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Effect of ergometrine on methamphetamine and apomorphine stereotypy in the guinea-pig

J. J. BALSARA*, T. R. BAPAT, V. P. GADA, N. V. NANDAL, A. G. CHANDORKAR, *Department of Pharmacology, V.M. Medical College, Solapur, India*

Pretreatment with the DAi receptor antagonist ergometrine (10, 20 mg kg^{-1} i.p.) significantly potentiated methamphetamine stereotypy and facilitated the induction of biting, gnawing or licking behaviour by amantadine. However, ergometrine (5–20 mg kg^{-1}) did not significantly influence the stereotyped behaviour induced by the DAe receptor agonist apomorphine. The results suggest that the DAi antagonist ergometrine is effective in modifying the behaviours induced by methamphetamine and amantadine, agents which through released DA simultaneously activate both DAe and DAi receptors, but fails to modify the stereotyped behaviour induced by apomorphine which specifically activates only DAe receptors. However, the possibility that ergometrine might have potentiated methamphetamine stereotypy and facilitated the induction of biting, gnawing or licking behaviour by amantadine through modulation of the activity of the central noradrenergic and 5-hydroxytryptaminergic systems, which are reported to influence DA-mediated behaviours, also needs to be considered.

Methamphetamine-induced stereotyped behaviour (SB) is considered to result from activation of postsynaptic striatal and mesolimbic dopamine (DA) receptors by released DA, whereas apomorphine stereotypy occurs as a result of direct stimulation of similar DA receptors by apomorphine (Randrup & Munkvad 1974; Wallach 1974). Recently, analogous to findings in the snail, *Helix aspersa*, Cools & Van Rossum (1976, 1980) have suggested that two types of DA receptors exist in the mammalian brain i.e. those mediating excitatory and those mediating inhibitory responses to DA. Further, those authors have stated that although the excitation-mediating (DAe) and inhibition-mediating (DAi) receptors are equally affected by DA, only the DAe receptors are specifically activated by apomorphine and inhibited by haloperidol, whereas the DAi receptors are selectively stimulated by 3,4-dihydroxyphenylamino-2-imidazoline (DPI) and inhibited by ergometrine. Since haloperidol, a DAe antagonist, antagonizes apomorphine and amphetamine-induced SB (Wallach 1974; Costall & Naylor 1975) it indicates that the DA agonist-induced SB results from activation of DAe receptors. We have investigated whether pretreatment with ergometrine, a DAi antagonist, influences the SB induced by apomorphine, a DAe agonist, and that induced by methamphetamine, an indirectly acting DA agonist which through released DA simultaneously activates both DAe and DAi receptors. We have also studied the interaction between ergometrine and amantadine, an antiparkinson drug, which acts by releasing DA (Bailey & Stone 1975) and through the released DA activates both DAe and DAi receptors.

* Correspondence.

Materials and methods

Male guinea pigs, 300–500 g, with free access to a standard diet and tap water were used once only. All observations were made between 10 and 17 h at 27–30 °C in a noiseless, diffusely illuminated room. During the experiments the animals were placed in individual cages made of wire netting, measuring 60 × 45 × 25 cm, 30 min before drug treatment to allow adaptation to the new environment. Cardboard screens were placed between the cages to prevent the animals from distracting each other.

Ergometrine maleate (Sigma), methamphetamine HCl (Burroughs Wellcome) and amantadine HCl (Ciba-Geigy) were dissolved in distilled water, and apomorphine HCl (Sigma) was dissolved in distilled water with 0·2 mg ml^{-1} ascorbic acid. All drugs were injected i.p., except apomorphine which was administered s.c. The volume of drug injection was 1 ml kg^{-1} except for ergometrine which was injected in a volume of 2 ml kg^{-1}. Doses refer to the salt. Ergometrine was injected 45 min before methamphetamine, apomorphine or amantadine. The control groups received vehicle (2 ml kg^{-1} i.p.) before methamphetamine, apomorphine or amantadine.

The effect of ergometrine pretreatment on the latency of onset, duration and intensity of SB induced by methamphetamine and apomorphine was determined. Observations were made blind with respect to the treatments used. Since close proximity of an observer disrupts the SB and the animal remains immobile and 'frozen' (Randrup & Munkvad 1967), the SB was observed without disturbing the animals. The intensity of SB was assessed over 30 s at 10 min intervals throughout its duration, using the scoring system of Costall & Naylor (1975) where periodic biting, gnawing or licking scores 1 and continuous biting, gnawing or licking scores 2. Inter-rater reliability was calculated from those simultaneous ratings made by two of the authors using the Pearson product-moment correlation (r). Inter-rater correlation coefficient r was found to be 0·97, indicating a high degree of inter-rater agreement. The maximum intensity of SB scored by each animal in

the group was taken to compute the mean value of the group.

Since in preliminary experiments amantadine did not induce SB, the ergometrine-amantadine interaction studies were conducted as follows. Following injection of amantadine the animals were observed for 1 h and note was made of the number of animals which exhibited biting, gnawing or licking behaviour.

Differences between experimental groups and their controls were analysed statistically by the two-tailed Student's *t*-test (latency of onset and duration of SB), Mann-Whitney U-Test (SB score) or Fisher's exact probability test (ergometrine-amantadine interaction data). The level of statistical significance chosen was $P < 0.05$.

Results

Ergometrine (5, 10, 20 mg kg^{-1} i.p.) did not induce locomotor stimulation or SB, on the contrary the animals appeared sedated and exhibited ptosis. However, animals given 10 and 20 mg kg^{-1} ergometrine, shortly after the injection, exhibited 'wet dog shake' behaviour similar to that seen in the rat, fore limb movement and extension spasm of hind limbs at rest, but the occurrence of these behaviours was infrequent, and depending upon the dose of ergometrine lasted for 30–40 min.

In preliminary experiments the SB patterns induced by methamphetamine (5–15 mg kg^{-1} i.p.) and apomorphine (0·5–2 mg kg^{-1} s.c.) in the guinea-pigs were found to be characterized by biting, gnawing or licking; the low intensity components of SB observed in the rat (sniffing and repetitive head and limb movements) were not induced in the guinea-pig. Methamphetamine at 5 and 10 mg kg^{-1} induced SB of score 1 intensity in 20% (n = 5) and 100% (n = 5) of the animals respectively. At 15 mg kg^{-1} it induced SB of score 2 intensity in 80% (n = 5) of the animals tested. For subsequent interaction studies methamphetamine was used in doses of 10 and 12·5 mg kg^{-1}. Apomorphine at 0·5 and 1 mg kg^{-1} induced SB of score 1 intensity in 20% (n = 5) and 80% (n = 5) of the animals respectively. At 2 mg kg^{-1} it induced SB of score 2 intensity in 100% (n = 5) of the animals tested. For subsequent studies apomorphine was used in doses of 1 and 1·5 mg kg^{-1}.

Pretreatment with 5 mg kg^{-1} ergometrine did not influence significantly methamphetamine (10, 12·5 mg kg^{-1}) stereotypy (Table 1). However, pretreatment with 10 and 20 mg kg^{-1} ergometrine not only decreased the time required for the onset of methamphetamine stereotypy ($P < 0.05$ or less), but also prolonged the duration and increased the intensity of methamphetamine-induced SB ($P < 0.05$) (Table 1). Pretreatment with ergometrine (5, 10, 20 mg kg^{-1}) however, did not influence apomorphine (1, 1·5 mg kg^{-1})-induced SB significantly (Table 1).

In preliminary experiments it was observed that amantadine (50–100 mg kg^{-1} i.p.) failed to induce SB in guinea-pigs. Doses greater than 100 mg kg^{-1} produced intermittent clonic convulsions and death so for subsequent studies amantadine was used in doses of 75 and 100 mg kg^{-1}. In animals pretreated with 5 mg kg^{-1} ergometrine, amantadine (75, 100 mg kg^{-1}) did not induce biting, gnawing or licking behaviour (Table 2).

Table 1. Effect of ergometrine (ERM) pretreatment on methamphetamine (MAM) and apomorphine (APO)-induced stereotypy in the guinea-pig. Ergometrine was injected i.p. 45 min before methamphetamine and apomorphine. Methamphetamine was injected i.p. whilst apomorphine was administered s.c. Each value represents the mean ± s.e.m. (n = 10). The drug effect for a particular parameter was compared with its respective control group. VEH = vehicle.

Treatment (mg kg^{-1})	Stereotypy Latency of onset (min)	Duration (min)	Maximum intensity (scored)
VEH + MAM (10)	36·2 ± 0·34	142·0 ± 3·54	1·0 ± 0·00
ERM (5) + MAM (10)	35·7 ± 0·26	147·0 ± 3·92	1·1 ± 0·10
ERM (10) + MAM (10)	23·9 ± 0·28*	175·0 ± 4·25*	1·6 ± 0·16**
ERM (20) + MAM (10)	18·6 ± 0·31*	192·0 ± 4·59*	1·9 ± 0·10**
VEH + MAM (12·5)	31·4 ± 0·32	154·0 ± 3·27	1·2 ± 0·13
ERM (5) + MAM (12·5)	30·8 ± 0·29	160·0 ± 3·75	1·4 ± 0·16
ERM (10) + MAM (12·5)	18·9 ± 0·27*	198·0 ± 4·12*	1·9 ± 0·10**
ERM (20) + MAM (12·5)	13·6 ± 0·24*	204·0 ± 4·34*	2·0 ± 0·00**
VEH + APO (1)	7·7 ± 0·22	32·9 ± 0·32	0·9 ± 0·10
ERM (5) + APO (1)	7·9 ± 0·16	33·3 ± 0·22	1·0 ± 0·00
ERM (10) + APO (1)	7·6 ± 0·18	34·1 ± 0·33	1·1 ± 0·10
ERM (20) + APO (1)	8·1 ± 0·20	33·6 ± 0·25	1·0 ± 0·00
VEH + APO (1·5)	5·9 ± 0·15	38·8 ± 0·34	1·3 ± 0·15
ERM (5) + APO (1·5)	5·7 ± 0·18	39·4 ± 0·32	1·5 ± 0·16
ERM (10) + APO (1·5)	6·1 ± 0·16	37·9 ± 0·29	1·2 ± 0·13
ERM (20) + APO (1·5)	5·8 ± 0·19	39·2 ± 0·26	1·4 ± 0·16

* Differs from vehicle treated, $P < 0.05$ or less (Student's *t*-test).
** Differs from vehicle treated, $P < 0.05$ or less (Mann-Whitney U-Test).

Table 2. Number of guinea-pigs exhibiting biting, gnawing or licking behaviour following administration of amantadine (AMA) to ergometrine (ERM)-pretreated animals. Ergometrine was injected i.p. 45 min before amantadine.

Pretreatment (mg kg^{-1} i.p.)	Treatment (mg kg^{-1} i.p.)	Number of guinea-pigs exhibiting biting, gnawing or licking behaviour		
		Number	%	P*
Vehicle	AMA (75)	0/6	0·0	
ERM (5)	AMA (75)	0/6	0·0	
ERM (10)	AMA (75)	4/6	66·6	0·03
ERM (20)	AMA (75)	6/6	100·0	0·001
Vehicle	AMA (100)	0/10	0·0	
ERM (5)	AMA (100)	0/10	0·0	
ERM (10)	AMA (100)	9/10	90·0	0·027
ERM (20)	AMA (100)	10/10	100·0	0·001

* Value for difference from vehicle-pretreated animals in each group. Statistical analyses performed using the Fisher exact probability test.

In animals pretreated with 10 mg kg^{-1} ergometrine, 75 and 100 mg kg^{-1} amantadine induced biting, gnawing or licking behaviour in 66·6% (n = 6) and 90% (n = 10) of the animals respectively (Table 2) while in animals pretreated with 20 mg kg^{-1} ergometrine, amantadine (75, 100 mg kg^{-1}) induced the SB in 100% (Table 2). The biting, gnawing or licking behaviour began about 15 min after injection of amantadine and occurred episodically for short intervals throughout the 1 h observation.

Discussion

After an initial delay of 30–40 min in which sedation and ptosis predominate, a bilateral injection of ergometrine into the n. accumbens of the rat, induces a strong and long-lasting locomotor stimulation, although an i.p. injection of ergometrine fails to do so (Pijnenburg et al 1973). In our study also i.p. administered ergometrine failed to induce locomotor stimulation. The 5-HT receptor agonist activity of ergometrine (Antkiewicz Michaluk 1977) might be responsible for the occurrence of 'wet dog shake' behaviour, fore-limb movements and extension spasms of hind legs at rest, behaviour reported to occur due to stimulation of central 5-HT receptors by directly or indirectly acting 5-HT agonists (Bedard & Pycock 1977; Trulson & Jacobs 1976).

Our observation that the SB induced by methamphetamine and apomorphine was characterized almost exclusively by biting, gnawing or licking agrees with that of Costall & Naylor (1975). There is disagreement among workers whether amantadine induces SB in rodents and those who think it does are agreed that it induces only low intensity SB in the rat characterized by sniffing (for reviews see Parkes 1974; Bailey & Stone 1975). Since this component is not induced in the guinea-pig no SB was seen following administration of amantadine to guinea-pigs.

The potentiation of methamphetamine stereotypy by ergometrine and the induction of biting, gnawing or licking behaviour by amantadine in ergometrine pretreated animals is explained by us as follows. Methamphetamine and amantadine act by releasing DA (Wallach 1974; Parkes 1974) which activates the DAe and DAi receptors. Ergometrine, by blocking the DAi receptors makes more DA available for interaction with the DAe receptors thereby potentiating methamphetamine stereotypy and inducting biting, gnawing or licking behaviour by amantadine. Also, since Cools & Van Rossum (1976, 1980) have suggested that the DAi system exerts an opposing influence on the DAe system, another possibility exists. The activation of DAi receptors by released DA might be opposing the manifestation of SB which occurs as a result of activation of DAe receptors. Ergometrine, by blocking the DAi receptors, counteracts this opposing inhibitory influence on the DAe system and permits full expression of methamphetamine's and amantadine's DAe receptor mediated behaviour, i.e. potentiation of methamphetamine stereotypy and induction of biting, gnawing or licking behaviour by amantadine. As apomorphine, unlike methamphetamine and amantadine, specifically activates only DAe receptors, its SB occurs as a result of activation of DAe receptors without concomitant activation of DAi receptors and opposition from DAi system. This might be the reason why ergometrine pretreatment failed to influence apomorphine stereotypy.

Furthermore, other neurotransmitter systems might have been operative in mediating the potentiating effect of ergometrine on methamphetamine stereotypy and in facilitating the induction of biting, gnawing or licking behaviour by amantadine in ergometrine-pretreated animals. Amphetamines release noradrenaline (NA) and 5-HT (Raiteri et al 1977), amantadine releases NA (Parkes 1974), and ergometrine acts as both agonist and antagonist of α-adrenoceptors and 5-HT receptors (Rall & Schleifer 1980). As the central NA (Mogilnicka & Braestrup 1976; Braestrup 1977) and 5-HT (Costall & Naylor 1974; Pycock et al 1978) systems modulate DA mediated SB, it is possible that ergometrine might have potentiated methamphetamine stereotypy and facilitated induction of biting, gnawing or licking behaviour by amantadine through modulation of the activity of the central NA and 5-HT systems.

In conclusion, the DAi receptor antagonist ergometrine was effective in potentiating methamphetamine stereotypy and in facilitating the induction of biting, gnawing or licking behaviour by amantadine but did not modify SB induced by the DAe agonist apomorphine.

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Intracisternal glycine activates the micturition reflex in urethane-anaesthetized rats

CARLO ALBERTO MAGGI*, MARTINO FURIO†, PAOLO SANTICIOLI, ALBERTO MELI, *Pharmacology Department, Smooth Muscle Division, Research Laboratories, A. Menarini Pharmaceuticals, Via Sette Santi 3,50131, Florence and †C.R.F. Via Tito Speri, Pomezia, Rome, Italy*

Intracisternal glycine, but not GABA, activates a series of neurogenic rhythmic contractions of the urinary bladder in urethane-anaesthetized rats. This effect of glycine was prevented by strychnine but not by bicuculline, indicating the involvement of specific glycinergic receptors. The effects of glycine were also prevented by either atropine or haloperidol suggesting an involvement of cholinergic and monoaminergic excitatory neurotransmission to the bladder.

Glycine, one of the major inhibitory neurotransmitters in the central nervous system, is widely distributed in the spinal cord where it serves as transmitter for presynaptic inhibition (Snyder 1975). Exogenous glycine inhibits firing of sacral parasympathetic neurons (De Groat, 1970). It has been proposed that endogenous glycine participates in the physiological regulation of the micturition reflex by suppressing the reflexly activated firing of preganglionic neurons in the sacral spinal cord (De Groat and Ryall 1968).

In the course of experiments aiming to investigate the effects of i.v. glycine on the micturition reflex in urethane-anaesthetized rats we observed that this amino acid, depending upon the dose and/or the degree of activation of the bladder, produces either excitatory or inhibitory effects on bladder motility.

* Correspondence.

In normal rats, as in cats and man, activation of a pontine centre induces micturition (Sato et al 1975, 1983; De Groat 1975; Satoh et al 1978). High levels of glycine (Aprison et al 1969) as well as high affinity uptake systems (Bennett et al 1973) and specific strychnine binding sites (Young & Snyder 1973) are present in the medulla oblongata-pons suggesting that glycine might serve as a neurotransmitter at this level (Snyder 1975).

Therefore it appeared worthwhile to determine whether the excitatory effects observed in some animals following i.v. glycine could be attributed to an effect on supraspinal centres involved in the regulation of the micturition reflex.

Materials and methods

Male albino rats, Wistar Morini strain, 340–360 g, were anaesthetized with subcutaneous urethane ($1{\cdot}2\ g\ kg^{-1}$) and the left jugular vein was cannulated for drug injection. Urethane was chosen as anaesthetic because it was shown to allow the study of both excitatory and inhibitory mechanism(s) on the micturition reflex (Maggi et al 1983, 1984a, b). The urinary bladder was exposed through a midline incision of the abdomen, emptied of urine by slight manual pressure, cannulated with a polyethylene tubing, and prepared for recording