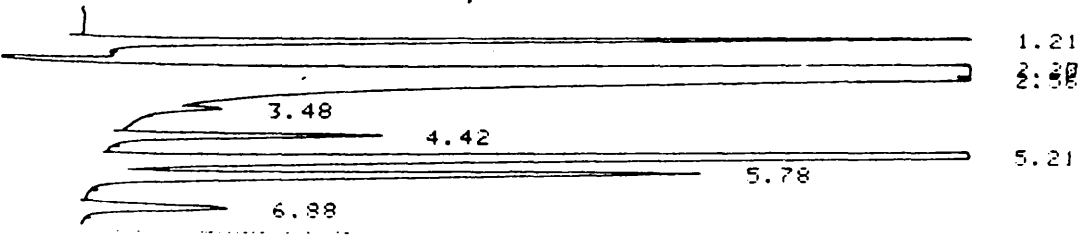
FIGURE 29   Chromatogram (h.p.l.c.) from simultaneous assay of trans-diol, 10-OH-CZ and oxcarbazepine itself. Assays are of standard preparations. In the top assay, these are of 2, 10, and 1 mg/L respectively. The lower assay is of 1.5, 7.5 and 0.75 mg/L standards.

standard radioimmunoassay [Connell et al. 1984a] and SHBG was analysed using a commercial immunofluorimetric assay (DELFIA, Pharmacia Ltd.). Urinary 6 beta-hydroxycortisol was measured by a specific radioimmunoassay method [Park 1978]. Thyroid hormone assays were performed by radioimmunoassay [Connell et al 1984b]. All hormone assays were kindly performed by Dr. GH Beastall of the Biochemistry Department, Glasgow Royal Infirmary. Mean interassay coefficients of variation for sex hormone assays were all between 5-10%, and for T4:6.5%, T3:5.9%, free T4:6.3% and TSH:8.9%.

Kinetics and Statistics

Kinetic parameters were calculated as described earlier. Trough concentrations during chronic dosing were predicted from the single-dose data using superposition [Gibaldi & Perrier, 1982] and compared with actual values.

The Wilcoxon matched-pairs signed-ranks test was used for direct comparisons, and Friedman two-way analysis of variance was employed for more than two groups.

RESULTS

OXC and metabolites

The concentration-time curves for OXC and 10-OH-CZ following single-dose OXC, using the mean of eight results at each time point, are shown in figure 30. Trans-diol levels were largely undetectable. The comparable curves following the 29th. dose are given in figure 31. All subjects demonstrated a higher AUC(0-12h) at steady state (mean ± SEM, 100 ± 15 mg.h/L) for 10-OH-CZ than the equivalent AUC(0-infinity) following
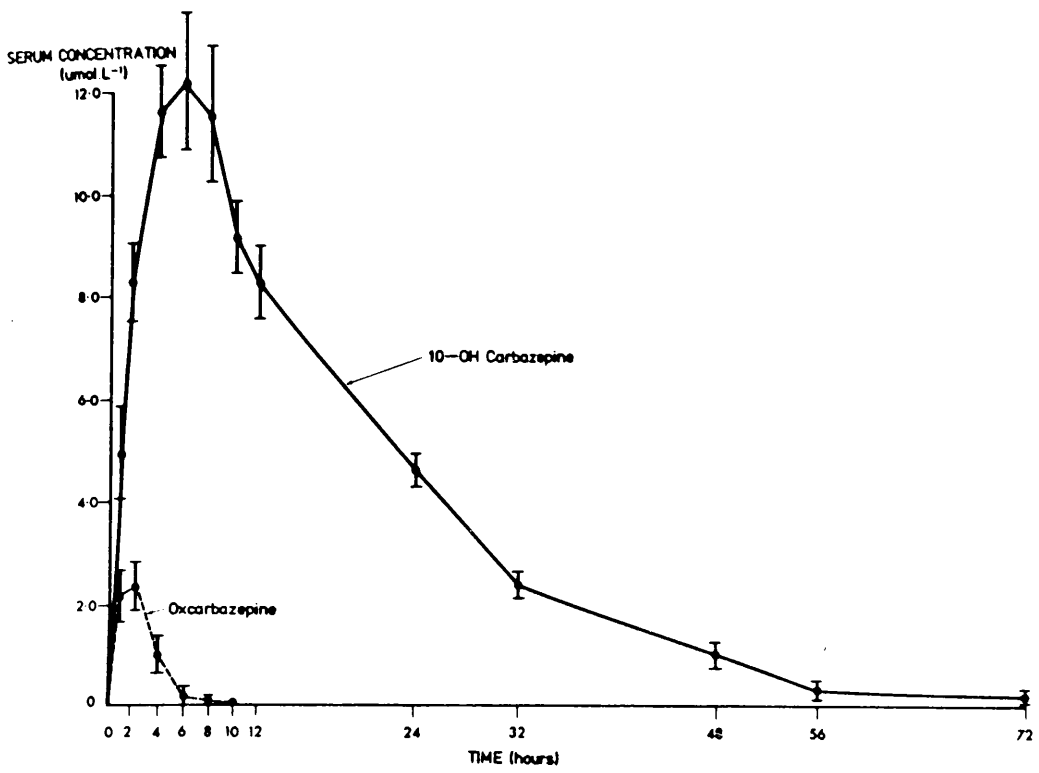
FIGURE 30   Concentration-time curves of oxcarbazepine and its metabolite 10-OH-CZ following 300mg oxcarbazepine by mouth in eight subjects (ϕ mean ±SEM).
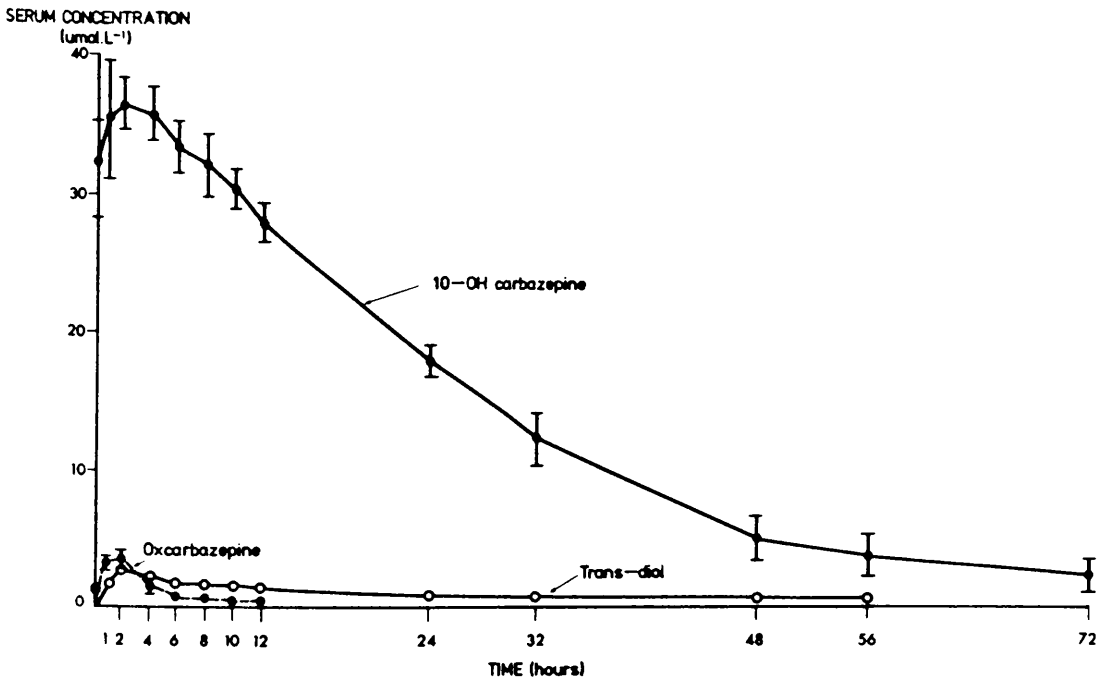
FIGURE 31   Concentration-time curves of oxcarbazepine and its metabolites 10-OH-CZ and trans-diol following the 29th dose of oxcarbazepine 300mg b.d. (ϕ mean ±SEM).
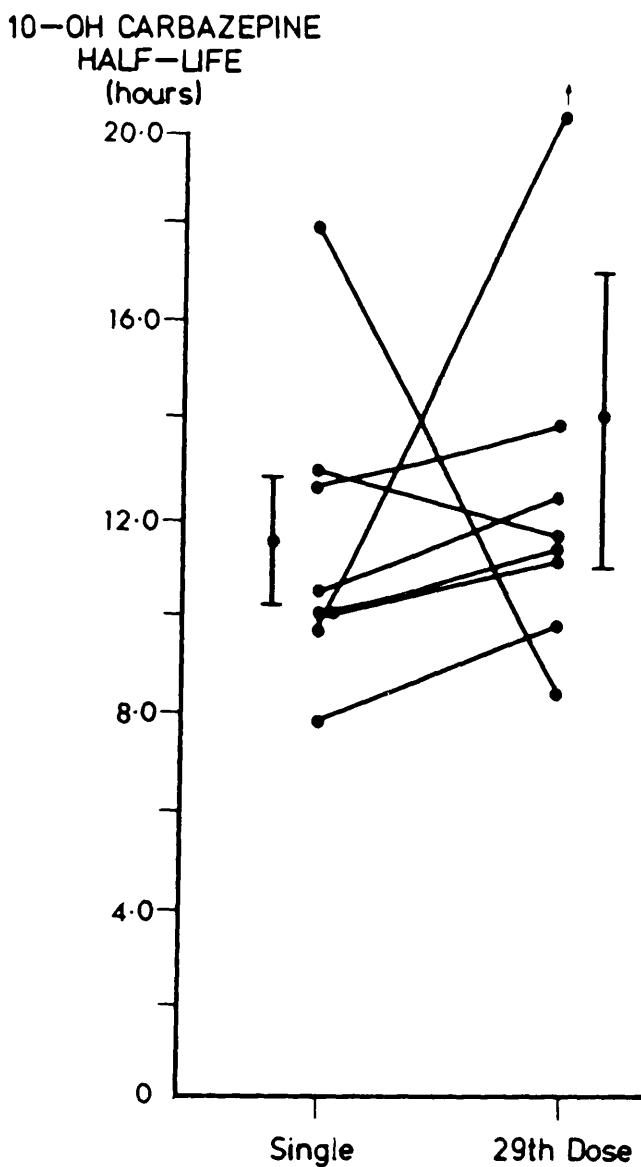
FIGURE 32   Half-life of 10-OH-CZ in eight subjects following single dose 300mg oxcarbazepine or after two weeks therapy (⬥ mean ±SEM).
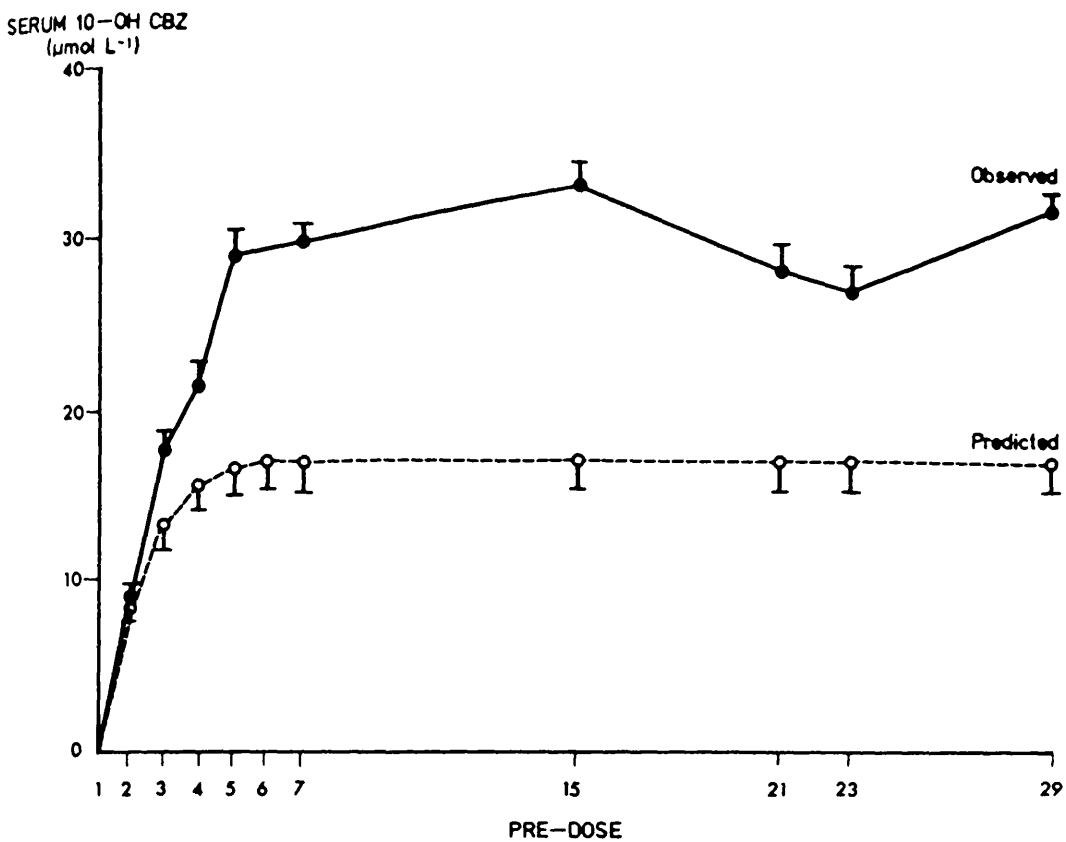
FIGURE 33   Mean pre-dosing serum concentrations of 10-OH-CZ in eight subjects taking oxcarbazepine 300mg b.d. Actual concentrations (●–●) and concentrations predicted from single-dose data (o--o) shown (mean ± SEM).
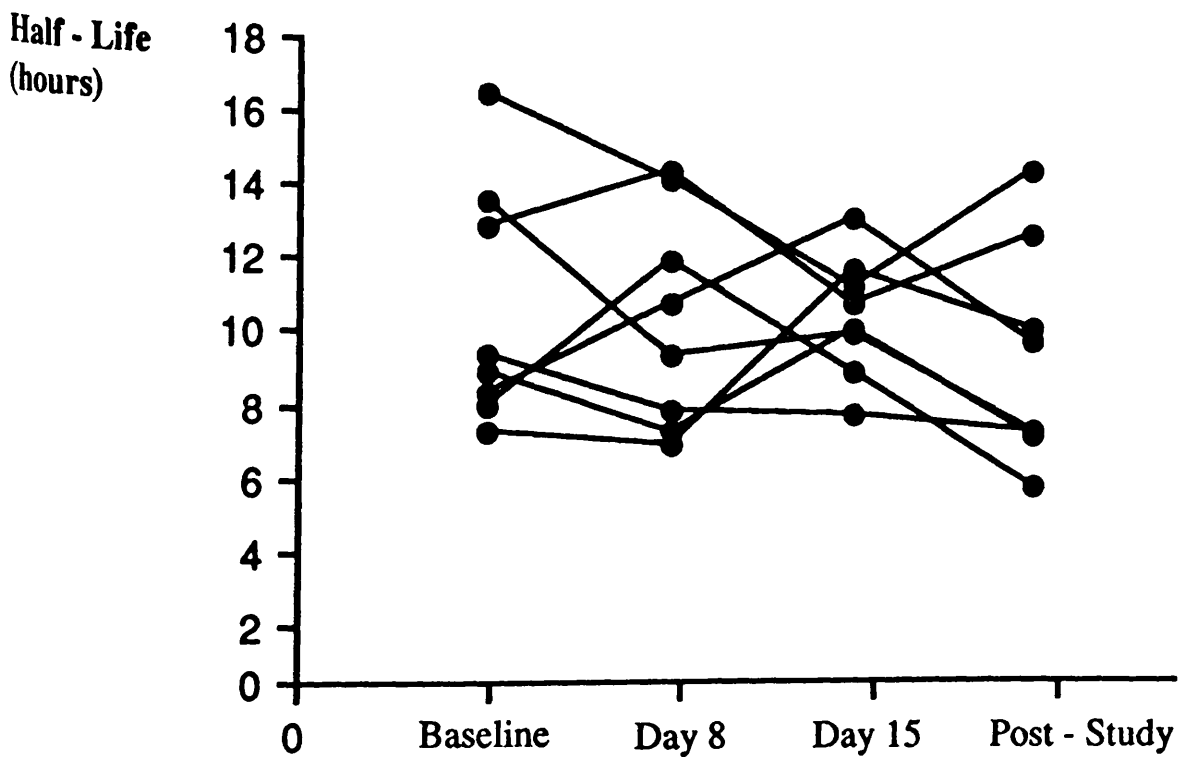
FIGURE 34   Individual antipyrine half-life values in eight subjects before, during (day 8 and day 15) and two weeks after two weeks therapy with oxcarbazepine 300mg b.d.

single dose (65 $\pm$ 15 mg.h/L, P<0.005). An increase was also seen for OXC (14.1 $\pm$ 6.4 mg.h/L vs. 8.0 $\pm$ 5.7mg.h/L) but these figures are less reliable owing to the small number of points on many curves. Elimination half-life of 10-OH-CZ was not significantly altered at day 15 from the baseline (Figure 32. 95% CI change -2.9 to +5h).

Mean predicted trough concentrations ($\pm$SD) from single-dose data and means of actual results are shown in Figure 33. Each individual subject had higher measured than predicted values at all steady-state time points.

Markers of Induction

Antipyrine half-life (95% CI change, -2.7 to +3.1h) and clearance (95% CI change, -0.1 to +0.29 ml/kg.min) and 6-beta OH-cortisol excretion (95% CI change, -151 to +197 ug/24h) were not altered by chronic dosing with OXC (Table 18). Individual antipyrine half-life results at all time points are shown in Figure 34. The hormone and SHBG levels showed no significant changes during OXC treatment (Table 19) although DHAS and FAI both showed a possible "rebound" increase post-study. The 95% CIs for the changes in hormones and binding protein between baseline and day 15 were: T (-5.9 to +2.3 nmol/L), SHBG (-9.6 to +5.6 nmol/L), AND (-4.1 to +4.5 nmol/L), DHAS (-3.1 to +1.9 umol/L), FAI (-14.9 to +10.7%), FSH (-2.3 to +5.6 U/L), LH (-1.7 to +3.6 U/L) and PRL (-19 to +44 mU/L).

DISCUSSION

The clinical use of carbamazepine may be complicated by its enzyme-inducing properties. In the present study its analogue, OXC, was found to be devoid of any auto-

TABLE 18 Antipyrine kinetics and urinary 6-beta hydroxycortisol excretion before, during and after two weeks' treatment with oxcarbazepine in 8 male subjects

	BASELINE	DAY 8	DAY 15	POST-STUDY
Antipyrine half-life (h)	10.6 (3.3)	10.3 (3.0)	10.4 (1.7)	9.3 (3.0)
Antipyrine clearance (ml/kg.min)	0.88 (0.20)	0.86 (0.20)	0.98 (0.15)	0.96 (0.23)
6-beta hydroxycortisol (ug/24h)	221 (180)	300 (129)	250 (149)	232 (129)

Values are mean (SD)

TABLE 19 Mean (SD) circulating hormones, sex hormone binding globulin concentrations and free androgen index before, during and after two weeks' treatment with oxcarbazepine in 8 male subjects

	BASELINE	DAY 8	DAY 15	POST-STUDY
Testosterone (nmol/L)	14.3 (3.8)	13.3 (2.4)	12.6 (3.8)	13.9 (2.4)
Sex hormone binding globulin (nmol/L)	24.9 (7.5)	25.4 (9.1)	22.9 (6.6)	22.4 (7.2)
Androstenedione (nmol/L)	7.3 (3.7)	5.3** (2.8)	7.5# (4.2)	6.7 (2.2)
Dehydroepiandrosterone sulphate (umol/L)	8.3 (2.6)	8.1 (2.5)	7.7 (2.0)	9.9## (2.3)
Free androgen index (%)	58.8 (10.5)	56.1 (16.6)	56.6 (13.0)	65.4## (15.3)
Luteinising hormone (U/L)	6.3 (2.2)	7.2 (1.7)	7.2 (2.7)	8.1* (2.5)
Follicle stimulating hormone (U/L)	3.2 (2.7)	4.2** (3.0)	4.6 (4.1)	3.7* (2.7)
+Prolactin (mU/L)	118 (28)	170** (41)	131 (28)	186** (64)

Friedman's analysis of variance + $P<0.05$

Wilcoxon matched pairs

* $P<0.05$ vs baseline

** $P<0.01$ vs baseline

\# $P<0.05$ vs day 8

\#\# $P<0.05$ vs day 15

inducing effects, as the half-life of the active metabolite remained unchanged after two weeks' administration - a time period sufficient for near-maximum induction by equivalent doses of CBZ [Rapeport et al. 1983, Mikati et al. 1989]. The confidence interval "excludes" a fall of 26% as opposed to the reduction in CBZ half-life of 40% in EXPT. 4 and of 38% in a similar previous report [Rapeport et al. 1983].

The standard markers of cytochrome P450 induction - antipyrine metabolism and 6-beta hydroxycortisol excretion [Roots et al. 1979, Park 1982, Rimmer et al. 1986] - were also unaffected by OXC therapy. Confidence intervals were against the likelihood of a reduction of 25% in antipyrine half-life (previous falls with CBZ - 35% in EXPT. 4, 33% [Connell et al. 1984a]); an increase of 33% in antipyrine clearance (CBZ - 39% [Connell et al. 1984a]), or a rise in 6 beta-hydroxycortisol excretion of 79% (CBZ - 178% [Rapeport et al. 1983]). These differences are illustrated graphically in Figures 35 & 36, while the effect of CBZ on hormone and binding protein concentrations is compared with that of OXC in Figure 37. The 95% CIs of the hormone measurements in the present study approximate the magnitude of changes previously shown with CBZ in SHBG, FAI and DHAS [Connell et al. 1984a], so changes of this magnitude were almost "excluded" at the 5% level. Thus, there was no evidence of OXC accelerating its own metabolism or that of any of the other indices of induction.

After two weeks' treatment with OXC, the dosage interval AUC of 10-OH-CZ was significantly larger than that to infinity following a single dose, and the observed steady-state concentration profile was also higher than predicted. Nevertheless, the time to reach steady-state was not affected, and the AUC ratio of 10-OH-CZ:OXC (where calculable) showed no overall change after repeated dosing. The elimination half-life of OXC (where calculable) was also unaffected by repeated dosing, suggesting a lack of autoinduction.
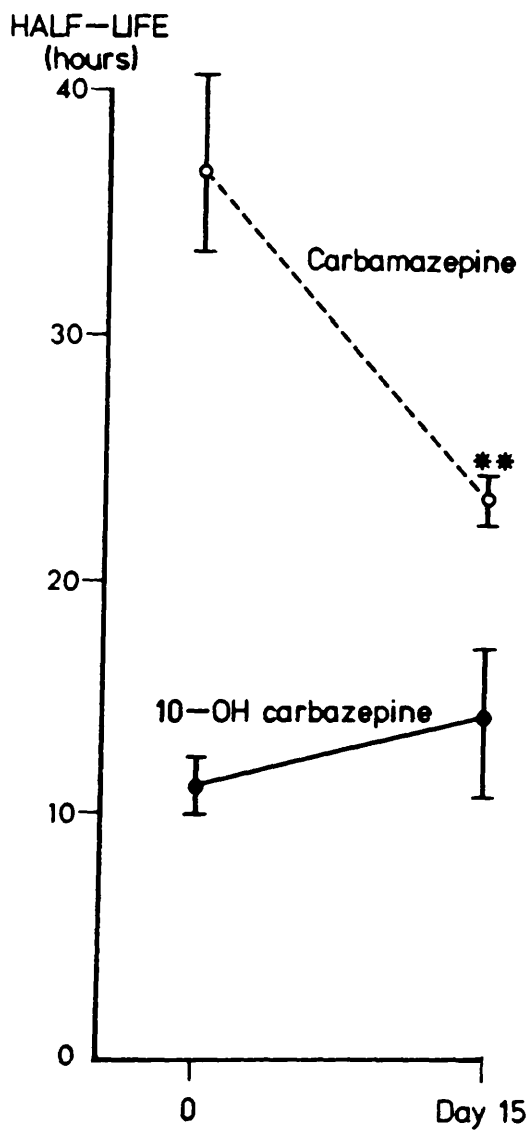
FIGURE 35   Effect of two weeks' oxcarbazepine on the half-life of its major metabolite 10-OH-CZ compared with the previously shown (EXPT. 4) effect of two weeks' carbamazepine on its own half-life. Mean ±SEM. ** P<0.01.
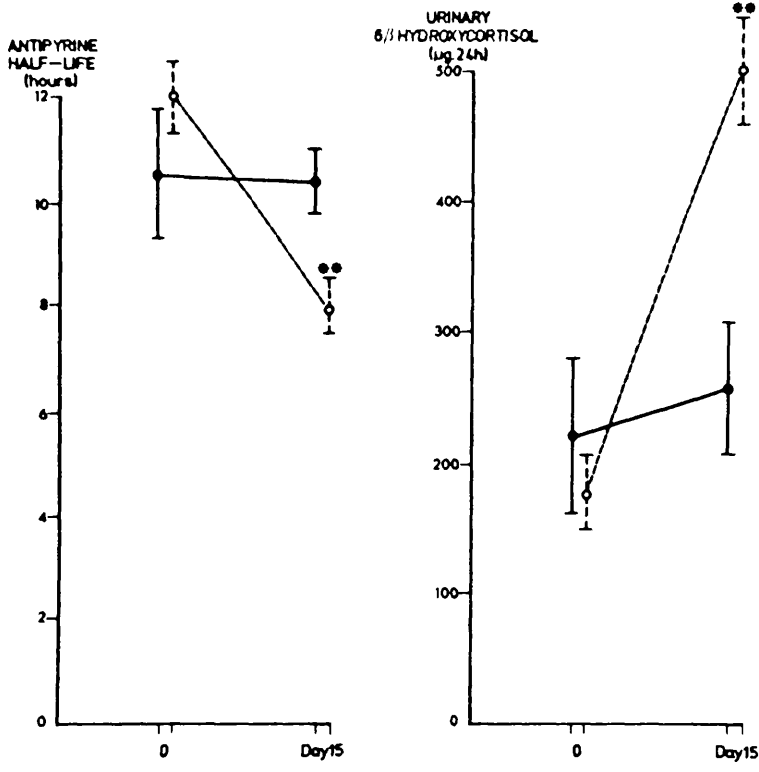
FIGURE 36   Comparison of effects of two weeks' oxcarbazepine (●—●) or
carbamazepine (o--o) on antipyrine half-life (cf. EXPT. 4) and 6-beta hydroxycortisol
excretion (cf. Connell et al 1984). ** P<0.01.
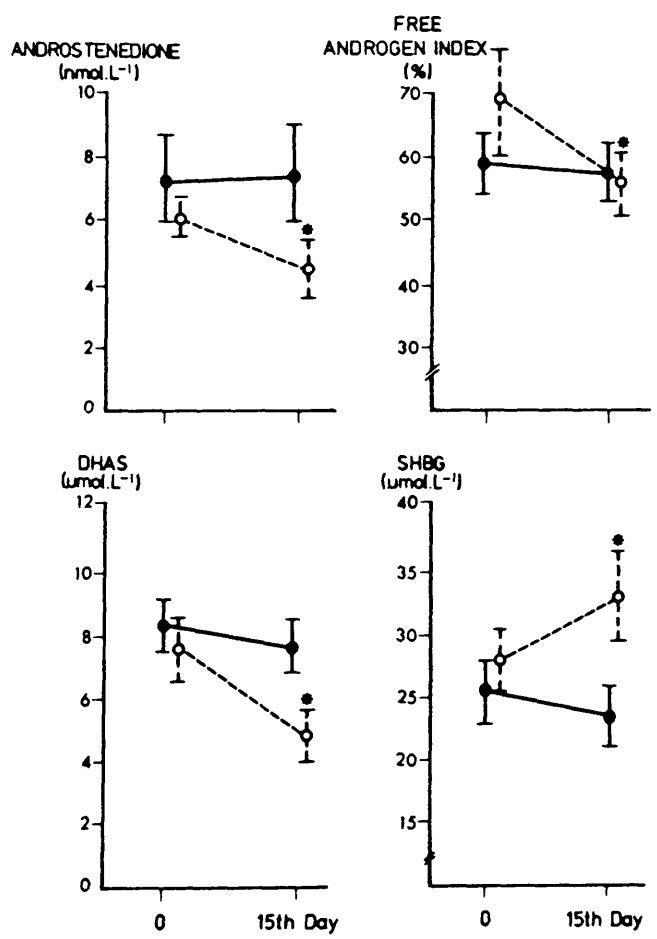
FIGURE 37   Comparison of effects of two weeks' oxcarbazepine (●—●) on sex
hormones and binding globulin with previously shown (cf. Connell et al 1984, Macphee
et al 1984) effects of carbamazepine (o--o). Mean ±SEM. * P<0.05.

The higher than predicted steady-state values require some comment. Recent work by the pharmaceutical company, Ciba-Geigy, has shown a 17% increase in the systemic availability of 10-OH-CZ when OXC is given after a meal [P Lloyd, personal communication]. Absorption of OXC itself may be slowed after a meal. In the present study, the single-dose and steady-state profiles were determined in fasted subjects, while steady-state trough levels were generally monitored under non-fasting conditions. This may account, at least in part, for the observed increase in serum levels of 10-OH-CZ. Another possible explanation is a putative "biphasic" elimination half-life for 10-OH-CZ. It has been suggested that the metabolism of OXC is saturable and so the elimination half-life may be prolonged at higher concentrations [Theisohn & Heimann 1982]. This could explain our findings, although analysis of the individual concentration-time curves showed only two which might better fit a biphasic model. The situation, therefore, remains unclear.

Of the hormonal measurements, only serum prolactin concentrations varied significantly during the study (Friedman's two-way analysis of variance, $P<0.05$). This difference is likely to be due to the use of indwelling cannulae for obtaining blood samples only for baseline and day 15 samples, which were the days when multiple sampling was performed. While none of the other hormones showed significant variation, it would be imprecise to ignore the trends for a rebound increase in AND, DHAS and FAI after an initial small fall and the trend for an increase in LH, since they are at variance with our hypothesis of lack of induction with OXC. These trends would be "statistically significant" if direct Wilcoxon tests were performed without the initial assessment by analysis of variance. Thus, while this may well be an effect of multiple analysis, we have not excluded some slight alteration of endogenous hormone metabolism caused by OXC.

If these small falls and rebound increases are indeed occurring, they could be due to increased LH, the negative feedback mechanism which would be expected to normalise

hormone values [Bentley 1980a] but which appeared inactive in a previous study with CBZ [Connell et al. 1984a]. This would suggest either that an unexplained rise in LH has occurred, or that a small fall in hormone levels is being caused by OXC. It is conceivable that, since T, AND, and DHAS are reduced as their first step in metabolism [Bentley 1980a], OXC - which is also reduced - might induce this pathway. This would explain a small effect on steroid metabolism, but no effect on the metabolism of 10-OH-CZ or that of markers of oxidative induction. A minor degree of induction by the OXC metabolite, 10-OH-CZ [Wagner & Scmid 1987], is another possible cause of a change in steroid metabolism, as the P450 cytochrome enzyme system is involved [Bentley 1980a] but this would not account for the discrepancy with other markers of oxidative metabolism. Significant induction of reducing enzymes is rare and unlikely to be of clinical significance [Bachur 1976].

Overall, we found no evidence of any major inducing effect of OXC on its own metabolism or those of other endogenous or exogenous markers of oxidation. By necessity, the dose of OXC was low and we cannot exclude at this stage a small induction effect in patients receiving a higher dose of the drug, as has very recently been suggested [Patsalos et al. 1990], however the difference between OXC and CBZ is obvious (Figures 35, 36, 37). OXC is a compound with similar anticonvulsant efficacy to CBZ, but which appears to have improved patient tolerability both with regard to allergic reactions and psychomotor impairment [Houtkooper et al. 1987, Reinikainen et al. 1987, Dam et al. 1989]. The latter potential advantage may be due to its lack of an epoxide metabolite, the role of which in the neurotoxic side-effects associated with CBZ has already been mentioned [Patsalos et al. 1985, Gillham et al. 1988].

It is this difference in metabolism which may also be responsible for the lack of monooxygenase induction with OXC demonstrated in the present study. This may give

OXC an advantage over CBZ in patients taking other drugs, particularly the contraceptive pill. However, a specific study to determine the effect of OXC on the metabolism and efficacy of oestrogen-containig contraceptives is indicated before we assume that no interaction exists.

It should be noted that the dosage of concomitant drugs will also require reappraisal if OXC is substituted for CBZ, as toxic concentraions of other AEDs could occur. As we have seen, the effect of enzyme induction on endogenous hormones remains unclear, and any elucidation of its importance may enhance the case of OXC as a possible first-line AED. Its place in the therapeutic armamentarium is as yet uncertain, but this should be clarified when OXC comes into more widespread use.

The problem of hyposexuality in epilepsy, and the relative importances of the treatment and the disease itself, remains a complex one. Perhaps it is significant that we have found, in EXPT 5, no change in the sex hormones in untreated epilepsy patients. Could hyposexuality be caused entirely by AEDs? While it may be argued that these are less severely affected patients, it is interesting to contrast the results of EXPT. 2, where a similar group of untreated epileptics had obvious psychomotor impairment compared with controls. Other workers have only rarely included untreated patients in their studies, and those who have done so, tend to find no differences between such a group and controls with regard to DHAS [Levesque et al 1986, Isojarvi et al 1988, 1989a], T, FT, SHBG, LH and FSH [Isojarvi et al 1988, 1989a]. Perhaps it is also significant that the concept of hyposexuality in epilepsy was not widely recognised until 1954 [Gastaut & Collomb] with very little mention of such problems in the pre-anticonvulsant era. Indeed, hypersexuality was considered as the norm, leading to such attempted treatments as clitoroidectomy and castration well into the nineteenth century [Duffy 1963]. Potassium bromide was first considered by Locock as a possible anticonvulsant because of its libido-reducing properties [Sieveking 1857].

It might, however, be too simplistic to suggest that hyposexuality in epilepsy is entirely due to the use of anticonvulsants. The early associations of hyposexuality with temporal lobe epilepsy, while not rigidly controlled, remain persuasive e.g. the finding by Hierons & Saunders [1966] of 15 cases of impotence - all amongst their TLE patients. They included a patient on no therapy, and Saunders & Rawson [1970] cite two further such cases. However, these were all severe cases of epilepsy following trauma. While the temporal lobe may have some part to play in sexual problems, it may be the underlying brain damage which was important in these patients, rather than the epilepsy itself. In the

normal epileptic population, it is more likely that social adaptation [Taylor 1969] and AED usage are the major factors in causing an increased incidence of hyposexuality. Certainly, the evidence for an intrinsic problem is less convincing than that with psychomotor impairment.

If drugs are to blame, enzyme induction may play an important part, though general sedative effects of AEDs may also contribute to the problem. It is clear that these anticonvulsants do alter hormone concentrations, although the mechanism - or order of events - is uncertain. An increase in SHBG is the most consistent finding in all of the studies performed, including EXPT. 5. It seems unlikely that this is a secondary phenomenon to an enzyme-induced fall in FT, as Toone suggests [1984], which leads to a "rise in LH with consequent increase in testosterone synthesis and so to an increase in bound testosterone and FT; and second to a rise in SHBG which in turn leads to a further increase in bound testosterone and to a fall in FT". It seems illogical that the homeostatic mechanisms compensating for a fall in FT should, with no other defect, result in a further lowering of FT.

Since studies which find no real change in total testosterone still find a markedly increased SHBG [Dana-Haeri et al 1982, EXPT. 5, Isojarvi et al 1989b], it is more likely that increased production of SHBG by the liver [Bentley 1980b] is a direct effect of enzyme induction. Free testosterone becomes decreased, and there is an LH response in an attempt to increase production of testosterone in the Leydig cells. The effectiveness of this feedback mechanism will obviously be hindered by an increase in T metabolism, and it may well be that the variation in findings with total and free testosterone is simply a reflection of the heterogeneity of patients' relevant homeostatic abilities. Under the stress of enzyme-inducing AEDs, some patients' mechanisms will cope, and some will not. In EXPT 5, while we failed to show a significant decrease in FT on AEDs, it should be

noted that 8 subjects had FT levels below 220pmol/L (6.6mg/dL) - and all of these were in the induced groups (3 CBZ, 3PHT, 2 polypharmacy). This level of FT has been described as a cut-off point below which patients tend to exhibit hyposexuality which improves with oral testosterone undecoanate therapy [Carani et al 1990]. These FT findings, although not "statistically significant" may represent clinically important findings in individual patients.

It appears, therefore, that any global assessment of epileptic patients will find evidence of hyposexuality, and evidence of hormonal changes. However, attempts to be more precise - looking at individual drugs or trying to relate hyposexuality to AED concentration - may fail simply because there is an intermediate variable factor, perhaps the patient's own homeostatic mechanisms, which dilutes the association. Some workers have managed to show, for instance, a positive correlation between CBZ concentration and PRL levels, and between low sexual drive and low FT, but have failed to take this a stage further and relate AED dosage or concentration with sexual function [Toone et al 1983]. A large scale study, looking at AED concentrations, hormone concentrations and a measure of sexual function is required, using groups of untreated patients, patients on different drugs, and controls.

Sexual function study

We have now designed and commenced such a study, where the patients' marital status, religion, social class, education and employment status are all recorded. The measure of sexual function used was the "sexual experience scale" (SES) as designed and validated by Frenken & Vennix [1981] at the University of Utrecht. Patients were recruited from the clinic, and controls from medical, laboratory and nursing staff, and from the patients' relatives, friends and spouses. Subjects answered questions in private using a video screen and user-friendly software prepared for an Apple II microcomputer. The questions are either of a yes/no variety, or allow answers on a three- to five-point scale between

two extremes. They fall into four different categories, and are designed to give an indication of the following aspects of sexual behaviour:

SES 1.   <u>Adherence to Restrictive Morality</u>

21 Questions: possible score 21-105.

High score = "high morality".

Example:

Q. It is acceptable that an engaged couple have sex together...

A. Completely agree...slightly agree...neither agree nor disagree...

...slightly disagree...completely disagree

SES 2.   <u>Allowance of Psychosexual Stimulation</u>

15 Questions: possible score 15-67.

Low score = high allowance of stimulation.

Example:

Q. I find watching a T.V. show with lots of nudity...

A. Very pleasant...fairly pleasant...a little pleasant...

...fairly unpleasant...very unpleasant.

SES 3.   <u>Attraction to Physical Sexual Stimulation</u> ("sexual motivation")

29 Questions: possible score 29-144

Low score = high sexual motivation.

Example:

Q. I find lengthy and extensive petting...

A. Very pleasant...fairly pleasant...a little pleasant...

...fairly unpleasant...very unpleasant

SES 4.   <u>Attraction to Marriage</u>

18 Questions; possible score 18-93

High score = high attraction to marriage.

Example:

Q. Are you satisfied with the affection and love your wife shows?

A. Not satisfied...could be better...completely satisfied

Scores for all questions in each category are summated to give an overall score for that category. Results may be divided into classifications - e.g. with regard to sexual motivation (SES3): "Appetitive" = 29-60, "Apathy" = 61-71, and "Aversion" =72-144. In this preliminary analysis, only the precise scores have been utilised.

Subjects supplied a venous blood sample for assay of sex hormones and anticonvulsant concentrations where appropriate. Antipyrine kinetics were also assessed in as many subjects as possible. These procedures were performed as previously outlined.

This is a long-term study with various facets, and has been conducted by Peter Milligan (pharmacist), Violet McKay (pharmacist), Jacqueline Blacklaw (research assistant), Carol Read (research assistant), Dr. Paul McKee and myself. The study is ongoing and involves male and female epileptic and healthy subjects. I have performed an interim analysis at various stages on males and females, the results of the first 50 males and females having been used as part of the M.Sc. theses of the first two named above. I will confine myself here to the data of the first 213 male subjects, of whom 96 have hormone assay results available (Table 20). This number is still too small for some comparisons, e.g. between individual drug groups, but much interesting information is already available.

TABLE 20 Details and mean (SD) Sexual Experience Scale scores of 213 subjects

	No	Mean Age	Further Education	Mean SES1	Mean SES2	No with SES3-4 results	Mean SES3	Mean SES4	Number with Hormone results
Controls	38	27.2 (7)	24	51.2 (11)	36.1 (7)	14	43.3 (10)	72.2 (4)	17
Untreated epileptics	31	28.2 (8)	14	55.5 (13)	41.7 (11)	13	49.6 (17)	70.3 (2)	15
Valproate	17	30.7 (11)	10	55.6 (18)	39 (8)	9	51.3 (21)	64.2 (10)	3
Carbamazepine	42	31.2 (9)	15	55.8 (14)	37.5 (8)	25	50.7 (12)	66.6 (11)	21
Phenytoin	28	32.1 (10)	13	58.7 (14)	42 (9)	15	56.7 (23)	68.5 (9)	16
Phenobarbitone	5	31.8 (6)	5	50.2 (11)	40.2 (11)	1	45	79	4
Polypharmacy	52	33.8 (6)	15	60.9 (14)	42.1 (9)	22	56.1 (16)	69.6 (7)	20

SES_1 : high score = 'high' morality. SES_2 : high score = low allowance psychosexual stimulation
SES_3 : high score = low allowance physical sexual stimulation. SES_4 : high score = high attraction to marriage

The most striking immediate finding further complicates the explanations of hyposexuality in epilepsy, as sufferers were shown to adhere to a more restrictive moral code than non-epileptics (P=0.0051). This might help explain their decreased affinity for psychosexual stimulation (P=0.0019) and physical stimulation (P=0.005), since a high morality was independently associated with low psychosexual (P<0.001) and physical sexual (P<0.001) appetite. The reasons for the "higher moral values" among epileptics may include a tendency for patients to spend more time under the "parental wing", or their decreased likelihood of going on to further education (P<0.001). The significance here is that, within the normal group (P<0.05) and within the epileptic group (P<0.0001), further education was independently associated with a lowered morality and a (?consequent) increase in psychosexual appetite (e.g. in epilepsy patients; P=0.0006). If we make allowance for this effect by studying further educated subjects in isolation, the difference in morality between epileptic and non-epileptic subjects becomes non-significant and all but disappears, but a trend to decreased physical appetite in epileptic patients (P=0.08) remains. Meanwhile, among subjects who have no further education, there is a definite decrease in sexual motivation among epileptics (P<0.01).

Other influential factors include religious affiliation (strong, moderate, weak) which affected morality (P<0.001) and allowance of psychosexual stimulation (P<0.01). It was also shown that untreated epileptic patients were less attracted to psychosexual stimulation than healthy controls (P=0.027), so it would appear that social conditioning or the disease itself must have some bearing on the hyposexuality, which cannot be an effect exclusively of the AEDs.

With regard to the hormone assay results, the drugs had similar effects to those in EXPT. 5, except that the CBZ effect on circulating PRL was not shown. The correlation of hormone concentrations with sexual function showed an interesting association between a raised SHBG and lowered physical appetite (r=0.498, P<0.01; Fig 38). The derived free

androgen index (r= -0.454, P<0.005) and freeT (r= -0.377, P<0.05) were also shown to correlate negatively with SES3 (i.e. positively with physical sexual appetite; Figures 39, 40).

So far, no significant differences have been seen between the drug groups, but one instance of making the link between drug, hormones and sexual function has been demonstrated. Free CBZ concentrations are inversely related to psychosexual appetite (r=0.633, P<0.02; Figure 41).

Clearly, when this database is complete, a substantial amount of information will be available, but it will require careful analysis. I have been able only to give a small preview of this, but what we can see is a further group of confounding factors - morality, education, social and religious background - which might influence sexuality among epileptic patients. Further education and separation from family ties seems to induce a more liberal morality, so, as a group, epileptic subjects may be relatively sexually inhibited.

It would appear that drugs are only one factor in the problem, although it may be true that they are more important than the physiological effects of the disease itself. While information from this massive study will hopefully prove the case, there is accumulating evidence that enzyme induction is a likely mechanism for the contribution of AEDs to altered sexuality in epileptic patients. It would seem appropriate, therefore, to use VPA in patients who complain of, or may suffer from, such problems. The advent of oxcarbazepine as a non-inducing "equivalent" of CBZ may give the clinician another valuable option.
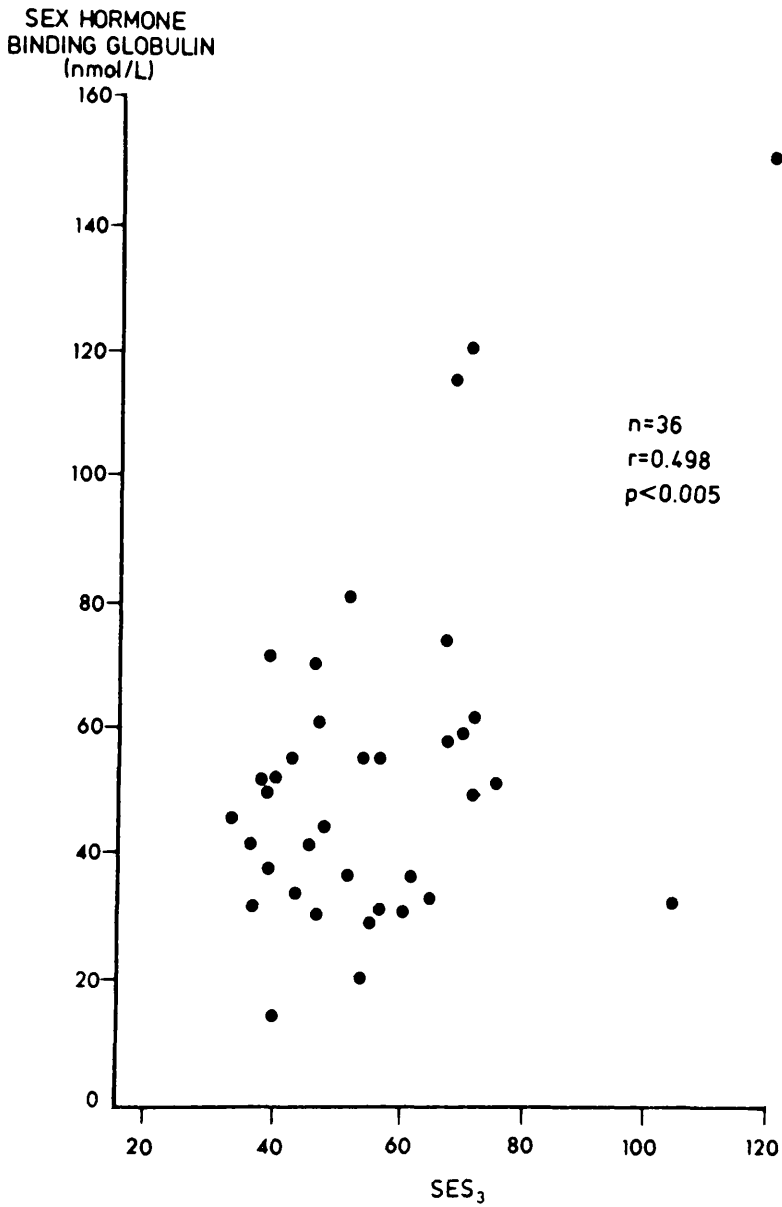
FIGURE 38   Correlation of Sex Hormone Binding Globulin concentrations and increasing SES3 scores (i.e. high SHBG, low sexual motivation) in 36 male subjects.

FIGURE 40 Negative correlation of free testosterone with increasing SES3 scores (i.e. high free testosterone, high sexual motivation).
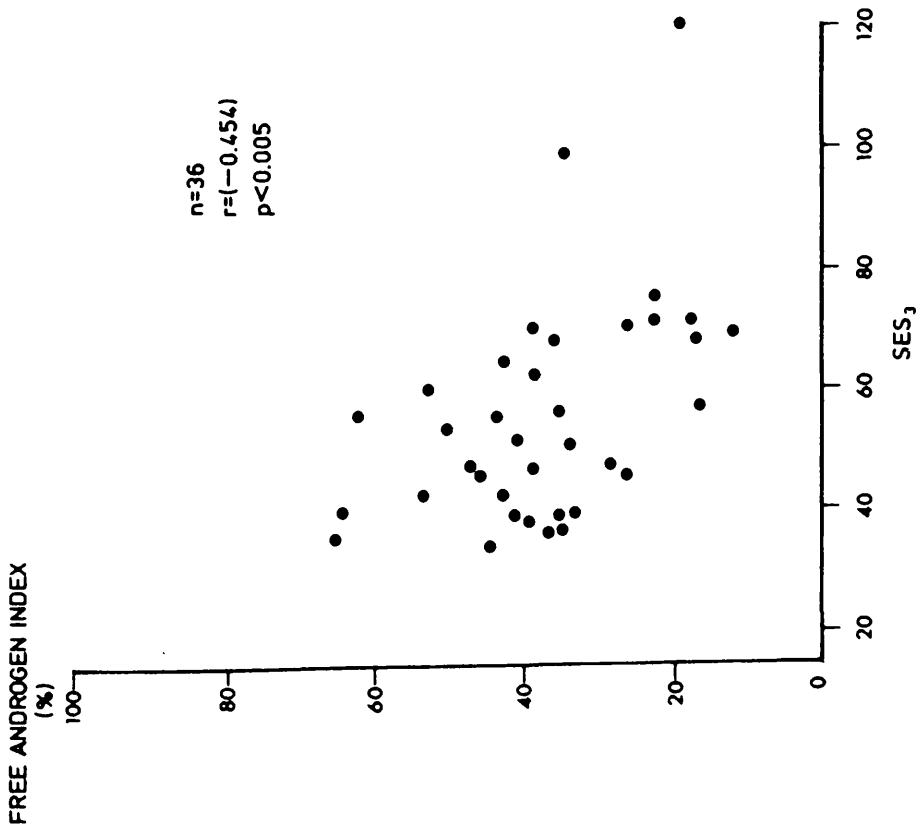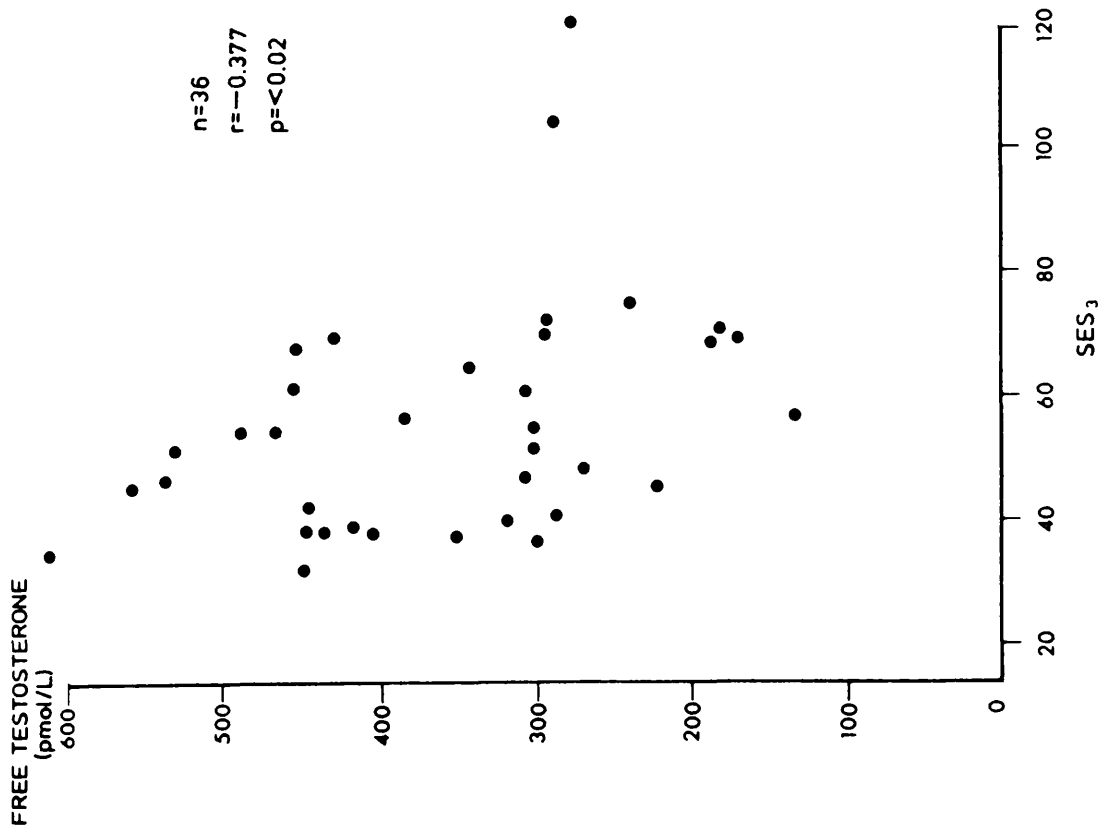
FIGURE 39 Negative correlation of Free Androgen Index with increasing SES3 scores (i.e. high FAI, high sexual motivation).
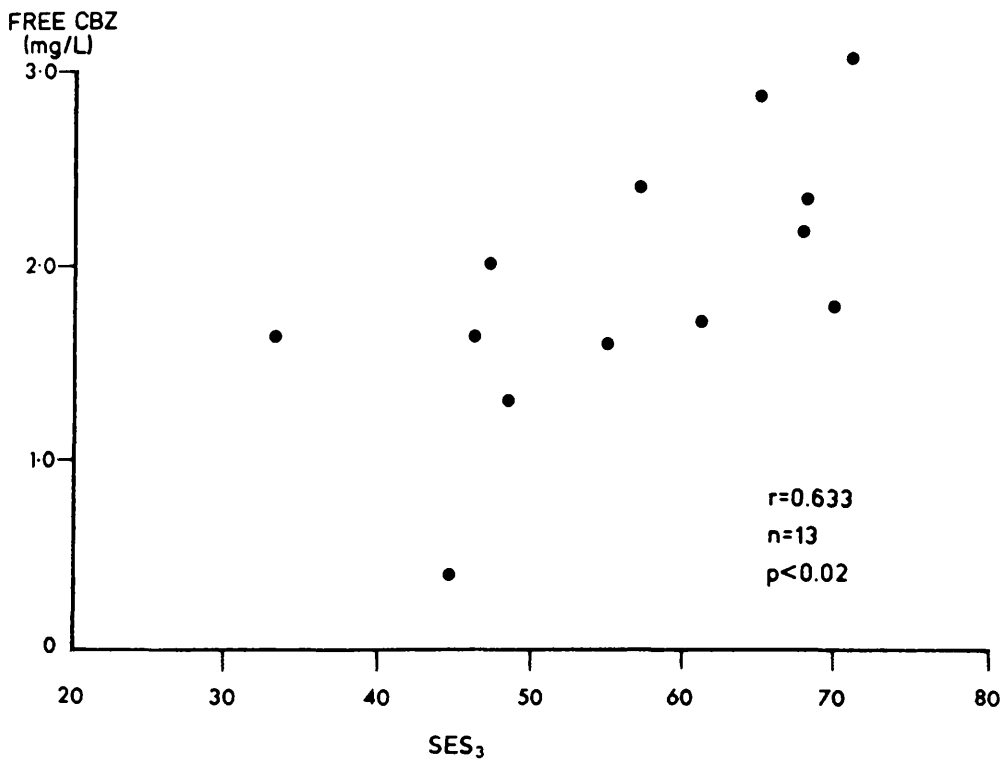
FIGURE 41 Correlation of serum free carbamazepine concentrations with increasing SES3 scores (i.e. high free carbamazepine levels, low sexual motivation) in 13 male epileptic patients.

PART 2

THE POSSIBLE ROLE OF CALCIUM ANTAGONISTS

The theoretical basis for the use of calcium antagonists in epilepsy will be discussed. Two drugs in the dihydropyridine group, nifedipine and nimodipine, are investigated. A preliminary open study of nifedipine (EXPT. 8) is followed by a pharmacokinetic assessment of these drugs in mice (EXPTS. 9 & 10). Further placebo-controlled studies of these drugs are presented (EXPTS. 11 & 12). The possible relevance of adenosine receptors is investigated using a radioligand binding assay in EXPT. 13. The present outlook and future avenues of research for these drugs are then discussed.

CHAPTER 4

CALCIUM ANTAGONISTS AND EPILEPSY

INTRODUCTION

In the first part of this thesis, I have demonstrated that many AEDs share the same side-effects - in particular psychomotor impairment and the effects of enzyme induction. Improvements in these factors with such drugs as slow-release CBZ and oxcarbazepine represent small steps forward in the treatment of epilepsy. However, a new drug, or group of drugs, which lacked the most important of these side-effects - concentration-dependent sedation - could presage a leap forward in epilepsy management. One possible such group of drugs is the calcium antagonists (CAs).

There are arguably more calcium antagonist drugs than there are AEDs. Verapamil was first investigated by Fleckenstein in 1964 [Fleckenstein 1977], and was used in cardiovascular disease for many years as a presumed "beta-adrenergic receptor antagonist" [Greenberg 1987] based on the finding that beta-agonists enhance calcium influx [Reuter 1965]. In 1977, however, Fleckenstein confirmed his initial suspicions that a direct action on calcium channels was the likely mode of action. By this time, nifedipine and diltiazem had appeared, heralding a shower of "me-too" drugs for use in angina and hypertension.

Classifications of the calcium antagonists are complex and may utilise pharmacological or clinical parameters [Vanhoutte & Paoletti 1987], but structurally the main groups at the time of starting this work were: the 1, 4-dihydropyridines (nifedipine, nimodipine, nicardipine, nitrendipine), the phenylalkylamines (verapamil, methoxyverapamil), the diphenylalkylamines (flunarizine) and the benzothiazepines (diltiazem) (Figures 42, 43).
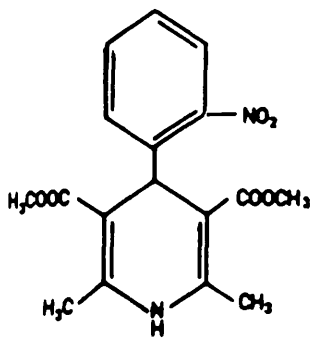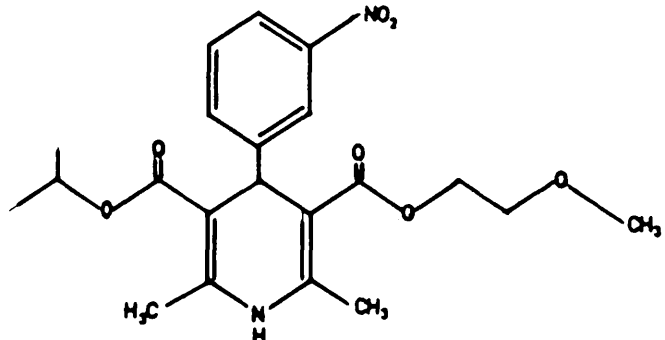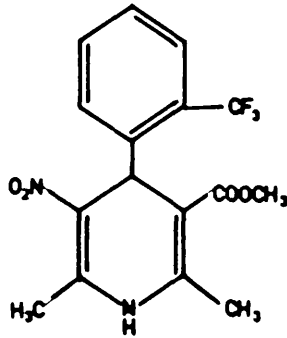
FIGURE 42   Dihydropyridine drugs. Nifedipine and nimodipine are calcium channel antagonists, while BAY K 8644 is a calcium agonist. Note similarities in structure of nifedipine and BAY K 8644.
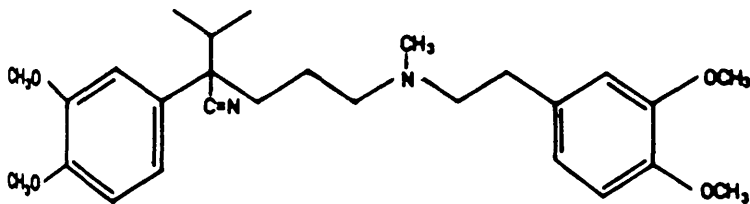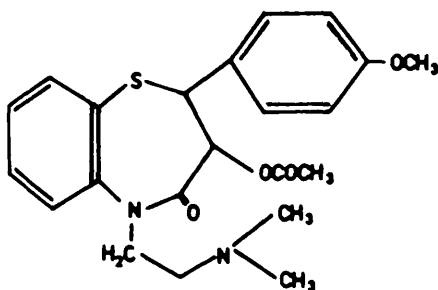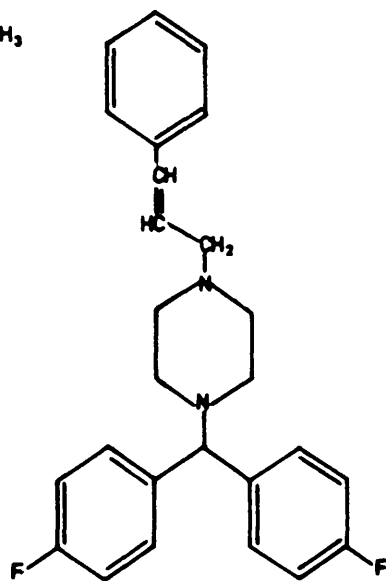
FIGURE 43   Other calcium antagonists including a phenylalkylamine (verapamil), a diphenylalkylamine (flunarizine) and a benzothiazepine (diltiazem).

Of these, only nifedipine, verapamil and diltiazem were being used to any great extent in this country. Indeed, these three were the only CAs available for clinical use in the USA [Greenberg 1987]. However, other drugs, including neuroleptics such as pimozide and thioridazine, were also known to have marked calcium antagonist properties [Snyder & Reynolds 1985].

The CAs have their effects on "calcium channels" - proteins spanning the cell membrane which, when "open", allow passive passage of the Ca^{++} ion from the mM extra-cellular concentrations to the nM intracellular concentrations [Greenberg 1987]. Other cations, such as Ba^{++} and Na^+ may also pass through the open channels, while others such as Cd^{++}, Mn^{++} and Mg^{++} may block the entry of Ca^{++}.

The channels themselves may be opened by changes in membrane potential such as that which occurs with depolarisation ("voltage-dependent calcium channels" - VDCCs) or by occupation of associated receptors ("receptor-dependent" or "ligand-gated" channels). The VDCCs may be classified into at least three different types. Long lasting (L) channels are activated by strong depolarisations, are resistant to inactivation, but are sensitive to blockage by Cd^{++}. Transient (T) channels are activated by weak depolarisation, readily inactivate and are less sensitive to Cd^{++}. A second transient channel (N) which shares the L-channel characteristics with regard to depolarisation and Cd^{++} blockage also exists [Greenberg 1987]. Calcium antagonist drugs (CAs) seem to exert their effects on the VDCCs, in particular the "L-channels" which produce a long-lasting inward calcium current [Greenberg 1987, Hirning et al 1988, Meyer 1989].

The VDCCs are almost ubiquitous in the body, being present in smooth, cardiac and skeletal muscle, and various other sites including neurones [Hagiwara & Byerly 1981]. The movement of calcium ions into axons during stimulation of neurones was first

shown by Hodgkin & Keynes in 1957. Studies with frog ganglion cells in a sodium-free medium soon demonstrated that depolarisation potentials could occur without Na^+ influx [Koketsu et al 1959a] but not without calcium [Koketsu et al 1959b]. The presence of VDCCs in the brain has led to the study of calcium antagonists in various neurological disorders [Raeburn & Gonzales 1988, Delumeau et al 1989]. Animal [Little et al 1986] and clinical [Koppi et al 1987] studies have suggested that they may relieve alcohol withdrawal symptoms. Schizophrenia has also been reported to improve with verapamil, though in an uncontrolled trial [Tourjman et al 1987] which has not been confirmed [Scheperlern & Koster 1987, Grebb et al 1986]. The gradual realisation that CAs may have a role to play in anticonvulsant therapy has resulted from a variety of observations which suggest that calcium influx across the neuronal membrane might play a part in the initiation and propagation of seizures.

Cellular Events in Epilepsy

The initiation of epileptogenic activity in the neurone is thought to concern the "intrinsic bursting" or "sustained repetitive firing" which may occur in normal invertebrate or vertebrate neurones and correlates with the excitability state of the neurone [MacDonald & McLean 1986, DeLorenzo 1988]. This can occur in isolated neurones, and may be due to a rapid repolarisation of the cell membrane caused by an outward K^+ current which is activated by the slower inward Ca^{++} current [Prince 1985, Hotson & Prince 1980]. Intrinsic bursting is inhibited by all AEDs active in GTCS and partial seizures [MacDonald & McLean 1986]. Similar burst-firing can be induced in e.g. hippocampal neurones by proconvulsants such as PTZ [Bingmann & Speckmann 1986] and penicillin [Wong & Prince 1979] and may lead to synchronous burst firing of multiple neurones. Synchronous bursting is almost certainly synapse-mediated [Meyer 1989] and thus calcium-dependent since the synaptosomal release of excitatory neurotransmitters is dependent on calcium influx [Katz & Miledi 1969] and not simply depolarisation

[Zucker & Lando 1986]. This action of calcium is probably mediated by a calcium/calmodulin-dependent kinase which inactivates an inhibitor of transmitter release by protein phosphorylation [DeLorenzo & Freedman 1977, Llinas et al 1985].

Normal intrinsic bursting does not usually precipitate synchronous burst firing. One possible cause of progression is a loss of inhibitory mechanisms (inhibitory post-synaptic potentials) which are GABA-mediated and are blocked in experimental models by proconvulsants such as penicillin [Prince 1985]. In man, it is possible that clinical epilepsy may occur when inhibiting fibres are damaged - perhaps preferentially by hypoxia [Sloper et al 1980]. Other possibilities include a decrease in electrical stability, as has been shown to occur in damaged neurones in crayfish [Kunada & Wine 1981] and cats [Kuno & Llinas 1970]. Whatever the reasons, the occurrence of "excessive repetitive discharge of neurones" has been described recently by Meyer as "the basic dysfunction at the cellular level (in clinical epilepsy)" [Meyer 1989]. The resemblance of this to Hughlings Jackson's description, so many years ago, of epilepsy as an "excessive synchronous discharge of nerve cells" [Jackson 1870] is striking.

Synchronous bursting may be the end-stage response of "stimulated" neurones, or it may progress to produce paroxysmal depolarising shifts (PDSs) which are abnormal, long-duration action potentials long recognised to be associated with the initiation of seizure activity [Goldensshon & Purpura 1963]. The PDS is the individual neurone's synchronised part of a large extracellular field potential which appears as the interictal "spike" on EEG tracings [Matsumoto & Marsan 1964].

It has been established that the PDS is calcium-dependent [Wong & Prince 1979], as is the associated burst firing in hippocampal neurones [Schwartzkroin & Slawsky 1977] which has been likened to the burst-firing of pacemaker cells, another calcium-dependent

phenomenon [Thompson & Smith 1976]. Application of various calcium channel blockers can inhibit burst firing [Bingmann & Speckmann 1989] and depress the amplitude and frequency of PDSs in hippocampal slices [Bingmann & Speckmann 1989] and in anaesthetised rats [Witte et al 1987, Speckmann et al 1986], while systemic administration of calcium antagonists e.g. by intracerebroventricular perfusion can depress interictal discharges [Walden & Speckmann 1988]. Conversely, the calcium agonist BAY K 8644 [Schramm et al 1983], a dihydropyridine which resembles nifedipine structurally (Fig. 42) can be shown to exhibit the opposite effects. It increases calcium influx in neurones [Walden & Speckmann 1987] and has been shown to enhance PDS activity in experiments similar to the above [Walden et al 1986], thus further confirming the importance of calcium influx and suggesting an anticonvulsant role for calcium antagonists.

The overall effect of calcium influx during epileptogenesis can be measured as a fall in extra-cellular calcium during chemical or electrical-induced seizures in the cortex of the cat [Heinemann & Louvel 1983, Heinemann et al 1977]. The decrease is in the order of 0.45-0.55 mM [Heinemann & Louvel 1983, Pumain et al 1983], a fall of almost 50%. It occurs just prior to the onset of burst firing, and accompanies intracellular accumulation of calcium in the mitochondria [Griffiths et al 1983].

Calcium Antagonism in AEDs

Further circumstantial evidence for the importance of calcium's role has come from investigation of conventional AEDs which has revealed that many of them have calcium antagonist properties. Sohn & Ferrendelli [1976] and later Ferendelli & Daniels-McQueen [1982] showed that phenytoin decreased synaptosomal uptake of calcium. The latter paper found similar results with CBZ which has since been shown to inhibit transmitter release by this mechanism [Crowder & Bradford 1987]. Benzodiazepines also

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inhibit calcium uptake [Leslie et al 1980] perhaps via a specific receptor [Taft & DeLorenzo 1984]. Actions of these drugs, however, may differ from those of conventional CAs. Phenytoin appears to act on more transient ("T") calcium channels than those affected by CAs [Twombly et al 1988], while some of the BZs' calcium antagonist activity may stem from inhibition of the intracellular calcium/calmodulin-dependent protein kinase [DeLorenzo et al 1981] rather than an effect on the calcium channels themselves. The relevance of these actions to the anticonvulsant properties of these drugs is not clear, but certainly the effects of the AEDs are not fully explained by other mechanisms. As an example, the relative binding of different BZs to the BZ central receptor correlates well with their relative anxiolytic properties and their inhibition of PTZ seizures, but not with the inhibition of electroshock seizures [Taft & DeLorenzo 1987]. It is possible that at least part of the action of established AEDs is due to calcium antagonism.

Animal Seizure Models

Studies of calcium antagonists in various animal models of epilepsy have furthered their case as possible AEDs. Flunarizine and cinnarizine were the first CAs to successfully inhibit electroshock seizures [Desmedt et al 1975] and pentylenetetrazol-induced seizures [Desmedt et al 1976] in rodents. Flunarizine was also found to be effective against seizures produced by direct application of D.L.-allylglycine onto rat cortex [Ashton & Wauquier 1979a], and in the more sophisticated model of "amygdaloid kindled" seizures - where regular subclinical electroshocks eventually "sensitise" the amygdala to produce seizures following very small shocks - in both rats [Ashton & Wauquier 1979b] and dogs [Wauquier et al 1979]. More recently, De Sarro et al [1986] tested flunarizine in models as diverse as audiogenic seizures in DBA/2 mice and photosensitive seizures in baboons. They found it to be effective, as did Pohl & Mares [1987] using a PTZ model. In the

latter study, only the tonic phase of major seizures was obviously decreased, a finding they describe as identical to previous results they had achieved with CBZ and PHT.

While Verapamil has occasionally shown some anticonvulsant activity [Walden & Speckmann 1988], it has proven ineffective in many studies [Meyer et al 1986a, De Sarro et al 1988, Wong & Rahwan 1989], and it is the dihydropyridines which have shown promise similar to that of flunarizine. Nimodipine was first noted by Meyer and his colleagues to inhibit ischaemic seizures in the white New Zealand rabbit [1986a]. Since nimodipine also improved cortical blood flow in ischaemic regions, they embarked on further experiments using other seizure models in the rabbit to determine whether nimodipine indeed had a separate anticonvulsant action [Meyer et al 1986a, Meyer et al 1986b, Meyer et al 1987]. These confirmed its efficacy in ischaemic, reperfusion and electroshock-induced seizures, and also in those induced by bicuculline and PTZ. Other workers had shown similar results in cefazolin-induced seizures [Morocutti et al 1986] and audiogenic seizures [De Sarro et al 1988]. This last paper of De Sarro is one of a number to show a proconvulsant effect with the calcium agonist Bay K 8644 [Shelton et al 1987, Dolin et al 1988].

Further confirmatory studies have been published during the work of the present thesis using PTZ [Dolin et al 1988, O'Neill & Bolger 1989], electroshock seizures [Meyer et al 1990] and kainic acid-induced seizures [Paczynski et al 1990]. Wong and Rahwan [1989] found nifedipine to be ineffective vs. maximal electroshock seizures but effective against PTZ seizures and inferred that the drug might only be active in "petit mal" attacks, since the standard PTZ threshold test [Krall et al 1978] has been suggested to be specific for drugs active against absence seizures. Various alterations in this test however, as discussed by Meldrum [1986] can change this supposed specificity either by varying the technique [Kent & Webster 1983] or the strain of mouse [Sugaya et al. 1986]. Since the method of Wong & Rahwan was not that of the "threshold test", their

inference is perhaps over-specific.

Clinical Trials

In the face of the above evidence, it is perhaps surprising that clinical trials of calcium antagonists in epilepsy remain in their infancy. At the time of commencing this thesis, only flunarizine had been tested in patients. Declerk & Wauquier initially reported some success in mentally retarded patients in trials performed in the late 1970s [Declerk & Wauquier 1980]. A double-blind placebo-controlled study by Overweg et al [1984] showed "a modest but statistically significant" fall in seizure frequency in 33 patients, 7 of whom had a seizure reduction of more than 50%. Blood levels of flunarizine were found to be rather low (enzyme induction by concomitant AEDs), and an open dose-ranging study followed [Binnie et al 1985] where 16 out of 47 patients had a 50% drop in seizures. The incidence of side-effects was acceptable, although 15 patients complained of drowsiness and one of these required withdrawal from the study. These, plus a further report from the same group [Overweg et al 1986], were the main reports in the English literature of trials with flunarizine. Work was in progress in Europe and Japan, and was reported at various clinical meetings. A recent review by Binnie [1989] recounts the marked success of many open trials, where overall, 74 of 197 (38%) patients achieved a 50% reduction in seizure frequency; and the promise of early crossover studies in which, again taken together, 30 (21%) patients improved by 50% on flunarizine, but only 6 (4%) on placebo.

Although the incidence of side-effects in the above studies was not particularly high, flunarizine is known to cause neurological abnormalities, including Parkinsonism and depression [Chouza et al 1986, Micheli et al 1987], and it seemed a logical step to consider other calcium antagonists as candidates for clinical trials. This is particularly

true since flunarizine has numerous actions including antihistaminic, antiserotonergic and antidopaminergic activity [Brodie 1990]. It also has an effect on sodium channels [Binnie 1989] similar to that of CBZ and PHT [Willow & Catterall 1982]. All of this leads to doubts over the importance of calcium antagonism in flunarizine's anticonvulsant activity [Binnie 1989].

Before the work of this thesis began, preliminary investigations with verapamil [Macphee et al 1986c] and diltiazem [Brodie & Macphee 1986] had been undertaken in our unit, but both these drugs were found to inhibit the metabolism of CBZ - which many patients were taking concurrently - thus increasing serum concentrations of CBZ and causing toxicity. The accumulating evidence for the efficacy of the dihydropyridines in animal models of epilepsy supported this group of drugs as an ideal choice for further investigation as possible AEDs.

In this chapter, the dihydropyridines nifedipine and nimodipine will be investigated. The former drug has received little attention in the epilepsy literature which has focussed more on its less well-recognised alternative. The reasons for this may be partly psychological - the avoidance of a drug established in other spheres - or perhaps even commercial, since the pharmaceutical companies may have little incentive to promote new research with an inexpensive drug now out of patent. Other considerations may have had more influence. Nifedipine, a less lipophilic drug than nimodipine, was not recognised to cross the blood-brain-barrier (BBB), and in 1986 flunarizine was still being quoted as the only calcium antagonist "known to enter the brain" [Overweg et al 1986]. However, nimodipine had been shown to cross the BBB in the gerbil [Heffez et al 1985], and was found to relax cerebral vessels more than nifedipine [Towart 1981] and increase both cerebral and coronary blood flow [Haws et al 1983]. It was also shown in animal EEG studies to possess psychotropic properties of a "mood-elevator" type [Itil et al

1984]. These findings may have led to its presumed superiority as a neurologically active drug. We decided to investigate these drugs, both clinically and in an animal model of epilepsy. Pharmacokinetic studies were also undertaken, in particular to assess the penetration of nifedipine into the brain.

The first study (EXPT 8) is an open clinical trial of nifedipine, performed to exclude any effect of this drug on the metabolism of CBZ, and to assess in an uncontrolled fashion, the possibility of its having anticonvulsant efficacy. Chronologically, the next studies performed were in mice (EXPTS 9 & 10) where blood and brain levels of nimodipine and nifedipine were measured at different time points after intraperitoneal (i.p.) injection, thus assessing blood and brain "half-lives" and BBB penetration. A PTZ seizure model was also utilised to compare anticonvulsant efficacy of the two drugs with each other at different dosages, and when used alone or in combination with CBZ. Finally, two placebo-controlled studies, one of each drug, were designed. One (of nimodipine) was performed by myself in the Western Infirmary, and the other (nifedipine) was designed in the Unit and performed in Lingfield Residential School. The implications of the disparity between the results of animal and clinical studies are discussed in the conclusion, with reference to the recent literature and future possibilities. The last study concerns a possible alternative mechanism for the antiepileptic properties of calcium antagonists and for any interaction with other AEDs - their effects on adenosine receptors.

Calcium Antagonists and Adenosine Receptors

Such is the complexity of neurologically active drugs, and such the magnitude of empirical drug use, that the theoretical basis for CAs in epilepsy outlined above may not be taken for granted, even if the drugs should prove successful. The tradition of empirical

usage of drugs with later attempts to discover mechanisms of action is well established, and only recently have "designer drugs" with specific actions such as vigabatrin (gamma-vinyl GABA, a GABA transaminase inhibitor [Jung et al 1977]) come to the fore [Editorial 1989]. Thus, while workers investigate possible mechanisms of action for such drugs as CBZ, we find the CAs being drawn into the argument. One such area is the effect of AEDs on adenosine - "the brain's natural anticonvulsant" [Dragunow & Faull 1988] - and its receptors.

Analogues of adenosine show anti-seizure activity [Rosen & Berman 1987] while antagonists have proconvulsant effects [Dragunow & Robertson 1987]. Paradoxically, CBZ is thought to be an adenosine antagonist [Lewin & Bleck 1977, Marangos et al 1987]. It inhibits adenosine binding to the A_1 receptor [Marangos et al 1983, Skerritt et al 1982] which responds to low concentrations ($10^{-8}M$) of adenosine and is likely to mediate its neurodepressant effects [Reddington 1982]. Part of the paradox may be explained by carbamazepine's specificity for inhibiting binding to the A_1 receptor, while other adenosine antagonists with strikingly dissimilar (proconvulsant) pharmacological effects (e.g. caffeine) affect both A_1 and A_2 receptors [Clark & Post 1989]. Furthermore, chronic dosing with CBZ has been reported to increase brain receptor numbers [Marangos et al. 1985], while similar adenosine receptor "upregulation" with theophylline [Choi et al 1988, Sanders & Murray 1988] has been associated with increased tolerance to proconvulsants [Sanders & Murray 1989]. Theophylline, meanwhile, interferes with the anticonvulsant activity of CBZ [Skerritt et al 1983b], futher complicating the picture. It may be that the acute effects of these drugs differ, while their chronic effects on receptor numbers may protect against seizures.

Calcium antagonists have been shown to inhibit binding to adenosine receptors [Murphy & Snyder 1982, Cheung et al 1987, Morgan et al 1987, Hu et al 1987] and share some pharmacological properties with adenosine [Morgan et al 1987] which also inhibits

calcium influx in the heart [Harder et al 1979] and synaptosomes [Ribeiro et al 1979]. The dihydropyridines appear to be the most active CAs at the adenosine receptor [Murphy & Snyder 1982, Hu et al 1987], although differences occur within the group, since only nifedipine inhibits adenosine agonist (cyclohexyladenosine) binding, whereas they all interfere with antagonist (diethylphenylxanthine) binding [Morgan et al 1987].

Thus, some authors have suggested that adenosine interactions may play a part in the anticonvulsant effects of CBZ [Skerritt et al 1983a, Marangos et al 1985] or even the CAs [Morgan et al 1987]. Alternatively, the effects on adenosine receptors may be an incidental finding, or a secondary response to anticonvulsant effects mediated by a separate mechanism. In the final experiment of this thesis, a binding assay for adenosine A_1 receptors is established, and the effect of chronic dosing with CBZ and CAs on these receptors in mice is assessed.

AIM

To ensure that nifedipine does not interfere with the metabolism of established AEDs, while assessing the possibility that it possesses anti-epileptic properties.

PATIENTS AND METHODS

Twelve patients agreed to take part in the study, and their clinical details are shown in Table 21. All had intractable epilepsy, and had attended the epilepsy clinic for more than a year, where they routinely completed monthly "seizure frequency" charts. No patient had any alteration in anticonvulsant medication during the previous three months.

Each patient was admitted to the Gardiner Institute for a total of 14 days. Erect and supine blood pressure (in duplicate) was measured 4-hourly. Anticonvulsant drugs were continued in unchanged dosage and their concentrations monitored. Blood for CBZ and VPA assay was drawn at 0800h ("trough"), 1100h ("peak") and 1600h for three consecutive days before nifedipine was commenced, and on days 1, 2, 3, 5, 7 and 10 of nifedipine therapy. Concentrations of PHT and PB, whose elimination half-lives exceed 24h, were obtained once daily. The mid-afternoon measurement was repeated as an outpatient at 2 and 4 months. Patients were treated with a standard nifedipine dose, namely 20mg thrice daily, in addition to their existing anti-epileptic therapy. Blood samples were centrifuged immediately and the serum stored at $-20^{o}C$ for batch analysis as in previous experiments.

TABLE 21   **EXPT.8 Clinical details of patients in study**

	Age	Type of epilepsy	Duration (years)	EEG abnormality	Anticonvulsants	Outcome
FEMALES						
1	28	Complex partial Secondary generalised	25	(L) Fronto-temporal	Carbamazepine	Completed
2	33	Complex partial	31	(R) Temporal	Carbamazepine	Completed
3	58	Generalised tonic-clonic	42	Centrencephalic	Sodium valproate Phenobarbitone	Completed
4	33	Complex partial	19	Centrencephalic	Carbamazepine	Completed
5	38	Complex partial Secondary generalised	25	(R) Fronto-temporal	Carbamazepine Phenobarbitone	Completed
6	20	Complex partial Secondary generalised	5	Centrencephalic	Carbamazepine	Completed
7	36	Complex partial	2	(L) Fronto-temporal	Carbamazepine	10 days - * headache
8	25	Complex partial Secondary generalised	7	None	Phenytoin	6 days - * headache
MALES						
9	32	Complex partial Secondary generalised	8	(R) Temporal	Carbamazepine	Completed
10	24	Complex partial Secondary generalised	16	(L) Fronto-temporal	Carbamazepine	Completed
11	28	Complex partial Secondary generalised	22	(R) Temporal	Phenytoin	Non- * compliance
12	36	Complex partial Secondary generalised	26	(R) + (L) Temporal	Carbamazepine	6 weeks - * stopped drugs

* Patients who withdrew from the study

NAME: _John Smith_   Month: _Jan_   Year: _1990_

	SEIZURES AM						SEIZURES PM						Possible Cause
Date	2	4	6	8	10	12	2	4	6	8	10	12	
1													
2													
3													
4											✓		? Alcohol
5													
6													
7													
8													
9													
10													
11													
12													
13					O								
14													
15													
16													
17													
18													
19													
20													
21													
22													
23								O	O				
24													
25													
26													
27													
28													
29													
30													
31													

Please mark: X MAJOR   O MINOR

TOTAL MAJOR = _____   TOTAL MINOR = _____

Treatment

Date	Drugs		Dose	AM				PM						Stopped
				6	8	10	12	2	4	6	8	10	12	

NOTE: A major seizure is one when you fall down and shake all over. Other names for
         it are grand mal and generalised seizure.
         A minor seizure is an absence event, a funny feeling or a staring turn. Other
         names include petit mal and partial seizure.

FIGURE 44   Seizure chart used by patients to record own seizures. Identical or similar
chart used in EXPTS. 8, 11, 12.

To assess the possible efficacy of nifedipine as an anticonvulsant, the patients completed seizure frequency charts for the three months before nifedipine was commenced. These were compared with those obtained for the next three months. In these charts, each generalised tonic-clonic and partial seizure was recorded separately each day by the patient and his/her family on a time grid divided into 2h segments (Figure 44).

Alterations in CBZ and CBZ-E concentrations and blood pressure measurements were compared using Student's t-test, with a calculation of 95% confidence intervals for the difference between pre- and post-nifedipine values. Seizure frequency before and after nifedipine introduction was examined using Wilcoxon matched pairs test for non-parametric data. Confidence intervals were also calculated for the differences in seizure frequency, assuming suitability for parametric analysis.

RESULTS

Patients 7 and 8 withdrew from the study after 10 and 6 days respectively because of headache and light-headedness, a possible consequence of the vasodilatory properties of nifedipine. Blood pressure, however, did not appear to be reduced in these patients, nor was it significantly affected in the group as a whole (Figure 45). Patient 11 was withdrawn because of non-compliance with drug therapy and seizure chart completion, and patient 12 spontaneously stopped taking all medication after 6 weeks. Thus, nine patients completed one month on nifedipine, and eight completed all three months of the trial.

In the nine patients on CBZ, trough, peak and mid-afternoon concentrations did not significantly change from baseline in the first 10 days of nifedipine therapy. The mean

TABLE 22 Mean (± SD) concentrations of total and free carbamazepine (CBZ), carbamazepine 10,11 epoxide (CBZ-E) and % unbound CBZ before and after seven days after introduction of nifedipine in 9 patients with intractable epilepsy

	BEFORE	AFTER	95% Confidence Intervals of change
TOTAL CBZ (mg/L)	12.4 ± 3.0	12.6 ± 2.7	(-2.6 - +3.1)
FREE CBZ (mg/L)	2.49 ± 0.5	2.68 ± 0.5	(-0.32 - +0.7)
FREE CBZ (%)	23.9 ± 2.1	24.4 ± 2.5	(-1.8 - +2.9)
CBZ-E (mg/L)	2.2 ± 1.2	2.0 ± 1.6	(-1.72 - +1.4)
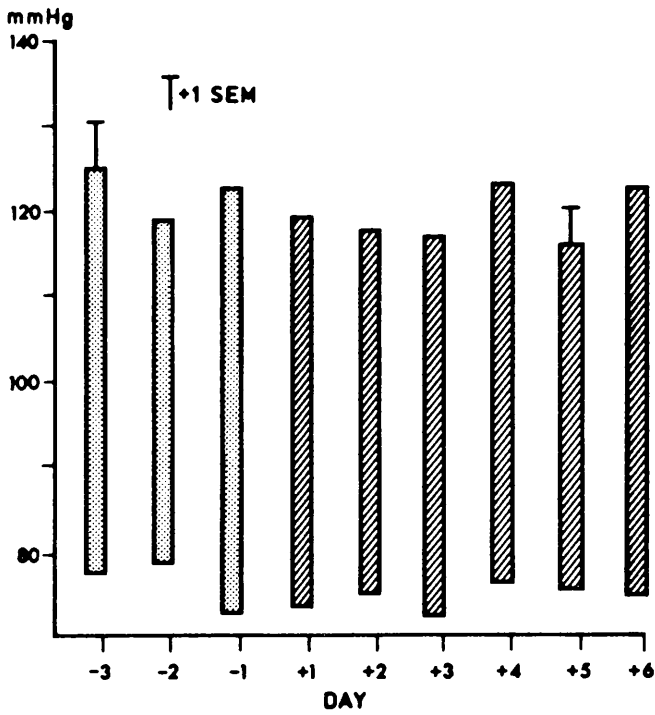
FIGURE 45   Mean (+2SEM) systolic and diastolic blood pressure in 12 patients on three days before and six days after commencing nifedipine retard 20mg b.d.
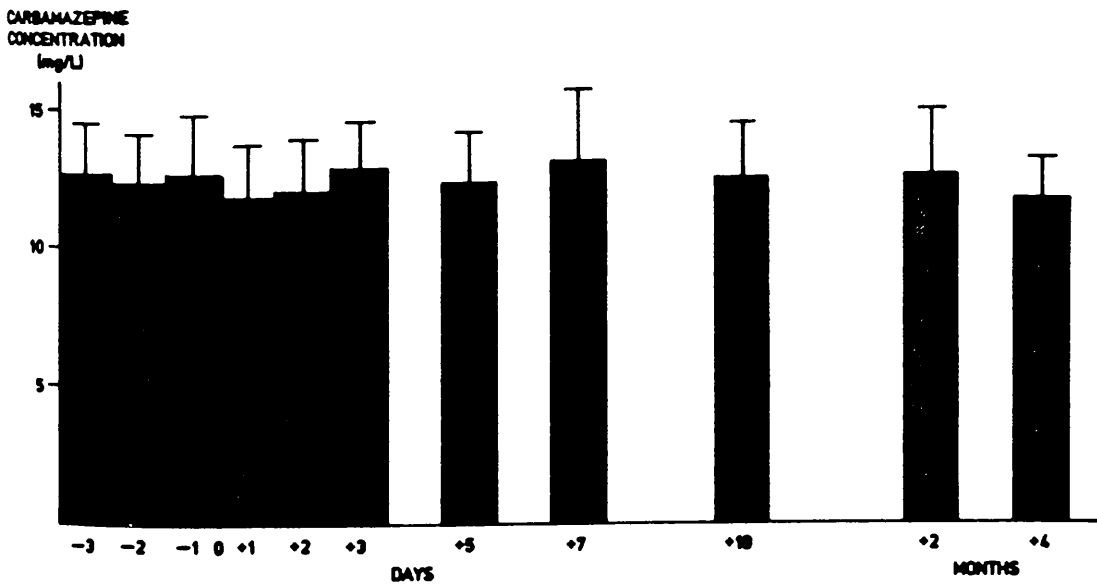
FIGURE 46   Mean (+2SEM) mid-afternoon carbamazepine concentrations for three days before and 10 days after nifedipine introduction in nine patients, and two and four months later in six patients.

FIGURE 47(a)   Total number of seizures in first month of
nifedipine therapy vs. previous month.

FIGURE 47(b)   Monthly seizure frequency in first three months
of nifedipine therapy vs. previous three months.

mid-afternoon levels are shown in Figure 46 to demonstrate the lack of any long term

effect in those continuing nifedipine for up to 4 months. Similarly, unbound CBZ and

extent of protein binding together with CBZ-E levels appeared unaffected by NIF

administration (Table 22). The 95% confidence interval demonstrates that NIF is very

unlikely to increase CBZ concentration by as much as 0.74 mg/L (6%). No overt effect

on circulating levels was seen in the 3 patients receiving other anticonvulsants.

Seizure frequency was significantly less at 1 and 3 months after commencing NIF

compared to the previous months (both P<0.01; Figures 47 a & b). To demonstrate

whether these results were affected by the withdrawal of patients who were doing badly,

the one-month comparison was repeated on an "intention-to-treat" basis and the

difference remained significant (P<0.02). Insufficient data was available from the

patients who withdrew or defaulted for such analysis at 3 months. Confidence intervals

for the change in seizure frequency suggest a decrease of 5-19 (23-36%) after one month,

and a reduction of 7-16 seizures (25-57%) per month at 3 months.

Five and 4 patients at 1 and 3 months respectively reported a >50% fall in seizures while

7 had a >25% reduction at both time points. All patients who claimed benefit requested

to continue taking NIF when the study was completed. Two patients remained seizure-

free for the whole 3 months - for the first time in many years. One of these (patient 2)

was still seizure-free one year later, while the other (patient 3) suffered a recurrence

under unusual circumstances after 4 months of NIF therapy.

Case Report

Mrs. G.Y., a 58 year old lady, suffered from GTCS from the age of sixteen. Serial EEGs

showed slow-wave activity in both hemispheres with a likely origin in centrencephalic

structures; CT scanning of brain was normal. All major anticonvulsants singly and in
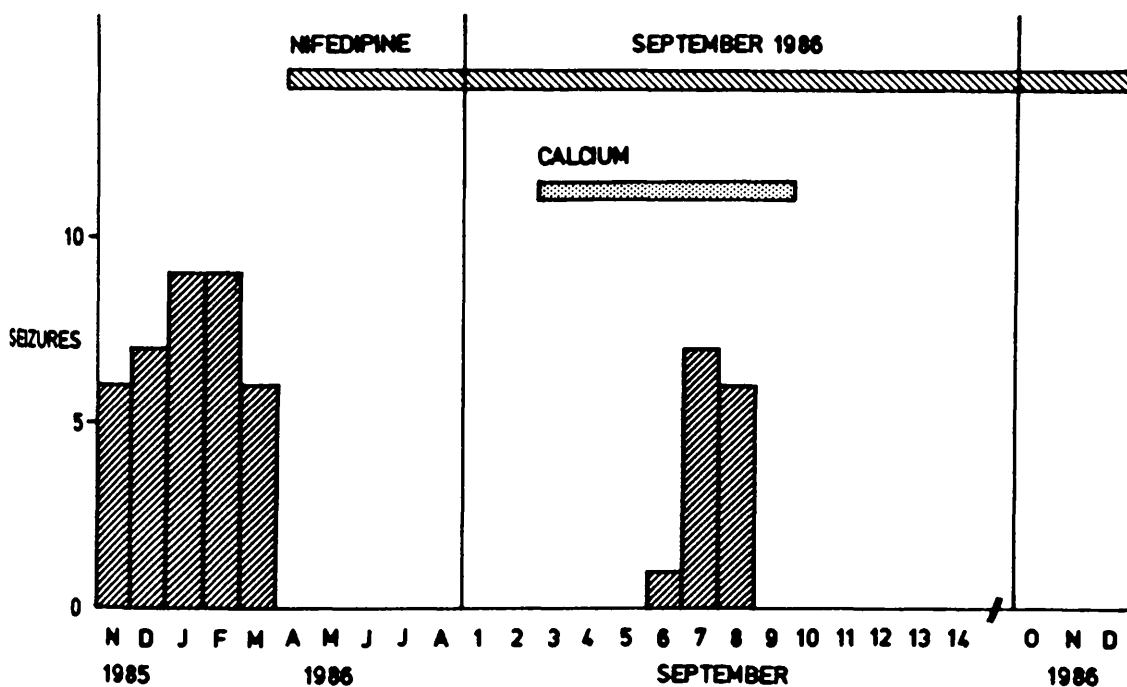
FIGURE 48   Schematic history of patient 3, showing the monthly seizure frequency in late 1985 and 1986, and the daily incidence of seizures in September 1986. The temporal relationships to nifedipine and calcium therapy are shown.

combination over the years had only partly controlled her seizure disorder. At the time of starting NIF (April 1986) she was taking VPA 500mg four times daily (serum level 129 mg/L) and phenobarbitone 60mg twice daily (serum level 20.9 mg/L), and was documenting 5-15 seizures per month.

For more than 5 months after commencing NIF, the patient remained seizure-free. On September 2nd, she was prescribed calcium supplements (Sandocal) by her general practitioner for treatment of low back pain, attributed to vertebral fractures due to osteoporosis. On September 6th, she had her first seizure since starting NIF therapy, which was followed by 13 more over the next 2 days (Figure 48). Sandocal was immediately discontinued and the patient became seizure-free for a further 4 months before her seizures gradually returned.

DISCUSSION

Both verapamil [Macphee et al 1986c] and diltiazem [Brodie & Macphee 1986] had previously been found to inhibit CBZ metabolism and increase serum concentrations of this drug. Both CAs were subsequently shown to impair the elimination of antipyrine, while NIF did not [Bauer et al 1986]. The results of the present study demonstrate that NIF does not substantially affect circulating concentrations of CBZ or of its active metabolite, either acutely or in the long term, making it a more realistic adjuvant therapy for intractable epilepsy.

The significant fall in seizure frequency with nifedipine was encouraging. This study did not control for the placebo effect of giving a new drug nor for that of a short hospital admission, but the improvement in control, particularly in the two patients who became

seizure-free, supports an anticonvulsant action for this calcium antagonist. The recurrence of seizures in the patient taking calcium supplements was not anticipated. This may have been a coincidence, although the chronology of events was quite striking. The results of this study marked out nifedipine as a possible anticonvulsant which required placebo-controlled evaluation, with measurements of circulating concentrations.

EXPT. 9+10   NIFEDIPINE AND NIMODIPINE IN MICE: BLOOD AND BRAIN
PHARMACOKINETICS AND EFFICACY AGAINST PENTYLENE-
TETRAZOL-INDUCED SEIZURES

AIM

To outline the pharmacokinetic parameters of NIF and NIM in mouse blood and brain,
and to assess their efficacy vs. PTZ-induced seizures when used alone or in combination
with CBZ.

METHODS

Male CF1 mice were obtained from Bantin & Kingman, Hull, weight 20g.
Pharmacokinetic and acute efficacy studies were performed in animals weighing 30g,
while those receiving chronic dosing weighed 30-35g at time of testing.

Materials

Nifedipine and nimodipine for intraperitoneal (i.p.) injection were freshly prepared as 1
mg/ml concentrations in 50/50 ethanol/water under sodium light. CBZ was made up as a
2 mg/ml solution in the same solvent by first dissolving it in the alcohol phase. The
ethanol/water vehicle was also used as a control injection, as ethanol itself has an
anticonvulsant effect in many seizure models [Mello et al 1990].

For EXPT. 9B, nifedipine and nimodipine were suspended in Tween 80
(polyoxyethylene sorbitan mono-oleate, Sigma) 0.5% in distilled water at a concentration
of 18.5 g/L.

Pentylenetetrazol was prepared as a 0.85% solution in 0.9% saline and kept at 37^{o}C. A 0.01 ml/g dose (85 mg/kg body weight), which will produce convulsions in 97% of mice [Swinyard & Woodhead 1982], was used.

H.p.l.c. calibration

Stock solutions of NIF, NIM and internal standard (nitrendipine) 100 mg/L in methanol were stored at -20^{o}C and were stable for several months. Internal standards were produced by diluting stock solutions in 50/50 ethanol/water. A calibration curve was constructed for both drugs in the range 0.2 - 5 ug/ml and was found to be linear (r=0.95). Limit of detection was 10 ng/ml in a 100 ul sample. A recording integrator compared peak area ratios of calcium antagonist and internal standard. When assaying homogenised brain samples, the individual brain weights were incorporated into the calculation to give these results as ug/g wet weight. Ether did not interfere with the assay. A Hewlett-Packard 1084B Liquid Chromatograph was used, and all steps were performed under sodium light, as the dihydropyridines rapidly break down under other artificial light sources or sunlight.

Sample Preparation

For drug assays, 100 uL of blood were sonicated before adding 300uL 1M NaOH and 200uL internal standard (2 ug/ml nitrendipine). Extraction was performed with hexane/diethylether (50/50) in a vortex mixer for one minute before centrifugation for 10 minutes at 2,000 rpm. The organic phase was transferred to a clean test-tube and evaporated to dryness at 40^{o}C under nitrogen, prior to reconstitution with methanol/water (70/30). Mouse brain was prepared, after weighing, by homogenising in 1.5ml 1M NaOH. 200uL internal standard were added and extraction proceeded as

above. All these procedures were carried out under sodium light by the technical staff in the Department of Medicine (and Therapeutics) laboratories.

Pharmacokinetic Studies

EXPERIMENT 9A:

Mice were injected i.p. with 6 mg/kg NIF or NIM (alcohol/water). The animals were sacrificed by overdosing with ether after 1, 5, 10, 20, 30 or 60 minutes. A truncal blood sample was obtained and the brain perfused with 0.9% saline by injection following double puncture of the heart before decapitation and removal.

EXPERIMENT 9B:

Mice were injected i.p. with 60mg/Kg NIF or NIM (Tween). The animals were sacrificed after 30, 45, 60, 90 or 120 minutes. As this experiment was designed simply to confirm that the drugs entered the brain regardless of whether an alcohol vehicle was used, only three animals were included in each group. Blood and brain samples were obtained as above.

PTZ seizure inhibition

EXPERIMENT 10A:

Mice (30g) were injected s.c. with a 97% convulsive dose of PTZ 10 minutes after i.p. administration of control solvent, NIF 3.3 mg/kg, NIM 3.3 mg/kg or CBZ 6.7 mg/kg. Each mouse was observed in isolation for features of the various components of PTZ seizures in rodents [Pohl & Mares 1987]. The time to the first clonic jerk was recorded as

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the seizure latency since, in preliminary experiments, it was consistent and was affected by CBZ - a feature not present in many other approaches to PTZ seizures [Meldrum 1986]. Recordings were made by one observer who was unaware of each animal's treatment.

EXPERIMENT 10B:

Since NIF has been suggested as an adjunctive therapy, a separate experiment was performed using pre-treatment with i.p. control, NIF, CBZ, or NIF in combination with CBZ in the same doses as above.

EXPERIMENT 10C:

The effect of different doses of NIF and NIM given by mouth on PTZ-seizures was investigated. All animals were fed a powdered diet (Beekay Feed No. 42, Banton & Kingman, Hull) and housed in separate cages. Groups of 5 test animals received daily doses of 1mg (33 mg/kg), 2mg (67 mg/kg) or 4mg (133 mg/kg) nifedipine or nimodipine for 2 weeks. Ten mice served as controls. One animal taking nimodipine died. All surviving mice gained weight. PTZ seizures were provoked and observed as previously desribed.

Statistics

An estimation of the elimination half-life of the CAs (alcohol/water) in blood and brain was obtained using the mean values at each time point with the STATIS2 statistical package (Clydesoft). A measurement of error was achieved using Jackknife analysis [Miller 1974] and 95% confidence intervals are shown.

Comparisons of seizure latency between groups of animals were made using the Kruskal-Wallis test to determine if significant differences were present, with follow-up Mann-Whitney "U" testing to define these differences. Pearson's product moment correlation coefficients are quoted.

RESULTS

Pharmacokinetics

EXPERIMENT 9A:

Both NIF and NIM readily crossed the blood-brain-barrier. Time courses of blood and brain concentrations of each CA are shown in figures 49 and 50, with each point the mean of at least 6 results. Estimates of pharmacokinetic parameters were made from the mean data and are shown in Table 23. The blood kinetics of NIM best fitted an intravenous model, while the behaviour of NIM in the brain and NIF in both blood and brain fitted an oral absorption model. The data at 1 minute were omitted from the half-life calculation. A correlation between blood and brain levels was seen at most time points (Figures 51 & 52) with an overall correlation of $r = 0.701$ ($P<0.001$) for NIF and $r = 0.572$ ($P<0.001$) for NIM.

EXPERIMENT 9B:

Concentration-time curves in blood and brain for the Tween preparations are shown in figures 53 and 54. As expected, absorption was prolonged and erratic, and no attempt at calculating pharmacokinetic parameters was made. Clearly, however, both drugs crossed the BBB. Nifedipine brain and blood concentrations were positively correlated ($P<0.02$,

TABLE 23 Pharmacokinetic parameters of nifedipine and nimodipine in mouse blood and brain

| | ELIMINATION HALF-LIFE (min) | | AREA UNDER CURVE (ug.min/ml) | |
	(Blood)	(Brain)	(Blood)	(Brain)
NIFEDIPINE	11.2	14.7	69.8	71.1
	±4.0	±2.4	±12.8	±19.4
NIMODIPINE	16.7	22.4	3.34	10.6
	±8.8	±7.2	±2.2	±3.0

Results ± 95% confidence limits
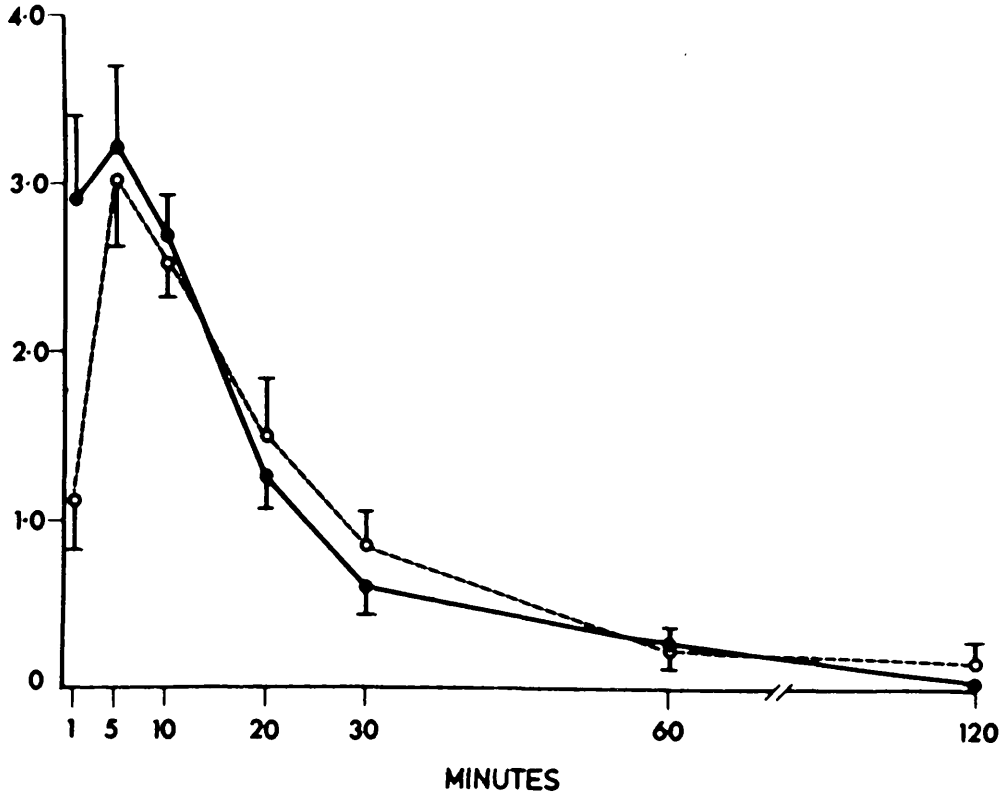
FIGURE 49   Concentration-time curves of mouse blood (●—●, ug/ml) and brain (o--o, ug/g) concentrations of nifedipine after i.p. 6 mg/kg nifedipine (alcohol/water). Vertical bars represent ±SEM.
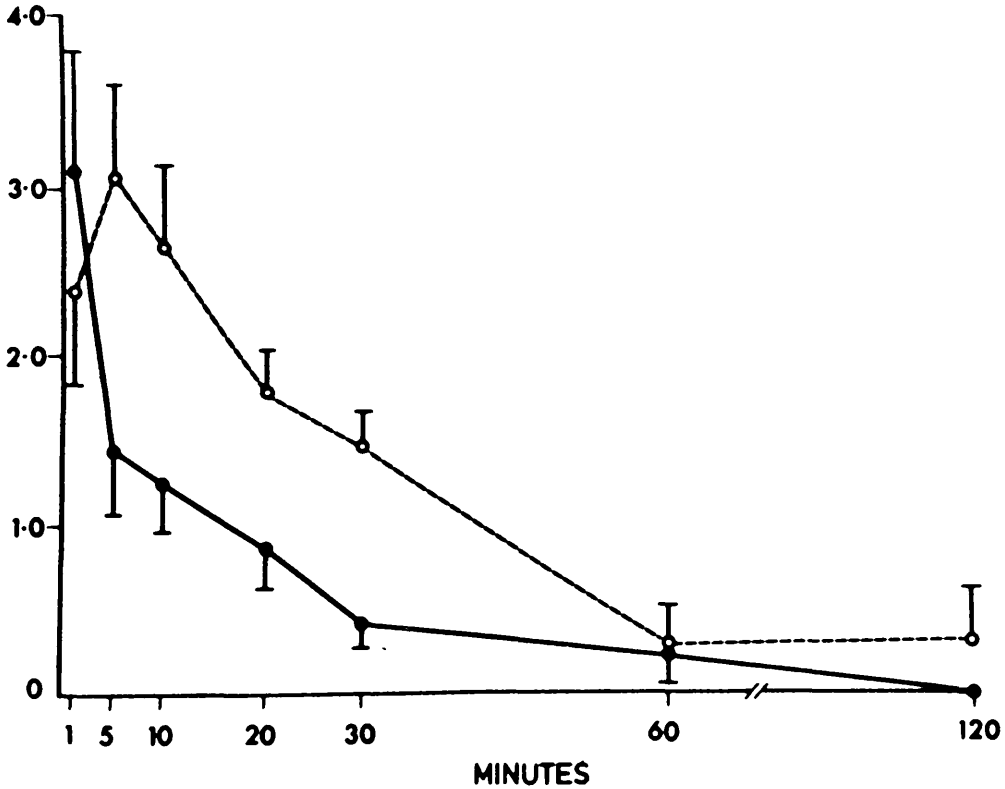
FIGURE 50   Concentration-time curves of mouse blood (●—●, ug/ml) and brain (o--o, ug/g) concentrations of nimodipine after i.p. 6mg/kg nimodipine (alcohol/water). Vertical bars represent ±SEM.
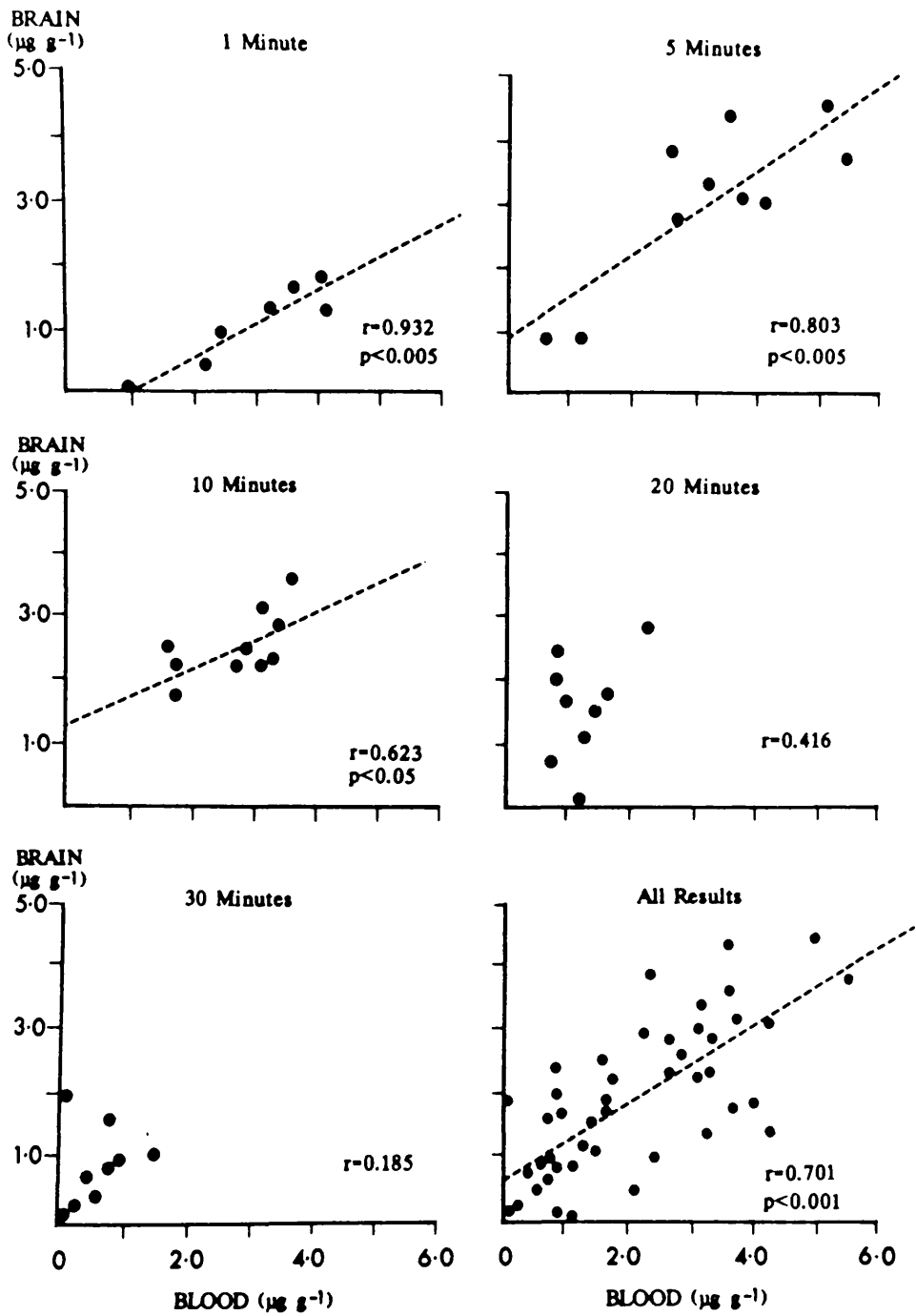
FIGURE 51   Nifedipine concentration in mouse blood and brain at stated times following i.p. 6 mg/kg nifedipine (alcohol/water).
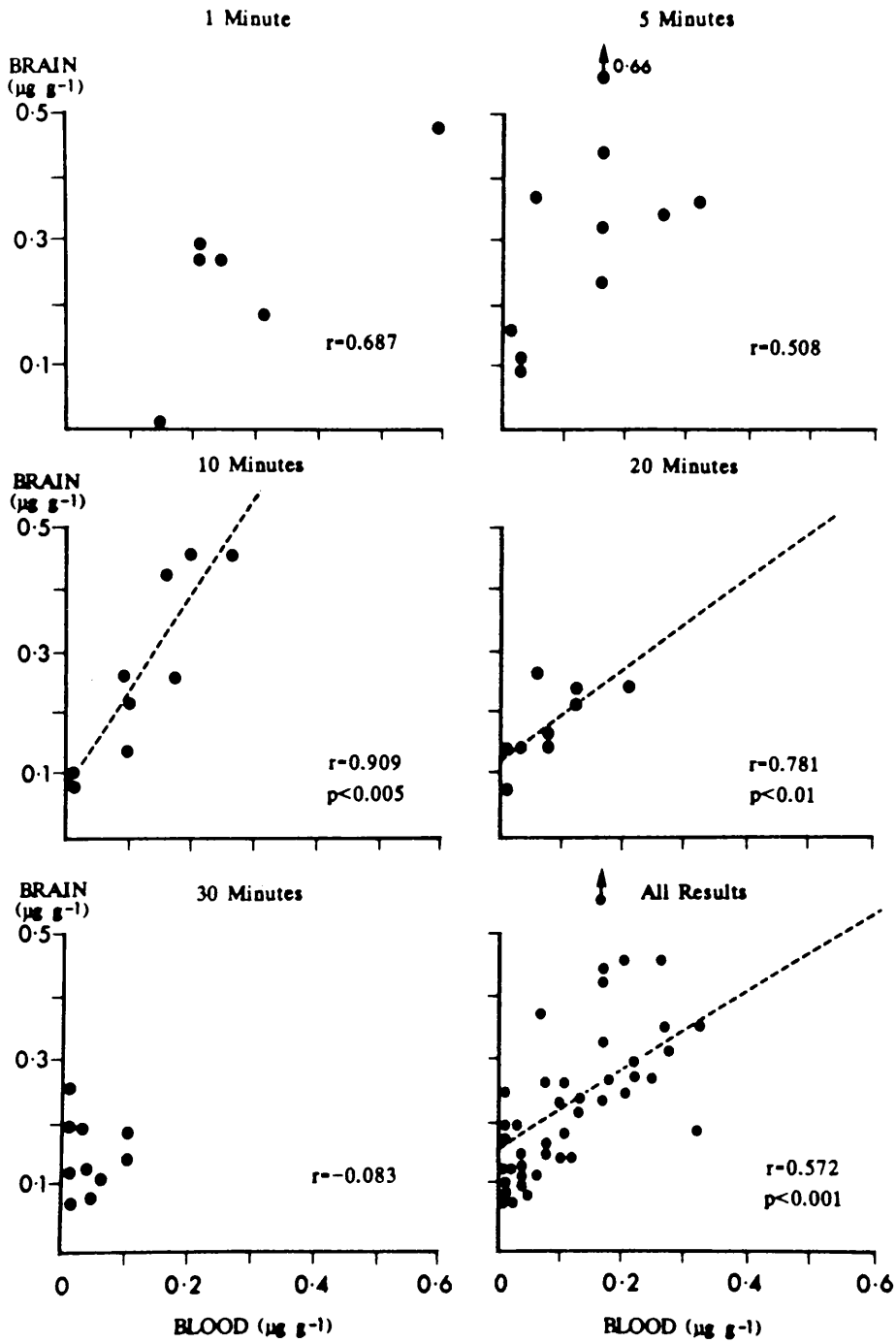
FIGURE 52   Nimodipine concentration in mouse blood and brain at stated times following i.p. 6 mg/kg nimodipine (alcohol/water).
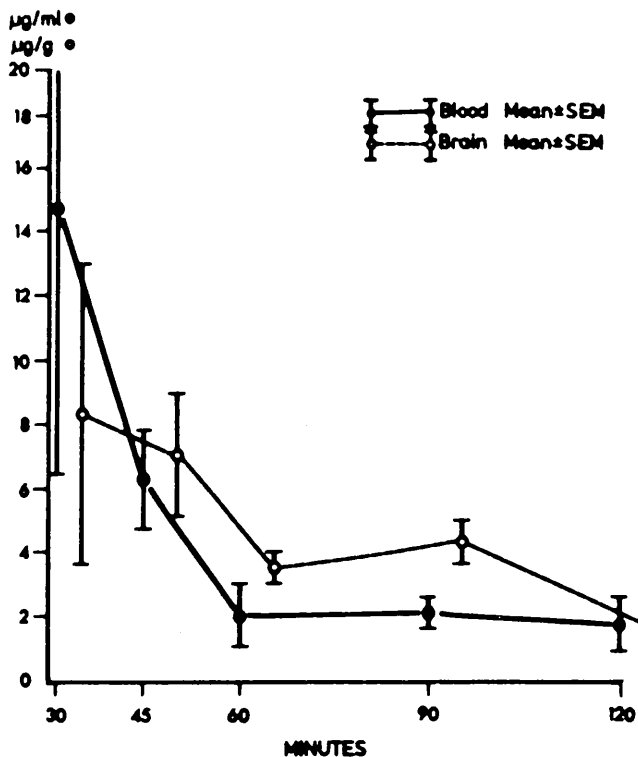
FIGURE 53   Concentration-time curves of blood (●—●, ug/ml) and brain (o--o, ug/g) concentrations of nifedipine after i.p. 60 mg/kg nifedipine (Tween). Vertical bars represent ±SEM.
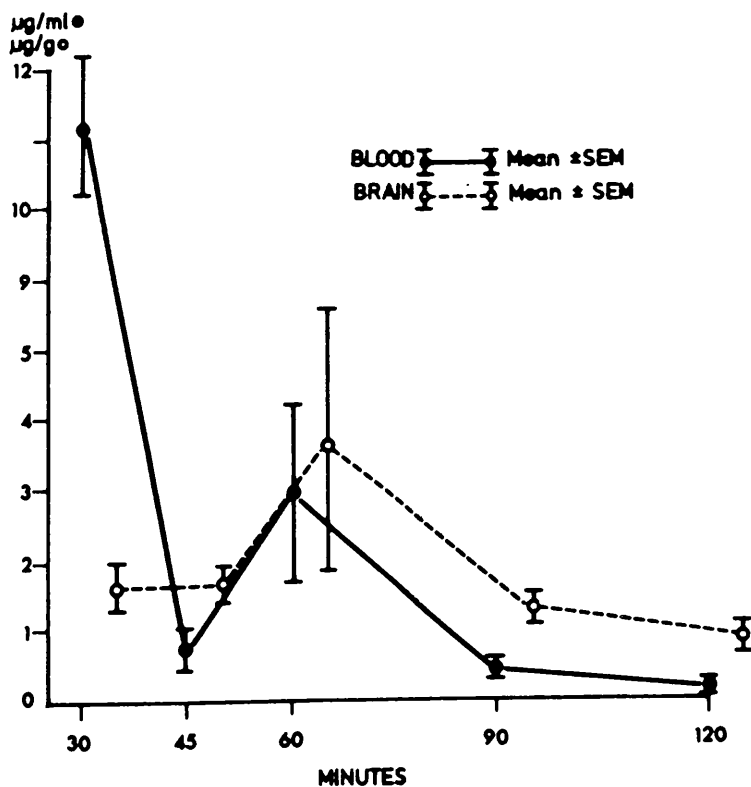
FIGURE 54   Concentration-time curves of blood (●—●, ug/ml) and brain (o--o, ug/g) concentrations of nimodipine after i.p. 60 mg/kg nimodipine (Tween). Vertical bars represent ±SEM.
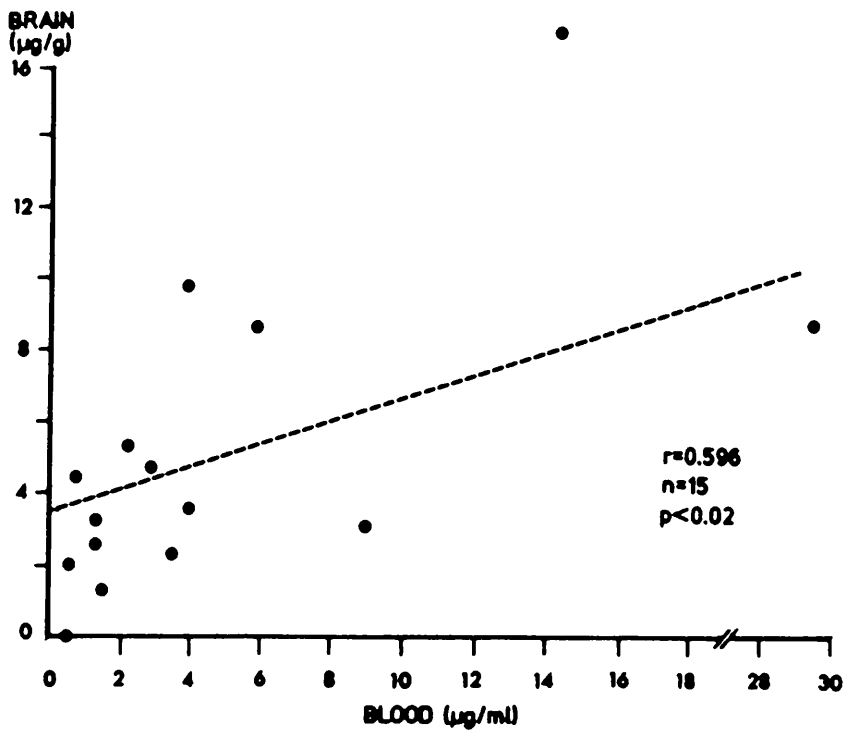
FIGURE 55   Nifedipine concentration in mouse blood and brain at all time-points after i.p. 60 mg/kg nifedipine (Tween).
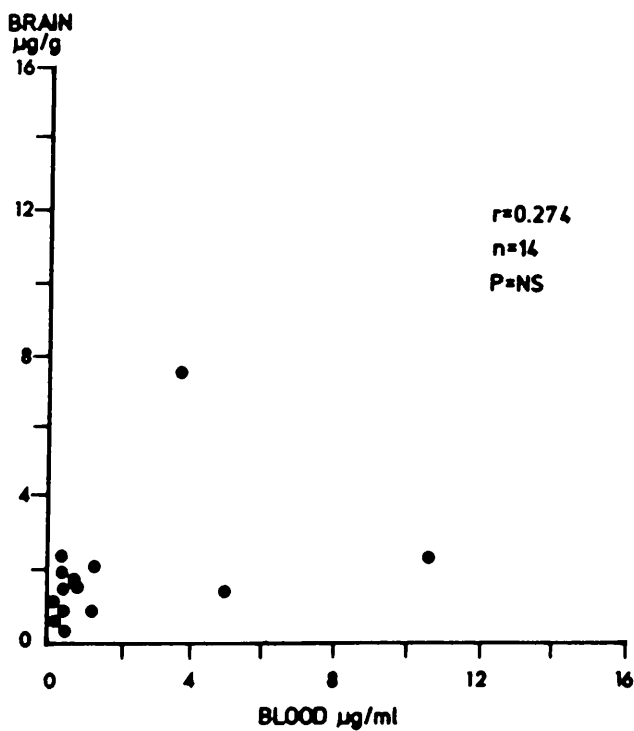
FIGURE 56   Nimodipine concentrations in mouse blood and brain at all time-points after i.p. 60 mg/kg nimodipine (Tween).

Figure 55), but no association was demonstrated with nimodipine (Figure 56).

PTZ seizure inhibition

EXPERIMENT 10A:

Mice treated with i.p. NIF ($P<0.001$), NIM ($P<0.02$) and CBZ ($P<0.001$) all exhibited increased seizure latency compared with controls (Figure 57). There were no significant differences between the drug groups.

EXPERIMENT 10B:

This experiment confirmed the beneficial effect of NIF ($P<0.001$) and CBZ ($P<0.01$) in this model (Figure 58). Combining NIF and NIM did not alter seizure latency significantly from control animals and was less effective than NIF given alone ($P<0.005$).

EXPERIMENT 10C:

Comparison of the drug groups (Figure 59) showed that both oral NIF ($P<0.002$) and oral NIM ($P<0.001$) increased seizure latency compared with controls. Results with NIF and NIM were amalgamated and the effects of different doses analysed. Daily doses of 1mg ($P<0.001$), 2mg ($P<0.001$) and 4mg ($P<0.02$) were all effective but the highest dose showed the least impressive prolongation of seizure latency (Figure 60). Indeed, the anticonvulsant effect of 4mg daily was significantly less than that of 1mg ($P<0.002$) or 2mg ($P<0.05$).

MINUTES
TO SEIZURE

Control   Nifedipine   Carbamazepine   Nifedipine +
                                          Carbamazepine

FIGURE 58   Fresh groups show increased latency with NIF (P<0.001)
and CBZ (P<0.01), but not with these drugs in combination.

MINUTES
TO SEIZURE

Control   Nifedipine   Nimodipine   Carbamazepine

FIGURE 57   All drug groups have longer seizure latencies than
controls (P<0.001, P<0.02, P<0.001 respectively).

FIGURES 57 & 58   Pentylenetetrazol seizure latency in mice following i.p. drugs. PTZ s.c. (85 mg/kg) given 10 min after i.p. drug (NIF,
NIM 3.3 mg/kg; CBZ 6.7 mg/kg) or control vehicle (alcohol/water). Values are mean ±SEM.

FIGURE 60   Increased seizure latency at all doses (P<0.001, P<0.001, P<0.02) but high dose poorer than lowest (P<0.002) or middle (P<0.02) doses.

FIGURE 59   Increased seizure latency after oral NIF (P<0.002) and NIM (P<0.001).

FIGURES 59 & 60   PTZ seizure latency in mice following oral drugs. PTZ s.c. (85 mg/kg) given after two weeks' NIF, NIM 33 mg/kg, 67 mg/kg and 133 mg/kg (= approx. 1, 2, 4mg daily), or control feeding. Values are mean ±SEM.

DISCUSSION

In this study, NIF and NIM were effective intraperitoneally and orally in extending latency of PTZ-induced seizures. In our version of the model, i.p. CBZ also increased the time to fitting. This is unusual as the standard PTZ threshold test [Krall et al 1978] has been suggested as specific for drugs active against absence seizures. However, various alterations in the test, as discussed by Meldrum [1986], can change this supposed specificity [e.g. Kent & Webster 1983, Sugaya et al 1986]. The procedure in the present study may allow for efficacy to be shown with CBZ and the other first-line AEDs.

It could be argued that the dihydropyridines were not acting as anticonvulsants per se, but were merely enhancing the action of ethanol present in the solvent. Dihydropyridines can prevent ethanol withdrawal seizures [Little et al 1986] and have been shown to augment its anaesthetic [Dolin & Little 1986] and psychomotor [Isaacson et al 1985] properties. However, they have not been shown to potentiate any anticonvulsant effect of ethanol, while nimodipine (although not nifedipine) is already known to possess an anticonvulsant action when given as a suspension [Dolin et al 1988] or in glycerol [Morocutti et al 1986] or Emulphor (5% alcohol) [O'Neill & Bolger 1989] preparations. In the present study, ethanol/water solutions were used to provide accurate pharmacokinetic data, as has been previously described for NIM in gerbils [Heffez et al 1985]. EXPT. 9B demonstrates that this would not be feasible with a Tween preparation. The alcohol/water vehicle was also used for EXP 10A and 10B. Any "synergism" with ethanol would not explain the efficacy of CBZ, nor the detrimental interaction between CBZ and NIF, and clearly, any effect of ethanol was not an issue in the oral experiment.

Nimodipine has been shown to be active against PTZ seizures both parenterally [Meyer et al 1986a] and orally [Meyer et al 1987]. This drug is known to cross the BBB [Heffez et al 1985], but after the publication of EXPT 8, doubts were expressed over whether the less lipophilic NIF possesses this ability [Wen 1988]. Recently it has been shown to do

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so in the rat [Janicki et al 1988] and the results of the present study confirm that this is also the case in the mouse. Both dihydropyridines entered the brain freely, reaching peak levels in approximately 5 minutes. NIM was particularly efficient in crossing the BBB following i.p. injection. However,in the present study NIF was as effective as NIM in delaying seizures. NIM concentrations in blood were much lower than those of NIF suggesting a larger apparent volume of distribution - and this concurs with the findings in man [Ramsch et al 1986].

It has been suggested, following preliminary communication of this work, that alcohol may be acting as a "carrier" across the BBB. EXPERIMENT 9B was subsequently performed using the Tween preparation in the high doses required for anticonvulsant efficacy [Dolin et al 1988]. It demonstrates that both NIF and NIM are able to cross the BBB without the assistance of alcohol in the vehicle. Blood:brain ratios were similar to those with the alcohol/water preparation. Absorption of the dihydropyridines in the latter solution, however, seems much faster and more consistent than the suspension in Tween, allowing the accurate determination of pharmacokinetic parameters not previously described. The relationship between blood and brain levels is more easily seen, as the erratic absorption and short half-life of the dihydropyridines conspire to render much of the Tween data uninterpretable.

The correlation between brain and blood levels of the dihydropyridines (alcohol/water) was close, particularly at the early time points. Those at one minute were "flatter" than the rest which suggests that equilibration between blood and brain had not yet taken place. The association becomes less obvious around 20-30 minutes presumably owing to the added complexity of drug elimination from the brain. Both dihydropyridines had longer elimination half-lives in brain than in blood.

The short half-life of NIF may explain a recent failure to demonstrate its efficacy against electroshock seizures when given 60 minutes before testing [Wong & Rahwan 1989], although NIF (prepared in 7.5% polyethylene glycol/7.5% alcohol) was active against PTZ seizures in that study. Their choice of a 60 minute delay probably relates to previous work showing protection from seizures over some hours with nimodipine [e.g. Dolin et al 1988], but such studies used suspensions and not solutions. The difference in absorption demonstrated in the present study explains why their solution may have had a shorter effect. The authors' inference that NIF's failure against electroshock seizures means that it might only be useful in absence seizures is perhaps less valid in view of the short half-life of NIF. The apparent efficacy of CBZ in the present study, using a model very similar to theirs, also argues against their results constituting proof of NIF's selectivity of effect. This demonstrates the importance of doing pharmacokinetic studies before performing and interpreting trials of efficacy.

The apparent loss of benefit when NIF was given with CBZ remains unexplained. Any pharmacokinetic interaction is presumably not due to an effect on CBZ concentrations, since such an interaction was virtually excluded (though in man) in the pilot study of NIF in epileptic patients (EXPT. 8). A pharmacodynamic interaction is another possibility, particularly as CBZ itself has calcium channel blocking properties [Crowder & Bradford 1987]. The possible involvement of adenosine receptors is discussed in EXPT. 13. Whatever the mechanism, if this lack of additional benefit is confirmed, it may be of some importance, since the major use of calcium antagonists in clinical practice is likely to be as adjuvant AEDs. It should be noted, however, that a recent study using electroshock seizures in mice [Czuczwar et al 1990] showed an enhancement of the anticonvulsant effects of CBZ and PHT when NIF was added. In this study, the dihydropyridine was used in subtherapeutic doses and appeared to act synergistically with the AEDs. Further dose-ranging studies using the dihydropyridines and AEDs are required to define the nature and extent of any interactions at different dosages.

Dose-ranging studies of the CAs in isolation are also indicated, particularly in view of the apparent diminishing effect with increasing dosage in the present study. It may be that the CAs exhibit a "therapeutic window" similar to that tentatively postulated for PHT [Troupin & Ojemann 1975, Osorio et al 1989] and this could have implications for their clinical use. Clinical trials must be carefully designed to allow for such dose-response relationships. Preliminary studies of a dose-ranging nature again may be required.

Both CAs were effective orally and parenterally against PTZ seizures. Both entered the brain freely and there were close correlations between brain and blood levels. This study further supported their consideration as AEDs for use in clinical practice, while warning of possible dose-response difficulties and pharmacological interactions. Placebo-controlled trials of these drugs in a clinical setting was the logical next step.

EXPT. 11   NIFEDIPINE IN EPILEPSY - A PLACEBO-CONTROLLED TRIAL

AIM

To assess the efficacy of nifedipine as an adjunctive anticonvulsant in epileptic patients in a double-blind crossover study.

PATIENTS AND METHODS

Twenty-two students (12 male, 10 female; aged 17-22 years) with refractory epilepsy attending Lingfield Hospital School, Surrey, England, were recruited into the study. Clinical details are shown in Table 24.

The trial was conducted as a balanced, placebo-controlled, crossover study with an eight week run-in period and an eight-week wash-out period between treatment phases, each of which also lasted eight weeks. Participants took either NIF tablets (Adalat Retard, Bayer) 20mg twice daily for four weeks, then 40mg twice daily for a further four weeks; or matched placebo in a similar fashion. Previous AEDs were continued throughout the study in unchanged timing and dosage. Partial and generalised tonic-clonic seizures were recorded by staff at the school for the duration of the trial.

At baseline and at 2 weekly intervals during the treatment phases, erect and supine blood pressures and heart rates were measured at 0900h and 1600h. Students also completed a side-effect profile. This consisted of questions on 15 specific symptoms either associated with NIF administration (e.g. flushing, headache, palpitations) or unassociated "dummy" symptoms (e.g. itching, dry mouth). These were graded as absent (0), mild (1), moderate (2), or severe (3).

TABLE 24 Exp.11 Clinical details of patients in nifedipine-placebo study

No	Sex M/F	Age (years)	Type of Epilepsy	Drugs	Trial Drug Order	Outcome
1	M	19	CxP + 2^{0}Gen.	CBZ	P/A	Completed
2	M	19	CxP + 2^{0}Gen.	CBZ+VPA+PHT	P/A	Completed
3	M	17	CxP + 2^{0}Gen.	CBZ,VPA,ETHOSUX.	A/P	* 8 weeks (irritability)
4	F	18	CxP + 2^{0}Gen.	CBZ+PHT	P/A	Completed
5	M	19	CxP + 2^{0}Gen.	CBZ+PHT+ETHOSUX.	P/A	Completed
6	M	19	CxP + 2^{0}Gen.	CBZ+ETHOSUX.	A/P	Completed
7	F	19	CxP	CBZ+VPA+PHT+ACET.	P/A	Completed
8	M	20	CxP + 2^{0}Gen.	CBZ+VPA+ETHOSUX.	A/P	* end of study (poor compliance)
9	M	22	CxP + 2^{0}Gen.	CBZ+VPA+ETHOSUX.	A/P	Completed
10	M	19	GTCS	CBZ+VPA	A/P	Completed
11	M	19	CxP + 2^{0}Gen.	CBZ+CLOBAZAM	P/A	Completed
12	F	19	CxP + 2^{0}Gen.	CBZ+VPA	A/P	Completed
13	F	18	GTCS	CBZ+VPA+ACET.	P/A	Completed
14	F	18	GTCS	CBZ,ACET,CLOBAZAM	P/A	Completed
15	F	17	CxP	CBZ,ACET,CLOBAZAM	P/A	Completed
16	F	21	CxP + 2^{0}Gen.	CBZ,PHT	A/P	Completed
17	F	19	GTCS	CBZ,VPA,ETHOSUX.	A/P	Completed
18	M	18	CxP + 2^{0}Gen.	CBZ,ETHOSUX.	A/P	Completed
19	M	19	GTCS	CBZ,PHT	P/A	Completed
20	F	18	CxP	CBZ,VPA	A/P	Completed
21	M	17	CxP + 2^{0}Gen.	CBZ,VPA	P/A	Completed
22	F	20	CxP + 2^{0}Gen.	CBZ,VPA,ACET	A/P	Completed

Cxp   - Complex partial seizures
2^{0}Gen - Secondary generalised seizures
GTCS - Generalised tonic-clonic seizures
*   - Withdrawn from study

ACET   - Acetazolamide
ETHOSUX - Ethosuximide
P   - Placebo
A   - Active nifedipine

Every two weeks, blood was withdrawn approximately 1 hour after the morning dose by venepuncture for assay of NIF concentrations. Samples were centrifuged immediately and the serum stored in black bags at $-25^{\circ}C$ until batch analysis. Nifedipine was measured by capillary gas chromatography using electron captive detection with nicardipine as internal standard by Dr. John Williams at the Department of Pharmacology & Therapeutics, University of Wales, Cardiff. All procedures involved in the extraction were performed under sodium light. The limit of detection was 1 ng/ml and inter-assay coefficients of variation at 2.5 ng/ml, 10 ng/ml and 20 ng/ml were 22.7%, 5% and 4.3% respectively.

A surface EEG was also performed every 2 weeks. These were graded by an experienced neurophysiologist in a blinded manner with reference to a "baseline" EEG for each student (+1/+2 = slight/definite improvement; -1/-2 = slight/definite deterioration; 0 = no change). These were summed to produce an overall score for the active and placebo phases of the trial.

Statistical comparisons between placebo and NIF phases of the trial were made using the Wilcoxon Rank Pairs test. Power calculations using the equivalent parametric test - the paired Student's t-test - suggested a power of 0.95 to pick up a fall in total seizure numbers (at $P<0.05$) of 50%, which was the figure recorded in the pilot study (EXPT. 8). The power to detect a 25% fall was 0.6. Calculations were also made to identify a 25% fall in GTCS (0.5), partial seizures (0.95) and seizure-days (0.95).

As this trial was performed at Lingfield, I did not take part in its day-to-day running. I was, however, involved in its conception and design, and was responsible for all of the collation, analysis and interpretation of data.

RESULTS

Twenty-one students completed the study. Eleven took placebo in the first leg, and ten received NIF first. One student was withdrawn after five weeks of treatment because of aggressive outbursts. He was found to have been receiving placebo. Another had very poor recording of seizures while on holiday and his data were omitted from the analysis of seizure frequency.

Total numbers of seizures in the two treatment groups are shown in Figure 61. Only in the first two weeks of the trial was seizure control better with NIF ($P<0.05$). Sub-group analysis suggested that this was due to a fall in the number of partial seizures (Figure 62), which was lost by the second month. There were fewer seizure days in the NIF phase during the first month of treatment (from a mean of 13.2 to 11.6, $P<0.05$) but this did not carry through to the second month of the trial. The number of patients reporting a 50% fall in seizures on each treatment is shown in Table 25. There were no differences between treatments for any seizure type. Blood pressure showed a small drop after two weeks of NIF and also after two weeks of placebo. This effect had settled by four weeks in both phases. There was no evidence of a significant postural drop in blood pressure in any of the students. Heart rate was not affected by either treatment.

"Severe" side-effects reported included headache in two students (both on active drug), dry mouth in two (one active, one placebo), drowsiness (one placebo) and poor memory (one active). Overall scores for all specific or all dummy side-effects did not differ between the NIF and placebo treatment phases. Scores for headache, however, were significantly higher with NIF ($P<0.02$).

Nifedipine concentrations are shown in Table 26. Students with maximum levels greater than 10 ng/ml (n=11) did no better in terms of seizure control than those with concentrations below this arbitrary figure. A weak correlation, however, was shown

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TABLE 25 Students reporting a 50% reduction in seizures following treatment with nifedipine and matched placebo for 2 months each

		FIRST MONTH	SECOND MONTH	TWO MONTHS
*Partial (n = 17)	Placebo	1	2	O
	Nifedipine	3	5	2
*Tonic-Clonic (n = 17)	Placebo	10	7	8
	Nifedipine	6	4	3
Total (n = 21)	Placebo	3	2	2
	Nifedipine	4	3	2

*   Each includes 4 students who had no seizures in this category and so could not have a 50% reduction

TABLE 26 Nifedipine concentrations (ug/L) in students with refractory epilepsy taking 20mg and 40mg of the drug twice daily for 4 weeks

	n	Mean (SD)	Median	Range
20mg nifedipine twice daily (at 4 weeks)	20	4.3±(5.3)	1.9	0-16.6
40mg nifedipine twice daily (at 8 weeks)	19	9.8±(11.3)	5.7	0-47
Maximum concentration	21	13.1±(10.4)	11.3	2.1-49
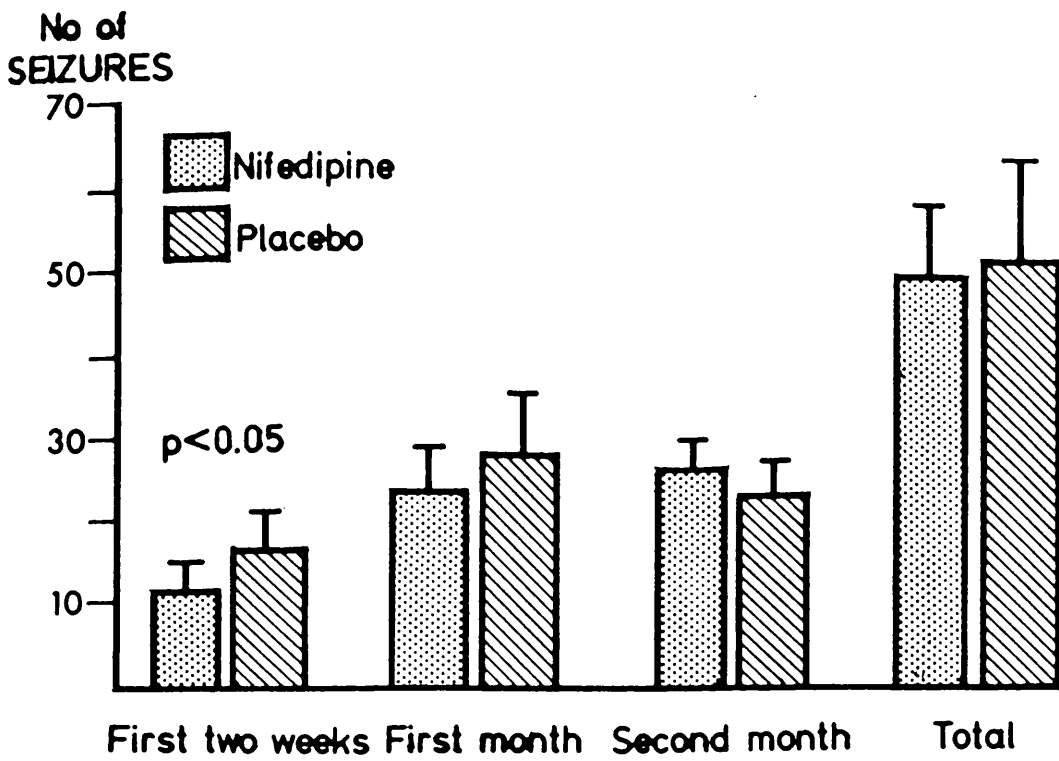
FIGURE 61   Total number of seizures in 20 students with refractory epilepsy taking nifedipine and placebo. Values are mean + SEM.
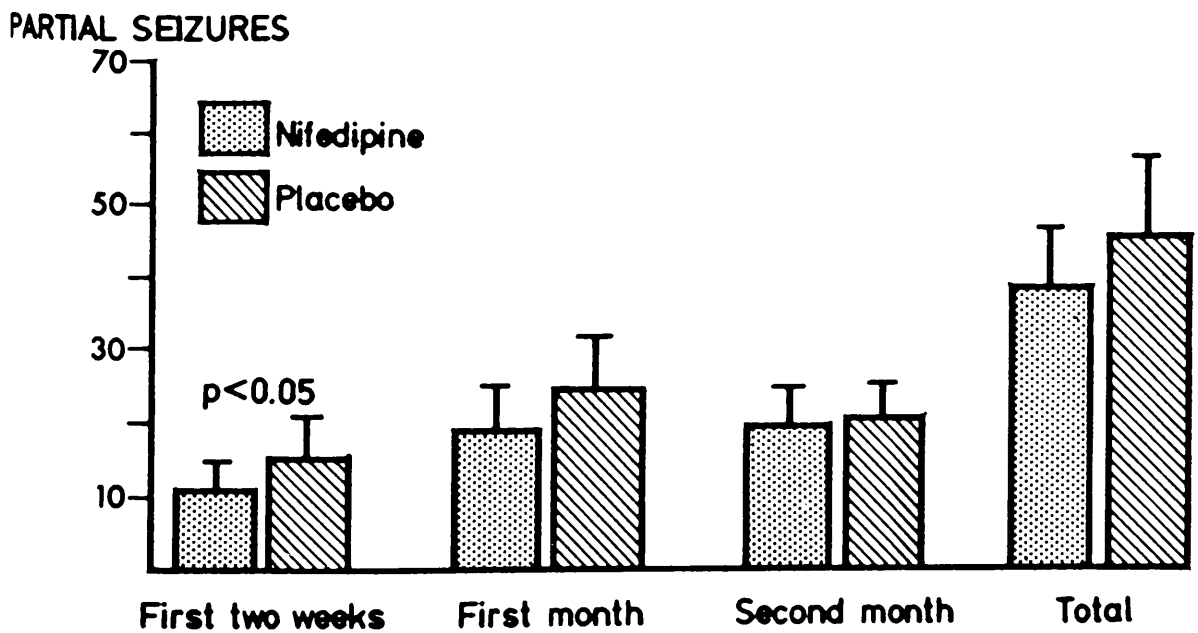
FIGURE 62   Number of partial seizures in 20 students with refractory epilepsy taking nifedipine and placebo. Values are mean + SEM.
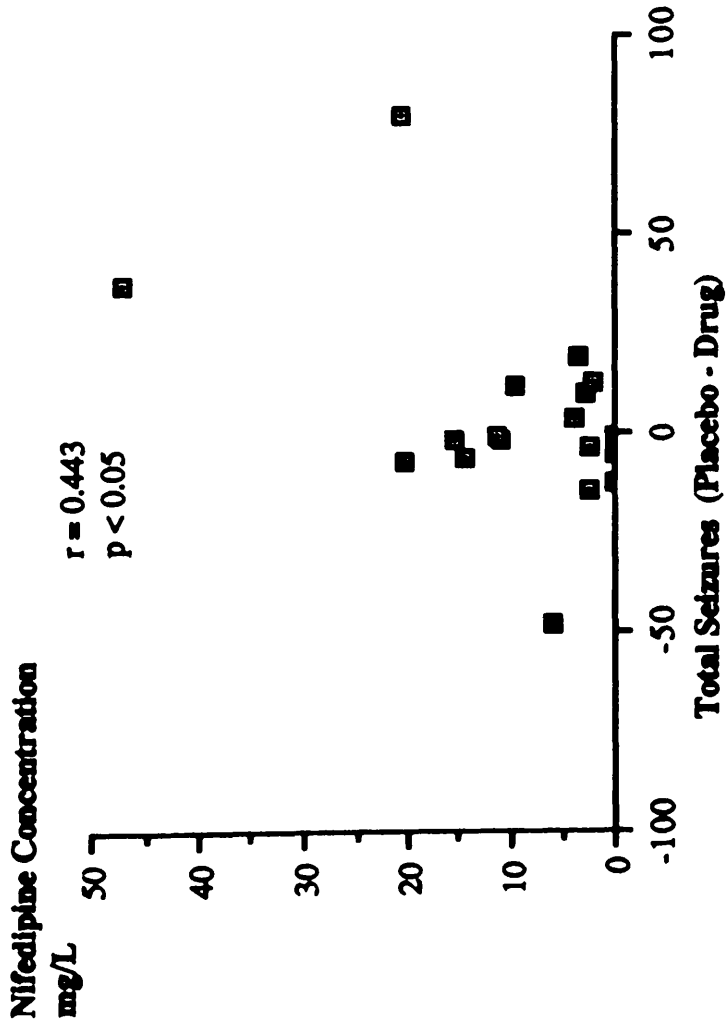
FIGURE 63   Correlation between improvement in total seizure
numbers and nifedipine concentrations following 8 weeks therapy.
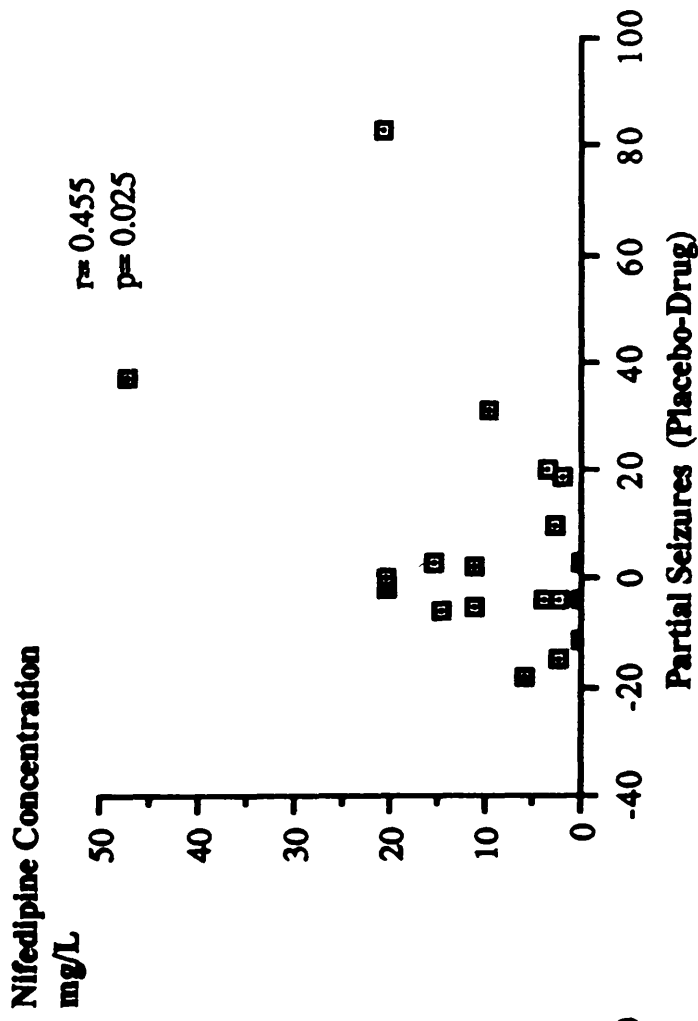
FIGURE 64   Correlation between improvement in partial seizure
numbers and nifedipine concentrations following 8 weeks therapy.
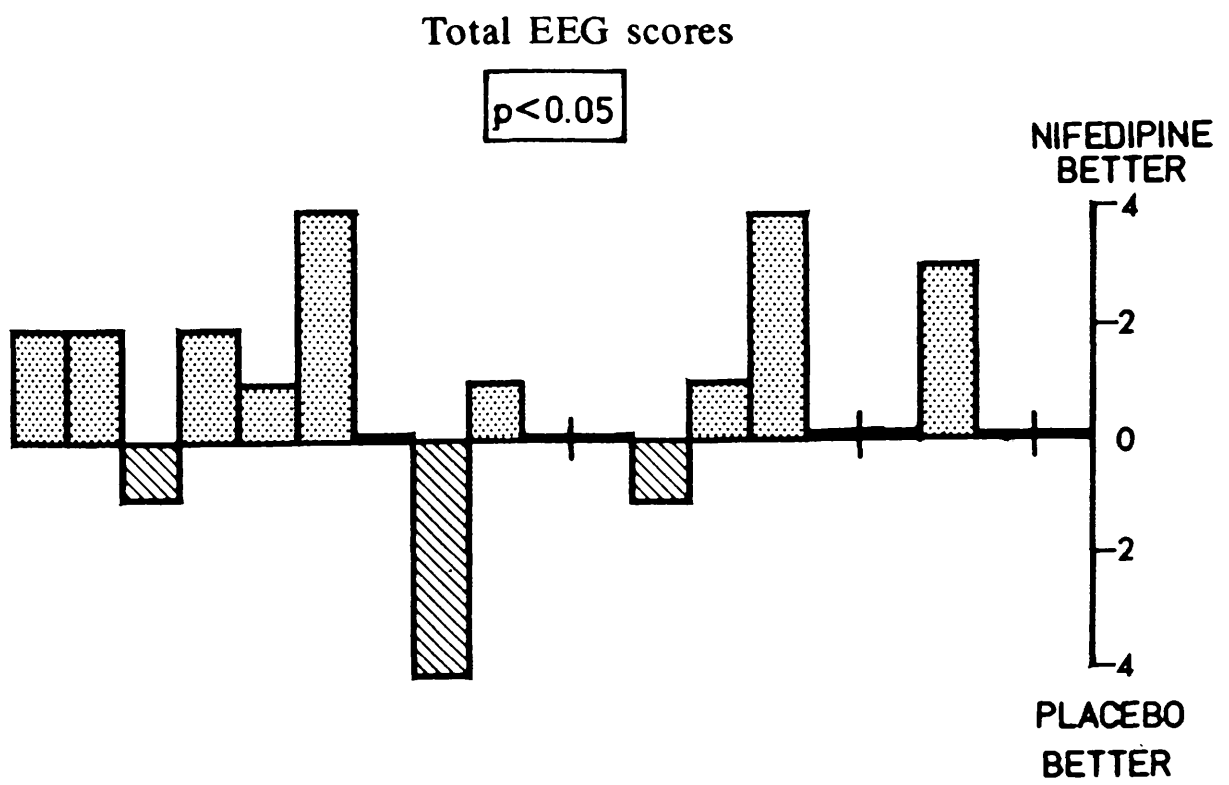
FIGURE 65   Comparison of summed 'EEG scores' during nifedipine and placebo phases of study in 19 students with refractory epilepsy.

between seizure improvement on NIF (seizure count on placebo - seizure count on NIF) and NIF concentrations at week 8 for total seizures (r = 0.443, P<0.05 one-tailed; Figure 63) and partial seizures (r = 0.455, P<0.025; Figure 64).

Total EEG scores for the four tracings during NIF treatment were compared with those on placebo. More students had improved scores while receiving the active drug (P<0.05, Figure 65)

DISCUSSION

The results from this study are not particularly promising. Although one or two "significant" improvements were seen with NIF, a large number of comparisons were made, and allowance for this makes their clinical relevance doubtful. Minor favourable changes were seen early in treatment, largely as a consequence of a decreased number of partial seizures. The EEG findings also suggest a positive pharmacological response to NIF.

If there is a genuine effect which is lost in the second month of treatment despite higher NIF doses, this may suggest the rapid development of tolerance. This could be receptor-mediated. Down-regulation of ^{3}H-nitrendipine sites in mice has been reported following treatment with NIF for 28 days [Panza et al 1985], due to a decrease in receptor numbers with no change in receptor affinity. Seizures themselves have also been shown to decrease [^{3}H]nimodipine binding in rat brain [Gleiter et al 1989] and to have variable effects on the cortex or the cerebellum of the cat [Bolger et al 1987]. The relevance of these findings to the clinical situation is unclear. Another possibility is the "therapeutic window" which was suggested in EXPT. 10C. This is, perhaps, unlikely, as there are

very few situations in clinical medicine where a true "window" exists. I have already mentioned that it has been postulated to occur with PHT [Gruber et al 1940, Troupin & Ojemann 1975], but others consider the phenomenon of doubtful significance [Osorio 1989]. In the present case, the particularly low concentrations of NIF may also argue against this explanation.

This disappointing study leaves an inconsistency which also requires an explanation. Why are CAs so impressive in animal models of epilepsy, yet apparently ineffective in epileptic patients? One possibility, of course, is that the models are inappropriate, but CAs have been effective in a large variety of animal seizures which have, over the years, been successful in predicting clinical activity [Meldrum 1986]. Another problem relates to the screening of potential AEDs in individuals with poorly controlled epilepsy despite treatment with multiple AEDs. The students in the present trial reported an average of nearly 30 seizures per month in the baseline period and were receiving, in the main, two or three established antiepileptic agents. It is, perhaps, not surprising that results with NIF are disappointing in such a population.

Next, the NIF dose must be taken into consideration. Circulating NIF concentrations in this study were low. Information on an "effective blood concentration" of NIF is limited, but single dose (10mg-20mg) studies usually achieve peak concentrations of 60-80 ng/ml [Taburet et al 1983, Renwick et al 1988] while a study of adjuvant NIF in angina suggested that effective levels were in the 30-40 ng/ml range [Challenor et al 1989]. Very few of our patients achieved these levels (Table 26 and Figure 63), as all were taking enzyme-inducing drugs which are likely to accelerate the break-down of NIF [Schellens et al 1989] which is extensively metabolised in the liver [Challenor et al 1987]. Half-life following a single dose of NIF is approximately 2.5 hours in healthy subjects [Ramsch et al 1986, Renwick et al 1988], and while this increases at steady-state [Ramsch et al 1986], it is likely that in induced patients the short half-life will make it

very difficult to maintain effective concentrations. This is true despite using the "Retard" preparation which we deliberately utilised in this study. A marked decrease in circulating concentrations of felodipine - another dihydropyridine - has been demonstrated in similar circumstances [Capewell et al 1988]. The slight correlation between seizure improvement and attained NIF concentrations also suggests that higher NIF concentrations may have improved the results, but one should be wary of reading too much into this correlation which depended largely on one or two results. Arguably, the significantly increased incidence of headache in patients taking NIF does hint at pharmacologically active concentrations in many individuals.

Pharmacological interactions, as suggested by EXPT. 10C would be another possible reason for failure of the nifedipine. Many of these factors also relate to nimodipine, the controlled study of which was taking place in our unit at the same time as the above.

EXPT.12   NIMODIPINE IN EPILEPSY - A PLACEBO-CONTROLLED TRIAL

AIM

To assess the aniconvulsant efficacy of adjuvant nimodipine therapy in epileptic patients using a placebo-controlled crossover study.

PATIENTS AND METHODS

Twenty-two patients (8 male, 14 female; aged 18-53 years) attending the epilepsy clinic with refractory epilepsy (each averaging > 3 seizures/month) were recruited into the study. Clinical details are shown in Table 27.

The trial was conducted as a balanced, placebo-controlled crossover study with a 4 week run-in period and a four week wash-out period after each treatment phase, both of which lasted 12 weeks. During each treatment phase, patients took nimodipine tablets 30mg tds (0800h, 1500h and 2300h) for four weeks, 60mg tds for the next four weeks, and 90mg tds for a further 4 weeks, or matched placebo in a similar fashion. During the washout period, patients took 60mg tds for one week followed by 30mg tds for a further week before stopping therapy (Figure 66). Twelve patients had placebo in the first treatment phase, and ten were given the active drug in the first phase. Previous anticonvulsant therapy was continued unchanged throughout the study.

Patients attended for review at weeks -4, 0, 4, 8, 12, 16, 20, 24, 28 and 32. At each visit, drug compliance was checked by way of a tablet count, and patients were supplied with a seizure chart (cf. Figure 44) which they used for recording each individual seizure during the subsequent month. At each visit, patients also completed a "tolerability profile"

TABLE 27   Exp.12 Clinical details of patients in nimodipine-placebo study

No	Sex M/F	Age (years)	Duration	Type of Epilepsy	Drugs	Trial Drug Order	Outcome
1	F	29	7	CxP + 2°Gen.	CBZ,PHT	P/A	Completed
2	M	26	18	CxP + 2°Gen.	CBZ,VPA	A/P	Completed
3	F	19	5	SCxP	VPA,PHT	A/P	Completed
4	M	41	10	CxP + 2°Gen.	CBZ	P/A	Completed
5	F	27	20	CxP + 2°Gen.	PHT	P/A	Completed
6	F	33	23	CxP + 2°Gen.	CBZ,VPA	A/P	* 16 weeks (weight loss)
7	M	38	10	CxP + 2°Gen.	CBZ	P/A	Completed
8	M	22	19	CxP + 2°Gen.	CBZ,PHT	A/P	Completed
9	F	33	24	CxP + 2°Gen.	CBZ,PRIM	A/P	Completed
10	M	25	13	CxP + 2°Gen.	CBZ	P/A	Completed
11	F	31	30	SCxP	CBZ,PRIM	A/P	Completed
12	F	19	19	GTCS	CBZ	P/A	Completed
13	F	37	12	CxP	PHT	P/A	Completed
14	M	26	26	CxP	PHT	A/P	Completed
15	F	32	21	CxP + 2°Gen.	CBZ	P/A	* 8 weeks (Nausea)
16	F	35	22	CxP + 2°Gen.	CBZ,VPA	A/P	Completed
17	F	42	23	CxP + 2°Gen.	VPA,PHT	A/P	* 20 weeks (Intercurrent pneumonia)
18	M	42	30	GTCS	PHT.PB	P/A	Completed
19	F	24	16	CxP + 2°Gen.	VPA	A/P	* 8 weeks (Headaches O.C.)
20	M	24	4	CxP	CBZ,VPA	P/A	Completed
21	F	30	17	CxP + 2°Gen.	CBZ,PB	P/A	* Completed study but non-compliant
22	F	53	46	CxP + 2°Gen.	CBZ	P/A	Completed

Cxp   - Complex partial seizures
SCxp   - Simple and complex partial seizures
2°Gen - Secondary generalised seizures
GTCS   - Generalised tonic-clonic seizures
*   - Withdrawn from study

PRIM   - Primidone
P   - Placebo
A   - Active nimodipine
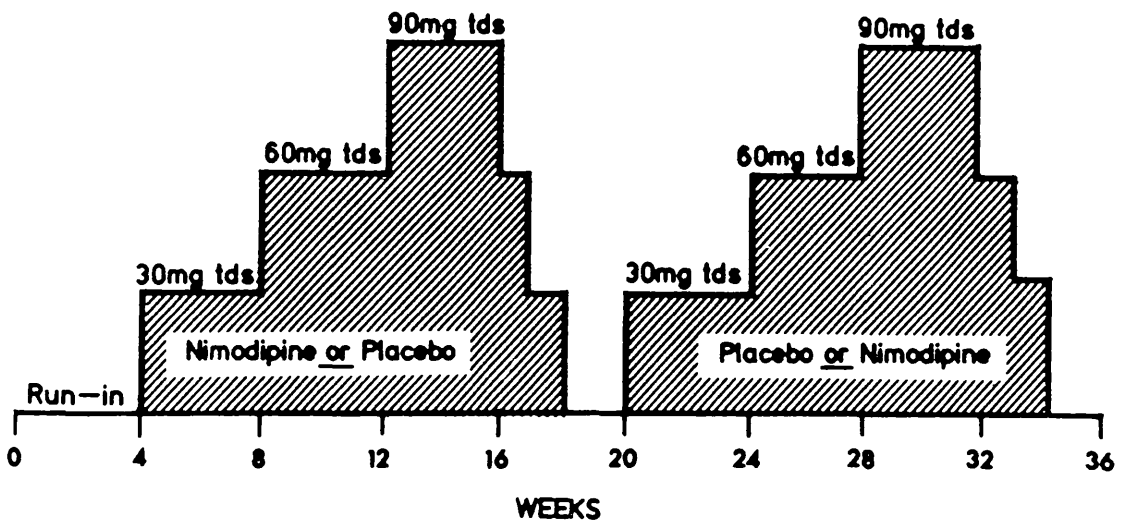
FIGURE 66   Schematic outline of balanced crossover trial of nimodipine in epilepsy
(EXPT. 12) showing drug dosage, including during "washout" periods (weeks 16-20 and
32-36)

consisting of 10 "visual analogue scale" lines of 10 cm length citing specific symptoms either associated with nimodipine administration (e.g. flushing, headache) or unassociated "dummy" symptoms (e.g. itching) and including possible side-effects from concomitant AEDs (e.g. sedation, double vision). A brief general medical examination was also performed. Erect and supine pulse and blood pressure were measured at 0900h, 1200h, and 1600h. Blood was withdrawn from an antecubital vein for nimodipine assay at 0900h ('peak'), 1500h ('trough') and 1600h ('peak'), and also for assay of other AEDs at 0900h and 1600h. Blood samples were centrifuged and the serum stored at -20°C for batch analysis. Haematological and biochemical parameters were also checked at each hospital visit.

Drug Assays

Carbamazepine, phenytoin and valproate were assayed by enzyme immunoassay as previously described. Nimodipine assays were performed in a blinded fashion by Bayer Laboratories using gas chromatography.

Statistics

Comparisons of seizure frequency between the nimodipine and placebo phases were performed using Wilcoxon rank pairs testing for non-parametric data. Power calculations (revised to allow for "drop-outs") performed as if using the equivalent parametric test - paired Student's t-test - suggested a power of >0.99 to pick up a fall of 50% in partial seizures, total seizures or seizure days in this group of patients. The power calculations to detect a 25% improvement were 0.79, 0.85 and 0.75 respectively. A 50% or 25% fall in GTCS had power calculations of 0.54 and 0.18.

RESULTS

Seventeen patients satisfactorily completed the study. Two were withdrawn because of possible side-effects while taking the active drug (1 weight loss, 1 nausea and vomiting) and one patient had an intercurrent pneumonia unrelated to therapy. Two patients were withdrawn because of inadequate compliance, one with drug therapy and one with seizure monitoring (see Table 27).

Median (range) numbers of GTCS and partial seizures at each stage of the trial are shown in Table 28. Median numbers of total seizures and seizure days are shown in Figures 67 and 68. There were no differences between nimodipine and the placebo preparation at any stage. The numbers of patients achieving a 50% or 25% drop in GTCS, partial seizures or seizure-days on either preparation is shown in Table 29. Again, there were no differences between active drug and placebo. Patient-preference was ascertained before coding was broken. Nine preferred nimodipine, 3 preferred placebo and 5 expressed no preference. This difference is not statistically significant (P=0.073, binomial test).

Mean concentrations of concomitant AEDs are shown in Table 30. Although all three drugs exhibited slightly higher concentrations while patients were taking nimodipine, no statistically significant differences were seen.

Nimodipine concentrations were generally low throughout the study. Afternoon peak concentrations on 60 mg tds were 6.77 $\pm$ 3.0 ug/L (mean $\pm$ SD, range 2.6 - 13.9 ug/L) while those at 90 mg tds were only marginally higher at 7.83 $\pm$ 2.0 ug/L (range 4.8 - 11.5 ug/L). There was no correlation between nimodipine concentrations and seizure control.

Mean systolic blood pressures were slightly lower at peak nimodipine concentrations (0900h, 1600h) than at similar times on placebo (difference = 3mm mercury) or at baseline (5-7 mm mercury), but these differences were not significant. There were no

TABLE 28 NIMODIPINE TRIAL
Median seizure frequency (range) in 17 patients on active drug and placebo

PARTIAL	ACTIVE	PLACEBO
1st month	4 (0 - 48)	4 (0 - 42)
2nd month	5 (0 - 75)	4 (0 - 43)
3rd month	7 (0 - 43)	6 (0 - 40)
TOTAL	18 (0 - 165)	14 (0 - 119)

TONIC-CLONIC		
1st month	1 (0 - 26)	0 (0 - 15)
2nd month	2 (0 - 19)	0 (0 - 30)
3rd month	0 (0 - 44)	1 (0 - 14)
TOTAL	5 (0 - 100)	2 (0 - 48)

Active drug (nimodipine 20mg tds, 60mg tds, 90mg tds each for a month) and matched placebo in ransom order for 12 weeks

TABLE 29   Patients achieving 25% (50%) fall in seizure frequency from baseline

| | 1ST MONTH | | 2ND MONTH | | 3RD MONTH | | TOTAL | |
	Active	Placebo	Active	Placbo	Active	Placebo	Active	Placebo
PARTIAL* SEIZURES	10 (6)	7 (6)	6 (6)	8 (5)	10 (7)	8 (4)	9 (7)	6 (5)
TONIC-** CLONIC	4 (4)	3 (3)	4 (1)	4 (3)	4 (2)	4 (1)	5 (1)	4 (1)
SEIZURE*** DAYS	9 (6)	7 (4)	5 (2)	6 (2)	7 (2)	6 (1)	7 (3)	7 (2)

*   15 appropriate patients finished study

**   12 appropriate patients finished study

***   20 appropriate patients finished study

TABLE 30 Anticonvulsant concentrations in 17 patients treated with adjuvant nimodipine or matched placebo

	b	Nimodipine	Placebo
CBZ	13	10.6 ± 3.6	9.2 ± 3.9
PHT	7	21.9 ± 8.5	19.3 ± 8.9
VPA	4	71.1 ± 35	61.6 ± 28

Results are from the 3rd month of treatment with the highest nimodipine dose.

TABLE 31 Tolerability Scores during baseline and high dose therapy of nimodipine and placebo

Complaint	Baseline	Active high dose	Placebo high dose
Sedation	5.96	5.75	4.94
Impaired concentration	6.68	5.55	6.78
Nausea	1.86	1.79	2.28
Headache	3.27	3.65	5.50
Itching	2.09	1.65	3.11
Agitation	3.27	3.00	3.67
Unsteadiness	2.27	3.20	3.11
Flushing	1.64	1.65	2.94
Palpitations	1.86	1.65	1.89
Double vision	2.91	3.00	1.56

Values are means of results on a scale from 1.0 to 20.0. Higher values suggest presence of side effect. There are no significant differences between drugs or between drug and baseline.
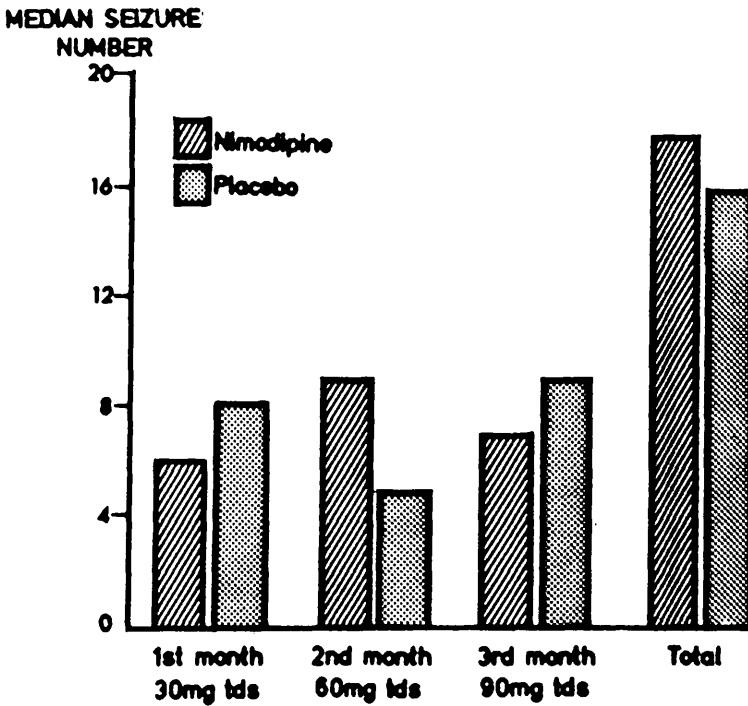
FIGURE 67   Comparison of total seizure numbers in 17 patients during nimodipine phase and placebo phase of the study. Values are medians.There are no significant differences.
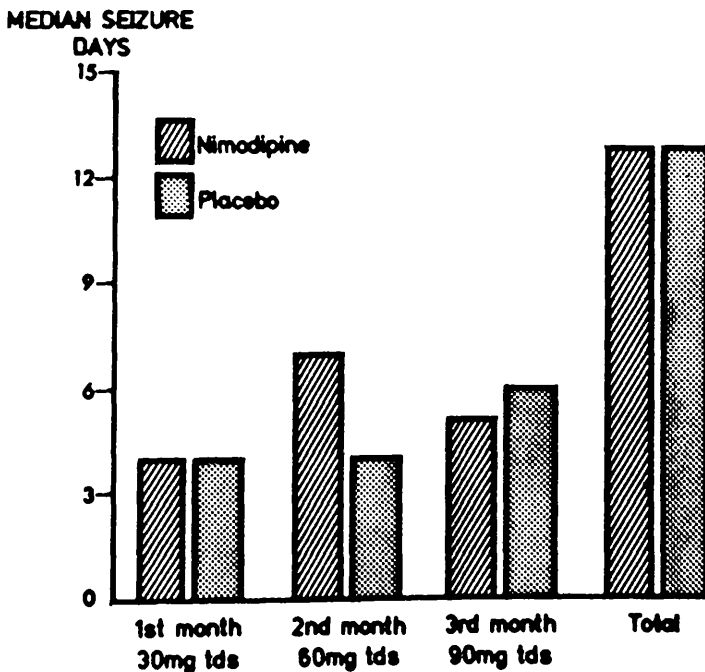
FIGURE 68   Comparison of seizure-days in 17 patients during nimodipine and placebo phases of the study. Values are medians. There are no significant differences.

incidences of postural hypotension. Haematological and biochemical indices were unchanged on nimodipine therapy, and "tolerability scores" did not reveal any signicant side-effects (Table 31).

DISCUSSION

The dihydropyridines have been a particularly effective group of CAs in a wide variety of animal models of epilepsy [Morocutti et al 1986, Meyer et al 1987, De Sarro et al 1988, Dolin et al 1988 etc.], and nimodipine has been prominent in most of these reports. In the present study, however, it failed to show any usefulness as an adjunctive therapy in a clinical setting. The power of this study, even allowing for withdrawals, was such that we would expect to detect an important action by NIM in this situation, but the results gave no suggestion of any effect, and indeed were more negative than those with nifedipine in EXPT. 11.

Again, there are many possible reasons for this disparity between efficacy in seizure models and in clinical practice. Firstly, the models may be inappropriate. Secondly, the dose regimen of NIM may not be optimal. Thirdly, there may be an interaction between NIM and other AEDs. Lastly, the anticonvulsant effect may be present, but insufficient to make a clinical impact as an adjuvant therapy in patients with refractory epilepsy.

With regard to the first possibility, it must be noted that NIM has been effective in a wide range of seizure models, including those chemically induced with PTZ [Meyer et al 1987, Dolin et al 1988, Moron et al 1989, O'Neill & Bolger 1989], cefazolin [Morocutti et al 1986], picritoxin [Thomas 1990], kainic acid [Paczynski et al 1990] and strychnine [O'Neill & Bolger 1989]. Other models include electroshock seizures [Meyer et al 1990]

and audiogenic seizures in DBA/2 mice [De Sarro et al 1988]. These and similar models
have proven useful in investigating anticonvulsant activity with other drugs [Meldrum
1986] and it seems unlikely that they are so inappropriate as to explain NIM's failure in
this study.

As with NIF, the pharmacokinetics may be a more likely explanation. The elimination
half-life of NIM in healthy subjects is only two hours [Ramsch et al 1986]. It has been
used as a 4-hourly regimen in the successful treatment of subarachnoid haemorrhage
[Allen GS et al 1983, Pickard et al 1989], but this would not be feasible in the long-term
management of epilepsy. Thrice-daily dosage, however, has been used successfully
following ischaemic stroke [Paci et al 1989] at a dosage of 40mg tds despite evidence
that very little accumulation occurs at this dosage [Ramsch et al 1985]. In the present
study, an attempt was made to overcome the rapid elimination of NIM by increasing the
dosage to the uppermost range of previous studies. However, even this manouevre seems
not to have achieved its aims. All patients were taking enzyme-inducing drugs along with
NIM. Since this drug undergoes hepatic metabolism with a large first-pass effect
[Ramsch et al 1986], enzyme induction would be likely to reduce circulating
concentrations in a similar fashion to the reported effects of AED induction on nifedipine
[Breimer et al 1989] and felodipine [Capewell et al 1988]. Patients with subarachnoid
haemorrhage who are given I-V infusions of NIM have been shown to run serum
concentrations of 36-72 ug/L [Ramsch et al 1985], while oral dosing (60 mg tds) gave
peak levels around 17-42 ug/L in the same study. Our subjects failed to achieve such
levels, with maximum concentrations approximating 12 ug/L. There was no correlation
between concentration and seizure control as was found with nifedipine in EXPT. 11, but
the circulating concentrations may have been too low in all patients to achieve any effect.

A more fanciful suggestion would be that the doses were perhaps too high. Both EXPT.
10C and another animal study [Thomas 1990] have shown some decrease in efficacy of

NIM with increasing dosage. Such an event could conceivably be related to an effect on receptors - dihydropyridine binding sites in mice are downregulated by chronic NIF therapy [Panza et al 1985] - but it seems an unlikely explanation.

In addition to the pharmacokinetic interactions with AEDs, a pharmacodynamic interaction is also a possibility. Phenytoin [Sohn & Ferrendelli 1976, Ferrendelli & Daniels-McQueen 1982] and CBZ [Ferrendelli & Daniels-McQueen 1982, Crowder & Bradford 1987] both have calcium antagonist activity, and a detrimental interaction - again perhaps receptor-mediated - cannot be excluded. This would be in keeping with the failure of the CBZ-NIF combination therapy in EXPT. 10B.

The final possibilty again refers to the present study as well as EXPT. 11. These patients were taking appropriate doses of established AEDs and achieving adequate serum concentrations (Table 30), yet were suffering an average of 18 seizures per month. It is very possible that a drug with genuine anticonvulsant activity might fail to impress in this situation.

In summary, this trial did not suggest that NIM will be a useful adjuvant anticonvulsant, at least not in these doses. Failure to achieve effective circulating concentrations seems the most likely explanation. Further dose increments may improve its efficacy, but there are obvious drawbacks in using such an approach to counteract the effects of enzyme induction from other AEDs. A slow-release preparation could be an alternative approach, but the disappointing results in EXPT. 11 using the nifedipine tablet which enhances its bioavailability, do not support a prediction of success.

EXPT. 13 ADENOSINE A_1 RECEPTORS IN MOUSE BRAIN: EFFECT OF ORAL CARBAMAZEPINE AND DIHYDROPYRIDINES

AIM

To investigate the effects of nifedipine, nimodipine and carbamazepine on adenosine A_1 receptors in mouse cortex, cerebellum and mid-brain, and assess the likely relevance of any effects to their anticonvulsive properties.

METHODS

CF1 mice (Bantin & Kingman, Hull) weighing 25g were used. Each was individually caged and given free access to food and water. They were allocated to one of four schedules - 1) normal feed (control); 2) added NIF: 0.3 g/kg per day; 3) added NIM: 0.3 g/kg per day; 4) added CBZ: 1 g/kg daily for three days followed by 2.25 g/kg for four days. These doses are similar to those effective against PTZ seizures in EXPT. 10C.

At the end of seven days' oral treatment, the animals were killed by decapitation and their brains removed and dissected as outlined by Glowinski & Iverson [1966]. Brain stem, cerebellum and cortex were stored separately at -70 $^{\circ}$C. Samples from two animals were pooled for each assay to ensure adequate protein. As all tissues could not be processed simultaneously, compensation for day-to-day variation was achieved by matching each treated pair of animals with a pair from each other group and a control pair.

Binding Assay

This was the first time that receptor assays had been performed in our laboratory. The assays were established "from scratch" by George Thompson (Senior Technician) and myself, from first principles of binding assays [Bennett & Yamamura 1985] and published papers. These principles, along with the mathematical theory behind them, are outlined in Appendix A. On occasions during the development of the assay and during the present study, I personally undertook each of the procedures involved in the assay. However, in general the assays were performed by Mr. Thompson and his staff - except for the computerised Scatchard and saturation isotherm analysis which I performed on all results.

Adenosine binding was assessed by a modification of the method of Marangos et al [1987] using tritiated cyclohexyladenosine ([^{3}H]CHA) 34.4 Ci/mmol (New England Nuclear). Brain tissue was thawed and homogenised (polytron setting 5) for 10 seconds in 25 volumes of 50 mM Tris-HCl buffer at pH 7.5. After centrifugation (30,000g at 4^{o}C for 20 min), the pellet was suspended in buffer and incubated with adenosine deaminase (2u/ml) at 22^{o}C for 30 min) before further centrifugation. The pellet was finally resuspended in approximately 25 volumes of buffer so that the protein concentration (measured by a modified method of Lowrie [Peterson 1977]) was 0.6-1.5 mg/L (final assay concentration 0.4-1 mg/L).

The receptor assay was performed by incubating 500 ul of protein solution and 150 ul [^{3}H]CHA with 100 uL buffer for total binding or with 100ul 37.5 uM "cold" CHA for non-specific binding.

Incubations at 6 different concentrations of [^{3}H]CHA (0.2 nM - 29 nM) were performed in duplicate for estimation of dissociation constant (Kd) and receptor numbers (Bmax).

The highest concentration (29nM) was included to ensure a suitable range [Bennett & Yamamura 1985] in the event of a marked increase in Kd in the treated groups, but was generally unneccessary. Assays were incubated for 2h at 22°C and the reaction was terminated by vaccuum filtration through GF-B filters using a Brandel cell harvester (3 washes with 4ml ice-cold buffer). Filters were dried overnight before counting in 3ml Emulsifier Safe Scintillin (Packard). Kd and Bmax were estimated using both Scatchard and Saturation isotherm analyses (Packard CA 2000 Combicept 1.1; see Appendix A).

Results were considered acceptable if correlation co-efficient on the Scatchard line was >0.85 and Scatchard and saturation isotherm analysis did not differ by >25%. The mean of Scatchard and isotherm analysis results was taken as the Kd or Bmax on each occasion. Preliminary experiments included eight separate assays performed on pooled control homogenised mouse brain which produced Kd (SD) values of 2.89 (0.24) nM (co-efficient of variance 8%) and Bmax (SD) of 661 (81) fmol/mg protein (co-efficient of variance 12%).

Statistics

Wilcoxon-Rank matched pairs analysis was used.

RESULTS

Despite tiered dosing, mice in the CBZ group were slightly lighter (mean 30.7 $\pm$ SD 1.2g, P<0.05) at the end of one week compared with the other groups (control 32.6 $\pm$ 3.6g, NIF 32.8 $\pm$ 2.3g, NIM 33.1 $\pm$ 1.6g).

In all, 120 assays were prepared, 10 from each anatomical area - brain stem, cerebellum and cortex - in each of the four treatment groups. Of these, 111 led to satisfactory results

by the criteria outlined. Most unsatisfactory assays (n=7) were in brain stem where protein concentration was sometimes too low.

Scatchard plot correlation co-efficients ranged from 0.902 to 0.999 (mean 0.983 $\pm$ 0.014). Correlation between Scatchard analysis and saturation isotherm analysis was also close for both Kd (r=0.96) and Bmax (r=0.99) results (Figures 69 & 70). Figure 71 shows the Scatchard and saturation isotherm analysis of the three brain areas in the control mice of the tenth and final run, while an actual print-out is shown as Figure 72.

Adenosine receptors were present in much higher numbers in the cortex than the cerebellum (P<0.02), which in turn contained more binding sites than brain stem (P<0.02). These differences are illustrated for the control mice in Figure 73, although they were present in all treated groups. The Kds were more closely matched, but the mean Kd in the cortex (P<0.05) was higher than in the other areas (Figure 73).

Table 32 shows the KDs and Bmax of all three brain areas in the four treatment groups, and these are shown graphically in Figures 74 and 75. The increased Kd with NIM in brain stem failed to reach statistical significance, but both NIF (P<0.005) and NIM (P<0.02) increased Kd in the cerebellum. There was a trend for CBZ to increase Kd in the cerebellum (0.05<P<0.1) while no effect was seen in the cortex. The increased Bmax in brain stem with NIM also failed to reach conventional levels of statistical significance. Treatment with CBZ produced an increase in receptor numbers but only in the cerebellum (P<0.02).

TABLE 32   Adenosine receptor Kd and Bmax in different brain areas in control mice and those treated with oral nifedipine, nimodipine and carbamazepine

	BRAIN STEM		CERERBELLUM		CORTEX	
	Kd	Bmax	Kd	Bmax	Kd	Bmax
CARBAMAZEPINE	2.09	188	2.39	280	3.12	449
	(0.31)	(26)	(0.2)	(24)	(0.28)	(54)
NIFEDIPINE	2.43	179	3.69**	317	3.22	371
	(0.36)	(27)	(0.35)	(24)	(0.29)	(54)

Values are means (SEM) of at least 7 animals.

Kd (nM) and Bmax (fmol/mg protein) were calculated using Scatchard and saturation isotherm analyses of [^{3}H]CHA binding at 0.2 - 29 nM concentrations.

* P<0.02   ** P<0.005 vs controls
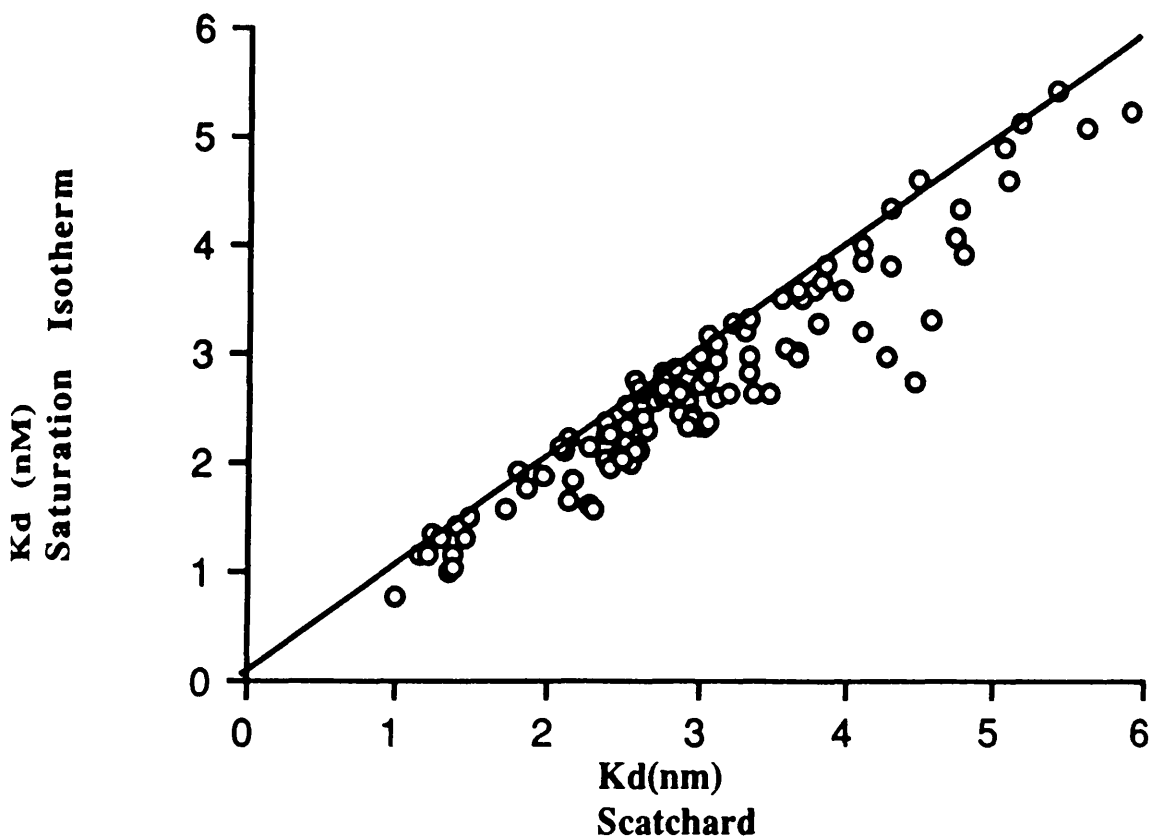
FIGURE 69   Adenosine A$_1$ receptors. Graph of dissociation constant (Kd) as calculated using saturation isotherm analysis vs. Scatchard analysis in 111 assays of mouse brain (line of identity shown). Results from cortex, cerebellum and brain stem of control mice and those treated with nifedipine, nimodipine or carbamazepine are shown.
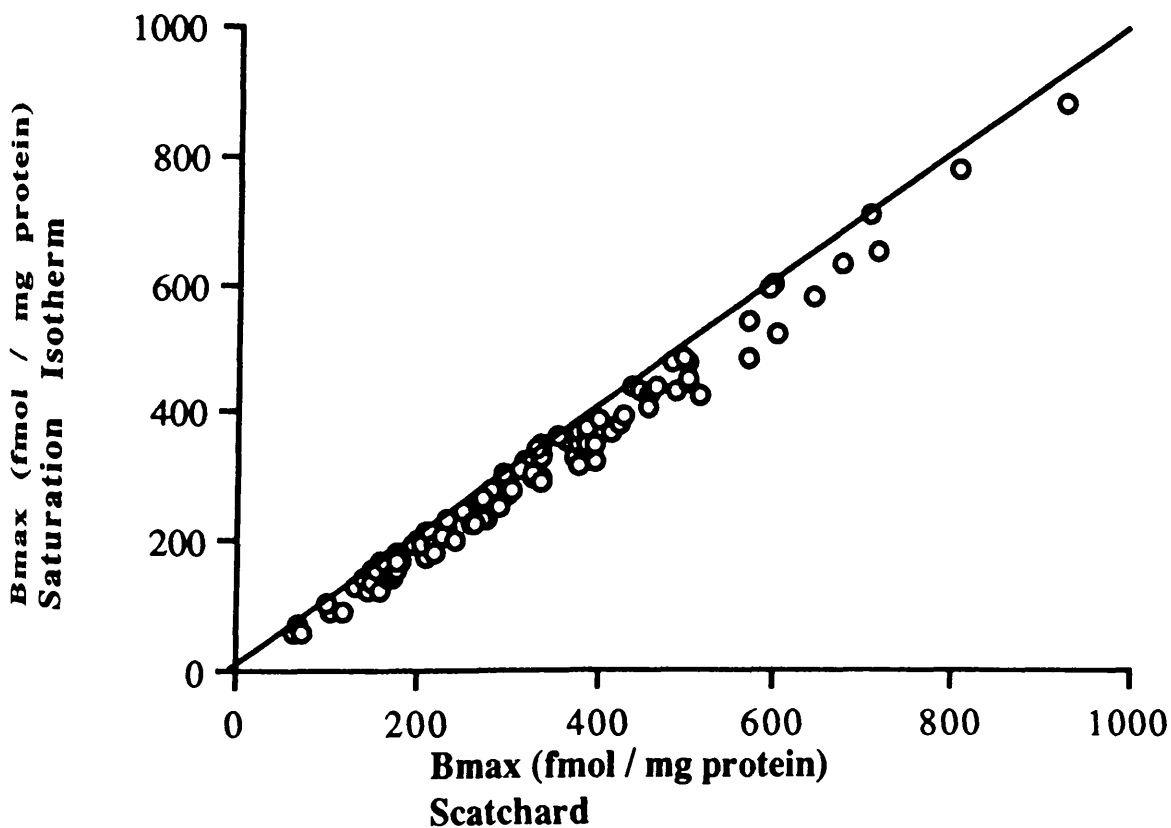
FIGURE 70   Adenosine A$_1$ receptors. Graph of total receptor number (Bmax) as calculated using saturation isotherm and Scatchard analysis as in Figure 69.
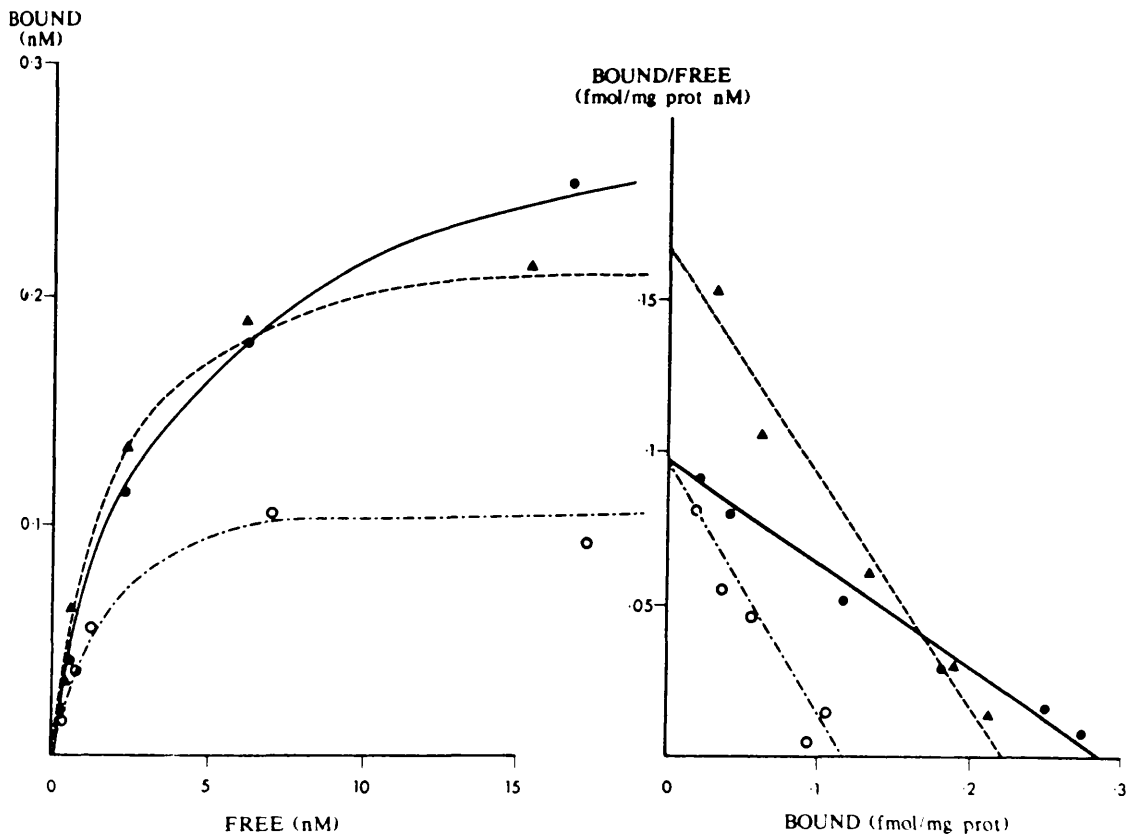
FIGURE 71   Saturation isotherm and Scatchard analysis of [^{3}H]CHA binding in a single control mouse brain stem (o--o), cerebellum (▲--▲) and cortex (●—●). Saturation isotherm analysis Kds respectively 1.16, 1.35 and 3.02nM; Bmax: 166, 337 and 430 fmol/mg protein. Scatchard analysis Kds 1.15, 1.15 and 2.73 nM; Bmax: 163, 298 and 391 fmol/mg protein.

SCATCHARD PLOT
B/F vs Bnd (nM)   ADEN
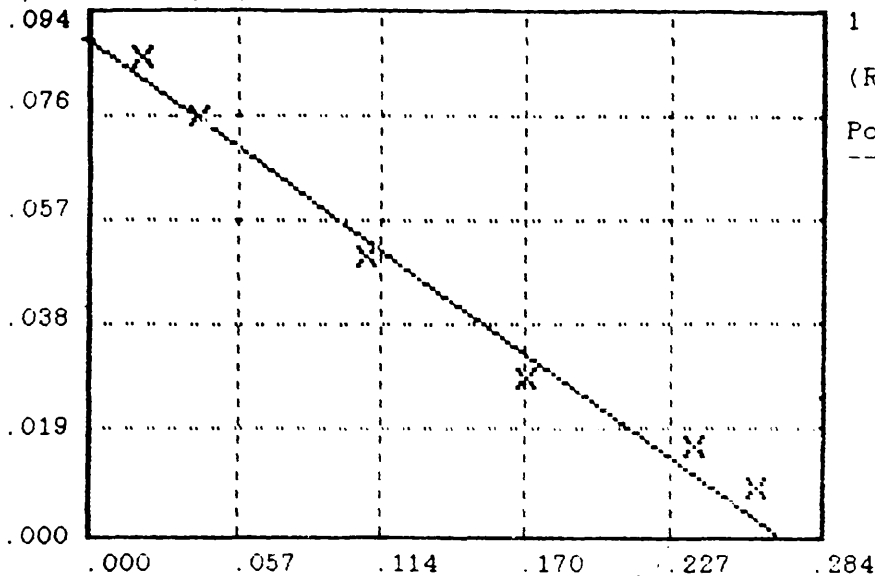
1 aden/12

(Rosenthal NSB corre

Point	B/F	
1	.0859	.0914
2	.0757	.0805
3	.0501	.0533
4	.0279	.0297
5	.0156	.0166
6	.0082	.0087

Protein concentration: P= .94 mg/mL
Dissociation constant: Kd= 3.02 nM
Binding site concentration: N= 404 fmol/mL Cytosol
                           or   430 fmol/mg Protein
Correlation coefficient: R= .9925

2ω

SATURATION CURVE
Sb vs St (nM)   ADEN
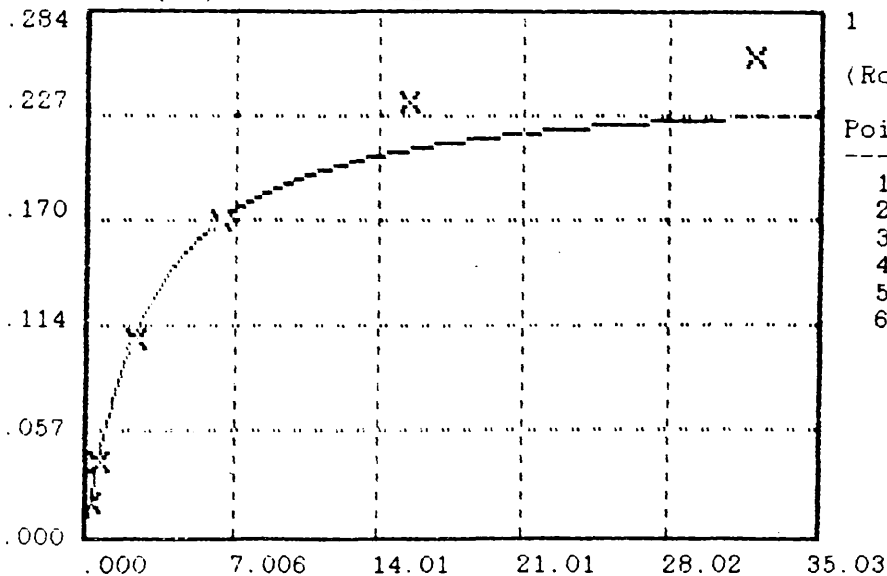
1 aden/12

(Rosenthal NSB corre

Point	Sb	
1	.0190	.0202
2	.0407	.0453
3	.1073	.1141
4	.1696	.1804
5	.2345	.2495
6	.2580	.2745

Protein concentration: P= .94 mg/mL
Dissociation constant: Kd= 2.73 nM
Binding site concentration: N= 372 fmol/mL Cytosol
                           or   396 fmol/mg Protein

COMBICEPT 1.1   (C) Copyright Packard Instrument Co. 1985,86   01-Mar-9

FIGURE 72 Printout from Packard CA2000. Scatchard and saturation isotherm analysis of [^{3}H]CHA binding in control mouse cortex.
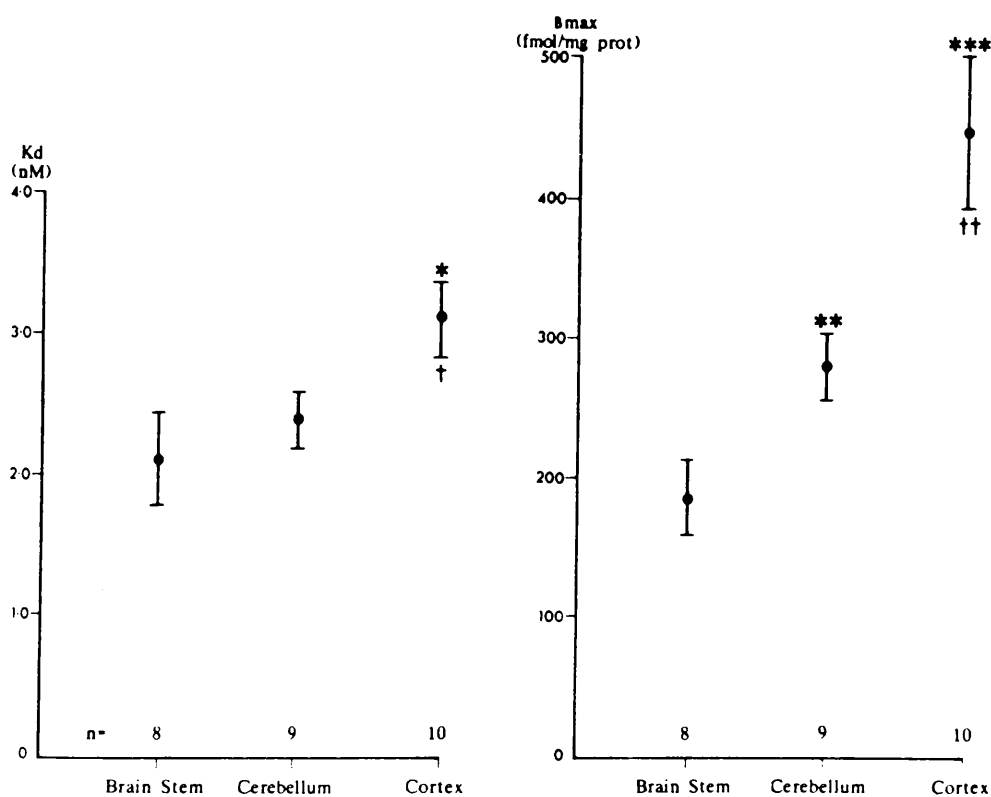
FIGURE 73   Adenosine A_1 receptor Kd and Bmax assayed by [^{3}H]CHA binding at
0.2-29nM. Mean of Scatchard and saturation isotherm analyses taken as result. Results
from control mouse brain stem, cerebellum and cortex. Vertical bars represent ±SEM.
* P<0.05; ** P<0.01; *** P<0.001   - vs. brain stem.
+ P<0.05; ++ P<0.01   - vs. cerebellum.
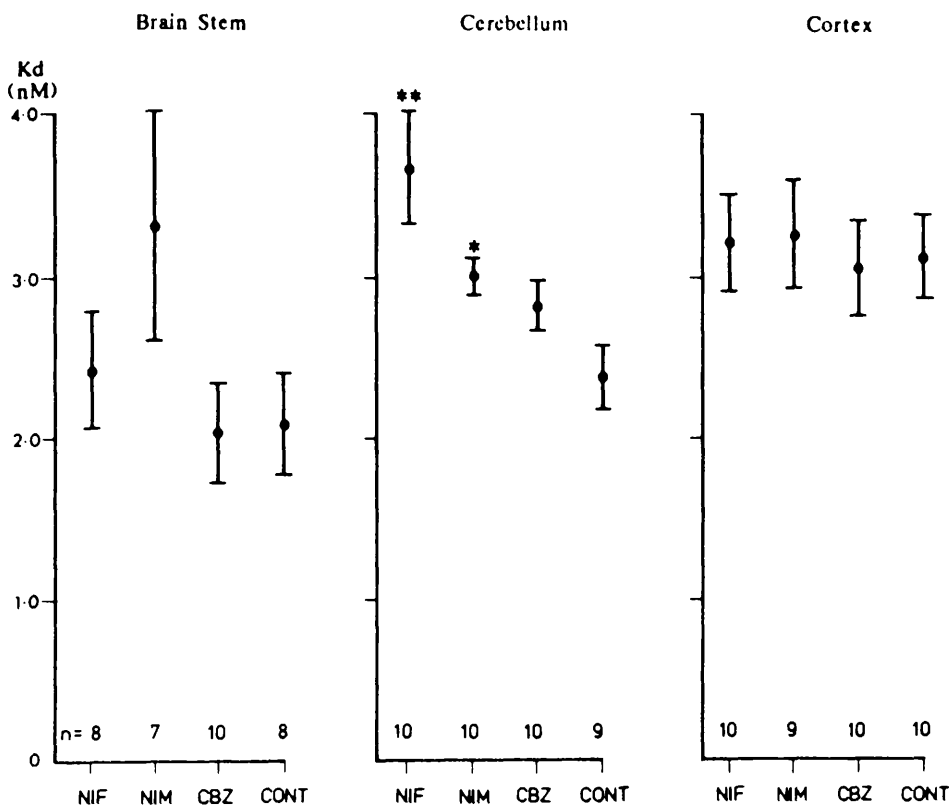
FIGURE 74 Adenosine A_1 receptor Kd assayed by [^{3}H]CHA binding at 0.2-29nM. Results from areas of mouse brain after treatment with nifedipine, nimodipine, carbamazepine or control feed. Vertical bars represent $\pm$SEM.
* P<0.05; ** P<0.01   - vs. controls.
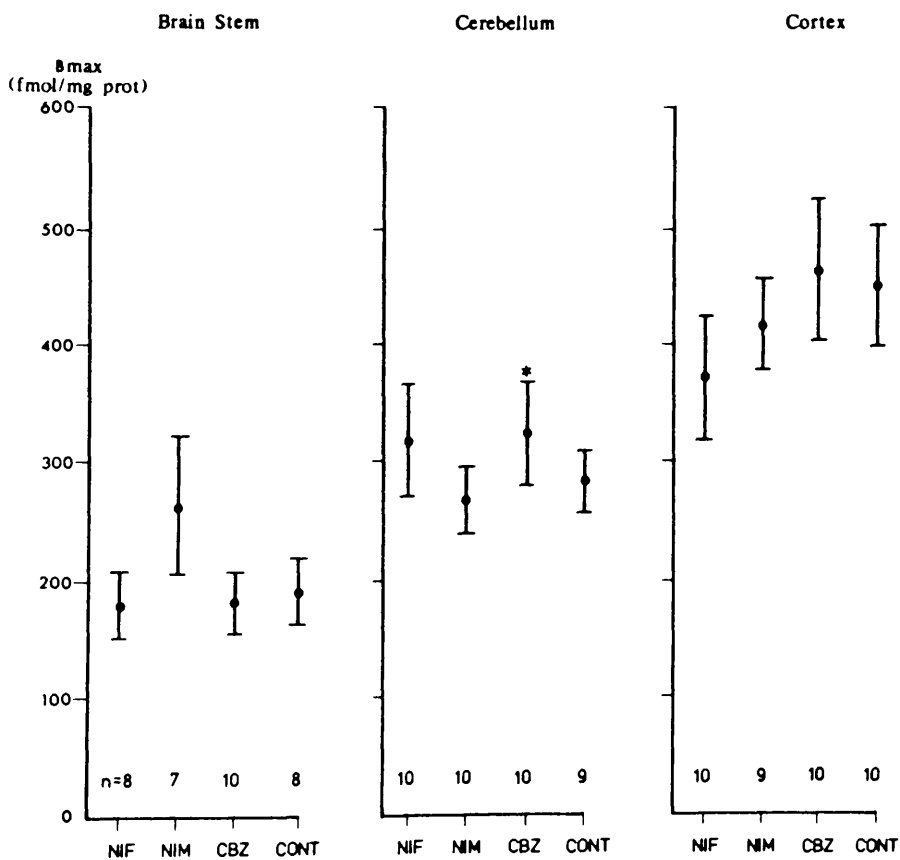
FIGURE 75 Adenosine A_1 receptor Bmax in areas of mouse brain assayed after treatment as in Figure 74.

DISCUSSION

Interaction with the adenosine receptor in vitro has been repeatedly shown with both CBZ [Marangos et al 1983, Skerritt et al 1982, Weir et al 1984] and CAs, particularly the dihydropyridines [Murphy & Snyder 1982, Morgan et al 1987, Cheung et al 1987, Hu et al 1987]. In vivo, chronic treatment with CBZ increases adenosine binding in rat cortex, cerebellum and brain stem [Marangos et al 1985, Marangos et al 1987] with Scatchard analysis suggesting that this was due to an increased Bmax rather than an alteration in Kd. Increased receptor numbers after CBZ in various brain regions have been confirmed by autoradiography [Daval et al 1989].

All of the above studies were performed in rat brain. Many used "specific binding" results at a single ligand concentration for comparisons between groups, with only the occasional Scatchard calculation of Kd or Bmax [Marangos et al 1985, Marangos et al 1987, Cheung et al 1987, Sanders & Murray 1989]. In the present study, Kd and Bmax for CHA binding in mice were calculated in all cases using the two outlined methods. Results obtained were in a similar range to those found in rats when Scatchard analysis has been performed. In particular, mean Bmax in cerebellum (297 fmol/mg protein) was comparable to the results of Marangos et al, 1987 (251 fmol/mg protein) and to the recent reports of Sanders & Murray (250 fmol/mg protein) and Daval et al (325 fmol/mg protein). The low receptor numbers in the brain stem are also similar to previous reports [Marangos et al 1985, Fastbom et al 1987]. The high adenosine receptor density in mouse cortex differs from one binding study [Marangos et al 1987], but agrees with autoradiographic findings [Fastbom et al 1987]. This may be explained by the higher Kd (lower affinity) in the cortex, as a single-point binding assay may underestimate the receptor density in this site relative to the cerebellum, as can be seen from the sample saturation curves in Figure 71. Calculation of Bmax, as in the present study, avoids this problem.

The effect of CBZ and calcium antagonists on receptor numbers seems less marked than in studies of rat brain. Only CBZ showed any significant effect on Bmax and then only on receptors in the cerebellum. Kd in this region, however, was altered by both NIF and NIM, and the "trend" with CBZ suggests that this drug may also produce this effect. The relevance of these changes is uncertain. Although only NIF is known to inhibit CHA binding in vitro [Morgan et al 1987], both dihydropyridines in the present study were seen to affect Kd in the cerebellum. Previous work would predict a change in Bmax rather than Kd [Marangos et al 1985]. However, adenosine agonists are known to induce a high-affinity receptor [Lohse et al 1984], and so the decrease in affinity which we have shown could possibly be associated with the antagonist action of CBZ, and an as yet unclassified action of the dihydropyridines.

Since the doses of the drugs used in the present study inhibit PTZ seizures in mice [EXPT 10C], the minimal changes in adenosine receptors suggest that such an effect is an incidental feature of these drugs and is not related to their anticonvulsant action. It is worth noting that Bay K 8644 - the calcium *agonist* which exacerbates seizure activity [Walden et al 1986] and antagonises some adenosine effects [Fredholm et al 1986] - has the same effect at the A_1 receptor as dihydropyridine *antagonists* which have the opposite pharmacological actions [Hu et al 1987]. This argues against the drugs' effects on A_1 receptors as being of major relevance to their main pharmacological actions. However, when we consider EXPT. 10C, where combination therapy of NIF an CBZ was poorer than either alone, and note the clinical failure of NIF and NIM in EXPTS. 11 and 12, it is interesting to speculate that any anticonvulsant effect of CBZ which is due to an increase in adenosine receptor numbers might be counteracted by a decreased affinity induced by the dihydropyridines.

It is also possible that our global assessment of cortical binding may miss important

localised changes. Daval et al [1989] have shown possible differentiation of CBZ effects on different areas of rat brain, but analysis of multiple small groups may have over-interpreted normal experimental variation. Further autoradiographic studies may confirm genuine differences in different brain regions and show a relationship between effects on adenosine receptors in electrophysiologically important brain areas and anticonvulsant efficacy. However, at present the association of adenosine receptor activity and anticonvulsant effect remains unproven.

CONCLUSION   CHAPTER 4

CALCIUM ANTAGONISTS AND EPILEPSY

The role of calcium antagonists in the drug management of epilepsy remains uncertain.
There is certainly a place for new AEDs, particularly if sedative side-effects can be
shown to be reduced, thus opening up the prospect of multiple drug therapy. Small doses
of "synergistic" drugs may increase efficacy while reducing toxicity. A long and barren
period for new AEDs is now ending with a clutch of potential anticonvulsants trying to
establish a place in the limited armamentarium. I have already mentioned the GABA
transaminase inhibitor vigabatrin, but other "designer drugs" have been successful in
early trials. Gabapentin is a further attempt to make use of the natural anticonvulsant
effects of GABA, although it now seems that its action may not be due to GABA effects
[Brodie & Porter 1990]. Lamotrigine attacks the other side of the excitation-inhibition
balance by blocking the release of excitatory neurotransmitters [Brodie & Porter 1990]
and a number of other such drugs are waiting in the wings.

As with these other drugs, there are good theoretical reasons why calcium antagonists
should be effective in epilepsy. The arguments for calcium involvement in the release of
excitatory neurotransmitters, with subsequent synchronisation of the "burst firing" of
neurones are persuasive [Meyer 1989]. The abolition of such events in vitro by the
calcium antagonists further enhances their prospects as logical anticonvulsants. It has to
be admitted, however, that the widespread and continuing [De Sarro et al 1990] success
of these drugs in animal models of epilepsy, including EXPT. 10, has not yet led to any
convincing application in the clinical setting. During the work of this thesis, a number of
clinical trials have been performed to assess the efficacy of flunarizine, the drug which
had shown such promise in the early work of Overweg and others [e.g. Overweg et al

1986]. One double-blind crossover study did show a small but significant drop in the seizure frequency of around 35% [Starreveld et al 1989], but other controlled studies have been less successful. Keene and his colleagues have studied young patients with refractory epilepsy [1989], and found more patients to have a 50% decrease in seizures on placebo than on the active drug. Similarly negative results were obtained in adult patients by Alving et al [1989]. An open study in Japan [Nakane et al 1989] also failed to show a convincing overall benefit, although two out of 64 patients apparently had an abolition of their seizures.

These findings do not necessarily mean that flunarizine has no effect on epilepsy. One of the negative trials [Keene et al 1989] suggested that the drug may be more effective against partial seizures, and the results do not exclude a small anticonvulsant effect from the drug. The trial of Alving and his colleagues, however, did exclude (at the 2.5% level) a 29% drop in seizure numbers being caused by the drug. It is likely that flunarizine does have some anticonvulsant efficacy, but in refractory patients it seems unable to make much impact on the disease.

The situation with the currently used dihydropyridines may well be similar. Following our successful open study of nifedipine in refractory epilepsy [EXPT. 8], other workers found little help from the drug in similar circumstances [Sander & Trevisol-Bittencourt 1990]. The results in the placebo-controlled trials of nifedipine [EXPT. 11] and nimodipine [EXPT. 12] in this thesis are also far from convincing. One might even be tempted to dismiss the open study as a manifestation of the placebo effect. As mentioned in the prologue, Esquirol in the 1840s showed similar results for up to three months with such remedies as bloodletting and cauterisation [Esquirol 1845]. Yet, there are one or two encouraging aspects of the placebo-controlled study of nifedipine. The possible short-term effect and the correlation between improved seizure control and serum

concentrations of the drug, taken together with the generally low levels of nifedipine measured in the study, might propose a failure to achieve adequate circulating concentrations as the reason for the drug's failure to significantly affect seizure control.

Nimodipine may have suffered to an even greater extent from this problem in EXPT. 12. Both dihydropyridines have short half-lives of well under ten hours. The enzyme induction caused by concomitant AEDs further reduces these, and will hinder attempts to achieve adequate levels. The high presystemic elimination of nimodipine may make its bioavailability particularly vulnerable to the effects of increased hepatic enzyme activity, and it is worth noting that the drug has been used successfully as an intravenous preparation in partial status epilepticus [Brandt et al 1988]. Using higher and higher doses of the dihydropyridine seems a simplistic approach to the problem of oral use. Even if peak levels could reach those required, the short half-life will result in a rapid fall from these levels, causing violent swings in concentrations during the day unless very frequent dosing is used. A slow-release preparation might possibly be expected to offset this effect, but the nifedipine retard preparation used in EXPT. 11 was, at best, only partially successful. However, the dosage of nifedipine used was not particularly high.

The question of whether these drugs will work as useful anticonvulsants requires an answer. Perhaps studies of high-dose slow-release nifedipine need to be tackled. Alternatively, the drugs may be more successful if used along with the non-inducing AEDs such as sodium valproate or oxcarbazepine. Another option is the use of dihydropyridines with a longer half-life, and such a drug - amlodipine - has recently been marketed by Pfizer.

Amlodipine has a half-life in man of approximately 35 hours [Reid et al 1988]. It appears to be efficacious in hypertension, with a duration of action of more than 24 hours [Webster et al 1987]. We have performed some preliminary studies in mice using our

PTZ model, and so far amlodipine (alcohol/water) does appear to be effective up to 6h after dosing. A very recent paper [O'Neill & Bolger 1990] also suggests that this drug may have similar anticonvulsant properties to the other dihydropyridines in animal models. We are in the process of setting up an h.p.l.c. assay for amlodipine, so that pharmacokinetic studies can be carried out in mice, and the relationship of blood and brain levels to any anticonvulsant effect ascertained. Hopefully, brief, dose-ranging pharmacokinetic studies in induced patients will presage a placebo-controlled study of this drug in a group of epileptic patients from our clinic. This should answer whether a simple pharmacokinetic interaction with inducing drugs is the reason for the relative failure of CAs as anticonvulsants in patients.

It is, however, possible that the problem is more complicated. The loss of efficacy with the combination of nifedipine and carbamazepine in EXPT. 10B (Figure 58) could not have been caused by enzyme induction as it was an acute study. A deliterious pharmacodynamic interaction may be taking place, which could involve the known calcium antagonist actions of CBZ [Crowder & Bradford 1987] or could involve the effects of the drugs on various neuroreceptors. In EXPT. 13, we were able to make a brief investigation of the effects of these drugs on adenosine receptors in mouse brain. While the importance of the CBZ effect on these receptors is only speculative, it is interesting to note that the effects of dihydropyridines differ from those of CBZ, and that the decrease in affinity caused by the calcium antagonists may go some way towards offsetting the increased numbers induced by CBZ.

Other receptors may be involved. The dihydropyridine receptor itself is, as expected, altered by chronic dihydropyridine therapy, which decreases its numbers [Panza et al 1985]. Electroshock seizures also alter these receptors [Bolger et al 1987], and it would

not be too fanciful to suggest that CBZ, with its calcium antagonist activity, might also alter the configuration or numbers of these binding sites, and thus influence the effects of the drugs. Studies are required of the effects produced by each of the line AEDs on these binding sites, both when used alone and when used in combination with a dihydropyridine. There may be a selective interaction between dihydropyridines and the AEDs known to have calcium antagonist properties (CBZ, PHT) but not with VPA - another reason why this drug might turn out to be the best co-therapy with calcium antagonists.

Clearly, much work has still to be done, to ensure that a potential major advance in epilepsy therapy is not discarded because of the indifferent performance of these drugs in early controlled clinical trials. It may be that the initial promise of the drugs will indeed come to naught. However, there are clear indications from the clinical and animal studies in the second part of this thesis that specific problems can be identified. By manipulating the drugs' pharmacokinetic profiles, or by considering the effects of concomitant AEDs, it is possible that these may be overcome. New dihydropyridines may be produced with the right pharmacokinetic profile, and provide an advance similar to that of oxcarbazepine over CBZ where innovations were made to improve one particular aspect of the drug's profile. Amlodipine is first in line for assessment in epilepsy.

The gains which could be made in the life-style of epileptic patients by the discovery of a new group of non-sedative AEDs are enormous. Investigations must continue to establish whether calcium antagonists will fit the bill.

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

Epilepsy is a potentially debilitating disease. While it is only rarely life-threatening, its impact on the "quality of life" of the sufferer is severe - not only through the physical problems and the social stigma which unquestionably remains, but also through damage to the patient's self-image.

Advances in understanding and treating epilepsy have been slow. A small group of drugs are in clinical use, and they only partially control the disease in a large minority of patients. In this thesis, many aspects of the pharmacology and therapeutics of epilepsy have been considered.

Therapeutic drug monitoring

Therapeutic drug monitoring is an attempt to rationalise the use of current antiepileptic drugs. However, care must be taken to avoid the thoughtless mechanisation of chasing "therapeutic ranges" which might paradoxically make dosage selection more irrational. An approach based on the concept that each patient has his own individual "therapeutic range", to which population studies can supply guidelines, is gaining ground, and this is reflected in EXPT. 1. However, the question of whether TDM improves control remains unanswered. No-one seems willing to tackle the necessary 'placebo'-controlled study, perhaps understandably in view of the inherent logistic difficulties. The attitudes of individual physicians, measures of outcome, financial aspects and drug toxicity are all problems requiring attention. Ethical considerations also make such a study difficult to design. It is perhaps for these reasons that we ourselves are simply following up our patients and comparing subsequent years with the original data presented, and have not undertaken a placebo-controlled trial.

Cognitive function

Impairment of cognitive function is ubiquitous with the currently used AEDs. The more recent drugs, such as CBZ and VPA, appear less troublesome on formal testing, but is this a true reflection of their effects? Five years'experience in the epilepsy clinic would suggest that many patients do suffer from increased tiredness, impaired memory, or slowness of thought and action while taking these drugs. As demonstrated earlier [EXPT. 2], the effect of the disease itself is a complicating factor, and the placebo side-effects of a patient expecting drug-induced drowsiness also requires consideration.

It may be, however, that the psychomotor tests we employ are rather stylised, unrepresentative of real life, and fail to detect subtle changes in patients' abilities to perform daily activities. While CBZ does not significantly alter one's appreciation of flickering diodes, it may convert the making of a plate of chips into a complicated and potentially hazardous procedure. A move towards "quality of life" testing is more than welcome and might show up some of the AEDs in a less favourable light. Newer drugs such as vigabatrin, lamotrigine, or possibly the calcium antagonists have hopefully even less neurotoxic side-effects than CBZ or VPA. Their use in combination may produce an improvement in seizure control at present unattainable in many patients.

The degree of cognitive impairment caused by the individual AEDs in epileptic patients remains difficult to ascertain. The proper placebo group of untreated epileptic patients with frequent seizures is ethically unachievable. Instead, various designs of study, each controlling for one aspect at a time, are performed, such as those in this thesis. Such studies should continue. They should include untreated epilepsy control groups as far as possible. They should also use patients as their own controls, changing therapies and allowing adequate time on each, so that short term effects (as in EXPT. 3) are not over-interpreted. The introduction of new tests, more relevant to everyday life, is also

advisable. One of our volunteers in the controlled-release CBZ study [EXPT. 4] performed all of his psychomotor testing beautifully, then rode his bicycle into the back of a parked car while returning home from the hospital.

Gradually, a "league-table" of cognitive impairment with AEDs could be built up. However, the lesson learned from TDM should be remembered. Each patient is different, and the least neurotoxic drug on a population basis might be the most toxic in any one individual, or vice-versa.

Enzyme induction

The enzyme induction caused by many AEDs is well known. The question here concerns its clinical relevance. Obviously, interactions with other drugs such as the contraceptive pill are worth avoiding, e.g. by using VPA or perhaps, in the future, oxcarbazepine. The effect of the latter drug when used long term in conjunction with the contraceptive pill requires assessment. The results of EXPT. 7 suggest that there may be no interaction, but specific confirmation that it does not induce oestrogen metabolism is needed before oxcarbazepine might be proposed as an ideal substitute for CBZ in many young female patients.

More doubtful is the importance of inducing AEDs' effects on the patients' endogenous hormones. It seems unlikely that thyroid function is affected to any appreciable extent, but the accumulating reports of hyposexuality in epileptic patients may indeed relate to AED usage. Although a preponderance of TLE patients amongst hyposexual epileptics has often been reported - including a few taking no therapy - we have shown no biochemical hormonal upset in untreated patients. The drugs, however, certainly decrease the effective circulating concentrations of many hormones, and it seems likely that the anecdotal report of the occasional patient in the clinic "having problems" does, in some

way, relate to AED therapy.

Our ongoing study of sexual function, mentioned in the conclusion to Chapter 3, does show that there are many complicating factors, including possible differences in the upbringing and education of people with epilepsy. This large study should pinpoint many of these factors, supplying new information per se, and perhaps facilitating a controlled study of the effects of AEDs on sexual function. Meantime, it is worth considering the substitution of VPA (or possibly oxcarbazepine) in patients complaining of sexual difficulties while taking one of the inducing drugs.

Calcium Antagonists

My conclusions concerning the calcium antagonists have been outlined in the previous section. It would be surprising if a group of drugs with such a good hypothetical basis, impressive record in animal seizures, and established clinical safety profile, did not produce one useful anticonvulsant. For various reasons, mainly pharmacokinetic, nifedipine and nimodipine may not be viable as AEDs, but the dihydropyridines remain a promising group, and amlodipine will be receiving close scrutiny in our unit in the near future.

REFERENCES

Aanderud S, Myking OL, Strandjord RE
The influence of carbamazepine on thyroid hormones and thyroxine binding globulin in hypothyroid patients substituted with thyroxine
Clinical Endocrinology 1981; 15: 247-252

Aanderud S, Strandjord RE
Hypothyroidism induced by anti-epileptic therapy
Acta Neurologica Scandinavica 1980; 61: 330-332

Aldenkamp AP, Alpherts WCJ, Moerland MC, Ottevanger N, Van Parys JAP
Controlled release carbamazepine: cognitive side-effects in patients with epilepsy
Epilepsia 1987; 28: 507-514

Allen GS, Ahn HS, Preziosi TJ et al
Cerebral arterial spasm - A controlled trial of nimodipine in patients with subarachnoid haemorrhage
New Eng J Med 1983; 308: 619-624

Allen JW, Oxley J, Robertson MM, Trimble MR, Richens A, Jawad SSM
Clobazam as adjunctive treatment in refractory epilepsy
Brit Med J 1983; 286: 1246-1247

Alving J, Kristensen O, Tsiropoulos I, Mondrup K
Double-blind placebo-controlled evaluation of flunarizine as adjunct therapy in epilepsy with complex partial seizures
Acta Neurol Scand 1989; 79: 128-132

Aman MG, Werry JS, Paxton JW, Turbott SH, Stewart AW
Effects of carbamazepine on psychomotor performance in children as a function of drug concentration, seizure type and time of medication
Epilepsia 1990; 31: 51-60

Anderson DC
Sex-hormone-binding globulin
Clin Endocrinol 1974; 3: 69-96

Andrewes DG, Bullen JG, Tomlinson L, Elwes RDC, Reynolds EH
A comparative study of the cognitive effects of phenytoin and carbamazepine in new referrals with epilepsy
Epilepsia 1986; 27: 128-134

Ashton D, Wauquier A
Behavioral analysis of the effects of 15 anticonvulsants in the amygdaloid kindled rat
Psychopharm (Berlin) 1979a; 65: 7-13

Ashton D, Wauquier A
Effects of some antiepileptic, neuroleptic and gabaminergic drugs on convulsions induced by D,L-allylglycine
Pharmacol Biochem Behav 1979b; 11: 221-226

Ashworth B, Horn DB
Evidence of osteomalacia in an outpatient group of adult epileptics
Epilepsia 1977; 18: 37-43

Bachur NR
Cytoplasmic aldo-keto reductases: A class of drug metabolising enzymes
Science 1976; 193: 595-597

Baltzer V, Schmutz M
Experimental anticonvulsive properties of GP47680 and of GP47779, its main human
metabolite; compounds related to carbamazepine
In: Advances in Epileptology 1977.
Meinardi H, Rowan AJ (Eds) Swets & Zeitlinger: Amsterdam 1978 pp 295-299

Barnes TC
Relationship of chemical structure to central nervous system effects of tranquilizing and
anticonvulsant drugs
J Am Pharm Assoc Sci Ed 1960; 49: 415-417

Barrett-Connor E, Khaw K, Yen SSC
A prospective study of dehydroepiandrosterone sulphate, mortality and cardiovascular
disease
New Eng J Med 1986; 315: 1519-1524

Bauer LA, Stenwall M, Horn JR, Davis R, Opheim K, Greene L
Changes in antipyrine and indocyanine green kinetics during nifedipine, verapamil and
diltiazem therapy
Clin Pharmacol Ther 1986; 40: 239-242

Bazemore RP, Zuckermann EC
On the problem of diphenylhydantoin-induced seizures
Arch Neurol 1974; 31: 243-249

Beardsley RS, Freeman JM, Appel FA
Anticonvulsant serum levels are useful only if the physician appropriately uses them:
An assessment of the impact of providing serum level data to physicians
Epilepsia 1983; 24: 330-335

Bennet JP jr, Yamamura HI
Neurotransmitter, hormone or drug receptor binding methods
In: Neurotransmitter Receptor Binding.
Yamamura HI, Enna SJ, Kuhar MJ (Eds) Raven Press: New York 1985 pp 61-89

Bentley PJ
Steroid hormones: Introduction
In: Endocrine pharmacology. Physiological basis and therapeutic applications
Cambridge University Press: Cambridge, London, NY, New Rochelle, Melbourne,
Sydney 1980a pp 139-147

Bentley PJ
The endocrinology and pharmacology of reproduction
In: Endocrine pharmacology. Physiological basis and therapeutic applications
Cambridge University Press: Cambridge, London, NY, New Rochelle, Melbourne,
Sydney 1980b pp 228-322

Bingmann D, Speckmann E-J
Actions of pentylenetetrazol on CA_3 neurons in hippocampal slices of guinea pig
Exp Brain Res 1986; 64: 94-104

Bingmann D, Speckmann E-J
Specific suppression of pentylenetetrazol-induced epileptiform discharges in CA_3
neurons (hippocampal slice, guinea pig) by the organic calcium antagonists flunarizine
and verapamil
Exp Brain Res 1989; 74: 239-248

Binnie CD
Potential antiepileptic drugs. Flunarizine and other calcium entry blockers
In: Antiepileptic Drugs (3rd Edition)
Levy R, Mattson R, Meldrum B, Penry JK, Dreifess FE (Eds) Raven Press: New York
1989 pp 971-982

Binnie CD, De Beukelaar F, Meijer JWA, Meinardi H, Overweg J, Wauquier A,
Van Wieringen A
Open dose-ranging trial of flunarizine - an add-on therapy in epilepsy
Epilepsia 1985; 26: 424-428

Birnie GG
Observations on drug metabolism and liver disease (MD thesis)
Appendix 4, pharmacokinetic programme 1
University of Glasgow 1986a

Birnie GG
Observations on drug metabolism and liver disease (MD thesis)
Appendix 4, pharmacokinetic programme 4
University of Glasgow 1986b

Blackshear JL, Schultz AL, Napier JS, Stuart DD
Thyroxine replacement requirements in hypothyroid patients receiving phenytoin
Ann Int Med 1983; 99: 341-2

Bogue JY, Carrington HC
The evaluation of mysoline - a new anticonvulsant drug
Brit J Pharmacol Chemother 1953; 8: 230-236

Bolger GT, Weissman BA, Bacher J, Isaac L
Calcium antagonist binding in cat brain tolerant to electro-convulsive shock
Pharmacol Bioch Behav 1987; 27: 217-221

Boobis AR, Brodie MJ, Kahn GC et al
Comparison of the in vivo and in vitro rates of formation of the three main oxidative
metabolites of antipyrine in man
Br J Clin Pharmac 1981; 12: 771-777

Bourgeois BFD
Anticonvulsant potency and neurotoxicity of valproate alone and in combination with
carbamazepine or phenobarbital
Clin Neuropharmacol 1988; 11: 348-359

Bourgeois BFD, Prensky AL, Palkes HS, Talent BK, Busch SG
Intelligence in epilepsy: A prospective study in children
Ann Neurol 1983; 14: 438-444

Brandt L, Saveland H, Ljunggren B, Andersson K-E
Control of epilepsy partialis continuans with intravenous nimodipine
J Neurosurg 1988; 69: 949-950

Breimer DD, Schellens JHM, Soons PA
Nifedipine: Variability in its kinetics and metabolism in man
Pharmac Ther 1989; 44: 445-454

Brodie MJ
Epilepsy octet: established anticonvulsants and treatment of refractory epilepsy
Lancet 1990; ii: 350-354

Brodie MJ, Feely J
Practical clinical pharmacology - therapeutic drug monitoring and clinical trials
Brit Med J 1988; 296: 1110-1114

Brodie MJ, McIntosh ME, Hallworth M
Therapeutic drug monitoring - the need for audit?
Scott Med J 1985; 30: 75-82

Brodie MJ, Macphee GJA
Carbamazepine toxicity precipitated by diltiazem
Brit Med J 1986; 292: 1170-1171

Brodie MJ, Porter RJ
New and potential anticonvulsants
Lancet 1990; 336: 425-426

Buchthal F, Svensmark O, Schiller PJ
Clinical and electroencephalographic correlations with serum levels of
diphenylhydantoin
Arch Neurol 1960; 2: 624-630

Buchthal F, Svensmark O, Simonsen H
Relation of EEG and seizures to phenobarbital in serum
Arch Neurol 1968; 19: 567-572

Burstein S, Klaiber EL
Phenobarbital-induced increase in 6-beta-hydroxycortisol excretion: Clue to its
significance in urine
J Clin Endocrinol Metab 1965; 25: 293-296

Bussey HI, Hoffman EW
A prospective evaluation of therapeutic drug monitoring
Ther Drug Monit 1983; 5: 245-248

Butler TC, Waddell WJ
Metabolic conversion of primidone (Mysoline) to phenobarbital
Proc Soc Exp Biol Med 1956; 93: 544-546

Capewell S, Freestone S, Critchley JAJH, Pottage A, Prescott LF
Reduced felodipine bioavailability in patients taking anticonvulsants
Lancet 1988; ii: 480-482

Carani C, Zini D, Baldini A, Della Casa L, Ghizzani A, Marrama P
Effects of androgen treatment in impotent men with normal and low levels of free
testosterone
Archiv Sexual Behaviour 1990; 19: 223-234

CCT ILAE (Commission on classification and terminology of the international league
against epilepsy)
Proposal for revised classification of epilepsies and epileptic syndromes
Epilepsia 1989; 30: 389-399

Cereghino JJ, Brock JT, Van Meter JC, Penry JK, Smith LD, White BG
Carbamazepine for epilepsy. A controlled prospective evaluation
Neurology 1974; 24: 401-410

Chadwick DW
Concentration-effect relationships of valproic acid
Clinical Pharmacokinetics 1985; 10: 155-163

Chadwick DW
Overuse of monitoring of blood concentrations of antiepileptic drugs
Brit Med J 1987; 294: 723-724

Challenor VF, Waller DG, Renwick AG, Gruchy BS, George CF
The trans-hepatic extraction of nifedipine
Br J Clin Pharmac 1987; 24: 473-477

Challenor VF, Waller DG, Renwick AG, George CF
Slow release nifedipine plus atenolol in chronic stable angina pectoris
Br J Clin Pharmac 1989; 28: 509-516

Charcot JM
In: Lectures on the diseases of the nervous system
Sigerson G (Trans/Ed), Adlard, London 1881 p290

Cheung W-T, Shi MM, Young JD, Lee C-M
Inhibition of radioligand binding to A_1 adenosine receptors by Bay K 8644 and nifedipine
Biochem Pharmacol 1987; 36: 2183-2186

Chin W, Schussler GC
Decreased serum free thyroxine concentration in patients treated with diphenylhydantoin
J Clin Endocrinol Metab 1968; 28: 181-186

Choi OH, Shamin MT, Padgett WL, Daly JW
Caffeine and theophylline analogues: Correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors
Life Sciences 1988; 43: 387-398

Chopra IJ, Solomon DH, Chopra U, Wu SY, Fisher DA, Nakamura Y
Pathways of metabolism of thyroid hormones.
Recent Progress in Hormone Research 1978; 34: 521-545

Chouza C, Caamano JL, Aljanati R, Scaramelli A, De Medina O, Romero S
Parkinsonism, tardive dyskinesia, akathisia and depression induced by flunarizine
Lancet 1986; i: 1303-1304

Clark M, Post RM
Carbamazepine, but not caffeine, is highly selective for adenosine A_1 binding sites
Eur J Pharmacol 1989; 164: 399-401

Connacher AA, Borsey DQ, Browning MCK, Davidson DLW, Jung RT
The effective evaluation of thyroid status in patients on phenytoin, carbamazepine or sodium valproate attending an epilepsy clinic
Postgrad Med J 1987; 63: 841-845

Connell JMC, Rapeport WG, Beastall GH, Brodie MJ
Changes in circulating androgens during short-term carbamazepine therapy
Brit J Clin Pharmacol 1984a; 17: 347-351

Connell JMC, Rapeport WG, Gordon S, Brodie MJ
Changes in circulating thyroid hormones during short-term hepatic enzyme induction with carbamazepine
Eur J Clin Pharmacol 1984b; 26: 453-456

Consensus statement
Febrile seizures: Long-term management of children with fever-associated seizures
Pediatrics 1980; 66: 1009-1012

Cooper AJ
Advances in the assessment of organic causes of impotence
Brit J Hosp Med 1986; 36: 186-192

Crowder JM, Bradford HF
Common anticonvulsants inhibit CA^{++} uptake and amino acid neurotransmitter release in vitro
Epilepsia 1987; 28: 378-382

Czuczwar SJ, Chodkowska A, Kleinrok Z, Malek U, Jagiello-Wojtowicz E
Effects of calcium channel inhibitors upon the efficacy of common antiepileptic drugs
Eur J Pharmacol 1990; 176: 75-83

Dalby MA
Behavioural effects of carbamazepine
In: Advances in Neurology Volume 11 (eleven)
Penry JK, Daly DD (Eds) Raven Press, New York 1975 p331-344

Dam M, Ekberg R, Loyning Y, Waltimo O, Jakobsen K
A double-blind study comparing oxcarbazepine and carbamazepine in patients with newly diagnosed, previously untreated epilepsy
Epilepsy Res 1989; 3: 70-76

Dana-Haeri J, Oxley J, Richens A
Reduction of free testosterone by anti-epileptic drugs
Brit Med J 1982; 284: 85-86

Dana-Haeri J, Oxley J, Richens A
Pituitary responsiveness to gonadotrophin-releasing and thyroid-releasing hormones in epileptic patients receiving carbamazepine or phenytoin
Clin Endocrinol 1984; 70: 163-168

Daval J-L, Deckert J, Weiss SRB, Post RM, Marangos PJ
Upregulation of adenosine A_1 receptors and forskolin binding sites following chronic treatment with caffeine or carbamazepine: A quantative autoradiographic study
Epilepsia 1989; 30: 26-33

Declerck AC, Wauquier A
Double blind study of the effectiveness of flunarizine in therapy-resistant epilepsy in mentally retarded children (abs)
In: Advances in Epileptology.
Wada JA, Penry JK et al (Eds) Raven Press: New York 1980 p356

Delaney RC, Rosen AJ, Mattson RH, Novelly RA
Memory function in focal epilepsy: A comparison of non-surgical, unilateral temporal lobe and frontal lobe samples
Cortex 1980; 16: 103-117

DeLorenzo RJ
Mechanisms of action of anticonvulsant drugs
Epilepsia 1988; 29 (suppl 2): S35-S47

DeLorenzo RJ, Burdette S, Holderness J
Benzodiazepine inhibition of the calcium calmodulin protein kinase system in brain membrane
Science 1981; 213: 546-548

DeLorenzo RJ, Freedman SD
Possible role of calcium-dependent protein phosphorylation in mediating
neurotransmitter release and anticonvulsant action
Epilepsia 1977; 18: 357-365

Delumeau JC, Bentue-Ferrer D, Saiag B, Allain H
Clinical neuropharmacology of calcium antagonists
Fundam Clin Pharmacol 1989; 3: 89S-102S

De Sarro G, De Sarro A, Federico F, Meldrum BS
Anticonvulsant properties of some calcium antagonists on sound-induced seizures in
genetically epilepsy prone rats
Gen Pharmac 1990; 21: 769-778

De Sarro GB, Meldrum BS, Nistico G
Anticonvulsant effect of some calcium entry blockers in DBA/2 mice
Br J Pharmacol 1988; 93: 247-256

De Sarro GB, Nistico G, Meldrum BS
Anticonvulsant properties of flunarizine on reflex and generalised models of epilepsy
Neuropharmacology 1986; 25: 695-701

Desmedt LKC, Niemergeers GJE, Janssen PAJ
Anticonvulsive properties of cinnarizine and flunarizine in rats and mice
Arzneimittel-Forsch 1975; 25: 1408-1413

Desmedt LKC, Niemergeers CJE, Lewi PJ, Janssen PAJ
Antagonism of maximal metrazol seizures in rats and its relevance to an experimental
classification of anti-epileptic drugs
Arzneimittel-Forsch 1976; 26: 1592-1603

Dodrill CB
Correlates of generalized tonic-clonic seizures with intellectual, neuropsychological,
emotional and social function in patients with epilepsy
Epilepsia 1986; 27: 399-411

Dodrill CB, Troupin AS
Psychotropic effects of carbamazepine in epilepsy: A double-blind comparison with
phenytoin
Neurology 1977; 27: 1023-1028

Dodson WE
Level off
Neurology 1989; 39: 1009-1010

Dolin SJ, Hunter AB, Halsey MJ, Little HJ
Anticonvulsant profile of the dihydropyridine calcium channel antagonists, nitrendipine
and nimodipine
Eur J Pharmacol 1988; 152: 19-27

Dolin SJ, Little HJ
Augmentation by calcium channel antagonists of general anaesthetic potency in mice
Brit J Pharmac 1986; 88: 909-914

Dragunow M, Faull RLM
Neuroprotective effects of adenosine
Trends in Pharmacological Sciences 1988; 9: 193-194

Dragunow M, Robertson HA
8-cyclopentyl 1,3-dimethylxanthine prolongs epileptic seizures in rats
Brain Red 1987; 417: 377-379

Dreifuss FE
Valproate toxicity
In: Antiepileptic Drugs (3rd Edn)
Levy R, Mattson R, Meldrum B, Penry JK, Dreifuss FE (Eds) Raven Press: New York
1989 pp 643-651

Ducharme JR, Collu R
Pubertal development: Normal, precocious and delayed
Clin Endocrinol Metab 1982; 11: 57-87

Duffy J
Masturbation and clitoroidectomy: A nineteenth century view
JAMA 1963; 186: 246-248

Editorial
Vigabatrin
Lancet 1989; i: 532-533

Eichelbaum M, Ekbom K, Bertilsson L, Ringberger VA, Rane A
Plasma kinetics of carbamazepine and its epoxide metabolite in man after single and
multiple doses
Eur J Clin Pharmacol 1975; 8: 337-341

Eichelbaum M, Tomson T, Tybring G, Bertilsson L
Carbamazepine metabolism in man. Induction and pharmacogenetic aspects
Clin Pharmacokinetics 1985; 10: 80-90

Esquirol E
"Epilepsy"
In: Mental Maladies. A Treatise on Insanity
Hunt EK (Editor/translator) Lea & Blanchard: Philadelphia 1845 pp 145-171

Evans RW, Gualtieri CT
Carbamazepine: A neuropsychological and psychiatric profile
Clin Pharmacol 1985; 8: 221-241

Faigle JW, Feldmann KF, Baltzer V
Anticonvulsant effect of carbamazepine. An attempt to distinguish between the potency
of the parent drug and its epoxide metabolite
In: Antiepileptic Drug Monitoring
Gardner-Thorpe C, Janz D, Meinardi H, Pippenger CE (Eds) Pitman Press: Avon 1977
pp 104-108

Fariello R, Smith MC
Valproate: Mechanisms of action
In: Antiepileptic Drugs (3rd Edition)
Levy RH, Mattson RH, Meldrum BS, Penry JK, Dreifuss FE (Eds) Raven Press: New
York 1989 pp 567-575

Fastbom J, Pazos A, Palacios JM
The distribution of adenosine A_1 receptors and 5' nucleotidase in the brain of some
commonly used experimental animals
Neuroscience 1987; 22: 813-826

Feely M, Calvert R, Gibson J
Clobazam in catamenial epilepsy: A model for evaluating anticonvulsants
Lancet 1982; ii: 71-73

Fenwick PBC, Mercer S, Grant R et al
Nocturnal penile tumescence and serum testosterone levels
Arch Sexual Behaviour 1986; 15: 13-21

Fenwick PBC, Toone BK, Wheeler MJ, Nanjee MN, Grant R, Brown D
Sexual behaviour in a centre for epilepsy
Acta Neurol Scand 1985; 71: 428-435

Ferrendelli JA, Daniels-McQueen S
Comparative actions of phenytoin and other anticonvulsants on potassium- and veratridine-stimulated calcium uptake in synaptosomes
J Pharmacol Exp Therap 1982; 220: 29-34

Fialip J, Aumaitre O, Eschalier A, Maradeix B, Dordain G, Lavarenne J
Benzodiazepine withdrawal seizures: Analysis of 48 case reports
Clinical Neuropharmacology 1987; 10: 538-544

Fleckenstein A
Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle
Ann Rev Pharmacol 1977; 17: 149-166

Franceschi M, Perego L, Cavagnini F et al
Effects of long-term antiepileptic therapy on the hypothalamic-pituitary axis in man
Epilepsia 1984; 25: 46-52

Fredholm BB, Hu PS, Lindgren E
The dihydropyridine calcium-channel agonist Bay K 8644 inhibits the presynaptic effects of R-phenylisopropyl adenosine in the rat hippocampus
Acta Physiolog Scand 1986; 128: 659-660

Frenken J, Vennix P
Sexuality experience scales manual
Swets & Zeitlinger Bv, 1981

Frey H-H
Primidone (Ch 15)
In: Antiepileptic Drugs
Frey H-H, Janz D (Eds) Springer Verlag: Berlin, Heidelberg, New York, Tokyo
1985 pp 449-477

Gallagher BB, Freer LS
Barbituric acid derivatives (ch 14)
In: Antiepileptic Drugs
Frey H-H, Janz D (Eds) Springer-Verlag: Berlin, Heidelberg, New York, Tokyo
1985 pp 421-447

Gallassi R, Morreale A, Lorusso S, Procaccianti G, Lugaresi E, Baruzzi A
Carbamazepine and phenytoin: Comparison of cognitive effects in epileptic patients during monotherapy and withdrawal
Arch Neurol 1988; 45: 892-894

Gallassi R, Morreale A, Lorusso S, Procaccianti G, Lugaresi E, Baruzzi A
Cognitive effects of valproate
Epilepsy Res 1990; 5: 160-164

Gannaway DJ, Mawer GE
Serum phenytoin concentration and clinical response in patients with epilepsy
Br J Clin Pharmac 1981; 12: 833-839

Gastaut H, Collomb H
Etude du comportement sexuel chez les epileptiques psychomoteurs
Annales Medico-Psychologiques 1954; 2: 657-696

Gibaldi M, Perrier D
In: Pharmacokinetics (2nd Ed)
Marcel Dekker Inc: New York & Basel 1982 pp 451-457

Gillham RA, Williams N, Weidmann K, Butler E, Larkin JG, Brodie MJ
Concentration-effect relationships with carbamazepine and its epoxide on psychomotor
and cognitive function in epileptic patients
J Neurol Neurosurg Psych 1988; 51: 929-933

Gillham RA, Williams N, Wiedmann KD, Butler E, Larkin JG, Brodie MJ
Cognitive function and anticonvulsant monotherapy
Epilepsy Res 1990; 7: 219-225

Gleiter CH, Cain CJ, Weiss S, Post RM, Marangos PJ
Differential effects of acute and repeated electrically and chemically induced seizures on
$[^3H]$nimodipine and $[^{125}I]$omega-conotoxin GVIA binding in rat brain
Epilepsia 1989; 30: 487-492

Glowinski J, Iversen LL
Regional studies of catecholamines in the rat brain - 1 The disposition of
$[^3H]$norepinephrine, $[^3H]$dopamine and $[^3H]$DOPA in various regions of the brain
J Neurochem 1966; 13: 655-669

Goldberg JB, Kurland AA
Dilantin treatment of hospitalised cultural-familial retardates
J Nerv Ment Dis 1970; 150: 133-137

Goldensohn ES, Purpura DP
Intracellular potentials of cortical neurons during focal epileptogenic discharges
Science 1963; 139: 840-842

Goode DJ, Penry JK, Dreifuss FE
Effects of paroxysmal spike-wave on continuous visual-motor performance
Epilepsia 1970; 11: 241-254

Gram L, Bentsen KD
Controlled and comparative trials of valproate performed in Europe and Asia
Epilepsia 1984; 25 suppl 1: 32-39

Gram L, Bentsen KD
Valproate: An updated review
Acta Neurol Scand 1985; 72: 129-139

Gram L, Jensen K
Carbamazepine: Toxicity
In: Antiepileptic Drugs (3rd Edition)
Levy RH, Mattson RH, Meldrum BS, Penry JK, Dreifuss FE (Eds) Raven Press: New
York 1989 pp 555-566

Grebb JA, Shelton RC, Taylor EH, Bigelow LB
A negative, double-blind, placebo-controlled clinical trial of verapamil in chronic schizophrenia
Biol Psychiatry 1986; 21: 691-694

Greenberg DA
Calcium channels and calcium channel antagonists
Ann Neurology 1987; 21: 317-330

Griffiths T, Evans MC, Meldrum BS
Intracellular calcium accumulation in rat hippocampus during seizures induced by bicuculline or L-allylglycine
Neuroscience 1983; 10: 385-395

Gruber CM, Harry VG, Drake ME
The toxic actions of sodium diphenylhydantoinate (Dilantin) when injected peritoneally and intravenously in experimental animals
J Pharmacol Exp Ther 1940; 68: 433-436

Gruber CM, Mosier JM, Grant P
Objective comparison of primidone and phenobarbital in epileptics
J Pharmacol (Exp Ther) 1957; 120: 184-187

Guest ADL, Duncan C, Lawther PJ
Carbon monoxide and phenobarbitone: A comparison of effects on auditory flutter fusion threshold and critical flicker fusion threshold
Ergonomics 1970; 13: 587-594

Hagiwara S, Byerly L
Calcium channel
Ann Rev Neurosci 1981; 4: 69-125

Haidukewych D, Rodin EA
Chronic anti-epileptic drug therapy: Classification by medication regimen and incidence of decreases in serum thyroxine and free thyroxine index
Ther Drug Monit 1987; 9: 392-398

Halstead H
Abilities and behaviour of epileptic children
J Ment Sci 1957; 103: 28-47

Hammond WA
"Epilepsy"
In: A treatise on diseases of the nervous system
Appleton & Trubner: New York & London 1871 pp 560-589

Hanson JW, Myrainthopoulos NC, Harvey MAS, Smith DW
Risks to the offspring of women treated with hydantoin anticonvulsants, with emphasis on the fetal hydantoin syndrome
J Pediatr 1976; 89: 662-668

Harder DR, Belardinole L, Speralakis N, Rubio R, Berue RM
Differential effects of adenosine and nitroglycerin on the action potentials of large and small coronary arteries
Circ Res 1979; 44: 177-182

Harding GFA, Alford CA, Powell TE
The effect of sodium valproate on sleep, reaction times and visual evoked potential in normal subjects
Epilepsia 1985; 26: 597-601

Hart RG, Easton JD
Carbamazepine and haematological monitoring
Ann Neurol 1982; 11: 309-312

Haws CW, Gourley JK, Heistad DD
Effects of nimodipine on cerebral blood flow
J Pharm Exp Therapeutics 1983; 225: 24-28

Heffez DS, Nowak TS, Passoneau JV
Nimodipine levels in gerbil brain following parenteral drug administration
J Neurosurg 1985; 63: 589-592

Heinemann U, Louvel J
Changes in (Ca^{2+}) and (K^+) during repetitive electrical stimulation and during
pentylenetetrazol induced seizures activity in the sensorimotor cortex of cats
Pflugers Arch 1983; 398: 310-317

Heinemann U, Lux HD, Gutnick MJ
Extracellular free calcium and potassium during paroxysmal activity in cerebral cortex of
the cat
Exp Brain Res 1977; 27: 237-243

Herzog AG
A hypothesis to integrate partial seizures of temporal lobe origin and reproductive
endocrine disorders
Epilepsy Res 1989; 3: 151-159

Herzog AG, Seibel MM, Schomer DL, Vaitukaitis JL, Geschwind N
Reproductive endocrine disorders in men with partial seizures of temporal lobe origin
Arch Neurol 1986; 43: 347-350

Heyma P, Larkins RG, Perry-Keene D, Peter CT, Ross D, Sloman JG
Thyroid hormone levels and protein binding in patients on long-term diphenylhydantoin
treatment
Clin Endocrinol 1977; 6: 369-376

Hierons R, Saunders M
Impotence in patients with temporal lobe lesions
Lancet 1966; ii: 761-764

Hindmarch I
Psychomotor function and psychoactive drugs
Brit J Clin Pharmacol 1980; 10: 189-209

Hindmarch I, Gudgeon AC
The effects of clobazam and lorazepam on aspects of psychomotor performance and car
handling ability
Br J Clin Pharmac 1980; 10: 145-150

Hirning LD, Fox AP, McClesky EW, Oliver BM, Thayer SA, Miller RJ, Tsien RW
Dominant role of N-type Ca^{2+} channels in evoked release of norepinephrine from
sympathetic neurons
Science 1988; 239: 57-61

Hirtz DG, Nelson KB
Cognitive effects of antiepileptic drugs
In: Recent Advances in Epilepsy 2.
Pedley TA, Meldrum BS (Eds) Churchill Livingstone: Edinburgh 1985 pp 161-181

Hodgkin AL, Keynes RD
Movements of labelled calcium in squid giant axons
J Physiol (London) 1957; 138: 253-281

Hoppener RJ, Kuyer A, Meijer JWA, Hulsman J
Correlation between daily fluctuation of carbamazepine serum levels and intermittent side-effects
Epilepsia 1980; 21: 341-350

Hotson JR, Prince DA
A calcium-activated hyperpolarisation follows repetitive firing in hippocampal neurons
J Neurophysiol 1980; 43: 409-419

Houtkooper MA, Lammertsma A, Meyer JWA et al
Oxcarbazepine (GP47680): A possible alternative to carbamazepine
Epilepsia 1987; 25: 693-698

Howell DC
Fundamental statistics for the behavioral sciences
Duxbury Press: Boston 1985

Hu PS, Lindgren E, Jacobson KA, Fredholm BB
Interaction of dihydropyridine calcium channel agonists and antagonists with adenosine receptors
Pharmacol and Toxicol 1987; 61: 121-125

Isaacson RL, Molina JC, Draski LJ, Johnston JE
Nimodipine's interaction with other drugs: 1. ethanol
Life Sci 1985; 36: 2195-2199

Isojarvi JIT, Pakarinen AJ, Myllyla VV
Effects of carbamazepine therapy on serum sex hormone levels in male patients with epilepsy
Epilepsia 1988; 29: 781-786

Isojarvi JIT, Myllyla VV, Pakarinen AJ
Effects of carbamazepine on pituitary responsiveness to Luteinising Hormone-Releasing Hormone, Thyrotropin-Releasing Hormone and metoclopramide in epileptic patients
Epilepsia 1989a; 30: 50-56

Isojarvi JIT, Pakarinen AJ, Myllyla VV
Effects of carbamazepine on the hypothalamic pituitary-gonadal axis in male patients with epilepsy: A prospective study
Epilepsia 1989b; 30: 446-452

Isojarvi JIT, Pakarinen AJ, Ylipalosaari PJ, Myllyla VV
Serum hormones in male epileptic patients receiving anticonvulsant medication
Arch Neurol 1990; 47: 670-676

Itil TM, Michael ST, Hoffmeister F, Kunitz A, Eralp E
Nimodipine, a calcium antagonist vasodilator with psychotropic properties (a controlled quantitative pharmaco-EEG study)
Curr Ther Res 1984; 35: 405-422

Jackson JH
"Investigation of Epilepsies" (1873)
In: Selected Writings of John Hughlings Jackson Vol 1. On Epilepsy & Epileptiform Convulsions.
Taylor J (Ed) Hodder & Stoughton: London 1931 pp 96,100,112

Jackson JH
"A Study of Convulsions" (1870)
In: Neurological Classics Wilkins RH, Brody IA (Eds)
Johnson Reprint Corp: New York and London 1973 p 136

Janicki PK, Siembab D, Paulo EA, Krzascik P
Single-dose kinetics of nifedipine in rat plasma and brain
Pharmacology 1988; 36: 183-187

Jones GL, Wimbish GH
Hydantoins (Ch 13)
In: Antiepileptic Drugs Frey H-H, Janz D (Eds) Springer-Verlag: Berlin, Heidelberg,
New York, Tokyo 1985 pp 351-419

Jung MJ, Lippert B, Metcalf BW, Bohlen P, Schechter PJ
Gamma-vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor
of gaba-T: effects on brain GABA metabolism in mice
J Neurochem 1977; 29: 797

Kapetanovic IM, Kupferberg HJ
Inhibition of microsomal phenobarbital metabolism by valproic acid
Fed Proc 1980; 39: 1099 (Abs)

Kasteleijn-Nolst Trenite DGA, Bakker DJ, Binnie CD, Buerman A, Van Raaij M
Psychological effects of subclinical epileptiform EEG discharges. I scholastic skills
Epilepsy Res 1988; 2: 111-116

Katz B, Miledi R
Spontaneous and evoked activity of motor nerve endings in calcium ringer
J Physiol (Lond) 1969; 203: 689-706

Keene D, Whiting S, Humphreys P, Jacob P
Flunarizine as a supplementary medication in refractory childhood epilepsy: A
double-blind crossover study
Can J Neurol Sci 1989; 16: 191-193

Kent AP, Webster RA
The evaluation of different types of anticonvulsant drug activity against leptazol-induced
epileptogenic activity in the anaesthetized rat
Brit J Pharmacol 1983; 78: 553-559

Kinnier Wilson JV, Reynolds EH
Translation and analysis of a cuneiform text forming part of a Babylonian treatise on
epilepsy
Medical History 1990; 34: 185-198

Koch KM, Ludwick BT, Levy RH
Phenytoin-valproic acid interaction in rhesus monkey
Epilepsia 1981; 22: 19-25

Koketsu K, Cerf JA, Nishi S
Effect of quaternary ammonium ions on electrical activity of spinal ganglion cells in
frogs
J Neurophysiol 1959a; 22: 177-194

Koketsu K, Cerf JA, Nishi S
Further observations on electrical activity of frog spinal ganglion cells in sodium-free
solutions
J Neurophysiol 1959b; 22: 693-703

Kolarsky A, Freund K, Machek J, Polak O
Male sexual deviation
Archives Gen Psychiatry 1967; 17: 735-743

Koppi S, Eberhardt G, Haller R, Konig P
Calcium-channel-blocking agent in the treatment of acute alcohol withdrawal -
caroverine versus meprobamate in a randomised double-blind study
Neuropsychobiology 1987; 17: 49-52

Krall RL, Penry JK, White BG, Kufferberg WJ, Swinyard EA
Antiepileptic drug development: II Anticonvulsant drug screening
Epilepsia 1978; 19: 404-428

Kramer G, Theisohn M, Stoll KD, Wendt G
Oxcarbazepin versus carbamazepin bei gesunden probanden, studien zur kinetik zu
metabolismus und vertraguchkeit
In: Kruse R (Ed) Epilepsie 84. Einhorn: Berlin 1985 pp 379-387

Kumps A
Simultaneous HPLC determination of oxcarbazepine, carbamazepine and their
metabolites in serum
J Liquid Curomatography 1984; 7: 1235-1241

Kunada JY, Wine JJ
Transient, axotomy-induced changes in the membrane properties of crayfish central
neurones
J Physiol 1981; 317: 435-461

Kuno M, Llinas R
Enhancement of synaptic transmission by dendritic potentials in chromatolysed
motoneurones of the cat
J Physiol 1970; 210: 807-821

Larsen PR
Thyroid-pituitary interaction, feedback regulation of thyrotropin secretion by thyroid
hormones
New Eng J Med 1982; 306: 23-32

Larsen PR, Atkinson AJ, Wellman HN, Goldsmith RE
The effect of diphenylhydantoin on thyroxine metabolism in man
J Clin Invest 1970; 49: 1266-1279

Leslie SW, Friedman MB, Coleman RR
Effects of chlordiazepoxide on depolarization-induced calcium influx into synaptosomes
Biochem Pharmacol 1980; 29: 2439-2443

Levesque LA, Herzog AG, Siebel MM
The effect of phenytoin and carbamazepine on serum dehydro-
epiandrosterone sulphate in men and women who have partial seizures with temporal
lobe involvement
J Clin Endocrinol Metab 1986; 63: 243-245

Levine M, McCollom R, Chang T, Orr J
Evaluation of serum phenytoin monitoring in an acute care setting
Ther Drug Monit 1988; 10: 50-57

Levy RH, Koch KM
Drug interactions with valproic acid
Drugs 1982; 24: 543-556

Lewin E, Bleck V
Cyclic AMP accumulation in cerebral cortical slices, effect of carbamazepine, phenobarbital and phenytoin
Epilepsia 1977; 18: 237-242

Liewendahl K, Majuri H, Helenius T
Thyroid function tests in patients on long-term treatment with various anticonvulsant drugs
Clin Endocrinol 1978; 8: 185-191

Little HJ, Dolin SJ, Halsey MJ
Calcium channel antagonists decrease the ethanol withdrawal syndrome
Life Sci 1986; 39: 2059-2065

Llinas R, McGuinness TL, Leonard CS, Sugimori M, Greengard P
Intraterminal injection of synapsin I or calcium/calmodulin dependent protein kinase II alters neurotransmitter release at the squid giant synapse
Proc Natl Acad Sci USA 1985; 82: 3035-3039

Lohse MJ, Lenschow V, Schwabe U
Two affinity states of Ri adenosine receptors in brain membranes. Analysis of nucleotide and temperature effects on radioligand binding
Molec Pharmacol 1984; 26: 1-9

Loiseau P, Duche B
Carbamazepine. Clinical use
In: Antiepileptic Drugs (3rd Edition)
Levy R, Mattson R, Meldrum B, Penry JK, Dreifuss FE (Eds) Raven Press: New York 1989 pp 533-554

Loscher W
Valproic acid
In: Antiepileptic Drugs. Frey H-H, Janz D (Eds)
Springer-Verlag: Berlin, Heidelberg, New York, Tokyo 1985 pp 507-536

Ludgate J, Keating J, O'Dwyer R, Callaghan N
An improvement in cognitive function following polyharmacy reduction in a group of epileptic patients
Acta Neurol Scand 1985; 71: 448-452

Luke 9: 37
In: The Jerusalem Bible, popular edition
Jones A (ed) Darton, Longman & Todd: London 1974

Lund L
Anticonvulsant effects of diphenylhydantoin relative to plasma levels: A prospective 3-year study in ambulant patients with generalised epileptic seizures
Arch Neurol Psych 1974; 31: 289-294

MacDonald RL, McLean MJ
Anticonvulsant drugs: Mechanisms of action
Advances in Neurology 1986; 44: 713-736

Macphee GJA, Brodie MJ
Carbamazepine substitution in severe partial epilepsy: implication of autoinduction of metabolism
Postgrad Med J 1985; 61: 779-783

Macphee GJA, Butler E, Brodie MJ
Intradose and circadian variation in circulating carbamazepine and its epoxide in epileptic patients: A consequence of auto-induction of metabolism
Epilepsia 1987; 28: 286-294

Macphee GJA, Goldie C, Roulston D et al
Effect of carbamazepine on psychomotor performance in naive subjects
Eur J Clin Pharmacol 1986a; 30: 37-42

Macphee GJA, McPhail EM, Butler E, Brodie MJ
Controlled evaluation of a supplementary dose of carbamazepine on psychomotor function in epileptic patients
Eur J Clin Pharmacol 1986b; 31: 195-199

Macphee GJA, Thompson GG, Scobie G et al
Effects of cimetidine on carbamazepine auto- and hetero-induction in man
Br J Clin Pharmacol 1984; 18: 411-419

Macphee GJA, Thompson GG, McInnes GT, Brodie MJ
Verapamil potentiates carbamazepine neurotoxicity: A clinically important inhibitory interaction
Lancet 1986c; i: 700-703

McKee PJW, Blacklaw J, Butler E, Gillham RA, Brodie MJ
Monotherapy with conventional and controlled-release carbamazepine: A double-blind, double-dummy comparison in epileptic patients
Brit J Clin Pharmacol 1991 (in press)

Marangos PJ, Montgomery P, Weiss SRB, Patel J, Post RM
Persistent upregulation of brain adenosine receptors in response to chronic carbamazepine treatment
Clinical Neuropharmacology 1987; 10: 443-448

Marangos PJ, Post RM, Patel J, Zander K, Parma A, Weiss S
Specific and potent interactions of carbamazepine with brain adenosine receptors
Eur J Pharmacol 1983; 93: 175-182

Marangos PJ, Weiss SRB, Montgomery P, Patel J, Narang PK, Cappabianca AM, Post RM
Chronic carbamazepine treatment increases brain adenosine receptors
Epilepsia 1985; 26: 493-498

Mark 9: 18
In: The Jerusalem Bible, popular edition
Jones A (ed) Darton, Longman & Todd: London 1974

Marshall EK jr
Experimental basis of chemotherapy in the treatment of bacterial infections.
The Ludwig Kast Lecture
Bull NY Acad Med 1940; 16: 722-731

Marty J, Fullinfaw R, Tuckett R, Trembath P, King J
Evaluation of the provision of rapid drug plasma assays in an outpatient anticonvulsant clinic
Ther Drug Monit 1981; 3: 253-258

Masala A, Meloni T, Alagna S, Rovasio PP, Mele G, Franca V
Pituitary responsiveness to gonadotrophin-releasing and thyrotrophin-releasing hormones in children receiving phenobarbitone
Brit Med J 1980; 281: 1175-1177

186

Masala A, Meloni T, Allagna S et al
Effect of long-term treatment with sodium valproate on gonadotrophin and prolactin secretion in paediatric patients
Br J Clin Pharmacol 1981; 12: 845-848

Matsumoto H, Marsan CA
Cortical cellular phenomena in experimental epilepsy: Interictal manifestations
Exp Neurol 1964; 9: 286-304

Mattson RH, Cramer JA
Epilepsy, sex hormones and antiepileptic drugs
Epilepsia 1985; 26 (suppl 1): S40-S51

Mattson RH, Cramer JA, Collins JF et al
Comparison of carbamazepine, phenobarbital, phenytoin and primidone in partial and secondary generalised tonic-clonic seizures
New Eng J Med 1985; 313: 145-151

Meador KJ, Loring DW, Huh K, Gallagher BB, King DW
Comparative cognitive effects of anticonvulsants
Neurology 1990; 40: 391-394

Meijer JWA
Antiepileptic drugs: Analytical techniques
In: Therapeutic Drug Monitoring. Richens A, Marks V (Eds)
Churchill-Livingstone: Edinburgh 1981 pp 349-369

Meldrum BS
Preclinical test systems for evaluation of novel compounds
In: Current Problems in Epilepsy 4: New anticonvulsant drugs
Meldrum BS, Porter RJ (Eds) John Libbey: London, Paris 1986 pp 31-48

Mello LEAM, Masur J, Formigoni MLOS, Kireeff W, Cavalheiro EA
Amygdaloid kindling and kindled seizures in rats receiving chronic ethanol administration
Epilepsy Res 1990; 7: 95-104

Mendoza DM, Flock EV, Owen CA jr, Paris J
Effect of 5,5'-diphenylhydantoin on the metabolism of L-Thyroxine -[131]I in the rat
Endocrinology 1966; 79: 106-118

Merritt HH, Putnam TJ
A new series of anticonvulsant drugs tested by experiments on animals
Arch Neurol Psychiatry 1938; 39: 1003-1015

Meunier H, Carraz G, Meunier Y, Eymard P, Aimard M
Proprietes pharmacodynamiques de l'acide n-dipropylacetique. 1er memoire: proprietes antiepileptiques
Therapie 1963; 18: 435-438

Meyer FB
Calcium, neuronal hyperexcitability and ischemic injury
Brain Research Reviews 1989; 14: 227-243

Meyer FB, Anderson RE, Sundt TM jr
Anticonvulsant effects of dihydropyridine Ca^{2+} antagonists in electrocortical shock seizures
Epilepsia 1990; 31: 68-74

Meyer FB, Anderson RE, Sundt TM jr, Sharbrough FW
Selective central nervous system calcium channel blockers. A new class of anticonvulsant agents
Mayo Clin Proc 1986a; 61: 239-247

Meyer FB, Anderson RE, Sundt TM jr, Yaksh TL, Sharbrough FW
Suppression of pentylenetetrazol seizures by oral administration of a dihydropyridine Ca^{2+} antagonist
Epilepsia 1987; 28: 409-414

Meyer FB, Tally PW, Anderson RE, Sundt TM jr, Yaksh TL, Sharbrough FW
Inhibition of electrically induced seizures by a dihydropyridine calcium channel blocker
Brain Res 1986b; 384: 180-183

Micheli F, Pardal MF, Gatto M, Torres M, Paradiso G, Parera IC, Giannaula R
Flunarizine- and cinnarizine-induced extrapyramidal reactions
Neurology 1987; 37: 881-884

Mikati MA, Browne TR
Comparative efficacy of antiepileptic drugs
Clinical Neuropharmacology 1988; 11: 130-140

Mikati MA, Browne TR, Collins JF
Time course of carbamazepine autoinduction
Neurology 1989; 39: 592-594

Mikkelsen B, Bergreen P, Joensen P, Kristensen O, Kohler O, Mikkelsen BO
Clonazepam and carbamazepine in psychomotor epilepsy: A randomised multicentre trial
Epilepsia 1981; 22: 415-420

Miller R, Nowosaid D, Warnes DM, De Wet JM
The role of therapeutic drug monitoring in the care of epileptic patients
SA Medical Journal 1982; 62: 512-515

Miller RG
The jackknife - a review
Biometrika 1974; 61: 1-15

Moreland TA, Park BK, Rylance GW
Microsomal enzyme induction in children: the influence of carbamazepine treatment on antipyrine kinetics, 6 beta-hydroxy-cortisol excretion and plasma gamma-glutamyltranspeptidase activity
Br J Clin Pharmac 1982; 14: 861-865

Morgan PF, Tamborska E, Patel J, Marangos PJ
Interactions between calcium channel compounds and adenosine systems in brain of rat
Neuropharmacology 1987; 26: 1693-1699

Morocutti C, Pierelli F, Sanarelli E, Stefano E, Peppe A, Mattioli GL
Antiepileptic effects of a calcium antagonist (nimodipine) on cefazolin-induced epileptogenic foci in rabbits
Epilepsia 1986; 27: 498-503

Moron MA, Stevens CW, Yaksh TL
Diltiazem enhances and flunarizine inhibits nimodipine's anti-seizure effects
Eur J Pharmacol 1989; 163: 299-307

Morris JC, Dodson WE, Hatelid JM, Ferrendelli JA
Phenytoin and carbamazepine, alone and in combination
Neurology 1987; 37: 1111-1118

Mucklow JC
Therapeutic progress: Review. IV. Therapeutic Drug Monitoring: can it improve the
treatment of epilepsy?
J Clin Hosp Pharmacy 1982; 7: 79-85

Murphy KMM, Snyder SH
Adenosine receptor binding and specific receptors for calcium channel drugs
In: Ca^{2+} entry blockers, Adenosine and Neurohumors
Merrell GF and Weiss HR (Eds) Urban and Schwarzenberg: Baltimore 1982 pp
295-306

Nakane Y, Seino M, Yagi K, Yamauchi T
Effects of flunarizine therapy on intractable epilepsy
Arzneimittel-Forschung/Drug Research 1989; 39: 793-798

Nanjee MN, Wheeler MJ
Plasma free testosterone - is an index sufficient?
Ann Clin Biochem 1985; 22: 387-390

Nielson OA, Johannessen AC, Bardrum B
Oxcarbazepine-induced hyponatremia, a cross-sectional study
Epilepsy Res 1988; 2: 269-271

Norton PRE
The effect of drugs on barbiturate withdrawal convulsions in the rat
J Pharm Pharmacol 1970; 22: 763-766

Ohnhaus EE, Park BK
Measurement of urinary 6 beta-hydroxycortisol excretion as an in vivo parameter in the
clinical assessment of the microsomal enzyme-inducing capacity of antipyrine,
phenobarbitone and rifampicin
Eur J Clin Pharmac 1979; 15: 139-145

Ohnhaus EE, Studer HA
Link between liver microsomal enzyme activity and thyroid hormone metabolism in man
Br J Clin Pharmacol 1983; 15: 71-76

O'Neill SK, Bolger GT
Anticonvulsant activity of MK-801 and nimodipine alone and in combination against
pentylenetetrazole and strychnine
Pharmacol Biochem & Behaviour 1989; 32: 595-600

O'Neill SK, Bolger GT
The effects of dihydropyridine calcium channel modulators on pentylenetetrazole
convulsions
Brain Res Bull 1990; 25: 211-214

Oppenheimer JH, Fisher LV, Nelson KM, Jailer JW
Depression of the serum protein-bound iodine level by diphenyl-hydantoin
J Clin Endocrinol 1961; 21: 252-262

Oppenheimer JH, Tavernetti RR
Displacement of thyroxine from human thyroxine-binding globulin by analogues of
hydantoin. Steric aspects of the thyroxine-binding site
J Clin Invest 1962; 41: 2213-2220

Orme ML'E
The clinical pharmacology of oral contraceptive steroids
Br J Clin Pharmac 1982; 14: 31-42

Osorio I, Burnstine TH, Remier B et al
Phenytoin-induced seizures: A paradoxical effect at toxic concentrations in epileptic patients
Epilepsia 1989; 30: 230-234

Overweg J, Ashton D, De Beukelaar F, Binnie CD, Wauquier A
Add-on therapy in epilepsy with calcium entry blockers
Eur Neurol 1986; 25 (suppl 1): 93-101

Overweg J, Binnie CD, Meijer JWA et al
Double-blind placebo-controlled trial of flunarizine as add-on therapy in epilepsy
Epilepsia 1984; 25: 217-222

Paci A, Ottaviano P, Trenta A et al
Nimodipine in acute ischaemic stroke: A double-blind controlled study
Acta Neurol Scand 1989; 80: 282-286

Paczynski RP, Meyer FB, Anderson RE
Effects of the dihydropyridine Ca^{2+} channel antagonist nimodipine on kainic acid-induced limbic seizures
Epilepsy Res 1990; 6: 33-38

Panza G, Grebb JA, Sanna E, Wright AG jr, Wright AG, Hanbauer I
Evidence for down-regulation of ^{3}H-nitrendipine recognition sites in mouse brain after long-term treatment with nifedipine or verapamil
Neuropharmacol 1985; 11: 1113-1117

Park BK
A direct radioimmunoassay for 6 beta-hydroxycortisol in human urine
J Steroid Biochem 1978; 9: 963-966

Park BK
Assessment of urinary 6 beta-hydroxycortisol as an in vivo index of mixed-function oxygenase activity
Br J Clin Pharmac 1981; 12: 97-102

Park BK
Assessment of the drug metabolism capacity of the liver
Br J Clin Pharmac 1982; 14: 631-651

Patsalos PN, Stephenson TJ, Korshna S, Elyas AA, Lascelles PT, Wiles CM
Side-effects induced by carbamazepine-10,11-epoxide
Lancet 1985; 8: 496

Patsalos PN, Josemire WASS, Oxley J, Lascelles PT
Immediate anticonvulsive drug monitoring in management of epilepsy
Lancet 1987; ii: 39 (Letter)

Patsalos PN, Zakrzewska JM, Elyas AA
Dose dependent enzyme induction by oxcarbazepine?
Eur J Clin Pharmacol 1990; 39: 187-188

Pellock JM, Carzo CG, Garnett WR
Carbamazepine in children: clinical and laboratory adverse effects
Ann Neurol 1984; 16: 392 (abs)

Pereira J
The elements of materia medica
Longman, Orme, Brown, Green & Longmans: London 1839 p2

Perucca E, Hedges A, Makki KA, Ruprah M, Wilson JF, Richens A
A comparative study of the relative enzyme inducing properties of anticonvulsant drugs in epileptic patients
Br J Clin Pharmacol 1984; 18: 401-410

Peterson GL
A simplification of the protein assay method of Lowry et al which is more generally applicable
Analytical Biochem 1977; 83: 346-356

Petruch F, Schuppel RVA, Steinhilber G
Effect of diphenylhydantoin on hepatic drug hydroxylation
Eur J Clin Pharmac 1974; 7: 281-285

Pickard JD, Murray GD, Illingworth R et al
Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial
Brit Med J 1989; 298: 636-642

Pitterle ME, Sorkness CA, Wiederholt JB
Use of serum drug concentrations in outpatient clinics
Amer J Hosp Pharmacy 1985; 42: 1547-1552

Pohl M, Mares P
Effects of flunarizine on metrazol-induced seizures in developing rats
Epilepsy Res 1987; 1: 302-305

Prince DA
Physiological mechanisms of focal epileptogenesis
Epilepsia 1985; 26: S3-S14

Pritchard PB III
Hypersexuality: A complication of complex partial epilepsy
Transactions of the American Neurological Association 1980; 105: 193-195

Pritchard PB III, Wannamaker BB, Sagel J, Nair R, Devillier C
Endocrine function following complex partial seizures
Ann Neurol 1983; 14: 27-32

Pumain R, Kurcewicz I, Louvel J
Fast extracellular calcium transients: Involvement in epileptic processes
Science 1983; 222: 177-179

Raeburn D, Gonzales RA
CNS disorders and calcium antagonists
Trends in Pharmac Sci 1988; 9: 117-119

Ramsch K-D, Ahr G, Tettenborn D, Auer LM
Overview on pharmacokinetics of nimodipine in healthy volunteers and in patients with subarachnoid haemorrhage
Neurochirurgia 1985; 28: 74-78

Ramsch K-D, Graefe KH, Scherling D, Sommer J, Ziegler R
Pharmacokinetics and metabolism of calcium-blocking agents nifedipine, nitrendipine and nimodipine
Am J Neurol 1986; 6 (Suppl 1): 73-80

Randall LO, Shallek W, Heise GA, Keit EF, Bagdon RE
The psychosedative properties of methaminodiazepoxide
J Pharmacol Exp Ther 1960; 129: 163-171

Rao ML, Froscher W, Bulau P
Influence of anticonvulsants and flunarizine on the metabolism of thyroid hormones:
speculation about the possible role of calmodulin
Clin Neuropharmacol 1987; 10: 356-364

Rao ML, Stefan H, Bauer J
Epileptic but not psychogenic seizures are accompanied by simultaneous elevation of
serum pituitary hormones and cortisol levels
Neuroendocrinology 1989; 49: 33-39

Rapeport WG, McInnes GT, Thompson GG, Forrest G, Park BK, Brodie MJ
Hepatic enzyme induction and leucocyte delta-aminolaevulinic acid synthase activity:
studies with carbamazepine
Br J Clin Pharmac 1983; 16: 133-137

Reddington M, Lee KS, Schubert P
An A_1 adenosine receptor, characterised by [^{3}H]cyclo-hexyladenosine binding, mediates
the depression of evoked potentials in rat hippocampal slice preparation
Neurosci Lett 1982; 28: 275-279

Reid JL, Meredith PA, Donnelly R, Elliott HL
Pharmacokinetics of calcium antagonists
J Cardiovasc Pharmacology 1988; 12 (suppl 7): S22-S26

Reinikainen KJ, Keranen T, Halonen T, Komulainen H, Riekkinen PJ
Comparison of oxcarbazepine and carbamazepine: a double-blind study
Epilepsy Res 1987; 1: 284-289

Renwick AG, Robertson DRC, Macklin B, Challenor V, Waller DG, George CF
The pharmacokinetics of oral nifedipine - a population study
Br J Clin Pharmac 1988; 25: 701-708

Reuter H
Uber die wirkung von adrenalin auf den cellularen CA-umsatz des
meerschweinchenvorhofs
Naunyn-Schmiedebergs Arch Exp Path Pharmacol 1965; 251: 401-412

Reynolds EH
Mental effects of antiepileptic medication: a review
Epilepsia 1983; 24(suppl. 2): S85-S95

Reynolds EH
Phenytoin: toxicity
In: Antiepileptic Drugs (3rd Edition)
Levy RH, Mattson RH, Meldrum BS, Penry JK, Dreifuss FE (Eds)
Raven Press: New York 1989 pp 241-255

Ribeiro JA, Sa-Almeida AM, Namorado JM
Adenosine and adenosine triphosphate decrease ^{45}Ca by synaptosomes stimulated by
potassium
Biochem Pharmac 1979; 28: 1297-1300

Richens A, Warrington S
When should plasma drug levels be monitored?
Drugs 1979; 17: 488-500

Ried LD, McKenna DA, Horn JR
Effect of therapeutic drug monitoring services on the number of serum drug assays ordered for patients: a meta-analysis
Ther Drug Monit 1989; 11: 253-263

Rimmer EM, Routledge PA, Tsanaclis LM, Richens A
Pharmacokinetics of antipyrine in epileptic patients
Br J Clin Pharmac 1986; 21: 511-514

Riva R, Albani F, Ambrosetto G et al
Diurnal fluctuations in free and total steady-state plasma levels of carbamazepine and correlation with intermittent side-effects
Epilepsia 1984; 25: 476-481

Rodin EA, Rim CS, Kitano H, Lewis R, Rennick PM
A comparison of the effectiveness of primidone versus carbamazepine in epileptic out-patients
J Nerv Ment Dis 1976; 163: 41-46

Rodin E, Subramanian MG, Gilroy J
Investigation of sex hormones in male epileptic patients
Epilepsia 1984; 25: 690-694

Roots I, Holbe R, Hovermann W, Nigam S, Heinemeyer G, Hildebrant AG
Quantitative determination by HPLC of urinary 6 beta-hydroxycortisol, an indicator of enzyme induction by rifampicin and antiepileptic drugs
Eur J Clin Pharmac 1979; 16: 63-71

Rosen JB, Berman RF
Differential effects of adenosine analogs on amygdala, hippocampus and caudate nucleus kindled seizures
Epilepsia 1987; 28: 658-666

Rosner W
A simplified method for the quantitative determination of testosterone estradiol binding globulin activity
J Clin Endocrinol Metab 1972; 34: 983-988

Sander JWAS, Trevisol-Bittencourt PC
Nifedipine as an add-on drug in the management of refractory epilepsy
Epilepsy Res 1990; 6: 82-84

Sanders RC, Murray TF
Chronic theophylline exposure increases agonist and antagonist binding to A_1 adenosine receptors in rat brain
Neuropharmac 1988; 27: 757-760

Sanders RC, Murray TF
Temporal relationship between A_1 adenosine receptor upregulation and alterations in bicuculline seizure susceptibility in rats
Neurosci Lett 1989; 101: 325-330

Sato S, White BG, Penry JK et al
Valproic acid versus ethosuxamide in the treatment of absence seizures
Neurology 1982; 32: 157-163

Saunders M, Rawson M
Sexuality in male epileptics
J Neurol Sci 1970; 10: 577-583

Schain RJ, Ward JW, Guthrie D
Carbamazepine as an anticonvulsant in children
Neurology 1977; 27: 476-480

Schellens JHM, Van Der Wart JHF, Brugman M, Breimer DD
Influence of enzyme induction and inhibition on the oxidation of nifedipine, sparteine, mephenytoin and antipyrine in man as assessed by a "cocktail" study design
J Pharmac Exp Ther 1989; 249: 638-645

Scheperlern S, Koster A
Verapamil in treatment of severe schizophrenia
Acta Psychiatr Scand 1987; 75: 557-558

Schmidt D
Phenobarbitone: Adverse Effects (Ch 27)
In: Antiepileptic Drugs. Frey H-H, Janz D (Eds)
Springer-Verlag: Berlin, Heidelberg, New York, Tokyo 1985, pp 791-829

Schmidt D, Haenel F
Therapeutic plasma levels of phenytoin, phenobarbital and carbamazepine: individual variation in relation to seizure frequency and type
Neurology 1984; 34: 1252-1255

Schobben F, Van Der Kleijn E, Vree TB
Therapeutic monitoring of valproic acid
Ther Drug Monit 1980; 2: 61-71

Schoeman JF, Elyas AG, Brett EM, Lascelles PT
Correlation between plasma carbamazepine-10,11-epoxide concentration and drug side-effects in children with epilepsy
Develop Med Child Neurol 1984; 26: 756-764

Schutz H, Feldmann KF, Faigle JW, Kriemler H-P, Winkler T
The metabolism of ^{14}C-oxcarbazepine in man
Xenobiotica 1986; 16: 769-778

Schramm M, Thomas G, Towart R, Franckowiak G
Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels
Nature 1983; 303: 535-537

Schwartzkroin PA, Slawsky M
Probable calcium spikes in hippocampal neurons
Brain Res 1977; 135: 157-161

Seidenberg M, Beck N, Geisser M et al
Academic achievement of children with epilepsy
Epilepsia 1986; 27: 753-759

Shakir RA, Johnson RH, Lambie DG, Melville ID, Nanda RA
Comparison of sodium valproate and phenytoin as single drug treatment in epilepsy
Epilepsia 1981; 22: 27-33

Shakir RA, Nanda RN, Lambie DG, Johnston RH
Caomparative trial of sodium valproate and clonazepam in chronic epilepsy
Arch Neurol 1979; 36: 301-304

Shannon JA
The study of antimalarials and antimalarial activity in the human malarias
Harvey Lect 1945-46; 41: 43-89

Shargel L, Cheung W, Yu ABC
High pressure liquid chromatographic analysis of antipyrine in small plasma samples
J Pharm Sci 1979; 68: 1052-1053

Shelton RC, Grebb JA, Freed WJ
Induction of seizures in mice by intracerebroventricular administration of the calcium channel agonist Bay K 8644
Brain Res 1987; 402: 399-402

Shorvon SD, Chadwick D, Galbraith AW, Reynolds EH
One drug for epilepsy
Brit Med J 1978; 1: 474-476

Shorvon SD, Reynolds EH
Reduction in polypharmacy for epilepsy
Brit Med J 1979; 2: 1023-1025

Siebelink BM, Bakker DJ, Binnie CD, Kasteleijn-Nolst Trenite DGA
Psychological effects of subclinical epileptiform EEG discharges in children. II.
General intelligence tests
Epilepsy Res 1988; 2: 117-121

Siegel S
Nonparametric statistics for the behavioural sciences
McGraw-Hill: Kogakusha, Tokyo 1956

Sieveking EH
Analysis of 52 cases of epilepsy observed by the author
Lancet 1857; (i): 527-528

Simon RP
Management of status epilepticus
In: Recent Advances in Epilepsy - 2
Pedley TA, Meldrum BS (Eds) Churchill Livingstone: Edinburgh, London, Melbourne, New York 1985 pp 137-160

Skerritt JH, Davies LP, Johnston GAR
A purinergic component in the anticonvulsant action of carbamazepine
Eur J Pharmacol 1982; 82: 195-197

Skerritt JH, Davies LP, Johnston GAR
Interactions of the anticonvulsant carbamazepine with adenosine receptors. 1
Neurochemical studies
Epilepsia 1983a; 24: 634-642

Skerritt JH, Johnston GAR, Chow SC
Interactions of the anticonvulsant carbamazepine with adenosine receptors. 2
Pharmacological studies
Epilepsia 1983b; 24: 643-650

Sloper JJ, Johnson P, Powell TPS
Selective degeneration of interneurones in the motor cortex of infant monkeys following controlled hypoxia: A possible cause of epilepsy
Brain Res 1980; 198: 204-209

Smith DB, Craft BR, Collins J et al
The VA cooperative study group 118. Behavioural characteristics of epilepsy patients compared with normal controls
Epilepsia 1986; 27: 760-768

Smith WL, Lowry JP
The effects of diphenylhydantoin on mental abilities in the elderly
J Am Geriatr Soc 1975; 23: 207-211

Smith PJ, Surks MI
Multiple effects of 5,5'-diphenylhydantoin on the thyroid hormone system
Endocrine Reviews 1984; 5: 514-525

Snyder SH, Reynolds IJ
Calcium-antagonist drugs. Receptor interactions that clarify therapeutic effects
New Eng J Med 1985; 313: 995-1002

Sohn RS, Ferrendelli JA
Anticonvulsant drug mechanisms
Arch Neurol 1976; 33: 626-629

Sommerbeck KW, Theilgaard A, Rasmussen KE, Lohren V, Gram L, Wulff K
Valproate sodium: evaluation of so-called psychotropic effect.
A controlled study
Epilepsia 1977; 18: 159-167

Spark RF, Wills CA, Royal H
Hypogonadism, hyperprolactinaemia and temporal lobe epilepsy in hyposexual men
Lancet 1984; i: 413-416

Speckmann E-J, Witte OW, Walden J
Involvement of calcium ions in focal epileptic activity of the neocortex
In: Calcium electrogenesis and neuronal functioning. Experimental brain research, series 14
Heinemann U, Klee M, Neher E, Singer W (Eds) Springer: Berlin, Heidelberg, New York, Paris, Tokyo 1986 pp 386-395

Spector RS, Park GD, Johnson GF, Vesell ES
Therapeutic drug monitoring
Clinical Pharmacol Ther 1988; 43: 345-353

Starreveld E, De Beukelaar F, Wilson AF, McLean DR, Findlay HP
Double-blind crossover placebo-controlled study of flunarizine in patients with therapy resistant epilepsy
Can J Neurol Sci 1989; 16: 187-190

Stevens LA
From spirits to electricity
In: Explorers of the Brain
Angus & Robertson: London, Sydney 1973 pp 10-26

Stilman N, Masdeu JC
Incidence of seizures with phenytoin toxicity
Neurology 1985; 35: 1769-1772

Stores G, Hart J, Piran N
Inattentiveness in school children with epilepsy
Epilepsia 1978; 19: 169-175

Sugaya E, Ishige A, Sekiguchi K et al
Pentylenetetrazol-induced convulsion and effect of anti-convulsants in mutant inbred strain E1 mice
Epilepsia 1986; 27: 354-358

Surks MI, Ordene KW, Kumara-Siri MH, Mann DN
Effect of diphenylhydantoin on TSH secretion in man and in the rat
J Clin Endocrinol Metab 1983; 56: 940-945

Swinyard EA, Woodhead JH
Experimental detection, quantification and evaluation of anticonvulsants
In: Antiepileptic Drugs
Woodbury DM, Penry JK, Pippenger CE (Eds) Raven Press: New York 1982 pp 111-126

Taburet A-M, Singlas E, Colin J-N, Banzet O, Thibonnier M, Corvol P
Pharmacokinetic studies of nifedipine tablet. Correlation with antihypertensive effects
Hypertension 1983; 5 (suppl II): 29-33

Taft WC, DeLorenzo RJ
Micromolar benzodiazepine receptors regulate voltage-sensitive calcium channels in nerve terminal preparations
Proc Natl Acad Sci USA 1984; 81: 3118-3122

Taft WC, DeLorenzo RJ
Regulation of calcium channels in brain: Implications for the clinical neurosciences
Yale J Biol Med 1987; 60: 99-106

Taylor DC
Sexual behaviour and temporal lobe epilepsy
Archives of Neurology 1969; 21: 510-516

Tedeschi G, Casucci G, Alloca S et al
Neurooccular side-effects of carbamazepine and phenobarbital in epileptic patients as measured by saccadic eye movement analysis
Epilepsia 1989; 30: 62-66

Temkin O
The sacred disease
In: The Falling Sickness (2nd Ed)
Johns Hopkins University Press: Baltimore & London 1971a pp 3-27

Temkin O
Epilepsy in ancient medical science
In: The Falling Sickness (2nd Ed)
Johns Hopkins University Press: Baltimore & London 1971b pp 28-81

Temkin O
Medieval medical theories
In: The Falling Sickenss (2nd Ed)
Johns Hopkins University Press: Baltimore & London 1971c pp 118-133

Temkin O
First period: 1800-1833
In: The Falling Sickness (2nd Ed)
Johns Hopkins University Press: Baltimore & London 1971d pp 255-277

Temkin O
Second period: 1833-1861
In: The Falling Sickness (2nd Ed)
Johns Hopkins University Press: Baltimore & London 1971e pp 278-299

Theisohn M, Heimann G
Disposition of the antiepileptic oxcarbazepine and its metabolites in healthy volunteers
Eur J Clin Pharmacol 1982; 22: 545-551

Theobald W, Kunz HA
Zur pharmakologie des antiepileptikums 5-carbamyl-5H-dibenzo(b,f)azepin
Arzneimittelforsch 1963; 13: 122-125

Thomas J
The effect of nimodipine on picrotoxin-induced seizures
Brain Res Bulletin 1990; 24: 11-15

Thompson P, Huppert F, Trimble M
Anticonvulsant drugs, cognitive function and memory
Acta Neurol Scand (suppl) 1980; 80: 75-81

Thompson P, Hubbert FA, Trimble M
Phenytoin and cognitive functions: effects on normal volunteers and implications for epilepsy
Br J Soc Clin Psycol 1981; 20: 155-162

Thompson PJ, Trimble MR
Sodium valproate and cognitive functioning in normal adults
Br J Clin Pharmacol 1981; 12: 819-824

Thompson PJ, Trimble MR
Comparative effects of anticonvulsant drugs in cognitive functioning
Br J Clin Pract 1982a; suppl 18: 154-156

Thompson PJ, Trimble MR
Anticonvulsant drugs and cognitive functions
Epilepsia 1982b; 23: 531-544

Thompson SH, Smith SJ
Depolarising afterpotentials and burst production in molluscan pacemaker neurons
J Neurophysiol 1976; 39: 153-161

Tomson T, Nilsson BY, Levi R
Impaired visual contrast sensitivity in epileptic patients treated with carbamazepine
Arch Neurol 1988; 45: 897-900

Tomson T
Interdose fluctuations in plasma carbamazepine determine
intermittent side-effects
Arch Neurol 1984; 41: 830-834

Tomson T, Bertilsson L
Potent therapeutic effect of carbamazepine-10,11-epoxide in trigeminal neuralgia
Archives of Neurology 1984; 41: 598-601

Toone BK
Sexual disorders in epilepsy
In: Recent Advances in Epilepsy. Pedley TA, Meldrum BS (Eds)
Churchill-Livingstone: Edinburgh 1985 pp 233-260

Toone BK, Edeh J, Fenwick P et al
Hormonal and behavioural changes in male epileptics
In: Advances in Epileptology XVth Epilepsy International Symposium
Porter RJ et al (Eds) Raven Press: New York 1984 pp 283-289

Toone B, Wheeler M, Fenwick P
Effects of anticonvulsant drugs on male sex hormones and sexual arousal
In: Psychopharmacology of Anticonvulsants. Sandler M (Ed)
Oxford University Press: Oxford 1982 pp 136-142

Toone BK, Wheeler M, Fenwick PBC
Sex hormone changes in male epileptics
Clin Endocrinol 1980; 12: 391-395

Toone BK, Wheeler M, Nanjee M, Fenwick P, Grant R
Sex hormones, sexual activity and plasma anticonvulsant levels in male epileptics
J Neurol, Neurosurgery, Psych 1983; 46: 824-826

Tourjman SV, Bloom DM, Nair NPV
Verapamil in the treatment of chronic schizophrenia
Psychopharmacol Bulletin 1987; 23: 227-229

Towart R
The selective inhibition of serotonin-induced contractions of rabbit cerebral vascular
smooth muscle by calcium-antagonist dihydropyridines
Circ Res 1981; 48: 650-657

Trimble MR
Anticonvulsant drugs and cognitive function: a review of the literature
Epilepsia 1987; 28 (suppl 3): S37-S45

Trimble MR
Serum prolactin in epilepsy and hysteria
Brit Med J 1978; 2: 1682

Trimble MR, Robertson MM
Clobazam
In: Current Problems in Epilepsy 4. New Anticonvulsant Drugs
Meldrum BS, Porter RJ (Eds) John Libbey: London, Paris 1986 pp 65-84

Troupin A, Ojemann LM, Halpern L et al
Carbamazepine - a double-blind comparison with phenytoin
Neurology 1977; 27: 511-519

Troupin AS, Ojemann LM
Paradoxical intoxication - a complication of anticonvulsant administration
Epilepsia 1975; 16: 753-758

Turnbull DM, Rawlins MD, Weightman D, Chadwick DW
Plasma concentrations of sodium valproate: their clinical value
Ann Neurol 1983; 14: 38-42

Twombly DA, Yoshii M, Narahashi T
Mechanisms of calcium channel block by phenytoin
J Pharmac Exp Ther 1988; 246: 189-195

Vanhoutte PM, Paoletti R
The WHO classification of calcium antagonists
Trends Pharm Sciences 1987; 8: 4-5

Vessell ES, Page JG
Genetic control of the phenobarbital-induced shortening of plasma antipyrine half-lives
in man
J Clin Invest 1969; 48: 2202-2209

Victor A, Lundberg PO, Johansson EDB
Induction of sex hormone binding globulin by phenytoin
Brit Med J 1977; ii: 934-935

Vining EPG, Mellits ED, Dorsen MM et al
Psychologic and behavioral effects of antiepileptic drugs in children: a double-blind
comparison between phenobarbital and valproic acid
Pediatrics 1987; 80: 165-174

Vozeh S
Cost-effectiveness of therapeutic drug monitoring
Clin Pharmacokinetics 1987; 13: 131-140

Wagner J, Schmid K
Induction of microsomal enzymes in rat liver by oxcarbazepine,
10,11-dihydro-10-hydroxy- carbamazepine and carbamazepine
Xenobiotica 1987; 17: 951-956

Walden J, Pockberger H, Speckmann E-J, Petsche H
Paroxysmal neuronal depolarisations in the rat motorcortex in vivo: intracellular
injection of the calcium agonist Bay K 8644
Exp Brain Res 1986; 64: 607-609

Walden J, Speckmann E-J
Calcium conductance in invertebrate neurons: effects of organic calcium antagonists and
agonists
In: Inactivation of Hypersensitive Neurons
Alan R Liss 1987 pp 399-405

Walden J, Speckmann E-J
Suppression of recurrent generalised tonic-clonic seizure discharges by intraventricular
perfusion of a calcium antagonist
Electroenceph Clin Neurophysiol 1988; 69: 353-362

Ward CD
Decision-making in refractory epilepsy: an analysis based on computer simulation
J Neurol Neurosurg Psychiatr 1986; 49: 781-784

Wauquier A, Ashton D, Melis W
Behavioral analysis of amygdaloid kindling in beagle dogs and the effects of
clonazepam, diazepam, phenobarbital, diphenyl-hydantoin and flunarizine on the seizure
manifestation
Exp Neurol 1979; 64: 579-586

Webster J, Robb OJ, Jeffers TA, Scott AK, Petrie JC, Towler HM
Once daily amlodipine in the treatment of mild-moderate hypertension
Br J Clin Pharmacol 1987; 24: 713-719

Wechsler D
Measurement and appraisal of adult intelligence
5th enlarged edition
Matarazzo JD (ed) Williams & Wilkins: Baltimore 1972 pp 204ff

Weir RL, Padgett W, Daly JW, Anderson SM
Interaction of anticonvulsant drugs with adenosine receptors in the central nervous system
Epilepsia 1984; 25: 492-498

Wen P
Nifedipine for epilepsy? (letter)
Brit Med J 1988; 296: 1069

Werk EE jr, MacGee J, Sholiton LJ
Effect of diphenylhydantoin on cortisol metabolism in man
J Clin Invest 1964; 43: 1824-1835

White PT, Plott D, Norton J
Relative anticonvulsant potency of primidone: a double-blind comparison
Arch Neurol 1966; 14: 31-35

Whiting B, Kelman AW, Bryson SM et al
Clinical pharmacokinetics: a comprehensive system for therapeutic drug monitoring and prescribing
Brit Med J 1984; 288: 541-545

Willow M, Catterall WA
Inhibition of binding of(^{3}H) batrachotoxinin A 20-alpha-benzoate to sodium channels by the anticonvulsant drugs diphenylhydantoin and carbamazepine
Mol Pharmacol 1982; 22: 627-635

Wing DS, Duff HJ
The impact of a therapeutic drug monitoring program for phenytoin
Therapeutic Drug Monitoring 1989; 11: 32-37

Witte OW, Speckmann E-J, Walden J
Motor cortical epileptic foci in vivo: actions of a calcium channel blocker on paroxysmal neuronal depolarisations
Electroenceph Clin Neurophysiol 1987; 66: 43-55

Wolff J, Standaert ME, Rall JE
Thyroxine displacement from serum proteins and depression of serum protein-bound iodine by certain drugs
J Clin Invest 1961; 40: 1373-1379

Wong RKS, Prince DA
Dendritic mechanisms underlying penicillin-induced epileptiform activity
Science 1979; 204: 1228-1231

Wong W-S, Rahwan RG
Examination of the potential antiepileptic activity of calcium antagonists with different sites of action
Gen Pharmac 1989; 20: 309-312

Woo E, Chan YM, Yu YL, Chan YW, Huang CY
If a well stabilised patient has a subtherapeutic antiepileptic drug level, should the dose be increased? A randomised prospective study
Epilepsia 1988; 29: 129-139

Yamaji T
Modulation of prolactin release by altered levels of thyroid hormones
Metabolism 1974; 23: 745-751

Yeo PPB, Bates D, Howe JG et al
Anticonvulsants and thyroid function
Br Med J 1978; 1: 1581-1583

Zucker RS, Lando L
Mechanism of transmitter release: voltage hypothesis and calcium hypothesis
Science 1986; 231: 574-579

APPENDIX A

RECEPTOR BINDING ASSAYS

Theory

The principle behind receptor binding assays is that the kinetics of ligand-receptor binding are similar to those of enzyme-substrate reactions i.e.

$$[L] + [R] \underset{k-1}{\overset{k+1}{\rightleftharpoons}} [LR] \qquad \text{--} \quad (1)$$

where
$$[L] = \text{free ligand concentration}$$
$$[R] = \text{unoccupied receptor concentration}$$
$$[LR] = \text{ligand-receptor complex concentration}$$

At equilibrium :

$$k+1 [L] [R] = k-1 [LR] \qquad \text{--} \quad (2)$$

A measure of the affinity of the receptor can be defined as the equilibrium association binding constant $= Ka$

$$Ka = \frac{K+1}{K-1} = \frac{[LR]}{[L] [R]}$$

or more usually as the dissociation constant $= Kd$

$$Kd = \frac{K-1}{K+1} = \frac{[L] [R]}{[LR]} \qquad \text{--} \quad (3)$$

Kd thus refers to the tendency for the ligand-receptor complex to come apart and from equation (3) we see that it will possess the units of concentration e.g. nM. We also see that a higher Kd (e.g. 100nM as vs. 10nM) suggests a receptor of <u>lower</u> affinity.

By measuring bound ligand [LR] at equilibrium using different ligand concentrations [L], we can essentially set up a series of simultaneous equations from which the Kd and the finite number of receptors which a tissue has (Bmax) can be derived viz :

Clearly $\qquad$ [LR] + [R] = Bmax

Multiply by [L] $\qquad$ ===> [L] [LR] + [L] [R] = Bmax [L]

Multiply segment ===> [L] [LR] + $\dfrac{[LR]}{[LR]}$ [L] [R] = Bmax [L]
by $\dfrac{LR}{LR} = 1$

$$===> \quad [LR] \left[[L] + \frac{[L]\ [R]}{[LR]} \right] = Bmax\ [L]$$

Substitute (3) $\qquad$ ===> [LR] [[L] + Kd] = Bmax [L]

$$===> \quad [LR] = \frac{Bmax\ [L]}{[L] + KD} \qquad \text{------------------------------ (4)}$$

More conventional terms for LR = bound ligand   = B
$\qquad\qquad\qquad\qquad\quad$ L = free ligand   = F

$$\therefore \quad B = \frac{Bmax\ F}{F + Kd}$$

Multiply out $\quad$ ===> BF + BKd = Bmax F

Divide by F $\quad$ ===> B + $B/_F$ Kd = Bmax

$$===> \quad B/_F = \frac{Bmax - B}{Kd} \qquad \text{----------------------------- (5)}$$

This is the Scatchard Equation. If the bound ligand is now assayed at, for example, six different concentrations of free ligand, then a graph of $B/_F$ (Y axis) vs B (x axis) can be constructed. By paraphrasing equation 5 we get :

$$B/_F = -1/_{Kd}\ B \quad + 1/_{Kd}\ Bmax$$

which, since Bmax is a relative constant, conforms to the general equation

y = ax + b which is a straight line with slope a. Therefore, the slope of the straight line formed by these points is $-1/_{Kd}$ (see Figure 71).

Further, where the line crosses the x axis y = 0, i.e. B/$_F$ = 0, and by substitution in (5).

$$O = \frac{Bmax - B}{Kd}$$

i.e. Bmax = B so the line crosses the x axis at Bmax.

Such Scatchard analysis has been the standard approach to interpreting binding assays to produce values for Kd and Bmax. There are obvious drawbacks, not least that 'B' is present on both sides of the equation, and that variations in the assay values at low ligand concentration (the most difficult assays) have a disproportionately large effect on the calculated Kd and Bmax.

An alternative approach correlates bound ligand at equilibrium directly with free ligand concentrations. As the latter increases, B also increases, but not in a straight line. The number of receptor sites is finite, so as ligand concentration increases, the percentage bound at equilibrium decreases as the dissociation reaction [L] ---> [L] + [R] be comes more prominent.

The curve of this 'Saturation Isotherm' therefore resembles those shown in Figure 71, where the slope of the curve at the origin is proportional to 1/Kd (steep slope = high affinity, low Kd) and the B at F = infinity, is equal to Bmax. The mathematics of fitting such a curve to experimental points is outwith the scope of this thesis, but can be performed by computer programmes such as that we have available with the Packard 2000 CA.

Assay

Experimental results which may be analysed as above are obtained using radioactive ligands which bind to receptors, such as tritiated cyclohexyladenosine ([^{3}H]CHA) which binds to adenosine A_1 receptors. The tissue to be assayed is homogenised, prepared, then incubated with a known concentration of the ligand to allow equilibration. At equilibrium, the reaction is abruptly stopped by centrifugation and washing, or by rapid filtration and washing. The pellet or filtered remnants are then assayed, e.g. using a liquid scintillation counter, to measure the amount of ligand which has become bound to

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the tissue. When this has been performed at 6 different ligand concentrations under identical conditions (usually simultaneously) Scatchard and isotherm analysis can be carried out as outlined above.

A mention should be made of 'non-specific binding' (NSB). As well as binding to genuine tissue receptors, the [^{3}H]CHA may bind non-specifically to glass, tissue binding sites or simply collect in various 'holes' in the tissue preparation. Such binding sites are clearly of low affinity, but are theoretically infinite so NSB increases in a linear fashion with increasing ligand concentration. To compensate for NSB, each reaction is duplicated but with the addition of 'cold' ligand i.e. non-tritiated CHA which being of much higher concentration (n x 1,000) than the [^{3}H]CHA displaces the radioligand from the receptors. However, the binding of [^{3}H]CHA to the 'limitless' NSB sites is relatively unaffected. This gives a result which is a measure of the NSB and can be subtracted from the assay reaction results to give a truer estimate of 'specific binding'. This final estimate is the one used in Scatchard and saturation isotherm analysis.

APPENDIX B

As the work of this thesis has progressed over four to five years, many extracts have been presented to learned societies , or published in scientific journals.

Presentations to Learned Societies	EXPT.

1. Anticonvulsant monitoring at the epilepsy clinic. A prospective study.
    Larkin JG*, McGuire GM, Percy-Robb I, Brodie MJ
    British Pharmacol Soc December 1987
    Abs. Brit J Clin Pharmacol 1988: 25; 627p
    — 1

2. Anticonvulsants and psychomotor function.
    Brodie MJ, McPhail E, Macphee GJA, Larkin JG*, Grey JMB
    British Pharmacol Soc December 1986
    — 2

3. Comparison of conventional and sustained-release carbamazepine in healthy subjects.
    Larkin JG*, McLellan AR, Munday A, Sutherland M, Butler E, Brodie MJ
    International League Against Epilepsy May 1987
    — 4

4. Hormonal abnormalities in male epileptic patients taking anticonvulsants.
    Macphee GJA*, Larkin JG, Beastall G, Brodie MJ
    Scottish Soc Exp Medicine May 1987
    — 5

5. Effects of antiepileptic drugs on thyroid hormones.
    Larkin JG*, Macphee GJA, Beastall GH, Brodie MJ
    British Pharmacol Soc December 1988 (Poster)
    Abs. Brit J Clin Pharmacol 1989: 27; 668p
    — 6

6. Evidence of lack of auto- and hetero-induction with the novel anticonvulsant oxcarbazepine in healthy volunteers.
    Larkin JG*, McKee PJW, Thompson GG et al
    British Pharmacol Soc January 1990 (Poster)
    Abs. Brit J Clin Pharmac 1990: 29; 643p
    — 7

7. Nifedipine in epilepsy - a preliminary report.
    Larkin JG*, Butler E, Brodie MJ
    Scottish Soc Exp Medicine February 1987
    — 8

8. Calcium antagonists and epilepsy.
    Larkin JG*, Thompson GG, Brodie MJ
    Scottish Society of Physicians
    — 8, 10abc

9. Nifedipine and nimodipine are effective against pentylene-tetrazol induced seizures in mice.
    Larkin JG*, Thompson GG, Brodie MJ
    British Pharmacol Soc December 1988
    Abs. Brit J Pharmacol 1989: 96; 138p
    — 10abc

10. Blood and brain pharmacokinetics of nifedipine and nimodipine in CF1 mice: efficacy against pentylenetetrazol (PTZ) seizures. 9ab, 10abc
Larkin JG*, Thompson GG, Brodie MJ
Medical Research Soc January 1990 (Poster)

11. A double-blind crossover, placebo-controlled trial of adjuvant nifedipine in refractory epilepsy. 11
Brodie MJ*, Larkin JG, Cox A, Besag FM
British Pharmacol Soc January 1990

12. Placebo-controlled trial of nimodipine in refracyory epilepsy. 12
Larkin JG, McKee PJW, Thompson GG, Brodie MJ*
North Europe Epilepsy Symposium September 1990

* denotes presenter

Publications   **EXPT.**

1.   Larkin JG, McGuire GM, Herrick A, Percy-Robb I,   1
        Brodie MJ
        Therapeutic drug monitoring at the epilepsy clinic
        Epilepsia 1991 (in press)

2.   Brodie MJ, McPhail E, Macphee GJA, Larkin JG,   2
        Gray JMB
        Psychomotor impairment and anticonvulsant therapy in adult
        epilepsy patients.
        Eur J Clin Pharmacol 1987: 31; 655-660

3.   Larkin JG, McLellan A, Munday A, Sutherland M,   4
        Butler E, Brodie MJ
        A double-blind comparison of conventional and controlled-
        release carbamazepine in healthy subjects.
        Brit J Clin Pharmacol 1989: 27; 313-322

4.   Macphee GJA, Larkin JG, Butler E, Beastall GH, Brodie MJ   5
        Circulating hormones and pituitary responsiveness in young
        epileptic men receiving long-term antiepileptic medication.
        Epilepsia 1988: 29; 468-475

5.   Larkin JG, Macphee GJA, Beastall GH, Brodie MJ   6
        Thyroid hormone concentrations in epileptic patients.
        Eur J Clin Pharmacol 1989: 36; 213-216

6.   Larkin JG, McKee PJW, Forrest G et al   7
        Lack of enzyme induction with oxcarbazepine (600mg daily)
        in healthy subjects.
        Brit J Clin Pharmacol 1991: 31; 65-71

7.   Larkin JG, Butler E, Brodie MJ   8
        Nifedipine for epilepsy? A pilot study.
        Brit Med J 1988: 296; 530-531

8.   Larkin JG, Thompson GG, Scobie G, Drennan JE, Brodie MJ   13
        Lack of major effects on mouse brain adenosine A_1
        receptors of oral carbamazepine and calcium antagonists.
        Epilepsia 1991 (in press)

Reprints of those papers published before the submission of this thesis are enclosed.

APPENDIX C

Some of my colleagues who helped to perform some of the work of this thesis have themselves presented their work for higher degrees.

EM McPhail
Effects of chronically administered anticonvulsants on psychomotor performance in epileptic subjects (alternative analysis of EXPT. 2)
M.Sc. University of Glasgow 1985

PA Milligan
Circulating androgens and sexual function in male epileptics (first 50 male subjects in **Sexual function study** p. 108)
M.Sc. University of Glsagow 1987

VC MacKay
Circulating oestrogens and sexual function in female epileptics (first 50 female subjects in **Sexual function study** p. 108)
M.Sc. University of Strathclyde 1988