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The role of dopaminergic systems in γ-hydroxybutyrate-induced electrocorticogram hypersynchronization in the rat

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The effects of dopaminergic agonists and antagonists on the duration of hypersynchronization induced in the electrocorticogram (ecog) by γ-hydroxybutyrate (γ-HB) were tested in rats. Apomorphine (0·2–8 mg kg^{-1}), piribedil (2·5–10 mg kg^{-1}) and haloperidol (0·5–1 mg kg^{-1}) had no influence on the duration of the hypersynchrony. Amphetamine (1·5–6 mg kg^{-1}) inhibited the hypersynchrony, while (3,4-dihydroxyphenylamino)-2-imidazoline (DPI; 5, but not 1, mg kg^{-1}) prolonged its duration. The lack of effect of the dopamine receptor agonists apomorphine and piribedil, and the dopamine receptor blocker haloperidol, on the γ-HB-induced hypersynchrony might indicate that the inhibition of the impulse flow in the nigrostriatal dopamine system by γ-HB is not involved in the generation of the hypersynchrony. DPI is thought to be an agonist at a dopamine receptor not sensitive to apomorphine, and its facilitatory effect on γ-HB-hypersynchrony can be interpreted in terms of a possible involvement of another dopamine system in the ecog hypersynchrony induced by γ-HB. The antagonism of γ-HB by amphetamine is possibly due to an indirect stimulatory effect on noradrenergic receptors.

γ-Hydroxybutyrate (γ-HB, 200 mg kg^{-1}) induces an electroencephalogram (eeg) with hypersynchronous spikes which can be antagonized, in the rat, by anti-petit mal drugs (Godschalk, Dzoljic & Bonta, 1976). The γ-HB-induced spikes appear simultaneously on both sides of the cortex and no convulsive movements are seen during the hypersynchrony, whether it occurs in bursts or continuously. Instead, the rats show a sudden arrest of motor behaviour during the periods of eeg hypersynchrony. It was suggested that this γ-HB-induced syndrome in the rat is reminiscent of absence (petit mal) epilepsy in man (Godschalk, Dzoljic & Bonta, 1977).

Administration of higher doses of γ-HB (750–1500 mg kg^{-1}) to rats causes an increase in intraneuronal dopamine concentrations in the striatum (Gessa, Vargiu & others, 1966; Aghajanian & Roth, 1970). This effect has been related to an inhibition of the impulse flow in the dopaminergic nigrostriatal axonal bundle (Stock, Magnusson & Andén, 1973). In lower doses (100–350 mg kg^{-1}), γ-HB inhibits the firing rate of the dopamine cells in the substantia nigra pars compacta, which send their axons to the striatum (Roth, Walters & Aghajanian, 1973).

In several animal models of epilepsy it has been suggested that dopamine receptor stimulation is implicated in antiepileptic activity, while blockade of dopamine receptors or a decreased availability of dopamine enhances epileptic phenomena (Stull, Jobe & others, 1973; Dow, Hill & McQueen, 1974; Meldrum, Anlezark & Trimble, 1975; Anlezark, Pycock & Meldrum, 1976; Cox & Lomax, 1976). In addition, recent data indicate that dopamine is also involved in cortical eeg activation (Kafi & Gaillard, 1976).

In view of these data we investigated the possibility that γ-HB-induced eeg hypersynchrony is due to inhibition of dopaminergic neurotransmission. To this end we studied the effects of dopamine-receptor agonists and blockers on γ-HB-induced hypersynchrony.

MATERIALS AND METHODS

Animals

Male Wistar albino rats, 160–180 g, were anaesthetized with Hypnorm (10 mg fluanison and 0·2 mg fentanyl ml^{-1}) in doses of 1·5 ml kg^{-1} subcutaneously, and implanted with 5 epidural silver screw electrodes (4 for bilateral fronto-parietal recording of the electrocorticogram (ecog) and one as reference) and two platinum ring electrodes in the neck muscles to record the electromyogram. The electrodes were connected to a contact fixed on the skull with dental acrylic cement. The rats were caged individually and had free access to water and food. After at least 8 days recovery the rats were

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used for several experiments. The period between consecutive experiments on the same rat was at least seven days.

Order of experiments

The experiments were in a sound proof cabin, into which 4 animals were placed in separate cages. Here the rats remained for at least 16 h before the experiment; they were maintained on the same day–night rhythm as in their home cages.

On the day of the experiment, about 09.30 h, water and food were removed and the rats were connected to a Grass eeg amplifier/recorder via a swivel. Between 10.30 h and 11.30 h a baseline ecog was taken. At 11.30 h one of the following compounds was administered intraperitoneally, in aqueous solutions, in a volume of 2 ml kg^{-1}: apomorphine HCl (0·2–8·0 mg kg^{-1}), 1-(2-pyrimidyl)-4-piperonyl piperazine (piribedil, ET-495; 2·5–10·0 mg kg^{-1}), (3,4-dihydroxyphenylamino)-2-imidazoline (DPI; 1–5 mg kg^{-1}), (+)-amphetamine sulphate (1·5–6·0 mg kg^{-1}), haloperidol (0·5–1·0 mg kg^{-1}) or 0·9% NaCl (saline). Piribedil and haloperidol were initially dissolved in acid solutions and then base and water were added until pH 6–6·5 was reached. After a time interval, fixed for every drug as specified in Table 1, γ-HB (200 mg kg^{-1}) was injected intraperitoneally. After the injection of γ-HB, the ecog was recorded continuously until its pattern had returned to normal, but for at least 2 h.

Experimental design

In a series of 4 experiments on 4 rats, each received 3 different doses of test compound and saline in randomized order.

With apomorphine, which was administered in more than three doses, each of three different groups of rats received three different doses plus a saline control treatment, and in the succeeding experiments the rats of the same group each alternately received the doses originally used in that group.

In the experiments with DPI and haloperidol, one rat received only DPI or haloperidol and not γ-HB. Therefore, only two doses of these compounds were tested in combination with γ-HB. The experiments with DPI were not all made on the same rats.

Evaluation

γ-HB (200 mg kg^{-1}) induces hypersynchronous ecog bursts, followed by continuous hypersynchrony and again hypersynchronous bursts, whereafter the normal eeg patterns reappear (Godschalk & others, 1977). The duration of the period from the first until the last hypersynchronous burst was measured, and the mean and standard error of the mean were calculated. The results were evaluated statistically with the paired Student's *t*-test.

RESULTS

Behaviour

Apomorphine in the dose range of 2–8 mg kg^{-1} and all doses of amphetamine used enhanced motor activity in the rats and induced turning and stereotyped behaviour. When apomorphine was followed by γ-HB the rats lay quiet during periods of ecog hypersynchrony. When a combination of amphetamine and γ-HB was followed by ecog 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 behaviour of the rats.

Electrocorticogram

Injection of apomorphine or amphetamine, alone, or followed by a saline injection, induced a continuous desynchronized ecog in the rats. The duration of this desynchronization was dose-dependent: after 0·5 mg kg^{-1} apomorphine it lasted for 30 min, 1 mg kg^{-1} of this compound induced a desynchronization for 60 min and after the highest dose used (8 mg kg^{-1}) it lasted for about 90 min. The duration of the desynchronized ecog after 0·2 mg kg^{-1} apomorphine was not different from that after a saline injection. The amphetamine-induced desynchronization lasted from 2 h after 1·5 mg kg^{-1} up to $3\frac{1}{2}$ h after 6 mg kg^{-1}.

The lower dose of DPI (1 mg kg^{-1}) induced a normal ecog. The higher dose (5 mg kg^{-1}) induced a mainly desynchronized ecog 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 ecog 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 γ-HB-induced bursts, those after DPI were lower and occurred less frequently while the peaks were closer to each other.

The ecog 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 ecog.

Pretreatment of rats with apomorphine, piribedil or haloperidol had no effect on the pattern of the γ-HB-induced ecog hypersynchrony, nor on its duration (Table 1). Amphetamine (1·5–6·0 mg kg^{-1}), however, antagonized the γ-HB-induced hypersynchrony in most rats (Table 1). In this case a continuous desynchronized ecog was seen and lasted for about 3 h.

The lower dose of DPI (1 mg kg^{-1}) had no influence on the effect of γ-HB; the higher dose (5 mg kg^{-1}) however, significantly prolonged the duration of the γ-HB-induced hypersynchrony (Table 1).

Table 1. *Duration of γ-HB-induced ecog hypersynchrony after pretreatment with different drugs.* Time refers to the interval between pretreatment and γ-HB-adminstration. Values are means ± s.e.m. Numbers of observations are given in brackets. Significance of differences (*vs* saline injected controls) was determined by paired two-tailed Student's *t*-test.

Treatment	Time (min)	Dose (kg mg^{-1})	Duration (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)

* $P < 0{\cdot}05$.

DISCUSSION

The most documented effect of γ-HB is inhibition of the impulse flow in the dopaminergic, nigrostriatal neuronal pathway, associated with an elevation of the dopamine concentration in the striatum. Apomorphine and piribedil are both direct dopamine receptor stimulants (Andén, Rubenson & others, 1967; Corrodi, Fuxe & Ungerstedt, 1971) and block γ-HB-induced accumulation of dopamine in rat whole brain (Handforth & Sourkes, 1975) and rat cingulate and frontal cortex (Pericic & Walters, 1976). The failure of both the dopamine receptor stimulants apomorphine and piribedil and blocker haloperidol to modulate the γ-HB-induced ecog hypersynchrony, in the present study, indicates that inhibition of the nigrostriatal dopaminergic system is not likely to be responsible for the γ-HB hypersynchrony. This is in accordance with observations that striatectomy has no influence on the eeg and behavioural effects of γ-HB in the cat (Marcus, Winters & Hultin, 1976) and that in the rat, after striatectomy, leptazol eeg seizures remain unaltered, while the threshold for convulsions is elevated (Avakyan, 1976).

Amphetamine is specifically effective in the treatment of absence epilepsy (Livingston, Kajdi & Bridge, 1948). Its inhibitory action on the γ-HB-induced ecog hypersynchrony, in the present study, is in accordance with the effect of other specific anti-absence drugs (Godschalk & others, 1976). This supports the hypothesis that some similarity exists between the effects of 200 mg kg^{-1} γ-HB in the rat and absence epeilepsy in man, and that this phenomenon might be a suitable tool for testing potential anti-absence drugs.

Amphetamine increases the availability of central catecholamines at 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 leptazol convulsions, appear to be mediated through indirect stimulation of noradrenaline receptors (Rudzik & Johnson, 1970; Riffee & Gerald, 1976). However, the present results do not permit definitive conclusions to be drawn concerning the mode of action of amphetamine in antagonizing the γ-HB-induced hypersynchrony. Nevertheless, indirect stimulation of noradrenergic receptors might be responsible for this antagonistic effect.

In the snail *Helix aspersa*, DPI has been found to be a selective agonist at dopamine receptors mediating neuronal inhibition; these receptors are pharmacologically distinct from excitation-mediating dopamine receptors, which are stimulated by

apomorphine (Struyker Boudier, Teppema & others, 1975). Two pharmacologically different dopamine systems have been proposed for many animals, including the rat (Fuxe, Agnati & others, 1975; Cools & van Rossum, 1976; Cools, Struyker Boudier & van Rossum, 1976). One of these systems is sensitive to apomorphine, the other to DPI (Cools & van Rossum, 1976; Cools & others, 1976). It is worthwhile emphasizing that a sufficiently high dose of DPI (5 mg kg^{-1}) prolonged the duration of the γ-HB-induced hypersynchrony. This might indicate that stimulation of DPI-sensitive receptors has a facilitatory effect on the generation of γ-HB-induced ecog hypersynchrony, and that possibly γ-HB and DPI act on the same system. Evidently, a more specific experimental approach is necessary to elucidate the role of dopamine systems in eeg and motor seizures. In particular, the possibility cannot be excluded that different dopamine systems might have different roles in epileptic phenomena.

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