EEG AND BEHAVIORAL EFFECTS OF GAMMA-HYDROXYBUTYRATE IN THE RAT: A POTENTIAL MODEL OF ABSENCE EPILEPSY

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TER VERKRIJGING VAN DE GRAAD VAN
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Rachel en Natalya

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א-ל מארץ מוציא תרופות
וגבר מבין אל ימאס בם.

יהושוע בן-סירא ל"ח:ד

God laat geneesmiddelen
uit de aarde voortkomen
en een verstandig man
versmade ze niet.

Jozua Ben-Sira 38:4

Several sections of this thesis are presented as papers:

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M. Godschalk, M.R. Dzoljic and I.L. Bonta. Slow wave sleep and a state resembling absence epilepsy induced in the rat by γ-hydroxybutyrate. Eur. J. Pharmacol. 44 (1977) in press.

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CONTENTS

ABBREVIATIONS

AAA	acetazolamide
AMPT	alpha-methyl-para-tyrosine
AOAA	amino-oxyacetic acid
COMT	catechol-O-methyl transferase
DA	dopamine, -ergic
DOPAC	3,4-dihydroxy-phenylacetic acid
DPA	n-dipropylacetate
DPH	diphenylhydantoin
DPI	(3,4-dihydroxyphenylamino)-2-imidazoline
EEG	electroencephalogram, -graphic
EMG	electromyogram
ESI	ethosuximide
GABA	gamma-aminobutyric acid
GABA-T	GABA-transaminase
GAD	glutamic acid decarboxylase
GBL	gamma-butyrolactone
GHB	gamma-hydroxybutyrate (sodium salt)
HA-966	1-hydroxy-3-amino-pyrrolidone-2
i.p.	intraperitoneal
i.v.	intravenous
MAO	monoamine oxidase
MPH	mephenytoin
PHB	phenobarbital
PS	paradoxical sleep
PSI	phensuximide
SEM	standard error of the mean
SNC	Substantia nigra, zona compacta
SWS	slow-wave sleep
TMD	trimethadione

CHAPTER 1. GENERAL INTRODUCTION

1.1. Properties of GHB

1.1.1. Chemistry and natural occurrence

Gamma-hydroxybutyric acid and its sodium salt gamma-hydroxybutyrate-Na (GHB) are short chain (4-carbon) fatty acid structure analogues (Saytzeff, 1874), just as the putative neurotransmitter substance, gamma-aminobutyric acid (GABA) (fig.1.1.). GHB is synthesized from its cyclic congener, gamma-butyrolactone (GBL), by alkaline hydrolysis (Chanlaroff, 1884).

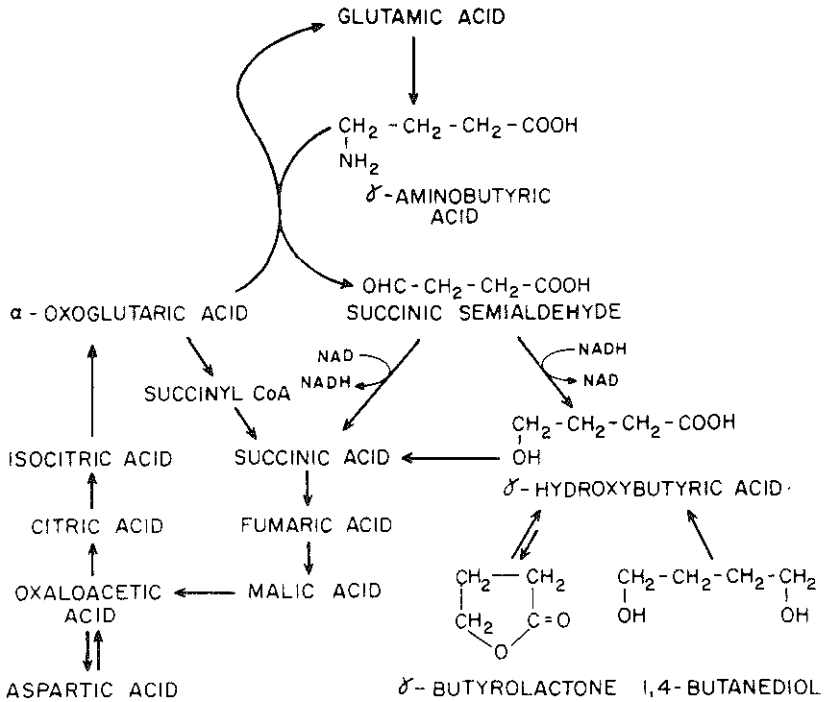


Fig. 1.1. Metabolic interrelationship between gamma-hydroxybutyric acid and other exogenous and endogenous compounds in brain (after Roth and Giarman, 1969).

The natural occurrence of GHB in mammalian brain was first proposed by Wolf (1960) and Bessman and Fishbein (1963), but final evidence was only obtained with the use of more sensitive techniques such as gas chromatography (Roth and Giarman, 1970). Thus far, GHB has been found to be present in the brains of the pigeon (Bessman and Fishbein, 1963), cow and cat (Roth and Giarman, 1970), rat (Bessman and Fishbein, 1963; Roth and Giarman, 1970; Doherty et al., 1975 a), guinea pig (Roth and Giarman, 1970; Doherty et al., 1976), rabbit (Doherty et al., 1975 a) and man (Bessman and Fishbein, 1963). Highest concentrations in the guinea pig brain were observed in the hippocampus, midbrain, diencephalon and cerebellum (Roth, 1970).

GHB was reported to be synthesized in vivo from GABA via succinic semialdehyde (Roth and Giarman, 1969; Roth, 1970) (fig. 1.1.). The reverse reaction, namely direct conversion of GHB to GABA, was proposed as the fate of GHB administered to the rat (Della Pietra et al., 1966) or mouse (De Feudis and Collier, 1970) and in rat brain tissue in vitro (Mitoma and Neubauer, 1968). Later reports have shown, however, that exogenous GHB is converted, in vivo, to succinate and thus enters into the tricarboxylic acid cycle (Doherty et al., 1975 b; Möhler et al., 1976) (fig. 1.1.).

GHB, GBL and some other structural analogues like 1,4-butanediol, appeared to have similar effects when injected into animals (Sprince, 1969; Marcus et al., 1976). GHB seems to be the active form and GBL and 1,4-butanediol probably act only after their conversion to GHB in vivo (Roth and Giarman 1966; 1968) (fig. 1.1.).

1.1.2. Sedative effects

In a study on the antibiotic effects of saturated and unsaturated lactones, Giarman (Giacomino) and his co-workers noted that butyrolactone, apart from exerting a positive therapeutic effect against influenza virus in mice (200 mg/kg) (Rubin and Giarman, 1947), demonstrated complete suppression of cortical EEG activity and respiratory failure at a slightly higher dose (545 mg/kg), while, after 300 mg/kg, the respiratory rate was half the initial value (Giacomino and Mc Cawley,

1947). 9 Years later it was reported that neutralized salts of short chain fatty acids, among them butyrate, induced "unconsciousness" (loss of righting reflex) in rats, dogs, guinea pigs, mice, frogs and chicks (Samson and Dahl, 1955; Samson et al., 1956); in rabbits, drowsy behavior was accompanied by a high amplitude, low frequency EEG (White and Samson, 1956). The only hydroxylated butyrate tested in these experiments, beta-hydroxybutyrate, did not change the behavior nor the EEG pattern of the animals (White and Samson, 1956; Samson et al., 1956). The authors regarded the butyrate-induced EEG as being similar to that seen during sleep and anesthesia (White and Samson, 1956), but the amplitude was higher than one would expect during physiological sleep or anesthesia.

In the search for a structural analogue of GABA able to cross the blood-brain-barrier, Jouany and co-workers synthesized, among other gamma-substituted butyrates, the hydroxylated product, gamma-hydroxybutyrate (sodium salt) (GHB) and reported its sedative action (Jouany et al., 1960 a,b). Injection of 500 mg/kg GHB in rats and non-restrained dogs induced an inhibition of the righting reflex, upon turning them on their side, for about 45 min; the authors considered this as sleep (Jouany et al., 1960a,b). In the rabbit, a slightly higher dose was needed to induce the same effect (Jouany et al., 1960 b). Similar results were obtained with GBL in the rat, pigeon and rabbit (Benda and Perlès, 1960). In the cat, 500 mg/kg GHB induced a high amplitude, slow wave electroencephalogram (EEG) (Jouany et al., 1960 b). These authors also reported that GHB (500 mg/kg in rat) postponed the convulsive and lethal effects of pentylenetetrazole, isoniazide and strychnine and, in the cat, it antagonized the hydroquinone-induced tremor and epileptic EEG.

The first experiments with GHB on humans were conducted by Laborit (Laborit et al., 1960). The dose required to induce a sedative state ("sleep") in man was much lower than in animals (60 mg/kg). Twenty minutes after administration of GHB, high amplitude, slow waves appeared in the EEG, but behavioral sedation was visible only after another 10-15 min. On the basis of this sedative action, these authors started to use GHB for inducing surgical anesthesia, although they described the effect of GHB as "physiological sleep, both clinically and electroen-

cephalographically" (Laborit et al., 1960). It was established that a premedication (generally pethidine and promethazine) was indispensible in order to obtain a stable anesthesia. However, with the correct premedication, GHB induced a state of anesthesia after a latency period of 10-20 min, which was accompanied by a slowing of the heart and respiration rate, muscular relaxation and myosis of the pupils. The duration of the anesthesia was dependent on the dose of GHB administered. When the effect of GHB ended, consciousness reappeared abruptly, accompanied by a sensation of pain. The authors initially reported the successful use of GHB-induced anesthesia in 40 cases of various kinds of operations (Laborit et al., 1960). Thereafter, GHB was used in neuroradiography and neurosurgery (Laborit et al., 1961) and in a wide variety of other types of operations (Blumenfeld et al., 1962; Solway and Sadove, 1965; and many other reports).

The sedative state induced by GHB, without premedication, was not a total anesthesia, because it was not possible to apply a surgical stimulus without producing reflex movements (Helrich et al., 1964). Hence, because of the induced state of unconsciousness accompanied by a high amplitude slow wave EEG and the possibility of inducing both behavioral and electrocortical arousal, when sufficiently strong stimuli were applied, many authors referred to the GHB-induced state as sleep (for references, see Pérez de la Mora and Tapia, 1970; Laborit, 1973).

There has been some discussion as to wether sleep observed after injection of GHB is induced by this compound itself or by a metabolite. In man the maximal sedative effect lagged behind the peak blood level of GHB (Helrich et al., 1964) whereas in rats sleep induced after the administration of GHB appeared simultaneously with the concomitant peak in GBL concentration in the brain and blood (Bessman and Skolnik, 1964). Furthermore, the effective dose of GBL was lower than that of GHB (Hosko and Gluckman, 1963). It was, thus, concluded that GBL was the active compound (Bessman and Skolnik, 1964). Shortly afterwards, however, Giarman and Roth (1964) reported the rapid conversion of GBL to GHB by a lactonase in the rat in vivo (Giarman and Roth, 1964) and in rat, cat and human blood and rat liver in vitro (Roth and Giarman, 1966). Therefore, they suggested that GHB was the active form. They also noted that GBL was inactive, when injected intra-cisternally in

the rat, or into the thalamus or hippocampus in the monkey, whereas GHB, administered along these routes, induced behavioral depression or a high amplitude EEG, respectively (Roth et al., 1966). This was explained by the absence of the lactonase metabolizing GBL in brain tissue, confirming the suggestion that GBL has to be metabolized to GHB in the blood before it can act. These authors also noted a high concentration of GBL in muscle tissue shortly after its intravenous administration (Roth and Giarman, 1966). They suggested that GBL, being non-polarized, is taken up more rapidly into muscle tissue than is the polarized GHB, and is only released slowly into the blood. This might explain the longer duration of action of GBL compared to GHB after intravenous administration, and also the lack of difference after intraperitoneal injection of these compounds, because then both are only released slowly into blood (Roth and Giarman, 1966).

1.1.3. Paradoxical sleep

Behaviorally, two states of vigilance can be distinguished in animals and man: wake and sleep. Electroencephalographically, the awake state is characterized by fast, low amplitude or "desynchronized" waves, whereas during sleep the electroencephalogram (EEG) shows slow, high amplitude ("synchronized") waves, interrupted, paradoxically, by desynchronized EEG activity as seen during wake (Klaue, 1937; Dement and Kleitman, 1957; Jouvet et al., 1959).

In man, rapid movements of the eyeballs were observed during behavioral sleep associated with desynchronized EEG; when the subjects were awakened during a period of rapid eye movements, they very often recalled having dreamed (Aserinsky and Kleitman, 1953; Dement and Kleitman, 1957). The muscular tone, already low during behavioral sleep associated with synchronized EEG, disappeared totally during the desynchronized phase of the sleep state (Jouvet et al., 1959), which enables differentiation to be made between the latter phase and the awake state, by means of the electromyogram (EMG) in combination with the EEG.

In view of these differences, the behavioral sleep state was di-

vided into two separate phenomena (Dement, 1958; Jouvet et al., 1959), which have been named according to the parameter thought to be most characteristic for each phenomenon. Thus, on the basis of the EEG, the sleep state characterized by slow, high amplitude waves has been called slow wave sleep (SWS) or synchronized sleep, to differentiate it from desynchronized or fast wave sleep. Because of the paradoxical appearance, during behavioral sleep, of a desynchronized EEG, characteristic of the active waking state, the desynchronized sleep state has also been called active or paradoxical sleep (PS). The obvious parallel of PS, orthodox sleep, has rarely been used as a synonym for SWS. On the basis of the occurrence of rapid eye movements during PS, this state has been designated REM sleep, as opposed to non-REM for SWS. Because the arousability during PS is lower than during SWS, the names deep and light sleep, respectively, have been introduced. The occurrence of dreams during PS, which of course can only be observed in man, gave rise to the use of the name dream sleep. Starting from the anatomical structure thought to be responsible for the triggering of PS, the rhombencephalon, this state has also been called -mainly in French literature- "phase rhombencéphalique du sommeil". We will use here the names SWS and PS, because they are most commonly used in the literature, at least as concerned to the rat.

Other phenomena observed during PS, are a regular 6-8 Hz rhythm in the hippocampal EEG, and monophasic peaks in EEG derivations from the pontine reticular formation (Jouvet et al., 1959), the lateral geniculate nucleus (Mikiten et al., 1961) and the occipital cortex (Mouret et al., 1963); these were called PGO-spikes.

The first experiments, leading to the discrimination between SWS and PS, were carried out in cats, rabbits and man. Later experiments showed that these sleep stages could also be distinguished in rats (Michel et al., 1961; Roldan and Weiss, 1962; Swisher, 1962). Most behavioral and electrographical phenomena characterizing PS in the cat were also observed in the rat, except PGO-spikes, which are absent in this species (Stern et al., 1974).

Intraperitoneal (i.p.) administration of GHB and GBL in low doses (50-100 mg/kg) was reported to induce PS in intact cats (Jouvet et al., 1961; Matsuzaki et al., 1964) and decorticate cats (Jouvet et al.,

1961). Sodium butyrate also induced PS in cats, when injected intravenously (i.v.) (Matsuzaki et al., 1964; Matsuzaki and Takagi, 1967 a) but not when injected i.p. (Matsuzaki and Takagi, 1967 a). Sodium butyrate induced PS in various cat brain-stem preparations as well (Matsuzaki and Takagi, 1967 b). Delorme et al., (1966) reported a similar induction of PS in the mesencephalic cat following i.v. injection of 200 mg/kg GHB. The consistency of this effect depended on the point at which the injection was made in the sleep cycle, since a 15-20 min "refractory period" was found to occur after a period of spontaneous PS. However, this was not confirmed by other authors (Vern and Hubbard, 1971). The latter authors also reported that true PS could be induced in cats, within 5 min of GHB administration (190 mg/kg i.v.), with only 9 out of 30 injections, whereas the rate of PS induction after control injections was 2 out of 30. Lower doses of GHB (40-100 mg/kg i.v.) had similar effects (Vern and Hubbard, 1971). Other authors either did not observe any PS in cats after i.v., i.p. or oral administration of 100 mg/kg GHB (Drakontides et al., 1962) or failed to observe a significant difference in periodicity or duration of PS after 60 mg/kg GHB, injected i.p., in this species (Winters and Spooner, 1965 a). Equivalent doses of GHB, GBL or butyrate, injected i.p. or i.v., failed to induce PS in rats (Marcus et al., 1967). Also, in chicks, PS was not induced by GHB (Osuide, 1972 b). Furthermore, PS was not induced in man after oral GHB (Metcalf et al., 1966) or i.v. GHB or GBL (Yamada et al., 1967). In human insomniacs, an increase in the amount of PS during the first third of the night was observed after administration of GHB, but the total PS per night was unchanged (Mamelak et al., 1973).

1.1.4. EEG hypersynchrony

Soon after the sedative effects of GHB and GBL were described and GHB came into use as an anesthetic adjuvant, it was reported that the EEG patterns, induced by these compounds in man, were different from those observed during physiological sleep or anesthesia (Benda et al., 1960; Solway and Sadove, 1965). These GHB-induced EEG patterns in man were called "epileptogenic" because of their high amplitude (Schneider

et al., 1963) and bear some similarity to the spike-wave complexes seen during absence (petit mal) epilepsy. Such high amplitude EEG patterns are often called "hypersynchrony", referring to the excessive synchronization of the neuronal population which is discharging, thus causing the high amplitude (Gastaut and Tassinari, 1975). In some cases, GHB produced tremors and clonic ("epileptiform") movements after rapid induction of anesthesia; these phenomena could be blocked by barbiturates (Laborit et al., 1961).

Large doses of GHB (about 1500 mg/kg) caused convulsions, with corresponding EEG effects, in rabbits (Cahn et al., 1960; Schneider et al., 1963) and chicks (Osuide, 1972 b), and caused convulsions in mice (Ban et al., 1967). In cats, GHB prolonged the evoked seizure activity in the hippocampus and amygdala (Drakontides et al., 1962).

Winters and his collaborators were the first to specifically point out that the central effects produced by GHB in cats (Winters and Spooner, 1965 a,b; 1966) and by GHB, GBL and short chain fatty acids in rats (Marcus et al., 1967) are epileptoid, rather than depressant, at all dose levels. They also pointed out that these non-convulsive epileptoid manifestations, appearing superficially as behavioral depression, might be misinterpreted. They described the induction, in the cat, by 700 mg/kg GHB, of "a progression of several types of epileptiform EEG patterns", ranging from intermittant hypersynchrony via continuous hypersynchrony to polyphasic bursts (i.e. bursts consisting of positive and negative spikes) with intermittent cortical silence. Thereafter the EEG passes through the same states in the reverse order until its final return to normal (Winters and Spooner, 1965 b). In the rat a similar sequence of EEG patterns was observed after 700 mg/kg GHB, with one difference: the continuous hypersynchrony consisted of two periods: first $2\frac{1}{2}$-3 c/s waves appeared and thereafter the frequency was reduced to 1-2 c/s (Marcus et al., 1967). Lower doses of GHB (200-600 mg/kg in cat or 250 mg/kg in rat) showed the same progression of EEG phenomena, though without the phase of polyphasic bursts with intermittent cortical silence (Winters and Spooner, 1965 a; Marcus et al., 1976). Recently, it was established that these changes in EEG pattern correlate well with the serum level of GHB (Snead et al., 1976).

During the continuous hypersynchrony and polyphasic bursts with intermittent cortical silence, the animals show a loss of righting reflex, and this is the phenomenon which lead other authors to refer to this state as "sleep". It was noted that the continuous EEG hypersynchrony in the cat shows similarities to the 3 c/s spike-and-wave pattern, characteristic for human absence epilepsy (Snead et al., 1976). Low doses did not induce convulsive or jerky movements, but following doses of 600 mg/kg GHB or higher, spontaneous and auditory stimulation-induced myoclonic movements were observed in cats (Winters and Spooner, 1965 a,b), rats (Marcus et al., 1967) and chicks (Osuide, 1972 b). Moreover, repetitive auditory stimuli in cats induced typical tonic-clonic convulsions, accompanied by hypersynchronous, high frequency EEG seizures (Winters and Spooner, 1965 b). GHB also enhanced the cortical evoked response induced by acoustic stimulation in cats (Winters and Spooner 1965 a) and rats (Kharkevich et al., 1971; Borbély and Huston, 1973), and photic stimulation and stimulation of the sciatic nerve in rats (Kharkevich et al., 1971).

From these observations, it was concluded that the phenomena induced by GHB are excitatory rather than depressive (Winters and Spooner, 1965 a; Marcus et al, 1967). This formed a part of the basis for a new theory concerning a continuum of excitatory states, anesthesia, hallucinosis and epileptic phenomena (Winters, 1976). It has also been proposed that GHB, because of its excitatory properties, might be used as an activating drug for clinical EEG diagnosis (Hirata et al., 1973).

The synthetic compound, 1-hydroxy-3-amino-pyrrolidone-2 (HA-966), is chemically related to the cyclic anhydric form of GABA and is thus a structure analogue of GHB and GBL (Havinga, Roorda and Kerling, 1959, unpublished). When injected into rats, rabbits and monkeys, HA-966 induced a sedative state and EEG hypersynchrony, which were similar to the phenomena induced by GHB and GBL (Bonta et al., 1971).

1.1.5. Dopaminergic neurotransmission

Gessa and his co-workers showed that administration of GHB, as well as GBL and 1,4-butanediol, both of which are converted in vivo to GHB (Roth and Giarman, 1966;1968), produced a selective, dose-dependent in-

crease in brain dopamine (DA) concentration (Gessa et al.,1966), with little or no effect on levels of other brain monoamines such as noradrenaline, serotonin or GABA (Giarman and Schmidt, 1963; Gessa et al., 1966; 1968). Fluorescence histochemistry has shown that the increased DA is located in "dots" in the nerve endings in brain areas that are also known to have a high DA content in control animals (e.g. nucleus caudatus, nucleus accumbens and tuberculum olfactorium) (Aghajanian and Roth, 1970). Recently, it has been found that, in the median eminence, though it is an area rich in DA, GBL does not induce increased DA levels (Gudelsky and Moore, 1976).

It was also established that GHB is not likely to act via inhibition of the metabolism of DA, since GHB does not affect the enzymes monoamine-oxidase (MAO) and catechol-O-methyl transferase (COMT), which are responsible for DA-breakdown (Gessa et al., 1968), and because of the fact that the increased DA-induced fluorescence after GHB administration has a different appearance (dotted) from the increased DA fluorescence (diffuse) caused by the MAO inhibitor pargyline (Aghajanian and Roth, 1970). It was demonstrated that the rise in DA concentration was, at least in part, dependent on de novo synthesis of this neurotransmitter, since inhibition of DA-synthesis by alpha-methyl-para-tyrosine (AMPT) prevented the increase in DA after GHB (Gessa et al., 1968). However, the decrease in DA level observed by Roth and Suhr after AMPT treatment was increased to control levels (but not higher) by the GHB precursor GBL. They suggested, therefore, that the increase in DA was caused, not only by increased synthesis, but also by decreased utilization or release of this compound (Roth and Suhr, 1970). The GHB-induced behavioral sedation, however, was not suppressed when AMPT inhibited the GHB-induced DA-increase (Gessa et al., 1968; Roth and Suhr, 1970). In fact, it was even significantly prolonged (Roth and Suhr, 1970). This might suggest that increased DA synthesis is not essential, at least, for inducing sedation.

GHB did not change the effects of AMPT on noradrenaline-containing neurons, once again indicating that GHB acts specifically on DA neurons (Gessa et al., 1968; Roth and Suhr, 1970). Moreover, GBL caused a selective increase in the specific activity of brain dopamine, but not of noradrenaline, when rats were injected with radiolabelled tyrosine

(Roth and Suhr, 1970). This selective action of GHB on newly synthesized DA was also confirmed by the ability of GHB to antagonize K^+-induced release of ^{3}H-dopamine from brain slices incubated with ^{3}H-tyrosine, but not when incubated with ^{3}H-DA; nor did GHB antagonize the release of ^{3}H-5-hydroxytryptamine after incubation with ^{3}H-tryptophan (Bustos and Roth, 1972). More important, however, was the indication, obtained from these results, that GHB might act via inhibition of DA-release.

Similar phenomena as those mentioned above, induced by GHB in whole brain (e.g. increased DA level, higher specific activity of DA after injection of ^{3}H-tyrosine together with GBL), were also observed in the rat corpus striatum (nucleus caudatus-putamen), a brain area rich in DA nerve terminals (Walters and Roth, 1972). This directed most further research efforts, concerning the mechanism of action of GHB on DA neurotransmission, towards the rat striatum.

Two groups of researchers simultaneously compared the effect of GHB with the results of hemisection (axotomy) of the nigro-striatal DA neurons. These manipulations exhibited several striking similarities: a rise in DA level; deceleration of AMPT-induced DA disappearance (Stock et al., 1973; Walters et al., 1973); blockade of the increase in DA concentration by intrastriatal injection of 25% KCl (Stock et al., 1973); increased DA fluorescence in the striatal nerve terminals on the lesioned side (Walters et al., 1973). This suggested that inhibition of neuronal impulse flow was the main cause of the GHB-induced rise in DA level. The most convincing evidence in this direction was supplied by Walters and her co-workers, who showed that GHB inhibited the firing rate of the dopaminergic neurons in the substantia nigra zona compacta, sending their axons to the striatum (Walters et al., 1972). Though a rise in DA level in rat brain was observed only after administration of GHB in doses higher than 750 mg/kg and was maximal after 1500 mg/kg (Stock et al., 1973), even a dose as low as 100 mg/kg GHB or GBL was sufficient to decrease the firing rate of nigral DA cells (Roth et al., 1973).

Starting from the premise that the inhibition of impulse flow was the main effect of GHB, these authors developed a theory explaining the simultaneous rise in DA level in terms of a sensitivity of tyrosine

hydroxylase to Ca^{++} ions. This theory stated that if, after inhibition of the impulse flow, the normal influx of Ca ions into the nerve ending is decreased, the tyrosine hydroxylase might change into a conformation for which the critical level of DA, necessary to inactivate the enzyme, is increased (Roth et al., 1974). The resulting enhanced DA synthesis, together with the cessation of the release, might thus be responsible for the increased DA concentration. Further evidence for the role of the impulse flow was produced recently when the GBL-induced increase in striatal DA was antagonized by electrical stimulation of the nigro-striatal pathway (Murrin and Roth, 1976).

The mechanism by which GHB induces inhibition of the impulse flow is not yet clear. Based on the facts that administration of GHB or GABA directly into the substantia nigra enhances striatal DA levels (Andén and Stock, 1973), that microiontophoresis of GABA produces inhibition of the firing rate of nigral neurones (Feltz, 1971), and on the structural similarity between GHB and GABA, it has been suggested that GHB acts synergistically with the action of putative inhibitory GABA-ergic nerve terminals in the substantia nigra (Andén and Stock, 1973). Another proposed mechanism for GHB in inducing an increase in striatal DA should be mentioned, namely an action on the storage granule membrane, for which the integrity of these granules is necessary (Menon et al., 1974).

A possible causal relationship between the effects of GHB or GBL on DA brain levels or neurotransmission on the one hand and GHB- or GBL-induced behavioral sedation on the other, has been discussed by several authors. Some of the arguments were:

(a) a good temporal correlation was obtained between the sedative action and the accumulation of brain DA after GHB administration in rabbits (Gessa et al., 1966; Walters and Roth, 1972);

(b) when GHB was injected into different brain areas in unanesthetized cats, the striatum, an area with high DA levels, was most sensitive to the actions of this drug (Gessa et al., 1967);

(c) AMPT acts synergistically with GHB in the sense that when the release of DA is partly inhibited by GHB, the amount of DA available for release is diminished by AMPT. It appeared that AMPT increases the duration of GHB-induced sedation (Roth and Suhr, 1970);

(d) amphetamine prevented the GHB-induced increase in DA level, probably due to release of newly synthesized DA (Andén et al., 1973); this compound also reduces GHB-induced sedation (Roth and Suhr, 1970).

Hutchins and co-workers excluded a causal relationship between GHB-induced sedation and DA-neurotransmission because they did not observe an increase in DA after GHB, when rats were pretreated with reserpine, but nevertheless sedation was induced (Hutchins et al., 1972). This might be due, however, to the relatively high dose of reserpine used (5 mg/kg), because a lower dose of reserpine (2 mg/kg), though depleting brain DA, did not prevent an increase in DA when followed by GHB (Gessa et al., 1968).

The synthetic compound HA-966, similar to GHB in structure and in its hypersynchronizing effect on the EEG (see 1.1.4), also exerts similar effects on dopaminergic neurotransmission. Intraperitoneal injection of HA-966 induced an increase in DA-concentration in the rat corpus striatum (Bonta et al., 1971). The AMPT-induced DA-depletion was counteracted by HA-966 (Bonta et al., 1971) and the intraneuronally-formed DA metabolite 3,4-dihydroxy-phenylacetic acid (DOPAC) was increased in the striatum, whereas the level of the DA metabolite homovanillic acid, which is dependent on the extraneuronally-located enzyme COMT, was increased (Hillen and Noach, 1971). These results suggested an analogy to the effects of GHB, namely that HA-966 inhibits the release of DA in rat striatum (Hillen, 1972). Further experiments revealed that these effects of HA-966 are caused by inhibition of the impulse flow in the nigro-striatal DA neurons (Van Valkenburg, 1976; Walters and Roth, 1976).

1.1.6. GABA-synthesis

GABA is synthesized in the brain by decarboxylation of glutamic acid, under the influence of the pyridoxal-phosphate-dependent enzyme, glutamic acid decarboxylase (GAD).

GHB appeared to inhibit GAD activity in vivo, in the mouse brain (Clifford et al., 1973) and rat brain (Godin and Mark, 1967;

Tunnicliff, 1976), and in the mouse brain in vitro it inhibited GAD competitively (Dye and Taberner, 1975). GHB was not reported to change brain GABA concentration in these and most other studies (Giarman and Schmidt, 1963; Godin and Mark, 1967; Mitoma and Neubauer, 1968; Clifford et al., 1973; Tunnicliff, 1976), except two observations in which the GABA level was said to be elevated (Wolleman and Dévényi, 1963; Della Pietra et al., 1966). However, one of these reports did not mention any significance in differences (Della Pietra et al., 1966); the other only stated that there was induction of a "slightly increased GABA content of rat and rabbit brain", without any quantitative evaluation (Wolleman and Dévényi, 1963). The latter authors, moreover, did not observe any change in GAD activity after GHB (Wolleman and Dévényi, 1963), which might be a consequence of the method used. It has also been noted that GHB induced an increased GAD activity, accompanied by decreased glutamate levels (Promislov and Solovjova, 1973).

HA-966 has been reported to inhibit GAD (Hillen et al., 1969) and decrease brain GABA concentration as well (Möhler et al., 1975).

1.1.7. Effects on serotonin, acetylcholine and excitatory amino acids

Administration of GHB (500 mg/kg) to mice induced an increase in the concentrations of the neurotransmitter substance serotonin, in whole brain (Benton et al., 1974) and brain stem (Clifford et al., 1973). Other authors did not observe any change in brain levels of serotonin (Giarman and Schmidt, 1963; Spano and Przegalinsky, 1973), but instead, increased synthesis and turnover rate of brain serotonin have been reported (Spano and Przegalinsky, 1973). It has not been established whether these changes are related to increased neuronal activity or to serotonin release.

Administration of GBL (750 mg/kg) increased acetylcholine concentrations in whole mouse brain, cortex and corpora quadrigemina (Giarman and Schmidt, 1963) and in whole rat brain, subcortex, striatum and, especially, hippocampus (Sethy et al., 1976). It was suggested that GHB inhibits the impulse flow in cholinergic neurons (Sethy et al.,

1976), analogous to the effect on DA neurons (see 1.1.5.). No temporal correlation was obtained between the increase in acetylcholine and the depth of "anesthesia" produced by GBL (Sethy et al., 1976).

The amino-acids L-glutamate and L-aspartate are putative excitatory neurotransmitters. GHB has been reported to increase brain levels of both glutamate (Promislov and Solovjova, 1973) and aspartate (Margolis, 1969). On the other hand, inhibition of the biosynthesis of these compounds by GHB has also been observed (Godin and Mark, 1967).

1.1.8. Anticonvulsive properties

GHB has been reported to induce epileptoid EEG and behavioral phenomena (see 1.1.4.) but, nevertheless, this compound antagonized convulsions elicited by chemical and electrical stimuli.

Pentylenetetrazol convulsions were antagonized by GHB in rats (Jouany et al., 1960 a), mice (Gluckman and Hosko, 1963), and chicks (Osuide, 1972 b), though other authors found no effect in mice and rats (Mayer, 1974). Strychnine convulsions were antagonized in rats (Jouany et al., 1960 a; Mayer, 1974) and mice (Ban et al., 1967; Mayer, 1974), but in chicks GHB potentiated the convulsive effect of strychnine (Osuide, 1972 b). GHB antagonized convulsions elicited by isoniazide in rats (Jouany et al., 1960 a) and by picrotoxin in chicks (Osuide, 1972 b). Semicarbazide convulsions were antagonized by GHB in mice (Ban et al., 1967) while other authors observed no significant protection in mice (Gluckman and Hosko, 1963; Mayer, 1974) and rats (Mayer, 1974). The doses of GHB, effective against these convulsant substances, were, as far as mentioned by the authors, 500 mg/kg in rodents (Ban et al., 1967; Jouany et al., 1960 a; Mayer, 1974) and 680 mg/kg in chicks (Osuide, 1972 b).

In mice, GHB antagonized electroshock convulsions (Gluckman and Hosko, 1963; Ban et al., 1967) in a dose of 500 mg/kg, but a dose of 300 mg/kg tended to increase the mortality rate (Ban et al., 1967).

1.1.9. Tranquillizing properties

GHB has been used successfully in the treatment of acute anxiety states in psychiatric patients (Du Couédic et al., 1964). In these experiments, GHB was administered in doses inducing a state of behavioral sedation and thus GHB does not necessarily possess a specific anxiolytic effect.

In mice, rats and cats, sub-sedative doses of GHB have been shown to decrease aggressive responses; it was concluded that GHB possesses specific tranquillizing properties (Pöschlová et al., 1974; Burov et al., 1976).

1.1.10 Other effects

GHB has been shown to block the uptake and accumulation of exogenous noradrenalin in the rat vas deferens but it had no effect on the level of the endogenous adrenergic mediator in this tissue (Arefolov and Panasyuk, 1975). At a dose of 1000 mg/kg, GHB has been reported to induce an increase in heart frequency and blood pressure in rats (Gomes et al., 1976). Administration of 400 mg/kg GBL to rats induced a triphasic change in body temperature, consisting of a decrease, followed by an increase and another decrease, whereupon, after about 6 h, the normal temperature is restored (Borbély and Huston, 1972).

Administration of GBL (500 mg/kg) to female rats, just prior to the proestrous critical period, significantly reduced serum LH and FSH levels and ovulation; it was suggested that these effects were mediated through inhibition of the neurotransmission in a central DA pathway (Beattie et al., 1976).

GHB has been shown to increase brain glucose levels in mice (Godin et al., 1968; Leonard and Watkinson, 1971), probably by enhancing glucose synthesis (Leonard and Watkinson, 1971). An increase in glucose-6-phosphate dehydrogenase activity has also been noted after GHB (Taberner et al., 1972).

1.2. Aims

In view of the reported sedative effects of GHB, to which many authors referred as a "sleep like" state (see 1.1.2.), we decided to compare the GHB-induced effects with natural sleep in the rat. Sleep was measured electrographically, using both EEG and EMG, in order to differentiate between wake and the two sleep states: SWS and PS. Thus, it was also possible to determine whether GHB induces PS in rats, as observed in cats (see 1.1.3.). It was established that administration of GHB, only in the doses lower than 100 mg/kg, was followed by EEG patterns similar to those observed in untreated animals. The effect of this dose range on the duration of the sleep stages was measured.

In addition, we analyzed behavioral and electrographical phenomena induced by 200 mg/kg GHB. Since we observed that these phenomena are reminiscent of generalized, non-convulsive epilepsy or absence (petit mal) epilepsy in man, we investigated whether or not these effects could be antagonized with specific anti-absence drugs.

In searching for a neurotransmitter system underlying the GHB-induced EEG phenomena we subsequently examined whether or not modulation of DA (see 1.1.5.) and GABA neurotransmission (see 1.1.6.) would affect these phenomena.

1.3. Materials and methods

1.3.1. Animals

All experiments were carried out on non-restrained male Wistar rats, obtained from the animal breeding farm of TNO (Zeist, The Netherlands).

1.3.2. Implantations

Cortical electrodes were implanted when rats had reached the weight of 160-180 g. The animals were anesthetized with Hypnorm(R) (10 mg fluanison and 0.2 mg fentanyl per ml; Philips-Duphar, Amsterdam, The

Netherlands) in doses of 1.5 ml/kg, injected either intraperitoneally or subcutaneously into the neck or the flank. Two kinds of preparations were used: chronically (Chapter 2-7) and semi-chronically (Chapter 8) implanted rats.

1.3.2.1. Chronic electrodes

When the rats ceased to respond to tactile stimuli, after Hypnorm injection, the skin over the skull was shaved and cleaned with 70% ethanol. The animals were then placed in a stereotactic apparatus and secured by ear-bars and an upper-incisor bar. A midline incision was made in the skin overlying the skull, from between the eyes backward until a point over the neck muscles, about 2 cm behind the skull. The uncovered periost was removed and then the nose-pressure-bar was fixed. The wound edges were drawn sidewards by small clamps and eventually after bleeding of the vessels in the bone was stopped, the skull was cleaned and dried with 70% ethanol.

Next, holes of 0.8 mm diameter were drilled in the skull, into which the EEG electrodes would be placed. The placement of the holes was as follows: one hole was drilled about 4 mm anterior to the bregma (the intersection point of the sagittal and coronal sutures) and about 1 mm laterally to the sagittal suture; this hole served as the site for the reference electrode. Two holes were drilled bilaterally, about 1 mm anterior to the coronal suture, or, in a few rats, about 1 mm posterior to the coronal suture, and about 2.5 mm laterally to the sagittal suture; these holes were placed over the frontal cortex. Two other holes were made bilaterally over the parietal cortex, about 5 mm posterior to the frontal holes and about 2.5 mm laterally to the sagittal suture. In a few rats, mentioned in Chapter 2, the holes on the right side were drilled as close as possible to the sagittal suture.

If necessary, the skull was cleaned and dried again after drilling the holes and then silver, or stainless steel, screw electrodes were secured in the holes. The diameter of the screw-thread was 1 mm and the length 1.5 mm; its tip was rounded and rested on the dura mater. The diameter of the head was 1.5 mm. A length of insulated copper wire

(about 1.5 cm) was soldered to the screw-head beforehand. The bone of the skull and the screws were then covered with an initial thin layer of dental acrylic cement (Durelon(R), ESPE GmbH, Seefeld/Oberbay, W.-Germany). This first layer was applied as early as possible in order to prevent bleeding during the further manipulations, which would otherwise decrease the attachment of the cement to the bone.

The copper wires from the screw electrodes were then soldered to an 8-pin miniature contact socket, to which two myogram electrodes were connected beforehand via insulated copper or stainless steel wire (fig. 1.2.). The socket was fixed to the skull with another application of

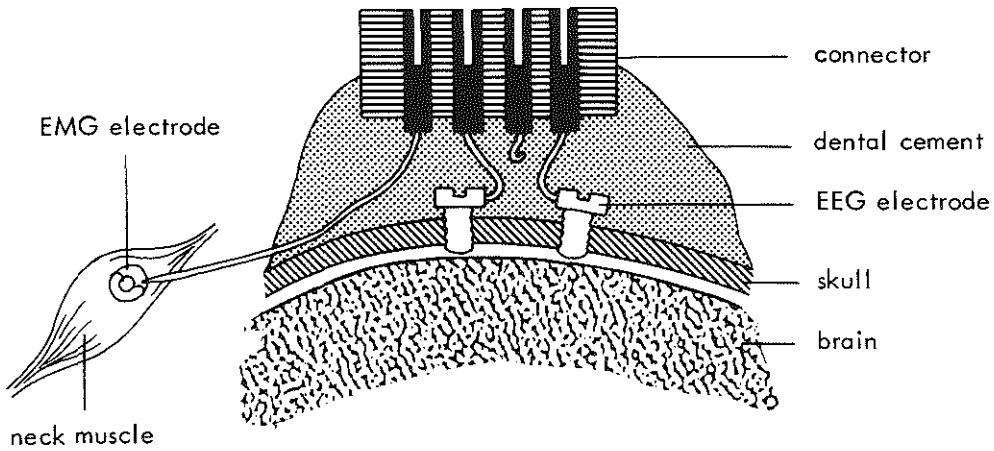


Fig. 1.2. Schematic drawing of chronically implanted electrodes and contact socket in a rat.

dental cement, covering the wires from the screw electrodes and with the wires to the myogram electrodes protruding from the posterior side. Then a small slit was torn in the two upper muscle layers on both sides of the neck, and at each side a myogram electrode was fixed, on the third muscle layer, with one or two stitches of surgical thread. The myogram electrodes consisted of a platinum ring or ball, 1 mm in diameter.

After application of penicillin powder (Penidural(R), Mycofarm, Delft, The Netherlands) into the wound, the skin was closed over the myogram wires and around the socket. The animals were warmed artificially until emerging from anesthesia.

Routinely, the EEG was derived bipolarly from the electrodes over the left frontal and parietal cortex. No difference in EEG pattern was noted, whether the electrode over the frontal cortex was placed either anterior or posterior to the coronal suture, or whether the electrodes over the right hemisphere were placed either close to the sagittal suture or more laterally (see also Chapter 2). The distance between both electrodes from which one signal was derived, influenced the mean amplitude of the EEG, but not the pattern. Thus, drug-induced changes in amplitude values in these preparations should be compared only to control circumstances in the same rat.

Initially, silver screw cortical electrodes were used, manufactured in the Central Research Workshop of the Medical Faculty, Erasmus University Rotterdam. Because this method was time consuming, stainless steel screws were purchased and used in subsequent experiments. No difference was observed between EEG patterns obtained with both types of electrodes.

The electromyogram (EMG) was derived bipolarly from both neck electrodes, and the prefrontal cortical electrode was used as reference. Initially, insulated copper wire was used to connect the electrodes with the socket. In a few weeks the copper corroded and as a consequence the quality of the EMG declined as movement artefacts appeared. Therefore, in later experiments, teflon insulated, twisted multistrand stainless steel wires were used. These produced a sustained, good quality EMG. The connection between the platinum EMG electrode and the wire caused some problems too. Initially, platinum rings were used, but they frequently became detached from the wire. A short piece of platinum wire with a ball-formed tip could be fixed more efficiently and rendered good EMG's for 2-3 months.

Rats with chronic electrodes were used for experiments from 8 days until about 3 months after implantation. Because the EMG generally deteriorated earlier, the rats were only used for about two months for experiments in which the EMG was necessary. After 3 months the cortical electrodes sometimes became detached, and therefore the rats were not used any longer.

1.3.2.2. Semi-chronic electrodes

Rats with semi-chronic electrodes were used only in lesion experiments (Chapter 8). The electrodes were adapted from a device described by De Vos and Bonta (1964) and consisted of one, prefabricated and reusable block. Five nail-shaped silver electrodes were connected to an 8-pin contact socket and then molded into a polymer resin, so that the socket protruded upward and the points of the electrodes downward. The diameter of the electrodes was 0.7 mm, they protruded 3 mm out of the plastic resin and their surface was ribbed. The placing of the electrodes is shown in fig. 1.3.

The rats were prepared and placed in the stereotactic apparatus as described for the implantation of chronic electrodes. When electrolytic lesions or sham lesions were made, holes were drilled into the skull at a point overlying the target site and needles were lowered into the brain. For lesioning, insect pins were used, varnish-isolated except for the tip. When a lesion was made, the needle served as anode and the ear-bars as cathode. A current of 1 mA was applicated for 20 s. Thereafter the needles were removed.

Then five holes (diameter 0.5 mm) were drilled in the skull. Aided by a jig they were placed such that they fitted to the conformation of the electrodes in the electrode block. The block was placed on the skull with the electrodes over the holes, and using slight pressure, the electrodes were pushed into the holes. The block was kept in place by the ribbed surface of the electrodes. After the application of penicillin powder, the edges of the incision were closed around the electrode block.

Rats implanted with this kind of electrode were used for only one experimental session, between 6 and 10 days after implantation. After about two weeks the electrode blocks loosened spontaneously. Routinely, a left bipolar parieto-occipital derivation was used, with the prefrontal electrode as reference. After using these rats, the place and extent of the lesions were verified histologically (see Chapter 8).

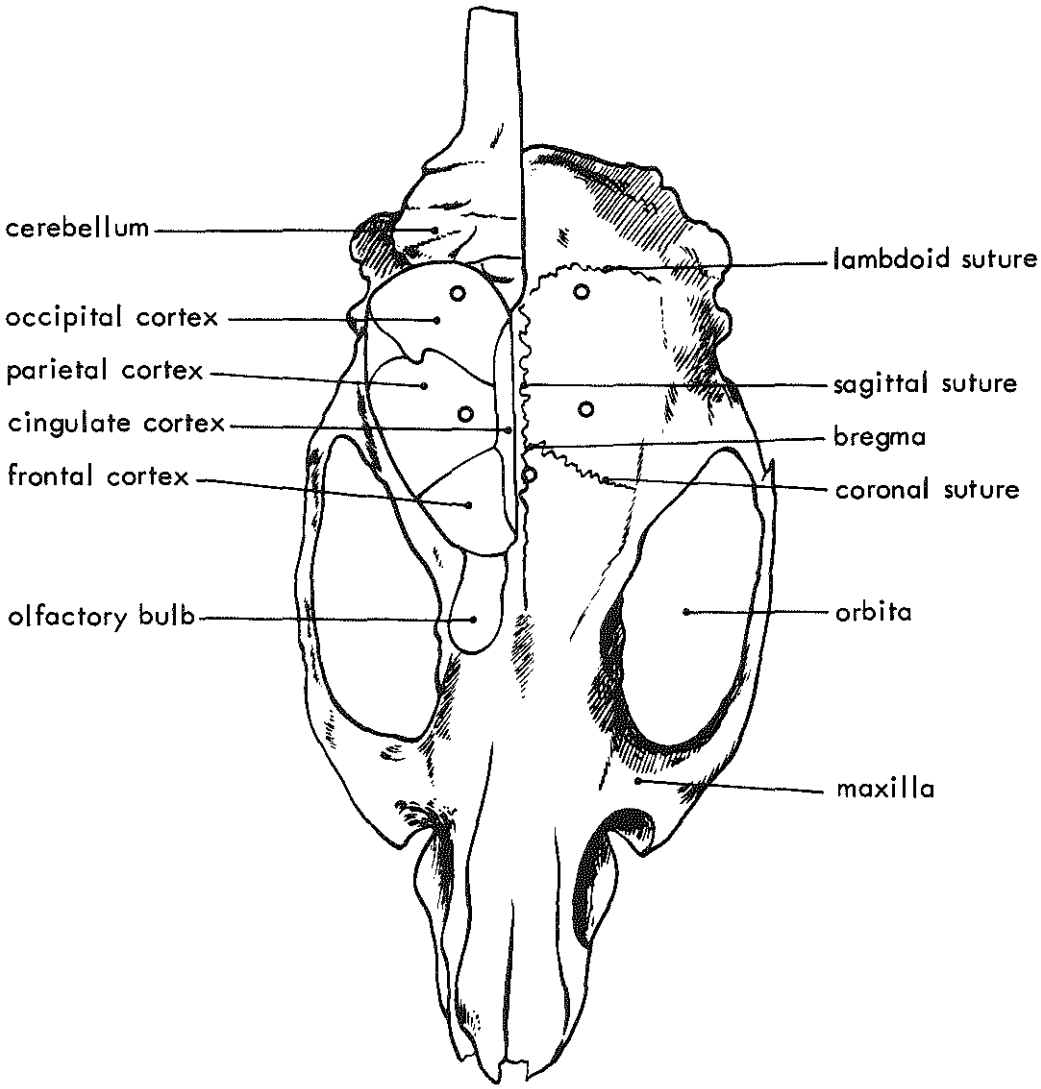


Fig. 1.3. Schematic drawing of the skull (right) and uncovered central nervous system (left) of a rat, showing the placing of semi-chronic electrodes over the cortex (circles).

1.3.3. Experimental set-up

Between the experimental sessions, the rats were caged individually, and received water and food ad libitum. The daily lighting period was from 07.00 h to 21.00 h. On the day before an experiment, the rats were weighed and placed in individual cages in a shielded, sound-proof cabin to adapt to the environment. They were kept on the same lighting schedule as in the previous cages, and received water and food ad libitum.

On the following day, at about 09.00 h, food and water were removed from the cages and an 8-pin miniature plug was plugged into the contact socket on the rat's head, connecting the electrodes with the EEG apparatus via a flexible cable. The cable was suspended from a swivel and was held up by a counterweight. Thus, the rats could move reasonably freely without becoming entangled or gnawing at the cable. However, when they were extremely active, as happened sometimes after administration of amphetamine or apomorphine (Chapter 5), the cables were twisted so many times that the rats had to be disconnected from the cables for a short period, in order to turn the cables back.

The animals were allowed to adapt to being connected to the cables, for at least one hour. Thereafter, the recording of the EEG and, in some experiments, the EMG, was started. Baseline signals were recorded for at least one hour before administration of drugs.

The signals were amplified by Grass model 7P511 amplifiers and registered on a Grass model 78 polygraph. Generally, the signals were simultaneously registered on a Philips Analog 7 magnetic tape recorder. The apparatus contained 6 amplifier channels. Thus, when both EEG and EMG had to be registered, as in the sleep experiments, a maximum of 3 rats could be used simultaneously. In other experiments, 4 rats were used in each session: in all rats unilateral EEG was registered, and the remaining two channels could be used for either contralateral EEG's or EMG's.

1.3.4. Route of drug administration

In practically all experiments the compounds were injected intraperitoneally (i.p.). As an alternative route of drug administration, intravenous (i.v.) injection into the tail vein was applied in rats with semi-chronic electrodes (Chapter 8). Oral administration was rejected as being unreliable because of the slower uptake and dependency on many variables.

The arguments for preferring i.p. over i.v. administration in most experiments were as follows:

a. i.p. administration is easier and can be made more rapidly;
b. i.p. administration causes less handling stress, because for i.v. administration the animals have to be restrained in a narrow cage;
c. in the literature concerning GHB, this compound was generally injected i.p.; thus, when comparing effects of certain dose-ranges of GHB, they should be administered along the same route;
d. rats bearing chronic electrodes were used for several months; during this period the skin over the tail thickened, and in the older rats it was very difficult to find the tail vein for i.v. injection. However, rats with semi-chronic electrodes were used only once, when they were young and i.v. injection was relatively easy. Therefore, in these rats, drugs were administered i.v. (Chapter 8).

1.3.5. Compounds used

The following compounds were administered to the rats:

- Acetazolamide (AAA). Source: Lederle. Dissolved in water.
- Amino-oxyacetic acid-hemihydrochloride (AOAA). Source: K & K Laboratories. Dissolved in water, neutralized with 1 N NaOH.
- Amphetamine-sulphate. Source : Brocacef. Dissolved in water.
- Apomorphine-hydrochloride. Source: Sandoz. Dissolved in water with added ascorbic acid 0.2 mg/ml, in order to prevent oxidation of the compound.
- (3,4-dihydroxyphenylamino)-2-imidazoline (DPI). Source: Boehringer-Ingelheim. Dissolved in water, neutralized with 0.1 N NaOH.

- Diphenylhydantoin-sodium (DPH). Source: Brocacef. Dissolved in propylene-glycol.
- Sodium-n-dipropylacetate (DPA). Source: Labaz. Dissolved in water.
- Ethosuximide (ESI). Source: Chemische Industrie Katwijk. Dissolved in water, neutralized with 0.1 N NaOH.
- Sodium-gamma-hydroxybutyrate (GHB). Source: Egic. Dissolved in water. In the experiments described in Chapters 2, 3 and (in part) 4, GHB was prepared by heating gamma-butyrolactone (Baker) in the presence of NaOH and neutralizing with HCl.
- 1-Hydroxy-3-amino-pyrrolidone-2 (HA-966). Source: Organon. Dissolved in water.
- Haloperidol. Source: Janssen Pharmaceutica. Dissolved in lactic acid and saline.
- Mephenytoin (MPH). Source: Brocacef. Dissolved in propylene-glycol.
- Phenobarbital-sodium (PHB). Source: Brocacef. Dissolved in water, neutralized with 0.1 N HCl.
- Phensuximide (PSI). Source: Chemische Industrie Katwijk. Dissolved in propylene-glycol.
- Piribedil. Source: Servier. Dissolved in 0.1 N HCl, neutralized with 0.1 N NaOH.
- Trimethadione (TMD). Source: Brocacef. Dissolved in water, neutralized with 0.1 N NaOH.

The solutions were administered in volumes of 2-5 ml/kg when dissolved in water, or 1-2 ml/kg when dissolved in propylene-glycol. In control experiments, equal amounts of saline (0.9% NaCl) were administered when drugs were dissolved in water, or of propylene-glycol when this solvent was used. If necessary, aqueous solutions were diluted in saline.

1.3.6. Frequency analysis

From characteristic EEG patterns, power and phase spectra were computed. An EEG fragment was designated "characteristic" when it possessed a recognizable pattern, appearing regularly (either spontaneously or after administration of a drug), and not being a short transition

phase between two other EEG patterns.

The computations were carried out off line, using a PDP 15 computer. After analog to digital conversion (50 Hz sampling frequency over blocks of 1024 points, thus each block covering 20 seconds), the spectra were computed using the Cooley-Tukey Fast Fourier Transform. The results were smoothed over 32 points, rendering a frequency resolution of (50 : 1024)×(32 : 2)= 0.8 Hz. In the figures shown, the spectra were computed over clusters of 1-7 consecutive blocks of 20 seconds each.

1.3.7. Statistical evaluation

Values of results are presented as means ± standard error of the mean (SEM). Where necessary, significance of differences was determined using two-tailed Student's t-test or paired two-tailed Student's t-test. The paired test was used in the experiments described in Chapters 5, 6 and 7; drug-induced effects were compared with control results in the same session.

CHAPTER 2. EFFECTS OF LOW DOSES OF GHB (12.5-100 mg/kg) ON SLEEP STAGES IN THE RAT

2.1. Introduction

The state of behavioral sedation, induced by GHB or GBL in several animals, has been called "sleep" by many authors (see 1.1.2.). The criterion for this state was, generally, the loss of the animal's righting reflex, which, however, points rather to an anesthetic state than to natural sleep. EEG phenomena discriminating between SWS and PS were not used in these experiments. Moreover, other authors using similar doses of GHB and GBL (400 mg/kg and higher) reported induction of EEG phenomena similar to the hypersynchronous patterns seen in convulsive disorders (see 1.1.4.). Thus, a discrepancy appeared to exist between the effects of GHB in these doses and naturally occurring sleep, both behaviorally and electrographically. We were interested in investigating whether lower doses of GHB, which do not change the normally-occurring EEG patterns, might influence the duration of sleep in the rat.

GHB was also reported to induce PS in cats (Jouvet et al., 1961; Matsuzaki et al., 1964). However, this effect was not observed by others in this species (Drakontides et al., 1962; Winters and Spooner, 1965 a; Vern and Hubbard, 1971), nor in the chick (Osuide, 1972 b), rat (Marcus et al., 1967) or man (Metcalf et al., 1966; Yamada et al., 1967). In view of these conflicting results, it was tempting to examine the effect of GHB on PS in the rat.

2.2. Materials and Methods

2.2.1. Experimental design

Rats with chronically implanted electrodes were used. The reported results were obtained from 9 rats in 11 experimental sessions.

In preliminary experiments, it was established that the highest dose of GHB which does not change normal EEG patterns, is 100 mg/kg. Therefore, in these experiments, GHB was administered in doses of 12.5-

100 mg/kg. Each dose was tested once only in any single rat. Each rat was used in 2-4 experimental sessions. Thus, no rat received all doses of GHB used. However, each rat received a control treatment (saline) at least once.

In each session, three rats were used. At about 11.30 h, saline was administered to one rat, and the two remaining rats each received a different dose of GHB. Thereafter, the EEG and EMG were registered continuously for at least 4 hours.

2.2.2. Evaluation of sleep stages

The states of vigilance of the rats were scored every 30 s for the first 4 h after the injection. Scoring was based on the registrations of EEG and EMG, according to the criteria of Timo-Iaria et al. (1970). These are that a 30 s period with a high EEG amplitude is scored as SWS, a low EEG amplitude combined with a high EMG amplitude is referred to as "wake" and a low amplitude of both EEG and EMG is designated PS.

Significance of differences was determined by Student's t-test. From characteristic EEG patterns, power spectra were computed (see 1.3.5.).

2.3. Results

2.3.1. States of vigilance in untreated rats

Aided by EEG and EMG, a clear discrimination was obtained between wake, SWS and PS (fig. 2.1.). The EMG was often contaminated by peaks from the electrocardiogram, which, however, did not hamper the evaluation of sleep stages. The ventroflexion, observed elsewhere in rats during PS (Khazan et al., 1967) was not always exhibited by our rats, possibly because they were hindered by the connecting cables.

During PS, a clear theta rhythm (6-8 Hz) was seen in the EEG (fig. 2.1.). A power spectrum of the EEG during the three states of vigilance confirmed the appearance of the theta rhythm especially during PS (fig. 2.2.). Derivation of both a lateral and a medial fronto-parietal

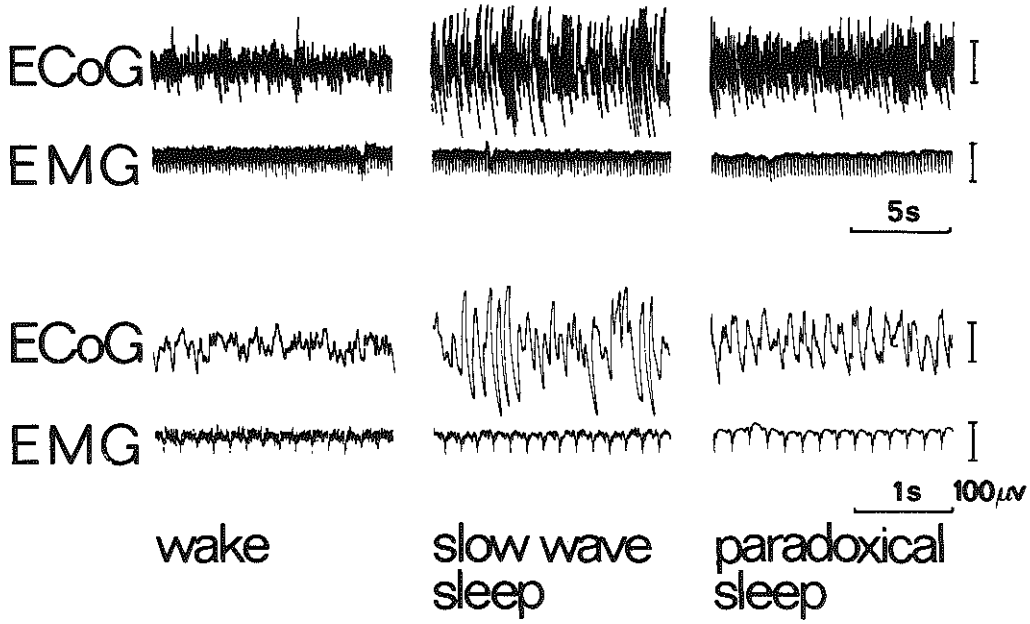


Fig. 2.1. States of vigilance as distinguished in the rat. ECoG = electrocorticogram; EMG = electromyogram. Note that the width of the solid band between the peaks in the EMG tracings represents the amplitude of the EMG; the peaks are contaminations by the electrocardiogram.

EEG in the same rat (fig. 2.2.b) and their coherence spectrum (fig. 2.3.) revealed that the theta rhythm during PS appears in a broad area in the cortex.

2.3.2. Effects of GHB on sleep stages

12.5 and 25 mg/kg GHB had no effect on the EEG nor on the sleep pattern of the rats. 50 and 100 mg/kg GHB caused a rise in the duration of SWS, which persisted for the whole 4 hour period of EEG

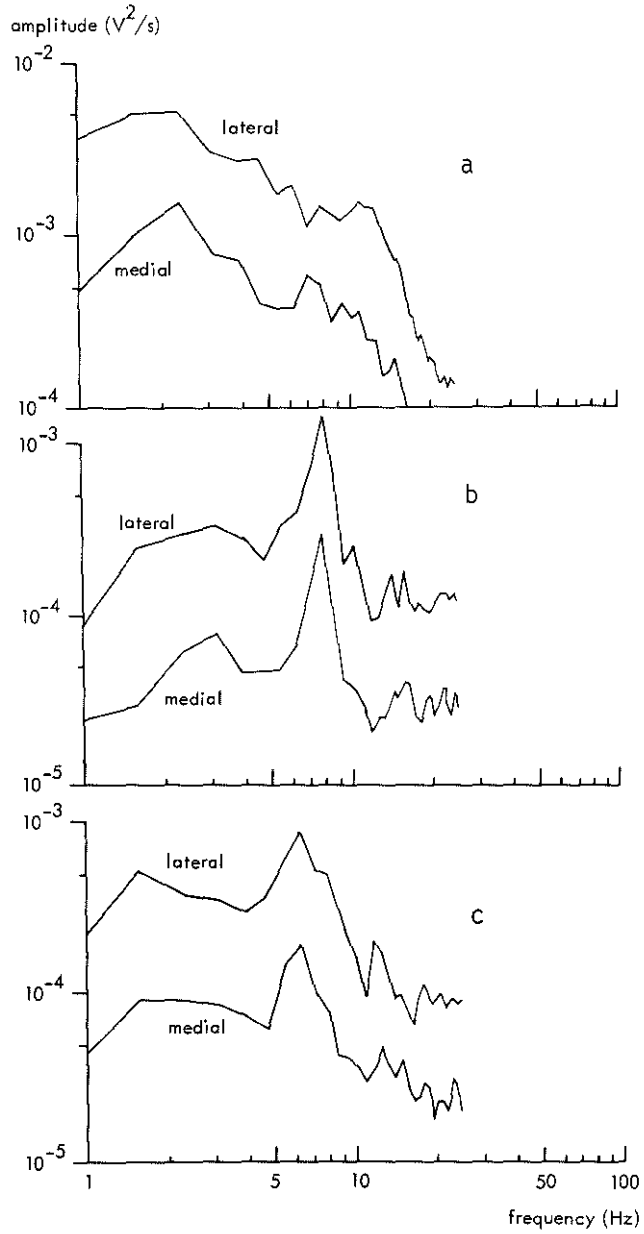


Fig. 2.2. Power spectra of representative EEG recordings, derived from the lateral and medial cortex, during SWS (a), PS (b), and wake (c).

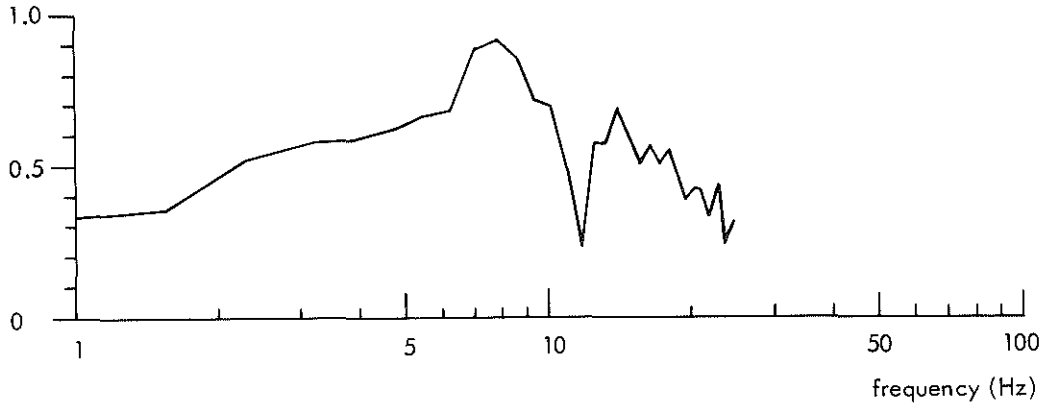


Fig. 2.3. Coherence spectrum of both recordings from fig. 2.2.b. The coherence, indicated along the abscissa in arbitrary units, is a degree for the correlation between two stationary, stochastic processes. The high level of coherence at 7-8 Hz indicates the equal appearance of theta rhythms during PS, in both medial and lateral cortex.

registration (fig. 2.4.). The SWS alternated regularly with PS and awake periods; during SWS, the rats could be aroused easily by tactile or acoustic stimuli. 50 mg/kg GHB caused a slight rise in PS during the third hour after injection, but this effect was not seen during the rest of the registration period.

2.4. Discussion

2.4.1. Theta rhythm in the cortical EEG

Visual and automatic analysis of the EEG during PS showed the occurrence of a theta rhythm in the cortex. This rhythm, generally registered in the rat hippocampus during PS (Roldan and Weiss, 1962; Timo-Iaria et al., 1970), is believed to be generated there and transmitted to the cortex (Timo-Iaria et al., 1970). Several authors registered theta rhythm in the rat cortex during PS (Michel et al., 1961; Swisher, 1962; Timo-Iaria et al., 1970). It was noted by Timo-Iaria

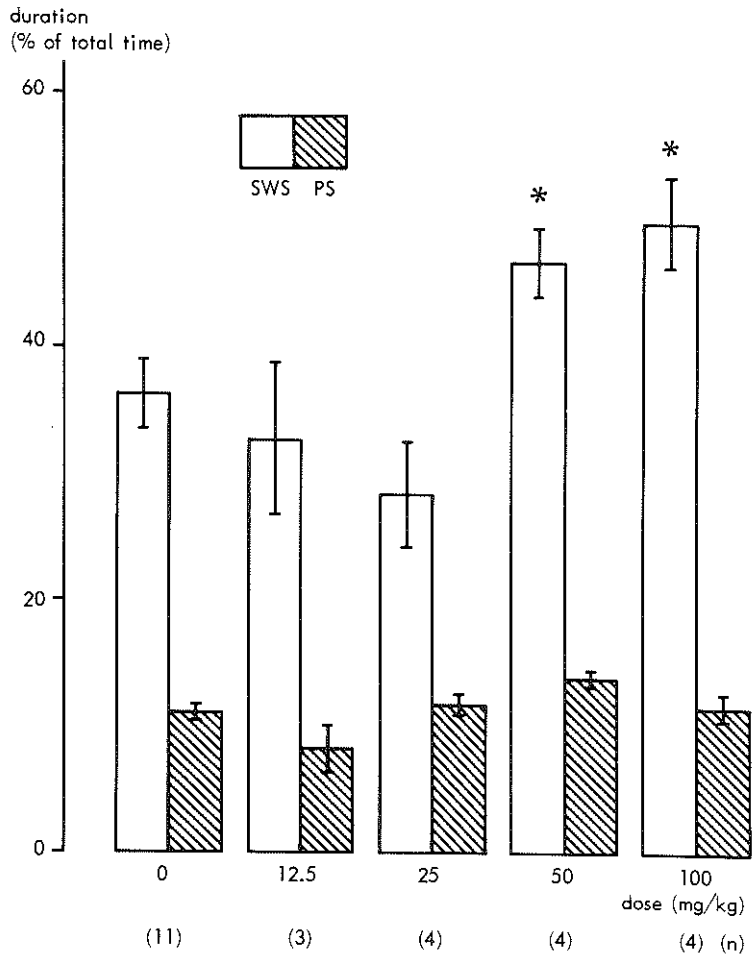


Fig. 2.4. Percentage of time spent in SWS or PS during the 4 hours after administration of GHB (mean ± SEM). Significance of differences (versus saline controls) was determined by Student's t-test. *$p<0.05$.

and co-workers (1970) that this pattern is not observed in all cortical registrations, but only in derivations from the medial parts of the hemispheres. Michel et al. (1961), however, reported the occurrence of theta rhythm in the frontal and parietal cortex, whereas Swisher (1962) observed theta rhythm in bipolar derivations from the frontal,

visual or motor cortex, both unilaterally and bilaterally, as long as the electrodes were not placed bilaterally symmetrical. Our results show that theta rhythm during PS is obtained from both medial and lateral cortical areas, in contradiction to the restrictions of Timo-Iaria et al. (1970), but in agreement with others (Michel et al., 1961; Swisher, 1962).

2.4.2. Effects of GHB on sleep stages

The results show that GHB, in the dose range of 50-100 mg/kg, may increase sleep, as measured electrographically. This GHB effect is different, in many respects, from the sedative state induced by higher doses of GHB (see 1.1.2.). The latter state is defined by a loss of righting reflex, which has nothing to do with natural sleep; the sedation is continuous for 1-2 hours, depending on the dose used, and the EEG observed during this state is described as being hypersynchronous (see 1.1.4.). On the other hand, during SWS after 50-100 mg/kg, the arousability of the animals, the sequence of sleep-wake stages and the EEG pattern were similar to those observed during natural sleep.

The increase in paradoxical sleep induced by 50-100 mg/kg GHB, as observed in the cat (Jouvet et al., 1961; Matsuzaki et al., 1964), was not reproduced in the rat. This confirms earlier observations in this species (Marcus et al., 1967), in chicks (Osuide, 1972 b) and in man (Metcalf et al., 1966; Yamada et al., 1967). This discrepancy is not likely to be due to a species difference, since, in the cat, conflicting results also exist (Drakontides et al., 1962; Winters and Spooner, 1965 a; Vern and Hubbard, 1971). Possibly, a difference in interpretation of EEG states is responsible for the discrepancy. This is probably the case with the "sws/PS" state, observed by Vern and Hubbard (1971) after administration of GHB in cats, which might be scored as either SWS or PS.

The mechanism of action underlying the enhancement of SWS by GHB is not at all clear. The serotoninergic neurons in the raphe system seem to play a role in the induction of SWS (Jouvet, 1972). It is not inconceivable that the reported increase in serotonin synthesis and

turnover rate produced by GHB (Spano and Przegalinsky, 1973) plays a role in the observed induction of SWS. It is more likely, however, that inhibition of the impulse flow in DA neurons by GHB (Roth et al., 1973) is responsible for the enhancement of SWS. This is because increased activity of DA neurons induce waking, and inhibition of these neurons diminish waking, both behaviorally (Lidbrink et al., 1973; Jones et al., 1973) and electrographically (Kafi and Gaillard, 1976).

An attempt has been made to determine whether changes occur in rat cerebrospinal fluid GHB levels between the awake and SWS state; however, no significant differences were observed (Tabakoff and Radulovacki, 1976), thus allowing no conclusion to be drawn concerning a possible role for naturally occurring GHB in SWS.

CHAPTER 3. INDUCTION OF HYPERSYNCHRONOUS EEG PATTERNS BY GHB (200 mg/kg) IN THE RAT

3.1. Introduction

The EEG pattern, observed during the state of behavioral sedation induced by GHB, has been reported to possess a very high amplitude and was described therefore as "hypersynchronous" or "epileptoid" (Winters and Spooner, 1965 a,b; 1966; Marcus et al., 1967). In preliminary experiments we found that the lowest dose of GHB, inducing an EEG differing from normal patterns in the rat, was 200 mg/kg. This was confirmed, recently, by Marcus and co-workers (1976). We were interested in investigating in detail the effects of 200 mg/kg GHB on EEG pattern and behavior in the rat. In particular, we wanted to know whether this EEG pattern was different from naturally occurring non-pathological patterns, and if so, which pathological state do the GHB-induced effects resemble.

3.2. Materials and methods

Rats with chronically implanted electrodes were used. GHB (200 mg/kg i.p.) was administered between 11.30 h and 12.30 h. The power spectra, as depicted here, were obtained from three rats; the duration of the EEG effects was determined from the mean of eight rats. In various series of experiments, not described in this Chapter, another 60 rats received the same dose of GHB, preceded by control injections (saline or propylene-glycol). The effects on EEG and behavior were similar, independently of the pretreatment.

3.3. Results

3.3.1. EEG and EMG

200 mg/kg GHB induced a sequence of EEG patterns, progressing from bursts of hypersynchronous waves (300-600 μV, 5-6 c/s) to continuous

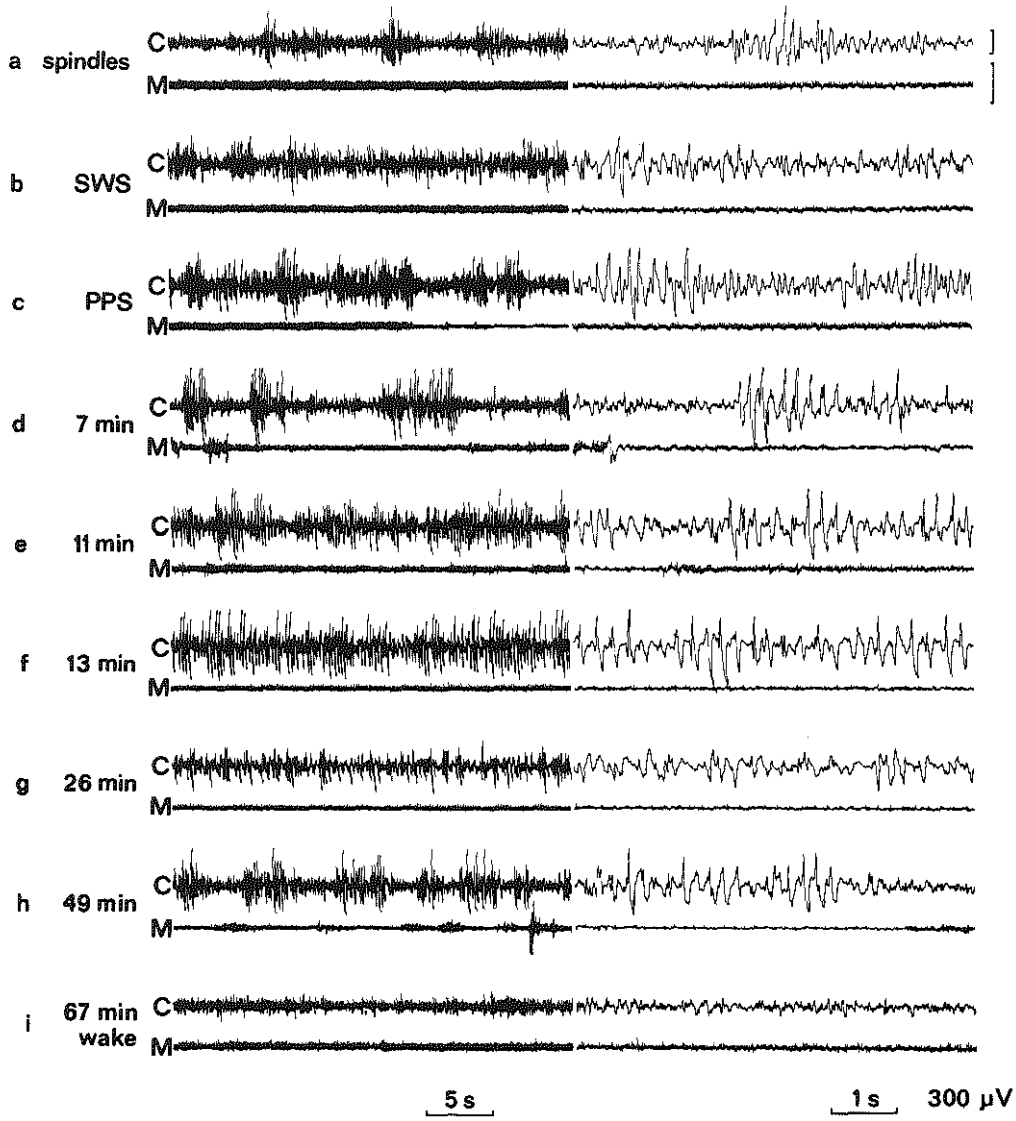


Fig. 3.1. Effect of GHB (200 mg/kg i.p.) on cortical EEG (C) and EMG (M) of a single rat. (a)-(c): baseline EEG patterns. (d): the first EEG response to GHB, administered 7 min earlier. (e)-(h): subsequent EEG phenomena induced by GHB. (i): the first period with continuous desynchronized EEG. SWS= slow wave sleep; PPS= preparadoxical sleep.

hypersynchrony (250-400 μV, 4-5 c/s); then, again, hypersynchronous bursts were observed and, finally, the normally occurring, alternating sleep-wake patterns reappeared.

The first hypersynchronous bursts appeared 5 min after administration of GHB and lasted 5-8 s each. During the periods between the bursts, a desynchronized EEG appeared. The duration of these intermediate periods was about 60 s between the first bursts and diminished gradually during the next 10 min (fig. 3.1. d,e). The neck EMG showed a low amplitude during the burst (associated with the immobile posture as described below) and a high amplitude during the intermediate periods. The continuous hypersynchrony which followed the bursts lasted for 23.38 ± 5.35 min (n=8). During this period the amplitude of the EMG was low. The continuous hypersynchronous EEG consisted of two subsequent patterns. During the first 10-15 min, the EEG pattern consisted of hypersynchronous spikes, followed by one or more slow waves (figs. 3.1.f and 3.2.). Thereafter, the amplitude of the spikes gradually decreased

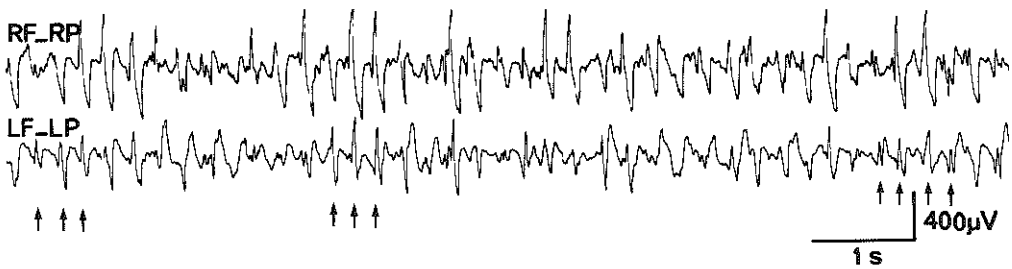


Fig. 3.2. EEG of a rat, 13 min after administration of GHB, showing right (RF-RP) and left (LF-LP) fronto-parietal patterns. Arrows indicate some of the spikes, followed by slow waves, as mentioned in the text.

to 200-300 μV (fig. 3.1.g). After the period of continuous hypersynchrony, bursts reappeared with a lower amplitude than before, separated by gradually lengthening intermediate periods with a desynchronized EEG (fig. 3.1.h). About 20 min later, a desynchronized wake EEG reappeared (fig. 3.1.i), followed by the other, usual, alternating states of vigilance. The total duration of the hypersynchronous phenomena was 72.13 ± 3.91 min (n=8). Thereafter, the rats showed a relatively high SWS duration, comparable to that induced by 50 or 100 mg/kg GHB. The PS

duration however was shorter.

A bilateral derivation of the EEG (fig. 3.2.) and its phase spectrum (fig. 3.3.) show that the spikes obtained during the continuous phase of the hypersynchrony were generated synchronously and symmetrically in the contralateral areas of the cortex.

In fig. 3.4. the power spectra of the EEG of 3 rats showing continuous hypersynchrony after GHB administration are compared with baseline SWS periods. During the continuous hypersynchrony, the power spectrum showed a higher energy, especially over the frequencies between 3 and 10 Hz.

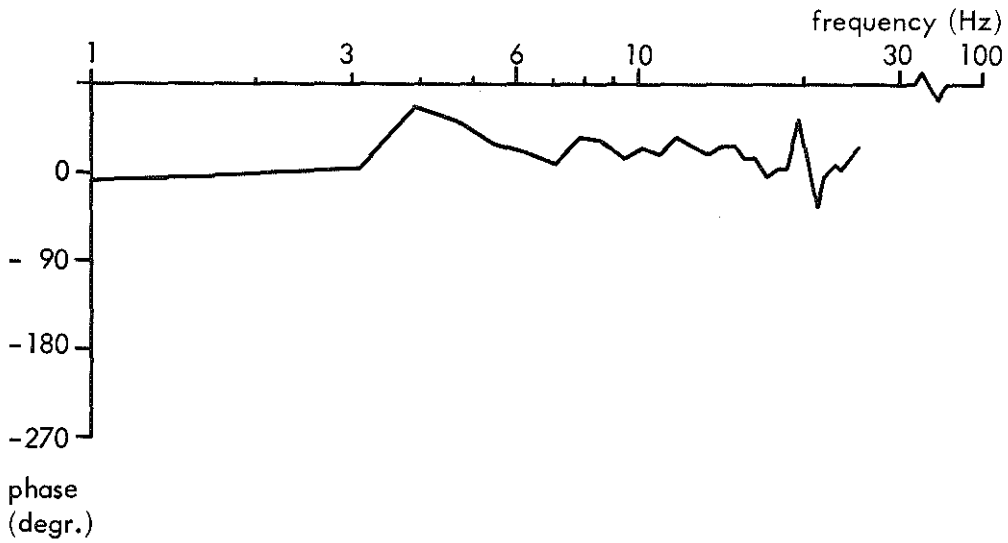


Fig. 3.3. Phase spectrum of the right and left fronto-parietal EEG's from fig. 3.2., showing the similarity in phase between the two derivations.

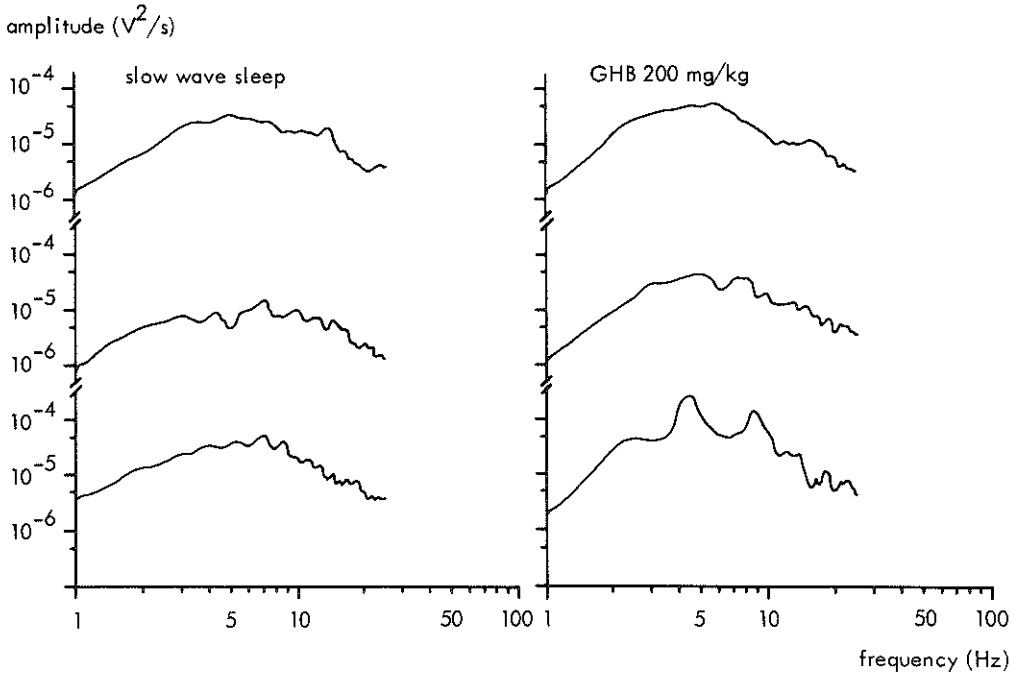


Fig. 3.4. Power spectra of characteristic EEG patterns induced by GHB (200 mg/kg), compared with naturally occurring slow wave sleep in 3 rats.

3.3.2. Behavior

The rats behaved normally until the appearance of the first hypersynchronous EEG burst after GHB administration; i.e. they were active and moved around the cage. At the onset of a burst they suddenly stopped moving and remained immobile, with their eyes open, for the duration of the burst. As soon as the burst stopped, the rats immediately resumed their previous motor activity until the next burst appeared. This can also be seen from the EMG (fig. 3.1. d,h). When the bursts became more frequent, only slight movements of the head were seen

during the short intermediate periods. When the hypersynchrony was continuous, the animals lay quietly with their eyes half closed. During and after the period of the GHB-induced changes in EEG no convulsions or other jerky movements were observed.

3.4. Discussion

Three high-amplitude EEG patterns have been described which occur naturally in the rat: spindling (fig. 3.1. a), SWS (fig 3.1. b) and preparadoxical sleep (PPS), the transitional state between SWS and PS (fig. 3.1. c) (Timo-Iaria et al., 1970). PPS, which is not distinguished as a separate stage by other authors, is similar to SWS in EEG, EMG and behavior, except that the amplitude of the EEG is slightly higher, and its high-frequency components are absent. Moreover, the EMG amplitude sometimes decreases already during PPS, to its low PS-level. Visual and automatic analysis of the EEG hypersynchrony, induced by 200 mg/kg GHB (fig. 3.1. d-h), showed that it was different from the naturally occurring high-amplitude patterns described above: the amplitude was higher, the peaks were sharper and they were separated by slow waves. This indicates that the behavioral sedation induced by 200 mg/kg GHB is dissimilar to natural sleep. It confirms the conclusions obtained by mere visual observation of the EEG, after the same dose of GHB in the rat (Marcus et al., 1976), or higher doses in man, rabbit, cat, rat and chick (Schneider et al., 1963; Winters and Spooner, 1965 a,b; Marcus et al., 1967; Osuide, 1972 b). These authors considered the GHB-induced EEG patterns to be different from those seen during sleep or anesthesia. Instead, they called them "epileptoid", because of their similarity to EEG patterns observed during human epileptic attacks and artificially induced convulsions in animals.

The synchronous generation of spikes on both sides of the cortex points towards a similarity to some type of generalized epilepsy: they were generated in a subcortical brain structure, and not in a cortical focus. Because convulsions did not occur, this state has been compared to "generalized non-convulsive epilepsy" or absence ("petit-mal") epilepsy (Winters and Spooner, 1965 a; Snead et al., 1976).

The most striking clinical symptom of absence epilepsy, absence episodes, can not be observed in animals. However, a motor parallel of absences has been described in the cat during insulin-induced hypoglycemia with hypersynchronous EEG phenomena (Waltregny, 1969). Also, the short periods of immobility, displayed by our rats during the hypersynchronous EEG bursts in the first 10-15 min after injection of GHB and after the continuous hypersynchrony, might parallel the absence episodes of human absence epilepsy.

Another characteristic of absence epilepsy is the appearance of 3/s spike-and-wave complexes in the EEG. Such a pattern was induced by GHB in the cat (Snead et al., 1976). In the rat, we observed spikes alternating with slow waves (figs. 3.1. f and 3.2.), but they appeared to be different from the classical spike-and-wave pattern, especially in their higher frequency of occurrence (4-5 c/s instead of 3). This difference might be due to species properties since spike-and-wave complexes are not produced in the rat as readily as in other species (McQueen and Woodbury, 1975).

Thus, in summary, the EEG and behavioral pattern induced by 200 mg/kg GHB is reminiscent of absence epilepsy with respect to the bilaterally symmetrical (generalized) hypersynchronous (epileptoid) EEG and the lack of convulsions, which appear to be replaced by an immobile posture during the bursts, possibly paralleling absences.

CHAPTER 4. EFFECTS OF ANTIEPILEPTIC DRUGS ON GHB-INDUCED EEG HYPERSYNCHRONY

4.1. Introduction

In view of the reminiscence of GHB-induced EEG hypersynchrony and behavior to absence epilepsy, as described in the previous Chapter, it was of interest to know whether this hypersynchrony can be antagonized by drugs which are effective in the treatment of absence epilepsy. To this end we pretreated rats with different types of antiepileptic drugs and observed the effects of these compounds on GHB-induced EEG hypersynchrony.

4.2. Materials and methods

Twelve rats with chronically implanted electrodes were used. The time table of the experiments and the arrangement of the injections are visualized in fig. 4.1. In each experimental session, 3 or 4 rats were used: one received an antiepileptic drug and saline, one was treated with the solvent of the antiepileptic drug and GHB (200 mg/kg), and both the antiepileptic drug and GHB were administered to the one or two remaining rats. The first injection (antiepileptic drug or its solvent) was made at about 11.30 h. The period between the first and the second injection (GHB or saline) varied for the different antiepileptic drugs, in order to allow the maximal effects of the drug and the GHB to coincide (table 4.1.). The antiepileptic drugs were administered intraperitoneally, in doses which were effective in antagonizing experimental convulsions (table 4.1.). After the second injection, the EEG was registered continuously for 2-5 h.

Each combination of an antiepileptic drug with GHB was administered not more than once only to any single rat. The time which elapsed between the administration of two consecutive combinations of an antiepileptic drug and GHB in any individual rat was at least seven days. Each rat received 1-4 times a combination of a different antiepileptic agent and GHB. The control treatment of a solvent

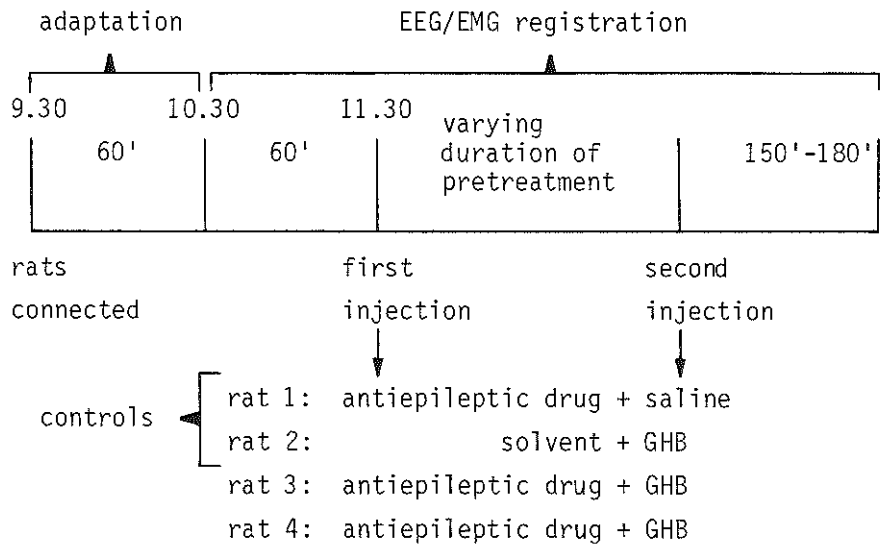


Fig. 4.1. Time table of the experiments.

followed by GHB, was administered 2-5 times to each rat.

4.3. Results

The effects of pretreatment with different antiepileptic drugs on GHB-induced hypersynchrony are presented in table 4.1.

Administration of an anticonvulsive dose of TMD followed by a saline injection had no pronounced influence on the EEG or the behavior of the rats. After a short period of excitation following the injection, the rats resumed their usual alternating states of vigilance. Sometimes, waves with a relatively high amplitude and low frequency appeared for short periods during SWS. The TMD pretreatment blocked the appearance of GHB-induced hypersynchrony in all 5 rats tested. These rats showed the same EEG pattern as observed after administration of TMD and saline.

Table 4.1. Effect of antiepileptic drugs on GHB-induced EEG hypersynchrony.

Drug	Dose (mg/kg)	Time[a] (min)	Clinical indication (type of epilepsy)	Number of experiments	
				Total	In which GHB-hypersynchrony was antagonized
Trimethadione (TMD)	300[b]	60	absence	5	5
Sodium-n-dipropylacetate (DPA)	300[c]	17	absence	4	4
Ethosuximide (ESI)	300[d]	60	absence	8	8
Phensuximide (PSI)	30-300[d]	60	absence[h]	6	0
Acetazolamide (AAA)	100-300[e]	60, 110	absence[h]	5	0
Sodium-phenobarbital (PHB)	30[f]	30, 60	convulsive[h]	6	2
Sodium-diphenylhydantoin (DPH)	135[b]	60	convulsive	6	0
Mephenytoin (MPH)	70-135[g]	60, 120	convulsive	4	0

a) Refers to the time lag between the first injection (antiepileptic drug or solvent) and the second one (GHB or saline) in each rat.

b) (Everett and Richards, 1944)

c) (Meunier et al., 1963)

d) (Chen et al., 1963)

e) (Chen et al., 1968)

f) (Truitt et al., 1960)

g) (Chen and Ensor, 1950)

h) The specificity or efficacy of these compounds is controversial (see 4.4.).

DPA alone had no influence on the EEG, but caused increased motor activity during the first 10 min after administration. Rats treated with DPA and GHB showed an EEG similar to that in untreated rats.

ESI caused a desynchronization of the EEG, lasting for about 110 min. During this period the rats were ataxic. Injections of ESI, preceding GHB-treatment, prevented the appearance of the hypersynchrony and the EEG remained desynchronized for 50 min after GHB administration.

PSI caused a desynchronization of the EEG and a decrease of muscle tone, resulting in ataxia. The intensity and duration of both phenomena were dose related and lasted for 30-180 min. When doses of 150 or 300 mg/kg PSI were followed by GHB, the hypersynchronous EEG was not seen, but instead, a pattern of polyphasic bursts with intermittent electrical silence appeared, starting 10-12 min after the GHB and lasting for 50-80 min. This pattern was similar to that observed after administration of 500-700 mg/kg GHB alone (Marcus et al., 1967; 1976). After the polyphasic bursts, a desynchronized EEG appeared which was followed by the alternating states of vigilance. Combination of 30 or 75 mg/kg PSI with GHB caused intermittent bursts starting 2-3 min after GHB administration. These bursts were less frequent and had a lower amplitude than those after control GHB injections. Five min after GHB, continuous hypersynchrony appeared, sometimes interrupted by a synchronous EEG for 3-5 s. 50-60 min after GHB the continuous hypersynchrony changed into intermittent bursts and 70 min after GHB the EEG showed the normal alternating states of vigilance once again.

AAA had no effect on EEG or behavior. When given in a relatively wide dose-range (100-300 mg/kg) and for different periods before GHB, it exerted no influence on the GHB-induced hypersynchrony.

Administration of PHB caused slight sedation in the rats, but had no influence on the EEG. In combination with GHB it antagonized the hypersynchronous EEG pattern in 2 out of 6 experiments, whereas in the remaining 4 experiments, GHB caused hypersynchrony, as in control experiments. For the time-limits used, the effect of PHB was independent of the duration of the period between the two injections.

DPH caused ataxia in all rats, but did not change the EEG pattern. It had no effect upon the GHB-hypersynchrony.

MPH in the highest dose used (135 mg/kg) caused severe ataxia. The lower dose (70 mg/kg) caused minor ataxia. Neither dose had any influence on the EEG when given alone. GHB, administered after MPH, induced polyphasic bursts with intermittent electrical silence, similar to the patterns observed after higher doses of GHB (500-700 mg/kg) or, as described above, after a combination of PSI and GHB (200 mg/kg).

4.4. Discussion

Three of the most specific anti-absence drugs currently used in therapy are ESI, DPA and TMD (Aicardi, 1975). These compounds completely antagonized the appearance of the hypersynchronous EEG pattern induced by GHB. DPH and MPH are effective in the treatment of generalized convulsive epilepsy (Coatsworth and Penry, 1972), but not against absences. Both drugs failed to exert an inhibitory influence on the GHB-induced hypersynchrony. In fact MPH seemed to potentiate the effect of GHB. These results suggest a specific sensitivity of the GHB-induced EEG pattern to anti-absence drugs.

The succinimides are generally classified as anti-absence agents (Coatsworth and Penry, 1972); however, PSI is less effective, clinically, against absence epilepsy than the structurally related ESI (Aicardi, 1975). In animal experiments, PSI showed a less specific anti-absence profile than did ESI and acted more like drugs that are effective against psychomotor epilepsy (Chen et al., 1963). This difference in activity has been attributed to the additional phenyl moiety in PSI as compared to ESI (Mercier, 1973). In our experiments, PSI did not antagonize and even facilitated the GHB-induced hypersynchrony. Conflicting views have been reported concerning the efficacy of AAA in controlling absence epilepsy. It has been reported that AAA is the most specific anti-absence agent (Millichap and Aymat, 1967), while others doubt its clinical effectiveness (Lombroso and Forxythe, 1960). In our experiments, AAA in a dose-range of 100-300 mg/kg, which covers the anticonvulsive dose (Chen et al., 1968), did not influence the GHB-

induced hypersynchrony. In view of the questionable anti-absence specificity of PSI and AAA, the results obtained with these compounds are not in contradiction to our suggestion that anti-absence drugs specifically antagonize GHB-induced hypersynchrony.

PHB showed an ambiguous effect in our experiments; in two cases GHB hypersynchrony was antagonized, whereas in the remaining 4 experiments, PHB had no effect on the EEG pattern. This is in agreement with our suggestion since PHB, though mainly used against convulsive epilepsy, is also reported to be sometimes effective in absence epilepsy (Coatsworth and Penry, 1972).

Of the many animal models used to test potential antiepileptic drugs, no single model can give a reliable answer concerning the anti-absence specificity of the investigated compound (Naquet and Lanoir, 1973). The most commonly used tests to evaluate possible anti-absence efficacy of drugs are seizures, elicited by either intravenous or subcutaneous administration of pentylenetetrazol (Swinyard, 1969). However, neither of these methods reproduces absence epilepsy, either behaviorally or electrographically, but instead they resemble convulsive epilepsy. Moreover, activity against pentylenetetrazol is not essential, nor constantly predictive, for efficacy in the treatment of absence epilepsy (Chen et al., 1963). Recently, two new laboratory models for the evaluation of anti-absence drugs have been proposed. One employs application of conjugated estrogens to the cerebral cortex of the cat, inducing 2-3 Hz spike-wave complexes (Fowler and Julien, 1974). The other uses photically evoked afterdischarges in the rat (Shearer et al., 1976); it is worthwile emphasizing here that photically evoked afterdischarges were enhanced by GHB (Kharkevich et al., 1971). Both phenomena were antagonized specifically by anti-absence drugs and therefore were claimed to be suitable models for testing this type of drugs (Fowler and Julien, 1974; Shearer et al., 1976). In view of the present results, the GHB-induced EEG may be an additional tool in testing potential anti-absence drugs.

This GHB effect is not only a possible functional model of absence epilepsy (i.e. useful in the evaluation of drugs), but also a phenomenological model of this type of epilepsy, imitating some characteristic phenomena of absence epilepsy. These phenomena, both electrographical

and behavioral, have been mentioned already in the previous chapter, and the specific sensitivity to anti-absence drugs may now be added as another similarity between the GHB-induced EEG hypersynchrony and absence epilepsy.

CHAPTER 5. THE ROLE OF THE DOPAMINERGIC SYSTEM IN GHB-INDUCED EEG HYPERSYNCHRONIZATION

5.1. Introduction

In view of the resemblance of GHB-induced EEG hypersynchrony to absence epilepsy, as discussed in Chapters 3 and 4, it was of interest to investigate the mechanism of action of GHB, at the level of neurotransmission, in generating the hypersynchrony.

The most documented effect of GHB on neurotransmission is the inhibition of the impulse flow in the nigro-neostriatal dopaminergic pathway (Walters et al., 1972; Roth et al., 1973; Stock et al., 1973). GHB prevents release of DA from these neurons, thus inducing an accumulation of DA in the nerve terminals of the neurons in the corpus striatum (Gessa et al., 1966; Aghajanian and Roth, 1970). A similar accumulation of DA was observed in nerve terminals situated in the nucleus accumbens and tuberculum olfactorium (Aghajanian and Roth, 1970).

In experiments with several animal models of epilepsy, it has been suggested that DA receptor stimulation is implicated in antiepileptic activity, whereas blockade of DA receptors or a decreased availability of DA at the receptor site enhances epileptic phenomena. These models include clonic convulsive seizures induced by electroshock in the rat (Stull et al., 1973), by auditory stimulation in a sensitive mouse strain (Anlezark et al., 1976) and by handling in Mongolian gerbils (Cox and Lomax, 1976); hypersynchronous EEG phenomena induced by cobalt-implantation in the rat cortex (Dow et al., 1974) and both hypersynchronous EEG spikes and myoclonies induced by light flashes in the photosensitive baboon Papio papio (Meldrum et al., 1975). In addition, recent data indicate that DA is also involved in cortical EEG desynchronization (Kafi and Gaillard, 1976).

In view of these data, we investigated the possibility that decreased stimulation of DA receptors, caused by inhibition of impulse flow in dopaminergic neurons, is implicated in GHB-induced EEG hypersynchrony. To this end we studied the effects of DA receptor agonists and a DA receptor blocker on GHB-induced hypersynchrony.

5.2. Materials and methods

22 rats with chronically implanted electrodes were used. Each rat was allowed to recover for at least 7 days between two consecutive experimental sessions. After adaptation to the environment and baseline EEG registration, at 11.30 h one of the following compounds was administered: apomorphine-HCl (0.2-8.0 mg/kg), 1-(2-pyrimidyl)-4-piperonyl piperazine (piribedil, ET-495; 2.5-10.0 mg/kg), (3,4-dihydroxyphenylamino)-2-imidazoline (DPI; 1-5 mg/kg), d-amphetamine sulphate (1.5-6.0 mg/kg), haloperidol (0.5-1.0 mg/kg) or saline. After a time interval, fixed for every drug as specified in table 5.1., GHB (200 mg/kg) was injected. The chosen time interval between the two injections was such that the maximal effects of the drugs would coincide. After the administration of GHB, the EEG (and in some cases the EMG too) was recorded continuously for either 2 hours or until its pattern had returned to normal, whichever effect occurred later.

In a series of 4 experimental sessions on any group of 4 rats, each of 3 rats initially received a different dose of test compound, the fourth rat receiving an injection of saline. In the succeeding 3 experiments the doses were randomized so that each rat had eventually received all 3 doses of test compound and saline by the end of the series.

In order to investigate the effect of apomorphine, 7 different doses were tested, and therefore three groups of rats were used. Thus, the rats of each group received three doses plus a saline control treatment alternately during 4 successive experimental sessions. In the experiments with DPI and haloperidol, one rat in each session received only DPI or haloperidol and not GHB. Therefore, only two doses of these compounds were tested in combination with GHB. The experiments with DPI were not all carried out on the same rats.

In preliminary experiments it appeared that some of the drugs did not totally antagonize the EEG effects of GHB. Moreover, it was expected that some drugs might enhance the effect of GHB. Thus, scoring the effects of drugs as "antagonizing" or "not antagonizing" the GHB hypersynchrony, as in the experiments mentioned in Chapter 4, was not a sensitive enough system for the present experiments. Therefore, we

measured the duration of the GHB-induced hypersynchronous EEG effects, from the appearance of the first hypersynchronous burst after GHB administration until the disappearance of the last burst. The results were evaluated statistically using the paired Student's t-test.

5.3. Results

5.3.1. Behavior

Apomorphine in the dose range of 2-8 mg/kg and all doses of amphetamine used, enhanced motor activity in the rats as observed visually, and induced turning and stereotyped licking and chewing. When apomorphine was followed by GHB the rats lay quiet during periods of EEG hypersynchrony. When a combination of amphetamine and GHB was followed by EEG hypersynchrony, the rats were less active than after injection of amphetamine alone. Following injection of DPI the rats showed piloerection and a state of apparent flaccid inactivity, though occasionally they walked across the cage for a short period. Also, after handling, they were active for about a minute. The other compounds used had no visible effect on the behavior of the rats.

5.3.2. EEG

Injection of apomorphine or amphetamine, alone, or followed by a saline injection, induced a continuous desynchronized EEG in the rats. The duration of this desynchronization was dose dependent: after 0.5 mg/kg apomorphine it lasted for 30 min, 1 mg/kg of this compound induced a desynchronization for 60 min and after the highest dose used (8 mg/kg) it lasted for about 90 min. The duration of the desynchronized EEG after 0.2 mg/kg apomorphine was not different from that after a saline injection. The amphetamine-induced desynchronization lasted from 2 h after 1.5 mg/kg up to 3½ h after 6 mg/kg.

The lower dose of DPI (1 mg/kg) induced an apparently normal EEG. The higher dose (5 mg/kg) induced a mainly desynchronized EEG for 2-3 h with occasional groups of hypersynchronous bursts. Such a group

consisted of 4-8 bursts and lasted for 1-2 min. Between the groups a continuous desynchronized EEG was seen for 3-5 min. Each burst consisted of 5-10 peaks (300-400 μV) and lasted for 2-3 s. When compared to the GHB-induced bursts, those after DPI were somewhat lower and occurred less frequently, while the peaks were closer to each other.

The EEG of piribedil- or haloperidol-injected rats was not different from that after a saline injection. Haloperidol, in the dose range used, had no visible synchronizing effect on the EEG.

Pretreatment of rats with apomorphine, piribedil or haloperidol had no effect on the pattern of the GHB-induced EEG hypersynchrony, nor on its duration (table 5.1.). Amphetamine (1.5-6.0 mg/kg), however, antagonized the GHB-induced hypersynchrony in most rats (table 5.1.). In this case a continuously desynchronized EEG was seen and lasted for about 3 h. The lower dose of DPI (1 mg/kg) had no influence on the effect of GHB; the higher dose (5 mg/kg) however, significantly prolonged the duration of the GHB-induced hypersynchrony (table 5.1.).

5.4. Discussion

Apomorphine and piribedil are both direct DA receptor stimulants (Ernst, 1967; Andén et al., 1967; Corrodi et al., 1971). Both compounds block GHB-induced accumulation of DA in rat whole brain (Handforth and Sourkes, 1975), rat neostriatum (Walters and Roth, 1974) and rat cingulate and frontal cortex (Pericic and Walters, 1976). This effect was suggested to be due to stimulation of presynaptic DA receptors (Walters and Roth, 1974). In the dose ranges used here, apomorphine and piribedil may have stimulated both pre- and postsynaptic DA receptors (Strömbom, 1976; Corrodi et al., 1972). Moreover, the present dose range of apomorphine covers the doses which are effective against experimental seizures (Stull et al., 1973; Dow et al., 1974; Meldrum et al., 1975; Anlezark et al., 1976; Cox and Lomax, 1976). The dose range of piribedil, antagonizing epileptic phenomena induced by photic stimulation in the baboon, Papio papio, (Meldrum et al., 1975) was covered by the doses we used. The failure of apomorphine and piribedil, and of the DA receptor blocker haloperidol, to modulate the GHB-

Table 5.1. Duration of GHB-induced EEG hypersynchrony after pretreatment with different drugs.

Pretreatment	Time[a] (min)	Dose (mg/kg)	Duration[b] (min)	
Control	0	-	62.8 ± 5.6	(12)
Apomorphine		0.2	39.8 ± 13.3	(4)
		0.5	60.3 ± 7.3	(4)
		0.7	55.3 ± 5.2	(4)
		1.0	66.0 ± 15.1	(4)
		2.0	71.4 ± 9.6	(8)
		4.0	65.0 ± 4.1	(4)
		8.0	73.0 ± 9.1	(4)
Control	20	-	66.0 ± 5.3	(4)
Piribedil		2.5	76.3 ± 9.1	(4)
		5.0	72.8 ± 6.5	(4)
		10.0	75.8 ± 2.7	(4)
Control	20	-	72.8 ± 2.5	(4)
DPI		1.0	78.0 ± 12.7	(4)
		5.0	136.8 ± 9.2*	(4)
Control	10	-	74.5 ± 5.0	(4)
Amphetamine		1.5	4.5 ± 2.6*	(4)
		3.0	18.5 ± 18.5*	(4)
		6.0	9.8 ± 9.8*	(4)
Control	30	-	70.0 ± 4.3	(4)
Haloperidol		0.5	88.3 ± 12.8	(4)
		1.0	77.3 ± 10.9	(4)

a) Time refers to the interval between the pretreatment with the drug in question, and GHB-administration.

b) Values are means ± SEM. Numbers of observations are given in brackets. Significance of differences (versus saline controls) was determined by paired, two-tailed Student's t-test. *: $p<0.05$.

induced EEG hypersynchrony in the present experiments, indicates that inhibition of the nigrostriatal dopaminergic system is not likely to be responsible for the GHB hypersynchrony. This is in accordance with the observations that striatectomy has no influence on the EEG or behavioral effects of GHB in the cat (Marcus et al., 1976) and that, in the rat, after striatectomy, pentylenetetrazol-induced EEG seizures remain unaltered, while the threshold for convulsions is elevated (Avakyan, 1976), indicating that the neostriatum is not involved in EEG hypersynchrony.

Amphetamine is specifically effective in the treatment of absence epilepsy (Livingston et al., 1948). Its inhibitory action on the GHB-induced EEG hypersynchrony, in the present study, is in accordance with the effect of other specific anti-absence drugs (see Chapter 4). This supports the hypothesis that some similarity exists between the effects of 200 mg/kg GHB in the rat and absence epilepsy in man, and that this phenomenon might be a suitable tool for testing potential anti-absence drugs.

Amphetamine increases the availability of central catecholamines for their receptor sites by enhancing their release and inhibiting their reuptake and biotransformation. Thus, amphetamine may, indirectly stimulate central catecholamine receptors. Anticonvulsive actions of amphetamine in two animal models of epilepsy, namely electroshock and pentylenetetrazol convulsions, appeared to be mediated through indirect stimulation of noradrenaline receptors (Rudzik and Johnson, 1970; Riffee and Gerald, 1976). However, the present results do not permit definitive conclusions to be drawn concerning the mode of action of amphetamine in antagonizing the GHB-induced hypersynchrony. Nevertheless, indirect stimulation of noradrenergic receptors might be responsible for this antagonistic effect.

DPI has become of interest only recently, and its pharmacological properties have not yet been studied extensively. In the snail Helix aspersa, DPI has been found to be a selective agonist at DA receptors mediating neuronal inhibition (DA_i receptors); these receptors are pharmacologically distinct from excitation-mediating DA receptors (DA_e receptors), which are stimulated by apomorphine (Struyker Boudier et al., 1975). Two anatomically different DA systems are proposed for

many animals, including the rat (Fuxe et al., 1975; Cools and Van Rossum, 1976; Cools et al., 1976). As far as the rat is concerned, DA_e receptors predominate in the neostriatum, whereas DA_i receptors, the type activated by DPI, might occur mainly in the limbic system (Cools and Van Rossum, 1976; Cools et al., 1976). It is worthwhile emphasizing that a sufficiently high dose of DPI (5 mg/kg) prolonged the duration of the GHB-induced hypersynchrony. This might indicate that inhibition of the mesolimbic DA system by stimulation of DA_i receptors has a facilitatory effect on the generation of GHB-induced EEG hypersynchrony, possibly by inhibition of a seizure-suppressing mechanism. On the other hand, also GBL, the precursor of GHB, was suggested to exert an inhibitory effect on the mesolimbic DA system (Fuxe et al., 1975) and may, thus, inhibit this seizure-suppressing mechanism. This might explain not only the excitatory effect of GHB, but also the synergism between GHB and DPI. This line of thought makes it also understandable that apomorphine did not antagonize GHB hypersynchrony, since both compounds would act on different DA-systems.

Another property of DPI is its ability to form a complex with pyridoxal phosphate (Th. De Boer, personal communication). Pyridoxal phosphate is a cofactor for many enzymes, including glutamic acid decarboxylase (GAD), the enzyme catalyzing the synthesis of GABA from glutamate (see fig. 6.1.). Thus, it is conceivable that DPI inhibits GAD activity, as do many other pyridoxal phosphate complexing agents (Tapia, 1974). GAD inhibition is an important factor in the generation of various epileptic phenomena (Wood and Peesker, 1974). Thus, it is conceivable that the enhancement, by DPI, of the present epileptic phenomenon, namely the absence-like effect of GHB, is originated by inhibition of GAD. Moreover, GHB itself is also a potent GAD inhibitor (Godin and Mark, 1967; Clifford et al., 1973; Dye and Taberner, 1975; Tunnicliff, 1976), and this property is probably involved in the generation of GHB hypersynchrony (see Chapter 6).

Evidently, a more specific experimental approach is necessary to elucidate the role of DPI in GHB-induced EEG hypersynchrony. The possibility cannot be excluded that different types of DA receptors might have different roles in epileptic phenomena, but in view of the results and conclusions of the next Chapter, it appears probable that the

enhancement of GHB-hypersynchrony by DPI can be explained by an inhibition of GABA synthesis.

CHAPTER 6. THE ROLE OF GABA METABOLISM IN GHB-INDUCED EEG HYPERSYNCHRONIZATION

6.1. Introduction

Evidence has been collated from many investigations, indicating that modulation of the availability of gamma-aminobutyric acid (GABA) for its receptor sites, either by inhibition of its metabolism or by blockade of its receptors, is directly related to epileptic phenomena (Meldrum, 1975). The activity of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) (fig. 6.1.) appeared to be the most important

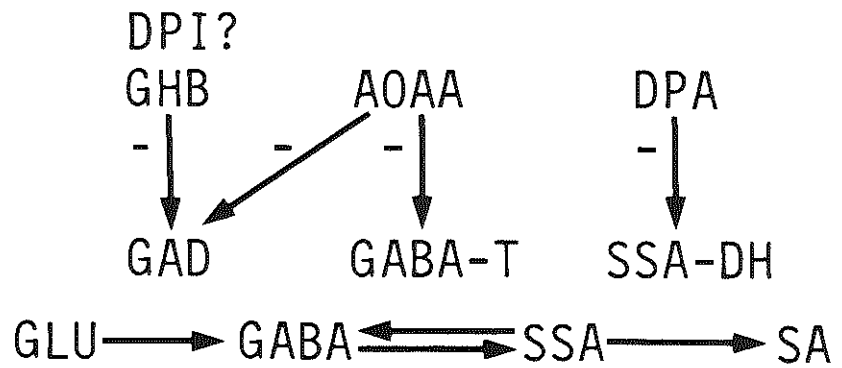


Fig. 6.1. Schematic outline of a part of the GABA-shunt of the citric acid cycle, showing synthesis and metabolism of GABA, the enzymes involved and some drugs inhibiting the enzymes. Abbreviations used: metabolites: GLU = glutamic acid; GABA = gamma-aminobutyric acid; SSA = succinate semialdehyde; SA = succinic acid; enzymes: GAD = glutamic acid decarboxylase; GABA-T = GABA-transaminase; SSA-DH = SSA-dehydrogenase; drugs: DPI = (3,4-dihydroxyphenylamino)-2-imidazoline; GHB = gamma-hydroxybutyric acid; AOAA = amino-oxyacetic acid; DPA = n-dipropylacetate.

factor in the antiepileptic action of the GABA-system, the continuous release of GABA being dependent on its synthesis (Tapia, 1974). Moreover, a good correlation exists between GAD inhibition and induction of convulsions by some drugs (Wood and Peesker, 1972; 1973; Tapia, 1974). Wood and Peesker (1974) developed an equation on an empirical basis providing a relationship between the excitable state of the brain and a function of GABA metabolism. The concept embodied in the equation is

that the excitable state of the brain is determined primarily by the rate of synthesis of GABA by GAD, but that the concentration of GABA in the cells also plays a role (Wood and Peesker, 1974).

GHB is an inhibitor of GAD activity in vivo in the brains of mice (Clifford et al., 1973) and rats (Godin and Mark, 1967; Tunnicliff, 1976), and in mouse brain tissue in vitro, GHB inhibited GAD competitively (Dye and Taberner, 1975). Thus, it is conceivable that the GHB-induced EEG hypersynchrony, reminiscent of absence epilepsy, is related to modulation of GABA metabolism.

In order to collect additional evidence for a relationship between GHB effects and the GABA system, we prevented GABA biotransformation by inhibiting the transaminating enzyme GABA-T (fig. 6.1.) with aminooxyacetic acid (AOAA) (Wallach, 1961; Baxter and Roberts, 1961), a substance with anticonvulsive properties in several animal models of epilepsy (Da Vanzo et al., 1961; Wood and Peesker, 1973). The effect of AOAA on the duration of GHB-induced EEG hypersynchrony was then measured.

6.2. Materials and methods

8 rats with chronically implanted electrodes were used, divided into two groups of 4 rats each. Between two consecutive experimental sessions, the rats were allowed to recover for at least seven days. In the first experiment of a series of 4, on each group, one rat received a saline injection and the 3 other rats were treated with AOAA-hemihydrochloride in doses of 7.5, 15 or 30 mg/kg intraperitoneally (i.p.). In the succeeding three experimental sessions, these pretreatments were randomized, so that eventually, the rats of each group received each pretreatment alternately. After a fixed period, 1 h 15 min in one group and 5 h 30 min in the other, the rats received GHB (200 mg/kg i.p.). In the group where the interval between both injections was 5 h 30 min, the rats received the first injection immediately at the onset of the session, without an initial adaptation period or baseline EEG registration. In both groups, after the GHB injection, the EEG was registered continuously for 2-3 h.

As in the previous chapter, the total duration of the EEG hypersynchrony after GHB administration was measured. Additionally, the duration of continuous hypersynchrony was also determined. Continuous hypersynchrony was defined as a period of at least one minute of uninterrupted EEG hypersynchrony. The total duration of such periods was then calculated. Thus, "continuous hypersynchrony" does not mean that during the whole period the EEG was uninterrupted by patterns of lower amplitude; in many cases several scattered periods of continuous hypersynchrony were observed within the total duration of hypersynchrony.

6.3. Results

AOAA in doses of 7.5 or 15 mg/kg, when injected alone, had no effect on the EEG nor on the behavior of the rats. 30 mg/kg induced a synchronous slow wave EEG; the rats behaved sluggishly.

The lowest dose of AOAA (7.5 mg/kg) had no effect on the duration of the GHB-induced hypersynchrony but the highest dose (30 mg/kg) prolonged the duration significantly in both series of experiments (fig. 6.2. A,B). The effect of 15 mg/kg AOAA was dependent on the duration of the pretreatment period: when injected 1 h 15 min prior to GHB, it tended to prolong the duration of the hypersynchrony (fig. 6.2. A), but when injected 5 h 30 min before GHB this dose significantly inhibited the duration of the hypersynchrony (fig. 6.2. B).

The continuous hypersynchrony followed the changes observed for the total duration of hypersynchrony, but never reached significant differences versus control treatments.

6.4. Discussion

The mechanism of action of AOAA possesses a dual character in two respects; the results, obtained with this compound, will be interpreted accordingly.

First, the antiepileptic action of AOAA involves two mechanisms,

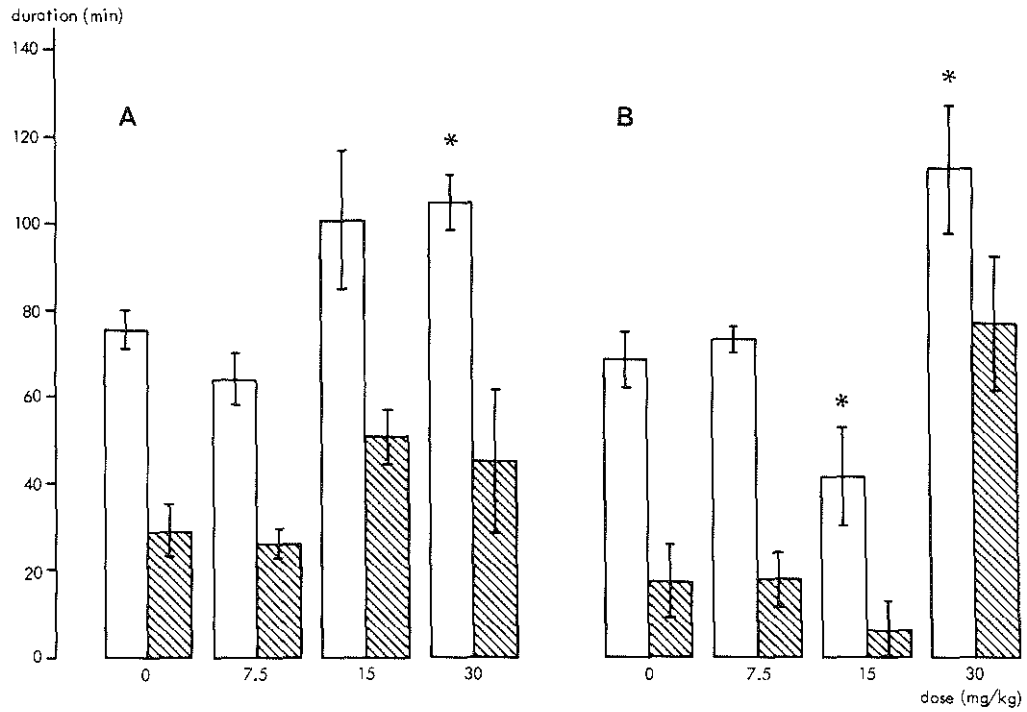


Fig. 6.2. Effect of AOAA, injected 1 h 15 min (A) or 5 h 30 min (B) prior to GHB (200 mg/kg), on the duration of GHB-induced EEG hypersynchrony. White bars: total duration of hypersynchrony; hatched bars: duration of the continuous phase of hypersynchrony. Values are means ± SEM from 4 rats. Significance of differences (versus saline-injected controls) was determined by paired, two-tailed Student's t-test. *$p<0.05$.

as described by Wood and Peesker (1976). One involves GABA metabolism and is maximally effective 6 h after AOAA administration. It is the sole mechanism counteracting convulsions induced by allylglycine. These convulsions are closely related to GAD inhibition by allylglycine (Fisher and Davies, 1976) and their antagonism by AOAA would seem to be mediated by elevation of GABA levels (Wood and Peesker, 1976). The other mechanism, not involving GABA metabolism, is maximally effective 1.5 h after AOAA injection and is absent after 6 h. It is the sole

factor in the action of AOAA operative against electroshock convulsions but its basic mechanism is unknown (Wood and Peesker, 1976). The present results show that the middle dose of AOAA (15 mg/kg) antagonized the GHB hypersynchrony if injected 6 h before the appearance of the maximal GHB effect. The same dose, however, administered 1 h 45 min before the maximal effect of GHB, did not reduce the duration of the GHB hypersynchrony, but rather tended to prolong it as it did the duration of GHB-induced loss of righting reflex (Benton et al., 1973). Interpreting these results in terms of the above theory, a temporal relationship emerges between the antagonism of GHB hypersynchrony by AOAA and the maximal involvement of GABA metabolism in the antiepileptic activity of AOAA. On the other hand, at the moment when the GABA-related antiepileptic mechanism of AOAA is submaximal, but the non-GABA mechanism is maximal, no inhibition of GHB hypersynchrony was observed. These results do not provide direct evidence indicating that the GHB-induced EEG hypersynchrony is mediated via the GABA system. They do indicate, however, that the GHB effects are very much influenced by changes in GABA metabolism.

The second dual mechanism of AOAA might explain why its antiepileptic action, 6 h after administration, is only exerted by the middle dose. AOAA, being an anticonvulsant in low doses (5-25 mg/kg in mammals, $\pm$ 5 mg/kg in chick), also shows convulsant actions at higher doses (Da Vanzo et al., 1961; Meldrum et al., 1970; Osuide, 1972 a; Wood and Peesker, 1973). This has been explained on the basis of its specific inhibition of GABA-T at lower doses, thus enhancing brain GABA concentration, while at higher doses (over 25 mg/kg), AOAA also inhibits GAD (fig. 6.1.) (Wood and Peesker, 1973), counteracting GABA formation and sensitizing the animal to epileptogenic stimuli (Meldrum, 1975). The latter mechanism might act synergistically with the GAD-inhibiting action of GHB. Thus, the prolongation of GHB hypersynchrony 6 h after pretreatment with 30 mg/kg AOAA may be due to synergistic GAD-inhibition by both compounds, whereas 15 mg/kg, a dose which particularly inhibits GABA-T, while having less influence on GAD, antagonized GHB. The effect of the lowest dose of AOAA, 7.5 mg/kg, on GABA metabolism is probably too small to influence GHB hypersynchrony.

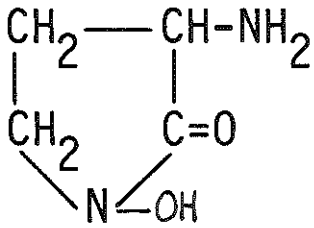
The specific anti-absence drug n-dipropylacetate (DPA) is a

blocker of the enzyme succinic acid semialdehyde dehydrogenase (Harvey et al., 1975; Anlezark et al., 1976), which is responsible for the second, rate-limiting step in GABA breakdown (fig. 6.1.). Our previous finding that DPA antagonizes the GHB-induced EEG hypersynchrony (see Chapter 4) might thus support our suggestion that GABA-metabolism is involved in GHB-hypersynchrony. The stronger inhibition of GHB hypersynchrony by DPA as compared to AOAA might be due to the fact that DPA blocks the rate-limiting step in GABA breakdown. The analogous finding that ethosuximide inhibits GABA-T activity (Sawaya et al., 1975) might explain the inhibition of GHB hypersynchrony by this specific anti-absence agent as reported in Chapter 4. Conversely, the enhancement of GHB-induced hypersynchrony by DPI (see Chapter 5.3.) might be attributed to its possible counteraction of GABA synthesis by GAD inhibition (fig 6.1.) through forming a complex with pyridoxal phosphate (see Chapter 5.4.).

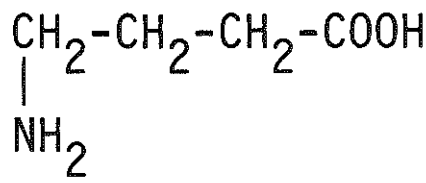
CHAPTER 7. THE EFFECT OF HA-966 ON GHB-INDUCED EEG HYPERSYNCHRONIZATION

7.1. Introduction

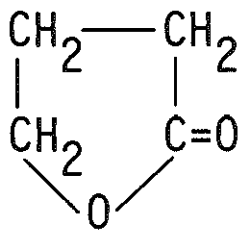
The synthetic compound 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) resembles GHB and GBL in many respects. Its chemical structure, possessing a 4-carbon chain, is related to the butyrate derivatives GABA, GHB and GBL (fig. 7.1.), though it might also fit the receptors of



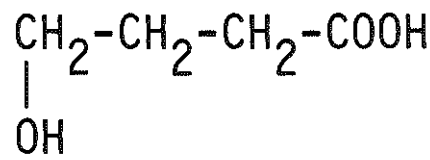
HA-966



GABA



GBL



GHB

Fig. 7.1. Structural relationship between HA-966 and the butyrate derivatives GABA, GHB and GBL.

putative amino acid neurotransmitters (Davies and Watkins, 1973). Administration of HA-966 to rats, rabbits and monkeys induced a sedative state and EEG hypersynchrony, similar to those caused by GHB and GBL (Bonta et al., 1971). HA-966 was shown to inhibit the impulse flow in

the nigro-neostriatal DA system (Van Valkenburg, 1976; Walters and Roth, 1976), antagonizing DA release from striatal nerve endings and thus inducing, like GHB and GBL, an increased DA concentration in the corpus striatum (Bonta et al., 1971; Hillen and Noach, 1971; Hillen, 1972). Another resemblance to GHB is the reported inhibition of GAD by HA-966 (Hillen et al., 1969) and a probably related decrease in brain GABA concentration (Möhler et al., 1975).

In view of these similarities, it was of interest to investigate the effect of HA-966 on GHB-induced EEG hypersynchrony.

7.2. Materials and methods

Four rats with chronically implanted electrodes were used in a series of 4 experimental sessions. In each session, after 1 h for adaptation to the connecting cables and 1 h baseline EEG registration, the 4 rats were pretreated with saline or 2.5, 5 or 10 mg/kg HA-966 respectively. 15 min later GHB (200 mg/kg) was administered to all the rats. The EEG was then registered continuously for 2-3 h. In the 4 successive sessions, the pretreatments were randomized, so that, eventually, each rat received each pretreatment once. Between the sessions, the rats were allowed to recover for at least 7 days.

As in the previous chapter, the total duration of EEG hypersynchrony and the duration of the continuous hypersynchrony were determined separately. The pattern of polyphasic EEG spikes with intermittent electrical silence was counted as continuous hypersynchrony.

7.3. Results

Pretreatment with HA-966 caused a dose-related increase in the duration of both total and continuous GHB-induced EEG hypersynchrony (fig. 7.2.). The values obtained after each dose of HA-966 were not only different from control values, but also from the effects of other doses. Exceptions to this were the duration of continuous hypersynchrony after 2.5 mg/kg HA-966, which was not different from the control

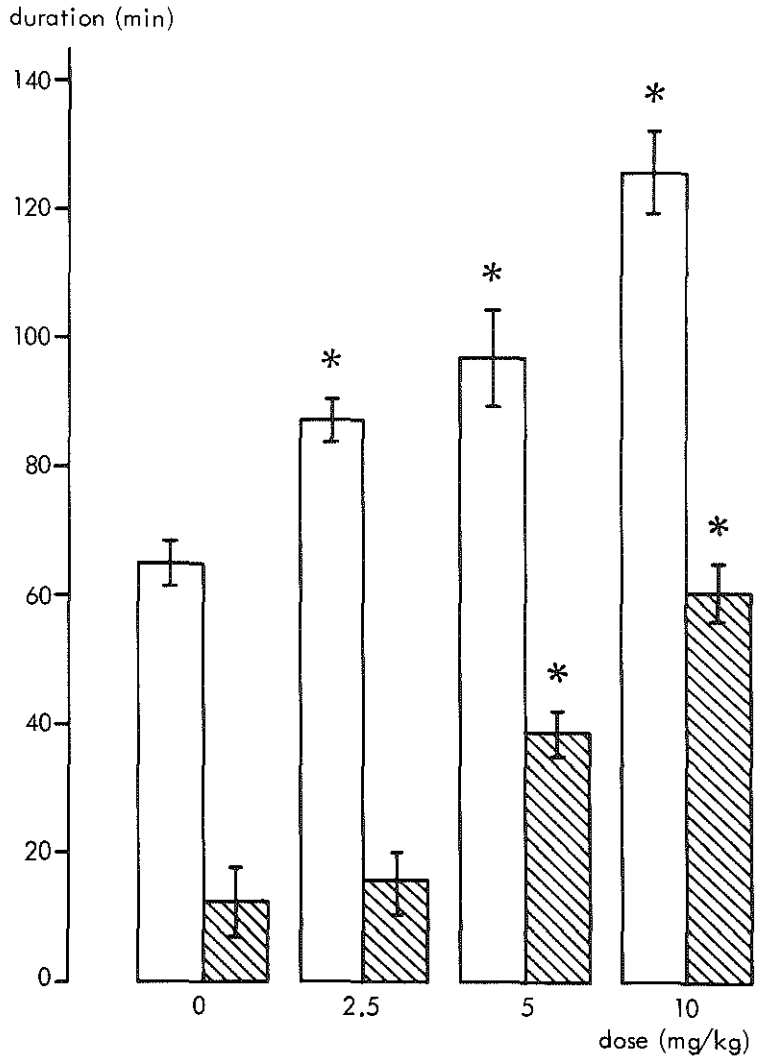


Fig. 7.2. Effect of HA-966, injected 15 min prior to GHB (200 mg/kg), on duration of GHB-induced EEG hypersynchrony. White bars: total duration of hypersynchrony; hatched bars: duration of the continuous phase of hypersynchrony. Values are means + SEM from 4 rats. Significance of differences (versus saline-injected controls) was determined by paired two-tailed Student's t-test. *$p<0.05$.

value, and the total durations of hypersynchrony after pretreatment

with 2.5 and 5 mg/kg, which were also not significantly different.

The highest dose of HA-966 used, 10 mg/kg, in combination with 200 mg/kg GHB, induced an EEG pattern consisting of polyphasic spikes separated by short isoelectric periods, as observed after sole administration of 500-700 mg/kg GHB in the rat (Marcus et al., 1967; 1976).

The behavior of the rats, pretreated with HA-966, was similar to that of animals which had received only 200 mg/kg GHB, on the understanding that the duration of the behavioral sedation was prolonged to the same measure as was the EEG hypersynchrony.

7.4. Discussion

The results clearly show the synergistic action of HA-966 and GHB. This might indicate a similar mechanism of action of these compounds. As stated in the introduction, HA-966 and GHB have in common both an antagonistic action on the nigro-striatal DA neurons and an inhibition of GABA synthesis. The present results, however, do not distinguish between either one or the other of these possibilities being involved in the hypersynchronizing effect of HA-966.

GHB has been reported to increase brain levels of the putative excitatory amino-acid neurotransmitters glutamate (Promislov and Solovjova, 1973) and aspartate (Margolis, 1969). As far as the latter compound is concerned, the increase seems to be caused by inhibition of its metabolism (Margolis, 1969). Therefore, a relationship has been suggested between the excitatory effect of GHB on the EEG and the enhancement of glutamate and aspartate levels by GHB (Marcus et al., 1976). One would then expect that GHB acts through enhancement of the excitatory action of glutamate and aspartate. HA-966, however, is a selective antagonist of neuronal excitation induced by glutamate and aspartate (Davies and Watkins, 1972; Curtis et al., 1973). The present results show that HA-966 and GHB act synergistically. Therefore, it seems unlikely that GHB induces EEG hypersynchrony by increasing glutamate or aspartate in brain.

CHAPTER 8. THE ROLE OF THE NIGROSTRIATAL SYSTEM IN SEDATION AND EEG SYNCHRONY, INDUCED BY HA-966

8.1. Introduction

The involvement of DA neurotransmission in EEG desynchronization and antiepileptic mechanisms has been emphasized in Chapter 5. Both GHB and HA-966 inhibit DA neurotransmission, at least in the nigrostriatal system, a bundle of DA neurons with their cell bodies in the substantia nigra compacta (SNC) and terminating in the corpus striatum. Therefore, it was conceivable that this inhibitory effect of GHB and HA-966 might be involved in the generation of EEG phenomena by these compounds. It appeared, however, that modulation of DA-neurotransmission did not influence the GHB-induced EEG hypersynchrony, suggesting that involvement of the DA system in this epileptoid feature is unlikely (Chapter 5). In order to investigate whether the synchronizing and sedating effects of HA-966, similar to those observed after GHB (Bonta et al., 1971), are mediated through its influence on the nigrostriatal DA system, we compared the effects of HA-966 in rats with bilaterally lesioned or intact SNC.

8.2. Materials and methods

Rats with semi-chronically implanted electrodes were used (see 1.3.1.2.). Lesions were made in the SNC, according to the atlas of König and Klippel (1963), using the following parameters: A (frontal plane) 2.4 mm; L (sagittal plane) 1.8 mm and H (horizontal plane) 2.1 mm. Immediately after lesioning, the cortical electrodes were implanted. Histological analysis of the lesions was performed and only the results from rats with a totally destroyed SNC were considered. In these rats some damage to the structures adjacent to the SNC was noted. The results described here were obtained from 4 intact and 8 SNC-lesioned rats.

Each rat was used once only for an experiment, 7-10 days after implantation. In each session, 1 intact and 4 lesioned rats were used.

Before the experiments, the rats were kept overnight in the sound-proof cabin. At 09.00 h, water and food were removed from the cages and the rats were connected with the cables to the EEG apparatus. At about 11.00 h, baseline registrations were started.

The EEG registrations were made as follows. Every 30 min the EEG was registered for 60 s. Arousal was then induced by acoustic stimulation, using a sound generator (200 Hz, 20 dB for 2 s). Thereafter, the EEG was registered for another 90 s. The amplitude of the registered EEG was averaged (by a Grass 7P3) and then integrated (by a Grass 7P10). The integrator was calibrated so that 20 resets in a 1 min period corresponded to 150 μV. The number of integrator resets was determined as a whole for the 60 s pre-stimulation period and separately for every 10 s interval of the post-stimulation period. The average voltage of each interval was then calculated.

Between 11.00 h and 13.00 h, 4 such pre- and post-stimulation EEG's were registered. The mean of the last 3 registrations only was used, in order to exclude variations due to adaptation. At about 13.00 h, HA-966 (10 mg/kg intravenously) was administered to all rats. At 30, 60 and 90 min after injection, acoustic stimulation was applied and the amplitude values of the EEG's registered during each of the pre- and post-stimulation intervals were calculated. The amplitude value of each interval was averaged over the three post-injection registrations. Thus, each rat served as its own control before and after drug administration. Finally, mean and SEM of the amplitude values were calculated for each interval and for each group of rats (lesioned or intact) before and after HA-966 injection.

After the experiment, the rats were killed and the brains of lesioned rats were immersed in a neutralized, 10% formalin solution for fixation. After at least a week the brains were embedded in 12% gelatin and again fixed in formalin for at least 2 days. After being immersed in 20% alcohol for 24 h, the brains were mounted on the freezing stage of a microtome. Serial transversal sections (30 μm in thickness) were collected from the region of the SNC and stained by Cresyl-violet. The site and extent of the lesion was then established. As mentioned before, only results from rats with totally destroyed SNC were used. A schematic drawing of a representative SNC lesion is shown in fig. 8.1.

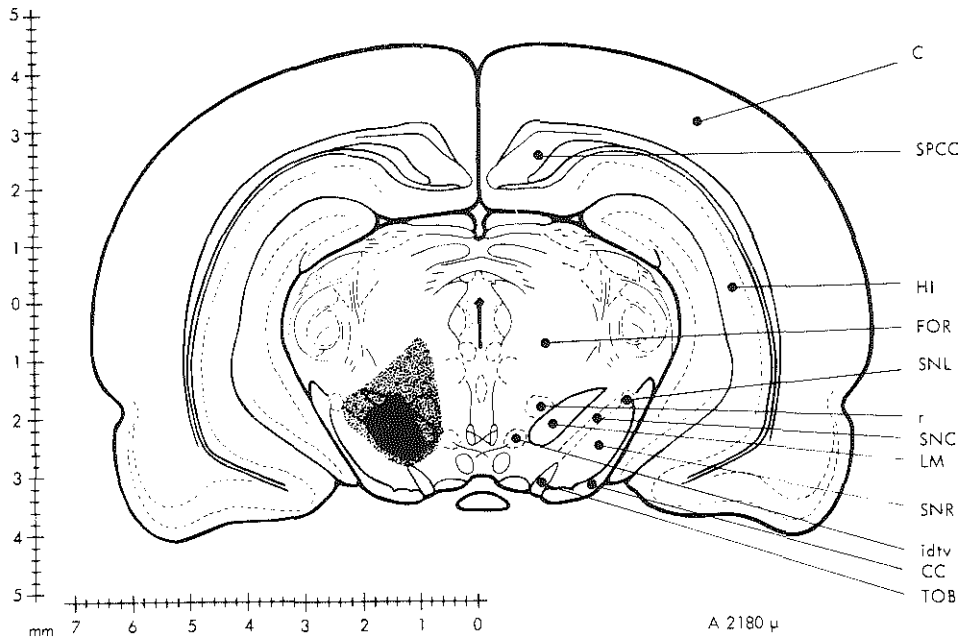


Fig. 8.1. Schematic drawing of a transversal section of a rat brain (A = 2180 µ according to the atlas of König and Klippel, 1963) showing the extent of the lesion in the Substantia nigra compacta in a representative rat. Black: totally destroyed; grey: proliferation of glia cells. Abbreviations used:

C	Cortex	SNR	Substantia nigra, zona reticulata
CC	Crus cerebri	SPCC	Splenium corporis callosi
FOR	Formatio reticularis	TOB	Tractus opticus basalis
HI	Hippocampus	idtv	Nucleus interstitialis decussationis tegmenti ventralis
LM	Lemniscus medialis	r	Nucleus ruber
SNC	Substantia nigra, zona compacta		
SNL	Substantia nigra, pars lateralis		

8.3. Results

Administration of HA-966 (10 mg/kg) induced inactive behavior, which lasted for about two hours.

The results, depicted in fig. 8.2., show that, before administration of HA-966, the acoustic stimulation caused a decrease in amplitude

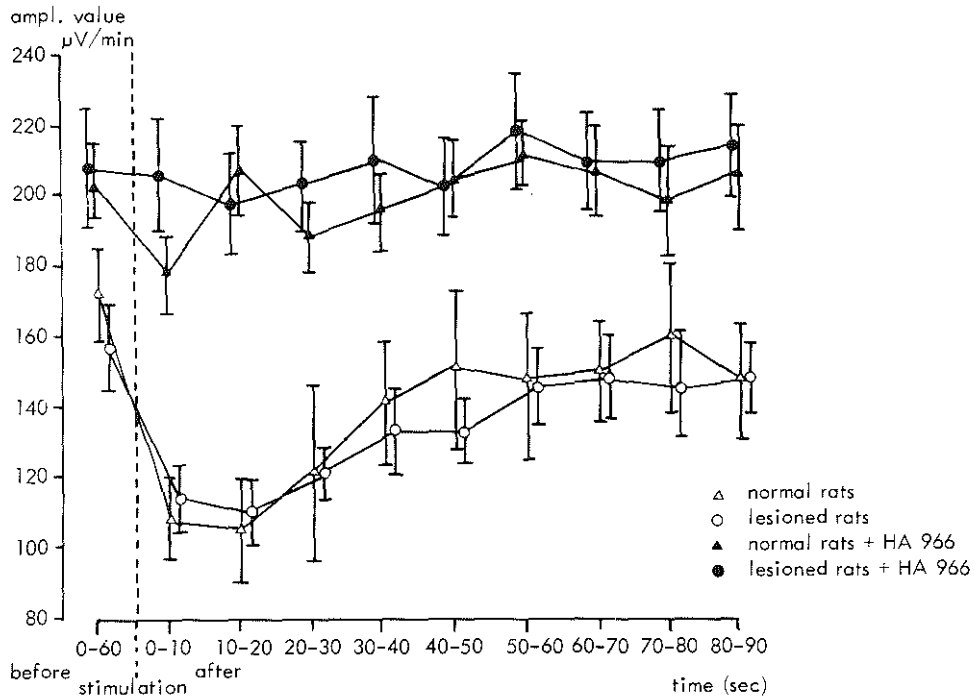


Fig. 8.2. Effect of HA-966 (10 mg/kg i.v.) on EEG amplitude before and after acoustic stimulation in rats with lesioned and intact SNC. Each point represents the mean ± SEM of 4 (intact) or 8 (lesioned) rats.

value, pointing towards an EEG desynchronization characteristic for arousal and lasting for 40-50 s. When HA-966 was injected, the amplitude value before stimulation was higher than in the absence of HA-966, thus indicating an EEG synchronizing effect of HA-966. The arousal induced by acoustic stimulation was prevented by HA-966, emphasizing

the sedating effect of this compound.

No significant differences were observed between normal and SNC-lesioned rats, either in the effect of an acoustic stimulus, or in the influence of HA-966.

8.4. Discussion

The present results confirm the synchronizing effect of HA-966 on the cortical EEG of the rat as observed by Bonta and co-workers (1971) and demonstrate its inhibitory influence on the EEG arousal response.

It was shown, however, that these effects of HA-966 do not depend on the integrity of the nigro-striatal DA system. This might indicate that the inhibitory effect of HA-966 on nigrostriatal neurotransmission is not involved in the generation of EEG synchronization. This parallels the effect of GHB, as suggested in Chapter 5, in that the EEG effects of neither of these compounds are influenced by modulation of DA receptor stimulation. This parallel might form another similarity between GHB and HA-966.

The question might be raised as to whether an electrolytic lesion is selective enough in this kind of experiment, because it always destroys brain structures adjacent to the target structure. Thus the theoretical possibility exists that deactivation of an adjacent structure compensates for the effect of the lesion in the target structure. In the last decade, the compound 6-hydroxy-dopamine (6-OHDA) has been used to produce specific lesions of catecholamine-containing nerve cells (Kostrzewa and Jacobowitz, 1974). It is, however, difficult to induce 6-OHDA lesions in DA neurons only, sparing noradrenalin-containing cells. Moreover, when injected locally to destroy a specific structure, 6-OHDA may leak to other parts of the brain and thus damage catecholamine cells elsewhere.

9.1. EEG and behavioral effects of GHB: resemblance to absence epilepsy

Several results obtained in the present series of investigations point towards a similarity between the phenomena induced by GHB and those characteristic of absence epilepsy.

The EEG pattern, induced by GHB in cats and rats, has been called "epileptoid" because of its high amplitude (hypersynchrony) and the occurrence of spikes (Winters and Spooner, 1965 a,b; Marcus et al., 1967). This was confirmed in the present experiments, using a power spectrum of the EEG. The simultaneous appearance of the hypersynchronous phenomena in both cortical hemispheres, observed both visually and in a phase spectrum of the EEG, indicated their generalized, non-focal origin. The lack of convulsions during the appearance of the EEG hypersynchrony made this GHB-induced syndrome reminiscent of generalized, non-convulsant epilepsy as observed in man. This syndrome is, preferably, referred to as absence epilepsy (Gastaut, 1973), but it is also called "petit mal" because of its less impressive character as compared to the convulsive "grand mal".

Absence epilepsy, as described by Marcus (1972), is characterized by short (5-30 s) interruptions of consciousness, during which general postural tone is relatively well preserved in most cases. During these periods, the patient is observed to stare, oblivious of stimuli introduced into his environment. He also interrupts his ongoing activities. This interruption is relatively abrupt in onset and in cessation. The patient's awareness and motor activities return promptly at the end of the electrical discharge in the EEG. Memory is defective only for the period of the seizure. It is as though the patient, although physically present, is "absent" with regard to his higher cortical functions for the brief period of the seizure. For this reason this form of generalized, non-convulsant epilepsy is called "absence epilepsy". Minor motor phenomena usually accompany the absence seizure. Of these, the most characteristic are eyelid opening, eyelid myoclonus, and at times repetitive ocular movements. Myoclonus of face and repetitive chewing

may also occur. Some minor loss of postural tone in neck muscles may occur with dropping of the head onto the chest. Automatic movements involving the hands may occur; at times these movements are appropriate to the environmental situation. When examined more closely, it is found that the impairment of awareness, of response capacity, and of motor activity are relative phenomena occurring to variable degrees. Thus, patients are usually unaware of phrases or numbers which are provided as auditory or visual stimuli during the episode; some patients are aware of these same stimuli and can even repeat them during the episode or during questioning following the episode. Some patients are able to continue a familiar recitation during the absence seizure. Whether a response to a stimulus occurs during these brief seizures may in part depend on the intensity of the stimulus, and on the motivation and the past experience of the patient (Marcus, 1972).

Besides these "typical" absences, associated with a generalized 3 c/s spike-and-wave EEG pattern, brief losses of consciousness may accompany certain localized or "partial" epileptic seizures, principally of temporal lobe origin. These losses of consciousness present clinically as absences (Gastaut, 1973). However, for various etiological, therapeutic and other reasons, they are not usually considered to be absences as such. Nevertheless, some authors continue to refer to them as "absences", qualified by an adjective denoting the region of origin (usually "temporal lobe absences"). This usage is not recommended (Gastaut, 1973).

As to the "typical absences": it is very difficult, if not impossible, to establish such short interruptions of consciousness and memory in animals. However, during the appearance of GHB-induced EEG hypersynchrony a behavioral pattern is observed which is reminiscent of the abrupt cessation and resumption of motor activity during absences in humans.

The EEG during absences consists of characteristic 3/s spike-wave complexes. A similar pattern is induced by GHB in the cat (Snead et al., 1976). In the rat after GHB we observed EEG spikes alternating with one or more slow waves. This pattern was, however, less regular and possessed a slightly higher frequency than the classical human spike-and-wave EEG. The difficulty to induce this pattern in the rat

was also reported by other authors (McQueen and Woodbury, 1975).

Besides these phenomenological similarities between the effects of GHB and absence epilepsy, it was observed that the EEG hypersynchrony after GHB was antagonized by trimethadione, n-dipropylacetate, ethosuximide and also amphetamine, drugs which proved to be effective in the treatment of absence epilepsy. Drugs effective against convulsive epilepsy, on the other hand, like diphenylhydantoin and mephenytoin, did not suppress the hypersynchrony. This points towards a pharmacological resemblance between the GHB effect and absence epilepsy.

The ability of GHB to induce a syndrome in animals which is reminiscent of absence epilepsy with regard to the EEG and the behavior as well as to its pharmacological properties is quite exceptional. Many artificially induced syndromes in several animal species, mimicking one or more aspects of absence seizures, have been proposed as models to study mechanisms underlying this type of epilepsy. However, all are imperfect.

During insulinic hypoglycemia in the cat, absence-like behavior was observed, accompanied by a generalized 3/s spike-and-wave EEG (Waltregny, 1969). However, in this model convulsions also occurred, differentiating it from the non-convulsive absence epilepsy. Moreover, to our knowledge, no antiepileptic drugs were tested.

Bilateral application of alumina cream, cobalt, pentylenetetrazol or conjugated estrogens to the cortex of cats and monkeys was found to induce spike-and-wave EEG patterns (Marcus, 1972). When conjugated estrogens were applied to the cortical anterior premotor area in the monkey, short staring spells, resembling absence seizures, were observed, together with the spike-and-wave EEG (Marcus, 1972). These models, however, are of focal origin, and are, thus, essentially different from the generalized absence epilepsy in man. Moreover, the sensitivity of these models to specific anti-absence drugs has not been tested, with one exception: EEG patterns induced by bilateral cortical application of conjugated estrogens appeared to be antagonized specifically by anti-absence drugs, and were proposed as a suitable model for testing potential drugs of this type (Fowler and Julien, 1974).

Systemic administration of various convulsant drugs in several animal species was reported to induce spike-and-wave EEG patterns. These

drugs include pentylenetetrazol (Huot et al., 1973), fluoroacetate (Chenoweth and St.John, 1947) and hexafluorodiethyl ether (Krantz et al., 1957). However, the spike-and-wave EEG pattern induced by these compounds is accompanied by convulsive movements, in contrast to the non-convulsive absence epilepsy. On the other hand, it is important to note that the convulsive syndrome elicited by one of these drugs, pentylenetetrazol, can be antagonized by several anti-absence drugs.

As mentioned above, none of these models mimicks absence epilepsy in the same measure as does the GHB-induced syndrome. Though the typical spike-and wave EEG pattern is elicited in the cat but not in the rat, the other effects of 200 mg/kg GHB in the rat, including the specific sensitivity to anti-absence drugs, make this syndrome one of the best matching animal models of absence epilepsy. Thus it might provide a useful model in studying the mechanisms underlying this type of epilepsy.

In view of the reported natural occurrence of GHB in mammalian brain (Roth and Giarman, 1970; Doherty et al., 1975 a), it is conceivable that this compound plays a role in the etiology of absence epilepsy in man. In this context, the suggestion of Hirata et al. (1973) that GHB could be used for clinical EEG diagnosis is noteworthy. These authors were able to detect patients with organic brain disorders who did not show definite EEG abnormality on routine examination, but showed marked deviation from normal after activation of the EEG by GHB (Hirata et al., 1973). One could imagine that administration of GHB, in subthreshold doses, during EEG diagnosis, might enable patients suspected of absence epilepsy to be selected with more certainty.

As suggested earlier, the GHB-induced EEG hypersynchrony might also be useful as a model to test the possible anti-absence efficacy of drugs. Though many animal models are used to test potential antiepileptic drugs, no single model can give a reliable answer concerning the anti-absence specificity of the investigated compound (Naquet and Lanoir, 1973).

The classical models for this purpose are convulsions induced by either intravenous or subcutaneous injection of pentylenetetrazol (Swinyard, 1969). As mentioned above, these seizures are antagonized by several anti-absence drugs. Conversely, it was suggested that

compounds counteracting pentylenetetrazol seizures would be effective in the treatment of absence epilepsy (Jenney and Pfeiffer, 1956). At present, pentylenetetrazol convulsions are the most commonly used model to test potential anti-absence drugs (Swinyard, 1969). However, activity against pentylenetetrazol is not essential for, nor constantly predictive of, efficacy in the treatment of absence epilepsy. For example, the anti-absence drug trimethadione is less effective against pentylenetetrazol than phenobarbital, generally used in convulsive epilepsy (Millichap, 1969). Recently, Desmedt and co-workers (1976) distinguished different groups of drugs according to their action against certain components of pentylenetetrazol convulsions. This division into groups correlated well with the antiepileptic profile of the drugs. However, notwithstanding their more accurate analysis, the authors confirmed the above conclusions of Millichap concerning the limited usefulness of pentylenetetrazol for testing anti-absence drugs (Desmedt et al.,1976).

Another model of epilepsy, which promises to be useful in testing anti-absence drugs, is light-stimulation-induced convulsions in photosensitive baboons of the species Papio papio (Woodbury, 1972). This suggestion was based on the inhibition of these seizures by several anti-absence drugs (Stark et al., 1970; Killam, 1976). The fact, however, that these seizures are also counteracted by diphenylhydantoin (Stark et al., 1970; Killam, 1976), a drug effective against convulsive epilepsy, decreases the specific value of this model in testing anti-absence drugs.

Another test model for potential anti-absence drugs based on light stimulation was proposed recently (Shearer et al., 1976). It employs photically evoked electrocortical afterdischarges in rats, which are significantly inhibited by the specific anti-absence drugs trimethadione and n-dipropyl-acetate but not by diphenylhydantoin (Shearer et al., 1974; 1976).

When mentioning these two functional models of absence epilepsy, both using responses elicited by photic stimulation, it is worthwhile emphasizing that GHB increased the amplitude and duration of cortical and subcortical potentials evoked by light stimulation in the cat (Kharkevich et al., 1971). This point of similarity between the effect

of GHB and both light stimulation-induced models is made more relevant by the fact that, in human absence patients, photosensitivity also exists and spike-and-wave patterns may be provoked by intermittent light stimulation (Bickford and Klass, 1969). Thus, in this respect also, some parallelism exists between the effects of GHB and absence epilepsy.

Two other models for predicting efficacy of drugs against absence seizures should be mentioned here. CO_2-withdrawal seizures in rats and the response to repetitive stimulation in the spinal cord of cats, both models of generalized epilepsy, are inhibited by trimethadione, whereas diphenylhydantoin is ineffective and even enhances the first type of seizures (Woodbury, 1972).

As no single model can provide a decisive answer concerning the anti-absence efficacy of a potential drug, it is necessary to use a combination of tests for screening new drugs. The specific sensitivity of GHB-induced EEG hypersynchrony to anti-absence drugs justifies its further evaluation as an additional model in the battery of tests for this type of drug. When used routinely, it might seem too laborious to implant electrodes into every rat to be used. On the other hand, however, these rats can be used several times. In the present experiments we used each rat for about 3 months in 8-10 sessions. Moreover, the rats can be used as their own control, excluding interindividual differences. Thus, it is possible both to reduce the number of rats required and to increase the reliability of the results.

9.2. Behavioral sedation induced by GHB: sleep, anesthesia or epilepsy?

For a long time since the discovery of the sedating effects of butyrate (Samson and Dahl, 1955; Samson et al., 1956; White and Samson, 1956) and GHB (Jouany et al., 1960 a,b; Laborit et al., 1960), the induced behavioral states have been looked upon as "sleep" or "anesthesia" (White and Samson, 1956; Jouany et al., 1960 a,b; Laborit et al., 1960; Pérez de la Mora and Tapia, 1970; Laborit, 1973). These conclusions, however, were largely based on observation of the behavior, and

to a lesser extent on the increased amplitude of the EEG. More precise examination of the GHB-induced EEG revealed that it was different from patterns seen during physiological sleep or anesthesia (Benda et al., 1960; Solway and Sadove, 1965). Instead, it was found to be similar to the hypersynchronous EEG patterns seen during epilepsy (Schneider et al., 1963; Winters and Spooner, 1965 a,b; 1966; Marcus et al., 1967). Moreover, higher doses of GHB (over 600 mg/kg) induced myoclonic jerks, and convulsions could be elicited by repetitive auditory stimulation (Winters and Spooner, 1965 a,b; Marcus et al., 1967; Osuide, 1972 b). This continuous drug-induced transition from a state of behavioral sedation via myoclonus to seizures was also observed after administration of the anesthetic agent ketamine (Ketalar$^{(R)}$) and some other, but not all, anesthetics (Winters et al., 1969; 1972). It inspired Winters to develop a theory concerning a multidirectional continuum of anesthetic states (fig. 9.1.), some represented by central nervous system (CNS) excitation and others by depression (Winters, 1976). According to this theory, the reticular activating system is influenced by all anesthetics; some inhibit its action (halothane, barbiturates; stage III) and some hyperexcite the system (nitrous oxide; stage IIC). Some agents traverse both excitation and depression (diethyl ether; stages I, II, III). Several compounds induce stage II and may proceed to further CNS excitation, manifested by seizures (GHB, ketamine, phencyclidine, alpha-chloralose, trichloroethylene, enflurane). This implies that the effects of GHB are related to CNS excitation and contradicts the interpretation of previous authors that the GHB-induced sedative state is due to CNS inhibition. Nevertheless, this GHB-induced sedation can be viewed as anesthesia according to the functional, pragmatic definition of Winters (1976), stating that surgical anesthesia is "a stage induced by a drug that makes the subject relatively unresponsive to painful stimuli, and amnestic".

Notwithstanding the excitatory effect of GHB, our results show that this compound may also enhance physiological sleep. This, however, does not support the opinion of Laborit and others, saying that the sedative state induced by 500-1500 mg/kg GHB i.p. was identical to physiological sleep (Jouany et al., 1960 a,b), since the increased duration of SWS observed in our experiments was caused by lower doses

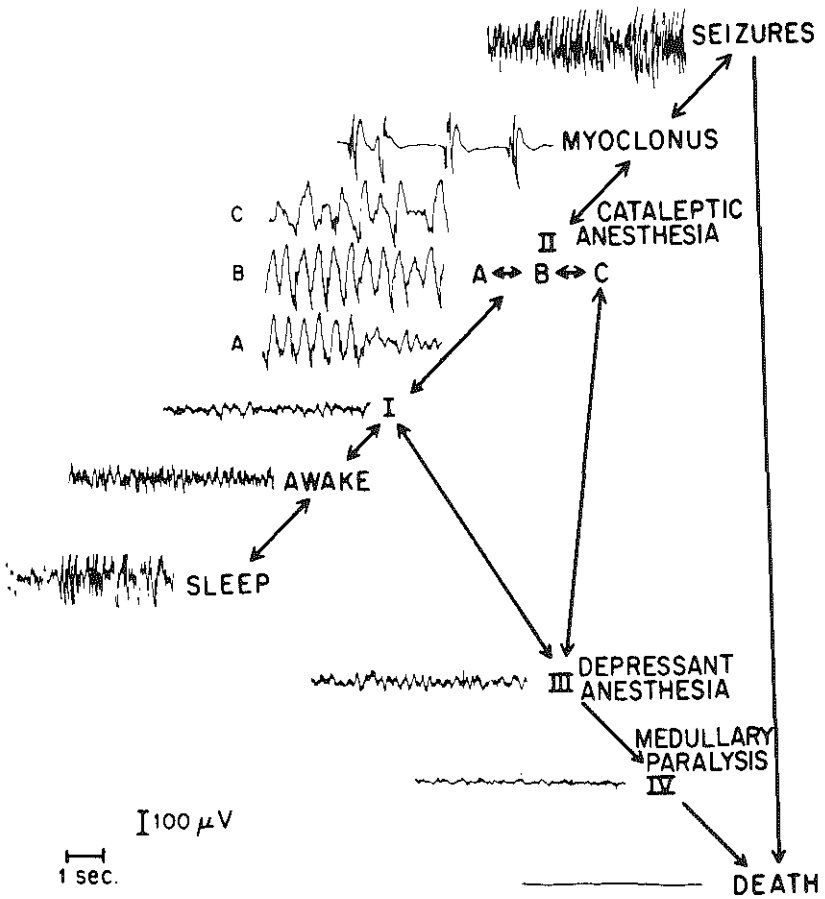


Fig. 9.1. Schematic representation of the stages of anesthesia. CNS excitation is implied above the awake level and CNS depression below. The cortical EEG (cat) representative of each stage is depicted (from Winters, 1976).

(50-100 mg/kg) than those used by these authors.

The influence of sleep stages on the susceptibility to generalized seizures has been the subject of many investigations and some review articles (Janz, 1962; Pompeiano, 1969; Passouant et al., 1975). The major epilepsies and tonic-clonic grand mal attacks are, in particular,

enhanced by SWS (Pompeiano, 1969; Passouant et al., 1975). Absence episodes, in case they would occur during sleep, can not be distinguished behaviorally. The EEG pattern, characteristic of absence epilepsy -3/s spike-wave complexes- however, was observed during various sleep stages. These EEG phenomena were facilitated during PS (Sato et al., 1973; Passouant et al., 1975) and also during the transition from one sleep stage to another, at sleep onset and upon awakening (Passouant et al., 1975).

It is possible that this relationship between absence epilepsy and PS plays a role in the mechanism of action of GHB, in view of the reported induction of PS by GHB in cats (see 1.1.3.). However, since this induction of PS could not be confirmed by several other authors, even in the same species, such a connection remains entirely speculative.

9.3. GHB-induced EEG hypersynchrony: the mechanism of action

An attempt was made to elucidate the neurotransmitter mechanisms underlying the induction, by GHB (200 mg/kg), of the syndrome reminiscent of absence epilepsy. To this purpose, the effects of several compounds on the duration of GHB-induced EEG hypersynchrony were measured.

We aimed our efforts at two well-documented CNS actions of GHB: cessation of impulse flow in the dopaminergic nigrostriatal pathway and inhibition of GABA synthesis by GAD.

In Chapter 5 we showed that modulation of DA receptor stimulation did not influence the duration of GHB hypersynchrony, suggesting that the DA system plays no major role in this mechanism. This suggestion was supported by experiments with HA-966, a synthetic compound similar to GHB in many respects. HA-966 antagonizes nigrostriatal neurotransmission, induces synchronization of the EEG and enhances the duration of GHB-induced EEG hypersynchrony, as was shown in Chapter 7. The synchronizing effect of HA-966 appeared to be unchanged in rats with an electrolytically lesioned substantia nigra compacta. This indicates that the integrity of the nigrostriatal system is not necessary for the

EEG effects of HA-966. Because of the similarity between GHB and HA-966, these results might support our previous suggestion, saying that DA plays no major role in the generation of hypersynchrony by GHB.

In the experiments described in Chapter 6, GHB was administered to rats pretreated with AOAA. The results were interpreted in view of the twofold dual mechanism of AOAA. This compound counteracted the effect of GHB, but only in a dose inhibiting GABA breakdown and not its synthesis, and only 6 h after administration of AOAA, when the antiepileptic activity of this compound is maximally related to the GABA system (Wood and Peesker, 1976). These results point towards an involvement of GABA metabolism in the EEG effects of GHB. This conclusion is supported by the findings that both HA-966 and DPI prolonged the action of GHB and that DPA inhibited it. For both former compounds, indications exist that they might inhibit GAD and thus act synergistically with GHB, whereas DPA inhibits GABA breakdown, abrogating GAD inhibition.

Additional experiments are necessary before final conclusions can be drawn concerning the mechanism of action of GHB. It is possible that, besides inhibition of GABA synthesis, other neurotransmitter mechanisms may also play a role. The reported inhibitory action of amphetamine on the effect of GHB was suggested to be a noradrenergic effect. Moreover, the possibility remains that two different DA systems are involved. Such a system, in turn, might interact with other neurotransmitters.

The involvement of subcortical brain structures in the generation of the EEG and behavioral effects of GHB has been studied only scarcely. Thus the way of action of GHB on the anatomical level is as yet unknown. Electrically evoked seizures in the hippocampus and amygdala of cats were prolonged by GHB (Drakontides et al., 1962), which might point at a role for these limbic structures in the propagation of GHB hypersynchrony.

The thalamus might also be of importance, since the thalamocortical recruiting response in cats was increased by GHB (Drakontides et al., 1962), as was the amplitude of the thalamic response upon sciatic nerve stimulation (Kharkevich et al., 1971). In this context it is noteworthy that a 3 c/s spike-and-wave pattern could be produced on stimulation of the midline nuclei or of the intralaminar system of the

thalamus (Jasper and Droogleever-Fortuyn, 1946). It was also demonstrated that stimulation of this area in unanesthetized animals produced an arrest of movement, reproducing the behavioral components of absences (Hunter and Jasper, 1949). Thus, a parallel exists between thalamic stimulation, inducing certain phenomena characteristic of absence epilepsy, and the effects of GHB, resembling absence epilepsy and enhancing the response to thalamic stimulation. This might suggest a role for the activity of certain thalamic nuclei in the generation of the effects of GHB. Further experimental evidence, however, is required to prove this suggestion.

In particular, it will be of interest to investigate the effects of GHB, when administered into discrete brain areas, upon single and multiple unit EEG registrations from various subcortical regions. This approach might, eventually, contribute to the understanding of the pathophysiological mechanisms underlying absence epilepsy.

The effects of sodium-gamma-hydroxybutyrate (GHB) on the cortical electroencephalogram (EEG) and behavior of the rat and the underlying neuronal mechanisms have been investigated.

In chapter 1 a review is presented of the literature concerning GHB and some related compounds, especially gamma-butyrolactone (GBL) and 1-hydroxy-3-amino-pyrrolidone-2 (HA-966). The state of behavioral sedation, induced by GHB and GBL, has been interpreted differently by several authors. Initially, this state was considered as "sleep" or "anesthesia". When administered in low doses (50-100 mg/kg) these compounds were reported to induce paradoxical sleep (PS) in cats. Registration of the EEG in animals and man after administration of higher doses of GHB and GBL (400-700 mg/kg), however, showed a high amplitude. Several authors considered this as a confirmation of the assumed induction of slow wave sleep (SWS) by these compounds. Closer observation of the amplitude and pattern of the EEG, however, brought to light a similarity between this phenomenon and epilepsy, though no spontaneous convulsions were observed, and myoclonic jerks only appeared after higher doses of both compounds.

These conflicting reports prompted the present investigation into the effects of GHB, in relatively low doses (12.5-200 mg/kg), on cortical EEG and behavior in the rat, with special reference to sleep and epileptoid phenomena.

The influence of GHB (12.5-100 mg/kg) on sleep stages in the rat is described in Chapter 2. Neither of these doses changed the normal EEG patterns. The lowest doses of GHB (12.5-25 mg/kg) did not modify the duration of the awake state, SWS or PS, as measured by combined examination of EEG and electromyogram (EMG). 50 and 100 mg/kg GHB, however, prolonged the duration of SWS by up to 4 h after administration, but the time spent in PS remained unchanged. This confirmed the suggestion of other authors that the reported induction of PS by GHB in cats is not a general phenomenon in all mammals. It is also reported that, during PS, a 6-8 Hz (theta) rhythm appears in the EEG of the rat and can be registered in several regions of the cortex.

The effects of a slightly higher dose of GHB, 200 mg/kg, are de-

cribed in Chapter 3. The induced EEG possesses a higher amplitude than the normal EEG. Its pattern was also different and contained high amplitude spikes, separated by one or more slow waves. These high amplitude or "hypersynchronous" phenomena, reminiscent of epilepsy in man or convulsive disorders in animals, appeared simultaneously on both hemispheres. No convulsions or other jerky movements were observed. This pointed towards a similarity between the effects of GHB and generalized, non-convulsive epilepsy or absence (petit mal) epilepsy. During the hypersynchrony, the rats were motionless, which was suggested to be a motor analogue of absences. The 3/s spike-and-wave EEG, typical of absence epilepsy, was not induced by GHB in the rat, contrary to the cat.

The suggested resemblance of the effects of 200 mg/kg GHB to absence epilepsy was strengthened by the influence of antiepileptic drugs on these phenomena, as presented in Chapter 4. Pretreatment of rats with the specific anti-absence agents ethosuximide, trimethadione and n-dipropylacetate antagonized the effects of a subsequent GHB injection. Diphenylhydantoin and mephenytoin on the other hand, drugs effective against convulsive epilepsy, did not counteract the GHB-induced EEG hypersynchrony. In view of these results it was proposed that this syndrome might be used as an animal model for screening potential anti-absence drugs.

In the following chapters an attempt was made to identify the neurotransmitter systems underlying the effects of GHB on the EEG. The most documented effect of GHB at the level of neurotransmission, namely inhibition of the impulse flow in the nigrostriatal dopaminergic (DA) system, was examined in this respect, as discussed in Chapter 5. It appeared that both specific DA receptor agonists apomorphine and piribedil, and the DA receptor blocker haloperidol, did not influence the duration of GHB-induced hypersynchrony. This argues against a role for the cessation of nigrostriatal impulse flow in the EEG effects of GHB.

Since GHB has been reported to block gamma-aminobutyric acid (GABA) synthesis, an investigation into a possible relation between the hypersynchronizing effect of GHB and the putative neurotransmitter GABA is described in Chapter 6. Amino-oxyacetic acid (AOAA) at a suitable dose and with a suitable pretreatment time, antagonized the duration of GHB

hypersynchrony. This effect was connected with the reported inhibition of GABA breakdown by AOAA under these conditions, an action which is also responsible for the antiepileptic activity of this compound. Thus, it was concluded that the hypersynchronizing effect of GHB is most probably related to inhibition of GABAergic neurotransmission.

In Chapter 7 a synergistic influence of HA-966 on the duration of GHB hypersynchrony was reported. In view of the many similarities between these compounds, it was suggested that a common mechanism of action might exist for both GHB and HA-966.

Impairment of the nigrostriatal DA pathway by electrolytic ablation of the Substantia nigra did not influence the amplitude of the EEG or the desynchronization upon acoustic stimulation. Neither did it modulate the EEG synchronizing effect of HA-966. These results, reported in Chapter 8, were interpreted as supporting evidence for the conclusion that the nigrostriatal DA system does not play a major role in the EEG effects of either HA-966 or GHB.

In Chapter 9 the practical consequences of the present results are discussed. It was emphasized that GHB-induced EEG hypersynchrony might serve as an additional model for testing the anti-absence efficacy of new drugs. Moreover, as GHB occurs naturally in the brains of several mammalian species, a possible role for GHB in the etiology of absence epilepsy was suggested.

In dit proefschrift is een onderzoek beschreven naar de invloed van gamma-hydroxybutyraat (GHB) op het electroencephalogram (EEG) en op het gedrag van de rat, en naar de neuronale mechanismen die daaraan ten grondslag liggen.

Hoofdstuk 1 geeft een overzicht van de literatuur over GHB en een aantal verwante stoffen, waaronder gamma-butyrolacton (GBL) en 1-hydroxy-3amino-pyrrolidon-2 (HA-966). Het bijzonder rustige gedrag, veroorzaakt door GHB en GBL is door een aantal onderzoekers verschillend uitgelegd. Aanvankelijk werd deze toestand beschouwd als "slaap" of "anesthesie". Toegediend in een lage dosering (50-100 mg/kg) bij katten, bleken deze stoffen paradoxale slaap (PS) te veroorzaken. Na toediening van GHB en GBL in hogere doses (400-700 mg/kg) bij proefdieren en mensen vertoonde het EEG echter een hoge amplitude. Verscheidene auteurs beschouwden dit als een bevestiging van hun veronderstelling dat deze stoffen rustige slaap of "slow wave sleep" (SWS) veroorzaken. Bij nadere beschouwing van amplitude en patroon van het EEG bleken er echter overeenkomsten te bestaan tussen dit verschijnsel en epilepsie ("vallende ziekte"), alhoewel er geen spontane convulsies (stuiptrekkingen) optraden en verspreide spiertrekkingen alleen waargenomen werden na toediening van veel hogere doses van deze stoffen.

Deze elkaar tegensprekende resultaten waren de aanleiding voor het onderhavige onderzoek naar de invloed van GHB, in lage doseringen (12.5-200 mg/kg), op EEG en gedrag van de rat. Daarbij werd bijzondere aandacht besteed aan slaap en op epilepsie lijkende verschijselen.

De invloed van GHB (12.5-100 mg/kg) op slaaptoestanden in de rat werd beschreven in Hoofdstuk 2. Geen van deze doseringen veranderde de normale EEG patronen. De laagste doseringen (12.5-25 mg/kg) brachten geen wijziging in de duur van de waaktoestand, SWS of PS, gemeten met behulp van een combinatie van EEG en electromyogram. Toediening van 50 of 100 mg/kg GHB verlengde daarentegen de tijdsduur doorgebracht in SWS, maar de duur van PS bleef onveranderd. Dit bevestigde de veronderstelling van andere auteurs dat de gevonden toename van PS, onder invloed van GHB in katten, geen algemeen verschijnsel is in alle zoogdieren. Daarnaast werd aangetoond dat, tijdens PS, een "theta" ritme

(6-8 Hz) in het EEG verschijnt en opgevangen kan worden op meerdere plaatsen op de hersenschors.

De uitwerking van een iets hogere dosis GHB (200 mg/kg) is beschreven in Hoofdstuk 3. Het veroorzaakte EEG heeft een hogere amplitude dan normaal. Het patroon was ook verschillend en vertoonde hoge pieken, gescheiden door één of meer langzame golven. Deze EEG verschijnselen met een hoge amplitude, ook wel hypersynchronie genoemd, doen denken aan epilepsie in de mens of convulsieve verschijnselen in proefdieren. De hypersynchronie verscheen gelijktijdig aan beide zijden van de hersenschors. Convulsies of verspreide spiertrekkingen werden werden niet waargenomen. Dit wees op een gelijkenis tussen de effecten van GHB en gegeneraliseerde, niet-convulsieve epilepsie, ook wel petit-mal of absence epilepsie genoemd. Tijdens de hypersynchronie waren de ratten bewegingloos, hetgeen was voorgesteld als een mogelijk analogon van absences. Het EEG met 3 piek-golf complexen per seconde werd niet veroorzaakt door GHB in de rat, dit in tegenstelling tot de kat.

De veronderstelde overeenkomst tussen de effecten van 200 mg/kg GHB en absence epilepsie werd versterkt door de invloed van anti-epileptische geneesmiddelen op deze verschijnselen, beschreven in Hoofdstuk 4. Voorbehandeling van ratten met de specifieke anti-absence stoffen ethosuximide, trimethadion en n-dipropylacetaat deed de invloed van een daaropvolgende GHB-toediening teniet. Diphenylhydantoïne en mephenytoïne daarentegen, stoffen die gebruikt worden in de behandeling van convulsieve vormen van epilepsie, gingen de door GHB veroorzaakte EEG hypersynchronie niet tegen. Gezien deze resultaten werd geopperd dat het EEG-effect van GHB gebruikt zou kunnen worden als een diermodel voor het schiften van nieuwe geneesmiddelen op hun mogelijke werking tegen absence epilepsie.

In de volgende hoofdstukken werd gepoogd vast te stellen welke neurotransmitter-systemen ten grondslag liggen aan de EEG-effecten van GHB. Het meest onderzochte effect van GHB op neurotransmissieniveau, namelijk het remmen van de prikkeloverdracht in het nigro-striatale dopaminerge (DA) systeem, werd centraal gesteld in dit verband, zoals beschreven in Hoofdstuk 5.
Het bleek dat zowel de specifieke DA receptor agonisten apomorfine en

piribedil, als de DA receptor blokker haloperidol, de duur van de GHB-hypersynchronie niet beïnvloedden. Dit pleit tegen een rol voor de remming van nigrostriatale prikkeloverdracht in het EEG effect van GHB.

Gezien de aanwijzingen dat GHB de synthese van gamma-aminoboterzuur (GABA) stillegt, werd gezocht naar een mogelijk verband tussen het hypersynchroniserende effect van GHB en GABA, waarvan verondersteld wordt dat het een neurotransmitter is. De resultaten werden beschreven in Hoofdstuk 6. Amino-oxy-azijnzuur (AOAA), in de juiste dosis en met een geschikte voorbehandelingstijd, verminderde de duur van de GHB-hypersynchronie. Dit effect werd in verband gebracht met de remming van de afbraak van GABA door AOAA onder gelijke omstandigheden. Diezelfde remming wordt ook verantwoordelijk geacht voor de anti-epileptische werking van deze stof. De resultaten leidden tot de gevolgtrekking dat de GHB hypersynchronie hoogst waarschijnlijk in verband staat met remming van de prikkeloverdracht in een GABA-systeem.

In Hoofdstuk 7 werd verslag gedaan van een synergistische invloed van HA-966 op de duur van de GHB hypersynchronie. Gezien de vele overeenkomsten tussen beide stoffen werd geopperd dat er mogelijk eenzelfde werkingsmechanisme bestaat voor GHB en HA-966.

Ontregeling van de nigrostriatale DA baan door electrolytische vernietiging van de Substantia nigra had geen invloed op de amplitude van het EEG, noch op de amplitudeverlaging door geluidsprikkeling. Bovendien werd ook de EEG hypersynchronie na toediening van HA-966 niet beïnvloed door zo'n letsel. Deze resultaten, beschreven in Hoofdstuk 8, werden beschouwd als een ondersteuning van de veronderstelling dat het nigrostriatale DA systeem geen belangrijke rol speelt in de beïnvloeding van het EEG door zowel HA-966 als GHB.

In Hoofdstuk 9 werd de praktische betekenis van de resultaten besproken. De EEG hypersynchronie zou van nut kunnen zijn als een toevoeging aan de serie tests die bebruikt worden voor het onderzoeken van mogelijke nieuwe geneesmiddelen voor absence epilepsy. Gezien het feit dat GHB normaal voorkomt in de hersenen van verscheidene zoogdiersoorten, werd bovendien de mogelijkheid geopperd dat GHB een rol speelt in het ontstaan van absence epilepsie.

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CURRICULUM VITAE

Mozes Godschalk werd geboren op 4 mei 1948 te Deventer. Het eindexamen HBS-B werd afgelegd in 1966 aan het Maimonides Lyceum te Amsterdam. Hij studeerde aan de Faculteit voor Landbouwwetenschappen van de Hebreeuwse Universiteit van Jeruzalem, waar in 1970 het B.Sc.-examen werd afgelegd. De M.Sc.-studie, met als hoofdrichting dierfysiologie, werd voltooid in 1973 met een onderzoek naar de regulatie van voedsel- en wateropname bij de kip. Vanaf maart 1973 was hij in dienst van de afdeling Farmacologie van de Erasmus Universiteit te Rotterdam, waar het onderzoek werd verricht dat tot dit proefschrift heeft geleid. Sinds 1 september 1977 is hij in dienst van de afdeling Neuroanatomie van de Erasmus Universiteit.