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The effects of apomorphine, (+)-amphetamine and L-dopa on maximal electroshock convulsions—a comparative study in the rat and mouse

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The anticonvulsant activity of apomorphine, (+)-amphetamine and pargyline + L-dopa was examined in the rat and mouse. Whereas all three treatments produced anticonvulsant effects in the rat, only (+)-amphetamine and L-dopa were anticonvulsant in the mouse. It is suggested that enhanced dopamine-like activity is responsible for the anticonvulsant activity of these three drugs in the rat. The anticonvulsant activity of (+)-amphetamine and L-dopa in the mouse may be unrelated to their pharmacological activity on dopaminergic neurons.

There is increasing evidence to support the hypothesis that apomorphine specifically activates dopamine receptors in the central nervous system (Ernst, 1965, 1969; Ernst & Smelik, 1966; Andén, Rubenson & others, 1967; Ungerstedt, Butcher & others, 1969; Roos, 1969). We have compared and contrasted the effects of apomorphine, (+)-amphetamine and L-dopa on maximal electroshock convulsions in the rat and mouse, with the view of finding additional information on the possible role of dopaminergic mechanisms in anticonvulsant activity in these two species.

METHODS

Sprague-Dawley, albino, male rats, 200–250 g, and Carworth Farms (CFI), white, male mice, 20–22 g, were used.

Maximal electroshock

Maximal electroshock treatment was performed using a Hans Seizure Apparatus and corneal electrodes. Rats received 150 mA and mice 50 mA for 0·2 s to which all control mice and 91% of control rats responded with the full seizure pattern described by Woodbury & Davenport (1952).

Dose-response relations for anticonvulsant activity were determined for apomorphine, (+)-amphetamine and pargyline + L-dopa. Diphenylhydantoin was used as the standard anticonvulsant agent. Animals were considered protected if the hind-limb extensor component was blocked. Ten to 30 animals were used for each dose of drug and ED50 values were calculated according to Miller & Tainter (1944).

Locomotor activity

The motor activity dose (MAD50)to produce a 50% increase in locomotor activity in rats and mice was determined for apomorphine, (+)-amphetamine and pargyline

+ L-dopa. Locomotor activity in mice was studied in 12 circular, six-beam, photocell activity cages (Woodward Research Corporation). Control and treated mice were housed, one per cage, and the cumulative counts/min were recorded simultaneously immediately after drug treatment and continued for 1–3 h. Experiments with rats were similarly conducted using Williamson activity cages (model HG4).

Toxicity

The effects of aggregation on the acute toxicity of apomorphine, (+)-amphetamine and pargyline + L-dopa were determined in mice. Aggregated animals were housed ten per cage. Animals were observed for 5 h after the drug treatment, and the number dead was recorded. Ten to 30 animals were used at each dose level. Dose-response curves were determined for all three treatments, and LD50 values were calculated (Miller & Tainter, 1944).

Compounds were prepared in the following diluents: apomorphine hydrochloride (0·001 N HCl); diphenylhydantoin sodium (0·001 N NaOH); (+)-amphetamine hydrochloride (saline); L-dopa (2·5% acacia for i.p. injections and 0·1 N HCl for s.c. administration); and pargyline (saline). All control animals received injections of the appropriate diluent.

RESULTS

Anticonvulsant activity in the rat

In the rat, apomorphine produced dose-related protection against maximal electroshock seizures (Fig. 1). The ED50 values for apomorphine, given 15 min, and diphenylhydantoin, given 60 min before electroshock, were calculated to be 2·4 and 9·2 mg/kg, respectively. On a molar basis, apomorphine was approximately 4 times more potent than diphenylhydantoin.

The time of peak anticonvulsant activity for apomorphine was approximately 15 min after the subcutaneous injection of 10 mg/kg; the duration of action of which

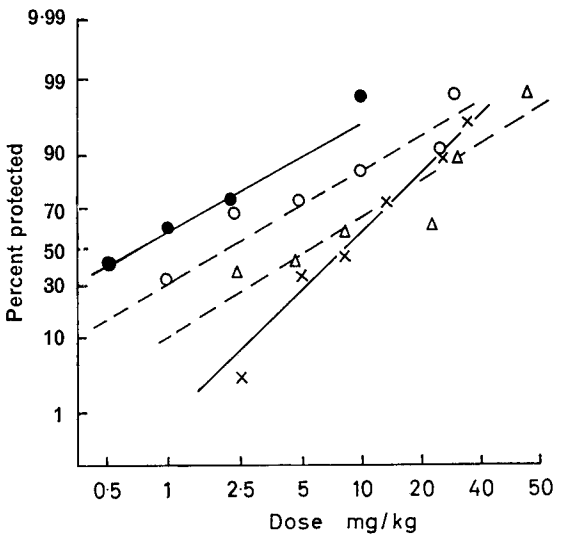

FIG. 1. Dose-response relations for anticonvulsant activity in the rat. (+)-Amphetamine, ●; apomorphine, ○; pargyline plus L-dopa, △; diphenylhydantoin, ×. Zero and 100% values were corrected according to Miller & Tainter (1944). 10 to 30 animals per point.

Table 1. *Relative potencies of apomorphine, (+)-amphetamine and* L-*dopa on maximal electroshock (MES), increased locomotor activity and toxicity in mice and rats (mg/kg ± s.e.).*

Compound	Mouse				Rat	
	MES ED50	Locomotor MAD50	Toxicity LD50 1/cage	Toxicity LD50 10/cage	MES ED50	Locomotor MAD50
Apomorphine s.c.	*	30 ± 5·0	165 ± 4·0	140 ± 4·0	2·4 ± 0·1	15 ± 2·0
(+)-Amphetamine s.c.	11·8 ± 0·9	0·75 ± 0·2	135 ± 9·0	17·0 ± 3·0	1·0 ± 3·0	1·2 ± 0·2
†Pargyline i.p. + ‡L-Dopa s.c.	26 ± 4·4	25 ± 3·0	480 ± 64	295 ± 51	9·0 ± 3·0§	20 ± 2·0§

*No effect up to 160 mg/kg; †Pargyline 100 mg/kg, i.p. in mice and 50 mg/kg, i.p. in rats given 2 h before L-dopa; ‡L-dopa administered 1 h before MES test; §L-dopa administered i.p.

was approximately 1 h. The per cent protected at 10, 15, 20, 30, 45 and 60 min respectively were: 75, 82, 75, 50, 50 and 25%.

(+)-Amphtamine had potent anticonvulsant activity in the rat (Fig. 1) with an ED50 of 1·0 mg/kg (s.c.) (Table 1). L-Dopa (100–300 mg/kg, i.p.), given 1 h before electroshock, did not show dose-related activity in the rat, but after pretreatment (3 h) with pargyline (50 mg/kg, i.p.), it protected animals in a dose-related fashion (Fig. 1) with an ED50 of 9·0 mg/kg (Table 1). Pargyline given alone (3 h previously), did not show anticonvulsant activity.

Anticonvulsant activity in the mouse

Apomorphine 2·5–160 mg/kg (s.c.), given 15–60 min before electroshock, did not protect mice against maximal treatment (Fig. 2). Similarly, (+)-amphetamine (1–5 mg, s.c.), given 30 min before electroshock, to aggregated mice, did not afford protec-

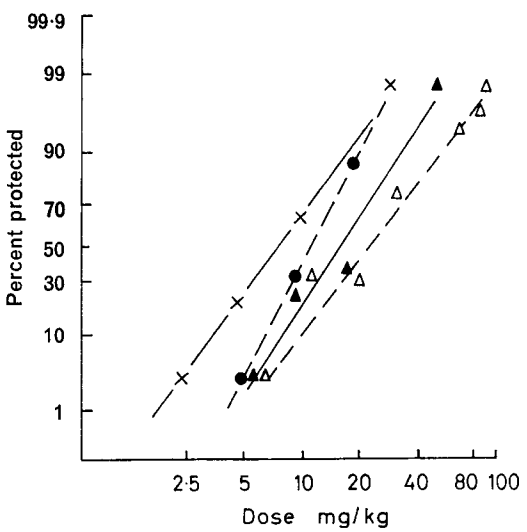

FIG. 2. Dose-response relations for anticonvulsant activity in the mouse. (+)-amphetamine, administered to aggregated (●) and individually housed animals (▲); pargyline + L-dopa, △; diphenylhydantoin, ×. Zero and 100% values were corrected according to Miller & Tainter (1944). 10 to 3 imals per point.

tion; higher doses (10 and 20 mg/kg) protected 40 and 90% of the animals, respectively, yielding an ED50 of 11·8 mg/kg (Table 1). This value was increased to 17 mg/kg by housing the animals individually (Fig. 2).

L-Dopa (50–800 mg/kg, s.c.) given 60 min before electroshock, did not show consistent or dose-related anticonvulsant activity in the mouse. After monoamine oxidase inhibition, L-dopa produced consistent anticonvulsant activity, with an ED50 of 26 mg/kg (Fig. 2, Table 1). Pargyline (100 mg/kg, i.p.), given 3 h before electroshock, had no anticonvulsant activity.

Diphenylhydantoin, given 3 h before electroshock, produced dose-related activity in the mouse, with an anticonvulsant ED50 of 10·0 mg/kg (i.p.) (Fig. 2).

Comparison of anticonvulsant ED50 and MAD50

A comparison of the anticonvulsant ED50 and the MAD50 for increased locomotor activity was made for apomorphine, (+)-amphetamine and pargyline + L-dopa (Table 1). In rats, the anticonvulsant ED50 was approximately equal to the MAD50 for (+)-amphetamine and 2–4 times lower than the MAD50 for apomorphine and pargyline + L-dopa. In contrast, in the mouse, the anticonvulsant ED50 was absent for apomorphine, 10 times greater than the MAD50 for (+)-amphetamine and approximately equal to the MAD50 for pargyline + L-dopa.

Acute toxicity in aggregated and non-aggregated mice

The LD50 of apomorphine in individually housed mice was 165 mg/kg (s.c.) and aggregation of animals reduced the LD50 to 140 mg/kg (Table 1). Aggregation of animals reduced the LD50 of (+)-amphetamine from 135 to 17·0 mg/kg (s.c.) and of L-dopa from 480 to 295 mg/kg. With L-dopa, toxicity was studied after pre-treatment with pargyline which by itself had an LD50 of 335 mg/kg (i.p.).

DISCUSSION

The calculated anticonvulsant ED50 for diphenylhydantoin in the rat was 9·2 mg/kg (s.c.) which is similar to that reported by Swinyard, Brown & Goodman (1952). However, to our knowledge, the present report is the first on the anticonvulsant activity of apomorphine.

The available information on the pharmacology and biochemistry of apomorphine (Ernst, 1965, 1969; Ernst & Smelik, 1966; Andén & others, 1967; Ungerstedt & others, 1969; Roos, 1969) supports the hypothesis that this drug may be a specific dopaminergic agonist. Therefore, it is presumed that the anticonvulsant activity of apomorphine is due to activation of central dopaminergic receptors.

(+)-Amphetamine and pargyline + L-dopa were effective in blocking the extensor seizure after maximal electroshock in rats. Both treatments affect the metabolism of dopamine in central dopaminergic neurons. (+)-Amphetamine increases the amount of unbound dopamine in the caudate nucleus of the cat (McKenzie & Szerb, 1968) and in rat isolated striatum (Besson, Cheramy & others, 1969) possibly through inhibition of the membrane amine pump (Coyle & Snyder, 1969) but more likely as a result of extragranular release of dopamine (Carlsson, Corrodi & others, 1966; Carlsson, Fuxe & others, 1969). On the other hand, L-dopa, particularly after monoamine oxidase inhibition, produces a marked increase in the concentration of brain dopamine in rabbits (Carlsson, Lindqvist & others, 1958) and rats (Scheel-

Krüger & Randrup, 1967), whereas, simultaneous changes in noradrenaline concentrations were absent or much less pronounced. Therefore, in the rat, the anticonvulsant activity of apomorphine, (+)-amphetamine and L-dopa could be related to their enhancing effects on central dopaminergic mechanisms.

In the mouse, apomorphine (2·5–160 mg/kg s.c.), did not produce anticonvulsant activity. Again, the anticonvulsant ED50 values for (+)-amphetamine and pargyline + L-dopa were 10 and 3 times larger respectively, than the corresponding ED50 values in the rat. These findings suggest that the anticonvulsant activity of high doses of (+)-amphetamine and L-dopa could be due to generalized neurotoxicity in the mouse. This is supported by the shift in the anticonvulsant ED50 of (+)-amphetamine from 11 mg/kg for aggregated mice to 17 mg/kg for non-aggregated mice. Again, the anticonvulsant ED50 for (+)-amphetamine is 10 times larger than the MAD50 for increased locomotor activity; a finding consistent with those of Rudzik & Johnson (1970). Finally, aggregation causes a marked lowering of the LD50 for (+)-amphetamine and L-dopa, as shown by Chance (1946) and Proctor, Greenfield & others (1966) whereas aggregation had only a small effect on the acute toxicity of apomorphine.

It is unlikely that the absence of anticonvulsant activity in the mouse after apomorphine is due to a rapid metabolic deactivation by this species, since increased locomotor activity, fighting and stereotyped movements were observed. Again, apomorphine (5–10 mg/kg s.c.), produces a marked potentiation of leptazol convulsions in the mouse and rat (Soroko & McKenzie, 1970).

Our results suggest that, in contrast to the rat, enhanced dopamine-receptor activity in the mouse does not confer anticonvulsant effects. Thus, the anticonvulsant activity of (+)-amphetamine and L-dopa, in the mouse, may be unrelated to their pharmacological activity on dopaminergic neurons.

The species difference in response to apomorphine in this study is consistent with the original hypothesis of De Schaepdryver, Piette & Delaunois (1962) that the anticonvulsant effects of L-dopa and (+)-amphetamine are due to increased dopaminergic activity in the rabbit. However, their hypothesis has been revised recently to include, for L-dopa, not only dopaminergic neurons but also noradrenergic neurons (Billet, Bernard & others, 1970). Our observations in the mouse are consistent with the concept of Rudzik & Johnson (1970) that convulsive thresholds to electroshock depend upon brain concentrations of noradrenaline; however, our results in the rat suggest that, in this species the dopaminergic system may play the dominant role in altering electroshock convulsions.

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