DOPAMINE RECEPTOR SUBTYPES:

INFLUENCES ON LOCAL CEREBRAL BLOOD FLOW

AND

FUNCTION-RELATED GLUCOSE UTILISATION.

by

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ERRATA

P11    line 5   should read:

"Specific uptake mechanisms for dopamine (Scatton et al 1985),
and high affinity binding sites for dopaminergic ligands
(Bernado and Prince 1982;   Bischoff et al 1980;   Dawson et al
1985: Ouimet et al 1984) have been found in the hippocampus.

P40    line 8   should read:

However, two recent studies have reported that electrical
stimulation of the fustigial nucleus results in widespread
increases in cerebral blood flow while having little effect on
cerebral glucose utilisation (Nagai et al 1983: Iadecola et
al 1985).   This apparent uncoupling of the flow/glucose use
relationship has been attributed to a direct neurogenic action
upon the cerebral vasculature.   Thus, both metabolic and
neurogenic factors can significantly affect the cerebral
circulation.

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During my course of study I have been helped and inspired by many people. It is with pleasure that I take this opportunity to acknowledge and thank them.

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It is said that there are "lies, dammed lies, ........ and statistics". I would like to thank Dr. Ian Ford and Mr. Graham Rowen of the University Department of Statistics for dispelling this myth by explaining the fundamentals of statistical analysis in language which even I could understand.

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SUMMARY

In recent years, two pharmacologically distinct subpopulations of dopamine receptor, designated $D_1$ and $D_2$ have been found in brain and vascular tissue. The development of new classes of chemical compounds which discriminate between the subpopulations of dopamine receptor now permits the examination of dopaminergic mechanisms with a precision hitherto impossible. Of these, the benzazepine derivative SKF 38393 possesses a high degree of selectivity for the $D_1$ receptor while the partial ergoline LY 141865 (or as its biologically active enantiomer LY 171555) is a selective $D_2$ agonist. These agents have been used extensively to examine anatomical distribution and neurochemical properties of the $D_1$ and $D_2$ subpopulations in vitro. However, little is known about the effects of pharmacological manipulation of the $D_1$ and $D_2$ receptor subpopulations upon the integrated activity of the brain in vivo.

The systemic administration of dopamine receptor agonists frequently results in pronounced cerebrovascular effects. However, the precise mechanism through which dopamine receptor agonists alter cerebral blood flow is unclear. Hitherto, two possible mechanisms have been proposed. According to one view dopamine receptor agonists may interact with specific dopamine receptive sites on cerebrovascular smooth muscle, altering the calibre of resistance vessels and thereby modify blood flow. Alternately, the agonists may modify the activity of neural tissue and, as a result of the homeostatic mechanisms which adjust the level of tissue perfusion to meet alterations in metabolic demand, indirectly modify cerebral

blood flow. In the studies which form the basis of this thesis I have examined the relative importance of the metabolic and neurogenic components of the cerebrovascular response to dopaminergic agonists.

The thesis also addresses the role of $D_1$ and $D_2$ receptors on the integrated functional activity of the brain. The experiments performed were threefold. The first series of experiments examined the vasomotor responses of individual cerebral arterioles lying on the pial surface of the anaesthetised cat brain to perivascular microinjections to dopamine receptor agonists and antagonists. The second set of experiments employed the 2-deoxyglucose quantitative autoradiographic technique to examine the influences exerted by dopamine $D_2$ receptor subtypes upon the integrated functional activity of the rat brain as reflected by altered rates of local cerebral glucose utilisation. Finally, a third series of investigations employed an analogous quantitative autoradiographic technique (using carbon 14 iodoantipyrine as tracer) to examine the effects of the selective $D_1$ and $D_2$ agonists SKF 38393 and LY 171555 upon local cerebral blood flow in the rat brain. These studies together with parallel 2-deoxyglucose investigations in rats, permitted the examination of the effects of $D_1$ and $D_2$ receptor activation upon the flow/glucose relationship. The direct perivascular microapplication of apomorphine and the selective $D_1$ and $D_2$ agonists SKF 38393 and LY 141865 provoked dose-related increases in pial arteriolar calibre. The rank order of potency, in terms of (the concentration producing 50% of the maximum response) SKF 38393 > apomorphine > LY 141865, consistent with the hypothesis that dilatation of the cerebrovasculature is mediated via the $D_1$ receptor subtype. This

view was supported by studies with the putatively selective $D_1$ antagonist SCH 23390. At the concentrations examined ($10^{-9}$ to $10^{-5}$M) SCH 23390 did not display any intrinsic vasomotor activity, nor did even high concentrations ($10^{-7}$M) of SCH 23390 affect the non-specific dilatory response elicited by acidic cerebrospinal fluid. However, the co-administration of low concentrations of SCH 23390 ($10^{-8}$M) markedly attenuated the cerebrovascular dilatations produced by apomorphine or SKF 38393.

The intravenous administration of LY 171555 (0.1 - 5 mg/kg) affected dose-related increases in glucose utilisation within 21 of the 59 brain regions examined. The stimulation of local cerebral glucose use occurred predominately within those brain areas which subserve somatosensory and motor functions (sensory motor and frontal cortices, ventral and intralaminar thalamic nuclei, spinal nucleus of the trigeminal nucleus, and basal ganglia inter alia). Following the administration of LY 171555, there was evidence that glucose use became topographically heterogeneous with dose-related increases occurring within the ventral portion of this nucleus. In only one brain area (the molecular layer of the hippocampus) was there evidence of a dose-related reduction in glucose use following the administration of this $D_2$ receptor agonist.

The putatively selective $D_1$ receptor agonist SKF 38393 (1 - 30 mg/kg) provoked concentration-dependent alterations in glucose use in only two of the 59 brain areas examined; the anterior cingulate cortex and anteriomedial thalamic nucleus. The parallel reductions in glucose use observed within these two brain areas suggest that activation of $D_1$ receptors may play a role in the activity of this limbic thalamo-cortical circuit.

The patterns of altered glucose use elicited by SKF 38393 and LY 171555 were compared to that of the classical dopamine receptor agonist apomorphine. Visual inspection of the autoradiograms suggested that the pattern of alterations in glucose use elicited by apomorphine was broadly similar to that produced by LY 171555 (markedly increased glucose use within frontal and sensory motor cortex, basal ganglia and other subcortical areas subserving somatosensory or motor function) but also contained features of the SKF 38393 response (reductions in glucose use within the anterior cingulate cortex and anteromedial thalamus). These data are consistent with the view that apomorphine is a mixed $D_1/D_2$ agonist. To permit a more critical evaluation of the metabolic responses elicited by these agonists a simple yet statistically rigorous system of data analysis has been devised (Ford et al. 1985). This method of data analysis describes the pattern of altered glucose for each drug treatment in terms of a rank order of regional responsiveness. This pharmacological 'fingerprint' of drug activity can be derived from the dose response relationship for each of the 59 brain regions examined using a simple arithmetic function. Evaluation of the functional profiles elicited by SKF 38393, LY 171555 and apomorphine revealed a markedly different pattern of response in response to each drug treatment.

The role of $D_1$ receptors on function-related glucose use in the CNS was further examined in a study of the effects of the selective $D_1$ antagonist SCH 23390. The intravenous injection of SCH 23390 (0.05 - 1 mg/kg) resulted in discrete alterations in local cerebral glucose utilisation. Following the administration of 0.1 mg/kg

significant increases in glucose use were found within the nucleus accumbens (+36%) and lateral habenular nucleus (+24%) while concomitant reductions occurred within the pars reticulata of the substantia nigra (+16%) and posterioventromedial nucleus of the thalamus (+14%). At the highest concentration examined (1 mg/kg) significant reductions in glucose use were within areas of the visual system and ventral thalamus. No significant alterations in glucose use were evident at 0.05 mg/kg. The prior administration of this low concentration of SCH 23390 (0.05 mg/kg) blocked the stereotyped gnawing and increases in glucose utilisation produced by LY 171555 (0.5 mg/kg), however, although neither drug significantly altered glucose use within the anterior cingulate cortex and anteromedial thalamus per se, the interaction of these agents significantly reduced function - related glucose utilisation within these brain areas. Together these data indicate that although focal alterations in local cerebral glucose utilisation is initiated in all but a few brain areas by the $D_2$ receptor population, the expression of these alterations require the presence of functional (unblocked) $D_1$ receptors.

Under normal circumstances there is a close relationship between local cerebral glucose utilisation and local cerebral blood flow such that areas of high glucose utilisation exhibit high levels of blood flow while areas of low metabolic activity receive correspondingly low levels of blood flow. However, it remains to be established whether this relationship is affected by the administration of dopamine receptor agonists.

The administration of the $D_2$ agonist LY 171555 (0.5 mg/kg) significantly increased local blood flow in those brain areas in which, enhanced rates of glucose use had been observed previously (viz. sensory motor and frontal cortices, basal ganglia and ventral tier of thalamus). Within each of these brain areas, the magnitude of the increases in blood flow and glucose use were proportionately similar.

SKF 38393 (30 mg/kg) provoked little change in local blood flow within the vast majority of brain regions examined. However, the administration of this $D_1$ agonist did provoke significant reductions in $\leftarrow$ local blood flow within the anterior cingulate cortex and anteromedial thalamic nucleus. These observations are of crucial importance in evaluating the relative importance of the vascular and metabolic components of the circulatory response to dopamine receptor agonists. Although, SKF 38393 a potent dilator of cerebral vessels in situ, it provokes parallel reductions in blood flow and glucose use within two brain areas which contain $D_1$ receptors. These data clearly indicate that local metabolic activity is the primary determinant of cerebral blood flow. This view was supported by an examination of the overall relationship between blood flow and glucose use in SKF 38393 and LY 171555 treated animals. Neither SKF 38393 nor LY 171555 significantly altered the overall relationship between blood flow and glucose use observed in saline treated control animals.

Further analysis revealed that both SKF 38393 and LY 171555 can produce focal uncoupling of the flow/glucose use relationship within particular subregions of the caudate nucleus. Disproportionately

high levels of blood flow (relative to glucose use) observed within the ventrolateral portion of the caudate nucleus suggest that $D_2$ receptors agonists may mediate vasodilatation by acting directly on the cerebral vasculature within this brain area.

SKF 38393 provoked significant reductions in blood flow at two of the three levels of the caudate nucleus exmined. In contrast SKF 38393 had little effect upon local glucose use in any of the subregions of the caudate nucleus examined. However, the mechanisms underlying these responses are unclear.

This thesis contributes insight into two aspects of dopaminergic function in vivo. Firstly, the studies comprising this thesis represent a comprehensive examination of the relative importance of the vascular and metabolic components of the circulatory response to dopamine receptor agonists. They clearly demonstrate that in the vast majority of brain areas it is the local rates of metabolic activity which is the primary determinants of tissue perfusion perfusion, however, within some brain areas (i.e. subregions of the caudate nucleus) other factors (vasomotor actions or non-specific drug effects) may play a significant role in determining levels of tissue perfusion. Secondly, the studies contribute to the understanding of the functional role of dopamine systems in the brain as these roles are reflected in local cerebral glucose utilisation.

## DECLARATION


    I declare that this thesis is based upon my own original studies and experiences and that it has not been presented previously as a thesis in any form.


John Sharkey,

September, 1986.

CHAPTER I


INTRODUCTION


1.   Dopamine Receptors in Cerebral and Vascular Tissues


From inauspicious beginnings, almost 80 years ago, the view of
dopamine has altered radically from being a precursor of
noradrenaline possessing weak sympathomimetic activity to its present
status as the most intensively studied of all the neurotransmitters,
possessing important physiological roles in the control of limbic and
motor function and in the maintenance of cardiovascular and endocrine
homeostasis.


1.1   Historical Perspective


Dopamine (B-(3,4-dihydroxyphenyl)ethylamine) was first
synthesised in 1910 (Barger and Ewins, 1910;  Mannich and Jacobsohn
(1910).  In that same year, 1910, Barger and Dale reported that
dopamine provoked pressor responses in the spinal cat, although it
proved to be fifty times less potent than noradrenaline.  Early
cardiovascular studies (Tainter, 1930) confirmed the weak
sympathomimetic actions of dopamine reported by Barger and Dale and
demonstrated that, like noradrenaline, the pressor actions of
dopamine could be potentiated by cocaine and inhibited with the
adrenergic blocking agent ergotoxine (Tainter, 1930).  However, in
the presence of ergotoxine, dopamine reduced blood pressure in the
spinal cat, an action which was similar to that provoked by
adrenaline in such preparations[1].

[1]FOOT NOTE. The vasodepressor effects of adrenaline in the presence
of ergot alkaloids was first reported by Dale (1906) and subsequently
shown to be due to an activation of dilatory B-adrenergic receptors

The first indications that dopamine may have a physiological role distinct from noradrenaline or adrenaline is generally attributed to Holtz and Credner (Holtz and Credner, 1942). These authors found that a vasodepressor response could be elicited in guinea-pigs and rabbits following low doses (<1 mg i.v.) of dopamine and that the pressor effects reported in earlier studies could be demonstrated only at high doses (>1 mg i.v.). This depressor effect was subsequently shown to be resistant to atropine, antihistamines and B-adrenergic blockers (McDonald and Goldberg, 1963; Hotlz et al. 1963, 1964; McNay and Goldberg, 1966). However, at the time the authors suggested that the depressor effects were produced not by dopamine, but by 3,4-dihydroxy-phenylacetaldehyde (DOPAC), the aldehyde product formed from dopamine by monoamine oxidase.

Some 15 years later Blaschko noted that dopamine constituted up to 50% of the total catecholamine content of the splenic nerve (Blaschko, 1957). These levels were evidently not consistent with the prevalent view that dopamine was merely a metabolic precursor of noradrenaline and led Blaschko to postulate that dopamine may possess physiological actions in addition to its role as a biosynthetic intermediate. This view was rapidly confirmed by Hornykiewicz who demonstrated that the depressor effects of dopamine in the guinea-pig were due not to the action of DOPAC as suggested by Holtz and Credner, but to specific action of dopamine on the vasculature (Hornykiewicz, 1958).

At about the same time it was shown that dopamine levels in the brain was similar to that of noradrenaline (Montagu, 1957; Carlsson et al. 1958). Moreover, the finding that dopamine was concentrated

within the basal ganglia (Bertler and Rosengren, 1959; Carlsson 1959) suggested that dopamine may have a neurotransmitter role in the CNS as well as in the peripheral vasculature.

In 1957 Carlsson reported that 1-dopa, a precursor of dopamine which unlike dopamine could cross the blood-brain barrier reversed the akinesia induced by neuroleptic doses of reserpine in mice (Carlsson et al. 1957). Later in a seminal paper, Carlsson (1959) demonstrated that administration of 1-dopa caused a marked increase in dopamine levels in the rabbit brain whereas noradrenaline levels were not markedly affected. He noted that reserpine, which depletes dopamine from the corpus striatum, produced a syndrome very similar to Parkinsonism, and was efficient in controlling the choreatic behaviours associated with lesions of the extrapyramidal system. The hypothesis that the "striatal dopamine system" was central to the pathophysiology of Parkinsonism was supported by clinical studies which identified profound reductions in striatal dopamine levels in patients suffering from idiopathic and postencephalitic Parkinsonism (Ehringer and Hornykiewicz, 1960) and by the demonstration of the beneficial effects of 1-dopa in these patients (Birkmayer and Hornykiewicz, 1961; Barbeau et al. 1961). The development of the Falck/Hillarp - histofluorescence technique demonstrated that dopaminergic neurones arise from the ventral mesencephalon to innervate the striatum (Anden et al. 1964; Bertler et al. 1964; Dahlstrom and Fuxe, 1966 a). With the development of more sensitive histochemical techniques (glyocylic acid, ALFA) and immunohistochemical techniques (Geffard et al. 1984) it has been possible to demonstrate the presence of a number of dopamine systems within the mammalian brain.

## 1.2    Distribution of Dopamine Systems in Cerebral Tissue

Central dopamine systems have been divided into three principle subsystems:  a large mesotelencephalic system which encompasses the nigrostriatal, mesocortical and mesolimbic pathways, and the smaller diencephalic system.  In addition dense plexus of dopamine neurons have been found within the amacrine cells of the mammalian and fish retina, and within the periglomerular cells of the olfactory bulb. Although, the dopamine systems of the rat brain have been the most extensibly studied, there is considerable evidence to suggest that the dopamine systems are essentially the same in all mammalian species (Lindvall and Björklund, 1984).

### 1.2.1  Mesotelencephalic Dopamine System

a)    Mesostriatal System

The principle dopamine projection system, accounting for approximately 80% of brain dopamine, arises from the catecholamine cell groups of the ventral mesencephalon.  The dopamine cells comprising this sytem are located predominately within the A9 cell group (of Dahlstrom and Fuxe, 1964) of the substantia nigra and the A10 cells of the ventral tegmental area (Anden et al. 1964, 1965; Bertler et al. 1964;  Dahlstrom and Fuxe, 1964).  The nigral neurons are found mainly within the pars compacta and pars lateralis of the substantia nigra, although a few perikayra are found scattered throughout the pars reticulata.  Dopamine cells are also located within the A8 (Figure 1) cells of the retrorubral nucleus, however,

# FIGURE 1

## The Anatomical Distribution of Central Dopamine Systems
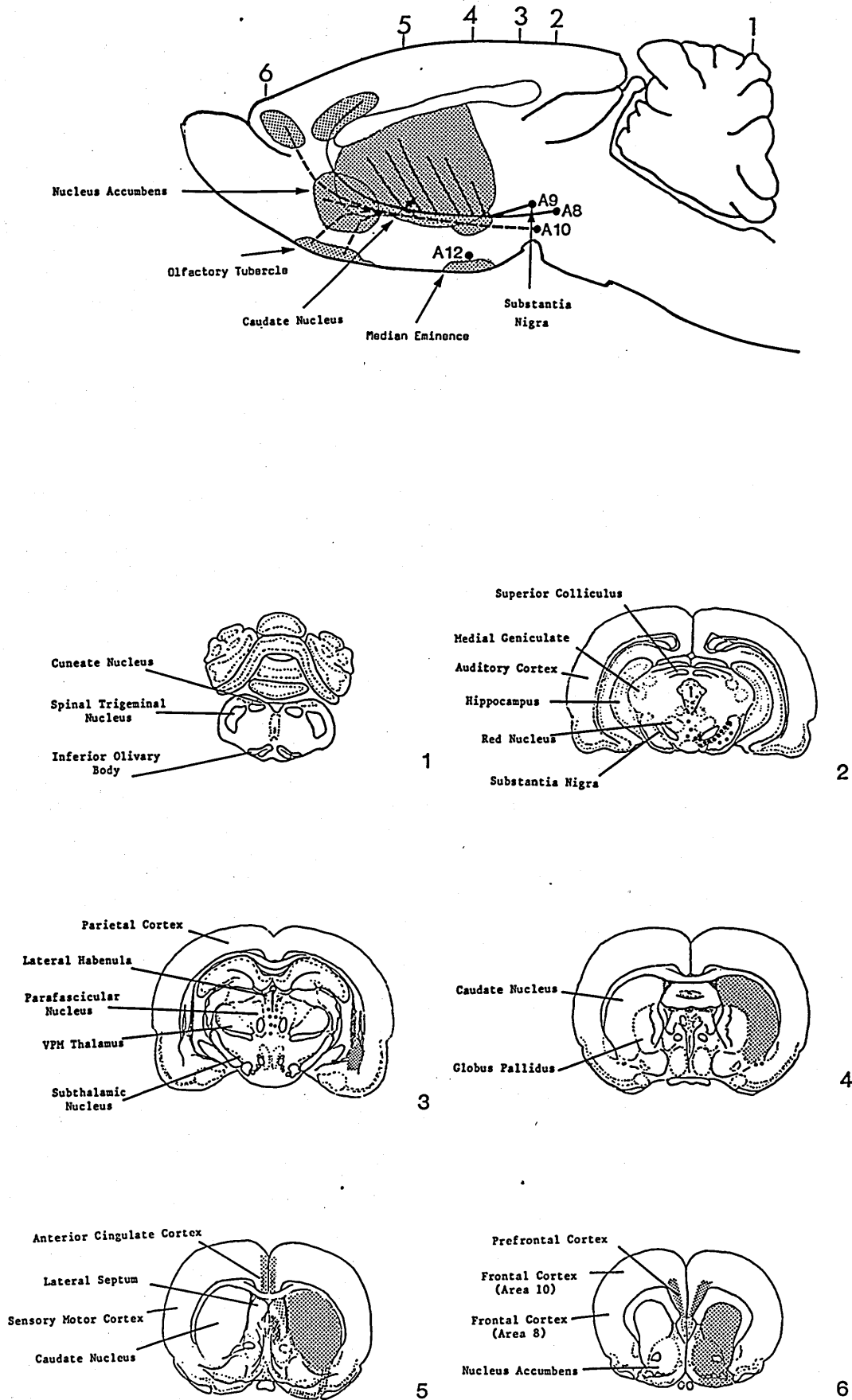
LEGEND TO FIGURE 1


Top.   A saggital representation of the principle dopaminergic

pathways of the rat brain.   Stipled areas indicate regions innervated

by dopaminergic neurons.   Dots – indicate the location of the major

sources of dopaminergic perikayra.   Figures 1 – 6.   Coronal

representation of the rat brain at 6 levels.  The distribution of

dopamine terminals (stipled) and cell bodies (dots) at each level are

indicated on the right hand side while the anatomical location of

some of the areas examined within the thesis are indicated on the

left.

since these cells project in the same manner as the nigral cells they are generally regarded as the caudal extension of the nigral dopamine cell group (Björklund and Lindvall, 1978, 1984; Ungerstedt, 1971; Nauta et al. 1978). Similarly, the non-nigral cells are not restricted to the ventral tegmental area but form a band medial to the substantia nigra which extends from the level of the supramammillary region caudal to the level of the superior cerebellar peduncles (Björklund and Lindvall, 1984). Recently it has been proposed that the separation of mesencephalic dopamine cells into nigral and non-nigral may be an artificial distinction (Björklund and Lindvall, 1984, see Table 1). These authors have argued that there are no clear anatomical boundaries between nigral and non-nigral cells. Moreover, a number of brain areas receive their dopaminergic input from both the substantia nigra and ventral tegmental area (Fallon and Moore, 1978; Björklund and Lindvall, 1978). The entire mesencephalic dopamine system is, therefore, considered to be an entity having a crude topographical order of projections to striatal, limbic and cortical areas (Lindvall and Björklund, 1984; Nauta and Domesick, 1984; Fallon and Moore, 1978; Loughlin and Fallon, 1984). However, it should be recognised that nigral cells send collaterals to a variety of brain regions, including the globus pallidus (Lindvall and Björklund, 1979, 1982) and subthalamic nucleus (Lindvall and Björklund, 1984; Campbell et al. 1985). By contrast, dopamine cells emanating from the ventral tegmental area appear to innervate only one brain area (Fallon 1981; Loughlin and Fallon, 1984).

Views of the mesencephalic dopamine system has recently been revised to encompass the concept of a 'ventral striatum' (Heimer and

Wilson, 1975). According to this view of the forebrain, the striatum can be regarded as comprising a dorsal (motor) division and a ventral (limbic) portion. The latter including not only the ventromedial portion of the caudate nucleus but also the nucleus accumbens, olfactory tubercle and portions of the amygdala lateral, central and basolateral nuclei inter alia (Heimer and Wilson, 1975; Kelly et al. 1982; Nauta and Domesick, 1984) (Table 1). Recent studies have suggested that the ventral striatum receives its dopaminergic innervation from the ventral tegmental area whereas the dorsal striatum is supplied by the A9 cells of the substantia nigra (Björklund and Lindvall, 1984).

b)    Mesolimbocortical Projections

The mesolimbocortical dopamine system comprises projections to limbic (septum, lateral habenular nucleus), allocortical (piriform cortex, amygdala and hippocampus) and neocortical (suprarhinal, supragenual pregenual and entorhinal cortices) brain areas.

Evidence for a catecholamine innervation of the lateral septum was first provided by Fuxe (1965). Though, this innervation was originally thought to be noradrenergic, more and more evidence has accrued which indicates that this innervation is in fact the terminal distribution of dopamine fibres originating in the  medial part of the ventral tegmental area (Assaf and Miller, 1977;  Carter and Fibiger, 1977;  Lindvall and Steveni, 1978). Almost all of the dopaminergic input to the septum is restricted to the lateral nucleus (Lindvall and Steveni, 1978). Morphologically, septal neurons appear to be of two distinct types:  one slender and varicose, that forms a dense band outlining the fornix, and the other, smooth with few

TABLE 1


ORIGINS AND PROJECTIONS OF THE MESOTELENCEPHALIC DOPAMINE SYSTEM.

| System | Origin(s) | Terminal Area |
|---|---|---|
| Nigrostriatal | | |
| Dorsal | A9 | Dorsal Caudate Nucleus |
| | | Globus Pallidus |
| | | Subthalamic Nucleus |
| Ventral | A8 | Ventroposterior Caudate Nucleus |
| | A10 | Anteromedial Caudate Nucleus |
| | | Olfactory Tubercle |
| | | Bed Nucleus of Stria Terminalis |
| | A10, A9, A8 | Nucleus Accumbens |
| Mesolimbocortical | | |
| | A10 | Lateral Septum |
| | A10, A9 | Amygdala |
| | | Olfactory Bulb |
| | | Piriform Cortex |
| | | Hippocampus |
| | A10, A8 | Entorhinal Cortex |
| | A10 | Lateral Habenula |
| | A10 | Dorsal Raphe Nucleus |
| | A10, A9 | Locus Coeruleus |
| | A10 | Suprarhinal Cortex |
| | | Pregenual Cortex |
| | A9 | Supragenual Cortex |

Modified from Björklund and Lindvall, 1984.

varicosites which form basket-like arrangements around the cell
bodies and proximal dendrites of the dorsal portion of the lateral
septum (Lindvall and Björklund, 1978 a,b). However, in a recent
immunocytochemical study no differences in the ultrastructure of the
dopamine immunoreactive profile of these two cells were observed
(Onteniente et al. 1984).

A discrete plexus of dopamine fibres terminates in the medial
portion of the lateral habenula nucleus (Lindvall et al. 1974;
Hökfelt et al. 1976; Phillipson and Pycock, 1982; Skagerberg et al.
1984). Studies with anterograde and retrograde tracers have
indicated that the dopaminergic innervation of the lateral habenula
originates in the ventral tegmental area (Herkenham and Nauta, 1977;
Phillipson and Griffith, 1980; Swanson, 1982; Skagerberg et al.
1984); a view which is supported by the loss of dopamine
fluorescence in the lateral habenula following lesions of the ventral
tegmental area (Kizer et al. 1976; Phillipson and Pycock, 1982;
Skagerberg et al. 1984). However, it should be emphasised that only
a relatively minor portion of the mesohabenular projection is
dopaminergic and that the habenula is also innervated by the
periventricular dopamine system. It is, however, important to note
that the lateral habenula is connected via polysynaptic pathways to a
number of brain areas which receive a more extensive dopaminergic
input (viz caudate nucleus, prefrontal and cingulate cortices and
nucleus accumbens).

Biochemical studies have shown that bilateral 6-hydroxydopamine
lesions of the noradrenaline input to the hippocampus resulting in an
almost complete depletion of hippocampal noradrenaline, has little

effect upon the levels of DOPAC within this allocortical structure
(Bischoff et al. 1979). However, if these lesions are combined with
destruction of the ventral mesencephalon a significant reduction of
DOPAC is observed in hippocampal tissue (Scatton et al. 1980). The
presence of specific uptake mechanism for dopamine (Scatton et al.
1985) and of high affinity binding sites for dopaminergic ligands
(Bernado and Prince, 1982; Bischoff et al. 1980; Dawson et al.
1985; Ouimet et al. 1984). However, so far, anatomical studies have
failed to demonstrate the presence of dopaminergic nerve terminals in
the hippocampus.

The existence of dopaminergic neurons in cerebral cortex was
first suggested by Thierry and her colleagues (Thierry et al. 1973
a), who found that lesions of cortical noradrenergic systems had
little effect on cortical dopamine levels. Subsequently, reports
from the same laboratory demonstrated the continued synthesis of
dopamine in neocortex following lesions of noradrenergic systems
(Thierry et al. 1973b, Berger et al. 1974). Shortly, thereafter,
specific high affinity dopamine uptake sites (Tassin et al. 1974) and
dopamine sensitive adenylate cyclase in cortical tissue (von Hungen
and Roberts, 1973; Bockaert et al. 1977) were demonstrated.

The dopaminergic innervation of frontal neocortex is formed
from the terminal ramifications of three mesotelencephalic projection
systems: suprarhinal, pregenual and supragenual systems (Berger et
al. 1976, 1985; Berger, 1977; Beckstead, 1976; Björklund and
Lindvall, 1978; Divac et al. 1978 a,b). It was soon recognised that
these cortical areas also receive major anatomical projections from
the mediodorsal thalamic nucleus and so correspond to areas of

prefrontal cortex (Divac et al. 1978 a,b). The dopaminergic innervation of prefrontal cortex has been observed in a variety of mammalian species, including man (Berger et al. 1977; Divac et al. 1978 a). However, recent behavioural and hodological studies have indicated that in the rat it is the pregenual cortex (the area corresponding to Broadmans´ area 32: Kreig, 1946) which corresponds to the primate prefrontal cortex whilst the supragenual cortex (Area 24) is more correctly termed anterior cingulate cortex (Leonard, 1969; Krettel and Price, 1977; Berger et al. 1985). The dopaminergic projection to pregenual cortex terminates predominately in the deep cortical areas (Berger et al. 1974, 1976; Lindvall et al. 1974, 1978), whereas the dopaminergic innervation of the anterior cingulate cortex terminates in two distinct fields; a deep subfield which appears to be the caudal extension of the prefrontal system, and a superficial subfield in cortical layers I-III (Hokfelt et al. 1974, 1977; Lindvall and Björklund 1974, 1978; Berger et al. 1985). Moreover, the dopaminergic innervation of the supra and pregenual cortices develop at different rates and arise from two anatomically distinct loci in the ventral mesencephalon (Lindvall et al. 1978; Lindvall and Björklund, 1984 a,b). On account of these differences it has been suggested that the prefrontal and supragenual dopamine systems may subserve different functional roles (Björklund and Lindvall, 1984; Berger, 1985).

The dopaminergic innervation of suprarhinal cortex bears many similarities to that found in the pregenual system: originating in the mesencephalic dopamine cells of the A10 area and terminating in the deep cortical layers of suprarhinal cortex. It too receives its

thalamic innervation primarily from the mediodorsal cortex and so is classified as prefrontal cortex (Divac et al. 1978 a,b).

A dopaminergic projection system from the ventral tegmental area and to a lesser extent from the retrorubral area (A8) terminates in a series of digit-like clusters in the ventral entorhinal cortex (Lindvall et al. 1974 a; Fallon and Moore, 1978 b; Swanson, 1982).

## 1.2.2 Diencephalic Dopamine System

While the mesotelencephalic system is generally associated with behavioural responsiveness and the initiation of movement, the dopamine cells of the diencephalon are believed to play an important role in neuroendocrine function (Björklund and Lindvall, 1984; Kaiser and Jain, 1985 for reviews). With the recent discovery of a diencephalospinal dopamine system it has been suggested that the dopamine systems of the diencephalon may also play a role in nociception, the regulation of sympathetic outflow, and the control of motor function at the level of the spinal cord (Björklund and Lindvall, 1984).

The descending diencephalospinal pathway originates primaily in the A11 cell group and terminates in clusters around preganglionic sympathetic neurones in the intermediate spinal gray and interomedialateral column of the spinal cord. High densities of dopamine neurones are also found within the central canal at the thoracic and upper lumber levels. Perikyra from the A11 cell group gives rise to ascending neurones innervating the habenula, dorsal hypothalamus and areas of the thalamus (pretectal area, parafascicular and paraventricular nuclei) (Lindvall et al. 1974 b, Björklund and Lindvall, 1984). From studies by Lindvall and his

associates (1974 b) it has been suggested that these ascending projections of the periventricular system may be formed from collaterals of the diencephalospinal system (Björklund and Lindvall, 1984).

A system of short intradiencephalic dopamine projections (incertohypothalamic system) has been described which arises in the zona incerta and areas of the hypothalamus (Lindvall and Björklund, 1974). The fibers of this system are distinguished from other diencephalic systems, being extensively arborised and weakly fluorescent. The similarities between the incertohypothalamic dopamine system and the dendritic network found in the substantia nigra has led to speculation that this system may represent the dendritic projections of the perventricular and spinal projection systems (Björklund and Lindvall, 1984).

The tuberoinfundibular systems arises in the arcuate nucleus and in the adjacent A12 cells of the paraventricular nucleus of the hypothalamus, and projects in an ordered manner to the median eminence and pituitary (pars intermedia and neural lobe) (Fuxe, 1976).

The tuberal dopamine system has been implicated in the regulation of a number of adenohypophyseal hormones, most notably prolactin and melanocyte stimulating hormone (MSH). Increases in the circulating concentrations of these hormones selectively stimulate the activity of tuberal dopamine neurones. In turn, dopamine exerts a direct inhibitory action on the release of prolactin from the anterior pituitary and MSH from the pars intermedia (see Björklund and Lindvall, 1984 for discussion). The tuberohypophyseal dopamine

system has also been implicated in the control of other pituitary hormones including, growth hormone, thyroid stimulating hormone, luteinizing hormone and gonadotropin releasing hormone (Kaiser and Jain, 1985; Björklund and Lindvall, 1984 for reviews).

Control of hormone release from the pituitary may also be exerted by dopamine systems outwith the tuberoinfundibular system. Recent electrophysiological and pharmacological studies have suggested that dopamine can act not only at the level of the posterior pituitary, but at the level to stimulate the release of vasopressin and oxytocin but also at the level of the cell bodies of these neurosecretory neurones (i.e paraventricular and supraoptic nuclei) (Björklund and Lindvall, 1984).

1.2.3    Dopamine Systems in the Cerebral Vasculature

The presence of dopamine receptors in the cerebral vasculature is widely accepted (Goldberg et al. 1978; Brodde, 1982; Goldberg and Kohli, 1983). However, whether cerebral vessels receive a functional innervation from dopaminergic neurons, or whether the dopamine receptor mechanisms found on cerebral arteries are of purely pharmacological interest remains to be established.

Dopamine is widely distributed in peripheral vascular tissue as a biosynthetic precursor of noradrenaline (Goldberg et al. 1978), Kaiser and Jain, 1985). In these vascular tissues endogenous dopamine accounts for less than 4% of the total catecholamine content (Bell and Gillespie, 1981). In contrast, dopamine accounts for up to 60% of the total catecholamine content of rat and bovine cerebral

microvessels (Head et al. 1980). Although the relatively high proportion of dopamine in cerebral vessels would be consistent with a dopaminergic innervation; the findings could equally be explained by the presence of non-innervated storage sites (i.e. mast cells). To obtain definitive evidence for a dopaminergic innervation of the cerebral vasculature would require the histochemical demonstration of processes from dopaminergic neurons making synaptic connections with cerebrovascular smooth muscle. Dendrites from monoamine-containing neurons in the primate substantia nigra and raphe nuclei have been shown to make direct contact with the basement membrane of small arterioles and capillaries (Felten and Crutcher, 1979). Unfortunately, the histofluorescence technique employed (that of Falck and Hillarp, 1964) does not discriminate between dopamine containing neurons and those containing noradrenaline or serotonin (Björklund and Lindvall, 1984). Since both serotoninergic and dopaminergic perikayra have been localised within both the substantia nigra and raphe nuclei (Steinbush, 1984) it is not possible to confirm the identity of these neurons.

The recent development of specific antibodies to dopamine (Geffard et al. 1984) and serotonin (Steinbush, 1984) offers the possibility of discriminating between catecholamine neurotransmitters. Specific antibodies raised against dopamine have been used to examine the possibility of a dopaminergic innervation of the rat cerebral vasculature. Preliminary reports have indicated that dopaminergic neurones within the substantia nigra and raphe nuclei do innervate cerebral arterioles (Steinbusch, 1986). Whether dopamine neurones in other brain areas innervate blood vessels remains to be determined.

## 1.3 Dopamine Receptor Pharmacology

### 1.3.1 Central and Peripheral Actions of Dopamine

Under normal physiological conditions dopamine does not cross the blood-brain barrier (Birkmayer and Hornykiewicz, 1961; Weiner, 1980; Kaiser and Jain, 1985). Consequently, the pharmacology of central dopamine system must be inferred from the actions of indirect sympathomimetics (1-dopa, methylphenidate, amphetamine) or agents which interact with dopamine receptors.

L-dopa, the metabolic precursor of dopamine readily crosses the blood-brain barrier into the brain where it is rapidly taken up into cells and converted into dopamine (Calne and Sandler, 1970; Holtz, 1959). In animals, the administration of 1-dopa evokes a characteristic range of responses. These actions include increased locomotor activity (Bartholini et al. 1969), and the reversal of reserpine-induced catalepsy (Carlsson et al. 1957) inter alia. In patients 1-dopa alleviates the motor deficits associated with Parkinson's disease although up to 1/3 of patients experience nausea and vomiting and abnormal involuntary movements, mainly buccolingual dyskinesias (Barbeau et al. 1971).

The indirect sympathomimetic agents amphetamine 1-dopa, and methylphenidate are thought to elicit their behavioural responses by an action primarily on central dopamine systems (Munkvad et al. 1968; Ross 1979). In rats the excitatory behaviours elicited by these agents is characterised by grooming, sniffing and licking at low concentrations with intense stereotyped gnawing and biting at higher concentrations. All these behavioural responses have been attributed

to the enhancement of dopaminergic activity within the striatum (Munkvad et al. 1968; Costall and Naylor, 1981; Seeman, 1981).

There is now considerable evidence to suggest that the aporphine derivative, apomorphine acts primarily as a dopamine receptor agonist (for reviews see Seeman, 1981; Neumeyer et al. 1981; Costall and Naylor, 1981; Kaiser and Jain, 1985; Kebabian and Calne, 1979; Creese et al. 1983 a,b).

Results from in vivo studies show that apomorphine provokes behavioural and biochemical responses characteristic of dopamine receptor activation (viz. contralateral rotation in 6-hydroxydopamine lesioned rats, stereotyped behaviours, emesis, reduction in the rate of dopamine turnover, and inhibition of prolactin release). In vitro, apomorphine displaces $^3$H dopamine from homogenates of rat and bovine striata (Seeman, 1981), inhibits the release of acetylcholine and activates dopamine sensitive adenyl cyclase (Greengard, 1974; Anden et al. 1967; Kebabian et al. 1972; Setler et al. 1978). Despite its potent behavioural and endocrine actions apomorphine displays only weak agonist effects on vascular dopamine receptors (Goldberg, 1978; Brodde, 1982) or in the dopamine sensitive adenyl cyclase assays (Kebabian et al. 1972; Greengard, 1974; Goldberg, 1978). Anomolous pharmacological profiles were also reported for the ergot derivatives bromocryptine and lergotrile (Lew et al. 1977; Spano et al. 1979). In addition to their marked adrenergic actions these agents possess many dopamine-like actions (stereotypy, reduction in prolactin release and contralateral rotation in rats with 6-hydroxy-dopamine induced lesioned). However, both bromocryptine and lergotrile failed to dilate the renal vasculature and behaved as antagonists of dopamine stimulated adenyl cyclase (Lew et al. 1977; Spano et al. 1977, 1979). Taken together these

findings appear inconsistent with the view that dopamine receptor agonists effect their behavioural and physiological actions through a single population of cyclase linked dopamine receptors. Anomolous actions on dopamine mechanisms have also been reported for the neuroleptic agents. In particular the substituted benzamide derivative, sulpiride (Spano et al. 1979) displays many of the characteristics of classical neuroleptics such as inhibition of apomorphine-induced changes in locomotor activity, production of catalepsy and increases in the brain concentrations of HVA and DOPAC have been reported for sulpiride. Yet this drug has minimal effects on dopamine sensitive adenylate cyclase (Spano et al. 1979).

1.3.2   Concept of Dopamine Receptor Subtypes

The concept of multiple dopamine receptors was first suggested by York In 1970. Subsequently, the existence of multiple classes of dopamine receptors has frequently been preferred to account for the often disparate actions of dopamine-like agents under a variety of experimental conditions. However, the variety of experimental approach employed has resulted in a bewildering array of dopamine receptor terminologies. Indeed in the intervening years there have been sone ten different classification systems, each porrecting from one to as many as four receptor subtypes (for reviews see Kebabian and Calne, 1979; Costall and Naylor, 1981; Goldberg and Kohli, 1981; Seeman, 1981). Fortunately, there is emerging a consensus of opinion that in both central and peripheral systems dopamine receptors exist as two pharmacologically distinct entities, and, although some dubiety has persisted as to whether the dopamine receptors of cerebral tissue are identical to those on vascular

smooth muscle (Goldberg and Kohli, 1981, 1983) there is now a growing acceptance that the receptors at both sites are the same (Kebabian et al. 1984; Goldberg et al. 1986).

As this thesis is primarily concerned with the effects of dopaminergic mechanisms on the relationship between cerebral blood flow and metabolic rate, and examines the characteristics of the dopamine receptors on both vascular and neural tissues it is imperative that there is conformity in the classification system used to describe both sites.

a)      Dopamine Receptors in Cerebral Tissue

In 1979 Kebabian and Calne presented evidence for the existence of two distinct subclasses of dopamine receptor in cerebral tissue based on the ability of dopamine receptor agonists and antagonists to interact with adenylate cyclase. This classification system was proposed by Kebabian in 1978 but using an alpha, beta terminology analogous to that of the adrenergic system. This terminology was discontinued to prevent confusion with the adrenergic system. In this scheme, the stimulation of one receptor, designated $D_1$, provoked and increase in adenyl cyclase activity whereas the second receptor, designated $D_2$, appeared to be unrelated to the activity of adenyl cyclase. This classification was subsequently modified to include the possibility of a dopamine $D_2$ receptor mediating inhibition of cyclase activity (Tsurata et al. 1981; Stoof and Kebabian, 1981). Release of parathyroid hormone was associated with the $D_1$ receptor activation whereas inhibition of prolactin release and apomorphine induced emesis in dogs were attributed to $D_2$ mediated effects. Based on these criteria the dopamine receptor agonist apomorphine behaved

as a weak agonist at the $D_1$ site while having full and potent agonist actions at the $D_2$ site, whereas the benzamide class of neuroleptics, particularly sulpiride, were potent inhibitors and considered to be prototype $D_2$ receptor antagonists.

Ligand bindings studies have obvious attractions. They are simple to perform and permit direct measurements of agonist – antagonist interactions. Furthermore, when used in conjunction with selective lesioning techniques binding studies permit the identification and separation of pre- and postsynaptic receptors and the examination of supersensitivity phenomena. Consequently, within six years of the introduction of $^3$H-haloperidol and $^3$H-dopamine (Seeman et al. 1975) as the first dopamine ligands, a "state of the art" review could report the binding characteristics of dopamine receptors as defined by 30 different ligands; encompassing over twelve hundred publications (Seeman, 1981). However, as Seeman observed the literature was far from consistent with some authors reporting up to five different dopamine receptors; a fact which was complicated further by the use of confusing and often contradictory terminologies (see Table 2).

Fortunately, much of the controversy has been resolved with the realisation that both the $D_1$ and $D_2$ can exist as two interconvertible states whose affinity can be regulated by guanyl nucleotides (Sibley et al. 1982; Leff and Creese, 1983; Caron et al. 1982). GTP-dependent alterations in affinity have also been reported in the adenylate cyclase system (Kebabian et al. 1979) and it has recently been reported that $D_2$ mediated inhibition of adenylate cyclase correlates with the high affinity $D_2$ site (Creese et al. 1983) in the anterior pituitary (McDonald et al. 1984).

Thus, there would appear to be a convergence between dopamine

TABLE 2

DOPAMINE RECEPTOR CLASSIFICATION SYSTEMS 1982-1986.

**1982**

| Authors | Approach | Classification | | | | |
|---|---|---|---|---|---|---|
| Kebabian and Calne | Cyclase | $D_1$ | | $D_2$ | | |
| Goldberg et al. | Drug Potency Series | | $DA_1$ | | | $DA_2$ |
| Creese et al. | Ligand Binding | $D_1$ | | $D_2$ | $D_3$ | $D_2^{high}$ |
| Seeman et al. | Ligand Binding | $D_1$ | $DA_1$ | $D_2$ | $D_3$ | $D_4$ |
| Sokoloff et al. | Ligand Binding | $D_1$ | | $D_4$ | $D_3$ | $D_2$ |

Adapted from Seeman, 1982.

**1986**

| Authors | Approach | Classification | | |
|---|---|---|---|---|
| Kebabian et al. | Cyclase | $D_1$ | | $D_2$ |
| Goldberg et al. | Drug Potency Series | $DA_1$? | | $DA_2$ |
| Creese et al. | Ligand Binding | $D_1^{low}$  $D_1^{high}$ | $D_2^{low} \rightleftarrows D_2^{high}$ | |
| Seeman et al. | Ligand Binding | $D_1^{low}$  $D_1^{high}$ | $D_2^{low} \rightleftarrows D_2^{high}$ | |
| Sokoloff et al. | Ligand Binding | $D_1$ | $D_2^{low} \rightleftarrows D_2^{high}$ | $D_4$ |

From Creese and Leff, 1986; Goldberg et al. 1986; Protais et al. 1986;

Seeman et al. 1985, 1986; Stoof and Kebabian, 1984.

receptor terminologies as characterised by ligand binding or cyclase
activation models (Table 2).


b)    Dopamine Receptors in the Peripheral Vasculature


The existence of specific dopamine receptors have been
demonstrated on vascular and gastrointestinal smooth muscle, on
postsynaptic sympathetic nerves and on autonomic ganglia (Goldberg et
al. 1978; Goldberg and Kohli, 1981). Dopamine receptors at these
sites have also been subdivided into two distinct populations by
examining the potency series of a large number of agonists and
antagonists (Goldberg and Kohli, 1979). Thus one receptor,
designated the $DA_1$ receptor, mediated smooth muscle relaxation while
the $DA_2$ subserved the presynaptic inhibition of noradrenaline release
from sympathetic nerve terminals (Golberg and Kohli, 1979).

Considerable overlap exists between the classification system
of Goldberg and Kohli (1979) and that proposed by Kebabian and Calne
(1979). The order of potency for agonists at the $DA_1$ receptor (A-
6,7,DTN = Dopamine > epinine > dipropyldopamine > apomorphine) is
similar to that reported for adenyl cyclase activation (Goldberg et
al. 1978; Kebabian and Calne, 1979; Goldberg and Kohli, 1984).
Apomorphine is a weak partial agonist at both $D_1$ and $DA_1$ while
exerting potent agonist effects at both $D_2$ and $DA_2$. Emesis and the
inhibition of prolactin secretion can also be mediated by drugs
acting via receptors designated $D_2$ and $DA_2$ in the two
classifications.

The major source of controversy between the two receptor
classification systems concerns the role of sulpiride in antagonising
the effects of dopamine. Sulpiride, a potent antagonist at the $D_2$

receptor has little effect on dopamine-stimulated adenyl cyclase (Kebabian and Calne, 1979;  Langer and Dubocovich, 1979).  In contrast, sulpiride inhibits both $DA_1$ and $DA_2$ receptors (Goldberg and Kohli, 1981).  The underlying reason for the discrepancy  between the two classification systems may lie in the different experimental approaches used to characterise the two receptor subtypes.  The classification system of Kebabian and Calne (1979) was based primarily on studies in vitro whereas that of Goldberg and Kohli (1979) is based upon investigations performed in vivo.  However, when sulpiride was examined in isolated vessel preparations it displayed extremely weak antagonistic properties at the $DA_1$ receptor (Hilditch and Drew, 1981;  Schmidt and Imbs, 1980).

1.3.3  Agents Discriminating Between $D_1$ and $D_2$ Receptors

The investigation of dopamine receptor subtypes has been greatly facilitated by the recent development of pharmacological agents which can discriminate between the two dopamine subpopulations.

Selective $D_1$ Receptor Agonists

The most selective $D_1$ receptor agonist presently available is 2,3,4,5, tetrahydro-7,8-dihydroxy-1-phenyl -1H-3-benzazepine (SKF 38393 (Figure 2).

In the dopamine sensitive adenyl cyclase system SKF 38393 displays a higher affinity than dopamine or the classical dopamine receptor agonist apomorphine (Setler et al. 1978;  Stoof and Kebabian, 1981, 1982;  Scatton, 1982);  however, like apomorphine,

FIGURE 2

## Dopamine Receptor Agonists
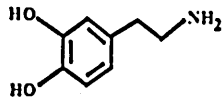


Dopamine
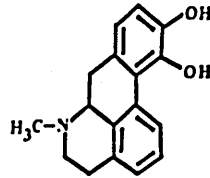
Apomorphine
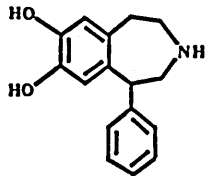
SKF 38393
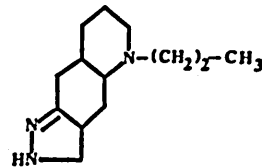
LY 141865

## Antagonists
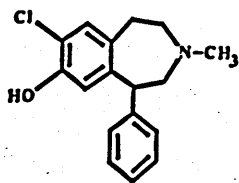


SCH 23390
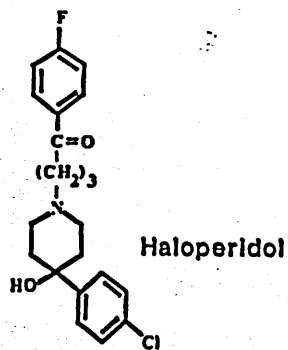
Haloperidol

Chemical structures of the dopamine receptor agonists and antagonists studied within this thesis.

SKF 38393 displays only partial agonist activity at the cyclase (Setler et al. 1978). Both SKF 38393 and apomorphine promote contralateral circling in rats with lesions of the nigrostriatal dopamine pathway (Setler et al. 1978; Gershanik et al. 1983). In contrast to apomorphine SKF 38393 does not promote the characteristic physiological or behavioural responses normally associated with the administration of dopamine receptor agonists (Setler et al. 1978), although a mild behavioural stimulation characterised by an increased incidence of sniffing and grooming has been reported (Waddington et al. 1982; Molloy and Waddington, 1983, 1984).

Biochemical studies, in vitro, have provided additional evidence in support of the $D_1$ selectivity of SKF 38393. Even at concentrations 1000 fold greater than required to stimulate adenyl cyclase, SKF 38393 fails to affect striatal acetylcholine turnover (Scatton, 1982; Stoof and Kebabian, 1982; Plantje et al. 1983). In vivo SKF 38393 has no effect on striatal dopamine or acetylcholine turnover at concentrations of 30 mg/kg).

Similarly, SKF 38393 relaxes vascular smooth muscle by acting as a partial agonist at the vascular $D_1$ receptor (Pendleton et al. 1978; Hahn and Wardell, 1980; Schmidt et al. 1982; Brodde, 1982). These vasomotor responses appear to be selectively mediated via the $D_1$ receptor as they are not affected by muscarinic, $\alpha$ or $\beta$-adrenergic, or histamine receptor antagonists (Hahn and Wardell, 1980; Pendleton et al. 1978).

A structural analogue of SKF 38393, fenoldopam (SKF 82526) is a full and potent agonist at the vascular dopamine receptor (Hahn et al. 1982; Lockhandwala and Steenberg, 1984); however, its poor penetration of the blood brain barrier and doubts as to its selectivity (Sibley et al. 1982; Stoof and Kebabian, 1984) make it

less suitable for investigating the effects on cerebral function of $D_1$ receptor stimulation.

## Selective $D_2$ Agonists

The partial ergdine derivative trans($\pm$)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-2H-pyrozolo-[3,4g] quinoline (LY 141865, Figure 3) displays many of the behavioural and biochemical actions characteristic of dopamine receptor agonists, however, unlike apomorphine LY 141865 does not stimulate adenylate cyclase (Table 3). LY 141865 also discriminates between the $DA_1$ and $DA_2$ in vascular tissues.

Studies in peripheral and neural tissues have demonstrated that LY 141865 displays little affinity for adrenergic, muscarinic, histamine and serotonin receptor (Hahn and McDonald, 1984 b; Hahn, et al. 1982; Ruffolo and Shaar, 1983; Wong et al. 1983; Cohen et al. 1984).

## Selective $D_1$ Antagonists

The first selective $D_1$ antagonist was SCH 23390, the 7-chloro derivative of SKF 38393 (Iorio et al. 1981). SCH 23390 displays many of the actions of a "typical" neuroleptic in behavioural tests. It suppresses conditioned avoidance responses in rats and primates (Iorio et al. 1983), blocks the stereotyped produced by a variety of dopamine receptor agonists (Iorio et al. 1983; Christensen et al. 1984 a,b; Mailman et al. 1984; Molloy and Waddington, 1984) and protects against lethal doses of methamphetamine (Iorio et al. 1983). However, unlike "classical" dopamine receptor antagonists SCH 23390

Selectivity of Dopamine Receptor Agonists

| | SKF 38393 | Apomorphine | LY 141865 |
|---|---|---|---|
| **Central Actions** | | | |
| Adenyl Cyclase | 75 nM (partial agonist)[29] | 810 nM (partial agonist)[29,10] | Inactive[33] |
| Dopac/HVA Turnover | Inactive[24,29,30] | Decrease[1,34] | Decrease[24,32] |
| Acetylcholine Turnover | Inactive[22,24,30] | Increase[11,25,23] | Increase[5,22,24,30] |
| Stereotypy | Inactive[29] | Stereotypy[19] | Stereotypy[32] |
| Emesis | Inactive[29] | Emesis[19] | Emesis[32] |
| Prolactin | Inactive[29] | Decrease[19] | Decrease[32] |
| **Ligand Binding** | | | |
| $^3$H-flupentixol (D$_1$) | 158 nM*[4] | 940 nM*[4] | Inactive[18] |
| $^3$H-Spiperone (D$_2$) | 33, 270 nM[20] | 400-500 nM[23,28] | 650 nM[21] |
| **Peripheral Actions** | | | |
| Vascular Relaxation (DA$_1$) | Partial Agonist[2,15,26] | Partial Agonist[2,6,15] | Inactive[8,14,16] |
| Presynaptic Inhibition of Synaptic Transmission | Inactive[26] | Active[3,7,16] | Active[12,13,14,17] |

*Low Affinity Conformation.

Cont./

# References

1. Bacopoulos and Roth, 1980.
2. Brodde, 1982.
3. Buylaert et al. 1977.
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5. Fuller et al. 1983.
6. Goldberg, 1972.
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8. Goldberg and Kohli, 1983.
9. Gower and Mariott, 1982.
10. Greengard, 1974.
11. Guyenet et al. 1975.
12. Hahn and McDonald, 1984 a.
13. Hahn and McDonald, 1984 b.
14. Hahn et al. 1982.
15. Imbs et al. 1981.
16. Lokhandwala and Steenberg, 1983.
17. Lokhandwala and Steenberg, 1984.
18. McKenzie and Zigmond, 1985.
19. Neumeyer et al. 1981.
20. O'Boyle and Waddington, 1984.
21. Rabey et al. 1981.
22. Plantje et al. 1983.
23. Scatton, 1981.
24. Scatton, 1982.
25. Scatton et al. 1982.
26. Schmidt et al. 1986.
27. Seeman, 1981.
28. Seiler and Markstein, 1984.
29. Setler et al. 1978.
30. Stoof and Kebabian, 1982.
31. Titeler, 1981.
32. Titus et al. 1983.
33. Tsurata et al. 1981.
34. Westerink, 1978.

does not cause hyperprolactinaemia or prevent apomorphine - induced emesis in dogs (Iorio et al. 1983; Christensen et al. 1984 a).

Biochemical studies indicated that SCH 23390 inhibits $D_1$ mediated cyclase activation at low nanomolar concentrations (Iorio et al. 1983; Hyttel, 1983; Itoh et al. 1984) whereas concentrations 100 fold higher are required to reverse $D_2$ mediated inhibition of adenyl cyclase (Itoh et al. 1984).

Ligand binding studies suggest that SCH 23390 is even more selective for $D_1$ receptors. Competititon studies using ^{3}H-piflutixol and ^{3}H-spiperone as ligands for the $D_1$ and $D_2$ receptor population respectively report a preference for the $D_1$ site which ranges from 500 to around 1500 fold (Hyttel et al. 1983; Christensen et al. 1984 a; Cross et al. 1983 b; O'Boyle et al. 1984). The $D_1$ selectivity of SCH 23390 is also attested by the report that chronic treatment with cataleptic doses of SCH 23390 up-regulate $D_1$ but not $D_2$ receptors within the rat striatum (Creese and Chen, 1985).

A similar degree of selectivity has been reported for vascular dopamine receptors. SCH 23390 is a potent inhibitor of the vascular $DA_1$ receptor (Hilditch et al. 1984; Goldberg et al. 1984) but is ineffective at $DA_2$ receptors at concentrations up to 200 fold higher (Hilditch et al. 1984).

In addition to being a very weak $D_2$ antagonist SCH 23390 displays little affinity for $a_1$ and B adrenergic receptors, muscarinic, histamine or benzodiazepine receptors (Hyttel 1983; Cross et al. 1983 b, Goldberg et al. 1984; Christensen et al. 1984 b). An affinity at $5HT_2$ and $a_2$ receptors has been demonstrated, but at concentrations more than 20 fold higher than that required to block the $D_1$ receptor (Hyttel et al. 1983; Cross et al. 1983; Ohlstein and Berkowitz, 1985).

## 1.3.4 Dopamine Receptors Subtypes in the Cerebral Vasculature

### a) In Vitro Studies

In both peripheral and cerebral vessels the contractile activity of dopamine is mediated via $\alpha$-adrenergic (Goldberg et al. 1978; Toda, 1976) and serotoninergic mechanisms (Edvinsson et al. 1978). However, if these contractile effects provoked by dopamine are blocked by high doses of phenoxybenzamine a dose-related relaxation can be demonstrated in the cerebral and in a number of other vascular beds (Goldberg and Toda, 1975; Goldberg et al. 1978; Brodde, 1982).

In the isolated cat middle cerebral artery pretreated with phenoxybenzamine, dopamine and a number of dopamine receptor agonists promote a dose-related relaxation of feline cerebral arteries (Edvinsson et al. 1978). The potency of these agonists was of the order; epinine > dopamine > apomorphine > piribedil; data consistent with a $D_1$ mediated relaxation. Furthermore, dopamine-induced relaxation could be attenuated by bulbocapnine, a predominately $D_1$ antagonist, thereby providing tentative evidence for the existence of a dopamine $D_1$ receptor mediating the relaxation of feline cerebral vessels.

Similarly, a dopamine $D_1$ receptor mediated relaxation of isolated human basilar arteries has been proposed (Forster et al. 1982), although in this preparation the selective $D_1$ receptor agonist SKF 38393 was found to be some 88 fold less potent than dopamine.

The evidence for a $D_1$-mediated relaxation of rabbit middle cerebral arteries is not compelling. Using the perfusion approach, Oudart and his colleagues (1981) found that the order of potency for

dopamine receptor agonists was; bromocryptine > dopamine = piribedil > apomorphine. Since both bromocryptine and piribedil have been shown to be inactive at other vascular $D_1$ sites (Goldberg and Kohli, 1981) it would appear that dopamine induced relaxation reported by Oudart et al. (1981) is probably not of the $D_1$ type. Moreover, the small concentrations of dopamine in rabbit cerebral microvessels (Head et al. 1980) would suggest that dopaminergic mechanisms may exert less influence in the cerebral circulation of the rabbit than in other species.

b)    Limitations of In Vitro Studies of Cerebrovascular Reactivity

The use of in vitro methods for investigating cerebrovascular dopamine systems is subject to severe criticism. Firstly, the physiological relevance of dopamine responses is questionable on the grounds that  the large extracerebral vessel used in isolated vessel preparations (middle cerebral or basilar) are not representative of cerebrovasculature as a whole as characterised by their morphological composition (McCulloch and Edvinsson, 1984;  Purves, 1972) or contribution to total cerebrovascular resistance (<7%;  Tuor and Farrar, 1985). Secondly, the necessary inclusion of high concentrations of alpha-adrenergic antagonists (i.e. phenoxybenzamine) in the bathing medium to inhibit contractile mechanisms, may fundamentally compromise attempts to characterise the dopamine receptor subtype mediating relaxation of cerebrovascular smooth muscle. For, in addition to its adrenergic and serotoninergic blocking properties, phenoxybenzamine can interact with a number of other receptor populations including both $D_1$ and $D_2$ dopamine receptors producing complete blockage of $D_2$ sites but only a

partially inhibition at $D_1$ receptors (Marchias and Bockaert, 1980; Creese et al. 1983).

The vasomotor reactivity of cerebral vessels in their normal physiological enrivonment was first examined in the mid 1930's (see Purves, 1972 for historical perspective). Indeed, until the advent of isolated vessel preparations more than 30 years later, cerebrovascular reactivity could be successfully examined only by studying the vessels in situ. However, despite the recent popularity of isolated vessel preparations, in situ studies still offer a number of significant advantages over in vitro methods. The normal physiological tone of vessels in situ, circumvents the need to preconstrict vessels when investigating dilator mechanisms. More importantly, by directly applying pharmacological agents onto small (<250 um diameter) pial arterioles it is possible to characterise receptor populations mediating vasomotor responses on resistance vessels; it is the small arterioles and not the major inflow tract vessels (the vessels most commonly used in isolated vessel preparations) which primarily determine cerebral blood flow.

In the present study, vasomotor responses of small individual arterioles lying on the pial surface of the cat brain to subarachnoid perivascular microapplication of the synthetic dopamine receptor agonists, SKF 38393, LY 141865 and apomorphine were investigated. The resulting hypothesis that dilatation of the feline pial arterioles is mediated via the $D_1$ receptor subtype was subsequently tested using the putative selective $D_1$ receptor antagonist SCH 23390.

## 2.    CEREBRAL BLOOD FLOW/OXIDATIVE METABOLISM AND BRAIN FUNCTION

### 2.1   BASIC CONCEPTS

a)    Blood Flow/Oxidative Metabolism and Brain Function

The vast array of electrical and chemical processes which
sustain the structural and functional integrity of all biological
systems are energy consuming. In most tissues the energy generating
substrate, ATP, can be derived from the oxidative catabolism of a
variety of substrates (viz glucose, lipids, protein). However, under
non-pathological conditions, the blood brain barrier excludes all
potential energy generating substrates, save glucose, from cerebral
tissue (Sokoloff, 1986), with the result that energy generation in
cerebral tissue is derived almost exclusively from the oxidative
catabolism of glucose. In cerebral tissue the reserves of glucose and
oxygen are not sufficient to sustain normal cerebral metabolism for
more than a few minutes (Siesjö, 1978). Consequently, the brain is
entirely dependent upon a continuous supply of substrates by the
blood. An adequate supply of substrate is ensured by homeostatic
mechanisms which adjusts the rate of tissue perfusion to meet
alterations in metabolic demand (Purves, 1972; Sokoloff, 1978 b;
Kuschinsky, 1982 for reviews). Thus the measurement of blood flow or
substrate utilisation (glucose or oxygen utilisation) can be used to
assess metabolic activity in cerebral tissue.

The concept that metabolic activity can be used as an index of
functional status in neural tissue was established by Gerard almost
60 years ago (Gerard, 1927). In studies on the isolated nerve
preparations Gerard demonstrated that oxygen consumption rose in

parallel with increasing firing rate. This relationship between
firing rate and metabolic rate has been established in a variety of
peripheral (Baker, 1965;  Shanes and Berman, 1955;  Larabee and
Klingman, 1962) and central (McIlwain and Bachelard, 1971) tissues in
vitro.  Similarly, qualitative studies in vivo have established that
blood flow is altered with changing functional status.  Illumination
of the retina increases blood flow within components of the visual
system, namely the lateral geniculate (Gerard, 1938) and visual
(occipital) cortex (Schmidt and Hendrix, 1938).  Similarly, increases
in blood flow have been observed within areas of the olfactory or
sensory motor systems following exposure to ammonia vapour or tactile
stimulation (Purves, 1972 for discussion).

The first quantitative evidence that cerebral blood flow (CBF)
and metabolic rate provided indices of functional status was provided
by the nitrous oxide method, the so-called Kety-Schmidt technique
(Kety and Schmidt, 1945, 1948 a).  This approach entailed the subject
inhaling nitrous oxide until equilibrium is reached between the
tissue and blood nitrous oxide concentrations.  Using the Fick
principle it was possible to calculate global cerebral blood from the
concentration of $N_2O$ at saturation and the arteriovenous differences
of the nitrous oxide during the process of saturation.  Steady state
cerebral oxygen consumption $CMR_{O2}$ (or glucose utilisation) could then
be calculated from the arteriovenous difference in oxygen (or
glucose) and the value derived for cerebral blood flow using equation
(1).

$$CMR_{O2} = CBF.(A - V)[O_2] \qquad\qquad (1)$$

In the late 1940's and in the 1950's this approach was widely used to

examine the effects of a variety of functional states upon cerebral blood flow and metabolic rate in man. These studies were able to demonstrate alterations in cerebral blood flow and oxygen consumption in association with gross changes in brain function; such as those associated with senility, diabetic coma, anaesthesia and convulsive states (Kety and Schmidt, 1948 b; Sokoloff and Kety, 1960; Lassen and Christensen, 1967). However, they were unable to detect the more subtle functional changes associated with sleep, sedation, schizophrenia, neuroleptic treatment and amphetamine induced psychosis (Kety, 1950, 1975; Mangold et al. 1955; Sokoloff, 1960). The apparent lack of change in cerebral metabolism under these conditions could be explained by highly focal alterations in functional and metabolic activity which were too small to be detected by global brain measurements (Kety, 1963 ). This view provided the impetus for the subsequent development of techniques for examining focal functional alterations in brain circulation and metabolism.

b)    Local Cerebral Blood Flow

The assumption that a freely diffusible tracer in blood would be in near instantaneous equilibrium with cerebral tissue permitted the measurement of regional levels of blood flow from the rate of accumulation of tracer in each brain region (Kety, 1951). This mathematical advance permitted the development of techniques for measuring hemispheric blood flow from the clearance of radioactive $^{85}Kr$ (Lassen and Ingvar, 1961) and later $^{133}Xe$ (Glass and Harper, 1963); regional blood flow with intracerebral hydrogen electrodes (Purves, 1972); or local cerebral blood flow based upon quantitative autoradiography (Landau et al. 1955; Freygang and Sokoloff, 1958;

Sakurada et al. 1978). In particular, the development of quantitative autoradiographic methods using at first trifluoroiodomethane (CF$_3$I) (Landau et al. 1958), then $^{14}$C-antipyrine (Reivich, 1969) and more recently $^{14}$C-iodoantipyrine (Sakurada et al. 1978) represented a major technological advance by permitting the rates of blood flow to be determined in all the structural components identifiable on the autoradiograms. Using CF$_3$I as a tracer, Sokoloff (1957) demonstrated that retinal stimulation increased local blood flow throughout the neuroanatomical components of the visual system (superior colliculus, lateral geniculate and visual cortex), thus providing the first images of local functional activity within the brain. Subsequently, local cerebral blood flow measurements have been used to detect functional alterations under a variety of physiological and pharmacologically-induced conditions (Sokoloff, 1978; McCulloch et al. 1982). However, it should be emphasised that blood flow is an accurate measure of functional status only if the normal relationship between blood flow and metabolic activity is maintained.

c)    Local Cerebral Oxidative Metabolism

A major disadvantage of the aforementioned local blood flow techniques is their inability to isolate the local venous outflow and so precludes the determination of local metabolic rates. Until the mid 1970's when the 2-deoxyglucose technique was developed no compatible autoradiographic method was available for measuring metabolism. The major problems in developing an autoradiographic technique for measuring local metabolic rates lie partly in the unsuitability of the normal substrates, oxygen and glucose as

radiolabelled tracers and partly in the complex kinetics of cerebral metabolism. The short half life of the radioisotopes of oxygen (t 1/2 = 123 seconds) its rapid conversion to $CO_2$ and equally rapid loss from the tissue makes oxygen a difficult species to use. Although a tomography - based technique employing $^{15}O$-oxygen as the tracer for measuring metabolic rate in humans has been developed (Frakowiak et al. 1980), the resolution capabilities of this technique (aproximately 1.8 $cm^3$ at present) are limiting, particularly when used in small laboratory animals.

A biochemical method for measuring local metabolic rates employing $^{14}C$-glucose was developed in the early 1970's (Hawkins et al. 1974) and subsequently adapted for use with quantitative autoradiography (Hawkins et al. 1979). However, despite a series of modifications to the original model the validity of this technique remains in doubt. The main limitation with this model is in the use of glucose as the indicator substance. Once it has been metabolised to $CO_2$ the radioactive label is rapidly lost from the tissue (Sacks, 1957). Loss of radiolabelled tracer from the could compromise the method by seriously underestimating glucose utilisation. In an attempt to minimise the loss of $^{14}C$ from the tissue proponents of this method have advocated that, the period of glucose use determination should be limited to around five minutes (Lu et al. 1983; Hawkins et al. 1985). It is the necessity to adopt short measurement periods which is the fundamental flaw in the Hawkins method. Quantitative autoradiographic methods of this type calculate reaction rates from the concentration of radiolabelled product which has accumulated in the tissue at the end of the experimental period and the integrated specific activity of the tracer in brain tissue over the measurement period. Since the integrated specific activity

of glucose in cerebral tissue cannot be measured readily in conscious animals the integrated specific activity must be calculated from measurements made in arterial blood and corrected for the lag time in equilibration between blood and brain. In the case of glucose the kinetic parameters associated with the transport of hexoses across the blood brain barrier and into the tissue precursor pool cannot be defined accurately as they vary from region to region and from one experimental condition to another. Over short measurement periods small inaccuracies in estimating the values of the kinetic constants would be of sufficient magnitude to compromise the accuracy of the method. In theory, the contribution of the kinetic constants to the overall measurement could be minimised by extending the measurement period. However, if the time-course of the experiment was extended the accuracy of the measurements would be severely compromised by the loss of $CO_2$ from the tissue. Thus the Hawkins method is of little value in measuring local rates of cerebral glucose utilisation.

The 2-deoxy-D-[$^{14}$C]-glucose molecule has proven to be a more suitable tracer for measuring local metabolic rate. The structural similarities between the molecules of 2-deoxyglucose (2-DG) and glucose enables 2-DG to enter the brain tissues and become metabolised through the first (rate limiting) step of the glycolytic pathway. However, once phosphorylated its metabolic product 2-deoxyglucose 6-phosphate is essentially trapped within the cell over the 45 minute measurement period. From a knowledge of the relative kinetic characteristics of glucose and deoxyglucose it is possible to calculate the local rates of glucose utilisation simultaneously in all components of the CNS visible on the autoradiograms (Sokoloff, 1977).

d)    The Relationship Between Cerebral Blood Flow and Glucose Use

Determination of local rates of cerebral blood flow (Sakurada et al. 1978) and glucose utilisation (Sokoloff et al. 1977) by quantitative autoradiography has established that there is a local relationship between cerebral blood flow and glucose utilisation which is maintained under a variety of experimental conditions (Sokoloff, 1978; McCulloch and Kelly, 1982; Kelly and McCulloch, 1982). However, recent studies have electrical stimulation of the fastigial nucleus results suggested that neuronal activation can effect  increases in cerebral blood flow which are independent of changes in oxidative  metabolism (Nagai et al. 1983;  Iadecola et al. 1985).  Thus, neurogenic influences may provide additional influences on the cerebral circulation.


2.2    Dopamine Systems, Cerebral Blood Flow and Metabolism


a)    Dopamine, Global  Cerebral Blood Flow and Metabolism

By the mid 1970's it had become established that dopamine increases blood flow in renal mesenteric and coronary vascular beds by interacting with specific dopamine receptors on vascular smooth muscle.  Using the $^{85}$Kr clearance approach in dogs von Esssen reported that,  blood flow increased in the cerebrovascular bed following the intravenous infusion of dopamine (von Essen, 1974). Since no concomitant increases in cerebral oxygen consumption could be demonstrated von Essen postulated that dopamine increased cerebral blood flow by acting primarily on the cerebral vasculature.  The discovery of specific dopamine receptors mediating relaxation of

cerebrovascular smooth muscle in the dog (Toda, 1976) and other species (Edvinsson et al. 1978, Forster et al. 1983, Toda, 1983) appeared to support the vascular hypothesis. However, it should be recognised that the von Essen study is flawed on a number of counts. First, as discussed previously, the physical and enzymatic components of the blood-brain barrier prevent dopamine from gaining access to receptors on cerebrovascular smooth muscle (Oldendorf, 1971). Secondly, the measurement of cerebral oxygen consumption requires the sampling of venous blood that is uncontaminated by extracerebral sources; however, the cerebrovascular anatomy of the dog with its numerous anastomotic connections between the intra and extracerebral circulations makes the sampling of uncontaminated venous blood virtually impossible (Purves, 1972). Finally, the variability of the data precluded statistical analysis.

Nevertheless, this study has become accepted into the body of literature and has been cited frequently as evidence that cerebrovascular dopamine receptors influence cerebral perfusion (Amenta et al. 1984; Carlsson and Johansson, 1978; Erkstrøm-Jodal et al. 1982; Goldberg, 1975; Toda, 1976).

In rats the systemic administration of the sympathomimetic agent amphetamine effected a 4-5 fold increase in cerebral blood flow. As this was far in excess of the 40% increase in oxygen consumption observed with this compound: a vascular action was proposed to account for the excessive perfusion (Carlsson et al. 1975; Berntman et al. 1978).

In contrast to the results obtained in the rat the increase in cerebral metabolism observed following the administration of amphetamine to primates was proportionately similar to that of blood flow, thereby implicating that the action of amphetamine was

primarily via alterations in cerebral metabolism (McCulloch and Harper, 1977 a). These authors provided further evidence for the metabolic hypothesis from studies with the dopamine receptor agonists apomorphine and piribedil (McCulloch and Harper, 1977 b; McCulloch and Edvinsson, 1980) demonstrated parallel time courses for the increases in blood flow and oxygen consumption were demonstrated following the administration of these agonists. However, the fact that the increase in cerebral blood flow was up to twice that of oxygen consumption was not commented upon.

The use of techniques to measure global cerebral blood flow and metabolic rate has, therefore, provided little insight into the relative contributions of the vascular and neural receptors to the blood flow response.

b)    Local Cerebral Glucose Utilisation

Application of the 2-deoxyglucose autoradiographic technique have provided the first images of the influences exerted by dopamine systems on integrated brain function. These studies employing a number of dopamine receptor agonists (apomorphine, bromocryptine, l-dopa, amphetamine and methylphenidate) antagonists (haloperidol, pimozide, fluphenazine), as well as contingent and non-contingent stimulation of the A9 and A10 cell groups of the ventral mesencephalon, and selective lesion studies have evoked remarkably consistent patterns of altered glucose use. These patterns have served to support some current concepts about dopaminergic influences on brain function, questioned the validity of others, and suggested dopaminergic influences on hitherto unknown neuronal systems.

According to one view based on behavioural and neuroanatomical

studies central dopamine systems exert a widespread influence upon cerebral function (Bloom, 1984; Björklund and Lindvall, 1984). Deoxyglucose studies have clearly demonstrated that this is not the case. The rate of glucose utilisation remains unaltered throughout the vast majority of brain areas irrespective of whether dopamine systems are manipulated by sytemic or intracerebral drug administration, stereotaxic lesions or electrical stimulation of the mesencephalic dopamine systems. A few studies have reported generalised reductions in glucose use following unilateral lesions of the nigrostriatal pathway (Schwartz et al. 1978; Kozlowski and Marshall, 1980). In the Schwartz study these global reductions probably reflect the extensive neuronal damage associated with large electrolytic lesions of the hypothalamus whilst the decreases observed in the Kozlowski and Marshall study were probably due to their inexplicable use of ether anaesthesia to facilitate the injection of deoxyglucose.

General anaesthesia is an integral feature of most electrophysiological and neurochemical studies performed in vivo. Indeed studies of cerebral dopamine systems in anaesthetised animals have contributed significantly to our current understanding of the influence of dopamine systems on brain function. However, general anaesthesia has profound effects on neural function (Siesjö, 1978; Fink, 1980), but more importantly the study of neuronal function in anaesthetised animals may result in misleading information about the effects of dopaminergic manipulations upon cerebral function in conscious animals.

The influence of general anaesthesia upon dopamine systems has been highlighted by a recent study of the effects of apomorphine upon function-related glucose utilisation in the substantia nigra.

Systemic administration of apomorphine results in the inhibition of electrical activity within the dopaminergic neurones of the substantia nigra pars compacta (Bunney et al. 1973; Bunney and Chiodo 1984; Skirboll et al. 1979; Gallagher et al. 1978; Walters et al. 1983, 1986). Apparently at variance with data from these electrophysiological studies is the increased glucose use observed within the pars compacta following the administration of apomorphine to conscious animals (Brown and Wolfson, 1978; McCulloch et al. 1982 b; Grome and McCulloch, 1981). However, if the apomorphine is administered to chloral hydrate anaesthetised rats a reduction in glucose use is observed within the pars compacta (Grome and McCulloch, 1981). In addition, the use of general anaesthesia reduces both the metabolic (Grome and McCulloch, 1981) and electrical activity (Bergstrøm et al. 1984) in the globus pallidus following apomorphine administration and to attenuate dopaminergic transmission in prefrontal and anterior cingulate cortices (Roth et al. 1986). Anaesthetic influences may also account for the apparently contradictory effects of dopamine agonists upon corticostriate transmission in anaesthetised (Brown and Arbuthnott, 1983) and encephale isole cat preparations (Norcross and Spehlman, 1978). These divergent responses highlight the difficulties and potential pitfalls of extrapolating data obtained from anaesthetised animals to the conditions prevalent in the conscious state and emphasise the need to perform investigations in unanaesthetised preparations.

In an attempt to obviate the need for general anaesthesia some investigators have examined the influence of dopaminergic agents upon electrical activity in paralysed artificially ventilated animals (Bergstrom et al. 1984; Gessa and Mereu 1984; Onali et al. 1984). However, the use of this preparation is of equivocal value since

paralysis produces severe stress in animals (Ohata et al. 1981; Lacombe and Seylaz, 1984), which in itself has profound influences on dopaminergic transmission (Thierry et al. 1984; Glowinski et al. 1984). The capacity to identify the functional consequences associated with the manipulation of dopamine systems humanely in conscious animals, constitutes a major advantage of the deoxyglucose method. The systemic administration of the dopamine receptor agonists apomorphine and bromocryptine and the sympathomimetic agents amphetamine, l-dopa and methylphenidate consistently results in pronounced increases in glucose within those brain areas involved in the integrated control of motor function (sensory motor cortex, basal ganglia, ventral thalamus, cerebellum and inferior olivary complex inter alia (Bell et al. 1982; Brown and Wolfson, 1978; McCulloch et al. 1982 a; Pizzolato et al. 1985; Warner et al. 1982; Weschler et al. 1979). From these studies it is clear that alterations in glucose use do not merely reflect the topographical distribution of dopaminergic innervation or receptor density, rather, these changes reflect the influences exerted by dopamine receptor stimulation upon functionally related neuronal circuits (McCulloch, 1982). This view is endorsed by studies of the effects of dopaminergic stimulation on thalamo-cortical function. The pharmacological activation of central dopamine systems provoke marked increases in glucose use within sensory motor and frontal cortices. Although these cortical areas are thought to be devoid of dopaminergic innervation and receptor populations (Björklund and Lindvall, 1984; Altar et al. 1985; Jastrow et al. 1984), they are anatomically connected, via polysynaptic neuronal pathways, with the caudate nucleus. The evidence that the alterations in cortical glucose use is initiated at the caudate nucleus is twofold. The systemic administration of

apomorphine and other dopaminergic agents such as bromocryptine, amphetamine, methylphenidate and l-dopa stimulates glucose utilisation within each component of the striatal → pallidal → ventrolateral thalamus → cortex pathway. Furthermore, the laminar pattern of cortical glucose use following apomorphine administration corresponds with the topographical distribution of the cortical projections of the ventral thalamus (McCulloch et al. 1979; McCulloch, 1982).

With the notable exceptions of the anterior cingulate cortex, anterior thalamic nucleus and lateral habenular nucleus, the anatomical components of the limbic system do not display consistent alterations in glucose use following the administration of dopamine receptor agonists (Pizzolato et al. 1985; McCulloch et al. 1982 a; Orzi et al. 1983; Weschler et al. 1979). Widespread alterations in glucose use have been recorded within the limbic system following electrical stimulation of the ventral mesencephalon (Savaki et al. 1983; Porrino et al. 1984 a; Esposito et al. 1984). However, the pattern of altered glucose use elicited by electrical stimulation may be viewed with circumspection as antidromic excitation of afferent fibres to the mesencephalon may stimulate non-dopaminergic components of the mesencephalic system, as well as the dopaminergic projections. In contrast, blockade of dopaminergic transmission, whether by neuroleptic treatment or selective lesions of the nigrostriatal pathway has generally had little effect upon glucose use within the extrapyramidal motor system, although some lesion studies have reported global reductions (Schwartz et al. 1976, 1978; Kozlowski and Marshall, 1980; Schwartzman and Alexander, 1985). Recently, the neurotoxin MPTP has been used in primates as a model of Parkinsonism (Schwartzman and Alexander 1985; Porrino et al. 1985). However,

unlike clinical Parkinsonism where minimal alterations in glucose use have been reported (Leenders et al. 1983; Rougement et al. 1983). MPTP treatment reduced glucose use within motor systems.

One consistent feature of dopamine receptor blockade has been observed within the lateral habenular nucleus. Elevated rates of glucose use occur within the lateral habenular nucleus following lesions of the nigrostriatal dopamine pathway (Wooten and Collins, 1981; Ferron et al. (1979)), following depletion of catecholamine stores with reserpine (Palacios and Wiederhold, 1984), or following blockade of dopamine receptors with fluphenazine (Wooten, 1981), haloperidol (McCulloch et al. 1980 b, 1982 c; Pizzolato et al. 1984), pimozide (Gomita and Gallistel, 1982; Gallistel et al. 1985), or YM09151-2 (Palacios and Wiederhold, 1985).

It is evident from the studies cited above that the manipulation of central dopamine systems produces characteristic profile of regions displaying altered rates of glucose use. Furthermore, a review of the literature on 2-deoxyglucose would suggest that the characteristic patterns of altered glucose use also produced by other classes of pharmacological agent (i.e. GABA agonists, α-adrenergic muscarinic, agonists and antagonists) and that each pattern appears to be unique for each class of compounds possessing similar pharmacological actions (Sokoloff et al. 1983; Muller and Martin, 1984). These characteristic "fingerprints" could be used to construct a data base with which test compounds could be compared. Such an approach could prove invaluable in drug development by predicting the effects, and possible side effects associated with novel compounds. However, at present the construction of such a system represents major problems of data handling and analysis. The dose response curves for large numbers of

brain regions generates vast quantities of data (for example, the studies comprising this thesis required over 96,000 individual densitometrical measurements). To simplify the analysis of such data a simple arithmetic function has been derived (Ford et al. 1985) and validated in this thesis which describes the dose response curve to drug administration for each brain region by a single value. These values can be ranked to reveal a "fingerprint" of responsiveness to each drug which can subsequently be compared with the profiles for other drug treatments.

c)    Local Cerebral Blood Flow and the Flow/Glucose Use Relationship

The use of quantitative autoradiographic methods for assessing local cerebral blood flow (using $^{14}$C-iodoantipyrine as tracer) and glucose utilisation (with $^{14}$C-2-deoxyglucose) have established that flow and glucose use remain tightly coupled under most physiological and pharmacologically-induced conditions (Sokoloff, 1978). However, despite the remarkable spatial resolution afforded by these techniques their use for studying the influences of dopaminergic agents upon the flow-metabolism relationship has only added to the controversy, with studies advocating a primarily metabolic action (McCulloch and Kelly, 1982; McCulloch et al. 1982 a), others a mixed vascular and metabolic response (Ingvar et al. 1983), and a third group a vascular action (Leenders et al. 1985).

According to one study the administration of apomorphine produced focal alterations in cerebral blood flow, characterised by increases in tissue perfusion within extrapyramidal and sensory motor areas and decreases within the anterior cingulate cortex and lateral habenular nucleus (McCulloch et al. 1982a). The alterations in blood

flow were similar, both in magnitude and directions to the changes in glucose use observed under the same experimental conditions, with the result that the overall relationship between flow and glucose use remained intact and unaltered. This study was porrected as unambiguous evidence that the changes in cerebral blood flow produced by dopamine receptor agonists are the indirect consequence of drug-induced disturbances in local metabolic activity (McCulloch, 1984). However, this view has been challenged by studies reporting widespread increases in blood flow irrespective of the underlying metabolic responses (Ingvar et al. 1983; Leenders et al. 1985). In a study by Ingvar and his associates the administration of apomorphine resulted in elevated blood flow throughout the CNS; including the anterior cingulate cortex. Moreover, the circulatory response to apomorphine was associated with a marked topographical heterogeneous response in the caudate nucleus; characterised by prominent increases in flow in the lateral caudate (164%) whilst the more medial portion remained relatively unaffected (+17%). As these findings did not concur with the highly focal alterations in glucose use it was suggested that apomorphine may increase blood flow by stimulating cerebrovascular dopamine receptors in some (anterior cingulate cortex, lateral caudate) if not all brain areas (Ingvar et al. 1983). This conclusion has been criticised on the grounds that a) no measurement of local cerebral glucose use was made under the same experimental conditions; and b) the comparison of local blood flow responses of anaesthetised rats with glucose use data from conscious animals is potentially flawed (vide infra).

Evidence for a direct vascular action of apomorphine has been obtained from studies in rats with lesions of the nigrostriatal pathway. The administration of apomorphine to lesioned animals

failed to significantly alter local cerebral glucose use within the caudate nucleus (Kozlowski and Marshall, 1980; Sagar and Snodgrass, 1980). In contrast the topographical heterogeneity observed within the caudate nucleus (under similar experimental conditions) was more pronounced in lesioned animals (Ingvar et al. 1983). Consequently, it has been suggested that the enhanced circulatory response in the denervated caudate was due to stimulation of supersensitive vascular dopamine receptors. A similar conclusion has also been reached on the basis of tomographic studies in man (Leenders et al. 1983, 1985). In control subjects the administration of l-dopa was associated with an increase in cerebral blood flow within the basal ganglia and overlying cortex; oxygen consumption in these areas remained relatively unaffected. This dissociation between flow and metabolism was more pronounced in patients suffering from striatal denervation as a result of Parkinsonism (Leenders et al. 1985). From the studies presented it is clear that, at present, no consensus exists concerning the relative importance of vascular dopamine receptors in the circulatory responses to systemically administered dopamine receptor agonists.

CHAPTER II

# METHODS

## 1.  PIAL VESSEL STUDIES

### 1.1  Surgical Preparation of Animals

Studies were performed on  cats, of either sex, weighing

between 2 and 4.5 kg.  Anaesthesia was induced by an intravenous

injection containing alphaxolone (6.75 mg/kg) and alphadolone (2.25

mg/kg).  After intubation, the animal was ventilated using a small

animal respirator (Harvard Instruments).  The right femoral artery

and vein were cannulated to permit, respectively, the continuous

monitoring of arterial blood pressure, and the administration of

fluid or anaesthetic agents.  During the subsequent course of the

experiment anaesthesia was maintained with alpha-chloralose;  an

initial infusion of 60 mg/kg with additional doses when necessary to

prevent the return of corneal reflexes.  End tidal concentration of

$CO_2$ was continuously monitored with an infra red capnograph (Godart-

Stratham, Model 17010) and arterial blood samples intermittently

withdrawn for blood gas analysis ($PCO_2$, $PO_2$, pH and oxygen tension).

In each cat mean arterial blood pressure was greater than 90 mm Hg.

Core temperature was maintained at $38^{\circ}C$ by a homeothermic heating

blanket (GFP Model 8140).

The cat was placed in a stereotaxic frame and the head shaved. A longitudinal incision made in the scalp and ligated onto a metal ring (diameter = 6.5 cm), enabled the scalp to be elevated and filled with mineral oil to form an intact pool over the calvarium (approximately 1.5 cm deep). After the left temporalis muscle had been retracted, a craniotomy (2.5 x 1.5 cm) was made over the left parietal cortex using a saline cooled dental drill. The exposed cortex was bathed by warmed mineral oil, maintained at $38^{\circ}C$. Thereafter, the dura was carefully removed with the aid of a stereomicroscope (Bausch and Schott magnification range x10 - x70), the field being illuminated by Schott fibre optic systems. Bleeding from the cut dural edges was halted by bipolar diathermy of the bleeding sites.


1.2     Measurement of Vessel Calibre


Vascular calibre was measured using the method of Baez (1966). Individual pial vessels, lying on the convexity of the brain were viewed through the microscope at a magnification of either x 40 or x 70. The image was passed through a Vickers AEI image splitting eyepiece to a close circuit television camera and visualised on a television monitor (Figure 3). Vascular diameter was measured from the degree of shear which has to be applied to the image splitting device in order to bring the two images of the vessel under investigation into apposition;  the degree of shear having been calibrated against lengths of wire and thread of known diameter.

FIGURE 3
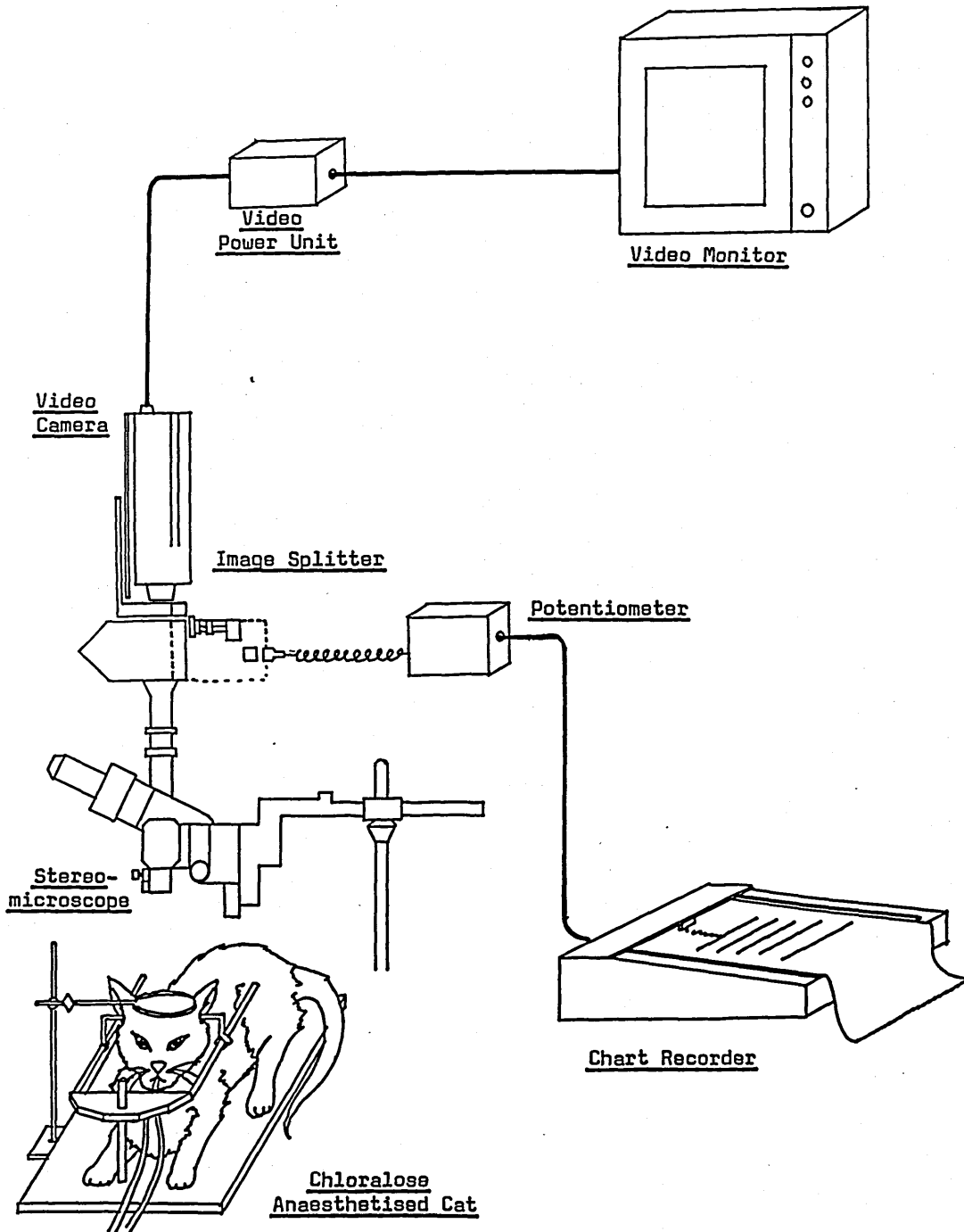


Diagrammatic Representation of the
Measurement of Pial Arteriolar Calibre in Situ

## 1.3   Administration of Drugs

Agents under investigation (with the exception of SCH 23390) were dissolved in artificial cerebrospinal fluid, (C.S.F.), the composition of which was -

| | |
|---|---|
| $Na^+$ | 145 mM |
| $K^+$ | 3 " |
| $Ca^{2+}$ | 2.5 " |
| $HCO_3$ | 11 " |
| $Cl^-$ | 142 " |

SCH 23390 was initially dissolved in 100 ul of a 5% ethanol/25% acetic acid solution and diluted in distilled water to form a stock solution of $10^{-2}$M. Serial dilutions were performed, using artificial CSF to yeild the desired injectate concentration. The ethanol/acetic solution was diluted in a similar manner to yeild the appropriate vehicle for comparison. Acidic CSF (pH 6.8) was prepared by altering the concentrations of $HCO_3^-$ and $Cl^-$ in the artificial CSF solution to 5 mM and 148 mM respectively. The pH of each solution was adjusted to 7.18 by aeration with $CO_2$, under oil, to minimise changes in pH. Micropipettes having a tip diameter of 8-10 uM, were filled under oil with the solution to be examined, immediately prior to use.

The tip of the micropipette was positioned close to a superficial pial arteriole, using a micromanipulator and using a micromanipulator inserted through the arachnoid into the perivascular space. Approximately 5 µl of the artificial CSF solution was injected over fifteen seconds and any resulting changes in arteriolar calibre monitored for up to three minutes after injection.

Microapplication of drugs was made at any particular site on only one occasion.

The maximum alteration in arteriolar calibre following drug administration was expressed as a percentage of the preinjection calibre.

## 1.4  Statistical Analysis

Drug-induced alterations in arteriolar calibre were compared with that evoked by artificial cerebrospinal fluid by Student's t-test with Bonferroni correction for multiple comparisons (Wallenstein et al. 1980).  This form of analysis does not take into account inter-animal variability in vasomotor reactivity.  However, analysis of variance on the data from each drug solution revealed that the variance observed within a single animal was invariably greater than that found between animals.  In fact no significant inter-animal differences could be demonstrated with any of the drug concentrations examined.

## 2.  AUTORADIOGRAPHIC STUDIES

### 2:1  Features Common to Local Blood Flow and Glucose Use Studies Using Quantitative Autoradiography

Quantitative autoradiographic techniques were employed for the determination of local rates of cerebral glucose utilisation and blood flow in conscious, lightly restrained rats.  The surgical preparation of these animals, the monitoring of the arterial concentrations of radiolabelled tracer, and the preparation and

analysis of autoradiograaams are features common to both experimental

approaches and so will be described separately.

## 2.1.1 Animal Preparation

### a) Housing

Specific pathogen free male Sprague-Dawley rats, approximately

ten weeks old (300 g) were obtained from registered animal suppliers

(Charles River, U.K.) and housed, three to a cage, in a holding unit.

Environmental conditions within the holding area were not strictly

controlled, the animals were subjected to a natural day/night cycle.

Food and water were freely available until the evening prior to

study. At this time animals to be studied were transferred to a

separate cage and fasted for a period of 12 to 15 hours prior to

surgery.

### b) Surgical Preparation

All surgical procedures were carried out under halothane

anaesthesia of sufficient depth to prevent reflex responses to strong

tactile stimuli. Anaesthesia was induced by placing the rat into a

clear perspex box into which flowed the anaesthetic gas mixture (5%

halothane in a nitrous oxide/oxygen mix: 70/30 v/v). Anaesthesia

was subsequently maintained throughout the period of surgery by 1-2%

halothane delivered to the animal through a face mask.

Femoral vessels were exposed through a small incision

(approximately 1 cm in diameter) made on either side of the groin

overlying the femoral triangle. Wounds were infiltrated with a local

anaesthetic solution (Xylocaine: 2%) and the femoral vessels freed from connective tissue. Polythene cannulae (Portex: external diameter 0.96 mm; internal diameter 0.58 mm), 15 cm long and filled with heparinised saline 10 I.U./ml) were inserted a distance of 2 cm into both femoral arteries and one femoral vein. The patency of each cannula was verified, the cannula secured and the wound sutured closed. Incision sites were smeared with a local anaesthetic gel, cushioned with gauze swabs, and the entire pelvic and abdominal area enveloped in a surgical stocking. A plaster of Paris bandage (Gypsona: 7.5 cm wide) applied over the pelvis and lower abdomen provided protection of the incision sites without encumbering normal respiration. The animal was lightly restrained by taping the plaster cast and the animals hindlimbs to a lead support brick. A temperature probe was inserted into the rectum to monitor core temperature and a pressure transducer (P23 ID Gould Stratham) attached to a chart recorder (Gould Stratham, Model 2202) was connected to one of the arterial femoral catheters to monitor blood pressure. Anaesthesia was discontinued and the rat allowed to recover for at least 2 hours prior to drug administration.

2.1.2 Experimental Analysis

a)    Preparation of Autoradiograms

At the end of the sampling period the animal was sacrificed by decapitation and the brain rapidly excised as follows. A longitudinal midline incision was made at the level of the snout and extended in a caudal direction to the level of the forelimb. Skin and extracranial muscle were reflected to expose the skull. The

dorsal cranium was removed and the dura reflected to facilitate excision of the intact brain. After all adhering hair and bone fragments had been removed the brain was frozen in isopentane which had been precooled to $-42^{\circ}$C in a mixture of acetone and dry ice. The time taken from sacrifice to immersion in isopentane did not exceed 5 minutes. Over a bed of dry ice, the olfactory bulbs were removed and the brain affixed to a swivel-headed microtome chuck with a plastic embedding matrix (Lipshaw). The brain was then sealed in a polysterene box and stored in a cryostat at $-22^{\circ}$C. From each brain approximately 900 coronal brain sections, precisely 20 µm thick, were cut in a cryostat. Of these, three sections in every ten were picked up onto glass cover slips and rapidly dried on a hotplate ($65^{\circ}$C). The remaining seven sections were discarded or where necessary stained with cresyl violet for the histological identification of brain regions. Histological staining of brain sections for acetyl-cholinesterase was performed using the Koell-Friedenwald thiocholine method as modified by Hardy et al. (1976). The coverslips were glued onto cards and placed, together with a set of $^{14}$C-labelled epoxy resin standards (18-1880 nCi/g tissue equivalents) in light-tight cassettes. Autoradiograms were obtained by exposing blue-sensitive x-ray film (Kodak - GRS-A) to the brain sections and standards for approximately 10 days. Films were then processed in a Kodak automatic processor, according to the manufacturers' instructions.

b)    Quantitative Densitometrical Analysis of Autoradiograms

Analysis of the resultant autoradiograms was performed using a computer-based microdensitometer system (Cambridge Instruments; Quantimet 720). With this system autoradiograms are magnified (x

5.6) and displayed on a video monitor. Optical density (OD) measurements are obtained either by placing a cursor (2.5 x $10^{-3}mm^2$ - $1800mm^2$) over the area of interest or circumscribing the region with a light pen attachment. Density values for each brain region are obtained by digitising the field of measurement into picture points (pixels, 2.5 x $10^{-3}mm^2$) with each pixel ascribed to one of 64 grey levels. Thereafter, a density value is obtained by integrating the grey level value of each pixel on each of four scans. Bilateral measurements were made on at least six autoradiograms in which each brain area appears. Identification of brain areas was made with reference to the stereotaxic atlases of Konig and Klippel (1967); Pellagrino et al. (1967); Paxinos and Watson (1982). The $^{14}C$ concentration for each brain region was calculated from the mean OD value, obtained from the bilateral measurement on at least six autoradiograms in which the region was defined, by reference to the calibration curve constructed from the optical density values of the $^{14}C$ standards.

A more detailed analysis of the effects of $D_1$ and $D_2$ receptor activation on local blood flow and glucose use in the caudate nucleus were obtained as follows. Densitometrical mesurements were made at three levels of the caudate nucleus; at the "head" (rostral) "body" (middle) and "tail" (caudal) of the nucleus (Figure 4). At each level bilateral measurements were made on each of six adjacent sections by placing a 10 x 10 pixel square over fixed anatomical loci. Anterior measurements were made at the level of the genu of the corpus callosum (A9410 : Konig and Klippel, 1963); middle level at the lateral processes of the anterior commissure (A7470) and posterior at the level of the globus pallidus (A6280 - A6360). At both the anterior and middle levels optical density measurements were

FIGURE 4
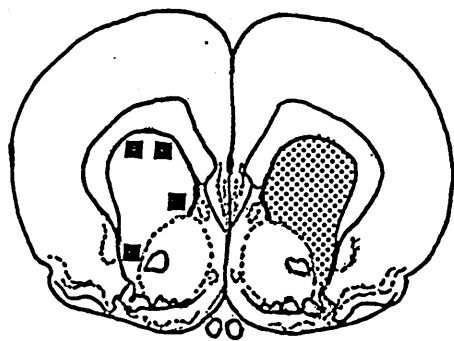


Level 6
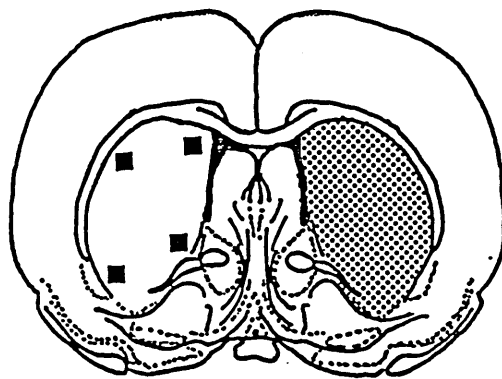
Level 5
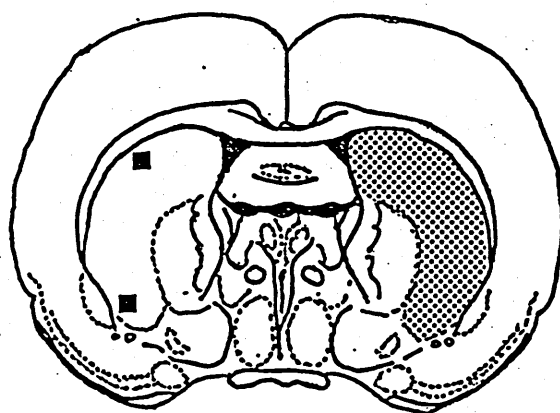
Level 4

Anatomical Criteria for Measuring Local Blood Flow and Glucose

Utilisation in Subregions of the Caudate Nucleus.

LEGEND TO FIGURE 4


Densitometrical analysis was performed at three anatomically
defined levels corresponding to the head (level 6), body (level 5)
and tail (level 4) of the caudate nucleus.  The caudate nucleus is
defined by stipling on the right side of each diagram.  The black
squares on the left side of each figure indicate the precise
locations at which densitometrical analysis was performed.

performed at fixed points on the four quadrants of the nucleus (Figure 4). The most caudal level (posterior) permitted only two measurements (dorsal and ventral).

Following the measurement of regional density levels, the mean density for the caudate nucleus at each level was calculated by encircling both caudata with a light-pen attachment.

c)    Liquid Scintillation Analysis

The time course of $^{14}$C-labelled tracer is arterial blood was determined by liquid scintillation analysis of the plasma (glucose use) or whole blood samples (blood flow) obtained during the measurement period.

Aliquots of arterial plasma (18.7 ± 0.1 microlitres) were pipetted into plastic scintillation vials containing 1 ml of distilled water. To ensure accurate delivery, the residual plasma was eluted from the pipette tip by repeated flushing with the distilled water. A 10 ml aliquot of scintillation fluid (Pico-fluor 30) was added to each vial and the radioactive emission analysed for periods of 4 minutes in a refrigerated liquid scintillation counter.

Raw data (cpm) for each sample were converted to disintegrations per minute (dpm) by reference to a quench calibration curve and the external standards ratio as described by Peng et al. (1977). A set of quench calibration standards analysed contemporaneously with each set of counts monitored the accuracy of the cpm to dpm conversion. For each experiment the mean of 4 temporally spaced sets of counts was used to calculate the $^{14}$C concentration in each sample.

Whole blood samples were bleached with hydrogen peroxide (0.4 ml of 30% w/v $H_2O_2$) for 30 minutes to reduce the counting errors associated with colour quenching. Scintillation fluid was added and the samples left to stand at room temperature overnight to allow complete elution of the isotope from the filter discs. The samples were then processed as described above.

## 2.1.3   Data Analysis

The pharmacological studies presented in this thesis fall into three main categories: a)  the analysis of drug effects on indices of brain function,  b)  examination of agonist/antagonist interactions on function-related glucose use,  and c)  the comparison of different drug treatments on the relationship between local rates of cerebral blood flow and glucose utilisation.

## a)   Analysis of Drug Responses

The Bonferonni method was used to compare the effects of single concentrations of two different drug treatments. This modified t-test adjusts for multiple comparisons by assigning a significance threshold of P/M, where P is the probability value obtained from the tables of t-distribution and M is the number of comparisons being made. thus, for an experimental situation involving two comparisons (i.e. SKF 38393 vs control and LY 171555 vs control) the significance threshold would be set at 0.025.

Situations involving more complicated comparisons (dose-response curves) were analysed using the Scheffe extension of analysis of variance (ANOVAR) (Scheffe, 1959). The analysis of

variance approach is a more conservative statistical test than the Bonferonni method and so is less susceptible to attributing false significance in studies involving large numbers of comparisons.

b)    Analysis of Drug Interactions

The interaction studies involving the $D_2$ agonist LY 171555 and the $D_2$ agonist LY 171555 and the $D_1$ antagonist SCH 23390 presented particular problems for statistical analysis.  In studies of this type the questions to be addressed are –

1)    Does antagonist pretreatment alter the response to the agonist.

If, Yes

2)    Is the effect of the antagonist pretreatment on the agonist respose homogeneous throughout the brain or is it restricted to a few brain regions.

They, therefore, require a more sophisticated analytical approach than was used to examine the effect of simple drug administration. An appropriate form of analysis would be to compare the differences in variance between a)  glucose use in the agonist-treated group and its control group (saline) and  b)  the agonist/antgonist group and its respective control group (antagonist alone) i.e.

(agonist treatment – saline control)  vs  (agonist/antagonist – antagonist alone).

Analysis of variance of this type can be performed using repeated measures approach (BMDP program P2V), treating the agonist and

agonist/antagonist groups as treatment variables, control or challenge groups as the grouping variables and the different brain regions as the trial factor. If inter-regional variability in response to pretreatment with antagonist is indicated by the ANOVAR study then the areas of interest can be identified by comparing the "interaction" group with the agonist treatment group for each brain areas using the students t-statistics.

c)   Analysis of the Relationship Between Blood Flow and Glucose Use

The quantitative autoradiographic techniques described provide the ideal tools for investigating the effects of drug administration of the fundamental relationship between local rates of metabolic activity and tissue perfusion throughout the brain. However, in many of the early studies naive and inappropriate statistical methods were used to analyse the relationship between blood flow and glucose use (Sokoloff, 1981; Kuschinsky et al. 1981 a,b; Mies et al. 1981; Ohata et al. 1981; Pickard 1981). Invariably, these authors analysed the relationship between local blood flow and glucose use by evaluating the correlation coefficient derived from regression analysis. However, fundamental to the use of regression analysis is the assumption that all data points are statistically independent. Patently this cannot be the case in autoradiographic studies where multiple measurements are made in each brain.

A rigorous statistical method has been developed specifically for the analysis of flow/glucose studies which takes into account the correlation between flow and glucose use in the different brain

regions and the influences of inter-animal variability (McCulloch et al. 1982 a). In practice this approach is similar to that described for the analysis of agonist/antagonist interactions. Analysis of variance (of repeated measures form) was performed (BMDP program P2V statistical package) with blood flow and glucose use treatments taken as the levels of grouping variable and the 59 brain regions as the trial factor.

## 2.2   Measurement of Local Cerebral Blood Flow

Local rates of cerebral perfusion were measured using the autoradiographic $^{14}$C-iodoantipyrine technique, as described by Sakurada et al. (1978).

### 2.2.1.  Theory

The $^{14}$C-iodoanipyrine technique, like many other methods for measuring cerebral blood flow, is based on the mathematical model developed by Kety for describing the exchange of inert gaseous tracer substances between blood and tissue (Kety, 1951, 1960). Kety's approach was to start with "the Fick principle" which in this context can be started as, the rate of change of a biologically inert freely diffusible tracer at a time (T), is related to the arteriovenous difference of the tracer and the flow per unit weight of tissue (F/W).

$$\frac{dC_i}{dt} = \frac{F}{W} (C_a - C_v) \qquad (3)$$

If it can be assumed that at the level of the capillary bed the tracer comes into instantaneous equilibrium betwen blood and tissue (Zuntz, 1897) then

$$(C_a - C_v) = m \left(C_a - \frac{C_i}{\lambda}\right) \qquad (4)$$

where $\lambda$ describes the tissue/blood partition coefficient and m is a constant between 0 and 1 which represents the extent to which diffusion equilibrium is achieved as blood passes through the capillary bed. In the absence of arteiovenous shunting mechanisms, the value of m for a freely diffusible tracer would be unity.

If the tissue compartment under investigation is homogeneous with respect to a) perfusion rate and b) tracer access then equations (1) and (2) can be combined and integrated to yield an expression (3) which relates a clearance constant K to the time course of the tracer in arterial blood over the period time (t) to time (T); the local concentration of tracer in the tissue at time T and the tissue/blood partition coefficient ($\lambda$).

$$C_i(T) = K \quad C_a e^{-K(T-t)} \, dt \qquad (5)$$

where the clearance K is defined as $\frac{mF}{\lambda}$. In the absence of arteriovenous shunts and limitations on free diffusion (i.e. m=1), then local rates of blood flow can be calculated from the clearance constant from the simple relationship

$$K = \frac{\text{flow per unit weight of tissue}}{\lambda} \qquad (6)$$

The Kety model was originally used in conjunction with the gaseous tracer ^{131}I-trifluoroiodomethane (Landau et al. 1955; Freygang and Sokoloff, 1958; Kety 1960), and later with ^{14}C-antipyrine (Reivich et al. 1969); however, neither compound proved to be satisfactory as a tracer substance. The short half life (8 days), and poor resolution characteristics of iodine labelled tracers make them unsuitable for routine use with quantitative autoradiography. Furthermore, the solubility of $CF_3I$ in blood varies with haematocrit and the tissue/blood partition coefficient varies markedly from region to region and from animal to animal (Reivich et al. 1969).

The introduction of ^{14}C-antipyrine as a tracer for measuring local cerebral blood flow appeared to overcome the problems of partition coefficient and isotope stability (Reivich et al. 1969), however, the uptake of ^{14}C-antipyrine into cerebral tissue diffusion limited (i.e. m considerably less than 1) (Sakurada et al. 1978; Siesjö, 1978). The diffusion limitations of ^{14}C-antipyrine were overcome by the introduction of the 4-iodo derivative of ^{14}C-antipyrine, iodoantipyrine (Sakurada et al. 1978). The high oil/water partition coefficient of iodoantipyrine make it a more freely diffusible tracer than ^{14}C-antipyrine (Sakurada et al. 1978). In addition the brain/blood partition coefficient is uniformly constant throughout the CNS (0.79) and does not vary with haematocrit (Sakurada et al. 1978). The ^{14}C-iodontipyrine technique was, therefore, adopted as the method of choice for determining local cerebral blood flow.

## 2.2.2 Practical Approach

The effects of saline, SKF 38393 and LY 171555 on local cerebral blood flow was determined in 18 rats by quantitative autoradiography using $^{14}$C iodoantipyrine as a tracer (Sakuruda et al. 1978). Rats were prepared for autoradiographic investigations as described previously. Local blood flow was determined 40 minutes after drug administration so as to coincide with the time of median specific activity of $^{14}$C-2-deoxyglucose in brain tissue in those experiments used for the determination of glucose use. $^{14}$C-iodoantipyrine (50 μCi in 0.7 ml of saline) was infused intravenously over the 60 second sampling period by on a "ramped" infusion schedule. The use of a progressively increasing infusion rate prevented the attainment of steady state arterial concentrations and so maximise the resolution of high flow and low flow areas on the autoradiogram. During the infusion period 15 to 18 timed blood samples were absorbed onto preweighed filter discs from blood which was allowed to flow freely from a partially occluded arterial catheter. The time at which each sample drop was collected was recorded and used together with the concentration of radioactivity in the sample to describe the history of arterial tracer throughout the experimental period. At the end of the sampling period the rat was sacrificed by decapitation, its brain removed and processed for autoradiography as described previously. The filter discs were returned to their respective scintillation vials and reweighed. Whole blood samples were bleached with hydrogen peroxides and processed for liquid scintillation analysis as described. The volume of whole blood for each sample was subsequently calculated from the sample weight, corrected for the specific gravity of whole blood

(1.05 g/ml) and used to compute the integral of $^{14}$C-concentration over the 60 second period.

Local tissue $^{14}$C concentrations for each of the 60 brain regions examined was determined by densitometrical analysis as described previously. Local values for blood flow were then calculated using the equation derived by Kety (1960).

## 2.3 The Measurement of Local Cerebral Glucose Utilisation

Local rates of cerebral glucose utilisation were measured using the autordiographic $^{14}$C-2-deoxyglucose technique (Sokoloff et al. 1977).

### 2.3.1 Theory

The unique biochemical properties of the 2-deoxyglucose molecule provides the theoretical basis for the 2-deoxyglucose technique. 2-deoxy-D-glucose, a chemical analogue of glucose shares many of the biochemical characteristics of the native substrate. Deoxyglucose competes for the same bidirectional transport mechanism which normally carries glucose across the blood-brain barrier (Bachelard, 1971; Oldendorf, 1971, Pardridge et al. 1982 a,b; Gjedde and Diemer, 1983). Once they have entered into cerebral tissue both 2-deoxyglucose and glucose are phosphorylated by hexokinase, the first and rate limiting enzyme of the glycolytic pathway (Sols and Crane, 1954). However, it is at this point that the metabolic fates of 2-deoxyglucose and glucose diverge. Glucose-6-phosphate is largely converted to fructose-6-phosphate by phosphohexoseisomerase and is ultimately oxidised to carbon dioxide

FIGURE 5
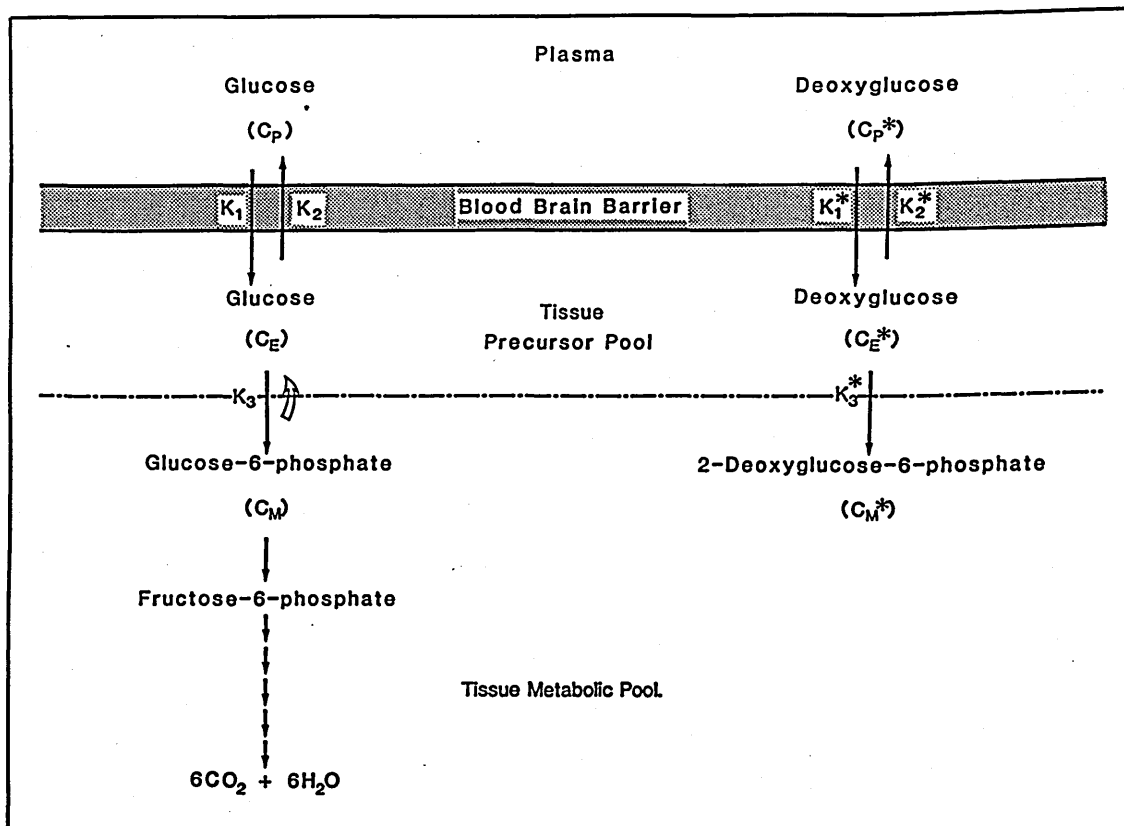


The model describes the fate of glucose and $^{14}C$-2-deoxyglucose in a single homogenous tissue of the brain. $^{14}C$-2-deoxyglucose and glucose share and compete for the same carrier facilitated transport mechanisms.

The constants $K_1^*$, $K_2^*$ and $K_3^*$ represent the rate constants for carrier mediated transport of $^{14}C$-2-deoxyglucose from plasma to tissue, for the carrier mediated transport back to plasma, and for the phosphorylation to 2-deoxyglucose-6-phosphate by hexokinase, respectively. $K_1$, $K_2$ and $K_3$ are the equivalent rate constants for the natural substrate, glucose.

and water via the citric acid cycle. A small proportion of glucose-6-phosphate (approximately 3%) is diverted to anabolic processes via the pentose phosphate (phosphogluconate) pathway (Siesjö, 1978).

In contrast the metabolism of 2-deoxyglucose essentially ceases after the hexokinase reaction (Tower, 1958; Horton et al. 1973). Deoxyglucose-6-phosphate is not a substrate for phosphohexose-isomerase nor glucose-6-phosphate dehydrogenase. Moreover, deoxyglucose-6-phosphate is not transported across the blood-brain barrier (Sokoloff et al. 1977; Nelson et al. 1986). It is a substrate for glucose-6-phosphatase (glucose-6-phosphohydrolase), however, the activity of this enzyme is low in vivo and the loss of 2-deoxyglucose negligible up to 1 hour after the administration of an intravenous pulse of deoxyglucose (Sokoloff et al. 1983). A proportion of deoxyglucose-6-phosphate is diverted into the production of glycogen and related compounds (Nelson et al. 1984; Sokoloff, 1986; Pentreath et al. 1982). However, these processes are slow in mammalian tissue (accounting for approximately 2% of total $^{14}$C - 45 minutes after deoxyglucose administration) and represent relatively stable secondary products of hexokinase activity (Sokoloff, 1986). Thus, so long as the interval of time following the intravenous administration of deoxyglucose is sufficiently short to allow for the assumption of negligible 2-deoxyglucose-6-phosphate loss (i.e. less than 1 hour), the accumulation of 2-deoxyglucose and its secondary products in brain tissue can be used to quantify the rate of glucose phosphorylation ($R_i$).

$R_i$ = <u>Concentration of Deoxyglucose Products at Time T</u>     (4)
[Isotope Correction Factor]    x    [Integrated specific activity in precursor pool].

This equation represents a specific application of the general equation for measuring rates of biochemical reactions with ratioisotopes. The isotope correction factor is a constant, composed of the Michaelis-Menten kinetic constants for glucose and deoxyglucose, which corrects for kinetic differences between the rate of phosphorylation of glucose and deoxyglucose by hexokinase.

Unfortunately, the integrated specific activity in the precursor pool cannot be measured readily and must be calculated from measurements made in arterial blood by correcting for the lag in the equilibration of the precursor pool with that of plasma. To apply this correction requires an acurate knowledge of the kinetic rate constants which describe the movement of 2-deoxyglucose into and out of the tissue precursor pool ($K_1^*$ and $K_2^*$) and its phosphorylation by hexokinase ($K_3^*$). These principles have been mathematically defined and incorporated into equations (5); the operational equation of the method (Figure 6). This method may be used to measure local rates of glucose utilisation under a variety of experimental conditions, provided that the following requirements are fulfilled.

1)    Deoxyglucose-6-phosphate, or its metabolites, remain trapped within cerebral tissue for the duration of the sampling period.

2)    Plasma glucose levels and local rates of glucose consumption remain constant throughout the experimental period.

3)    Tracer theory is upheld with respect to 2-deoxyglucose and 2-deoxyglucose-6-phosphate.

FIGURE 6

Operational Equation for the 2-Deoxyglucose Method.

$$R_i = \frac{C_i^*(T) \quad - \quad k_1^* \; e^{-\left(k_2^*+k_3^*\right)T} \displaystyle\int_0^T C_p^* \; e^{\left(k_2^*+k_3^*\right)t} \, dt}{LC \cdot \left[\displaystyle\int_0^T \left(\frac{C_p^*}{C_p}\right) dt \; - \; e^{-\left(k_2^*+k_3^*\right)T} \displaystyle\int_0^T \left(\frac{C_p^*}{C_p}\right) e^{\left(k_2^*+k_3^*\right)t} \, dt\right]}$$

where LCGU is the local rate of glucose consumption in a homogenously perfused area of brain tissue $C_1^*$ is the total $^{14}C$ concentration in tissue ($C_E^* + (M^*)$), T represents the time at which the animal is sacrificed. The "lumped constant", LC, converts the steady state rate of $^{14}C$-2-deoxyglucose phosphorylation to rates of glucose phosphorylation and is composed of the relative distribution ratios between brain tissue and arterial plasma ($\lambda^*$), the michaelis-menten kinetic constants of glucose and 2-deoxyglucose, and a factor $\emptyset$ which corrects for the fraction of glucose-6-phosphate which is lost from the metabolic pool. The other symbols are the same as defined in Figure 5.

## 2.3.2 A Critical Appraisal of the 2-deoxyglucose Method

The assumption that the products of 2-deoxyglucose metabolism, once formed, remain trapped within brain tissue is fundamental to the validity of the 2-deoxyglucose method. This premise appeared reasonable since 2-deoxyglucose-6-phosphate and its metabolites (glycogen, glycolipids and glycoproteins) are relatively stable products which remain trapped within cerebral tissues. It was recognised that 2-deoxyglucose-6-phosphate is a substrate for glucose-6-phosphatase. However, substantial biochemical evidence indicated that the activity of this enzyme is exceedingly low in brain tissue (Hers and De Duve, 1950; Hers, 1957; Raggi et al. 1960; Prasannan and Subrahmanyan, 1968) with the result that the loss of 2-deoxyglucose-6-phosphate is negligible over the 45 minute experimental period (Sokoloff et al. 1977).

The validity of the 2-deoxyglucose method as a measure of function related glucose utilisation has been called into question by reports of significant phosphatase in cerebral tissue in vivo (Hawkins and Miller, 1978; Sacks et al. 1983; Huang and Veech, 1982). However, careful examination of these studies reveals fundamental flaws in each.

In a study by Sacks et al. (1983), the arteriovenous difference in ^{14}C following a bolus injection of ^{14}C-2-deoxyglucose was shown to be negative. Thus, they concluded that since no ^{14}C-2-deoxyglucose-6-phosphate could be detected in the blood samples, there must be significant phosphatase activity in brain tissue (Sacks et al. 1983).

Studies of arteriovenous differences in 2-deoxyglucose can be used to assess phosphatase activity only if the pools of 2-deoxyglucose in plasma and in brain tissue are in equilibrium (Gjedde, 1984; Nelson et al. 1986). Since the study by Sacks et al. (1983) was performed under conditions where arterial tracer concentrations were rapidly declining, their conclusions concerning phosphatase activity were invalid.

In the study by Hawkins and Miller (1978) the authors compared the actual concentration of deoxyglucose-6-phosphatase in brain tissue with that predicted by the Sokoloff equation. Since the predicted value was some 2 to 3 fold higher than the actual deoxyglucose-6-phosphate concentration, Hawkins and Miller concluded that the discrepancy between the measured predicted values was due to high phosphatase activity. This conclusion has been challenged by Sokoloff (1982) and others (Nelson et al. 1986) who argued that the lumped constant used by Hawkins and Miller (1978) to predict deoxyglucose-6-phosphate levels (i.e. 1.10) was too high. Indeed if the calculations were performed using the appropriate lumped constant (0.483) the predicted values would be indistinguishable from that measured by biochemical assay (Sokoloff, 1982; Nelson et al. 1986).

In 1982, Huang and Veech reported that the half life of glucose-6-phosphate in brain tissue was approximately 20 minutes. Obviously, such high levels of phosphatase activity, if correct, would completely invalidate the 2-deoxyglucose method. The principle behind the study by Huang and Veech is simple but sound. Metabolism of 2-$^3$H-glucose as far as fructose-6-phosphate results in the loss of the tritum label. By contrast, the $^{14}$C-moiety is retained at this stage of the glycolytic pathway. A proportion of the fructose-6-

phosphate is converted back to glucose-6-phosphate which then could be returned to the glucose pool by glucose-6-phosphatase. Significant phosphatase activity would, therefore, result in an increase in the $^{14}C/^3H$ ratio in the glucose pool. The validity of this approach depends upon purification techniques which yield glucose which is uncontaminated by products of glucose metabolism. However, recent studies have shown that the separation methods used by Huang and Veech result in a glucose fraction which is contaminated by glucose metabolites which have lost the $^3H$, but not the $^{14}C$ label (Nelson et al. 1985, 1986). Indeed a repetition of the Huang and Veech study, but with more rigorous purification techniques has failed to demonstrate significant phosphatase activity in brain tissue (Nelson et al. 1985).

Recent cytochemical studies have suggested that the glucose-6-phosphatase enzyme is located on the luminal side of the endoplasmic reticulum, or microsomes derived therefrom (Arion et al. 1980). Tissues displaying high phosphatase activity (viz. the liver), possess a translocase enzymes which serves to ferry substrate from the cytoplasm to the phoshatase in the lumen of the endoplasmic reticulum (Arion et al. 1980). This translocase enzyme appears to be absent from cerebral tissue (Fishman and Karnovski, 1986), with the result that the phosphatase has to rely on passive diffusion to obtain substrate. Thus, not only is activity of glucose 6-phosphatase low in brain tissue but the enzyme may also be physically separated from its substrates.

Although, the weight of evidence would support the view that the phosphatase activity is not sufficient at 45 minutes to

compromise the method, it is recognised that phosphatase is not zero, and may become an important limitation if the measurement period exceeds 1 hour (Sokoloff, 1982, 1986). The underestimation of glucose use due to phosphatase activity is of particular relevance to clinical studies using [18]Fluoro-deoxyglucose in conjunction with positron tomission - tomography where the measurement period can extend to 2 hours (Reivich et al. 1979). To overcome this problem the Sokoloff equation has been revised to include a $K_4$ term which represents the rate of loss of phosphorylated label (Sokoloff, 1982). However, for experiments of 45 minutes duration the original Sokoloff equation may suffice (Sokoloff et al. 1983).

In theory, the 2-deoxyglucose method is applicable only to those experiments where plasma glucose and local rates of glucose utilisation remain constant throughout the experimental period. A subsequent modification of the operational equation permits the 2-deoxyglucose method to be used under conditions of moderate dynamic fluctuations in plasma glucose (Savaki et al. 1980). Interestingly, a comparison of the Savaki model with the original equation has revealed that the original method can accommodate moderate dynamic changes ($\pm$ 10%) in plasma glucose without seriously impairing the accuracy of the measurement (Sharkey and McCulloch - unpublished observations).

The assumption that in conscious animals local rates of glucose use remain constant throughout the 45 minute experimental period is unrealistic. However, although rates of glucose utilisation are measured over 45 minutes, the results are weighted towards the early part of the experiment, particularly over the period between 5 and 15

minutes after 2-DG administration. Consequently, studies should be designed so that the behavioural state under investigation will be sustained for at least 15 minutes (Sokoloff et al. 1983). As the drugs used in this thesis produced sustained alterations in behaviour they do not present any major problems for glucose use determination.

The lumped constant, corrects for the relative abilities of glucose and 2-deoxyglucose to enter the tissue metabolic pool. As it has to be determined in a separate group of animals it represents a potential source of error in the estimation of local rats of glucose phosphorylation. To date, the value of the lumped constant has been determined in the rat, cat, dog, sheep, monkey and human (for review see Sokoloff, 1986). These studies have indicated that although interspecies differences in the value of the lumped constant do occur (0.344 in monkey to 0.568 in adult human) the values within each species remain relatively constant over a wide range of experimental conditions (Sokoloff, 1979, 1986; Crane et al. 1981; Gjedde, 1984). Indeed, the only conditions where significant alterations in the lumped constant have been observed have been in severe hypoglycaemia (plasma glucose less than 5 mM) and hyperglycaemia (plasma glucose greater than 17 mM) and in studies involving pathological alterations in brain structure (Suda et al. 1981; Schuier et al. 1981; Ginsberg and Reivich, 1979). These constraints are of particular relevance to studies involving dopamine receptor agonists. The administration of dopamine receptor agonists can result in marked increases in plasma glucose (McCulloch et al. 1982 a; Tyce, 1976). In the case of LY 141865 plasma glucose levels can exceed 24 mM (Figure 7). At these levels the 2-deoxyglucose method would be compromised by significant

alterations in the rate constants and lumped constant (Schuier et al. 1981; Sokoloff et al. 1983). The hyperglycaemic effects of LY 141865 can be largely attenuated by fasting (Figure 7), however, care must be taken to ensure that the period of starvation does not exceed 18 hours as it has been shown that longer periods of fasting result in the significant use of ketone bodies as an energy substrate (Gjedde and Crone, 1975; Ruderman et al. 1974). For this reason the animals participating in the present studies were fasted for a period of 12 – 15 hours prior to surgery.

## 2.3.3 Practical Considerations

Measurement of local cerebral glucose utilisation was initiated thirty minutes after drug administration by an intravenous pulse of $^{14}$C-2-deoxy-D-glucose (50 uCi in 0.7 ml of saline, infused over 30 seconds). Over the succeeding forty-five minute sampling period fouteeen timed arterial blood samples were withdrawn for the determination of plasma $^{14}$C and glucose profiles. Plasma was then prepared by the immediate centrifugation of arterial whole blood samples for approximately thirty seconds in a table-top microcentrifuge (Beckman). Aliquot of plasma (18.7 microlitres) from each sample were pipetted into plastic scintillation vials and processed for liquid scintillation analysis. A further ten microlitres of plasma was taken from each sample for the determination of plasma glucose levels in a semiautomated glucose analyser. At the end of the sampling period the animal was

FIGURE 7
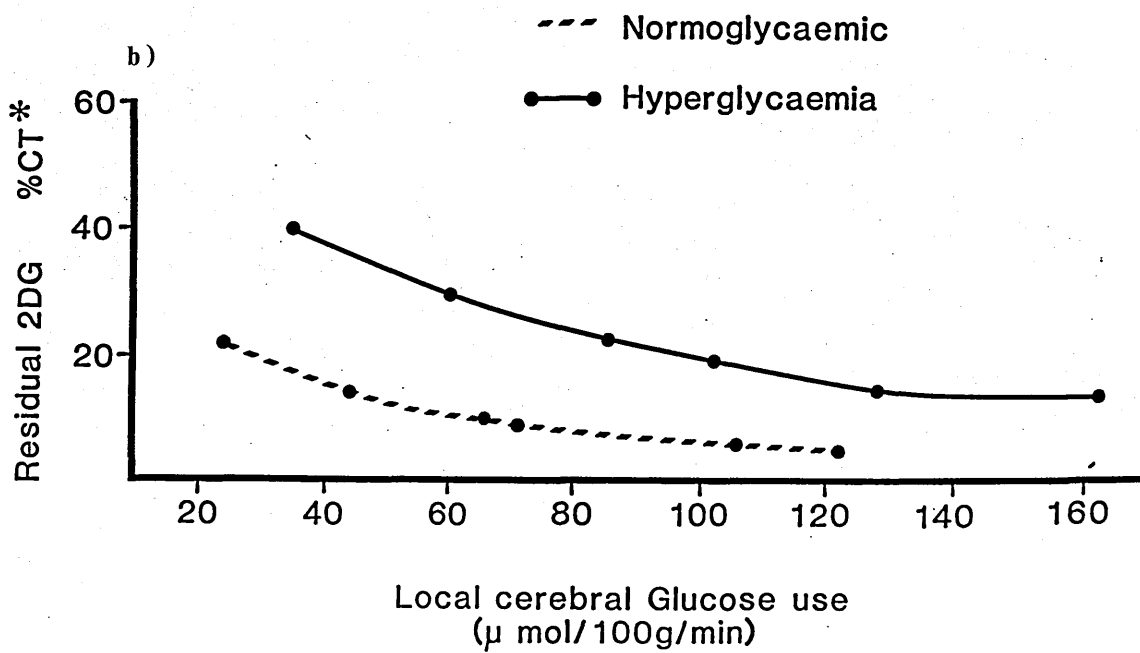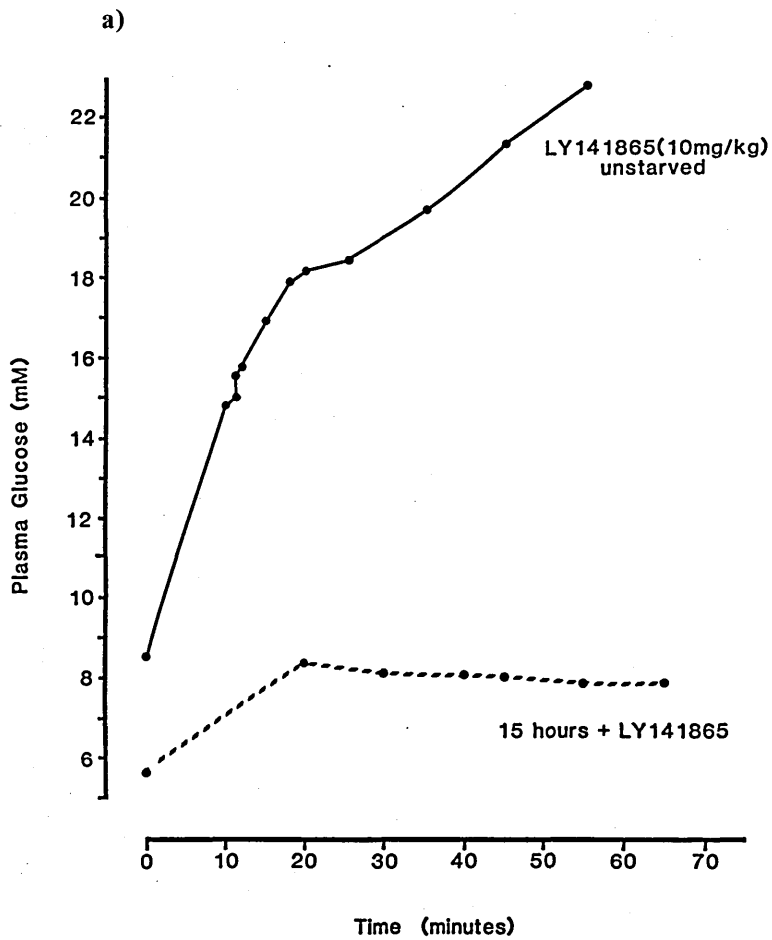
LEGEND TO FIGURES 7a and 7b


7a.    Plasma glucose levels in fasted (dashed line) and unfasted
       (solid line) rats following the intravenous administration of
       the dopamine $D_2$ receptor agonist LY 141865 (10 mg/kg).


7b.    Calculated levels of unphosphorylated 2-deoxyglucose expressed
       as a fraction of total brain isotope ($CT^*$) in six grey and
       white matter regions of the rat brain, 45 minutes after the
       intravenous delivery of tracer to the fasted (normoglycaemic)
       and unfasted (hyperglycaemic) rats.

sacrificed by decapitation and the brain processed for autoradiography.

Rates of glucose utilisation for each brain region were derived from a) the local tissue $^{14}C$ concentration, b) the plasma histories of $^{14}C$ and glucose and c) the appropriate rate constants for 2-deoxyglucose in grey and white matter by means of the operational equation as derived by Sokoloff and his associates (Sokoloff, 1977). The values of the rate constants $K_1^*$, $K_2^*$, $K_3^*$ and the lumped constant were as follows:-

|  | Grey Matter | White Matter |
|---|---|---|
| $K_1^*$ | 0.189 $(min^{-1})$ | 0.079 $(min^{-1})$ |
| $K_2^*$ | 0.245 $(min^{-1})$ | 0.133 $(min^{-1})$ |
| $K_3^*$ | 0.052 $(min^{-1})$ | 0.02 $(min^{-1})$ |

(Sokoloff et al. 1977)

Lumped constant = 0.483.

## 2.4   A Data Handling System for Fingerprinting Neuropharmacological

Responses.

### 2.4.1   The Model

The method proposed involves ranking brain regions according to their responsiveness over a chosen concentration range.  Hierarchies of regional responsiveness are derived from a function 'f' which is defined as -

$$f = \sum_{j=1}^{J-1} \left( X_c - X_j \right)^2 \qquad (6)$$

where $X_c$ and $X_j$ are the natural logarithms of the mean glucose use values obtained for the control group and $j^{th}$ drug treatment group, respectively.  $J-1$ describes the number of concentrations examined for the drug under investigation (usually 3 or 4).

The function offers a number of advantages over alternative ranking procedures (percentage changes or degree of statistical significance).  First, the model makes use of all data from the dose response curve for each brain region, and not just one selected concentration.  Second, the use of logged data serves to minimise the distorting influences associated with heteroscedasticity, and third, squaring the differences permits the analysis of bidirectional changes and serves to increase the sensitivity of the method.

## 2.4.2 Evaluation of the Fingerprinting Model

If the fingerprinting model is to be of value, the system of ranking ´f´ values must be reliable and reproducible.

### a)   Reliability of the Fingerprinting Method

The reliability of the fingerprinting approach was evaluated by means of the Bootstrap approach (Efron, 1982) in which the data set for each drug is compared with 100 simulated data sets, each having the same overall mean and variance structure as the original data. In order to perform analysis of this type a model (of repeated measures form) was constructed which describes observations made in a given brain area in terms of its contribution to the overall mean value of the region.

### The Repeated Measures Model

$$X_{ijk} = U_{ij} + A_{jk} + E_{ijk} \qquad (7)$$

where  —  $X_{ijk}$, denotes the value in region (i) of animal (k) which has received drug treatment (j), $U_{ij}$, is the mean response for region (i) after drug treatment (j), $A_{jk}$, represents "the animal effect"; the contribution made by animal k to the overall response to treatment (j). $E_{ijk}$ denotes the background variability of the data.

If it is assumed that there is constant variance between animals $(\tau^2)$ and within animals $(\sigma^2)$, independent of the type of observations being studied and the magnitude of $U_{ij}$ then

$$A_{jk} \quad N (0_1 \tau^2)$$

$$E_{ijk} \quad N (0_1 \sigma^2)$$

where $N (0_1 \tau^2)$ denotes the normal distribution (N) with mean (0) and variability $(\tau^2)$.

The assumption of constant variance is common to all the repeated measures analyses used in this thesis (drug interactions, flow/glucose use relationships and drug fingerprinting procedures). At first sight this assumption would appear to be invalid since obvious differences in variance exists, particularly between regions of high and low numerical value (if SEM of $\pm$ 26 for control auditory cortex blood flow with a SEM of $\pm$ 1 was observed in white matter). However, the assumption of exact equality of variance is not considered to be critical unless the variances are substantially different (Scheffe, 1959; McCulloch et al. 1982a). In practice this problem of heteroscedasticity can be largely overcome by employing logged data (McCulloch et al. 1982a).

In order to stimulate new data sets estimates of $U_{ijk}$, $\tau^2$ and $\sigma^2$ were obtained using the MINITAB computing package. The variance parameters $\tau^2$ and $\sigma^2$ were estimated via two way analysis of variance of each drug concentration and pooled to yield overall estimates of variance for each drug response (see Appendix 1). The calculated estimates of mean values ($U_{ijk}$) and variance parameters ($\hat{\tau}^2$ and $\hat{\sigma}^2$) were used subsequently to generate a series of 100 simulated data

sets of exactly the same format as the original data. The 'f' values
were then calculated for each of the simulations and compared with
the original data set via correlation. For simplicity correlations
were calculated between 'f' values rather than ranking position as
the same results should be obtained by both procedures. The
robustness of the method was further tested by repeating the
Bootstrap procedure both with increased variance (up to 16 fold). A
second correlation program analysed the relationship between the
simulated data of two different drugs. This procedure allows a
comparison to be made between simulated correlations and the actual
correlation obtained from the original data set and so gives an
estimate of the reliability about the original correlation.


b)    Reproducibility


An investigation of the reproducibility of the fingerprinting
model was performed by comparing the responses observed in the same
animals by two independent investigators. The original
autoradiograms and blood data for the apomorphine studies of
(McCulloch et al. 1982 b) were reanalysed using the same
densitometrical criteria used for the studies of LY 17155 and SKF
38393. In a series of preliminary investigations, the responses
measured by the two investigators were compared by the correlation
programs and the reliability assessed as before.


c)    Application of the Fingerprinting Model


Having established that the model is indeed reliable, and
reproducible (Appendix 1), it was used to compare the effects of SKF

38393 and LY 171555 on function-related glucose use with those produced by apomorphine (generated for the reproducibility study).

The reproducibility of the method also permitted the comparison of the functional responses evoked by SCH 23390 with published data for putatively $D_2$ selective concentrations of haloperidol (McCulloch et al. 1982 c).


3.   DRUGS AND TRACERS


The following pharmacological agents were kindly donated for the purpose of these studies:- SKF 38393 (Smith, Kline and French, Welwyn Garden City, England); LY 141865 and LY 171555 (Eli Lilly, Indianapolis, USA); SCH 23390 (Schering - Plough, Bloomfield New Jersey, USA). Other pharmacological agents used were -
Alpha Chloralose (BDH Chemical, Poole, England); Apomorphine (Janssen Pharmaceuticals, Belgium); Halothane (May and Baker, Dagenham, England); Heparin (Paines and Byrne, Greenford, England); Saffan (Glaxovet, Harefield, England); Xylocaine (Astra Pharmaceuticals, Kings Langley, England).

2[$^{14}$C]-deoxy-D-glucose and 4[N-methyl-$^{14}$C]-iodontipyrine were obtained at a specific activity of 280 mCi/mmol and 60 mCi/mmol from New England Nuclear (Boston, Massachusetts, USA).

RESULTS

1. DOPAMINE RECEPTOR SUBTYPES IN CEREBRAL ARTERIOLES

1.1 Vasomotor Responses of Feline Pial Arterioles in Situ to Dopamine Receptor Agonists.

The injection of approximately 5 microlitres of artificial cerebrospinal fluid (CSF) into the perivascular space surrounding individual pial arterioles (diameter 43 - 230 um) had no significant effect on arteriolar calibre (Table 4).

Perivascular microapplication of artificial CSF containing apomorphine ($10^{-8}$M to $10^{-4}$M) resulted in a dose-related increase in the calibre of pial arterioles (Table 4, Figure 8). Significant increases in pial arteriolar calibre were observed following microinjections of $10^{-6}$M apomorphine (23% increase with respect to preinjection calibre) with the maximum response (31% increase) at $10^{-5}$M. At the highest concentration examined, $10^{-4}$M, the vasomotor response to apomorphine was not significantly different from that produced by the administration of CSF.

The $D_1$ agonist SKF 38393 ($10^{-9}$M to $10^{-4}$M) provoked dose-dependent dilatations of pial arterioles. Significant increases in arteriolar calibre were observed at $10^{-8}$M SKF 38393 (15% increase in

Alterations in Feline Pial Arterioles Following Subarachnoid Perivascular

Microapplication of Putative Dopamine Receptor Agonists.

| Drug Concentration | APOMORPHINE | SKF 38393 | LY 141865 |
|---|---|---|---|
| 0 | (12) 1.1 ± 2.3 | (14) 0.0 ± 2.1 | (13) 0.2 ± 2.3 |
| $1 \times 10^{-9}$ | - | (10) 2.1 ± 2.9 | - |
| $1 \times 10^{-8}$ | (7) 2.5 ± 4.0 | (13) 14.8 ± 3.9* | (5) 3.0 ± 2.5 |
| $1 \times 10^{-7}$ | (11) 8.3 ± 1.8 | (9) 23.8 ± 3.0** | (6) 5.6 ± 1.6 |
| $1 \times 10^{-6}$ | (15) 23.1 ± 4.1** | (15) 20.0 ± 5.8** | (8) 11.6 ± 5.7 |
| $1 \times 10^{-5}$ | (9) 30.6 ± 5.8** | (12) 8.8 ± 3.0 | (8) 18.1 ± 3.7** |
| $1 \times 10^{-4}$ | (7) 3.3 ± 3.8 | (9) 4.1 ± 2.3 | (7) 24.6 ± 6.1** |

Data are presented as mean increase in arteriolar calibre $\pm$ SEM for the n arterioles tested with each solution. Each concentration was tested in 2-6 cats. $\hat{\Delta}$ % - Alteration in arteriolar calibre expressed as a per cent of preinjection diameter. *$p < 0.05$; $p < 0.01$ (derived from Student's t-statistic with Bonferroni correction for multiple comparisons).

FIGURE 8
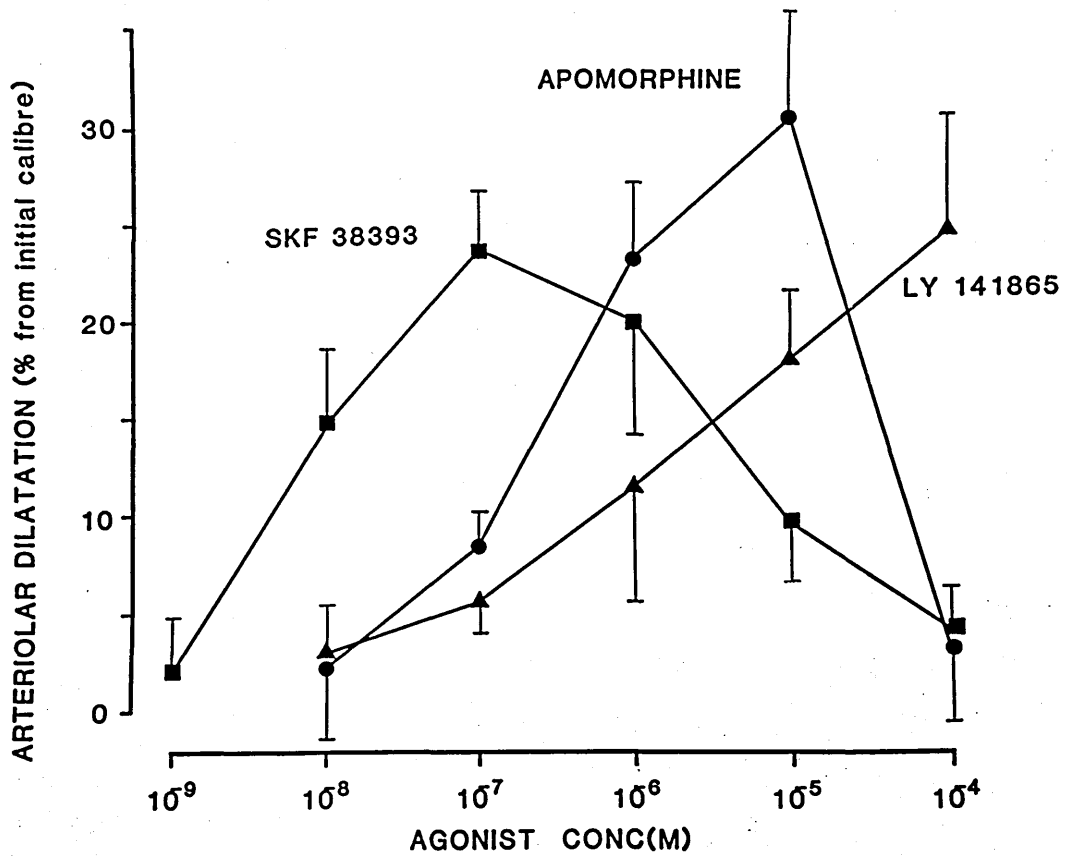


Alterations in pial arteriolar calibre produced by the perivascular microapplication of the dopamine receptor agonists apomorphine (●), SKF 38393 (■), and LY 141865 (▲). Data are presented as mean ± SEM.

calibre) with the maximum dilatations occuring at $10^{-7}$M (+24%). The dose-response curve to SKF 38393 was also biphasic with concentrations in excess of $10^{-7}$M provoking progressively smaller increases in arteriolar calibre.

The $D_2$ agonist LY 141865 was examined at five concentrations ($10^{-8}$M to $10^{-4}$M). Significant dilatations of pial arterioles occurred only at the highest concentrations tested ($10^{-5}$M and $10^{-4}$M) with the largest increases in calibre (+25%) occurring at $10^{-4}$M.

The rank order of potency, with respect to threshold and approximate $ED_{50}$ values (SKF 38393 > apomorphine > LY 141865) was consistent with a $D_1$ mediated dilatation of the cerebral vasculature. No significant correlation could be demonstrated between the magnitude of the dilatory response elicited by any of the agents tested and the preinjection calibre of the vessel.

## 1.2   Antagonism of Vasomotor Responses

The perivascular microapplication of the putatively selective $D_1$ receptor antagonist SCH 23390 ($10^{-9}$M – $10^{-5}$M) did not significantly alter the calibre of pial arterioles (Figure 9a). The concomitant administration of SCH 23390 potently antagonised the arteriolar dilatation produced by apomorphine ($10^{-6}$M) or SKF 38393 ($10^{-7}$M) – (Figure 9b), with $pA_2$ values between 8 and 9. SCH 23390, even at high concentrations ($10^{-7}$M) did not significantly alter the non-specific vasodilatatory response produced by acidic CSF (pH 6.8) (calibre increase by acidic CSF was 32.9 ± 4.2%, n=9 as compared to 31.1 ± 4.3%, n=8 for acidic CSF containing $10^{-7}$M SCH 23390).

FIGURE 9



a)

b)

Inhibition of the dilatation
produced by:-  ● SKF 38393

○ Apomorphine
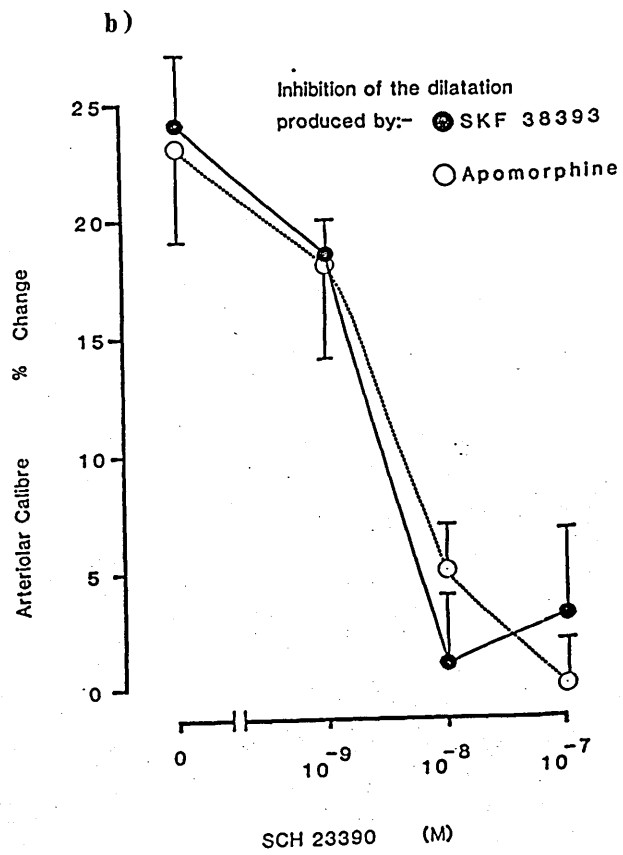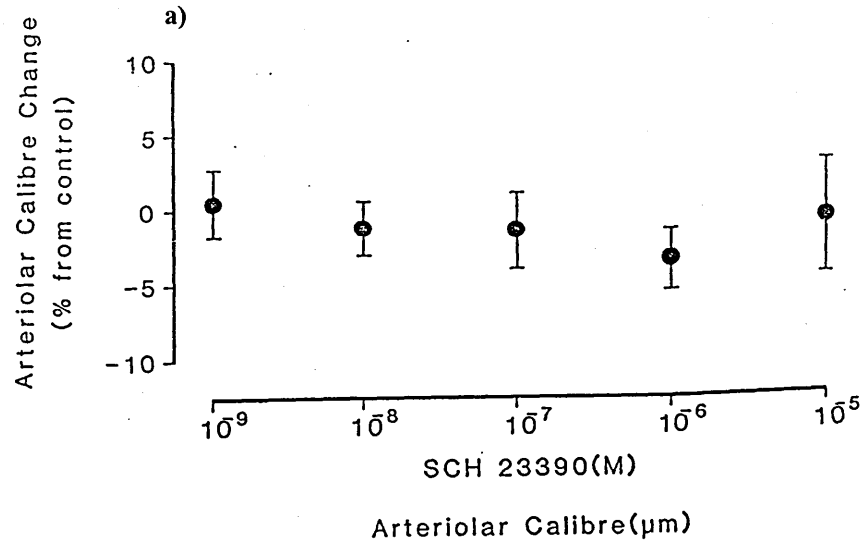
LEGEND TO FIGURES  9 a and 9 b

9a.  Alterations in pial arteriolar calibre following the perivascular microapplication of SCH 23390 ($10^{-9}$ – $10^{-5}$M). There was no significant alterations in arteriolar calibre at any of the concentrations examined. Data are presented as mean calibre change $\pm$ SEM.

9b.  The effect of the concomitant microapplication of SCH 23390 ($10^{-9}$M – $10^{-7}$M) on the arteriolar dilatation produced by apomorphine ($10^{-6}$M) or SKF 38393 ($10^{-7}$M). The dilitatory response to both agonists is attenuated significantly by SCH 23390 ($10^{-8}$M and $10^{-7}$M). Data are presented as mean calibre change $\pm$ SEM.  *$p < 0.05$ with respect to agonist alone.

2.    LOCAL CEREBRAL GLUCOSE UTILISATION

2.1   Effects of the Putatively Selective Dopamine Receptor Agonists

      SKF 38393 and LY 171555 Upon Local Cerebral Glucose Utilisation

      in the Rat.

2.1.1  General Results and Observations

       The intravenous administration of the putatively selective $D_1$

agonist SKF 38393 (1-30 mg/kg) provoked distinctive behavioural and

physiological responses in experimental animals.  Overt behavioural

responses behaviours were characterised by frequent bouts of grooming

and sniffing with occasional episodes of dyskinetic jaw movements

interspersed with periods of quiescence (particularly at 10 and 30

mg/kg).   Animals receiving SKF 38393 (1 mg/kg) appeared more alert

between bouts of grooming and sniffing as compared to animals treated

with larger doses of the drug. At no time during the measurement did

any animal receiving SKF 38393 display gnawing or any other

stereotyped behavioural response. There was a transient, increase in

arterial pressure immediately after administration of SKF 38393 with

blood pressure returning rapidly to pre-drug levels (Appendix 2.1).

At the time of administration of 2-deoxyglucose mean arterial

pressure was not significantly different from control values. No

significant alterations in core temperature, arterial blood gases or

plasma glucose concentration were detected at any concentration of

SKF 38393 examined.

Administration of the putatively selective $D_2$ agonist LY 171555 (0.1-5 mg/kg) was associated with characteristic changes in the behavioural and physiological status of the treated animals. Small doses of LY 171555 (0.1 mg/kg) resulted in intermittent episodes of sniffing and yawning. With increasing doses the sniffing behaviour was replaced by marked hyperactivity interspersed with intense and protracted gnawing of the lead support bricks. These behaviours were most marked with LY 171555 (0.5 mg/kg) with the intensity of the response subsiding at the higher doses. In addition to the behavioural changes described, low doses of LY 171555 (0.1 and 0.5 mg/kg) provoked a mild hypotensive response which tended to persist throughout the experimental period at the 0.5 mg/kg dose (Appendix 2.1). LY 171555 administration resulted in a dose-related increase in plasma glucose concentrations. However, even at the highest concentration of LY 171555 examined, (5 mg/kg) the increase in plasma glucose was small (<4mM) and remained relatively constant throughout the period of measurement.

There were minimal alterations in respiratory variables following the administration of SKF 38393 or LY 171555, with the exception of a slight acidosis at the lowest concentration of the $D_2$ receptor agonist.

## 2.1.2 Local Cerebral Glucose Utilisation

The effects of the selective dopamine receptor agonists SKF 38393 and LY 171555 upon function-related glucose utilisation in 59 anatomically distinct regions of the rat brain are presented in

Appendix 2. There were a number of distinctive features in the distribution and dose dependency of the alterations elicited by each of these dopamine receptor agonists.

The administration of the $D_1$ receptor agonist SKF 38393 failed to significantly modify the rate of glucose use in the majority of brain regions at any of the concentrations examined. In two brain areas (anterior cingulate cortex and anteromedial thalamic nucleus) SKF 38393 provoked dose-dependent reductions in glucose utilisation (Figure 10). In addition glucose use within the inferior olivary body and ventrolateral thalamic nucleus were affected by various concentrations of SKF 38393 although there was little evidence of true dose dependency in these responses (Figure 11). In 9 brain regions (including the stratum superficiale of the superior colliculus, the rostral neocortex and trigeminal nucleus) SKF 38393 significantly increased glucose use at a single concentration, most commonly 1 mg/kg (Figure 11). These significant alterations in glucose use, however, were not dose-dependent. There was a tendency for similar though non-significant increases in glucose utilisation in many brain regions of animals receiving SKF 38393 (1 mg/kg). These may reflect the mild behavioural stimulation observed in these animals.

The administration of the $D_2$ agonist LY 171555 (0.1 - 5 mg/kg) failed to significantly alter glucose use in the majority of brain regions investigated. In 24 of the 59 regions examined LY 171555 failed to provoke any significant change in glucose use at any of the concentrations examined. However, in each of these 24 areas small reductions in glucose use (approximately 10%) were noted with

FIGURE 10



Concentration of SKF 38393 ( mg/kg )

Concentration of SKF 38393 ( mg/kg )

Anterior Cingulate Cortex

Anteromedial Thalamic
Nucleus

Glucose Utilisation
( % change from control )
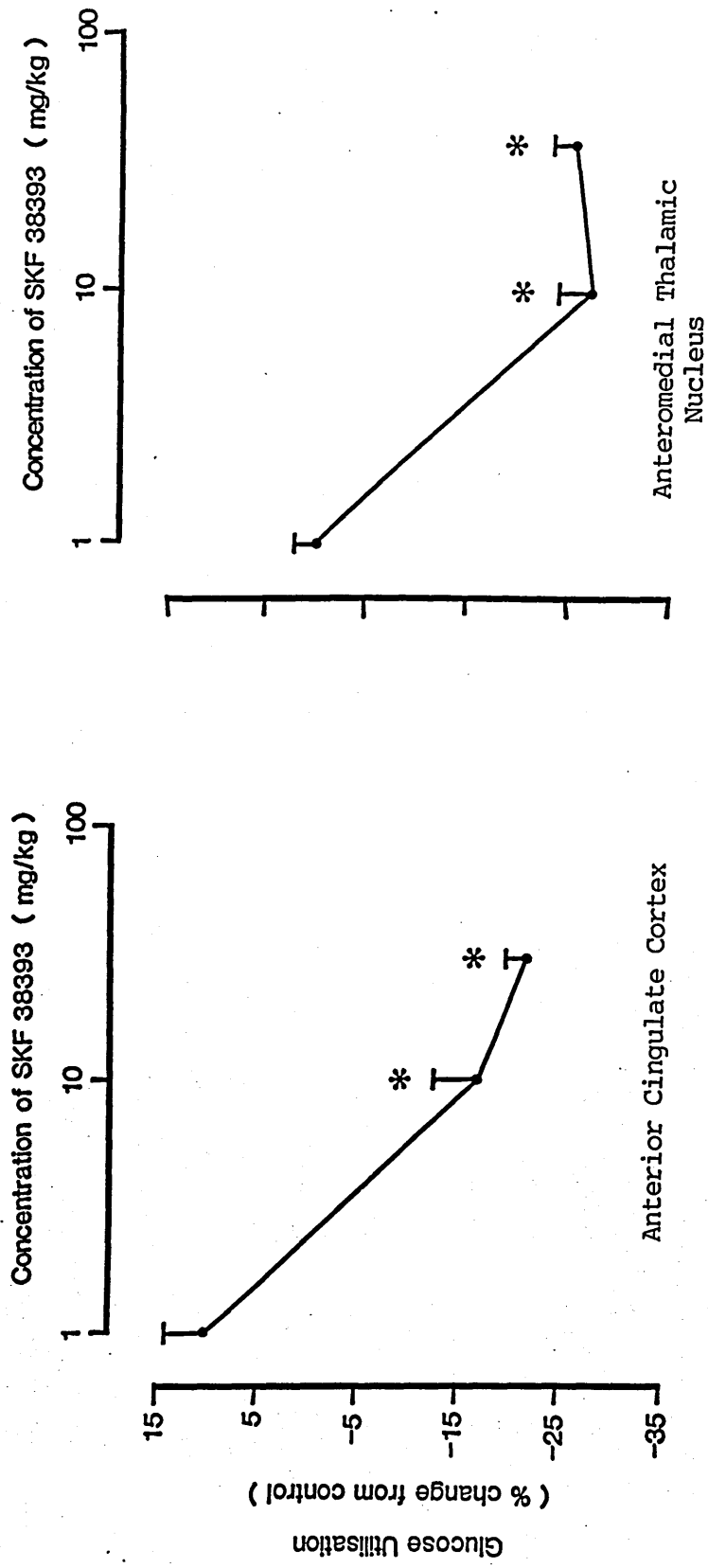
Log-dose response curves from two areas of the rat brain which displayed significant reductions in glucose use following the administration of SKF 38393. Data are presented as mean percentage change ± SEM.

FIGURE 11
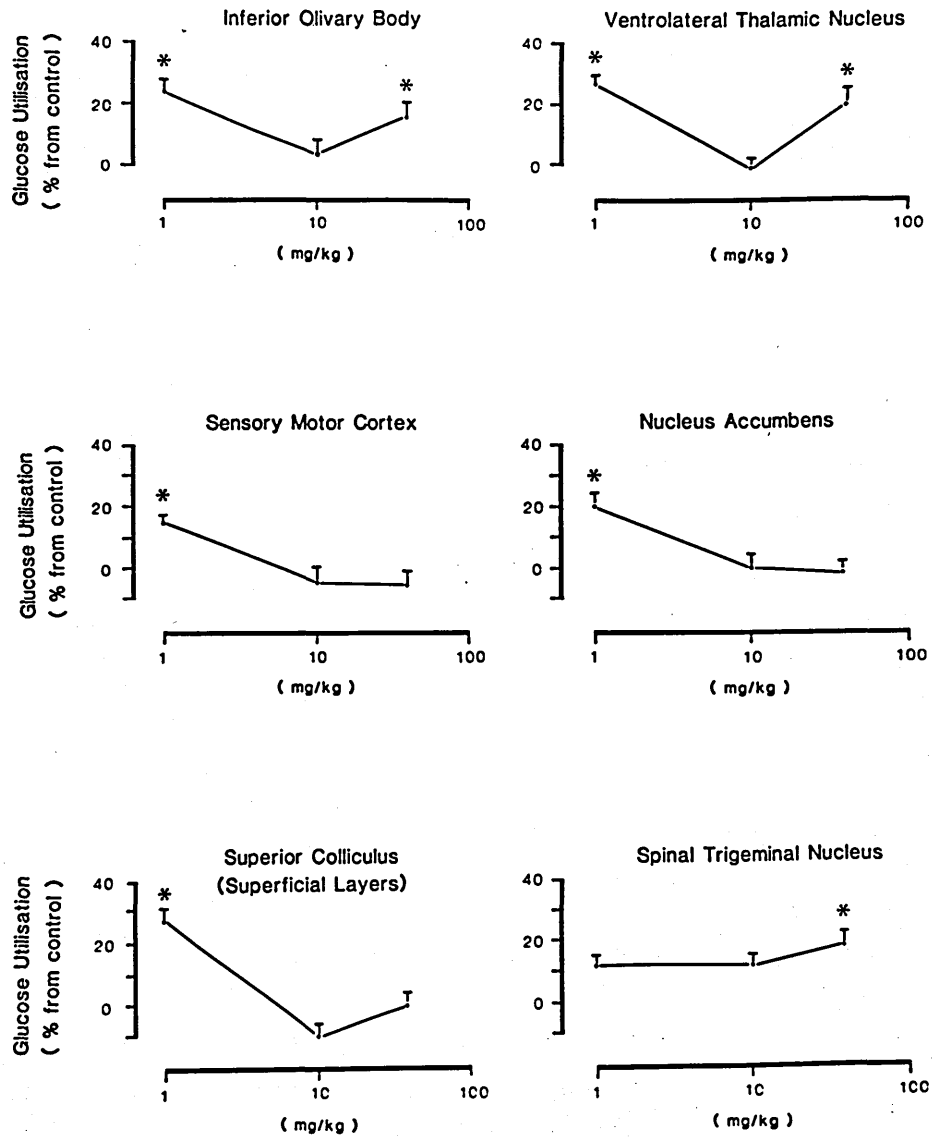


Log-dose response curves from six brain area of the rat brain in which the rate of glucose utilisation was significantly increased by the intravenous injection of SKF 38393.

Data are presented as mean percentage change ± SEM.  *p<0.05.

high concentrations of this $D_2$ agonist (Figure 12). In a further 13 brain areas no significant alterations in glucose use were observed in those animals receiving 0.1 - 1.5mg/kg, although significant reductions (approximately 24%) were evident at the largest concentration examined (Figure 13). These 37 brain regions, in which glucose use was insensitive to the $D_2$ agonist, LY 171555, encompassed most of the brain and included the dorsal caudate nucleus, lateral habenula, all nuclei involved in the processing of auditory information, the anterior cingulate cortex and caudal neocortex.

In 22 brain areas LY 171555 consistently effected marked and dose-dependent alterations in glucose utilisation. Of these 22 regions, 21 exhibited an increased glucose use whereas a reduction in glucose utilisation was observed only within the molecular layer of the hippocampus (Figure 14). The threshold for all significant increases in glucose use was in excess of 0.1 mg/kg with the maximal responses occurring at 0.5 mg/kg. At the largest concentration examined (5 mg/kg), the increase in glucose use provoked by LY 171555 within these 21 areas were generally less than the maximal response occurring at 0.5 mg/kg. This tendency towards depressed glucose utilisation was observed throughout the CNS, both in regions which were sensitive and in those which were insensitive to modification by this $D_2$ receptor agonist.

The administration of LY 171555 was associated with dose-related increases in glucose utilisation particularly in those areas of cortex and subcortex which subserve motor function (Figure 15). Within cortex, dose-related increases in glucose use were restricted

FIGURE 12



Parietal Cortex · Lateral Habenular Nucleus · Lateral Amygdala · Hypothalamus
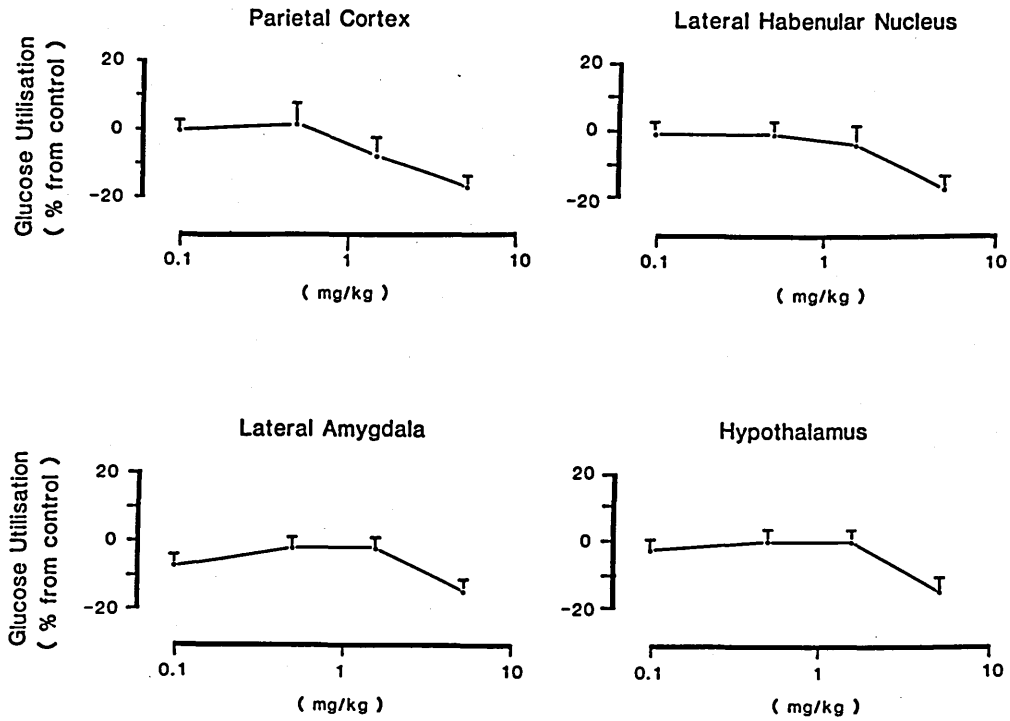
Log-dose response curves from four areas of the rat brain in which the rate of glucose utilisation was not significantly affected by LY 171555 at any of the concentrations examined (0.1 - 5 mg/kg). Data are presented as mean percentage change ± SEM.

FIGURE 13
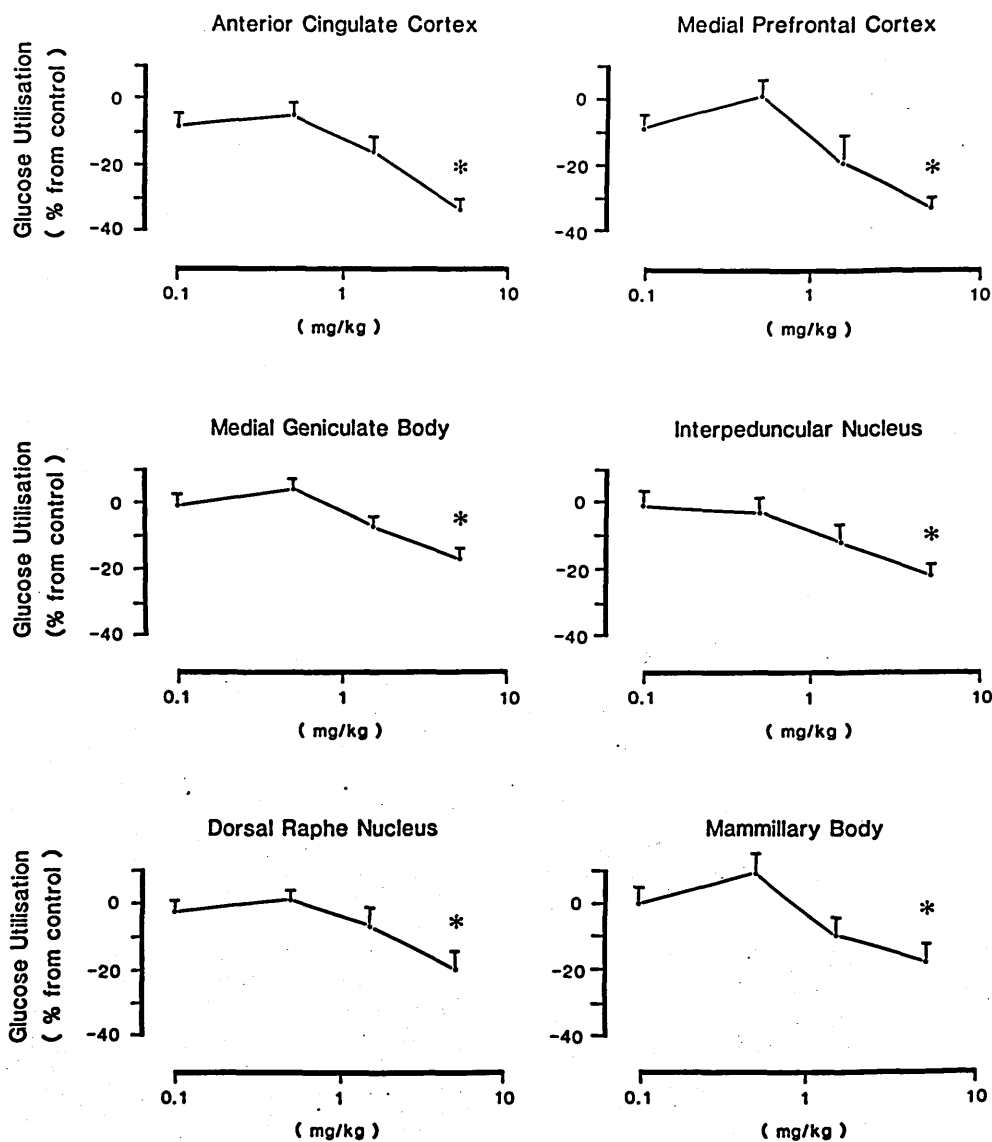


Log-dose response curves from six areas of the rat brain which displayed significant alterations in glucose use at only the highest concentration (5 mg/kg) of LY 171555 examined. Data are presented as mean percentage change from control ± SEM. *p<0.05.
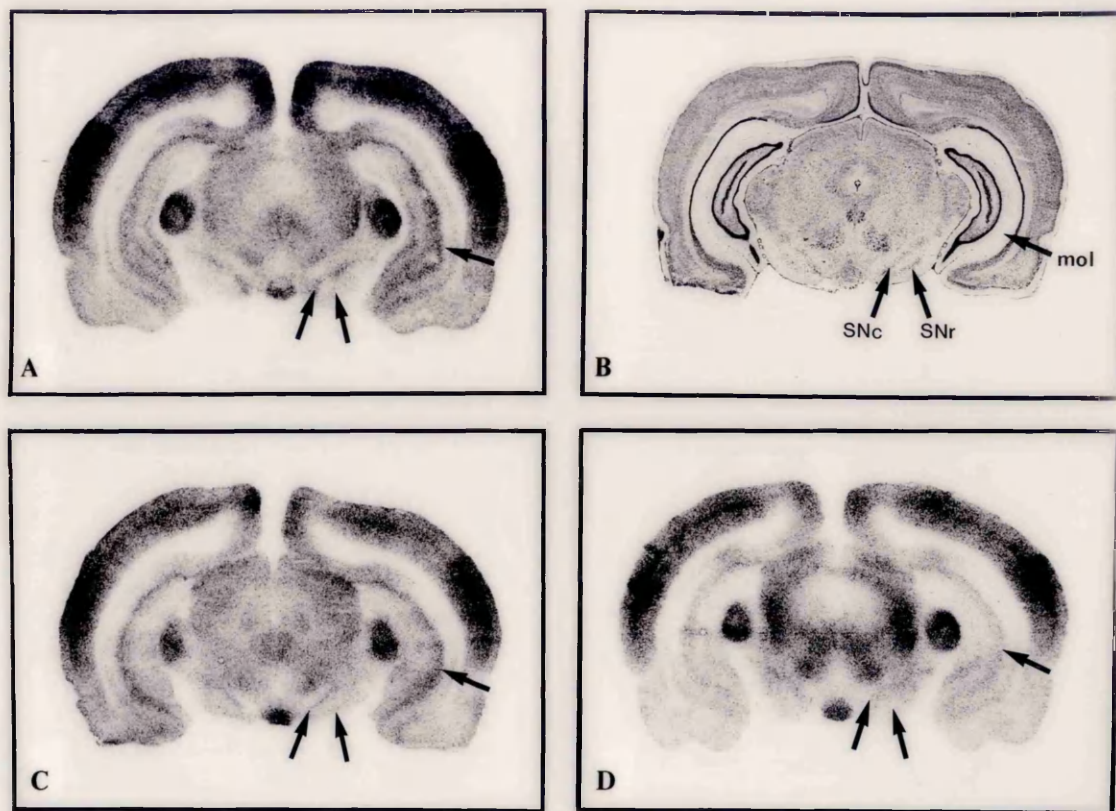
Representative autoradiographs from coronal sections of rat brain at the level of the medial geniculate bodies. Glucose use is proportional to relative optical density in the autoradiograms. A cresyl violet stained section is included (Fig. B) for the histological identifications of the molecular layer of the hippocampus (mol), the pars compacta (SNc) and pars reticulata (SNr) of the substantia nigra. (A) Saline control: the molecular layer of the hippocampus and the pars compacta of the substantia nigra appear as well defined bands of high optical density with respect to surrounding tissue. (C) SKF 38393 (30 mg/kg): pars compacta of the substantia nigra and molecular layer of the hippocampus remain well defined. (D) LY 171555 (0.5 mg/kg): a focal decrease in glucose use within the molecular layer of the hippocampus results in the loss of the distinctive band of high optical density observed in controls. A similar loss of definition within the pars compacta of the substantia nigra is due to a relatively more pronounced increase in glucose use in the adjacent pars reticulata of the substantia nigra (see Fig. 4).

FIGURE 15
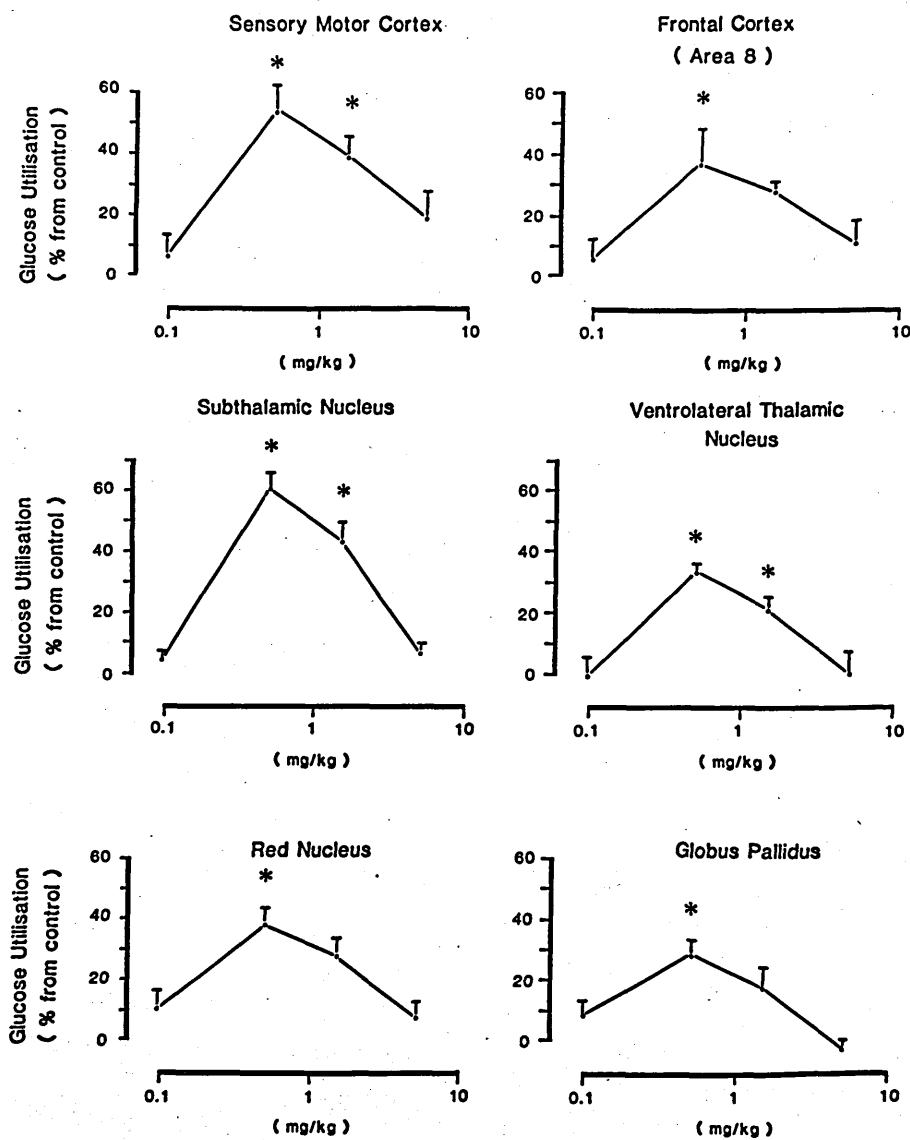


Log-dose response curves from six areas of the rat brain which displayed significant increases in glucose utilisation following the intravenous administration of low concentrations (0.5 mg/kg) of LY 171555. Data are presented as mean percentage change from control ± SEM. *p<0.05.

to sensory motor (by 54% following the administration of 0.5 mg/kg LY 171555) and area 8 of frontal cortex (37%). These alterations were organised in a columnar fashion and were confined not only to layer IV but also to layer VI (see Figure 16). Marked increases in glucose use were also evident within the basal ganglia and motor nuclei of the thalamus. Of these, the largest increases in glucose use were found within the subthalamic nucleus (by 64%: Figure 17) ventrolateral thalamic nucleus (45%) and red nucleus (39%: Figure 14). Proportionately smaller increases in glucose use occurred in the globus pallidus (30%) and pars reticulata of the substantia nigra (33%: Figure 18a). In contrast glucose use in the pars compacta of the substantia nigra was not significantly altered (Figure 18a). Within the caudate nucleus glucose utilisation was topographically heterogeneous. Dose-dependent increases occurred in the ventral aspect of the caudate nucleus (19%) whilst the dorsal portion remained relatively insensitive to LY 171555 (Figure 18b).

Some of the most pronounced increases in glucose use following LY 171555 administration were found within the medulla, in particular the spinal nucleus of the trigeminal nerve (64%) and cuneate nucleus (44%: Figure 19).

Small punctate areas of increased glucose use were observed in cerebellar cortex after the administration of this $D_2$ receptor agonist (see Figure 19). However the variability in the anatomical position of these alterations precluded accurate densitometrical analysis.

FIGURE 16
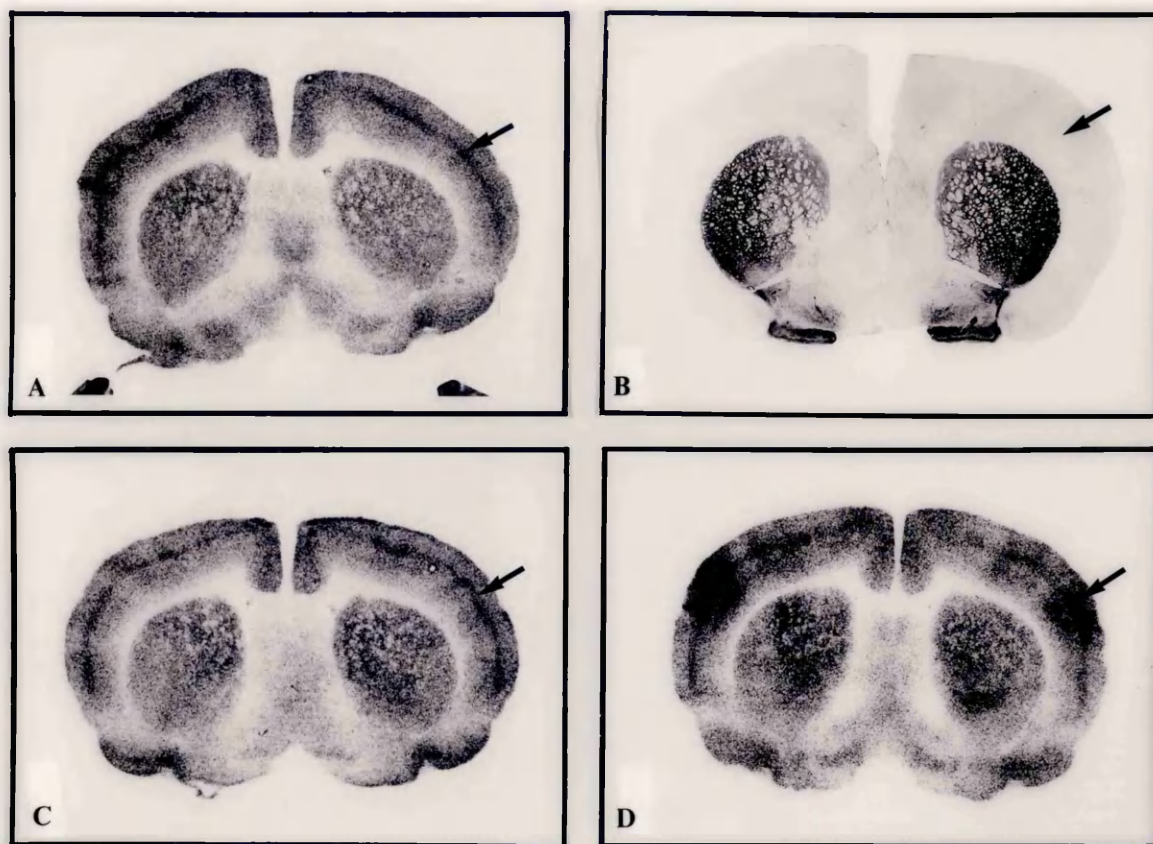


Representative autoradiographs from coronal brain sections at the level of the caudate nucleus. Glucose use is proportional to relative optical density within the autoradiograms. The acetylcholinesterase stained section (Fig. B) demonstrates the heterogenous nature of acetylcholinesterase activity within the caudate nucleus. (A) Saline controls: within sensory motor cortex layer IV appears as a band of uniformly high optical density relative to surrounding cortical layers. Optical density within the caudate appears relatively homogenous. (B) SKF 38393 (30 mg/kg): no marked alterations in relative optical density in caudate nucleus and layer IV of sensory motor cortex relative to saline controls. (D) LY 171555 (0.5 mg/kg): note the laminar bands of relatively increased optical density (and glucose use) which traverse sensory motor cortex. These increases in relative optical density are evident both in layer IV and in layer VI of cortex. In addition, the caudate nucleus appears regionally heterogenous with marked increases in glucose use (and relative optical density) within the ventromedial portion).
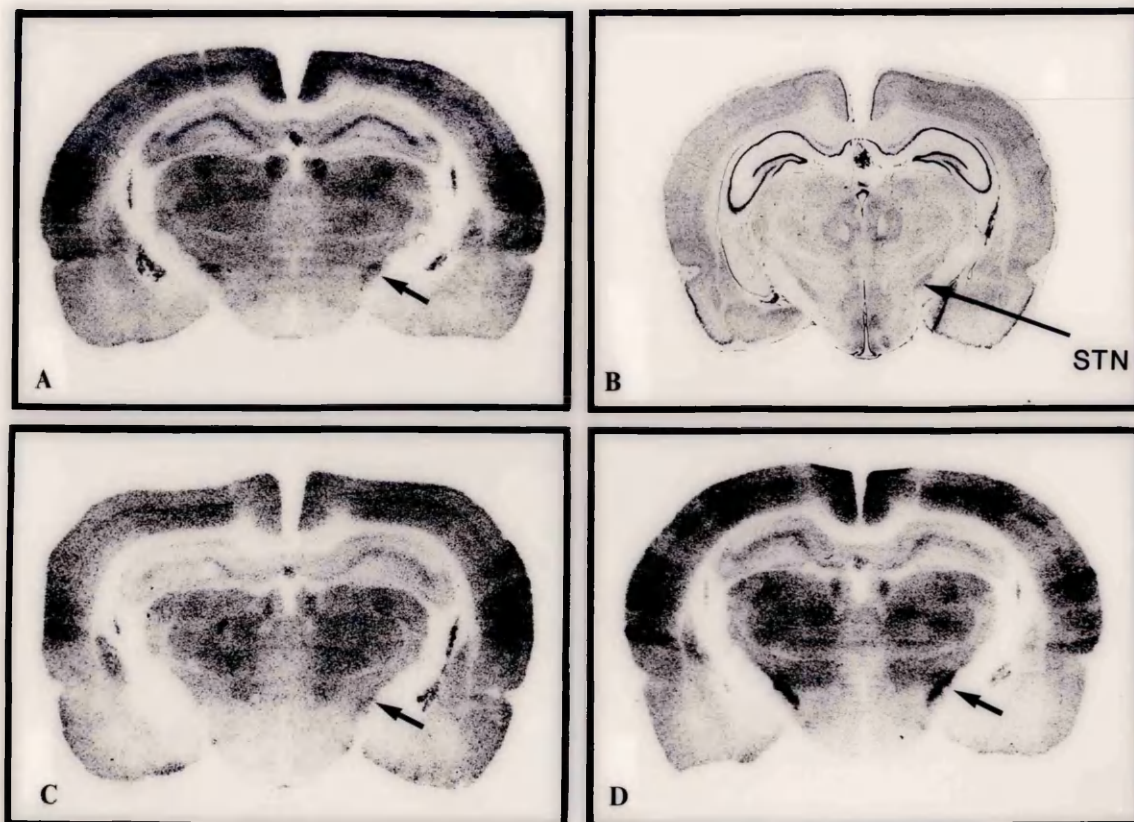
Representative autoradiographs obtained from coronal sections of rat brain at the level of the subthalamic nucleus. Glucose use is proportional to relative optical density autoradiographs. A cresyl violet stained section (Fig. B) is included for the histological identification of the subthalamic nucleus (STN). (A) Saline control: optical density of the subthalamic nucleus appears similar to the adjacent thalamus. (C) SKF 38393 (30 mg/kg): no significant alterations in relative optical density relative to controls are evident. (D) LY 171555 (0.5 mg/kg): the marked elevation in glucose use within the subthalamic nucleus is evidenced by a relative increase in optical density within this area.

FIGURES 18 a and b
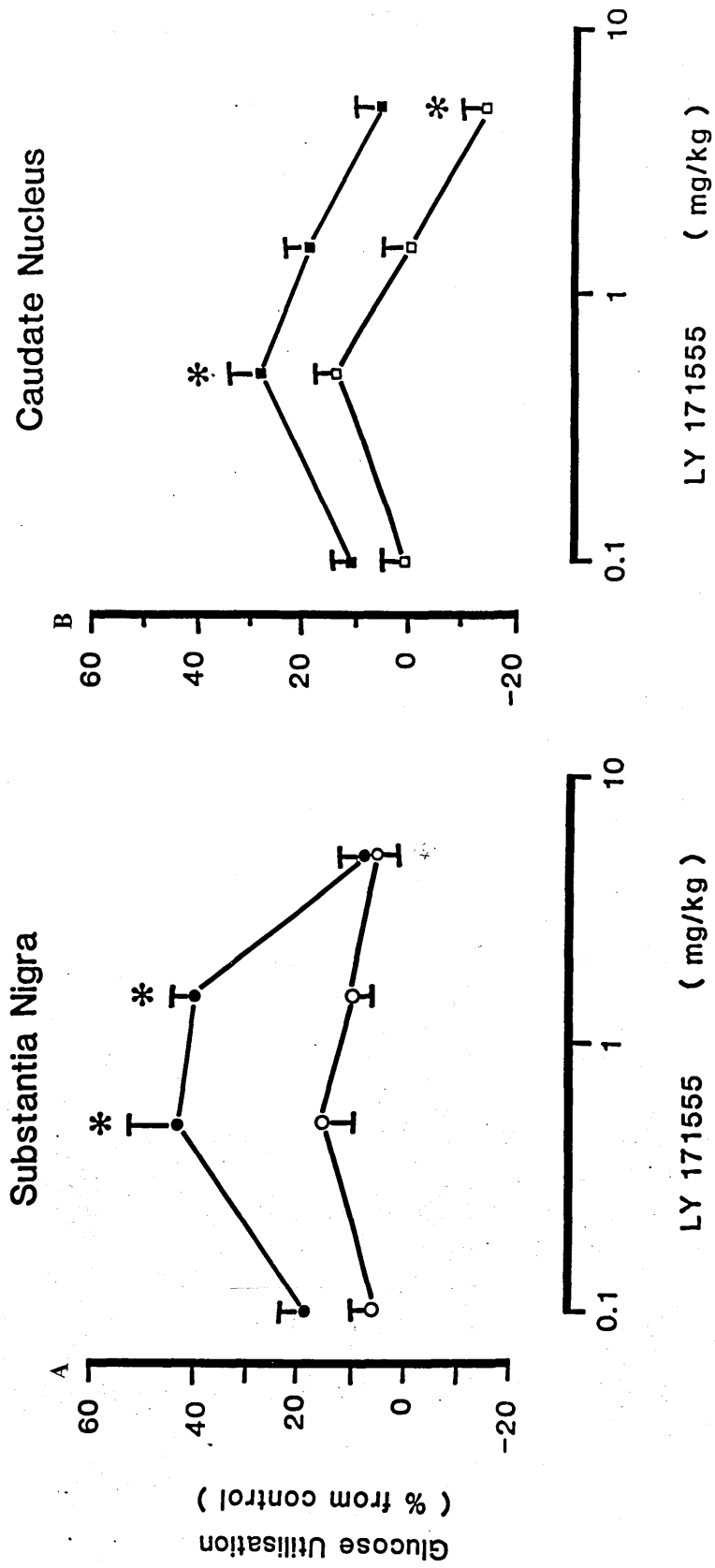
LEGEND TO FIGURES 18 a and 18 b


18a. Log-dose response curves from the substantia nigra pars compacta ( O ) and pars reticulata ( ● ) following the intravenous administration of LY 171555.


18b. Log-dose response curves from the dorsal [ □ ] and ventral ■ portions of rat caudate nucleus following the intravenous adminstration of LY 171555.

FIGURE 19
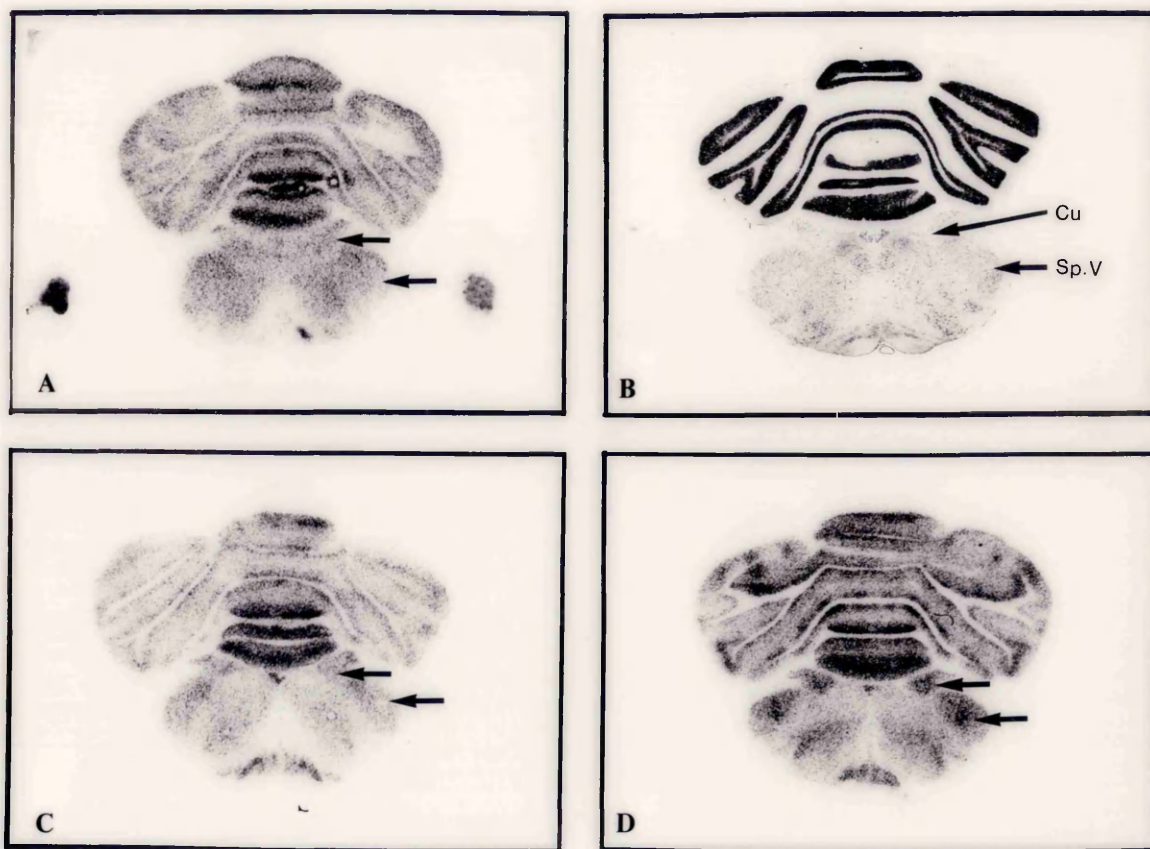


Representative autoradiograms from coronal brain sections at the level of posterior cerebellum. Glucose utilisation is proportional to relative optical density within the autoradiogram. A cresyl violet stained section (Fig. B) is included for the identification of the cuneate nucleus (Cu), and the spinal nucleus of the trigeminal nerve (Sp.V). (A) Saline control: both the spinal trigeminal and cuneate nuclei are indistinguishable from the surrounding medullary tissue. Cerebellar cortex appears uniform in optical density. (C) SKF 38393 (30 mg/kg). The slight increase in relative optical density of the spinal trigeminal and cuneate nuclei reflect the mild stimulation in glucose use in these areas noted at this concentration of SKF 38393. Cerebellar cortex remains relatively uniform in optical density. (D) LY 171555 (0.5 mg/kg). Marked increases in glucose use within the cuneate and trigeminal nuclei are evidenced by the high optical density of these nuclei relative to surrounding tissue. In addition note the small punctate areas of increased optical density within cerebellar cortex.

2.2    Effects of the Putatively Selective Dopamine $D_1$ Antagonist, SCH

23390 on Local Cerebral Glucose Utilisation in the Rat.


2.2.1  General Results and Observations


The intravenous injection of the putatively selective $D_1$ receptor antagonist SCH 23390 (0.05 - 1 mg/kg) failed to produce significant alterations in any of the cardiovascular or respiratory variables monitored as compared to vehicle treated control animals (Appendix 3). Animals that received the largest concentration of SCH 23390 (1 mg/kg) displaced significantly greater arterial plasma glucose concentrations than vehicle treated rats. Since the elevated glucose levels were within the "normal" physiological range and did not vary significantly over the period of glucose use determination, the principles of the 2-deoxyglucose method were upheld.

The administration of SCH 23390 resulted in dose-dependent reductions in spontaneous motor activity. Immediately following infusion of SCH 23390 spontaneous motor activity ceased while the animal remained quiet, its eyes closed and ears pricked. In animals receiving 1 mg/kg this behaviour persisted throughout the entire experimental period (85 minutes). In contrast, appparently normal locomotor behaviour had returned after 30 minutes in those animals receiving 0.05 mg/kg of SCH 23390.


2.2.2  Local Cerebral Glucose Utilisation


The effects of SCH 23390 on local rates of glucose utilisation

in 59 anatomically discrete regions of the rat brain are presented in Appendix 3.

The rate of glucose use, within 50 of the 59 brain regions examined, was minimally altered by SCH 23390 at any of the concentrations tested. Highly focal alterations in glucose utilisation were elicited by 0.1 mg/kg SCH 23390, with prominent increases in glucose use evident within the lateral habenula (24%: Figure 20) and nucleus accumbens (36%), and these were evident in the autoradiograms. In addition significant reductions in glucose use were observed within the posterioventromedial nucleus of the thalamus (-13%) and pars reticulata of the substantia nigra (-12%).

The largest concentration of SCH 23390 examined (1 mg/kg) provoked increases in glucose use within the nucleus accumbens and the lateral habenula which were measurably less than those produced by 0.1 mg/kg. This tendency towards depressed glucose use (approximately 10%) was evident throughout all brain structures examined but was most prominent and attained significance within the thalamus [posterior nucleus (-13%), ventromedial nucleus (-20%), posterioventromedial nucleus (-16%), medial geniculate body (-20%) the superficiale layers of the superior colliculus (-16%)], visual cortex (-19%) and the pars reticulata of the substantia nigra (-14%) (Figure 21).

No significant alterations in glucose use were evident following 0.05 mg/kg of SCH 23390 in any brain region which was examined.

FIGURE 20
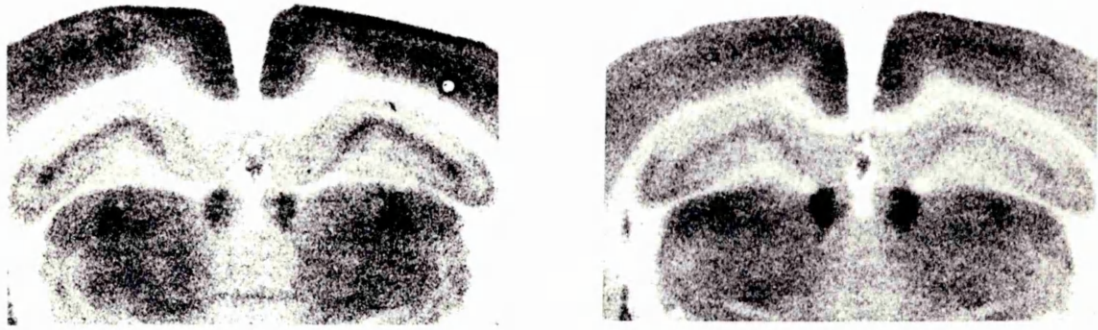


Saline
Control

SCH 23390
( 0.1mg/kg )
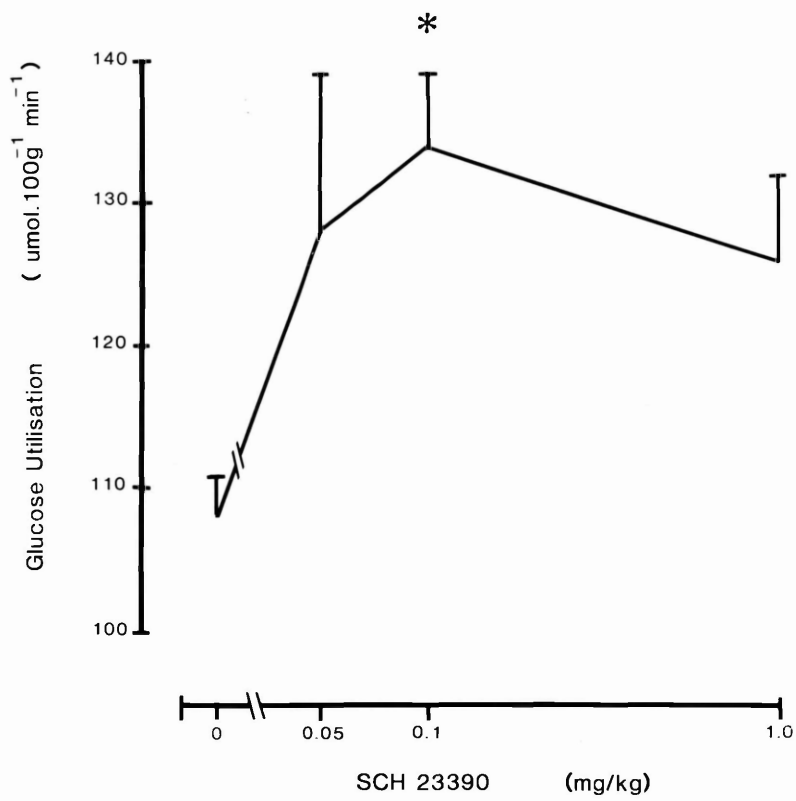


Effect of SCH 23390 upon Local Glucose Utilisation in
the Lateral Habenular Nucleus.

LEGEND TO FIGURE 20

Upper Left. Autoradiograph at the level of the habenular nuclei of a saline-treated control rat. The lateral habenula can be identified as the well defined band of relatively high optical density, lying on top of the medial thalamus. The optical density of the lateral habenula appears similar to that of the overlying cortex.

Upper Right. Autoradiograph at the level of the habenular nuclei of a rat which had received 0.1 mg/kg, SCH 23390. Glucose use is markedly increased and appears much darker on the autoradiograph than the overlying cortex.

Lower. Log-dose response curve of glucose utilisation in the lateral habenular nucleus following the administration of SCH 23390. Data are presented as mean glucose use ( $umol.100g^{-1}min^{-1}$) $\pm$ SEM. *p<0.05.

FIGURE 21



Nucleus Accumbens

Posterioventromedial Thalamic
Nucleus
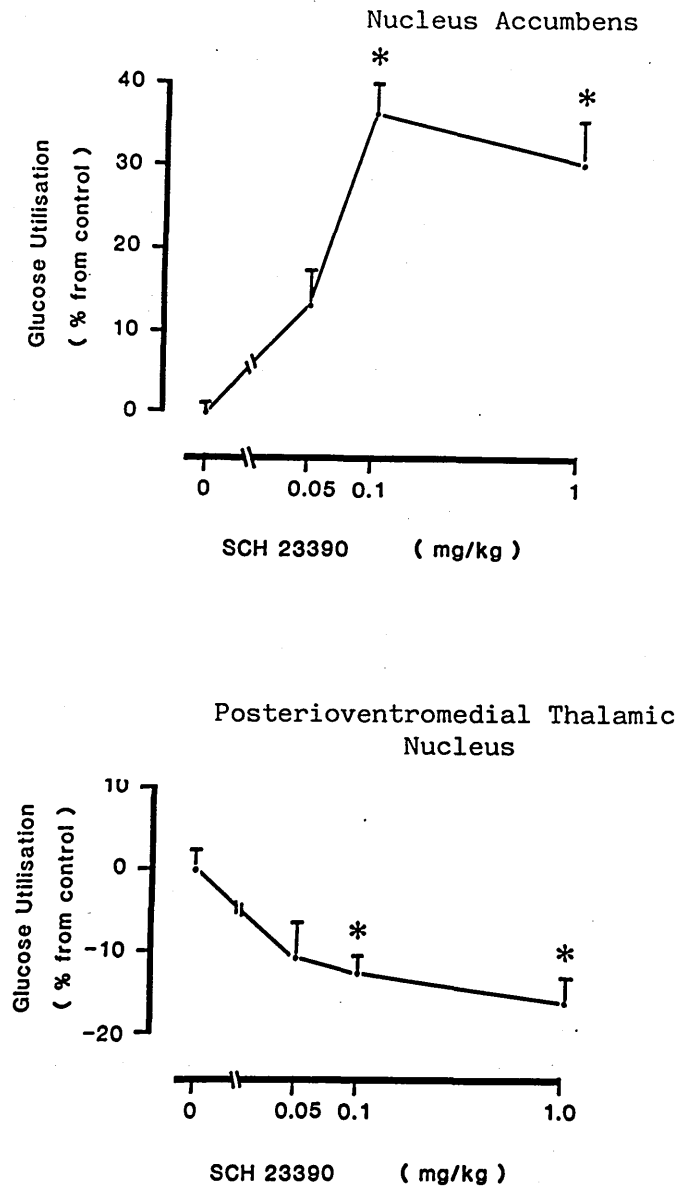
Log-dose response curves from 2 areas of the rat brain (nucleus accumbens and posterioventromedial thalamic nucleus) which displayed significant alterations in rates of glucose use following the administration of SCH 23390. Data are presented as mean percentage from control ± SEM. *p<0.05.

## 2.3  Interaction Between LY 171555 and SCH 23390

### 2.3.1  General Results and Observations

The prior administration of SCH 23390 (0.5 mg/kg) completely abolished the stereotyped behaviour produced by LY 171555 (0.5 mg/kg). Upon receiving LY 171555 rats became transiently hyperactive with jerking and side-to-side movements of the upper body. Thereafter, the hyperactive response was progressively replaced by extended periods of quiescence interspersed with bouts of sniffing. No animal receiving both drugs displayed the gnawing or biting behaviours characteristic of $D_2$ agonist administration at any time following LY 171555 administration.

Cardiovascular and respiratory parameters were unaffected by interaction of SCH 23390 and LY 171555 (Appendix 3.1).

### 2.3.2  Local Cerebral Glucose Utilisation

The effects of the interaction between the $D_2$ receptor agonist LY 171555 (0.5 mg/kg) and the $D_1$ antagonist SCH 23390 on local cerebral glucose utilisation are presented in Appendix 3.

Analysis of co-variance (of repeated measured form) revealed that pretreatment with SCH 23390 significantly altered the effect of $D_2$ agonist LY 171555 on local cerebral glucose utilisation (P = 0.0001). However, inter-regional analysis demonstrated that SCH 23390 pretreatment did not affect uniform changes in glucose use

throughout the brain (Greenhouse Geisser probability = 0.0079).
Further analysis of this regional heterogeneity performed by means of
a Student's-t comparison between the LY 171555 (agonist) and the SCH
23390 + LY 171555 (interaction) groups (Appendix 3) revealed that the
prior administration of SCH 233900 significantly affected glucose use
in 29 of the 59 brain regions examined.

SCH pretreatment significantly attenuated the widespread
increases in glucose utilisation produced by LY 171555 in those areas
of cortex and subcortex which subserve somatosensory and motor
functions (Figure 22). Of these, the most pronounced reductions in
glucose use were observed within sensory motor cortex (-40% with
respect to LY 171555 treatment value), ventrolateral thalamus (-34%)
and subthalamic nucleus (-32%). In addition, SCH 23390 pretreatment
produced significant reductions in glucose utilisation within a
number of brain areas which were not sensitive to LY 171555 or SCH
23390 alone. In particular, the prior administration of SCH 23390
depressed glucose use in all rostral cortical areas examined,
including the anterior cingulate cortex (-19%). The decrease in
glucose use within the anterior cingulate cortex was accompanied by
a proportionately similar reduction in the anteromedial thalamus
(-20%). Reduced functional activity within frontal cortical areas
was also reflected in lower rates of glucose utilisation within the
white matter tracts of the corpus callosum. The prior
admininstration of SCH 23390 significantly increased glucose use in
only 3 of the 59 areas examined, namely the nucleus accumbens (+24%),
lateral habenular nucleus (+23%) and superior olivary body (+21%).

FIGURE 22
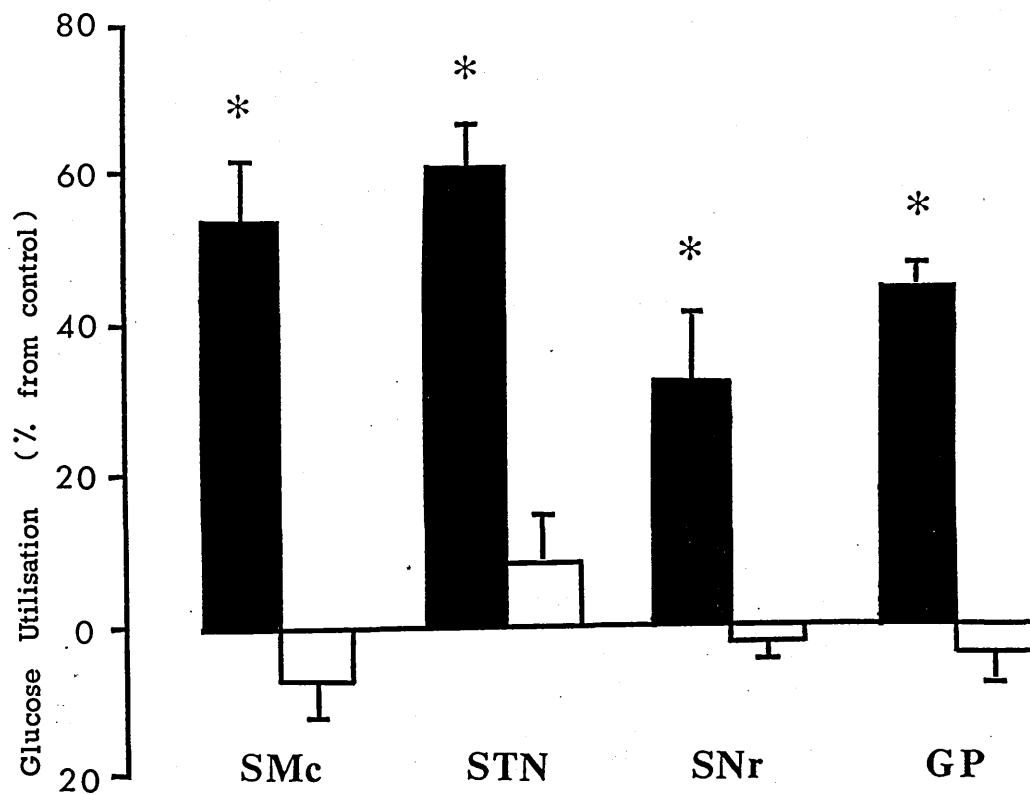


Attenuation of the increases in glucose use produced by LY 171555 (0.5 mg/kg, ■ ) by the prior administration of the $D_1$ antagonist SCH 23390 (0.05 mg/kg □ ) in four brain areas; sensory motor cortex (SMc), subthalamic nucleus (STN), pars reticulata of the substantia nigra (SNr) and globus pallidus (GP). In each of the brain areas the antagonist pretreatment significantly attenuated the glucose use response. Data are presented as mean percentage change from saline-treated control values ± SEM.

## 3.  FUNCTIONAL PROFILES OF DOPAMINERGIC ACTIVITY

Having established the reproducibility and reliability of the 'fingerprinting' approach for drug analysis (see Appendix 1), this method was used to examine and compare the effects of dopamine receptor agonists, and antagonists upon local cerebral glucose utilisation.

### 3.1  Agonist Studies

In this study the data sets for the SKF 38393, LY 171555 and apomorphine (generated for the reproducibility study in Appendix 1) treatments were analysed using the 'f' function and hierarchies of regional 'f' values constructed. In the previous section it was noted that widespread reductions in glucose use were observed at the highest concentrations of LY 171555 examined (5 mg/kg). As these widespread reductions in glucose use occurred at concentrations which were higher than that required to promote stereotypy (a response normally associated with $D_2$ receptor activation), they may reflect the action of LY 171555 on mechanisms other than via dopamine $D_2$ receptors. Non-specific drug effects would confound the interpretation of the drug's actions and so the 5 mg/kg concentration was omitted from the analysis.

The intravenous administration of the $D_1$ receptor agonist, SKF 38393 resulted in a relatively narrow range of 'f' values (0.18 - 0.005). Inspection of the hierarchy of 'f' values resulted from SKF 38393 (1 - 30 mg/kg) administration confirmed the view that the anteromedial thalamus and anterior cingulate cortex (ranked 59 and 58 respectively) were profoundly influenced by this $D_1$ receptor agonist,

whereas sensory motor cortex (ranked 8 of the 59 areas) and subthalamic nucleus (5) were minimally affected (Table 5). As noted previously the majority of brain areas were insensitive to SKF 38393 administration. As a consequence, those areas in which significant, though non-dose dependent, alterations in glucose use that were observed following Scheffe analysis (Appendix 2) were generally found to occupy high ranking positions (i.e. trigeminal nucleus (57), ventrolateral thalamus (55), superficial layers of the superior colliculus (51).

Administration of the $D_2$ agonist LY 171555 (0.1 - 1.5 mg/kg) produced a wider range of ´f´ values (0.49 - 0.0006) (Table 6). In accordance with Scheffe analysis (Appendix 2) the most prominent responses, and consequently highest ranking positions, were found in brain areas subserving motor function; trigeminal nucleus (59) subthalamic nucleus (58), sensory motor cortex (56). Anteromedial thalamus (43) and anterior cingulate cortex (30) possessed intermediate ranking positions whereas the lateral habenula was towards the bottom of the hierarchy (2).

Of the three dopamine agonists examined, apomorphine produced the most marked behavioural and metabolic responses (Appendix 4). Consequently, the range of ´f´ values (2.003 - 0.005) was greater than than produced by the other two agents. Inspection of the hierarchy of regional responsiveness to apomorphine administration (Table 7) revealed that the pattern of alterations in glucose use produced by this mixed $D_1/D_2$ receptor agonist contained elements of both the $D_1$ and $D_2$ agonist responses. Hence, glucose use was increased within motor areas as was observed following LY 171555 administration. Similarly, reductions in functional activity within the anterior cingulate cortex and anteromedial thalamus associated

with SKF 38393 administration also occurred following apomorphine administration. However, the action of apomorphine upon local cerebral glucose use was not merely a composite of the SKF 38393 and LY 171555 responses. Although, both apomorphine and LY 171555 increased glucose use within the basal ganglia (substantia nigra, caudate nucleus, globus pallidus, subthalamic nucleus and red nucleus inter alia), the hierarchy of regional responses produced by these two agonists were markedly different.

The reliability of each data set was examined by comparing the original values with 100 simulated data sets (f1 - f100) having precisely the same mean and variability structures (Efron, 1982). For each drug the correlation between the original and simulated data was high; indicating that the hierarchy constructed for each drug was reliable.

Correlation $(F_i, f_i)$, i = 1-100

SKF 38393   = 0.92

LY 171555   = 0.99

Apomorphine = 0.94

The initial impression that the three dopamine receptor agonists provoked markedly different patterns of regional responsiveness was borne out by plots of the ´f´ values (Figure 23). Correlation analysis between the sets of ´f´ values confirmed this view.

Correlation (SKF 38393, LY 171555)   = 0.085

Correlation (SKF 38393, Apomorphine)   = 0.161

Correlation (LY 171555, Apomorphine)   = 0.241

FIGURE 23
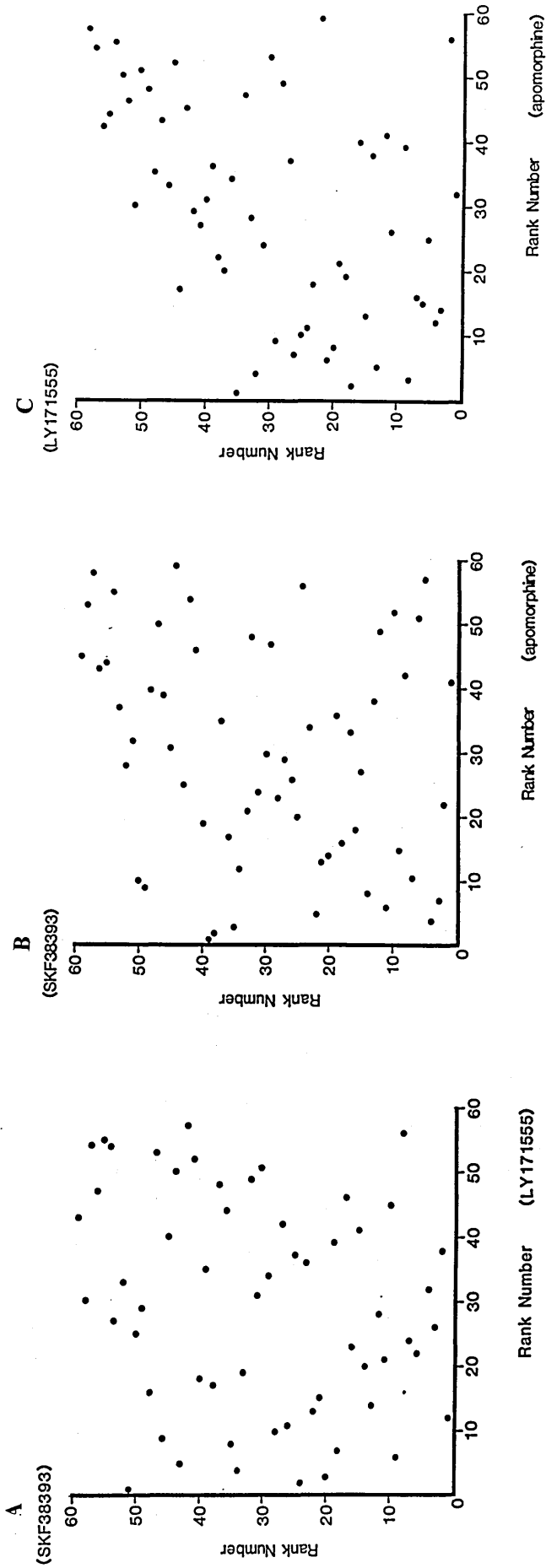


LEGEND TO FIGURE 23

Relationships between the hierarchies of regional responsiveness to SKF 38393, LY 171555 and apomorphine. Each data point represents the rank number of the 'f' value generated from the dose response curves of a single structure in response to a) SKF 38393 and LY 171555, b) SKF 38393 and apomorphine and c) LY 171555 and apomorphine.

The reliability of these correlation values were examined by the Bootstrapping approach and revealed that simulated correlations were close to, and normally distributed about these measured correlation values. Hence, the measured correlation values are probably good approximations of the true relationship.

3.2    Antagonist Studies

The 'fingerprinting' approach was found to be sufficiently robust to permit the comparison of deoxyglucose data generated by different investigators (Appendix 1). The method, therefore, was used to compare the pattern of altered glucose use provoked by the putatively selective $D_1$ receptor antagonist SCH 23390 (0.05 - 1 mg/kg: Table 8) with that generated for the classical neuroleptic haloperidol (0.01 - 1 mg/kg:  Table 9) from the published data of McCulloch et al. 1982.

The administration of $D_1$ selective concentrations of SCH 23390 had little effect on local cerebral glucose utilisation in the 49 of the 59 brain regions examined (Table 8). Consequently, most of the 'f' values were tightly grouped around the median value of 0.02 (Figure 24a). However, a few brain areas did display moderate/minimal sensitivity to SCH 23390 (Table 8). Of these, the most prominent responses occurred within the nucleus accumbens (f = 0.177, rank number 59), lateral habenular nucleus (f = 0.088, rank number 58) and motor nuclei of the thalamus (Table 8).

The pattern of altered glucose use provoked by haloperidol was markedly different from that produced by SCH 23390 (Figure 24). Haloperidol provoked more widespread alterations on function related glucose use, than SCH 23390 with 21 of the 35 regions examined displaying some degree of sensitivity to this $D_2$ antagonist (Table 9). The brain region in which glucose use was most affected by haloperidol administration was the genu of the corpus callosum (f = 0.343). By contrast SCH 23390 had little effect upon glucose use within this brain area (f = 0.028). The lateral habenular nucleus, ventrolateral thalamus and nucleus accumbens were among the most sensitive brain areas to alteration by haloperidol or SCH 23390 (Tables 8 and 9). However, although haloperidol and SCH 23390 were equipotent in provoking increases in glucose use within the nucleus accumbens, the $D_1$ antagonist proved to be only 1/3 as potent as haloperidol in the lateral habenula and ventrolateral thalamus (Figure 25 a).

Marked differences in responses elicited by SCH23390 and haloperidol were also evident within the substantia nigra. In the pars compacta the overall response to haloperidol was some 15 fold greater than to SCH 23390 (f = 0.007), while in the pars reticulata glucose use was preferentially affected by the $D_1$ antagonist, SCH 23390 (f = 0.043 for SCH 23390, f = 0.021 for haloperidol (Figure 25 b).

The reliability of the SCH 23390 and haloperidol data sets were determined by the Bootstrapping approach (with the measured 'f' values compared with 100 simulations of the data sets). The

FIGURE 24
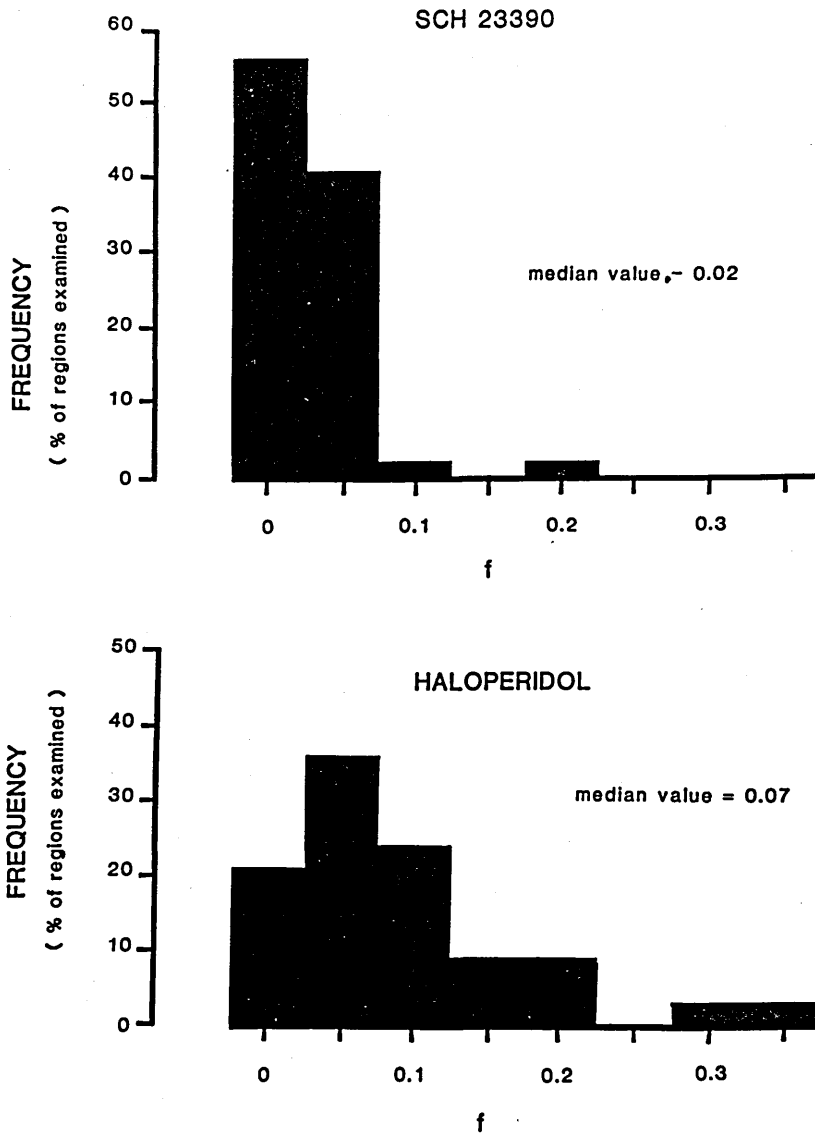


Distribution of 'f' values following the administration of SCH 23390 and haloperidol.

FIGURE 25
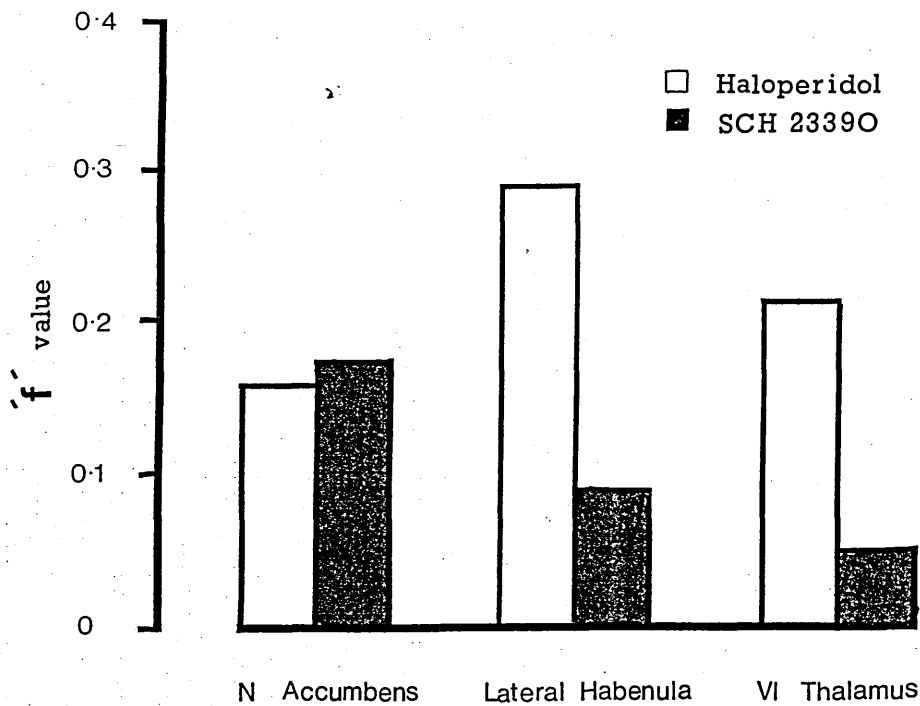


Comparison of the effects of the dopamine receptor antagonists SCH 23390 and haloperidol upon local cerebral glucose utilisation in three brain; the nucleus accumbens, lateral habenula and ventrolateral thalamic nucleus.

resulting correlations between the simulated and measured data sets:

$$\text{Correlation } (F_i, f_i), \; i = 1\text{--}100$$

$$\text{SCH 23390} = 0.704$$

$$\text{Haloperidol} = 0.668$$

These analyses revealed that the hierarchy was well maintained, although in each case the product moment correlation coefficient was less than would have been expected.

A plot of the ´f´ values for the 35 brain regions common to both the SCH 23390 and haloperidol studies suggested that the hierarchy of altered glucose use produced by these agents were dissimilar (Figure 26 a): a view confirmed by correlation analysis (correlation coefficient = -0.005). Evaluation of this correlation value by the Boostrapping approach revealed that the simulated values were close to (median value -0.009) and roughly normally distributed about the measured value for the SCH 23390 haloperidol comparison (Figure 26 b).

FIGURE 26

A



f ( HALOPERIDOL )

f ( SCH 23390 )

B



FREQUENCY
( no. of simulations )

Correlation ( f SCH 23390, f HALOPERIDOL )
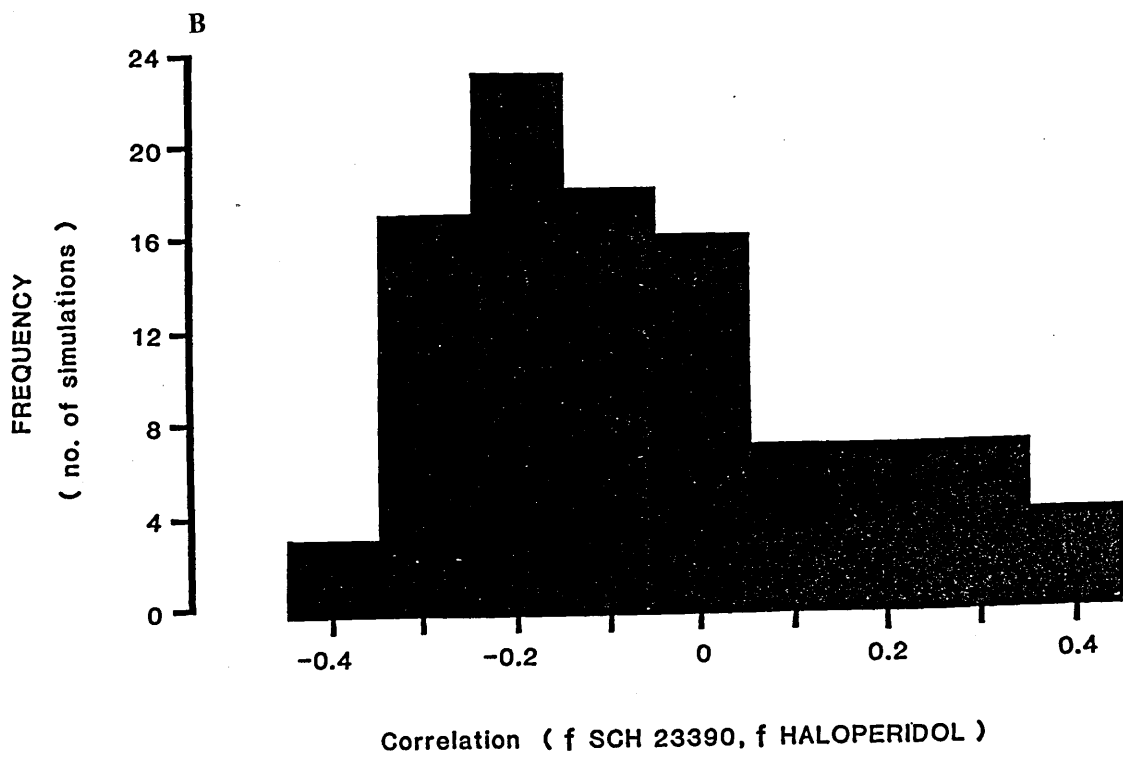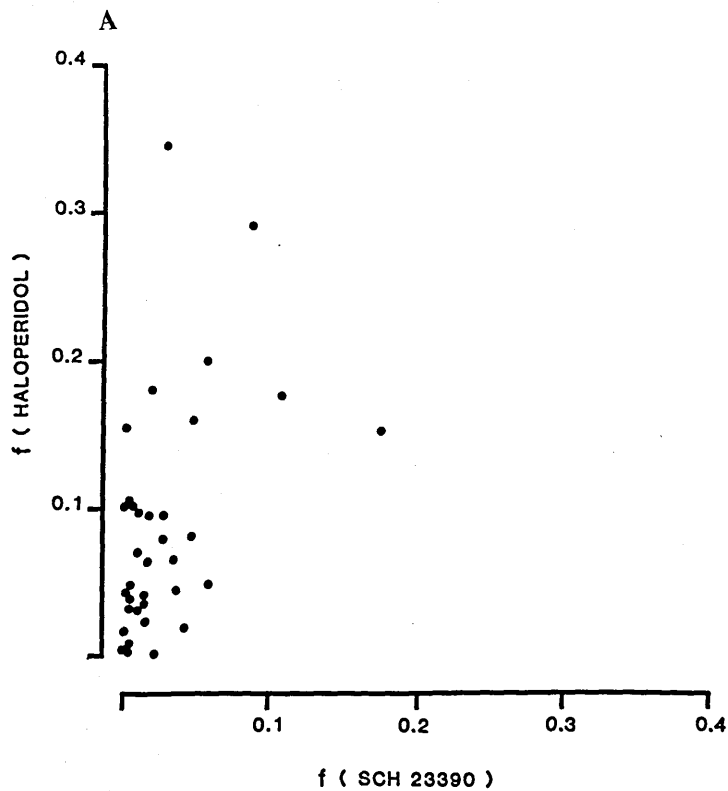
LEGEND TO FIGURES 26 a and 26 b


26a.    Relationship between the hierarchies of regional responsiveness

to SCH 23390 and haloperidol.  Each data point represents the

´f´ value generated from the dose response curves of a single

brain region to haloperidol and SCH 23390.


26b.    The reliability of the (´f´ SCH 23390, ´f´ haloperidol)

correlation, as analysed by the Bootstrapping procedure.  The

frequency and distribution curve was constructed and 100

correlation estimates obtained using simulations of the SCH

23390 and haloperidol data sets (see pages 87-89 for

description).

LEGEND TO TABLES 5 - 9


Hierarchies of responsiveness were constructed for each drug treatment by ranking the values of the ´f´ function for each brain region. Brain regions were assigned to one of the five interval groups.


Extremely Sensitive    :    values of ´f´ greater than 0.4

Sensitive              :    values of ´f´ less than 0.4

Moderately Sensitive   :    values of ´f´ less than 0.2

Minimally Sensitive    :    values of ´f´ less than 0.1

Insensitive            :    values of ´f´ less than 0.05


Brain areas are ranked from least sensitive (rank number 1) to most sensitive.

TABLE 5

HIERARCHY OF RESPONSIVENESS TO SKF 38393
DISTRIBUTION OF 'f' VALUES

| Extremely Sensitive | Sensitive | Moderately Sensitive | Minimally Sensitive | Insensitive |
|---|---|---|---|---|
| | | (59) Anteromedial Thalamus | (55) Ventrolateral Thalamus | (48) Superior Colliculus (D) |
| | | (58) Anterior Cingulate Cortex | (54) Cuneate Nucleus | (47) Posterioventromedial Thalamus |
| | | (57) Trigeminal Nucleus | (53) Superior Colliculus (S) | (46) Genu |
| | | (56) Inferior Olivary Body | (52) Lateral Geniculate | (45) Pontine Reticular |
| | | | (51) Hypothalamus | (44) S. Nigra Reticulata |
| | | | (50) Cochlear Nucleus | (43) Parietal Cortex |
| | | | (49) Inferior Colliculus | (42) Zona Incerta |
| | | | | (41) Red Nucleus |
| | | | | (40) Dentate Gyrus |
| | | | | (39) N. Accumbens |
| | | | | (38) Mediodorsal Thalamus |
| | | | | (37) Posterior Thalamus |
| | | | | (36) Anterior Pretectal Area |
| | | | | (35) Superior Olivary Body |
| | | | | (34) Medial Geniculate Body |
| | | | | (33) Piriform Cortex |
| | | | | (32) Parafascicular Nucleus |
| | | | | (31) Caudate Nucleus (V) |
| | | | | (30) Frontal Cortex (Area 8) |
| | | | | (29) Prefrontal Cortex |
| | | | | (28) Visual Cortex |
| | | | | (27) Pontine Nuclei |
| | | | | (26) Internal Capsule |
| | | | | (25) Hippocampus Moleculare |
| | | | | (24) Lateral Habenula |
| | | | | (23) Cerebellar Nucleus |
| | | | | (22) Dorsal Tegmental Area |
| | | | | (21) Frontal Cortex (Area 10) |
| | | | | (20) Dorsal Raphe Nucleus |
| | | | | (19) Ventromedial Thalamus |
| | | | | (18) Corpus Callosum |
| | | | | (17) Lateral Septum |
| | | | | (16) Auditory Cortex |
| | | | | (15) Cerebellar White |
| | | | | (14) Caudate Nucleus (D) |
| | | | | (13) Mammillary Body |
| | | | | (12) Ventral Tegmental Area |
| | | | | (11) Vestibular Nucleus |
| | | | | (10) Globus Pallidus |
| | | | | (9) Lateral Amygdala |
| | | | | (8) Sensory Motor Cortex |
| | | | | (7) Lateral Lemniscus |
| | | | | (6) S. Nigra Compacta |
| | | | | (5) Subthalamic Nucleus |
| | | | | (4) Median Raphe |
| | | | | (3) Basolateral Amygdala |
| | | | | (2) Lateroposterior Thalamus |
| | | | | (1) Interpeduncular Nucleus |

TABLE 6

HIERARCHY OF RESPONSIVENESS TO LY 171555

DISTRIBUTION OF 'f' VALUES

| Extremely Sensitive | Sensitive | Moderately Sensitive | Minimally Sensitive | Insensitive |
|---|---|---|---|---|
| (59) Trigeminal Nucleus | (56) Sensory Motor Cortex | (52) Cingulate Cortex | (37) Hippocampus - moleculare | (19) Cerebellar Nucleus |
| (58) Zona Incerta | (55) Ventrolateral Thalamus | (51) Red Nucleus | (36) Caudate Nucleus (D) | (18) Auditory Cortex |
| (57) Subthalamic Nucleus | (54) Cuneate Nucleus | (50) Prefrontal Cortex | (35) Anterior Pretectal Area | (17) Parietal Cortex |
| | (53) Posterio Ventromedial Thalamus | (49) Frontal Cortex (Area 8) | (34) Dentate Gyrus | (16) Caudate N. (V) |
| | | (48) Parafascicular Nucleus | (33) Inferior Colliculus | (15) Superior Colliculus (D) |
| | | (47) S. Nigra Reticulata | (32) N. Lateral Lemniscus | (14) Lateral Habenula |
| | | (46) Anteromedial Thalamus | (31) Pontine Nuclei | (13) Medial Geniculate Body |
| | | (45) Posterior Thalamus | (30) Interpeduncular Nucleus | (12) Ventral Tegmental Area |
| | | (44) Median Raphe Nucleus | (29) Cerebellar White | (11) Lateral Amygdala |
| | | (43) Inferior Olivary Body | (28) Lateral Geniculate Body | (10) Superior Olivary Body |
| | | (42) Dorsal Tegmental Nucleus | (27) Cochlear Nucleus | (9) Superior Colliculus (S) |
| | | (41) Lateral Septum | (26) Ventromedial Thalamus | (8) Basolateral Amygdala |
| | | (40) Piriform Cortex | (25) Frontal Cortex (Area 10) | (7) S. Nigra Compacta |
| | | (39) Lateroposterior Thalamus | (24) Mammillary Body | (6) Vestibular Nucleus |
| | | (38) Globus Pallidus | (23) Dorsal Raphe Nucleus | (5) Mediodorsal Thalamus |
| | | | (22) Nucleus Accumbens | (4) Internal Capsule |
| | | | (21) Pontine Reticular | (3) Corpus Callosum |
| | | | (20) Visual Cortex | (2) Hypothalamus |
| | | | | (1) Genu |

TABLE 7

HIERARCHY OF RESPONSIVENESS TO APOMORPHINE.
DISTRIBUTION OF 'f' VALUES.

| Extremely Sensitive | Sensitive | Moderately Sensitive | Minimally Sensitive | Insensitive |
|---|---|---|---|---|
| (59) S. Nigra Reticulata | (49) Ventral Tegmental Area | (41) Interpeduncular N. | (35) Posterior Thalamus | (24) Caudate Nucleus (VM) |
| (58) Trigeminal Nucleus | (48) Parafascicular Nucleus | (40) Superior Colliculus (D) | (34) Cerebellar Nucleus | (23) Visual Cortex |
| (57) Subthalamic Nucleus | (47) Prefrontal Cortex | (39) Genu | (33) Lateral Septal Nucleus | (22) Lateroposterior Thalamus |
| (56) Lateral Habenula | (46) Red Nucleus | (38) Mammillary Body | (32) Hypothalamus | (21) Piriform Cortex |
| (55) Cuneate Nucleus | (45) Anteromedial Thalamus | (37) Superior Colliculus (S) | (31) Pontine Reticular | (20) Hippocampus Moleculare |
| (54) Zona Incerta | (44) Ventrolateral Thalamus | (36) Ventromedial Thalamus | (30) Frontal Cortex (Area 8) | (19) Hippocampus Dentate Gyrus |
| (53) Cingulate Cortex | (43) Inferior Olivary Body | | (29) Pontine Nuclei | (18) Auditory Cortex |
| (52) Globus Pallidus | (42) Sensory Motor Cortex | | (28) Lateral Geniculate | (17) Anterior Pretectal Area |
| (51) S. Nigra Compacta | | | (27) Cerebellar White | (16) Corpus Callosum |
| (50) Posterioventromedial Thalamus | | | (26) Internal Capsule | (15) Amygdala Lateral N. |
| | | | (25) Parietal Cortex | (14) Dorsal Raphe N. |
| | | | | (13) Frontal Cortex (Area 10) |
| | | | | (12) Medial Geniculate |
| | | | | (11) N. Lateral Lemniscus |
| | | | | (10) Cochlear N. |
| | | | | (9) Inferior Colliculus |
| | | | | (8) Caudate N. (DL) |
| | | | | (7) Amygdala Basolateral N. |
| | | | | (6) Vestibular N. |
| | | | | (5) Dorsal Tegmental N. |
| | | | | (4) Medial Raphe N. |
| | | | | (3) Superior Olivary Body |
| | | | | (2) Mediodorsal Thalamus |
| | | | | (1) Nucleus Accumbens |

TABLE 8

HIERARCHY OF RESPONSIVENESS TO SCH 23390
DISTRIBUTION OF 'f' VALUES

| Extremely Sensitive | Sensitive | Moderately Sensitive | Minimally Sensitive | Insensitive |
|---|---|---|---|---|
| | | (59) Nucleus Accumbens | (58) Lateral Habenula<br>(57) Lateral Lemniscus<br>(56) Posterioventromedial Thalamus<br>(55) Superior Colliculus (D)<br>(54) Medial Geniculate<br>(53) Dorsal Tegmental Nucleus<br>(52) Ventrolateral Thalamus<br>(51) Parafascicular Nucleus<br>(50) Ventromedial Thalamus | (49) Hypothalamus<br>(48) Visual Cortex<br>(47) Superior Colliculus (S)<br>(46) S. Nigra Reticulata<br>(45) Globus Pallidus<br>(44) Inferior Colliculus<br>(43) Posterior Thalamus<br>(42) Pontine Nuclei<br>(41) Lateroposterior Thalamus<br>(40) Red Nucleus<br>(39) Genu<br>(38) Corpus Callosum<br>(37) Piriform Cortex<br>(36) Caudate Nucleus (V)<br>(35) Anterior Pretectal Area<br>(34) Cuneate Nucleus<br>(33) Caudate Nucleus (D)<br>(32) Basolateral Amygdala<br>(31) Parietal Cortex<br>(30) Frontal Cortex (Area 8)<br>(29) Sensory Motor Cortex<br>(28) Anteromedial Thalamus<br>(27) Internal Capsule<br>(26) Cochlear Nucleus<br>(25) Dorsal Raphe Nucleus<br>(24) Ventral Tegmental Area<br>(23) Trigeminal Nucleus<br>(22) Subthalamic Nucleus<br>(21) Lateral Septum<br>(20) Pontine Reticular<br>(19) Cerebellar White<br>(18) Auditory Cortex<br>(17) Frontal Cortex (Area 10)<br>(16) Hippocampus Moleculare<br>(15) Prefrontal Cortex<br>(14) Medial Raphe Nucleus<br>(13) Cerebellar Nucleus<br>(12) S. Nigra Compacta<br>(11) Interpeduncular Nucleus<br>(10) Lateral Amygdala<br>(9) Superior Olivary Body<br>(8) Mediodorsal Thalamus<br>(7) Mammillary Body<br>(6) Vestibular Nucleus<br>(5) Zona Incerta<br>(4) Cingulate Cortex<br>(3) Lateral Geniculate<br>(2) Dentate Gyrus<br>(1) Inferior Olivary Body |

TABLE 9

HIERARCHY OF RESPONSIVENESS TO HALOPERIDOL
DISTRIBUTION OF 'f' VALUES

| Extremely Sensitive | Sensitive | Moderately Sensitive | Minimally Sensitive | Insensitive |
|---|---|---|---|---|
| (35) Genu | (34) Habenula<br>(33) Ventrolateral Thalamus | (32) Parietal Cortex<br>(31) Auditory Cortex<br>(30) Hypothalamus<br>(29) Dentate Gyrus<br>(28) Nucleus Accumbens<br>(27) Medial Geniculate<br>(26) Lateral Geniculate<br>(25) S. Nigra Compacta | (24) Subthalamic Nucleus<br>(23) Sensory Motor Cortex<br>(22) Corpus Callosum<br>(21) Visual Cortex<br>(20) Red Nucleus<br>(19) Hippocampus Moleculare<br>(18) Frontal Cortex<br>(17) Globus Pallidus<br>(16) Lateral Lemniscus<br>(15) Superior Olive | (14) Vestibular Nucleus<br>(13) Inferior Colliculus<br>(12) Mediodorsal Thalamus<br>(11) Internal Capsule<br>(10) Pontine Reticular<br>(9) Cochlear Nucleus<br>(8) Cerebellar Nucleus<br>(7) Lateral Septum<br>(6) S. Nigra Reticulata<br>(5) Inferior Olive<br>(4) Superior Colliculus<br>(3) Amygdala<br>(2) Cingulate Cortex<br>(1) Caudate Nucleus |

4. <u>EFFECTS OF SKF 38393 AND LY 171555 UPON LOCAL CEREBRAL BLOOD

FLOW AND THE FLOW/GLUCOSE USE RELATIONSHIP</u>

4.1 General Results and Observations

Administration of the selective $D_1$ agonist SKF 38393 and $D_2$ agonist LY 17155 resulted in the characteristic alterations in behaviour, as described previously: SKF 38393 (30 mg/kg) provoked an increase in the frequency and intensity of episodic sniffing and licking behaviour whereas the administration of LY 171555 produced intense and protracted gnawing of the lead support bricks. With both agents the characterisitic behavioural patterns persisted throughout the experimental period. Neither SKF 38393 nor LY 171555 affected significant alterations in cardiovascular or respiratory status at the time of blood flow measurement (Appendix 4:1). Rectal temperature was maintained close to $37^{o}C$ by means of a heating lamp.

4.2 Local Cerebral Blood Flow

The effects of the putatively selective $D_1$ agonist SKF 38393 (30 mg/kg) and $D_2$ receptor agonist LY 171555 (0.5 mg/kg) on the local levels of blood flow in 59 anatomically discrete brain regions are presented in Appendix 4.

The $D_2$ receptor agonist LY 171555 affected significant increases in local blood flow within 18 of the 59 brain areas examined. Of these 18 regions, 15 primarily subserve motor function

(viz. sensory motor cortex, basal ganglia and motor thalamic nuclei).
In addition to these areas, significantly elevated levels of blood
flow were found within the visual cortex, basolateral nucleus of the
amygdala and cerebellar white matter (Figure 27).

SKF 38393, the selective $D_1$ receptor agonist failed to provoke
significant alterations in local blood flow in 52 of the 59 brain
regions under investigation. Following SKF 38393 administration
blood flow was significantly reduced in 7 areas (anterior cingulate
cortex, anteromedial thalamus, nucleus accumbens, mamillary body,
visual cortex) and (the lateral nucleus of the amygdala).


4.3  Relationship Between Local Blood Flow and Glucose Utilisation


As discussed previously there exists a close correlation
between local levels of blood flow and metabolic rate under normal,
physiological conditions. Thus, areas of highest metabolic activity
(viz. primary auditory structures) receive the highest levels of
blood flow whilst areas of intermediate (medial geniculate body) and
low metabolic rate (white matter) receive correspondingly
intermediate and low levels of perfusion.

By plotting average blood flow against average glucose use
values for each of the 59 brain regions examined revealed, as
expected, a close linear relationship between these two parameters in
saline treated control animals (Figure 28a). The ratio of average
blood flow to glucose use, obtained from the gradient of the best
fitting straight line was 1.78. Using the same procedure ratios of
1.77 and 1.92 were calculated for the SKF 38393 and LY 171555 treated
groups respectively (Figure 28 b,c).

FIGURE 27
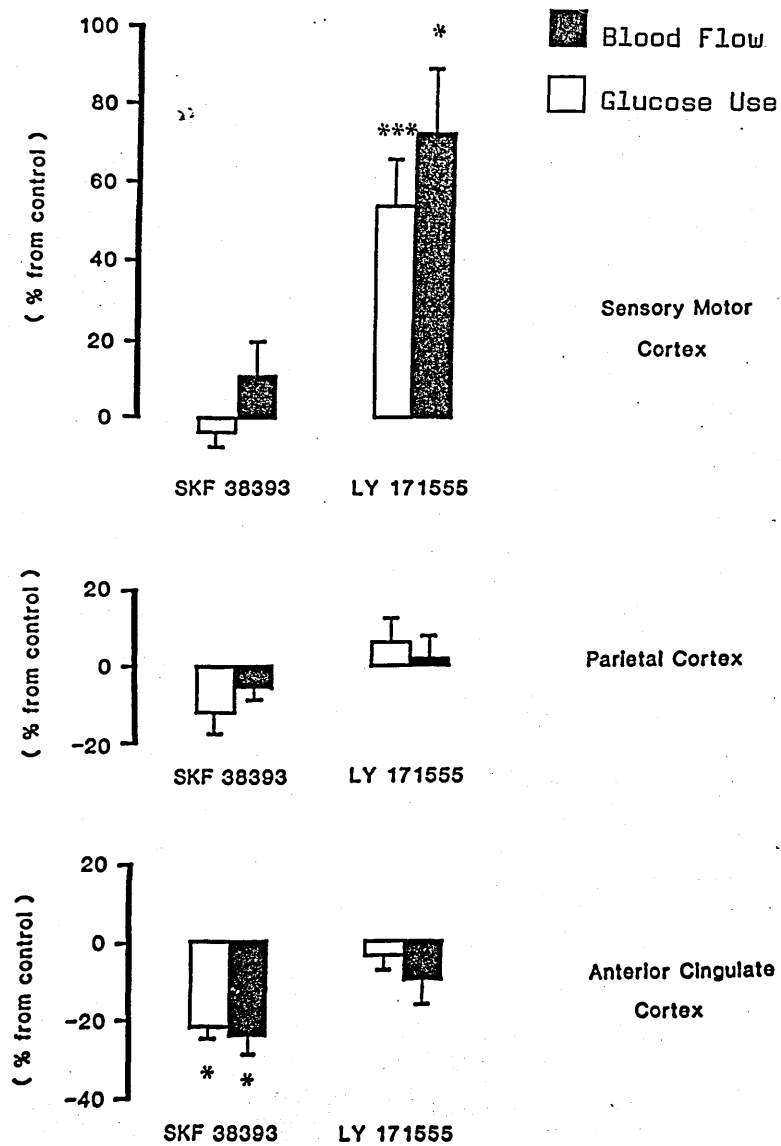


Effects of SKF 38393 (30 mg/kg) and LY 171555 (0.5 mg/kg) administration upon local blood flow and glucose utilisation in neocortex. Data are expressed as mean percentage change from control ± SEM.

LEGEND TO FIGURE 27

Upper:

Significant increases in blood flow and glucose use within the sensory motor cortex following the administration of the $D_2$ agonist LY 171555. By contrast, SKF 38393 had little effect on blood flow or glucose use in sensory motor cortex.

Middle:

Local blood flow and glucose use in parietal cortex are minimally altered by SKF 38393 and LY 171555.

Lower:

Parallel reductions in blood flow and glucose use within the anterior cingulate cortex following the administration of the $D_1$ agonist SKF 38393. By contrast, LY 171555 had little effect upon blood flow or glucose use within the anterior cingulate cortex.

FIGURE 28
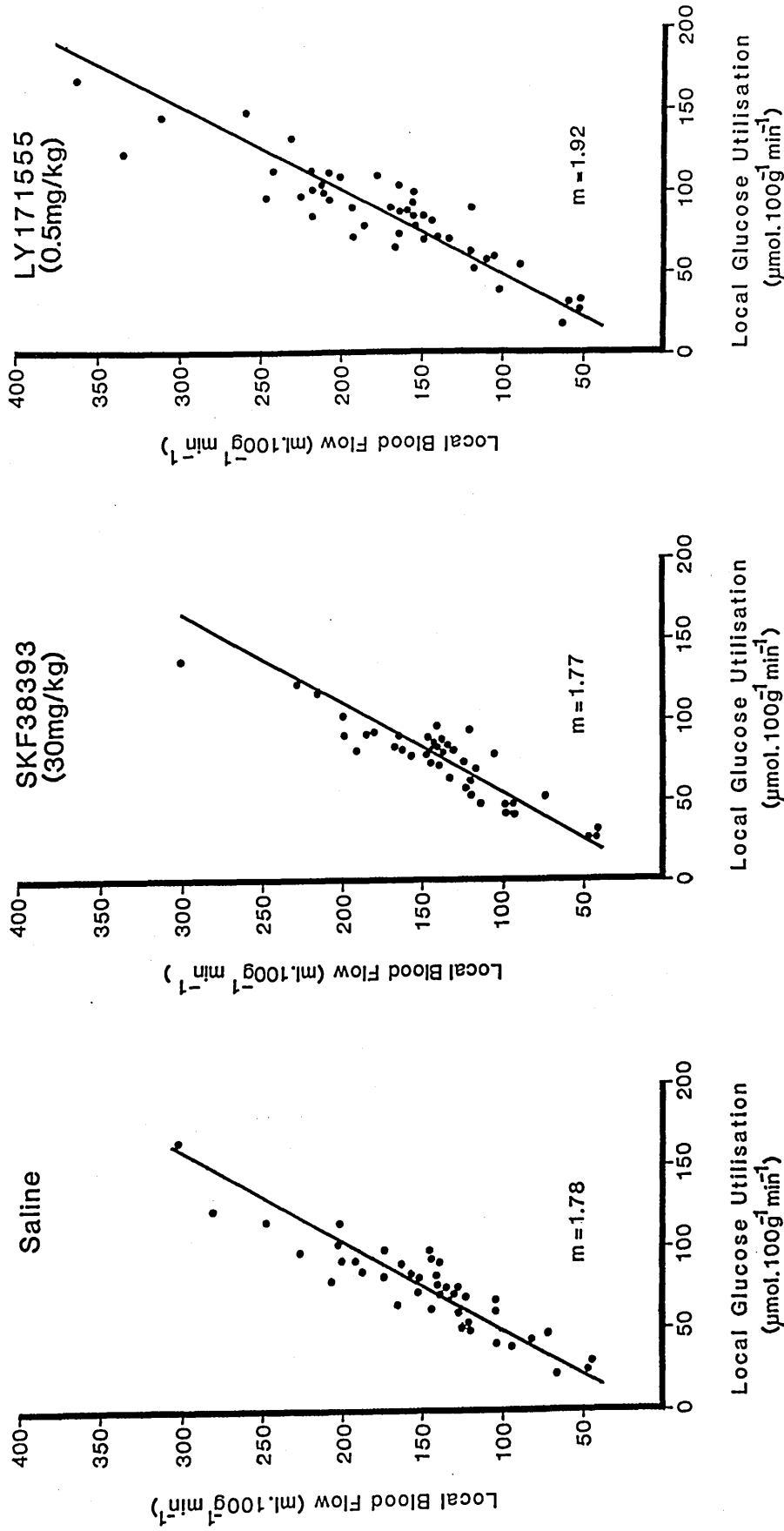
Relationship between mean local cerebral blood flow and mean local cerebral glucose utilisation in 59 anatomically discrete brain regions of the conscious rat, following the intravenous administration of a) saline, b) SKF 38393 (30 mg/kg) and c) LY 171555 (0.5 mg/kg). Each point represents the data from a single brain region. For each treatment the relationship between blood flow and glucose use is described by a best fitting straight line of gradient m.

The non-independent nature of the data sets generated by these autoradiographic techniques precludes the use of linear regression analysis to test the relationship between blood flow and glucose use (McCulloch et al. 1982). A more appropriate form of statistical analysis has been described recently (McCulloch et al. 1982). To minimise the problem of heteroscedasticity this model requires that data sets are transformed to their logarithms. A plot of transformed data reveals a linear relationship between mean flow and glucose use having unit slope and intercept (Figure 29). Thereafter, comparisons of drug treatments on the flow-glucose reltionship can be performed by comparison of the values for each treatment.

Analysis of variance between the treatment groups revealed that neither SKF 38393 nor LY 171555 significantly altered the relationship between flow and glucose use. Moreover, analysis of the variance within each group failed to identify any brain region where the flow-glucose use relationship deviated significantly from derived line. Thus, the fundamental relationship between blood flow and glucose utilisation remained intact and unaltered by the administration of selective $D_1$ or $D_2$ receptor agonists.

FIGURE 29
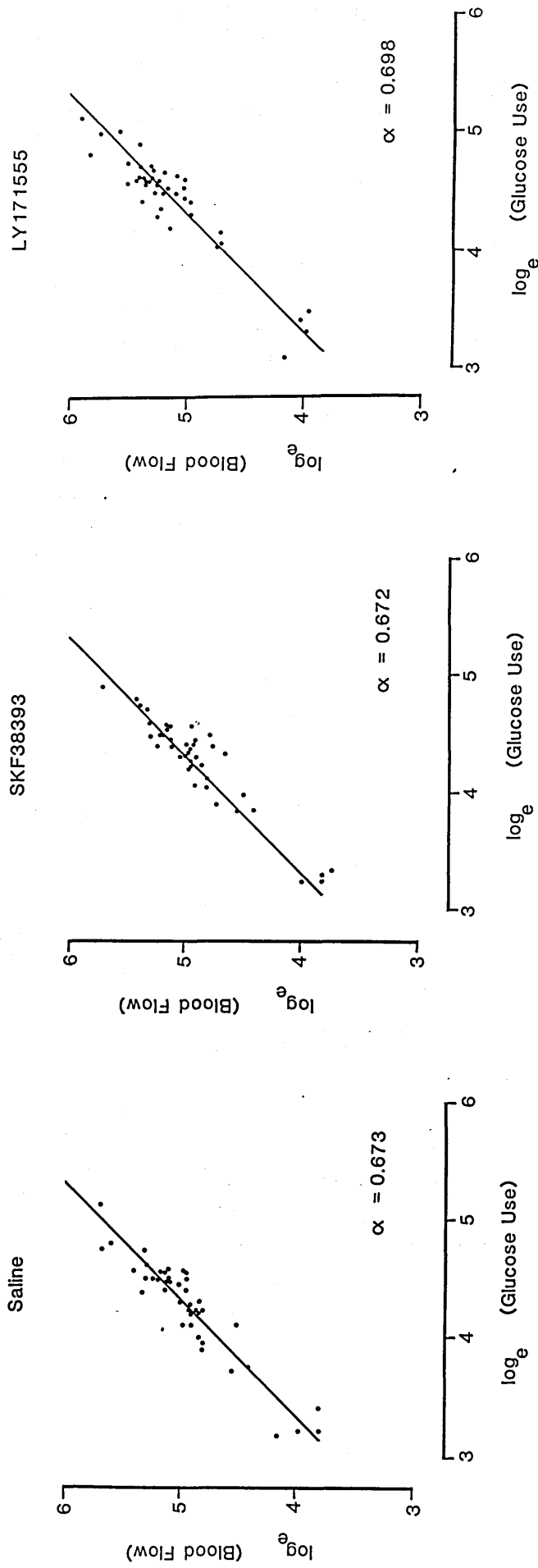


Relationship between local cerebral blood flow and glucose utilisation presented as a logarithmic transformation of mean vales for each of 59 anatomically discrete brain regions of the conscious rat. The relationship is described by a line of gradient 1 and intercept for each treatment.

## 4.4 Relationship Between Local Blood Flow and Glucose Utilisation in the Caudate Nucleus

The effects of the putatively selective $D_1$ agonist SKF 38393 (30 mg/kg) and $D_2$ agonist LY171555 (0.5 mg/kg) upon local rates of blood flow and glucose utilisation within 10 subregions of the caudate nucleus are presented in Table 5:8.

The administration of SKF 38393 failed to alter glucose use significantly in any of the 10 subregions of the caudate nucleus examined. In contrast, significant reductions in blood flow were observed in two of the three levels (middle and anterior). At each of these two levels blood flow was reduced in all four quadrants, although the reductions in tissue perfusion were most prominent within dorsomedial portions (Figure 30).

LY 171555 administration failed to provoke significant alterations in mean values of glucose use or blood flow in any of the three levels examined. However, each level the alteration in glucose use was not uniformly distributed throughout the nucleus. Dose-related increases in glucose use within the ventral, but not dorsal, caudate nucleus following LY 171555 administration, a significant increase in glucose use was observed within the ventromedial aspect (+19%). Proportionately similar increases in glucose use were observed within the ventrolateral quadrant (+15%) at the same level and in the ventral portion (+19%) of the more caudal, (peripallidal) level although in both areas the alteration in glucose use just failed to attain statistical significance after Bonferroni correction for multiple comparisons. There was no evidence of medial-lateral

FIGURE 30
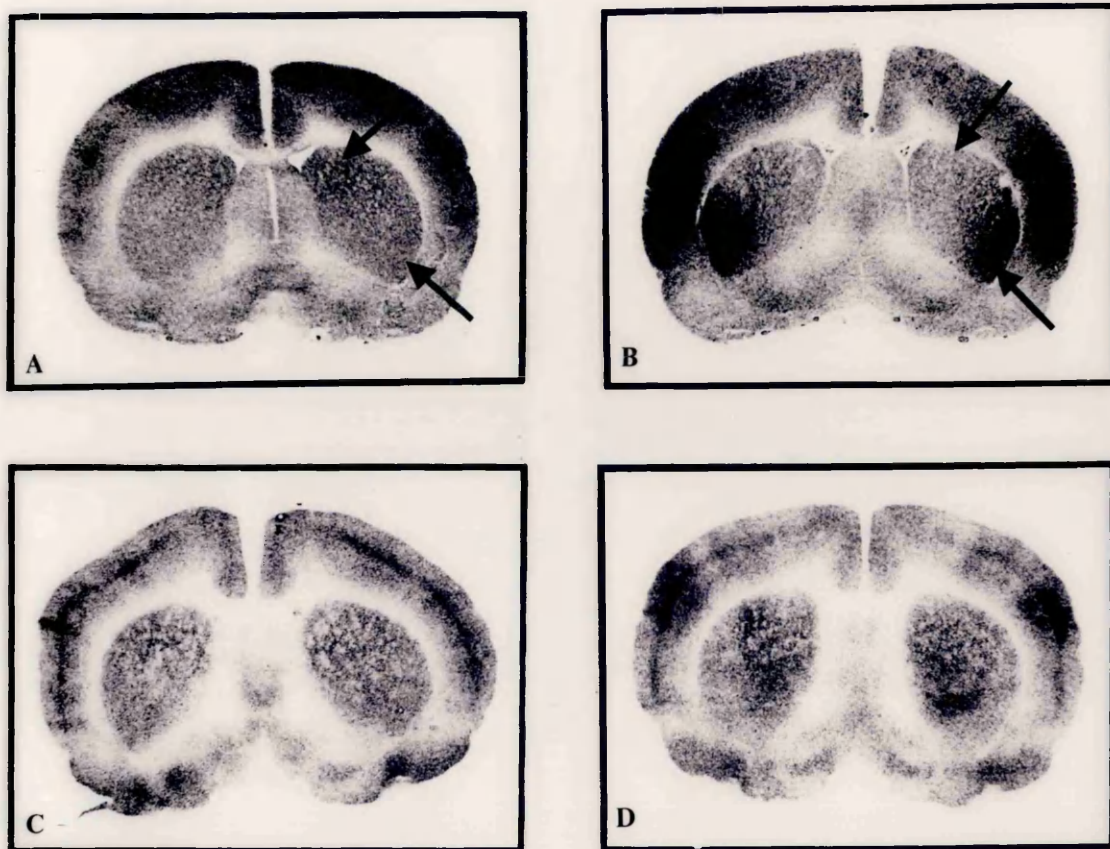


Representative autoradiographs from coronal sections of rat brain at the mid caudate level.

A) Saline treated control animal (Blood Flow). Optical density (and blood flow) appears relatively homogeneous throughout the caudate nucleus.

B) LY 171555 (0.5 mg/kg) treated animal (Blood Flow). Marked heterogeneity in optical density (and blood flow) within the caudate nucleus, with the ventrolateral portion notably darker than the rest of the nucleus.

C) Saline treated control animal (Glucose Use). Optical density (and glucose use) appear relatively homogeneous throughout the caudate nucleus.

D) LY 171555 (0.5 mg/kg) treated animal (Glucose Use). Heterogeneous pattern of optical density evident within the caudate nucleus with marked increases in optical density (and Glucose Use) within the ventromedial but not ventrolateral portions of the nucleus. This pattern of altered glucose use is evidently different from the blood flow response evoked by LY 171555 within the caudate nucleus (B).

differences in glucose use following LY 171555 administration. In contrast marked medial to lateral differences in blood flow were immediately apparent upon visual inspection of the autoradiograms of LY 171555 treated animals (Figure 31). At the middle caudate level LY 171555 provoked a large (+57%) focal increase in blood flow in the ventrolateral quadrant but failed to significantly affect perfusion rate in the adjacent ventromedial portion (Figure 31). Similarly, at the most rostral level LY 171555 a significant reduction (-18%) in blood flow within the mediodorsal portion while increasing flow in dorsolateral quadrant (Figure 32).

An examination of the flow-glucose use relationship within the various subregions of the caudate nucleus revealed a complex pattern of responses (Figure 32). In six of the ten areas examined neither flow nor glucose use were significantly affected following LY 171555 administration. In two subregions (ventral and ventrolateral portions at the caudal and medial levels respectively) both flow and glucose use increased, however, in both areas the relative circulatory response was some threefold greater than the increase in metabolism. In a third region LY 171555 affected significant reductions in local blood flow but failed to alter glucose use. Similarly, in the one area where glucose use was significantly increased (the ventromedial quadrant of the middle level), blood flow was unaffected. These data suggest indicate that uncoupling of the flow-glucose use relationship may occur within discrete subregions of the caudate nucleus. This view was borne out by analysis of covariance between the drug and saline treated groups. In saline-treated animals the relationship between blood flow and glucose use

was maintained. Furthermore, the flow/glucose use relationship did not deviate significantly from the derived line in any of the caudate subregions examined. In contrast, no significant correlation could be demonstrated between blood flow and glucose use in the caudate of LY 171555 or SKF 38393 treatment groups.

Thus, although the overall flow-glucose use relationship is maintained, within the caudate nucleus and in the brain as a whole focal uncoupling occurs within discrete subregions of the caudate nucleus following the administration of SKF 38393 or LY 171555.

FIGURE 31
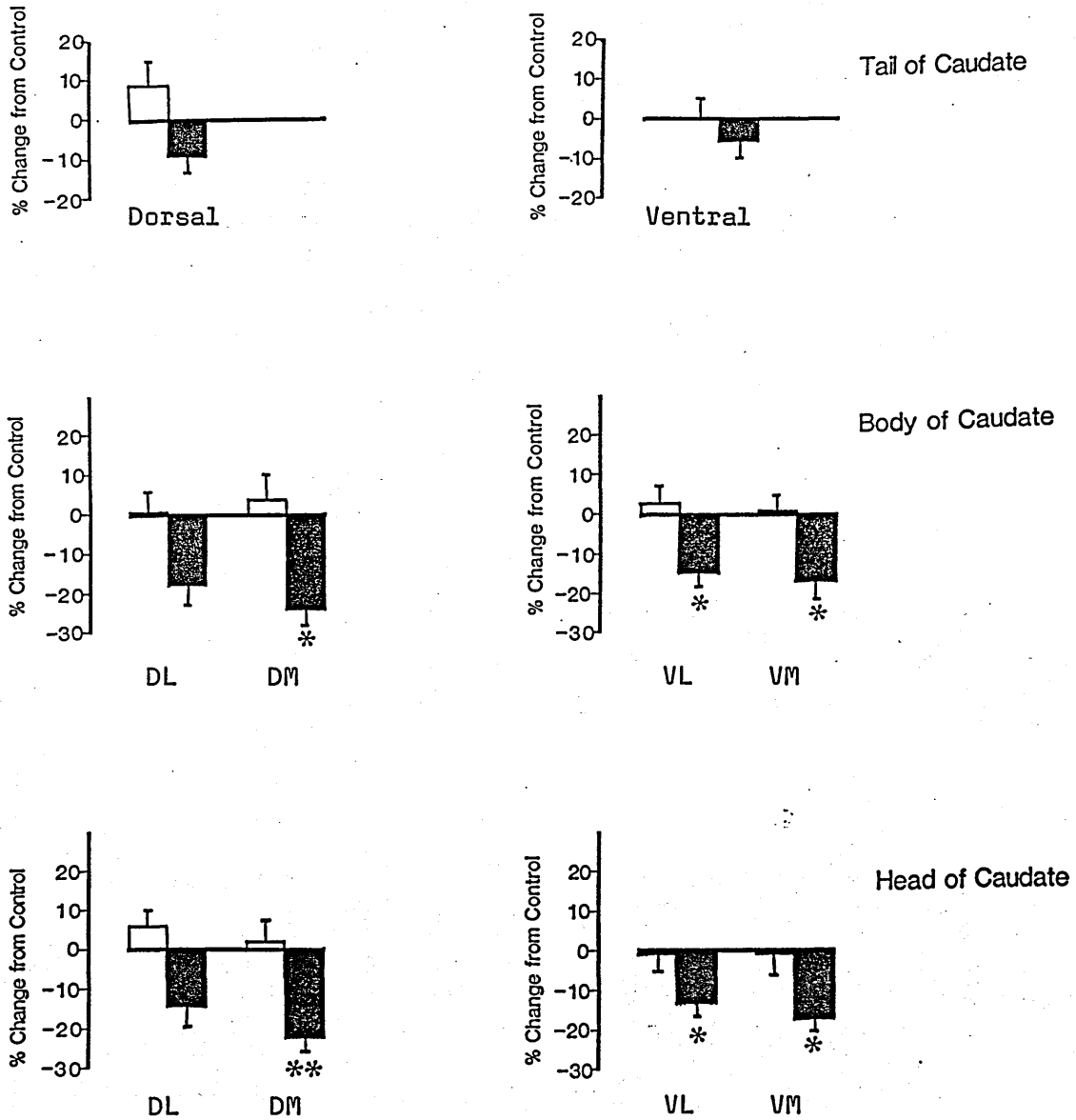
The effects of SKF 38393 (30 mg/kg) administration upon local cerebral blood flow (■) and glucose utilisation (□) within subregions of the caudate nucleus. Data are presented as mean percentage change from control levels ± SEM. *p<0.05.

FIGURE 32
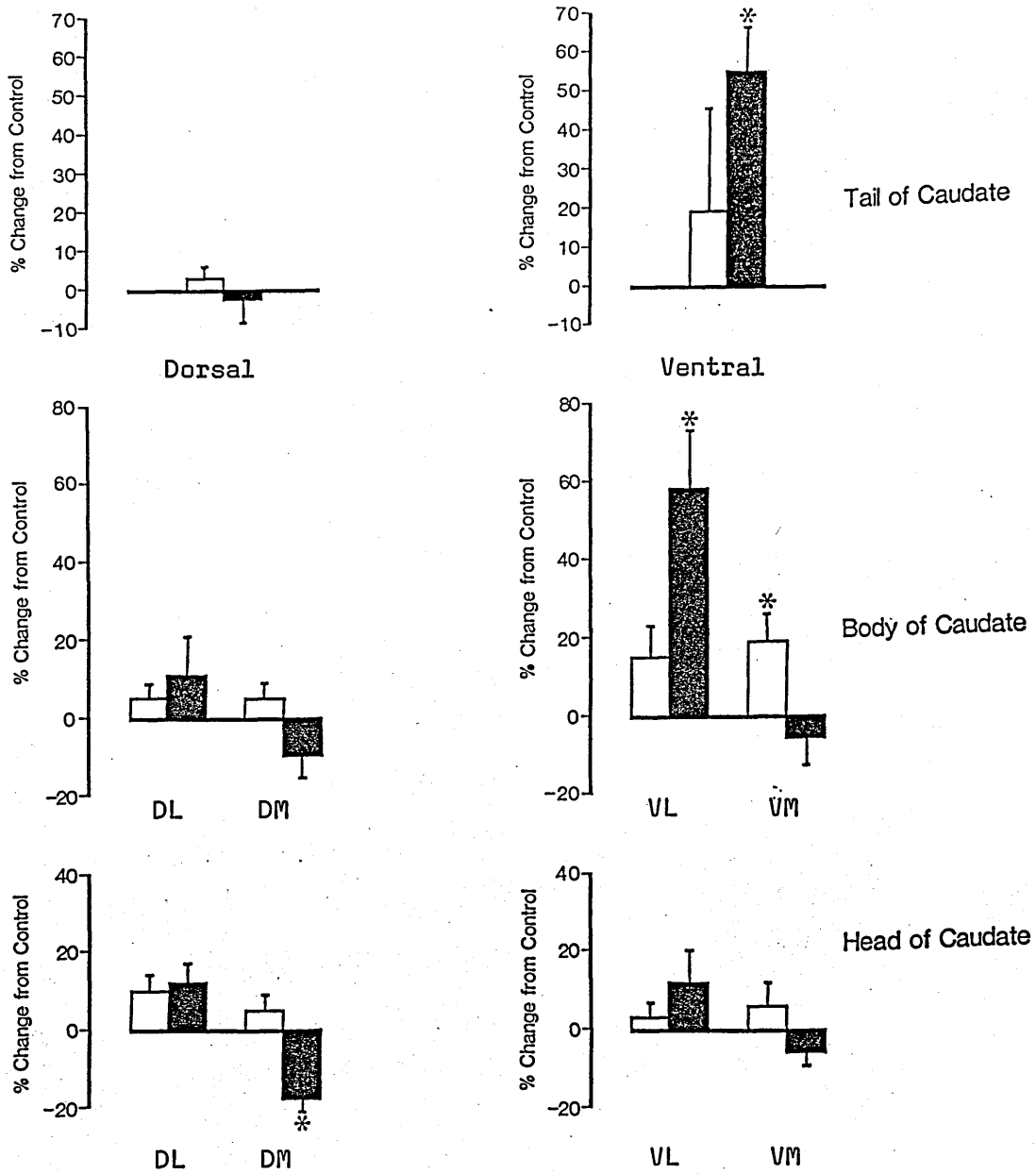


The effects of LY 171555 administration upon local cerebral blood flow (■) and glucose utilisation (□) within subregions of the caudate nucleus. Data are presented as mean percentage change from control levels ± SEM. *p<0.05.

CHAPTER IV

DISCUSSION

The concept that cerebral blood flow is regulated to meet the
metabolic requirements of the brain was first proposed almost a
century ago (Roy and Sherrington, 1890). This close relationship
between cerebral blood flow and metabolic rate has been demonstrated
under a wide variety of physiological and drug-induced conditions
(Purves, 1972; Sokoloff, 1981; Des Rosiers et al. 1974; McCulloch
and Kelly, 1982; Kelly and McCulloch, 1983 c). However, in recent
years it has been demonstrated that a number of pharmacologically
diverse agents (anaesthetics, calcium antagonists, neurotoxins and
prostaglandin synthetase inhibitors) can fundamentally alter the
relationship between flow and metabolism by acting directly on the
cerebral vasculature (Celik et al. 1982; Mies et al. 1981; Mohammed
et al. 1985; McCulloch et al. 1982 d).

Dopamine receptors mediate relaxation of vascular smooth
from renal, mesenteric and splenic beds (Goldberg and Toda 1975;
Schmidt and Imbs, 1980; Brodde, 1982; Schmidt et al. 1986). In
these peripheral vascular beds the administration of dopamine
receptor agonists is associated with increases in tissue blood flow
(McDonald and Golberg, 1963; Goldberg et al. 1986). The presence of
dopamine neurons innervating cerebral arterioles would suggest that
dopamine receptor agonists may act directly on receptors on the
cerebral vasculature to increase cerebral blood flow. Indeed, a

direct vasomotor action has been advocated to account for the excessive increases in cerebral blood flow relative to metabolic rate that have been observed by many authors following the administration of dopamine receptor agonists (Carlsson et al. 1975; Nahorski and Rogers, 1976; Berntman et al. 1978; Ingvar et al. 1983; Lindvall et al. 1982; Leenders et al. 1985). However, this view has been challenged by authors who have reported alterations in blood flow parallel to those of metabolic rate, both in magnitude and time course (McCulloch et al. 1982 a). These latter authors advocate that the alterations in cerebral blood flow observed following dopamine receptor agonist administration occur as an indirect consequence of drug-induced changes in metabolic rate.

In this thesis I have sought to examine systematically the relative importance of the cerebrovascular and metabolic components of the blood flow response elicited by new and highly selective dopamine receptor agonists. The studies performed were threefold. In the first series of experiments, I examined the vasomotor responses elicited by the putatively selective $D_1$ agonist SKF 38393 and $D_2$ receptor agonist LY 141865 upon feline pial arterioles in situ. In the second series of experiments the 2-deoxyglucose quantitative autoradiographic technique was used to characterise the functional consequences associated with the pharmacological manipulation of $D_1$ and $D_2$ receptor subpopulations. Finally, in the third section the influence exerted by dopamine receptors upon local cerebral blood flow and on the relationship between flow and glucose use was investigated.

1.  DOPAMINE RECEPTOR SUBTYPES AND THE CEREBRAL CIRCULATION:

    ACTIONS ON CEREBROVASCULAR SMOOTH MUSCLE


Dopamine receptors mediating relaxation of cerebrovascular smooth muscle have been demonstrated in isolated cat middle cerebral arteries (Edvinsson et al. 1978) and on the cerebrovasculature of a variety of other species including man (Toda, 1976, 1983; Forster et al. 1983; Oudart et al. 1981, 1982). The presence of dopamine receptors mediating vasodilatation of the cerebrovasculature is fundamental to the argument that dopamine receptor agonists increase cerebral blood flow primarily through an action on cerebrovascular (Berntman et al. 1978; Toda 1983; Leenders et al. 1983, 1984, 1985; Ingvar et al. 1983; Carlsson et al. 1975). However, by equating the vasomotor responses produced by dopamine receptor agonists in isolated vessel preparations, with the alterations in cerebral tissue perfusion observed in vivo, the studies advocating direct vascular responses are open to criticism.

Inherent in the vascular hypothesis is the assumption that the vessels under study are representative of the cerebral circulation as a whole (i.e. changes in vessel calibre are concomitant with alterations in cerebral blood flow). Several lines of evidence suggest that the large inflow tract vessels (basilar artery and major vessels of the Circle of Willis), commonly used in isolated vessels preparations are not characteristic of the intraparenchymal vasculature (Purves, 1982; McCulloch and Edvinsson, 1984). These large vessels do not exhibit many of the responses which are consistent with the circulatory response to physiological stimuli (autoregulation to changes in systemic pressure, hypercapnia on

hypoxia) (Purves, 1972). Moreover, recent studies have shown that inflow tract vessels contribute little (<7%) to total cerebrovascular resistance (Tuor and Farrar, 1985). Thus, even large changes in vessel calibre would have minimal effects on cerebral blood flow. By contrast pial vessels constitute a more relevant portion of the cerebral vasculature for studying vasomotor reactivity by virtue of their significant contribution to cerebrovascular resistance. However, in vivo studies have shown that the changes in pial vessels calibre to hypercapnia and altered blood pressure are consistent with the observed changes in cerebral tissue perfusion (Purves, 1972; McCulloch and Edvinsson, 1984). The use of large concentrations of phenoxybenzamine to inhibit adrenergic and serotoninergic mediated contraction may fundamentally compromise attempts to characterise the dopamine receptor mediating relaxation of cerebrovascular smooth muscle. In addition to its potent adrenergic and serotoninergic blocking properties high concentrations of phenoxybenzamine can also interact with other receptor populations, including both $D_1$ and $D_2$ dopamine receptors. Moreover, ligand binding studies have shown that, at the high concentrations employed in isolated vessel studies, phenoxybenzamine differentiates between the two dopamine receptor subpopulations; blocking all $D_2$ receptor sites while sparing a proportion of the $D_1$ sites (Marchias and Bockaert, 1980; Walton et al. 1978; Hamblin and Creese, 1982). Hence, the present studies which demonstrate the dopamine receptor agonists provoke dilatation of pial arterioles, in situ, and without the need for preconstriction or adrenergic blockade provides a more relevant basis for the vascular hypothesis (i.e. that dopamine receptor agonists can alter cerebral blood flow by interacting directly with dopamine receptors on cerebral vascular smooth muscle).

In the present study the hierarchy of agonist potency in dilating pial arterioles (SKF 38393 > apomorphine > LY 141865) is indicative of a $D_1$ mediated relaxation of cerebrovascular smooth muscle. A similar conclusion has been reached by studies demonstrating the presence of dopamine sensitive adenylate cyclase activity within the intraparenchymal and extracerebral vessels of the cat and rat (Nathanson and Glaser, 1979; Amenta et al. 1984; Baca and Palmer, 1978). Although an increase in intracellular cAMP is associated with the action of a dopamine and other vasodilator substances (adenosine, papaverine, serotonin and B-adrenergic agonists) there is, as yet, no evidence linking cAMP generation to cerebrovascular dilatation.

A $D_1$ mediated relaxation of isolated human basilar arteries has been proposed by Forster et al. (1982), although in this preparation SKF 38393 was found to be a weak partial agonist. The reason for the lack of efficacy of SKF 38393 in the Forster study is unclear. One possible explanation may lie in the use of an incubation cocktail containing indomethacin, propranolol, phenoxybenzamine and $PGF_2$ (Forster et al. 1982), for in isolated vascular preparations where a similar incubation mixture was used the efficacy of SKF 38393 was markedly reduced (Brown et al. 1980; Woodman et al. 1980; Hilditch and Drew, 1981) while in studies where only phenoxybenzamine and $PGF_2$ were employed, SKF 38393 observed that SKF 38393 had a much higher affinity for dopamine receptors (Schmidt et al. 1982; Brodde et al. 1981).

Further evidence that dopamine $D_1$ receptors mediate relaxation of cerebrovascular smooth muscle was obtained using SCH 23390; the most potent selective $D_1$ receptor antagonist presently available (Goldberg et al. 1984; Kebabian et al. 1984). It was found that SCH

23390, at concentrations of 1-10 nM inhibits the vasodilator responses of pial arterioles to apomorphine and SKF 38393. These values are in close agreement with the concentration of SCH 23390 required to inhibit dopamine stimulated adenylate cyclase ($IC_{50}$: 4-11 nM: Iorio et al. 1983; Hyttel et al. 1983: Itoh et al. 1984) or to competively antagonise ^{3}H-flupentixol binding at $D_1$ sites $IC_{50}$=1-4 nM: Cross et al. 1983 a,b; Hyttel et al. 1983; Christensen et al. 1984), and so provide strong evidence that SKF 38393 and apomorphine dilate pial arterioles by interacting with dopamine $D_1$ receptors.

The mechanisms underlying the weak dilator activity of LY 141865 are unclear. A $D_1$ mediated dilatation is unlikely since LY 141865 failed to stimulate dopamine sensitive adenylate cyclase, even at the high concentrations ($10^{-4}$M) (Scatton, 1982). However, it has been reported that high concentrations of LY 141865 can interact with histamine – $H_2$ receptors ($ED_{50}$ = 1.2 uM: Ruffolo and Shaar, 1983), whose stimulation effects dilatation of pial arterioles in situ (Wahl and Kuschinsky, 1979).

On the basis of the data presented it is proposed that the dopamine receptor mediating dilatation of feline pial arterioles is of the $D_1$ subtype. Moreover, the presence of $D_1$ receptors in small cerebral arterioles suggests that agents stimulating $D_1$ receptors could increase CBF by a direct action on cerebrovascular dopamine receptors.


2.   DOPAMINE RECEPTOR SUBTYPES AND FUNCTION-RELATED GLUCOSE UTILISATION

Inherent in the concept of function-related glucose utilisation is the premise that alterations in the rate of 2-deoxyglucose

phosphorylation are, in some way, indicative of changes in functional activity. What evidence supports this view?

Some of the most elegant demonstrations of the relationship between local functional activity and glucose utilisation have been obtained from studies of specific sensory systems under controlled experimental conditions. By far the most extensively studied sensory system has been the primary visual system. In primates and cats the 2-deoxyglucose method has been used to define orientation and ocular dominance columns in visual (striate) cortex (Kennedy et al. 1976; Horton and Hubel, 1981; Schoppman and Stryker, 1981; Tieman and Tumosa, 1983). Moreover, combined electrophysiological and 2-deoxyglucose investigations have found that the columns in visual cortex observed in deoxyglucose studies correspond precisely with the orientation columns described by electrophysiologists (Schoppmann and Stryker, 1981). The 2-deoxyglucose method has also been used to differentiate between the different cortical patterns evoked by colour and black and white stimuli (Crawford et al. 1982). Studies in rats have demonstrated quantitative relationships between the frequency and intensity of retinal stimulation and local rates of glucose utilisation within components of the visual system (Miyaoka et al. 1979; Toga and Collins, 1981). Although less extensively studied function-related alterations in glucose use have been mapped following manipulation of the olfactory and somatosensory systems (Collins, 1980; Sokoloff, 1986 for reviews).

2-deoxyglucose autoradiography has also been used to map the tonographic organisation of the auditory system (Ryan et al. 1982; Webster et al. 1984; Servier et al. 1984). Combined electrophysiological and 2-deoxyglucose studies have confirmed that the pattern of increased glucose use observed within the cat inferior

colliculus following the stimulation of the auditory system by isofrequency sound corresponds with the isofrequency contours observed in electrophysiological studies (Serviere et al. 1984). Furthermore, studies in the avian homologue of the auditory cortex (the auditory neostriatum) have demonstrated that the rate of 2-deoxyglucose uptake following tone stimuli parallels the rate of spike activity observed within this region (Theurich et al. 1984). The relationship between electrical activity and metabolic rate was observed in isolated nerve preparations 60 years ago (Gerard, 1927). More recent studies have demonstrated a quantitative relationship between spike frequency and glucose utilisation in neural tissue in vivo (Yarowski et al. 1983, 1985; Kadekaro et al. 1985). The increase in glucose use in response to increased impulse activity is thought to be related to the activation of $Na^+/K^+$ ATPase the enzyme which restores the ionic distribution across the nerve membrane following an action potential. Thus, increases in the electrical activity of a neuron would precipitate larger ionic fluxes across the cell membrane and, therefore, would require greater ATPase activity to restore the ionic balance. In accordance with this view Mata et al. (1980) found that electrical stimulation or pharmacological depolarisation (by veratridine, an alkaloid which increases sodium conductance) of posterior pituitary cells enhanced 2-deoxyglucose uptake. This increase in glucose utilistion could be blocked by preventing depolarisation with tetrodotoxin or blocking ATPase activity with ouabain (Mata et al. 1980).

Together the studies cited provide a sound basis for the hypothesis that alterations in local cerebral glucose utilisation reflect changes in functional activity and not merely substrate utilisation.

1.    Effects of Dopamine Receptor Agonists


Stimulation of central dopamine systems results in marked alterations in glucose utilisation within a limited number of neuroanatomically discrete brain areas. It is important to emphasise that function related alterations in glucose use are not indicative exclusively of the activation of dopamine receptors within the region being investigated.  Rather, the anatomical distribution of altered rates of glucose utilisation is indicative of polysynaptic neuronal pathways in which activity can be modified by dopamine receptor activation (McCulloch, 1982;  Savaki, 1984).  In the present study, the distribution of dose-dependent alterations in glucose use following the stimulation of different dopamine receptor subtypes suggests that the neuronal systems most markedly influenced by dopamine receptors involve the basal ganglia, thalamus and cerebral cortex.  With the exception of a single limbic thalamo-cortical circuit, it is the $D_2$ dopamine receptor subtype rather than the $D_1$ subtype which is responsible for dose-dependent modifications in glucose use.  Although the stimulation of central dopamine receptors alters glucose use in a number of brain areas, in the majority of brain regions examined, the activation of dopaminergic mechanisms failed to modify function related glucose use.

Faradic or pharmacological stimulation of dopaminergic systems results in marked increases in glucose use within the basal ganglia (caudate nucleus, globus pallidus, subthalamic nucleus and substantia nigra) (Brown and Wolfson 1978; McCulloch et al. 1982 b;  Weschler et al. 1979;  Porrino et al. 1984 b;  Esposito et al. 1984;  Savaki et al. 1983).  Each of these basal ganglia structures possesses both subtypes of dopamine receptor and are interconnected by extensive

neuronal pathways (Figure 33).  Until now, no systematic attempt has been made to identify the dopamine receptor subtype responsible for the functional disturbances in the basal ganglia.

The present data suggest that the increased functional activity within the basal ganglia associated with dopamine receptor activation is mediated via the $D_2$ receptor population. The importance of $D_2$ receptors within the basal ganglia is further supported by the demonstration that increased glucose use within the basal ganglia is closely related to the promotion of stereotypy (Porrino et al. 1984); a behaviour which can be initiated only by $D_2$ receptor agonists (Seeman, 1981).  However, the hypothesis that dopaminergic influences on the basal ganglia are selectively mediated via $D_2$ receptors may be too simplistic.  Behavioural investigations have demonstrated that apomorphine or LY 171555 induced stereotypy can be blocked not only by the $D_2$ antagonist metochlopramide but also by the selective $D_1$ antagonist SCH 23390 (Molloy and Waddington, 1985; Iorio et al. 1983).

The importance of dopamine $D_2$ receptor in eliciting local alterations in glucose use has been proposed on the basis of semiquantitative investigations of 2-deoxyglucose uptake (Palacios and Wiederold, 1985). Although the distribution of altered 2-deoxyglucose uptake resulting from the administration of $D_2$ agonists (Palacios and Wiederhold, 1985) displays many similarities to the alterations in glucose use elicited by LY 171555 in the present study (e.g. significant changes within the subthalamic nucleus, neocortex and thalamus) there are a number of inherent weaknesses in the use of semiquantitative deoxyglucose uptake as a measure of local cerebral glucose utilisation. Radioisotope levels in the CNS at time of sacrifice comprise 2-deoxyglucose-6-phosphate (the index of metabolic

FIGURE 33
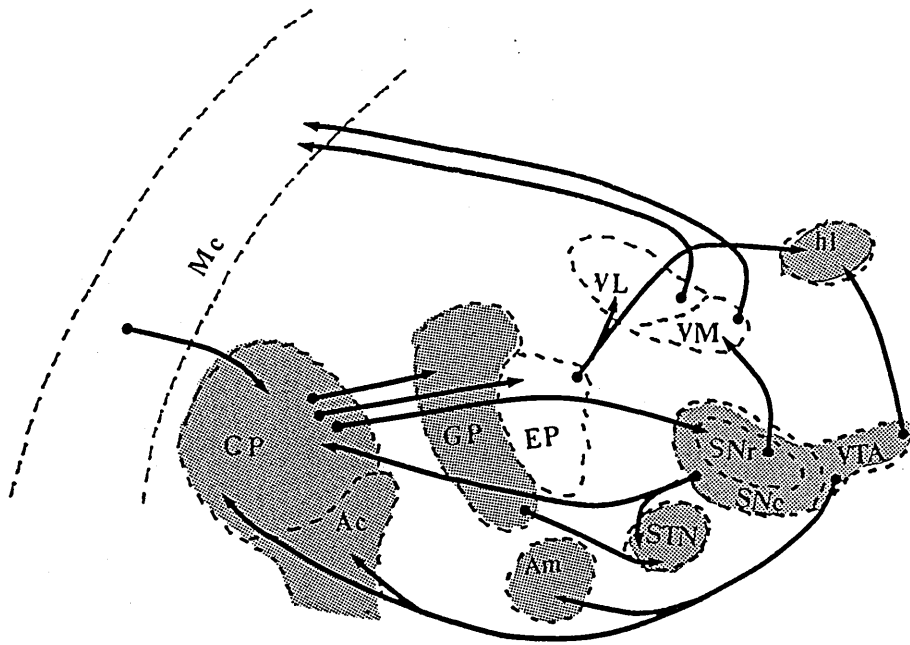


Diagrammatical Representation of Some of the

Connections of the Basal Ganglia

Mc: motor cortex, CP: caudate-putamen, Ac: nucleus accumbens, Am: amygdaloid complex, SNc, Snr: substantia nigra pars compacta, pars reticulata, hl: habenula, VTA: ventral tegmental area, VL, VM: ventrolateral and ventromedial nuclei of the thalamus. Areas receiving dopaminergic projections are shaded.

activity) and deoxyglucose (which is related to residual plasma levels). If $^{14}$C-2-deoxyglucose is administered intravenously and plasma glucose levels are maintained within the normal range, then deoxyglucose-6-phosphate constitutes the major portion (approximately 95%) of isotope within the CNS after 45 minutes (Sokoloff et al. 1977; Kelly and McCulloch, 1983 a (Figure 7a)). However, the administration of dopamine receptor agonists can increase plasma glucose levels (Tyce 1976; McCulloch et al. 1982 b) (e.g. up to 22 mM with LY 141865, 10 mg/kg, in non-fasted animals; see Figure 7b). The consequence of hyperglycaemia and the intraperitoneal route of administration of 2-deoxyglucose tracer is that total 2-deoxyglucose uptake into the CNS poorly reflects actual rates of glucose use (Kelly and McCulloch, 1983 a). These errors are further compounded by the use of optical density ratios (Palacios and Wiederhold, 1985) to derive a semiquantitative estimation of 2-deoxyglucose uptake (Kelly and McCulloch, 1983 b). The methodological differences between the measurement of local rates of glucose utilisation (present study) and local indices of 2-deoxyglucose uptake (Palacios and Wiederhold, 1985) are the most probable basis for key the differences between the two studies (e.g. the failure to demonstrate differences in glucose use within the anterior cingulate cortex and trigeminal nerve, and their demonstration of reduced rates of glucose use within the superficial layers of the superior colliculus; a region in which no significant change in glucose use occurred in the present study). Moreover, the present study provides a more comprehensive pharmacological (the effects of multiple doses of each drug explicitely described) and neuroanatomical description of the effects of dopamine receptor agonists with a rigorous model for determining function related glucose use.

Although the neuroanatomical location of dopamine receptors responsible for initiating functional alterations in the basal ganglia cannot be determined by the present approach alone, there exists a body of evidence which points to the caudate nucleus as being the main site of initiation. Firstly, intrastriatal injections of dopamine can provoke increases in glucose use within the substantia nigra and subthalamic nucleus similar to that provoked by systemically administered dopamine agonists (Brown and Wolfson, 1983). Secondly, systemic administration of apomorphine and LY 171555 (but not SKF 38393) results in an increase in the firing rate of nigral and pallidal neurons whereas their local microapplication onto cells in the pallidus or substantia nigra failed to provoke significant alterations in their firing rates (Walters et al. 1983).

The neostriatum possesses marked topographical heterogeneity in its anatomical connections (Goldman and Nauta, 1979), indices of neurotransmitter distribution (Herkenham and Pert 1981; Graybiel et al. 1981; Graybiel, 1984) and functional response to pharmacological challenge or electrical stimulation (McCulloch et al. 1983; Sharp and Evans, 1982). There is evidence that within the caudate the topographical distribution of $D_1$ receptors is relatively uniform (Scatton and Dubois, 1985) whereas the $D_2$ population appears relatively heterogeneous with gradients of increasing densities in both the caudal-rostral and medial-lateral planes (Joyce et al. 1985; Altar and Marshall, 1985). Furthermore, the topographical distribution of both dopamine $D_2$ binding sites and cholinergic neurons appear to be in close register (Joyce and Marshall, 1985; Marshall et al. 1983). The heterogeneous alterations in glucose use provoked by the selective $D_2$ agonist LY 171555 with significant increases restricted to the ventral portion of the caudate nucleus

are consistent with the known antomical distribution of $D_2$ receptors in the striatum. These results are similar to the findings of earlier studies in which apomorphine administration provoked increased glucose use within the acetylcholinesterase rich areas of the ventral striatum (McCulloch et al. 1983). They further demonstrate the importance of the $D_2$ receptor population of the ventral striatum, particularly as the ventral striatum has been implicated as a site for the initiation of dopamine-induced oral stereotypies (Iversen and Koob, 1977; Arnt, 1985).

In contrast to LY 171555, the selective $D_1$ receptor agonist SKF 38393 failed to provoke significant changes in glucose use within the striatum. This observation supports the conclusions of biochemical investigations where activation of $D_2$ but not $D_1$ receptors within the striatum modulates cholinergic transmission both in vitro (Stoof and Kebabian, 1981, 1982; Plantje et al. 1983) or in vivo (Scatton, 1982).

The dopaminergic innervation of cerebral cortex is limited to a few areas associated with limbic function, such as prefrontal, anterior cingulate, and piriform cortices (Lindvall and Björklund, 1984). In contrast to most dopaminergically innervated regions, which contain both dopamine $D_1$ and $D_2$ receptor populations, there is increasing evidence that cerebral cortex may possess only the $D_1$ population (Thierry et al. 1984; Jastrow et al. 1984; Altar et al. 1985). Anterior cingulate cortex was the only region in which consistent alterations in glucose utilisation were elicited by the $D_1$ receptor agonist SKF 38393. In contrast glucose use proved relatively insensitive to the $D_2$ agonist LY 171555 in this brain area. The function related alterations in glucose use in anterior cingulate cortex (and in the anterior thalamus nucleus with which it

has extensive reciprocal connections) provide evidence for a functional role of dopamine $D_1$ receptor upon limbic mechanisms via these two brain areas. Administration of the selective $D_2$ agonist LY 171555 provoked dose-dependent increases in local glucose use within sensory motor and frontal cortices. Since these areas appear to contain few, if any, dopamine $D_2$ receptors (Alter and Marshall, 1985) and receive no substantial dopaminergic innervation (Lindvall and Björklund, 1984) the increases in glucose use within sensory motor and frontal cortices probably reflect alterations in thalamo-cortical function initiated at the level of the striatum (McCulloch et al. 1979; McCulloch, 1982; Savaki, 1984; Collins, 1980). The laminar and columnar organisation of the enhanced cortical glucose use following $D_2$ receptor activation and the parallel increases in glucose utilisation within the sensory and motor relay nuclei of the thalamus (VM, VL) following LY 171555 administration emphasise the importance of the thalamus in mediating dopaminergic influence of cortical function.

Although extensive dopaminergic projections to the limbic system exist (Ungerstedt, 1971; Lindvall and Björklund, 1982), the consequences of dopamine receptor activation, as assessed by alterations in function-related glucose use, have in general been small and highly variable (McCulloch, 1982). The role of dopamine as a neurotransmitter in the hippocampus remains controversial. Originally the presence of low concentrations of dopamine in the hippocampus was attributed to its role as a precursor of noradrenaline (Lindvall et al. 1974 b). However, there is now evidence that dopamine may play a true neurotransmitter role within the hippocampus as shown by the presence of both $D_1$ (Bischoff et al. 1980; Bernado and Prince, 1982; Dawson et al. 1985, Ouimet et al.

1984) and $D_2$ receptor sites (Bischoff et al. 1980) and of specific dopamine uptake mechanisms (Scatton et al. 1985). In the present investigation we found that the $D_2$ receptor agonist LY 171555 provoked a dose related decrease in glucose use within the molecular layer of the hippocampus while, in contrast, the $D_1$ receptor agonist SKF 38393 failed to significantly affect glucose use. In the electrophysiological investigations, dopamine induced hyperpolarisation of hippocampal pyramidal cells was mediated by an increase in adenyl cyclase, and by definition $D_1$ receptor mediated (Bernado and Prince 1982). However, it should be noted that the latter studies were focused on the CA1 pyramidal cells of the dorsal hippocampus whereas, in the present studies, glucose use was measured in the molecular layer of the ventral hippocampus at the level of the medial geniculate body. Therefore, the findings of Bernado and Prince (1982) and the present study are not strictly comparable on neuroanatomical grounds.

An indirect dopaminergic action via the septohippocampal pathway may underlie the reductions in glucose use observed in the ventral hippocampus following the administration of LY 171555. The septohippocampal pathway is a major source of cholinergic imput to the hippocampus (Shute and Lewis, 1961). Cholinergic neurons within the medial septum and nucleus of the diagonal band project via the fimbria to the hippocampus and dentate gyrus. Ligand binding studies have demonstrated the existence of distinct populations of muscarinic and nicotinic receptors within both the dorsal and ventral hippocampus. $\alpha$-bungarotoxin labelled nicotinic receptors are located primarily within the stratum lancunosum moleculare of the ventral hippocampus while ^{3}H-QNB (3-quinuclidinyl benzilate) labelled

muscarinic sites have been found within all levels of the hippocampus
(see Rotter et al. 1984 for review).

Neurochemical studies have indicated that dopamine neurons
within the septum may influence the activity of cholinergic fibres
projecting to the hippocampus. Inhibition of dopaminergic activity
within the septum (whether by local injections of haloperidol into
the septum or by 6-hydroxydopamine lesions of the ventral tegmental
area) results in an increase in the turnover of acetylcholine (Ach)
within the hippocampus (Robinson et al. 1978 a), while conversely,
the administration of apomorphine reduces Ach turnover within the
hippocampus (Robinson et al. 1978 b). It has, therefore, been
proposed that dopaminergic neurones within the septum may tonically
inhibit cholinergic neurones of the septohippocampal pathway
(Robinson et al. 1978 a). This inhibition of cholinergic
transmission in the hippocampus may underlie the alterations in
glucose use observed following the administration of LY 171555.

In the present studies, administration of the $D_2$ agonist LY
171555 provoked dose-related increases in glucose use within the
lateral septal nucleus which were accompanied by significant
reductions in glucose use within the stratum lancunosum moleculare of
the ventral hippocampus. In contrast significant reductions in
glucose use within the dentate gyrus were observed only at the
highest concentrations examined. The specificity of the action of LY
171555 upon glucose use within this nicotinic-receptor rich layer of
the hippocampus would appear to indicate that the activation of $D_2$
receptors within the septum may underlie the reduction in glucose use
observed within this molecular layer of the hipocampus. However,
interpretation of the present study involving systemic drug
administration should be viewed with circumspection as this route of

drug administration does not provide sufficient information on the site of action of any agent. Nevertheless, the possibility of $D_2$ mediated effects upon nicotinic transmission with the hippocampus should receive further investigation.

The nucleus accumbens and lateral habenula represent two of the major points of interaction between the limbic and motor systems (Nauta and Domesick, 1984). Both receive a discrete dopaminergic innervation from the ventral tegmental area (Phillipson and Griffith, 1980; Phillipson and Pycock, 1982; Skagerberg et al. 1984; Lindvall and Björklund; 1978), and both areas possesss both $D_1$ and $D_2$ receptor populations (Dawson et al. 1985; Jastrow et al. 1984). The present observations, where increased glucose utilisation in the nucleus accumbens was observed at a single concentration of each agonist, provide little insight into the extent to which dopamine receptors can influence glucose use in this region or into which receptor subtype may be involved. Manipulation of central dopamine systems generally, but not invariably, result in alteration in glucose use within the lateral habenula nucleus (McCulloch et al. 1980 b; Brown and Wolfson, 1983; Pizzolato et al. 1984, 1985). Systemic administration of dopamine receptor agonists such as apomorphine and amphetamine decreased glucose use within the habenula (Wescher et al. 1979; McCulloch et al. 1980 b) while neuroleptics such as haloperidol increase glucose use (McCulloch et al. 1980 b; Pizzolato et al. 1984, 1985). However, in the present study we were unable to demonstrate significant changes in glucose use following administration of the $D_1$ agonist SKF 38393 or any but the highest dose of the $D_2$ agonist LY 171555. The apparent lack of efficacy of

these selective $D_1$ and $D_2$ agents upon glucose use within the lateral habenula constitutes one major difference with respect to the dopamine receptor agonists examined hitherto. The minimal effect of $D_1$ and $D_2$ agonists upon glucose use in the habenula is in excellent accord with the minimal functional altertions which are associated with electrical stimulation of mesencephalic dopamine systems (Porrino et al 1984 a). Similarly, the large increases in glucose utilisation within the substantia nigra pars reticulata following the administration of apomorphine or amphetamine contrast sharply with the relatively modest elevation achieved with LY 171555.

The present report provides a comprehensive description of the distribution of changes in function related glucose use associated with the selective activation of dopamine receptor subtypes. These observations emphasise the importance of the $D_2$ receptor subtype in the functional disturbances associated with the activation of central dopamine systems. Although the distribution of altered glucose use following the activation of $D_2$ receptors with LY 171555 is broadly similar to that evoked by apomorphine, a number of clear differences exist. In conclusion we have demonstrated that the administration of selective $D_1$ and $D_2$ dopamine agonists provoke distinct alterations in local cerebral glucose use. The marked increases in glucose use associated with the stimulation of central dopamine systems appears to be predominately mediated via the $D_2$ receptor population as they are provoked by the $D_2$ agonist LY 171555 but not by the $D_1$ agonist SKF 38393. Activation of the $D_1$ subpopulation with SKF 38393 results in focal decreases in glucose use within the anterior cingulate cortex and in its projection field, the anterior thalamic nucleus.

## 2. Effects of Dopamine Receptor Antagonists

Prior to the development of SCH 23390, antipsychotic activity in man and the antagonism of dopaminergically induced behavioural changes in animals were generally believed to be mediated via the $D_2$ receptor popultion (Seeman, 1981; Costall and Naylor, 1981). Indeed the poor correlation between adenylate cyclase activity and dopamine-related functions fostered the view that the $D_1$ receptor was a "receptor looking for a role" (Stoof and Kebabian, 1984). However, in the light of studies with the novel $D_1$ antagonist SCH 23390 views concerning the relative importance of $D_1$ and $D_2$ receptors in brain function may have to be revised. For, despite displaying more than 1000 fold selectivity for $D_1$ receptors over $D_2$ receptors in radioligand binding and adenyl cyclase assays in vitro, SCH 23390 produces catalepsy and potently inhibits the stereotyped behaviours produced by apomorphine and other dopaminomimetics in vivo (Iorio et al. 1983; Christensen et al. 1984; Mailman et al. 1984; Molloy et al. 1984), actions previously thought to be mediated solely by the $D_2$ receptor (Seeman, 1981). The studies comprising this part of the thesis addressed three aspects of the actions of SCH 23390 on brain function: 1) how are the actions of SCH 23390 expressed in terms of alterations in function-related glucose utilisation? 2) how do the actions of SCH 23390 compare with those of the classical neuroleptic and potent $D_2$ antagonist haloperidol? and 3) how does SCH 23390 affect the pattern of altered glucose use produced by the $D_2$ agonist LY 171555?

The selectivity of SCH 23390 as an antagonist of $D_1$ receptors is based primarily on in vitro biochemical studies (Iorio et al. 1981, 1983; Christensen et al. 1984; O'Boyle and Waddington, 1984;

Plantje et al. 1984; Schulz et al. 1985). It is, therefore, appropriate to question the $D_1$ selectivity of this agent in vivo. Over the concentration range examined in the present studies SCH 23390 did not prevent the electrical activity produced by presynaptic doses of apomorphine in dopamine cells of the substantia nigra (Mereu et al. 1985). Nor did it affect tyrosine hydroxylase activity or l-dopa accumulation in the striatum (Onali et al. 1985; Iorio et al. 1983), actions thought to be mediated via $D_2$ receptor activation. Furthermore, at concentrations as high as 3 mg/kg SCH 23390 protects $D_1$ but not $D_2$ receptors from inactivation by the neurotoxic agent N-ethoxycarbonyl-2-ethoxy 1, 2-dihydroquinoline (Meller et al. 1985). Thus, over the concentration range examined in this thesis, SCH 23390 would be expected to act primarily on $D_1$ receptors. However, the possibility that non-dopaminergic effects, particularly serotoninergic (Ohlstein and Berkowitz, 1985) cannot be excluded.

In the present studies SCH 23390 provoked highly focal alterations in glucose use, with significant alterations evident in only 4 of the 59 brain regions examined (lateral habenula, nucleus accumbens, pars reticulata of the substantia nigra and the posterioventromedial thalamic nucleus). These findings are consistent with the view that the blockade of central dopamine systems does not provoke widespread alterations in function-related glucose utilisation (McCulloch, 1982). However, it is the highly focal alterations in glucose use which provide insight into the mechanisms of action of SCH 23390.

An increase in local glucose use within the lateral habenular nucleus has been demonstrated following the administration of every neuroleptic hitherto examined irrespective of its dopamine receptor subtype specificity (Table 11). Although a number of non-neuroleptic

agents have been shown to increase glucose use within the lateral habenula (oxytremorine, caffeine estrogen) this response may prove useful in predicting neuroleptic activity of test compounds.

There is evidence that, although the lateral habenula nucleus receives direct dopaminergic input from the ventral tegmental area (Kizer et al. 1976; Phillipson and Pycock, 1982), other studies indicate that the function-related alterations in glucose use are initiated in the caudate nucleus and expressed via the internal segment of the globus pallidus (entopeduncular nucleus). Firstly, reduction of nigrostriatal activity 6-hydroxydopamine lesions of the nigrostriatal pathway or kainate lesions of the striatum result in increased glucose use within the lateral habenula (Ferron et al. 1979; Wooten 1981; Kozlowski and Marshall, 1983; Kelly et al. 1982) while intrastriatal dopamine or apomorphine reduce metabolic activity within the habenula (Kozlowski and Marshall, 1980; Brown and Wolfson, 1983). Furthermore, the increase in glucose use in the habenula produced by lesions of the nigrostriatal pathway is markedly attenuated by lesions of the entopeduncular nucleus (Wooten, 1981). In contrast faradic stimulation of the ventral tegmental area did not significantly alter glucose use within this nucleus (Porrino et al. 1984; Esposito et al. 1984), thereby signifying that the activation of the mesohabenular pathway is not responsible for the metabolic changes observed in this diencephalic nucleus following manipulation of central dopamine systems. Thus, it would appear that dopamine-related alterations in habenular glucose use, like the effect upon the basal ganglia, are initiated at the level of the caudate nucleus.

The increase in glucose use within the lateral habenula associated with reduced dopaminergic transmission suggests that the pathway is under a tonic inhibitory influence by the striato-

entopeduncular-habenular pathway. This inhibitory influence appears to require the presence of functional $D_1$ and $D_2$ receptor subtypes since the increase glucose use selective $D_1$ and $D_2$ antagonists produce increases in glucose use within the habenula while only mixed $D_1/D_2$ agonists reduce metabolic activity within this nucleus (Table 11). The necessary activation of both $D_1$ and $D_2$ receptors populations would also explain why neither SKF 38393 nor LY 171555 significantly altered glucose use within the lateral habenular. At variance with this view is the reduction in habenular glucose use produced by the putatively selective $D_2$ agonist bromocryptine (Pizzolato et al. 1985). It should be emphasised, that although bromocryptine has a low affinity for $D_1$ receptors, it does interact with adrenergic and serotonin receptors (Lew et al. 1977) particularly at the high concentrations employed by Pizzolato and his associates (20-400 mg/kg).

Specific lesion techniques combined with adenylate cyclase or ligand binding assays have indicated that the $D_1$ and $D_2$ receptor populations within the substantia nigra may be differentially located. Several groups have reported that 6-hydroxydopamine lesions of the substantia nigra or median forebrain bundle does not significantly alter cyclase activity which is localised in the pars reticulata (Spano et al. 1976; Kebabian and Saavedra, 1976; Gale et al. 1977). Cyclase activity, however, was largely abolished by the destruction of the gabaergic/substance P-ergic striato-nigral projection system (Spano et al. 1976, 1977; Gale et al. 1977). In contrast ligand binding studies have demonstrated that ^{3}H-spiperone ($D_2$ receptor) binding is significantly reduced by 6-hydroxydopamine lesions of the mesencephalic dopamine neurones but is largely unaffected by destruction of the descending striato-nigral projection

# TABLE 11

Dopminergic Influences Upon Local Glucose Utilisation

in the Lateral Habenular Nucleus

| | Receptor Preference | Metabolic Activity in the Lateral Habenula | Reference |
|---|---|---|---|
| **Inhibition** | | | |
| SCH 23390 | $D_1$ | Increase | Present Study. |
| YM 09151-2 | $D_2$ | Increase | Palacios and Wiederhold, 1985. |
| Sulpiride | $D_2$ | Increase | Palacios and Wiederhold, 1984. |
| Clozapine | $D_2$ | Increase | Palacios and Wiederhold, 1984. |
| Haloperidol | $D_2 > D_1$ | Increase | McCulloch et al. 1980. Pizzolato et al. 1984. |
| Pimozide | $D_2 > D_1$ | Increase | Gomita and Gallistrel, 1982. Gallistrel et al. 1985. |
| Droperidol | $D_2 > D_1$ | Increase | Oguchi et al. 1982. |
| Fluphenazine | $D_2 / D_1$ | Increase | Wooten, 1979. |
| Reserpine | $D_1 / D_2$ | Increase | Palacios and Wiederhold, 1984. |
| Nigral Lesion | $D_1 / D_2$ | Increase | Wooten and Collins, 1981. |
| Striatal Lesion | $D_1 / D_2$ | Increase | Kelly et al. 1982. |
| Lesion Entopeduncular | | Increase | Wooten, 1979. |
| **Stimulation** | | | |
| SKF 38393 | $D_1$ | No Change | Present Study. |
| LY 171555 | $D_2$ | No Change | Present Study. |
| Apomorphine | $D_1 / D_2$ | Decrease | McCulloch et al. 1982. |
| Amphetamine | $D_1 / D_2$ | Decrease | Weschler et al. 1979. |

system (Murrin et al. 1979; Quik et al. 1979).

These data suggest that $D_1$ receptors are localised mainly on the terminals of striato-nigral fibres in the pars reticulata of the substantia nigra whilst $D_2$ receptors occur primarily on the dopamine neurons of the pars compacta. In the present study the administration of the $D_1$ antagonist, SCH 23390, reduced glucose utilisation within the pars reticulata of the substantia nigra while glucose use within the pars compacta was minimally affected. These findings contrast with the pronounced increases in glucose use observed within the pars compacta following administration of $D_2$ selective concentrations of haloperidol (McCulloch et al. 1982 c). Furthermore, although significant reductions in glucose use were observed in the pars reticulata of haloperidol-treated animals, the concentration required was some 1000 fold greater than that required to increase glucose use within the pars compacta (McCulloch et al. 1982 c). Thus, SCH 23390 would appear to selectively alter function related glucose use within the $D_1$ rich cells of the pars reticulata of the substantia nigra whilst haloperidol differentially interacts with cells from the pars compact. This concept is supported by biochemical and electrophysiological studies performed on the substantia nigra of <u>unanaesthetised</u> rats, (the distorting influences of anaesthesia within the substantia nigra has been discussed previously, see Introduction). In these studies SCH 23390 increased cell firing rate of cells within the pars reticulata in a manner similar to that produced by other neuroleptics (Mereu et al. 1985; Onali et al. 1984; Walters et al. 1986), but unlike haloperidol SCH 23390 did not reverse the inhibition produced by low (presynaptic)

concentrations of apomorphine in the dopamine cells of the pars compacta (Mereu et al. 1985).

In a recent semiquantitative study Palacios and Wiederhold reported that oral administration of SCH 23390 failed to significantly alter 2-deoxyglucose uptake in any of the brain regions examined. The reason for the discrepancies between the present quantitative study and the semiquantitative studies of Palacios and other has been addressed previously (see Methods Section, previous discussion). However, one additional factor may underly the lack of efficacy of SCH 23390 in the Palacios study, namely the use of oral administration of SCH 23390. Several groups have remarked on the low efficiency of orally administered SCH 23390, as compared to parenterally administered (Iorio et al. 1983; Christensen et al. 1984; Mailman et al. 1984). A large "first pass" metabolism of SCH 23390, resulting in subthreshold concentration of SCH 23390 reaching the brain has been proposed to account for the low efficacy of enteral administration SCH 23390 (Mailman et al. 1984).

The present studies have demonstrated that pretreatment with low concentrations of the $D_1$ antagonist SCH 23390 abolishes the stereotyped gnawing produced by the $D_2$ agonist LY 171555; findings which are consistent with reports that SCH 23390 can block the stereotyped behaviours produced by the $D_2$ agonist RU24213 (O'Boyle et al. 1984 b) apomorphine (Iorio et al. 1983; Christensen et al. 1984; Mailman et al. 1984; Molloy and Waddington, 1985), 6,7 ADTN (Christensen et al. 1984) methylphenidate (Christensen et al. 1984) and amphetamine (Iorio et al. 1983; Mailman et al. 1984; Christensen et al. 1984) as well as the selective $D_1$ agonist SKF 38393 (Molloy and Waddington, 1984).

Underlying the inhibition of stereotypies were widespread reductions in glucose use, particularly within motor systems, i.e. those areas most affected by LY 171555 administration (see agonist section). As discussed previously the increases in glucose use within the striatum are localised within the ventral striatum. In keeping with this finding the present study revealed that SCH 23390 is more effective in reducing glucose use in the ventral rather than the dorsal striatum. This idea is supported by a recent study which claimed that the stereotyped behaviours produced by apomorphine could be selectively blocked by the intracerebral microinjection of a variety of neuroleptics, including SCH 23390 or sulpiride into the ventral striatum (Arnt, 1985).

Recently a number of authors have suggested that the efferent pathways from the striatum are under the control of different receptor subpopulations (Ungerstedt et al. 1983) and in dopamine receptor agonists Herrera-Marschitz and Ungerstedt 1984 a,b; Waszczak et al. 1984). Behavioural studies in rats 6-hydroxydopamine lesions of the nigrostriatal pathway have demonstrated that SCH 23390 selectively blocks the rotational response induced by SKF 38393 but not that produced by pergolide whereas the selective $D_2$ antagonist spiroperidol attenuated the pergolide response but not that produced by SKF 38393 (Arnt and Hyttel 1984; Arnt, 1985). The rotation evoked by the mixed $D_1/D_2$ agonist, apomorphine could not be completely blocked by SCH 23390 or haloperidol, however the administration of these antagonists successfully blocked the apomorphine response (Arnt, 1985). Since kainic acid lesions of the substantia nigra in 60HDa treated rats reverses the direction of

apomorphine while having little effect on pergolide induced rotation Herrera-Marschitz and Ungerstedt, 1984 a) postulated that the nigrostriatal pathway is controlled by $D_1$ receptor mechanisms whereas striopallidal pathways are affected by $D_2$ mechanisms (Herrera-Marschitz and Ungerstedt, 1984 a; Ungerstedt, 1983). A similar view has been proposed by Waszcak and her colleagues who reported that systemically administered apomorphine provoked a uniform increase in the firing rate of pallidal neurones while producing no consistent alterations within the pars reticulata of the substantia nigra. If these two striatal efferent systems are controlled by different receptor mechanisms then the co-administration of SCH 23390 and LY 171555 should selectively block the strio-nigral pathway, leaving the striopallidal system relatively unaffected. However, it was found that glucose use within the globus pallidus and pars reticulata were depressed by a proportionately similar amount (24% and 27% respectively). Thus, the present studies do not support the concept that striatonigral and striatopallidal pathways are influenced by different receptor mechanisms.

3. Evaluation of Dopaminergic Influences Upon Function-Related Glucose Use by the Fingerprinting Approach

The ability to quantify changes in functional status simultaneously in every anatomical component of the brain visible on the autoradiograms with 2-deoxyglucose, provides a unique perspective on the effects of drug administration upon brain function. Nevertheless, the 2-deoxyglucose technique has failed to make a major impact on the field of neuropharmacology.

One of the principle problems with pharmacological studies employing 2-deoxyglucose autoradiography is associated with the interpretation and analysis of the vast quantities of data generated (i.e large numbers of brain areas and multiple drug concentrations). Hitherto, the effects of drug administration upon function-related glucose use has been interpreted on the basis of the acquisition of some notional level of statistical significance or on the percentage change in glucose use at a single drug dose. However, in each case only a small proportion of the data generated is used in the interpretation of the drug response. Furthermore, the acquisition of notional levels of statistical significance can be influenced by the form of analysis adopted. For example, McCulloch and his associates (McCulloch et al. 1982) reported significant alterations in local glucose use in 18 of the 43 brain areas examined following the administration of apomorphine to rats. However, an independent reanalysis of the same autoradiograms (Appendix 4) revealed significant alterations in glucose use in only 7 of the 43 brain areas investigated by McCulloch et al. This latter form of analysis would have resulted in a completely different interpretation of the effects of apomorphine on local cerebral glucose utilisation. For example, one of the major findings of the apomorphine studies by McCulloch et al. (1982) was that alterations in glucose use within specific thalamic nuclei were accompanied by parallel changes within their cortical projection areas. Apomorphine administration results in parallel increases in glucose use within ventrolateral thalamus and sensory motor cortex, while concomitantly producing proportionately thalamic nucleus and in its cortical projection area,

the anterior cingulate cortex. However, when the autoradiograms from these apomorphine treated animals were reanalysed using the densitometrical criteria and statistical procedures adopted within this thesis, no significant alterations could be demonstrated in 3 of these 4 brain areas.

The fingerprinting approach offers considerable advantages over percentage change ranking at a single drug concentrations or acquisition of notional levels of statistical significance in that analysis is performed on all the data available for each brain area. Furthermore, the reliability studies described in Appendix 1 have shown that the hierarchies for apomorphine generated by independent investigators using different densitometrical criteria are highly correlated.

The value of the fingerprinting approach in analysing the distribution of metabolic changes in response to drug administration has been highlighted recently in a study of the effects of diazepam and the gaba agonists muscimol and 4,5,6,7-tetrahydroisoxazolo-[-5, 4c]pyridin-3-ol (THIP) on local cerebral glucose utilisation (Ford et al. 1985). Each agent was found to reduce glucose use in almost every region of the brain. Fingerprinting analysis revealed a close correlation between the regional hierarchies generated for muscimol and THIP. However, these patterns of regional responsiveness differed markedly from that produced by diazepam. Thus, although gaba and benzodiazepine receptors may interact within the same receptor complex to reduce functional activity, the fingerprinting approach was able to discriminate between agents producing similar pharmacological effects, via different receptor mechanisms.

In the present studies the fingerprinting approach was used to examine the more complex pattern of altered glucose use produced by pharmacological manipulation of central dopamine systems. From the pattern of altered glucose use produced by apomorphine, it would appear that this dopamine receptor agonist possesses actions which are similar to those evoked by SKF 38393 (reduction in glucose use within anterior cingulate cortex-anteromedial thalamic circuit) and LY 171555 (increase in glucose use within areas of the brain associated with motor function). These findings are consistent with the view that apomorphine can interact with both $D_1$ and $D_2$ receptor populations (Seeman et al. 1981). However, the pattern of glucose use provoked by apomorphine could not be viewed simply as the cumulative effects of SKF 38393 and LY 171555. Stimulation of somatosensory and motor areas of the brain is associated with $D_2$ receptor activation (Walters et al. 1983, 1986; Palacios and Wiederhold, 1985) and these areas feature prominently within the hierarchies of regional responsiveness to apomorphine and LY 171555. However, even within these areas, the sensitivity of these brain areas to apomorphine was markedly different than that to LY 171555.

For example, if we examine the hierarchy of responsiveness for four regions of the rat brain which are involved in motor function (substantia nigra pars reticulata, globus pallidus and sensory motor cortex) we find that in apomorphine treated animals the hierarchy of responsiveness is (SNr > STN > GP > SMc). However, in LY 171555 treated animals the hierarchy is (STN > SMc > SNr > GP). Neuroleptics are characterised by an increase in local glucose utilisation within the lateral habenula. Consistent with this view

are the findings that the lateral habenula is among the most sensitive brain regions to both the $D_1$ antagonist SCH 23390 and $D_2$ antagonist haloperidol. The fingerprinting approach also provided support for the view, based on electrophysiological studies (Mereu et al. 1985), that haloperidol and SCH 23390 may have differential effects within the pars compacta and pars reticulata of the substantia nigra. In the present studies, it was found that the ´f´ value generated for haloperidol adminstration to the pars compacta of the substantia nigra was some fifteenfold greater than that produced for SCH 23390. Conversely, the ´f´ value for SCH 23390 in the pars reticulata was twice that for haloperidol. These findings are consistent with the neuroanatomical localisation of $D_2$ receptors on the pars compacta and $D_1$ receptors in the pars reticulata of the substantia nigra (Gale et al. 1977; Seeman, 1981).

An important feature of the SCH 23390 response was that the ´f´ values for all but a few key brain areas were tightly grouped around zero. The high specificity of the response to SCH 23390 would sugest this potent neuroleptic may exhibit fewer clinical side effects than haloperidol.

DOPAMINE RECEPTOR SUBTYPES AND THE CEREBRAL CIRCULATION: INFLUENCES ON LOCAL CEREBRAL BLOOD FLOW AND THE FLOW/GLUCOSE USE RELATIONSHIP

On the basis of the studies undertaken in the first two sections of this thesis it is proposed that dopamine-induced dilatation of the cerebral vasculature is mediated via the $D_1$

receptor subtype while alterations in function-related glucose utilisation are initiated in all but a few discrete brain areas by the $D_2$ receptor population. This apparent difference in the receptor characteristics of cerebral vascular and neural tissues may be used to examine the relative importance of the vascular and metabolic components of the circulatory response to dopamine receptor agonists. For the administration of a selective $D_1$ agonist would be expected to dilate the cerebral vasculature while having minimal effects on glucose utilisation. Conversely, the administration of a selective $D_2$ agonist would elicit marked metabolic effects while having little effect on the vascular receptor. This hypothesis was subsequently tested using SKF 38393 and LY 171555.

The administration of the selective agonist LY 171555 (0.5mg/kg) significantly increased local blood flow within those brain areas in which elevated rates of glucose use had been observed in the glucose use studies (sensory motor and frontal cortices, basal ganglia and ventral thalamus inter alia). Moreover, the increases in blood flow were in general similar in magnitude to the changes in glucose use observed following the administration of this $D_2$ agonist. Administration of the $D_1$ agonist SKF 38393 effected little change in local cerebral blood flow, with the exception of a few discrete brain areas (e.g. anterior cingulate cortex and anteromedial thalamus). These observations are of crucial importance in delineating the functional significance of the vascular dopamine receptor in mediating the increases in cerebral blood flow associated with the systemic administration of dopamine agonists. For despite being a potent vasodilator of the pial vasculature in situ, this $D_1$ agonist

failed to stimulate cerebral blood flow and, in fact, provoked parallel reductions in blood flow and glucose use within the anterior cingulate cortex, an area rich in $D_1$ receptors.

The reason why a number of authors observe disproportionately large increases in cerebral blood flow relative to metabolic activity following the administration of dopamine receptor agonists while other studies using the same agents do not, has never been adequately addressed. The results of the studies presented in this thesis clearly indicate that a direct vasomotor action is unlikely to be the primarily mediator of large increases in blood flow. However, a number of alternative explanations exist.

With the notable exception of the clinical studies of Leenders and his associates (Leenders et al. 1984, 1985) every study which has reported an excessive increase in cerebral perfusion have been performed under conditions of pronounced hypertension. It is widely accepted that homeostatic mechanisms ensure that cerebral perfusion remains relatively constant over a wide range of blood pressures (Harper, 1966; Purves, 1972; Strandgaard and Paulson, 1984). However, recent evidence has suggested that the administration of dopamine receptor agonists prevents the cerebral circulation from autoregulating at elevated blood pressures, with the result that the mechanical stress on the cerebral vasculature produced by the high intraluminal pressure (at 150 mm Hg) is sufficient to disrupt the blood-brain barrier (Carlsson and Johansson, 1978; Tuor and McCulloch, 1986) and significantly alter the normal flow-glucose use relationship (Tuor and McCulloch, 1985). Thus, in studies of this type the increases in cerebral blood flow are more likely to be due to mechanical stress rather than a $D_1$ mediated dilatation.

In a recent clinical study by Leenders et al. (1984, 1985) the infusion of 1-dopa was reported to significantly increase blood flow within the basal ganglia and overlying frontal cortex while having minimal effects upon local metabolic activity within these brain areas. In contrast to the aforementioned studies the investigations of Leenders were performed under normotensive conditions. However, their conclusion that 1-dopa increases cerebral blood flow by stimulating cerebrovascular dopamine receptors appears to be unfounded as the circulatory response to 1-dopa could be blocked by bulbocapnine, a potent dopamine receptor antagonist which does not readily cross the blood-brain barrier. Thus, Leenders own data would argue against a direct vascular action of 1-dopa.

It would appear, therefore, that the excessive increases in blood flow, observed by some investigators is the results of mechanisms other than via the stimulation of vascular dopamine receptors. Furthermore, the results of the present studies with SKF 38393 and LY 171555 and of the previous studies with apomorphine (McCulloch et al. 1982 a) clearly show that metabolic activity is the primary determinant of cerebral blood flow following the administration of dopamine receptor agonists.

This view would appear to be challenged by the detailed studies of the caudate nucleus. Although, LY 171555 effected focal alterations in blood flow and glucose use particularly within the ventral caudate nucleus, inspection of the autoradiographs suggested that the pattern of altered blood flow within the caudate nucleus did not match that of glucose use. This impression which was subsequently borne out by analysis of the flow/glucose relationship in 10 subregions of the caudate nucleus. These findings are clearly at variance with the close coupling of flow to glucose use observed

throughout the rest of the brains of these same animals.

An apparent uncoupling of the flow/glucose use relationship may result from experimental artefact (due to differences in the temporal resolution characteristics of CBF and glucose use studies) or from drug effects resulting in hypertension or systemic hypercapnia. Ingvar and his associates (1983) have argued that any transient increases in glucose utilisation might be "diluted out" over the 45 minute sampling period, while the secondary increases in blood flow would be readily detected over the shorter 1 minute blood flow experiment. However, in the present studies the possibility of transient drug effects resulting in an apparent uncoupling of flow to metabolism is unlikely since blood flow and glucose use measurements were initiated 40 and 30 minutes after drug administration. Rigorous control of the cardiovascular and respiratory status of experimental animals (Appendix 5.1) also served to obviate the possible complicating effects of hypertension and hypercapnia on cerebral blood flow.

LY 171555 significantly elevated local blood flow and glucose utilisation in the ventral caudate nucleus, while having only minimal effects upon the more dorsal aspects of the nucleus. However, the pronounced medial to lateral difference in blood flow was not accompanied by corresponding changes in glucose use. Thus, although similar increases in glucose use were observed within both the medial and lateral parts of the ventral caudate, following LY 171555 administration, the circulatory responses within these two areas were markedly different. A possible explanation for the distinctive circulatory responses of the medial and lateral caudate nucleus to LY 171555 may lie in the peculiar vascular anatomy of the striatum.

Anatomical studies have shown that the more lateral portions of the caudate nucles are supplied by branches of the middle cerebral artery whereas more medial portions are supplied by branches of the anterior cerebral artery (Yamori et al. 1976; Rieke et al. 1981). Differential reactivity of vessels within the middle cerebral and anterior cerebral artery territories may underlie the different blood flow responses observed between the medial and lateral caudate nucleus. Supporting this view are recent reports of differential sensitivity in the major cerebral vessels to a variety of neurotransmitters (see McCulloch and Edvinsson, 1984 for review), including preliminary evidence for dopamine (Hamel et al. 1985). Testing the hypothesis, at present, is not possible. The anatomical positioning of the anterior cerebral artery precludes the examination of vasomotor responses by the pial vessel approach and in vitro investigators using rat middle and anterior cerebral arteries have proved to be impracticable (Edvinsson – personal communication).

Following the administration of LY 171555, the area displaying the largest increases in local blood flow (ventrolateral portion at the middle caudate level) corresponds to the subregion of the caudate nucleus containing the highest concentration of $D_2$ receptors (Jastrow et al. 1984; Altar et al. 1985; Nock et al. 1986). Within this subregion of the caudate nucleus the mgnitude of the circulatory response to LY 171555 was almost four times greater than the observed increase in glucose use. These observations would suggest that LY 171555 is increasing blood flow within the ventrolateral caudate nucleus via $D_2$ receptor mechanisms on, or close to, cerebral vessels.

A similar pattern of heteogeneous blood flow has been reported following the administration to intact and 6-hydroxy-dopamine lesioned rats (Ingvar et al. 1983). On the grounds that the

circulatory response to apomorphine was enhanced within the lateral caudate nucleus by lesions of the nigrostriatal pathway while glucose use in this area remained relatively unaffected, Ingvar and his associates postulated that this increase in blood flow was probably due to the stimulation of supersensitive vascular dopamine receptors. However, the precise location of these receptors remains obscure. A direct stimulation of dopamine receptors on the cerebral vasculature would be the most obvious site of action for this dilatory response. However, both in vitro (Foster et al. 1983) and in situ (present pial vessel studies) investigations have indicated that it is the $D_1$ and not the $D_2$ receptor which mediates dilatation of cerebral vessels. Nevertheless, the existence of $D_2$ receptors subserving dilatation of resistance vessels within the ventrolateral caudate nucleus cannot, at present, be excluded.

A $D_2$ mediated inhibition of sympathetic neurons innervating cerebral vessels could underlie the observed increase in local perfusion within the ventrolateral caudate nucleus. Indeed, a $D_2$ mediated inhibition of sympathetic activity has been reported to subserve the vasodilatory actions of LY 171555 in peripheral vascular tissues (Hahn and McDonald, 1983, 1984; Lokhandwala and Steenberg, 1984). However, in contrast to the situation pertaining in peripheral vascular beds, the cerebral vasculature does not receive a tonic constriction by the sympathetic system (Edvinsson and MacKenzie, 1977; Harper and McCulloch, 1984). Consequently, even if a $D_2$ mediated inhibition of the sympathetic innervation of the cerebral vasculature does occur, it would not result in an increase in local blood flow.

Despite displaying pronounced vasodilator activity in the pial vessel preparation, SKF 38393 administration resulted in a generalised decrease in blood flow within the head and body of the caudate nucleus. As there was no evidence of parallel reductions in glucose use at these levels it would appear that SKF 38393 was acting directly on the microvasculature within the rostral caudate nucleus to reduce blood flow. Significant reductions in local blood flow within the caudate nucleus have also been reported following electrical stimulation of the nigrostriatal pathway or the release of tissue dopamine stores by amphetamine (Lavyne et al. 1977). Thus, the reductions in local blood flow observed in the present study would appear to indicate that, in this area at least, $D_1$ receptors mediate constriction of the local microvasculature. However, the possibility that the reductions in local blood flow observed in the present study and that of Lavyne et al. (1977) may be mediated via non-dopaminergic mechanisms cannot be discounted. It is well recognised that in cerebral, as well as in peripheral vessel preparations, dopamine provokes a dose-related constriction by stimulating $\alpha$-adrenergic receptors (Goldberg and Toda, 1975; Toda 1976; Edvinsson et al. 1978). SKF 38393 is generally thought to exhibit low adrenergic activity (Pendleton et al. 1978; Hahn and Wardell, 1980). However, Lang and Woodman (1982) have demonstrated that SKF 38393 provokes a dose-related decrease in coronary blood flow; a response which could be abolished by $\alpha$-adrenoceptor blockade by phentolamine of yohimbine. A similar $\alpha$-adrenoceptor mediated vasoconstriction of caudate microvessels would provide an equally plausible explanation for the reductions in blood flow observed in the present study.

At present it is not possible to determine whether the observed reductions in blood flow within the caudate nucleus are mediated via adrenergic or dopaminergic receptor mechanisms. The small calibre and inaccessability of intraparenchymal vessels preclude the examination of vasomotor responses by current in vitro or in situ approaches. Furthermore, detailed pharmacological studies of cerebral perfusion in vivo would not be feasible due to the complexity of an experimental design involving multiple concentrations of various agonists and antagonists and their interactions.

It is recognised that $D_1$ receptors mediate relaxation of vascular smooth muscle from renal, mesenteric and splenic beds ( Brodde 1982, Schmidt et al 1986 ) and that the administration of $D_1$ agonists can provoke increased local blood flow within these areas. However in 58 of the 59 brain regions examined in the present studies, neither SKF 38393 nor LY 171555 provoke independant vascular responses with the result that the flow/glucose use remained unaltered. Moreover, even within the one brain area where cerebrovascular dopamine receptors may contribute significantly to the level of local tissue perfusion (viz the caudate nucleus) it was the $D_2$ receptor agonist rather than the $D_1$ agonist which provoked the increase in local blood flow. Thus the present studies demonstrate that vascular $D_1$ receptors do not contribute significantly to the circulatory responses elicited by dopamine receptor agonists. This absence of a significant increase in blood flow following the administration of SKF 38393 calls into question the relevance of studying the vasomotor responses of cerebral vessels. In contrast to dopaminergic actions on the peripheral vasculature, the response

of individual cerebral arterioles to dopamine receptor agonists provides little insight into the circulatory responses to these agents in vivo. The dichotomy between the vasomotor responses elicited by pharmacological agents in vitro and their circulatory responses in vivo is not unique to dopamine systems. A similiar situation also occurs following the pharmacological manipulation of other monoamine neurotransmitter systems within the central nervous system ( gaba and noradrenergic systems). Despite the demonstration of noradrenergic and gaba mediated vasomotor responses on cerebral vessels (Edvinsson and McKenzie 1977, Edvinsson and Krause 1979) the stimulation of these systems by systemic drug administration does not alter the flow/metabolism relationship.

In conclusion, the studies within this thesis clearly demonstrate that, in the vast majority of brain areas, local metabolic activity is the primary determinant of tissue perfusion. However, within a few brain regions, other factors may play a significant role in determining levels of blood flow. The precise role of the cerebrovascular dopamine receptor is unclear and is worthy of further investigation.

APPENDIX 1

VALIDATION OF THE ´FINGERPRINTING´ METHOD

# APPROACH

A reproducibility study on the 'fingerprinting' method was carried out on glucose use data generated by two independent investigators from the same animals.

The 2-deoxyglucose autoradiograms from a series of 19 control and apomorphine (0.1 - 5 mg/kg) treated animals were analysed by Dr. J. McCulloch in Bethesda (USA) and by J. Sharkey in Glasgow (UK) using different densitometrical approaches and on different densitometers. Local cerebral glucose use values calculated for each data set were subsequently used to investigate the reproducibility of the fingerprinting model.

In the initial analyses, systematic differences between the USA and UK data sets were examined. For each animal the USA/UK difference for each of 39 brain regions were obtained. Regional and inter-animal differences between the two data sets were examined by two-way ANOVAR differences.

Hierarchies of regional responsiveness were constructed for each data set and compared via correlation analysis. The reliability of the hierarchies and of the product moment correlation coefficient were determined using the principles of the Boostrapping method (i.e. comparison of the original data set with 100 simulated data sets having the same overall mean and variability structure) as described previously (see Methods section).

RESULTS


Preliminary Analysis


Plots of local cerebral glucose use values for each of the 19 animals revealed a strong linear relationship betweeen the UK and USA data, but with the USA data generally numerically higher than the UK measurements. However, the close correlation was marred by a few brain regions which consistently deviated from the line of identity. The anomolous regions included prefrontal cortex, amygdala, cerebellar hemisphere and vermis (Figure 34).

Interval estimates for USA/UK differences revealed values significantly greater than zero in 14 of the 19 animals. The positive trend for the USA/UK differences would suggest that the USA data was higher than that generated in the UK. This impression was confirmed by a two-way analysis of variance on the USA/UK differences which revealed significant regional and inter-animal differences between the two data sets.


Analysis of Fingerprinting Model


Analysis of the 'f' values generated by the two investigators confirmed the close linear correlation (0.875) between the two data sets, although the value was somewhat lower than would be expected from the same autoradiograms.

The reliability of the rankings generated by both investigators were good, with the correlation coefficients between the original and 100 simulated data sets being 0.96 and 0.94 for the USA and UK analyses respectively.

FIGURE 34
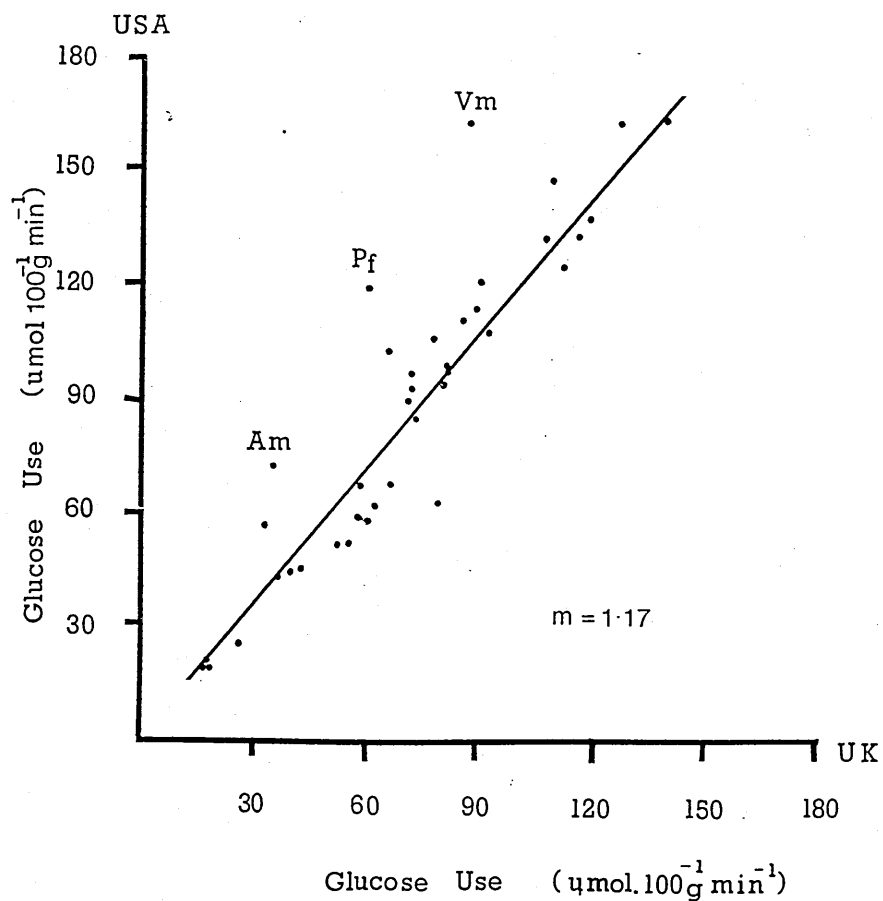


Influences of different densitometrical approaches upon local cerebral glucose use values of rats which received apomorphine (5mg/kg). Each data point represents the mean glucose use value for a single brain region. The best fitting straight line of gradient (m) is given. Am: lateral amygdala, Pf: prefrontal cortex, Vm: cerebellar vermis.

Simulations of the USA vs UK correlation were close to, but generally less than, the calculated value of 0.875. This biasing of highly correlated data downwards is predicted by the Bootstrapping method and indicates that the true correlation coefficient for these two data sets is higher than the measured value of 0.875. Therefore, taking into account the downward biasing of the correlation coefficient and the inclusion of data from anomolous brain regions (i.e. prefrontal cortex) the reproducibility of the fingerprinting model would appear to be adequate to permit the comparison of data generated by different investigators.

COMMENTARY

The studies presented in this section indicate that a neuropharmacological 'fingerprint' of a drug could, indeed be reliably constructed from ranking of brain regions according to their functional responses to drug administration. Moreover, this hierarchy could be reproducibly constructed by independent investigators using different densitometrical criteria. Bearing in mind that the reproducibility studies were performed on the same autoradiograms (and with the same blood data) the correlation between the two data sets (0.875) was lower than would have been expected. The relatively low correlation between the two data sets probably reflects the different densitometrical criteria adopted by the two investigators.

The USA data set was generated by placing a small (3 x 3 pixel) measuring frame over the darkest portion of the region under examination, irrespective of its precise anatomical position within the region of interest. This approach confers a high degree of sensitivity to the densitometry but with a loss of anatomical precision and an increase in the variability of the data (as a result of the small number of pixels used to evaluate the optical density of the region). In contrast, the UK analysis approach was to increase the anatomical precision of the measurements by adopting more rigid anatomical criteria and to increase the accuracy by using a larger measuring frame, (up to 10 x 10 pixels in large brain areas) wherever possible. The use of larger measurements areas on a predetermined position within a brain area would be expected to dilute highly focal alterations in optical density and so yield glucose use values which were systematically lower than those produced by the USA approach. Moreover, if focal alterations in optical density were not consistently found within the same subregion the ´fixed position´ measurement approach dilute or even fail to detect marked alterations in optical density. These expectations were borne out by two-way analysis of variance on the USA/UK differences which revealed systematically lower (approximately 12%) glucose use values in the UK data set. Moreover, in a number of large brain regions where focal alterations in glucose use occurred (i.e. cerebellum cortex and vermis, see Figure 19) the response was not marked in the UK data.

The different responses observed by the two investigators within prefrontal cortex was caused by the use of different anatomical criteria to define this brain region. Traditionally,

prefrontal cortex is defined as that area of neocortex which receives

its principle thalamic projections from the mediodorsal thalamic

nucleus (Rose and Woolsey, 1948). However, in the rat the

mediodorsal thalamus projects to two distinct cortical fields; a

perirhinal area located on the dorsal bank of the rhinal sulcus; and

an anteromedial field located rostral and dorsal to the genu of the

corpus callosum (Divac et al. 1978 a,b). In the original (USA)

apomorphine studies prefrontal cortex was examined in the sulcar

region whereas the UK study analysed the anteromedial subdivision.

However, it should be emphasised that despite the different

densitometrical approaches the correlation between the two data sets

was high and the hierarchy of regional responsiveness well preserved.

Local Rates of Glucose Utilisation Following the Administration

of

SKF 38393 and LY 171555.

Table 2:1

Cardiovascular and respiratory variables for each treatment group.

Tables 2:2 - 2:7

Local cerebral glucose utilisation was measured in conscious rats following the intravenous injection of saline (n=6), SKF 38393 at the concentrations of 1 mg/kg (n=5), 10 mg/kg (n=5), and 30 mg/kg (n=4) or LY 171555 at the concentrations of 0.1 mg/kg (n=6), 0.5 mg/kg (n=5), 1.5 mg/kg (n=5), and 5 mg/kg (n=5).

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) $\pm$ SEM.

$*p < 0.05$.

TABLE 2:1

CARDIOVASCULAR AND RESPIRATORY VARIABLES

| | SALINE | SKF 38393 | | | LY 171555 | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 mg/kg | 10 mg/kg | 30 mg/kg | 0.1 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5.0 mg/kg |
| Rectal Temperature (°C) | 37.2±0.1 | 37.0±0.1 | 37.4±0.2 | 37.5±0.3 | 37.1±0.1 | 37.0±0.1 | 37.0±0.1 | 37.3±0.1 |
| Arterial $pCO_2$ (mm Hg) | 35.1±1.2 | 38.0±0.7 | 36.1±1.6 | 38.9±1.3 | 39.9±1.6 | 38.7±1.7 | 37.1±1.2 | 38.3±0.9 |
| $pO_2$ (mm Hg) | 90±7 | 95±3 | 96±1 | 91±5 | 95±4 | 99±4 | 103±3 | 103±3 |
| pH | 7.44±0.01 | 7.41±0.01 | 7.43±0.02 | 7.42±0.02 | 7.38±0.01* | 7.41±0.01 | 7.43±0.01 | 7.41±0.01 |
| $HCO_3^-$ (mM) | 23.5±0.5 | 24.1±0.1 | 24.0±0.7 | 24.9±0.6 | 23.4±0.7 | 24.5±0.9 | 24.3±0.6 | 23.7±0.9 |
| Plasma Glucose (mM) | | | | | | | | |
| Pre-Drug | 7.1±0.3 | 5.9±0.2 | 6.8±0.5 | 6.4±0.4 | 6.0±0.2 | 6.5±0.3 | 6.9±0.3 | 6.9±0.3 |
| Drug + 30 mins. | 7.1±0.2 | 7.6±0.2 | 7.7±0.3 | 7.3±0.4 | 8.1±0.5 | 8.1±0.6 | 10.2±0.4* | 9.1±0.4* |
| MABP (mm Hg) | | | | | | | | |
| Pre-Drug | 120±4 | 110±3 | 118±5 | 122±2 | 117±1 | 115±1 | 115±4 | 129±1 |
| Drug + 1 min. | 116±3 | 140±3* | 154±6* | 159±6* | 104±3 | 101±6 | 127±3 | 130±8 |
| Drug + 30 mins. | 120±4 | 112±2 | 119±3 | 118±4 | 117±2 | 109±3 | 127±2 | 124±4 |

Data are presented as mean ± SEM.

*$p < 0.05$ for the comparison with saline treated control animals.

Mean arterial blood pressure (MABP).

## GLUCOSE UTILISATION IN CEREBRAL CORTEX.

| | SALINE | SKF 38393 | | | LY 171555 | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 mg/kg | 10 mg/kg | 30 mg/kg | 0.1 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
| Parietal Cortex | 89 ± 2 | 104 ± 3 | 81 ± 6 | 83 ± 3 | 89 ± 2 | 91 ± 5 | 82 ± 5 | 74 ± 3 |
| Sensory Motor Cortex | 94 ± 2 | 109 ± 2* | 91 ± 4 | 90 ± 3 | 101 ± 6 | 145 ± 8* | 131 ± 7* | 112 ± 9 |
| Frontal Cortex (Area 8) | 89 ± 2 | 103 ± 4* | 87 ± 5 | 93 ± 3 | 95 ± 6 | 122 ± 10* | 114 ± 3 | 99 ± 7 |
| Frontal Cortex (Area 10) | 86 ± 3 | 99 ± 4 | 82 ± 5 | 86 ± 2 | 86 ± 3 | 98 ± 4 | 84 ± 3 | 71 ± 4 |
| Anterior Cingulate Cortex | 95 ± 4 | 105 ± 4 | 79 ± 4* | 74 ± 2* | 88 ± 3 | 91 ± 3 | 80 ± 4 | 64 ± 2* |
| Medial Prefrontal Cortex | 98 ± 5 | 106 ± 3 | 91 ± 4 | 88 ± 4 | 90 ± 4 | 99 ± 4 | 81 ± 7 | 67 ± 2* |
| Pyriform Cortex | 97 ± 4 | 113 ± 6 | 91 ± 4 | 97 ± 4 | 92 ± 4 | 103 ± 4 | 86 ± 3 | 72 ± 3* |

[+]Measurements were carried out on cortical layer IV.

Data are presented as mean glucose use ( $\mu$moles.$100g^{-1}min^{-1}$) $\pm$ SEM.

*$p < 0.05$ for the comparison with saline treated control values.

TABLE 2:3

GLUCOSE UTILISATION IN AUDITORY AND VISUAL STRUCTURES

| | SALINE | SKF 38393 | | | LY 171555 | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 mg/kg | 10 mg/kg | 30 mg/kg | 0.1 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
| Visual Cortex | 96 ± 5 | 106 ± 3 | 86 ± 5 | 91 ± 2 | 98 ± 2 | 107 ± 7 | 94 ± 4 | 79 ± 2 |
| Superior Colliculus | | | | | | | | |
| Superficiale | 71 ± 2 | 91 ± 2* | 64 ± 2 | 71 ± 2 | 72 ± 2 | 83 ± 3 | 66 ± 3 | 68 ± 5 |
| Profundum (Medial) | 68 ± 3 | 80 ± 2 | 68 ± 4 | 76 ± 4 | 72 ± 2 | 73 ± 2 | 75 ± 6 | 59 ± 1 |
| Lateral Geniculate Body | 72 ± 3 | 87 ± 4* | 67 ± 2 | 75 ± 2 | 71 ± 3 | 88 ± 2* | 74 ± 3 | 61 ± 2 |
| Pretectal Area | 82 ± 5 | 98 ± 4 | 79 ± 4 | 82 ± 2 | 85 ± 3 | 107 ± 4* | 93 ± 5 | 77 ± 6 |
| Auditory Cortex | 124 ± 7 | 141 ± 4 | 123 ± 4 | 123 ± 6 | 125 ± 4 | 146 ± 8 | 123 ± 5 | 109 ± 1 |
| Inferior Colliculus | 166 ± 7 | 181 ± 10 | 151 ± 3 | 137 ± 10 | 165 ± 8 | 167 ± 9 | 138 ± 8 | 132 ± 6 |
| Medial Geniculate Body | 93 ± 2 | 108 ± 4 | 87 ± 4 | 91 ± 6 | 92 ± 3 | 97 ± 2 | 87 ± 2 | 78 ± 3* |
| Lateral Lemniscus | 93 ± 5 | 103 ± 7 | 92 ± 5 | 97 ± 8 | 99 ± 5 | 89 ± 5 | 80 ± 5 | 74 ± 4 |
| Superior Olivary Body | 116 ± 3 | 137 ± 7 | 118 ± 8 | 116 ± 7 | 126 ± 5 | 109 ± 5 | 117 ± 6 | 100 ± 5 |
| Cochlear Nucleus | 117 ± 7 | 148 ± 8* | 119 ± 6 | 117 ± 7 | 130 ± 5 | 132 ± 6 | 124 ± 3 | 98 ± 4 |

Data are presented as mean glucose use ( $\mu$moles.100g$^{-1}$min$^{-1}$) $\pm$ SEM.

*p<0.05 for the comparison with saline treated control values.

TABLE 2:4

GLUCOSE UTILISATION IN EXTRAPYRAMIDAL AND SUBCORTICAL MOTOR AREAS

| | SALINE | SKF 38393 | | | LY 171555 | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 mg/kg | 10 mg/kg | 30 mg/kg | 0.1 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
| Trigeminal Nucleus | 50 ± 2 | 56 ± 1 | 56 ± 1 | 59 ± 2* | 56 ± 3 | 82 ± 4* | 81 ± 5* | 66 ± 6* |
| Cuneate Nucleus | 50 ± 2 | 55 ± 3 | 55 ± 3 | 58 ± 2* | 52 ± 4 | 72 ± 2* | 66 ± 3* | 55 ± 3 |
| Inferior Olivary Body | 61 ± 3 | 76 ± 2* | 64 ± 3 | 71 ± 4* | 64 ± 3 | 77 ± 2* | 80 ± 4* | 65 ± 1 |
| Cerebellar Nucleus | 80 ± 3 | 92 ± 3 | 76 ± 2 | 82 ± 3 | 79 ± 3 | 95 ± 1* | 91 ± 3* | 79 ± 2 |
| Vestibular Nucleus | 97 ± 3 | 109 ± 3 | 98 ± 4 | 103 ± 4 | 98 ± 4 | 112 ± 3 | 103 ± 3 | 93 ± 2 |
| Pontine Nucleus | 52 ± 4 | 59 ± 1 | 48 ± 3 | 49 ± 2 | 54 ± 3 | 65 ± 4 | 60 ± 3 | 48 ± 2 |
| Red Nucleus | 59 ± 3 | 70 ± 2 | 60 ± 4 | 63 ± 4 | 66 ± 2 | 82 ± 3* | 76 ± 3 | 64 ± 3 |
| Substantia Nigra Pars Compacta | 53 ± 2 | 56 ± 2 | 55 ± 4 | 54 ± 2 | 51 ± 2 | 62 ± 3 | 54 ± 2 | 51 ± 2 |
| Substantia Nigra Pars Reticulata | 42 ± 1 | 46 ± 1 | 44 ± 2 | 49 ± 3 | 46 ± 2 | 56 ± 4* | 55 ± 2* | 41 ± 2 |
| Subthalamic Nucleus | 69 ± 2 | 73 ± 2 | 65 ± 3 | 71 ± 2 | 80 ± 2 | 111 ± 4* | 100 ± 4* | 81 ± 3 |
| Zona Incerta | 63 ± 2 | 72 ± 2 | 59 ± 3 | 72 ± 2 | 71 ± 2 | 99 ± 4* | 93 ± 6* | 78 ± 4 |
| Globus Pallidus | 44 ± 1 | 49 ± 2 | 45 ± 2 | 46 ± 1 | 48 ± 2 | 57 ± 2* | 52 ± 3 | 43 ± 1 |
| Caudate Nucleus – Dorsal | 81 ± 3 | 91 ± 2 | 78 ± 2 | 82 ± 4 | 74 ± 4 | 85 ± 3 | 73 ± 5 | 62 ± 4* |
| Caudate Nucleus – Ventral | 75 ± 3 | 83 ± 3 | 70 ± 2 | 79 ± 5 | 76 ± 3 | 89 ± 5* | 82 ± 4 | 72 ± 4 |

Data are presented as mean glucose use ($\mu$moles.100g$^{-1}$min$^{-1}$) ± SEM.

*$p < 0.05$ for the comparison with saline treated control values.

GLUCOSE UTILISATION IN LIMBIC AND RELATED AREAS

| | SALINE | SKF 38393 | | | LY 171555 | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 mg/kg | 10 mg/kg | 30 mg/kg | 0.1 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
| Pontine Reticular Formation | 47 ± 1 | 52 ± 1 | 48 ± 2 | 55 ± 3 | 51 ± 2 | 57 ± 2* | 53 ± 1 | 45 ± 1 |
| Median Raphe Nucleus | 78 ± 2 | 85 ± 1 | 75 ± 3 | 79 ± 5 | 69 ± 2 | 73 ± 3 | 68 ± 2 | 57 ± 2* |
| Dorsal Raphe Nucleus | 72 ± 2 | 84 ± 2 | 73 ± 3 | 71 ± 4 | 70 ± 1 | 73 ± 1 | 67 ± 4 | 58 ± 3* |
| Interpeduncular Nucleus | 90 ± 4 | 92 ± 3 | 86 ± 4 | 85 ± 4 | 89 ± 3 | 87 ± 4 | 80 ± 3 | 71 ± 3* |
| Dorsal Tegmental Nucleus | 85 ± 4 | 97 ± 4 | 79 ± 4 | 87 ± 4 | 83 ± 4 | 89 ± 4 | 76 ± 1 | 61 ± 4* |
| Ventral Tegmental Area | 48 ± 1 | 53 ± 4 | 49 ± 3 | 51 ± 1 | 44 ± 2 | 56 ± 3 | 47 ± 1 | 46 ± 4 |
| Hippocampus Molecular Layer | 67 ± 2 | 70 ± 2 | 64 ± 4 | 58 ± 3 | 59 ± 2 | 61 ± 4 | 57 ± 3* | 54 ± 1* |
| Dentate Gyrus | 56 ± 2 | 58 ± 2 | 50 ± 3 | 49 ± 1 | 51 ± 1 | 53 ± 2 | 51 ± 2 | 43 ± 1* |
| Mammillary Body | 86 ± 5 | 92 ± 3 | 78 ± 1 | 89 ± 5 | 86 ± 4 | 94 ± 5 | 78 ± 4 | 71 ± 4* |
| Hypothalamus | 39 ± 2 | 41 ± 2 | 39 ± 2 | 42 ± 2 | 38 ± 1 | 39 ± 1 | 39 ± 1 | 34 ± 1 |
| Amygdala – Basolateral Nucleus | 63 ± 3 | 66 ± 2 | 62 ± 4 | 58 ± 3 | 62 ± 2 | 70 ± 3 | 72 ± 3 | 64 ± 2 |
| Amygdala – Lateral Nucleus | 39 ± 1 | 41 ± 1 | 38 ± 2 | 42 ± 1 | 36 ± 1 | 38 ± 1 | 38 ± 1 | 33 ± 1 |
| Lateral Septal Nucleus | 42 ± 1 | 45 ± 2 | 37 ± 2 | 44 ± 3 | 43 ± 2 | 57 ± 4* | 48 ± 3 | 45 ± 1 |
| Nucleus Accumbens | 64 ± 2 | 77 ± 3* | 64 ± 3 | 63 ± 2 | 69 ± 3 | 78 ± 3* | 66 ± 3 | 59 ± 1 |

Data are presented as mean glucose use ( $\mu$moles.$100g^{-1}min^{-1}$) ± SEM.

*p<0.05 for the comparison with saline treated control values.

GLUCOSE UTILISATION IN DIENCEPHALON

| | SALINE | SKF 38393 | | | LY 171555 | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 mg/kg | 10 mg/kg | 30 mg/kg | 0.1 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
| Lateral Habenula | 93 ± 3 | 105 ± 4 | 89 ± 4 | 99 ± 6 | 92 ± 3 | 92 ± 3 | 89 ± 5 | 77 ± 3 |
| **Thalamus** | | | | | | | | |
| Parafascicular Nucleus | 71 ± 1 | 79 ± 1 | 74 ± 5 | 79 ± 4 | 76 ± 4 | 97 ± 3* | 91 ± 5* | 79 ± 3 |
| Mediodorsal Nucleus | 91 ± 4 | 108 ± 5 | 88 ± 4 | 95 ± 1 | 88 ± 4 | 103 ± 7 | 87 ± 7 | 80 ± 5 |
| Posteriolateral Nucleus | 76 ± 2 | 80 ± 3 | 72 ± 3 | 77 ± 4 | 69 ± 2 | 89 ± 6 | 87 ± 9 | 62 ± 3 |
| Posterior Nucleus | 72 ± 2 | 81 ± 3 | 69 ± 3 | 80 ± 5 | 74 ± 3 | 94 ± 2* | 94 ± 7* | 74 ± 5 |
| Posterioventromedial Nucleus | 70 ± 3 | 80 ± 2 | 73 ± 3 | 86 ± 1* | 73 ± 5 | 96 ± 5* | 96 ± 6* | 71 ± 4 |
| Ventromedial Nucleus | 91 ± 5 | 102 ± 4 | 89 ± 4 | 97 ± 3 | 89 ± 5 | 113 ± 4* | 98 ± 3 | 87 ± 4 |
| Ventrolateral Nucleus | 69 ± 2 | 88 ± 2* | 69 ± 2 | 83 ± 1* | 76 ± 4 | 100 ± 2* | 91 ± 3* | 76 ± 5 |
| Anteriomedial Nucleus | 102 ± 6 | 102 ± 2 | 74 ± 4* | 76 ± 2* | 83 ± 4 | 103 ± 3 | 84 ± 4 | 78 ± 5* |

Data are presented as mean glucose use ( $\mu$moles.100g$^{-1}$min$^{-1}$) $\pm$ SEM.

*p<0.05 for the comparison with saline treated control values.

TABLE 2:7

GLUCOSE UTILISATION IN MYELINATED FIBRE TRACTS

| | SALINE | SKF 38393 | | | LY 171555 | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 mg/kg | 10 mg/kg | 30 mg/kg | 0.1 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
| Cerebellar White Matter | 25 ± 1 | 27 ± 1 | 25 ± 1 | 28 ± 2 | 26 ± 1 | 30 ± 2 | 30 ± 1 | 26 ± 1 |
| Internal Capsule | 24 ± 1 | 26 ± 2 | 22 ± 1 | 26 ± 1 | 22 ± 1 | 24 ± 2 | 26 ± 1 | 22 ± 1 |
| Body of Corpus Callosum | 30 ± 1 | 31 ± 2 | 26 ± 2 | 29 ± 2 | 28 ± 1 | 32 ± 1 | 29 ± 1 | 27 ± 2 |
| Genu of Corpus Callosum | 26 ± 1 | 27 ± 2 | 23 ± 1 | 26 ± 2 | 24 ± 1 | 28 ± 1 | 26 ± 1 | 24 ± 1 |

Data are presented as mean glucose use ( $\mu$moles.100g$^{-1}$min$^{-1}$) ± SEM.

*p<0.05 for the comparison with saline treated control values.

Local Rates of Glucose Utilisation Following the Administration

of

SCH 23390.

Table 3:1

Cardiovascular and respiratory parameters for each treatment group.

Table 3:2 - 3:7

Local cerebral glucose utilisation was measured in conscious rats following the intravenous injection of saline (n=5) or SCH 23390 at concentrations of 0.05 mg/kg (n=3), 0.1 mg/kg (n=5) and 1 mg/kg (n=4). In a further four animals the effects of SCH 23390 (0.05 mg/kg) pretreatment on the glucose use responses elicited by LY 171555 (0.5 mg/kg) was examined.

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) $\pm$ SEM.

*$p < 0.05$.

Cardiovascular and Respiratory Variables

| Physiological Variable | Vehicle | SCH 23390 | | | LY 171555 0.5 mg/kg | LY 171555 0.5 mg/kg + SCH 23390 0.05 mg/kg |
|---|---|---|---|---|---|---|
| | | 0.05 mg/kg | 0.1 mg/kg | 1 mg/kg | | |
| Rectal Temperature (°C) | 36.9 ± 0.1 | 37.3 ± 0.4 | 37.3 ± 0.2 | 37.0 ± 0.1 | 37.0 ± 0.1 | 36.5 ± 0.2 |
| Arterial pCO$_2$ (mm Hg) | 37.5 ± 0.1 | 36.0 ± 1.4 | 38.5 ± 0.6 | 37.5 ± 1.2 | 38.7 ± 1.7 | 38.6 ± 2.0 |
| pO$_2$ " | 89 ± 3 | 90 ± 4 | 87 ± 1 | 97 ± 3 | 99 ± 4 | 101 ± 3 |
| pH | 7.41 ± 0.01 | 7.41 ± 0.01 | 7.41 ± 0.01 | 7.41 ± 0.01 | 7.41 ± 0.01 | 7.40 ± 0.0 |
| HCO$_3$$^-$ (mM) | 23.6 ± 0.4 | 23.3 ± 1.0 | 24.3 ± 0.3 | 23.6 ± 0.5 | 24.5 ± 0.9 | 23.8 ± 1.0 |
| Plasma Glucose (mM) | | | | | | |
| Pre- Drug | 6.3 ± 0.2 | 6.6 ± 0.1 | 5.7 ± 0.4 | 6.6 ± 0.2 | 6.5 ± 0.3 | 6.7 ± 0.4 |
| Drug + 40 min | 6.7 ± 0.5 | 6.9 ± 0.2 | 7.1 ± 0.5 | 8.5 ± 0.5* | 8.1 ± 0.6 | 8.4 ± 0.9 |
| MABP (mm Hg) | | | | | | |
| Pre- Drug | 120 ± 8 | 120 ± 3 | 116 ± 2 | 120 ± 4 | 115 ± 1 | 120 ± 5 |
| Drug + 40 min | 115 ± 3 | 125 ± 3 | 120 ± 3 | 118 ± 1 | 109 ± 3 | 115 ± 3 |

Data are presented as mean ± SEM.

*p<0.05 for the comparison with saline treated control values.

Mean arterial blood pressure (MABP).

TABLE 3:2

Glucose Utilisation in Cerebral Cortex

| | SALINE | SCH 23390 | | | LY 171555 0.5 mg/kg | LY 171555 0.5 mg/kg + SCH 23390 0.05 mg/kg |
|---|---|---|---|---|---|---|
| | | 0.05 mg/kg | 0.1 mg/kg | 1 mg/kg | | |
| Parietal Cortex[+] | 105 ± 5 | 104 ± 6 | 101 ± 4 | 92 ± 5 | 91 ± 5 | 90 ± 5 |
| Sensory Motor Cortex[+] | 112 ± 4 | 108 ± 6 | 106 ± 3 | 102 ± 2 | 145 ± 8 $^\mathsf{¢}$ | 87 ± 5°°° |
| Frontal Cortex (Area 8)[+] | 112 ± 5 | 106 ± 7 | 107 ± 3 | 100 ± 4 | 122 ± 10 $^\mathsf{¢}$ | 83 ± 4° |
| Frontal Cortex (Area 10)[+] | 97 ± 6 | 99 ± 6 | 91 ± 2 | 90 ± 2 | 98 ± 4 | 77 ± 4°° |
| Anterior Cingulate Cortex | 105 ± 4 | 106 ± 6 | 103 ± 4 | 110 ± 6 | 91 ± 3 | 74 ± 4° |
| Medial Prefrontal Cortex | 121 ± 3 | 114 ± 7 | 114 ± 3 | 116 ± 5 | 99 ± 4 | 83 ± 3° |
| Pyriform Cortex | 111 ± 4 | 99 ± 4 | 103 ± 2 | 102 ± 5 | 103 ± 4 | 88 ± 2° |

[+]Measurements were carried out on cortical layer IV.

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) ± SEM.

$^\mathsf{¢}$ p<0.05 for the comparison with respective control values.

*p<0.05 for the comparison with saline treated animals.

°p<0.05, °°p<0.01, °°°p<0.001 for the comparison with LY 171555 treatment values.

Glucose Utilisation in Auditory and Visual Structures

| | SALINE | SCH 23390 | | | LY 171555 0.5 mg/kg | LY 171555 0.5 mg/kg + SCH 23390 0.05 mg/kg |
| --- | --- | --- | --- | --- | --- | --- |
| | | 0.05 mg/kg | 0.1 mg/kg | 1 mg/kg | | |
| Visual Cortex | 114 ± 3 | 115 ± 9 | 110 ± 3 | 92 ± 5* | 107 ± 7 | 100 ± 6 |
| Superior Colliculus Superficiale | 86 ± 3 | 77 ± 3 | 85 ± 3 | 72 ± 2* | 83 ± 3 | 73 ± 4 |
| Superior Colliculus Profundum | 86 ± 5 | 79 ± 2 | 77 ± 1 | 70 ± 3 | 73 ± 2 | 74 ± 6 |
| Lateral Geniculate Body | 85 ± 4 | 86 ± 6 | 89 ± 3 | 84 ± 3 | 88 ± 2¢ | 72 ± 3 |
| Pretectal Area | 98 ± 6 | 92 ± 6 | 92 ± 1 | 86 ± 4 | 107 ± 4¢ | 92 ± 7 |
| Auditory Cortex | 146 ± 7 | 137 ± 5 | 140 ± 4 | 136 ± 6 | 146 ± 8 | 120 ± 7 |
| Inferior Colliculus | 164 ± 4 | 185 ± 13 | 179 ± 1 | 185 ± 4 | 167 ± 9 | 172 ± 7 |
| Medial Geniculate Body | 113 ± 5 | 105 ± 6 | 107 ± 3 | 90 ± 3* | 97 ± 2 | 91 ± 3 |
| Lateral Lemniscus | 111 ± 4 | 104 ± 9 | 95 ± 4 | 92 ± 5 | 89 ± 5 | 95 ± 4 |
| Superior Olivary Body | 138 ± 3 | 141 ± 12 | 140 ± 2 | 129 ± 7 | 109 ± 5 | 132 ± 7° |
| Cochlear Nucleus | 152 ± 4 | 161 ± 11 | 151 ± 3 | 137 ± 4 | 132 ± 6 | 137 ± 4 |

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) ± SEM.

¢ $p < 0.05$ for the comparison with respective control values.

*$p < 0.05$ for the comparison with saline treated animals.

°$p < 0.05$, °°$p < 0.01$, °°°$p < 0.001$ for the comparison with LY 171555 treatment values.

**TABLE 3:4**

Glucose Utilisation in Extrapyramidal and Subcortical Motor Areas

| | SALINE | SCH 23390 | | | LY 171555 0.5 mg/kg | LY 171555 0.5 mg/kg + SCH 23390 0.05 mg/kg |
|---|---|---|---|---|---|---|
| | | 0.05 mg/kg | 0.1 mg/kg | 1 mg/kg | | |
| Trigeminal Nucleus | 53 ± 2 | 53 ± 4 | 49 ± 1 | 49 ± 1 | 82 ± 4¢ | 52 ± 3ᵒᵒᵒ |
| Cuneate Nucleus | 61 ± 2 | 55 ± 1 | 55 ± 2 | 59 ± 2 | 72 ± 2¢ | 58 ± 2ᵒᵒ |
| Inferior Olivary Body | 72 ± 1 | 72 ± 1 | 70 ± 3 | 71 ± 2 | 77 ± 2¢ | 71 ± 3 |
| Cerebellar Nucleus | 94 ± 2 | 94 ± 6 | 93 ± 3 | 86 ± 4 | 95 ± 1¢ | 87 ± 3ᵒ |
| Vestibular Nucleus | 111 ± 2 | 115 ± 5 | 110 ± 2 | 107 ± 4 | 112 ± 3 | 100 ± 3 |
| Pontine Nucleus | 60 ± 3 | 54 ± 3 | 54 ± 2 | 54 ± 4 | 65 ± 4¢ | 52 ± 2ᵒ |
| Red Nucleus | 74 ± 3 | 73 ± 7 | 64 ± 2 | 68 ± 4 | 82 ± 3¢ | 64 ± 2ᵒᵒ |
| Substantia Nigra Pars Compacta | 64 ± 1 | 62 ± 1 | 61 ± 3 | 60 ± 2 | 62 ± 3 | 47 ± 3ᵒᵒ |
| Substantia Nigra Pars Reticulata | 49 ± 1 | 47 ± 3 | 43 ± 1* | 42 ± 1* | 56 ± 4¢ | 41 ± 1ᵒ |
| Subthalamic Nucleus | 77 ± 3 | 75 ± 4 | 72 ± 2 | 71 ± 4 | 111 ± 4¢ | 75 ± 4ᵒᵒᵒ |
| Zona Incerta | 74 ± 1 | 73 ± 6 | 71 ± 2 | 72 ± 2 | 99 ± 4¢ | 70 ± 4ᵒᵒᵒ |
| Globus Pallidus | 54 ± 3 | 50 ± 2 | 50 ± 2 | 46 ± 1 | 57 ± 2¢ | 43 ± 3ᵒ |
| Caudate Nucleus Dorsal | 94 ± 4 | 93 ± 6 | 94 ± 4 | 81 ± 3 | 85 ± 3 | 75 ± 6 |
| Caudate Nucleus Ventral | 89 ± 4 | 87 ± 3 | 90 ± 3 | 76 ± 2 | 89 ± 5¢ | 69 ± 6ᵒ |

Data are presented as mean glucose use ( $umol.100g^{-1}min^{-1}$ ) $\pm$ SEM.

¢ $p < 0.05$ for the comparison with respective control values.

* $p < 0.05$ for the comparison with saline treated animals.

ᵒ $p < 0.05$, ᵒᵒ $p < 0.01$, ᵒᵒᵒ $p < 0.001$ for the comparison with LY 171555 treatment values.

TABLE 3:5

Glucose Utilisation in Limbic and Related Areas

| | SALINE | SCH 23390 | | | LY 171555 0.5 mg/kg | LY 171555 0.5 mg/kg + SCH 23390 0.05 mg/kg |
|---|---|---|---|---|---|---|
| | | 0.05 mg/kg | 0.1 mg/kg | 1 mg/kg | | |
| Pontine Reticular Formation | 56 ± 1 | 55 ± 3 | 52 ± 1 | 52 ± 3 | 57 ± 2 | 53 ± 3 |
| Median Raphe Nucleus | 88 ± 3 | 92 ± 3 | 88 ± 2 | 81 ± 3 | 73 ± 3 | 74 ± 5 |
| Dorsal Raphe Nucles | 84 ± 4 | 85 ± 3 | 82 ± 1 | 75 ± 5 | 73 ± 1 | 72 ± 5 |
| Interpeduncular Nucleus | 100 ± 3 | 102 ± 1 | 101 ± 5 | 92 ± 3 | 87 ± 4 | 76 ± 1 |
| Dorsal Tegmental Nucleus | 104 ± 4 | 101 ± 9 | 91 ± 4 | 85 ± 4 | 89 ± 4 | 81 ± 4 |
| Ventral Tegmental Area | 54 ± 2 | 52 ± 4 | 60 ± 1 | 55 ± 3 | 56 ± 3 | 42 ± 2$^{o}$ |
| Hippocampus Molecular Layer | 72 ± 4 | 78 ± 3 | 76 ± 1 | 72 ± 3 | 61 ± 4 | 60 ± 3 |
| Hippocampus Dentate Gyrus | 66 ± 2 | 66 ± 3 | 64 ± 2 | 64 ± 2 | 53 ± 2 | 50 ± 2 |
| Mammillary Body | 98 ± 6 | 101 ± 6 | 99 ± 3 | 95 ± 3 | 94 ± 5 | 85 ± 6 |
| Hypothalamus | 43 ± 1 | 43 ± 2 | 52 ± 3 | 48 ± 3 | 39 ± 1 | 34 ± 1$^{o}$ |
| Amygdala Basolateral Nucleus | 72 ± 3 | 77 ± 4 | 81 ± 3 | 69 ± 1 | 70 ± 3 | 64 ± 4 |
| Amygdala Lateral Nucleus | 45 ± 1 | 45 ± 1 | 44 ± 2 | 42 ± 3 | 38 ± 1 | 36 ± 1 |
| Lateral Septal Nucleus | 49 ± 1 | 50 ± 2 | 51 ± 2 | 54 ± 1 | 57 ± 4[¢] | 44 ± 3 |
| Nucleus Accumbens | 70 ± 1 | 79 ± 3 | 95 ± 3* | 91 ± 4* | 78 ± 3[¢] | 59 ± 3$^{oo}$ |

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) $\pm$ SEM.

[¢] $p < 0.05$ for the comparison with respective control values.

*$p < 0.05$ for the comparison with saline treated animals.

$^{o}p < 0.05$, $^{oo}p < 0.01$, $^{ooo}p < 0.001$ for the comparison with LY 171555 treatment values.

TABLE 3:6

Glucose Utilisation in Diencephalon

| | SALINE | SCH 23390 | | | LY 171555 0.5 mg/kg | LY 171555 0.5 mg/kg + SCH 23390 0.05 mg/kg |
| --- | --- | --- | --- | --- | --- | --- |
| | | 0.05 mg/kg | 0.1 mg/kg | 1 mg/kg | | |
| Lateral Habenula | 108 ± 3 | 128 ± 11 | 134 ± 5* | 121 ± 6 | 92 ± 3 | 113 ± 8° |
| Thalamus | | | | | | |
| Parafascicular Nucleus | 86 ± 3 | 81 ± 4 | 74 ± 3 | 73 ± 3 | 97 ± 3¢ | 75 ± 5°° |
| Mediodorsal Nucleus | 105 ± 5 | 102 ± 4 | 102 ± 4 | 100 ± 6 | 103 ± 7 | 95 ± 9 |
| Posteriolateral Nucleus | 97 ± 4 | 103 ± 8 | 101 ± 4 | 83 ± 4 | 89 ± 6 | 71 ± 4 |
| Posterior Nucleus | 89 ± 2 | 83 ± 5 | 81 ± 2 | 77 ± 3* | 94 ± 2¢ | 73 ± 4°°° |
| Posterioventromedial Nucleus | 88 ± 2 | 78 ± 4 | 77 ± 2* | 74 ± 3* | 96 ± 5¢ | 65 ± 3°° |
| Ventromedial Nucleus | 107 ± 6 | 101 ± 6 | 105 ± 3 | 86 ± 1* | 113 ± 4¢ | 93 ± 7 |
| Ventrolateral Nucleus | 86 ± 3 | 75 ± 5 | 79 ± 2 | 72 ± 2 | 100 ± 2¢ | 66 ± 3°°° |
| Anteromedial Nucleus | 114 ± 4 | 110 ± 7 | 108 ± 4 | 102 ± 6 | 103 ± 3 | 82 ± 3° |

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) ± SEM.

¢p<0.05 for the comparison with respective control values.

*p<0.05 for the comparison with saline treated animals.

°p<0.05, °°p<0.01, °°°p<0.001 for the comparison with LY 171555 treatment values.

**TABLE 3:7**

Glucose Utlisation in Myelinated Fibre Tracts

| | SALINE | SCH 23390 | | | LY 171555 0.5 mg/kg | LY 171555 0.5 mg/kg + SCH 23390 0.05 mg/kg |
|---|---|---|---|---|---|---|
| | | 0.05 mg/kg | 0.1 mg/kg | 1 mg/kg | | |
| Cerebellar White Matter | 29 ± 2 | 32 ± 1 | 29 ± 1 | 28 ± 2 | 30 ± 2 | 26 ± 2 |
| Internal Capsule | 24 ± 1 | 27 ± 2 | 25 ± 1 | 24 ± 1 | 24 ± 2 | 21 ± 1 |
| Body of Corpus Callosum | 30 ± 1 | 35 ± 2 | 32 ± 1 | 30 ± 2 | 32 ± 1 | 22 ± 1[ooo] |
| Genu of Corpus Callosum | 29 ± 2 | 25 ± 3 | 27 ± 1 | 30 ± 2 | 28 ± 1 | 23 ± 1[o] |

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) ± SEM.

$p < 0.05$ for the comparison with respective control values.

*$p < 0.05$ for the comparison with saline treated animals.

[o]$p < 0.05$, [oo]$p < 0.01$, [ooo]$p < 0.001$ for the comparison with LY 171555 treatment values.

Local Rates of Glucose Utilisation Following the Administration

of

Apomorphine.

Glucose utilisation was measured in conscious rats following the intravenous injection of saline (n=6), or apomorphine at concentrations of 0.15 mg/kg (n=3), 0.5 mg/kg (n=4), 1.5 mg/kg (n=3) and 5 mg/kg (n=3).

Data are presented as mean glucose use $(\mu mol.100g^{-1}min^{-1})$ $\pm$ SEM.

$*p < 0.05$.

TABLE 4:1

Glucose Utilisation in Cerebral Cortex

| | SALINE | APOMORPHINE | | | |
|---|---|---|---|---|---|
| | | 0.15 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5.0 mg/kg |
| Parietal Cortex | 89 ± 4 | 86 ± 2 | 85 ± 9 | 79 ± 5 | 73 ± 3 |
| Sensory Motor Cortex | 95 ± 5 | 94 ± 12 | 121 ± 22 | 128 ± 7 | 120 ± 16 |
| Frontal Cortex (Area 8) | 95 ± 5 | 92 ± 6 | 105 ± 9 | 113 ± 3 | 113 ± 8 |
| Frontal Cortex (Area 10) | 87 ± 5 | 82 ± 2 | 87 ± 10 | 78 ± 7 | 79 ± 11 |
| Anterior Cingulate Cortex | 93 ± 4 | 75 ± 4 | 67 ± 7* | 64 ± 4* | 53 ± 3* |
| Medial Prefrontal Cortex | 94 ± 5 | 81 ± 5 | 73 ± 8 | 70 ± 2 | 61 ± 3* |
| Pyriform Cortex | 84 ± 5 | 91 ± 6 | 77 ± 7 | 98 ± 4 | 89 ± 3 |

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) $\pm$ SEM.

*$p < 0.05$ for the comparison with saline treated control values.

Glucose Utilisation in Auditory and Visual Structures

| | SALINE | APOMORPHINE | | | |
|---|---|---|---|---|---|
| | | 0.15 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5.0 mg/kg |
| Visual Cortex | 88 ± 6 | 91 ± 5 | 81 ± 7 | 80 ± 6 | 74 ± 5 |
| Superior Colliculus | | | | | |
| Superficial | 62 ± 3 | 81 ± 3 | 76 ± 10 | 76 ± 9 | 75 ± 6 |
| Profundum (Medial) | 56 ± 2 | 74 ± 1 | 63 ± 7 | 67 ± 5 | 72 ± 2 |
| Lateral Geniculate Body | 66 ± 4 | 82 ± 2 | 73 ± 8 | 69 ± 5 | 67 ± 5 |
| Pretectal Area | 78 ± 7 | 90 ± 6 | 83 ± 9 | 73 ± 4 | 75 ± 10 |
| Auditory Cortex | 109 ± 7 | 127 ± 6 | 117 ± 6 | 109 ± 6 | 117 ± 6 |
| Inferior Colliculus | 137 ± 8 | 140 ± 8 | 150 ± 7 | 131 ± 11 | 140 ± 6 |
| Medial Geniculate Body | 85 ± 7 | 94 ± 10 | 92 ± 8 | 89 ± 1 | 91 ± 1 |
| Lateral Lemniscus | 80 ± 5 | 79 ± 3 | 75 ± 2 | 87 ± 5 | 78 ± 8 |
| Superior Olivary Body | 106 ± 6 | 107 ± 6 | 100 ± 7 | 111 ± 2 | 109 ± 5 |
| Cochlear Nucleus | 120 ± 6 | 122 ± 4 | 126 ± 6 | 117 ± 2 | 109 ± 12 |

Data are presented as mean glucose use ( $\mu$mol.100g$^{-1}$min$^{-1}$) ± SEM.

*p<0.05 for the comparison with saline treated control values.

TABLE 4:3

Glucose Utilisation in Extrapyramidal and Subcortical Motor Areas

| | SALINE | APOMORPHINE | | | |
| --- | --- | --- | --- | --- | --- |
| | | 0.15 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5.0 mg/kg |
| Trigeminal Nucleus | 47 ± 2 | 63 ± 11 | 89 ± 15* | 102 ± 8* | 83 ± 5 |
| Cuneate Nucleus | 45 ± 2 | 62 ± 6 | 75 ± 9 | 75 ± 1* | 82 ± 7 |
| Inferior Olivary Body | 63 ± 2 | 69 ± 1 | 80 ± 3 | 86 ± 8* | 82 ± 7 |
| Cerebellar Nuclei | 75 ± 2 | 83 ± 7 | 93 ± 6 | 90 ± 3 | 87 ± 1 |
| Vestibular Nucleus | 94 ± 2 | 95 ± 3 | 98 ± 8 | 103 ± 4 | 93 ± 3 |
| Pontine Nucleus | 56 ± 2 | 64 ± 6 | 65 ± 3 | 64 ± 4 | 58 ± 5 |
| Red Nucleus | 55 ± 2 | 69 ± 6 | 77 ± 10 | 79 ± 4 | 71 ± 8 |
| Substantia Nigra Pars Compacta | 49 ± 3 | 61 ± 6 | 67 ± 10 | 70 ± 1* | 83 ± 3* |
| Substantia Nigra Pars Reticulata | 38 ± 2 | 58 ± 5 | 67 ± 8* | 84 ± 4* | 92 ± 8* |
| Subthalamic Nucleus | 65 ± 5 | 90 ± 7 | 109 ± 9* | 134 ± 10* | 128 ± 8* |
| Zona Incerta | 63 ± 4 | 81 ± 8 | 101 ± 11 | 108 ± 13* | 99 ± 9 |
| Globus Pallidus | 39 ± 3 | 48 ± 4 | 59 ± 6 | 58 ± 6 | 60 ± 8 |
| Caudate Nucleus - Dorsal | 79 ± 5 | 80 ± 10 | 78 ± 8 | 85 ± 5 | 85 ± 4 |
| Caudate Nucleus - Ventral | 71 ± 4 | 75 ± 5 | 78 ± 10 | 85 ± 2 | 66 ± 11 |

Data are presented as mean glucose use ($\mu$mol.100g$^{-1}$min$^{-1}$) ± SEM.

*$p < 0.05$ for the comparison with saline treated control values.

TABLE 4:4

Glucose Utilisation in Limbic and Related Areas

| | SALINE | APOMORPHINE | | | |
| --- | --- | --- | --- | --- | --- |
| | | 0.15 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5.0 mg/kg |
| Pontine Reticular Formation | 45 ± 2 | 50 ± 4 | 56 ± 2 | 51 ± 4 | 56 ± 5 |
| Median Raphe Nucleus | 72 ± 2 | 69 ± 3 | 71 ± 3 | 67 ± 3 | 69 ± 4 |
| Dorsal Raphe Nucleus | 67 ± 3 | 69 ± 3 | 70 ± 5 | 59 ± 7 | 71 ± 3 |
| Interpeduncular Nucleus | 77 ± 4 | 83 ± 8 | 58 ± 4 | 62 ± 9 | 59 ± 9 |
| Dorsal Tegmental Nucleus | 76 ± 2 | 78 ± 7 | 78 ± 4 | 72 ± 2 | 70 ± 5 |
| Ventral Tegmental Area | 43 ± 2 | 44 ± 3 | 55 ± 9 | 62 ± 1 | 67 ± 9 |
| Hippocampus Molecular Layer | 56 ± 2 | 62 ± 5 | 56 ± 6 | 47 ± 4 | 58 ± 10 |
| Hippocampus Dentate Gyrus | 43 ± 3 | 48 ± 4 | 40 ± 5 | 39 ± 1 | 41 ± 5 |
| Mammillary Body | 84 ± 5 | 82 ± 4 | 84 ± 8 | 62 ± 8 | 69 ± 11 |
| Hypothalamus | 35 ± 2 | 36 ± 2 | 44 ± 4 | 38 ± 3 | 37 ± 4 |
| Amygdala – Basolateral Nucleus | 34 ± 2 | 34 ± 4 | 37 ± 3 | 38 ± 4 | 36 ± 6 |
| Amygdala – Lateral Nucleus | 59 ± 5 | 63 ± 4 | 64 ± 5 | 62 ± 7 | 58 ± 10 |
| Lateral Septal Nucleus | 34 ± 3 | 40 ± 7 | 39 ± 5 | 41 ± 3 | 34 ± 2 |
| Nucleus Accumbens | 62 ± 3 | 67 ± 3 | 64 ± 5 | 58 ± 1 | 63 ± 11 |

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) $\pm$ SEM.

*p<0.05 for the comparison with saline treated control values.

Glucose Utilisation in Diencephalon

| | SALINE | APOMORPHINE | | | |
| --- | --- | --- | --- | --- | --- |
| | | 0.15 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5.0 mg/kg |
| Lateral Habenula | 86 ± 4 | 65 ± 6 | 60 ± 4* | 45 ± 6* | 43 ± 6* |
| Thalamus | | | | | |
| Parafascicular Nucleus | 63 ± 4 | 81 ± 6 | 89 ± 7* | 91 ± 2* | 88 ± 9 |
| Mediodorsal Nucleus | 82 ± 6 | 88 ± 7 | 83 ± 8 | 83 ± 8 | 80 ± 7 |
| Posteriolateral Nucleus | 63 ± 5 | 69 ± 2 | 60 ± 5 | 55 ± 2 | 57 ± 8 |
| Posterior Nucleus | 67 ± 5 | 77 ± 5 | 85 ± 12 | 76 ± 5 | 73 ± 12 |
| Posterioventromedial Nucleus | 64 ± 4 | 81 ± 6 | 86 ± 8 | 99 ± 7* | 86 ± 11 |
| Ventromedial Nucleus | 83 ± 5 | 90 ± 10 | 101 ± 3 | 105 ± 8 | 95 ± 1 |
| Ventrolateral Nucleus | 67 ± 6 | 74 ± 7 | 87 ± 15 | 96 ± 10 | 81 ± 10 |
| Anteromedial Nucleus | 91 ± 6 | 80 ± 3 | 71 ± 9 | 62 ± 8 | 70 ± 8 |

Data are presented as mean glucose use ($\mu mol.100g^{-1}min^{-1}$) ± SEM.

*$p < 0.05$ for the comparison with saline treated control values.

Glucose Utilisation in Myelinated Fibre Tracts

| | SALINE | APOMORPHINE | | | |
|---|---|---|---|---|---|
| | | 0.15 mg/kg | 0.5 mg/kg | 1.5 mg/kg | 5.0 mg/kg |
| Cerebellar White Matter | 24 ± 2 | 27 ± 4 | 28 ± 3 | 28 ± 2 | 26 ± 3 |
| Internal Capsule | 16 ± 2 | 15 ± 1 | 17 ± 2 | 13 ± 2 | 18 ± 4 |
| Body of Corpus Callosum | 18 ± 2 | 21 ± 3 | 19 ± 2 | 19 ± 2 | 17 ± 3 |
| Genu of Corpus Callosum | 17 ± 3 | 21 ± 4 | 20 ± 3 | 22 ± 3 | 18 ± 3 |

Data are presented as mean glucose use ( $\mu mol.100g^{-1}min^{-1}$ ) ± SEM.

*$p < 0.05$ for the comparison with saline treated control values.

# APPENDIX 5

Effects of SKF 38393 and LY 171555

on

Local Cerebral Blood Flow and Glucose Use.

## Table 5:1

Cardiovascular and respiratory status for the three groups of animals in which local cerebral blood flow was determined.

## Table 5:2 - 5:7

Blood flow and glucose use were measured in different groups of rates following the intravenous administration of saline, SKF 38393 (30 mg/kg) or LY 171555 (0.5 mg/kg).

The data are presented as mean blood flow $(ml.100g^{-1}min^{-1})$ ± SEM and mean glucose use ( $\mu mol.100g^{-1}min^{-1}$) ± SEM.

Statistical analysis was performed using Student´s t-test with Bonferroni correction for multiple comparisons.

*$p<0.05$.

Cardiovascular and Respiratory Variables

| Physiological Variable | Saline | SKF 38393 | LY 171555 |
|---|---|---|---|
| Rectal Temperature (°C) | 37.2 ± 0.1 | 37.0 ± 0.1 | 37.0 ± 0.1 |
| Arterial $PCO_2$ (mm Hg) | 39.2 ± 0.5 | 38.1 ± 0.6 | 38.7 ± 1.1 |
| $PO_2$ ( " ) | 87 ± 2 | 88 ± 1 | 91 ± 4 |
| pH | 7.405 ± 0.008 | 7.405 ± 0.007 | 7.419 ± 0.005 |
| $HCO_3^-$ (mM) | 24.4 ± 0.4 | 24.5 ± 0.5 | 25.1 ± 0.8 |
| **MABP** | | | |
| Pre-drug ( " ) | 117 ± 2 | 117 ± 3 | 109 ± 5 |
| Drug + 40 min ( " ) | 120 ± 3 | 117 ± 3 | 115 ± 4 |
| | n=5 | n=7 | n=6 |

Data are presented as mean ± SEM (n=5-7).

Statistical analysis, (Student's t-test with Bonferroni inequality for multiple comparison), failed to demonstrate significant differences between saline control and either drug treatment.

TABLE 5:2

Local Cerebral Blood Flow and Glucose Utilisation in Cerebral Cortex

| | BLOOD FLOW | | | GLUCOSE USE | | |
|---|---|---|---|---|---|---|
| | Saline | SKF 38393 | LY 171555 | Saline | SKF 38393 | LY 171555 |
| Parietal Cortex | 167 ± 3 | 146 ± 8 | 177 ± 11 | 89 ± 2 | 83 ± 3 | 91 ± 5 |
| Sensory Motor Cortex | 180 ± 2 | 200 ± 16 | 310 ± 30* | 94 ± 2 | 90 ± 3 | 145 ± 8*** |
| Frontal Cortex (Area 8) | 166 ± 5 | 181 ± 12 | 339 ± 32** | 89 ± 2 | 93 ± 3 | 122 ± 10* |
| Frontal Cortex (Area 10) | 157 ± 9 | 167 ± 12 | 189 ± 14 | 86 ± 3 | 86 ± 3 | 98 ± 4 |
| Anterior Cingulate Cortex | 174 ± 9 | 132 ± 7* | 156 ± 10 | 95 ± 4 | 74 ± 2* | 91 ± 3 |
| Medial Prefrontal Cortex | 172 ± 8 | 166 ± 11 | 155 ± 14 | 98 ± 5 | 88 ± 4 | 99 ± 4 |
| Pyriform Cortex | 146 ± 6 | 175 ± 9 | 164 ± 4 | 97 ± 4 | 97 ± 4 | 103 ± 4 |

Data are presented as mean ± SEM.

Blood flow is expressed in $ml.100g^{-1}min^{-1}$; glucose use in $\mu mol.100g^{-1}min^{-1}$.

Statistical analysis was performed using Student's t-test with Bonferroni correction for multiple comparisons.

*p<0.05,  **p<0.01,  ***p<0.001.

TABLE 5:3

Local Cerebral Blood Flow and Glucose Utilisation in Auditory and Visual Structures

|  | BLOOD FLOW | | | GLUCOSE USE | | |
|---|---|---|---|---|---|---|
|  | Saline | SKF 38393 | LY 171555 | Saline | SKF 38393 | LY 171555 |
| Visual Cortex | 143 ± 4 | 120 ± 4*** | 177 ± 10* | 96 ± 5 | 91 ± 2 | 107 ± 7 |
| Superior Colliculus: |  |  |  |  |  |  |
| Stratum - Superficiale | 132 ± 7 | 127 ± 7 | 149 ± 8 | 71 ± 2 | 71 ± 2 | 83 ± 3* |
| Stratum - Profundum | 139 ± 9 | 136 ± 9 | 141 ± 7 | 68 ± 3 | 76 ± 4 | 73 ± 2 |
| Lateral Geniculate Body | 138 ± 5 | 135 ± 5 | 164 ± 8 | 72 ± 3 | 75 ± 2 | 88 ± 2* |
| Pretectal Area | 174 ± 10 | 165 ± 4 | 200 ± 9 | 82 ± 5 | 82 ± 2 | 107 ± 4*** |
| Auditory Cortex | 281 ± 26 | 227 ± 15 | 260 ± 9 | 124 ± 7 | 123 ± 6 | 149 ± 8 |
| Inferior Colliculus | 303 ± 20 | 301 ± 10 | 363 ± 37 | 166 ± 7 | 137 ± 10 | 167 ± 9 |
| Medial Geniculate Body | 200 ± 9 | 182 ± 7 | 211 ± 11 | 93 ± 2 | 91 ± 6 | 97 ± 2 |
| Lateral Lemniscus | 179 ± 11 | 173 ± 13 | 195 ± 8 | 93 ± 5 | 97 ± 8 | 89 ± 5 |
| Superior Olivary Body | 203 ± 17 | 208 ± 15 | 200 ± 8 | 116 ± 3 | 116 ± 7 | 109 ± 5 |
| Cochlear Nucleus | 249 ± 11 | 215 ± 16 | 229 ± 11 | 117 ± 7 | 117 ± 7 | 132 ± 6 |

Data are presented as mean SEM.

Blood flow is expressed in $ml.100g^{-1}min^{-1}$; glucose use in $\mu mol.100g^{-1}min^{-1}$.
Statistical analysis was performed using Student's t-test with Bonferroni correction for multiple comparisons.
*p<0.05, **p<0.01, ***p<0.001.

TABLE 5:4

Local Cerebral Blood Flow and Glucose Utilisation in Extrapyramidal and Subcortical Motor Areas

| | BLOOD FLOW | | | GLUCOSE USE | | |
|---|---|---|---|---|---|---|
| | Saline | SKF 38393 | LY 171555 | Saline | SKF 38393 | LY 171555 |
| Trigeminal Nucleus | 121 ± 6 | 136 ± 8 | 217 ± 12*** | 50 ± 2 | 59 ± 2* | 82 ± 4*** |
| Cuneate Nucleus | 122 ± 7 | 123 ± 8 | 191 ± 7*** | 50 ± 2 | 58 ± 2* | 72 ± 2*** |
| Inferior Olivary Body | 145 ± 5 | 145 ± 8 | 186 ± 11* | 61 ± 3 | 71 ± 4* | 77 ± 2** |
| Cerebellar Nucleus | 208 ± 14 | 189 ± 10 | 246 ± 15 | 80 ± 3 | 82 ± 3 | 95 ± 1** |
| Vestibular Nucleus | 226 ± 18 | 200 ± 12 | 243 ± 10 | 97 ± 3 | 103 ± 4 | 112 ± 3* |
| Pontine Nucleus | 123 ± 5 | 113 ± 4 | 169 ± 7*** | 52 ± 4 | 49 ± 2 | 65 ± 4* |
| Red Nucleus | 128 ± 6 | 122 ± 8 | 144 ± 6 | 59 ± 3 | 63 ± 4 | 82 ± 3*** |
| Substantia Nigra Pars Compacta | 98 ± 1 | 90 ± 4 | 110 ± 6 | 53 ± 2 | 54 ± 2 | 62 ± 3 |
| Substantia Nigra Pars Reticulata | 96 ± 4 | 95 ± 8 | 113 ± 7 | 42 ± 1 | 49 ± 3 | 56 ± 4** |
| Subthalamic Nucleus | 134 ± 6 | 141 ± 5 | 205 ± 7*** | 69 ± 2 | 71 ± 2 | 111 ± 4*** |
| Zona Incerta | 134 ± 5 | 140 ± 5 | 195 ± 6*** | 63 ± 2 | 72 ± 2 | 99 ± 4*** |
| Globus Pallidus | 82 ± 6 | 82 ± 3 | 109 ± 7* | 44 ± 1 | 46 ± 1 | 57 ± 2*** |
| Caudate Nucleus - (Dorsolateral) | 142 ± 8 | 117 ± 7 | 157 ± 14 | 81 ± 3 | 82 ± 4 | 85 ± 3 |
| Caudate Nucleus - (Ventromedial) | 127 ± 6 | 105 ± 5* | 119 ± 9 | 75 ± 3 | 79 ± 5 | 89 ± 5* |

Data are presented as mean ± SEM.

Blood flow is expressed in ml.100g$^{-1}$min$^{-1}$; glucose use in μmol.100g$^{-1}$min$^{-1}$.

Statistical analysis was performed using Student's t-test with Bonferroni correction for multiple comparisons.

*p<0.05, **p<0.01, ***p<0.001.

<u>TABLE 5:5</u>

Local Cerebral Blood Flow and Glucose Utilisation in Limbic and Related Areas

| | BLOOD FLOW | | | GLUCOSE USE | | |
|---|---|---|---|---|---|---|
| | Saline | SKF 38393 | LY 171555 | Saline | SKF 38393 | LY 171555 |
| Pontine Reticular Formation | 104 ± 3 | 120 ± 9 | 126 ± 2*** | 47 ± 1 | 55 ± 3 | 57 ± 2* |
| Median Raphe Nucleus | 140 ± 10 | 133 ± 5 | 149 ± 2 | 78 ± 2 | 79 ± 5 | 73 ± 3 |
| Dorsal Raphe Nucleus | 152 ± 6 | 125 ± 5* | 164 ± 4 | 72 ± 2 | 71 ± 4 | 73 ± 1 |
| Interpenduncular Nucleus | 163 ± 12 | 141 ± 3 | 158 ± 6 | 90 ± 4 | 85 ± 4 | 87 ± 4 |
| Dorsal Tegmental Nucleus | 153 ± 4 | 136 ± 5 | 168 ± 6 | 85 ± 4 | 87 ± 4 | 89 ± 4 |
| Ventral Tegmental Area | 72 ± 12 | 74 ± 2 | 89 ± 7 | 48 ± 1 | 51 ± 1 | 56 ± 3 |
| Hippocampus Molecular Layer | 107 ± 5 | 102 ± 4 | 119 ± 3 | 67 ± 2 | 58 ± 3 | 61 ± 4 |
| Dentate Gyrus | 109 ± 5 | 99 ± 3 | 119 ± 4 | 56 ± 2 | 49 ± 1 | 53 ± 2 |
| Mammillary Body | 188 ± 13 | 145 ± 5* | 188 ± 6 | 86 ± 5 | 89 ± 5 | 94 ± 5 |
| Hypothalamus | 93 ± 2 | 99 ± 4 | 102 ± 4 | 39 ± 2 | 42 ± 2 | 39 ± 1 |
| Amygdala-Basolateral Nucleus | 104 ± 5 | 106 ± 2 | 135 ± 3*** | 63 ± 3 | 58 ± 3 | 70 ± 3 |
| Amygdala-Lateral Nucleus | 96 ± 4 | 95 ± 2 | 104 ± 3 | 39 ± 1 | 42 ± 1 | 38 ± 1 |
| Lateral Septal Nucleus | 103 ± 6 | 92 ± 5 | 107 ± 5 | 42 ± 1 | 44 ± 3 | 57 ± 4* |
| Nucleus Accumbens | 167 ± 5 | 132 ± 3*** | 152 ± 4 | 64 ± 3 | 63 ± 2 | 78 ± 3* |

Data are presented as mean ± SEM.

Blood flow is expressed in $ml.100g^{-1}min^{-1}$; glucose use in $\mu mol.100g^{-1}min^{-1}$.

Statistical analysis was performed using Student's t-test with Bonferroni correction for multiple comparisons.

*p<0.05, **p<0.01, ***p<0.001.

TABLE 5:6

Local Cerebral Blood Flow and Glucose Utilisation in Diencephalon

| | BLOOD FLOW | | | GLUCOSE USE | | |
|---|---|---|---|---|---|---|
| | Saline | SKF 38393 | LY 171555 | Saline | SKF 38393 | LY 171555 |
| Lateral Habenula | 192 ± 22 | 177 ± 21 | 210 ± 16 | 93 ± 3 | 99 ± 6 | 92 ± 3 |
| Thalamus | | | | | | |
| Parafascicular Nucleus | 139 ± 5 | 144 ± 6 | 212 ± 10*** | 71 ± 1 | 79 ± 4 | 97 ± 3*** |
| Mediodorsal Nucleus | 184 ± 18 | 173 ± 13 | 214 ± 12 | 91 ± 4 | 95 ± 1 | 103 ± 7 |
| Posteriolateral Nucleus | 149 ± 4 | 148 ± 11 | 182 ± 12 | 76 ± 2 | 77 ± 4 | 89 ± 6 |
| Posterior Nucleus | 136 ± 4 | 141 ± 5 | 192 ± 10*** | 72 ± 2 | 80 ± 5 | 94 ± 2*** |
| Posterioventromedial Nucleus | 123 ± 6 | 136 ± 6 | 225 ± 7*** | 70 ± 3 | 86 ± 1* | 96 ± 5** |
| Ventromedial Nucleus | 140 ± 5 | 140 ± 10 | 218 ± 18** | 91 ± 5 | 97 ± 3 | 113 ± 4* |
| Ventrolateral Nucleus | 130 ± 7 | 138 ± 17 | 218 ± 18** | 69 ± 2 | 83 ± 1* | 100 ± 2*** |
| Anteriomedial Nucleus | 201 ± 13 | 157 ± 8* | 203 ± 15 | 102 ± 6 | 76 ± 2* | 103 ± 3 |

Data are presented as mean ± SEM.

Blood flow is expressed in $ml.100g^{-1}min^{-1}$; glucose use in $\mu mol.100g^{-1}min^{-1}$.

Statistical analysis was performed using Student's t-test with Bonferroni correction for multiple comparisons.

*$p<0.05$, **$p<0.01$, ***$p<0.001$.

Local Cerebral Blood Flow and Glucose Utilisation in Myelinated Fibre Tracts

| | BLOOD FLOW | | | GLUCOSE USE | | |
|---|---|---|---|---|---|---|
| | Saline | SKF 38393 | LY 171555 | Saline | SKF 38393 | LY 171555 |
| Cerebellar White Matter | 45 ± 2 | 45 ± 2 | 59 ± 2* | 25 ± 1 | 28 ± 2 | 30 ± 2 |
| Internal Capsule | 65 ± 4 | 54 ± 4 | 64 ± 3 | 24 ± 1 | 26 ± 1 | 22 ± 2 |
| Body of Corpus Callosum | 45 ± 2 | 41 ± 3 | 52 ± 4 | 30 ± 1 | 29 ± 2 | 32 ± 1 |
| Genu of Corpus Callosum | 54 ± 1 | 46 ± 2* | 53 ± 2 | 26 ± 1 | 26 ± 2 | 28 ± 1 |

Data are presented as mean ± SEM.

Blood flow is expressed in $ml.100g^{-1}min^{-1}$; glucose use in $\mu mol.100g^{-1}min^{-1}$.

Statistical analysis was performed using Student's t-test with Bonferroni correction for multiple comparisons.

*p<0.05, **p<0.01, ***p<0.001.

TABLE 5:8

Local Cerebral Blood Flow and Local Cerebral Glucose Utilization Within Subregions of the Caudate Nucleus.

| | | BLOOD FLOW | | | GLUCOSE USE | | |
|---|---|---|---|---|---|---|---|
| | | Saline | SKF 38393 | LY 171555 | Saline | SKF 38393 | LY 171555 |
| Dorsal Caudate | Anterior DL | 151 ± 7 | 131 ± 7 | 169 ± 9 | 81 ± 2 | 86 ± 2 | 89 ± 3 |
| | Anterior DM | 151 ± 6 | 118 ± 4** | 124 ± 5* | 83 ± 3 | 85 ± 5 | 87 ± 3 |
| | Middle DL | 142 ± 8 | 117 ± 7 | 157 ± 14 | 81 ± 3 | 82 ± 4 | 85 ± 3 |
| | Middle DM | 147 ± 8 | 112 ± 6* | 133 ± 8 | 76 ± 3 | 79 ± 5 | 80 ± 3 |
| | Posterior D | 112 ± 7 | 102 ± 4 | 110 ± 8 | 66 ± 3 | 72 ± 4 | 68 ± 2 |
| Ventral Caudate | Anterior VL | 145 ± 4 | 126 ± 4* | 162 ± 11 | 75 ± 2 | 74 ± 3 | 77 ± 3 |
| | Anterior VM | 149 ± 4 | 124 ± 4** | 140 ± 4 | 78 ± 3 | 77 ± 4 | 83 ± 5 |
| | Middle VL | 150 ± 7 | 127 ± 4* | 235 ± 24* | 73 ± 3 | 75 ± 3 | 84 ± 6 |
| | Middle VM | 127 ± 6 | 105 ± 5* | 119 ± 9 | 75 ± 2 | 76 ± 3 | 89 ± 5* |
| | Posterior V | 141 ± 9 | 132 ± 6 | 218 ± 16** | 75 ± 2 | 75 ± 4 | 89 ± 7 |
| Mean | Anterior | 139 ± 2 | 123 ± 4* | 148 ± 7 | 78 ± 2 | 78 ± 3 | 83 ± 4 |
| | Medial | 139 ± 7 | 112 ± 5** | 151 ± 10 | 76 ± 3 | 76 ± 4 | 82 ± 4 |
| | Posterior | 121 ± 5 | 111 ± 4 | 149 ± 10 | 68 ± 2 | 70 ± 2 | 76 ± 3 |

Data are presented as mean ± SEM.

Blood flow is expressed in ml. $100g^{-1}min^{-1}$; glucose use in $\mu mol.100g^{-1}min^{-1}$. Statistical analysis was performed using Student's t-test with Bonferroni correction for multiple comparisons.

*$p<0.05$, **$p<0.01$.

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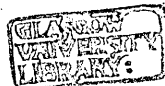ZUNTZ N (1897). Zur pathogenese und therapie dur durchrasche luftdruckanderungen erzeugten krankheiten.
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# PUBLICATIONS

## REVIEWS

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## PAPERS

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# ABSTRACTS

1.  McCULLOCH J, KIRKHAM D, MacPHERSON H, SHARKEY J (1983).
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