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ERGOMETRINE INDUCED LOCOMOTOR ACTIVITY FOLLOWING INTRACEREBRAL INJECTION INTO THE NUCLEUS ACCUMBENS

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SUMMARY

Ergometrine was injected in rats intraperitoneally or intravenously and also directly into different parts of the brain. After peripheral injection the animals showed a characteristic crawling behaviour. This behaviour was reversed by apomorphine.

Following bilateral injection of ergometrine into the nucleus accumbens, the rats showed a biphasic response. After a period, during which sedation and ptosis predominated, there followed a strong and long-lasting locomotor stimulation, which started and finished in most cases rather abruptly. When the doses of ergometrine were lowered the locomotor activity remained at a constant high level, but the duration was shorter. Bilateral injection of ergometrine into the caudate nucleus or into the septum had only little or no stimulant effect on locomotor activity.

The strong locomotor stimulation following injection of ergometrine into the nucleus accumbens was inhibited by low doses of haloperidol and pimozide given intraperitoneally.

Pretreatment with a-methyl-p-tyrosine had no influence on this motor activity.

Ergometrine injected unilaterally into the nucleus accumbens and into the caudate nucleus caused only a slight enhanced locomotor activity and turning to the contralateral side.

The possible mode of action of ergometrine is discussed.

INTRODUCTION

Ergometrine has been used widely in therapy as a uterus spasmogen since its pharmacological properties were described in detail by Brown and Sir Henry Dale.
in 1935. They also described a central stimulant action of ergometrine with the production of 'sham rage'. A similar effect was reported by Gaddum and Vogt\textsuperscript{13} after the injection of 2 mg ergometrine into the lateral ventricle of conscious cats.

Recently ergometrine has been shown to be a potent antagonist of the inhibitory action of dopamine on neurones of the snail \textit{Helix aspersa}\textsuperscript{23,26}. The effect of dopamine on these \textit{Helix aspersa} neurones appears to be mediated by true dopamine receptors\textsuperscript{25}.

In the mammalian brain dopamine plays an essential role\textsuperscript{14}. We have considered the possibility that some of the central actions of ergometrine in mammals could be brought about by an action on dopamine receptors. For this reason we decided to investigate some of the central actions of this compound in rats by intracerebral injections. In this paper we report a strong locomotor stimulant action of ergometrine.

\textbf{METHODS}

\textit{Intraperitoneal and intravenous injections}

Male Wistar rats were used, weighing 150–200 g, housed in groups of 3, with free access to food and water.

In some experiments groups of 4 rats, each subjected to drug treatment, were placed in a semicircular wooden cage with a glass front. The cage had a radius of 40 cm and a height of 60 cm. In another similar cage 4 untreated or vehicle treated animals were placed for comparison. The behaviour of the animals was observed for up to 4 h following the injection.

In other experiments individual animals were placed in a motor activity cage (see under apparatus) and locomotor activity was recorded for up to 6 h after the injection.

Ergometrine maleate, dissolved in saline, was injected intraperitoneally in doses of 5.0 and 10.0 mg/kg or intravenously (tail vein) in doses of 1.0, 2.0 and 5.0 mg/kg, either alone or followed by apomorphine HCl in a dose of 1.0 mg/kg i.p. about 20 min later.

\textit{Injections directly into the brain}

Male Wistar rats were used, weighing 200–220 g at the time of operation. Under sodium pentobarbital anaesthesia (40 mg/kg i.p.) double barrelled stainless steel cannulae were implanted bilaterally into different regions of the brain. The injection sites aimed for were: the head of the caudate nucleus in 4 rats (coordinates A 9.4, L 2.0, H 1.0 according to the atlas of König and Klippel\textsuperscript{16}); the nucleus accumbens in 6 rats (coordinates A 9.4, L 1.2, H – 0.6) and the septum in 3 rats (coordinates A 8.0, L 0.5, H 0.6). The cannulae placed in the caudate nucleus and the nucleus accumbens were brought in from the lateral side at an angle of 10° in order to avoid the ventricles, and were fixed on to the skull with acrylic dental cement (Paladur). The cannulae placed in the septum were brought in from the contralateral side at an angle of 12.5° and also at an angle of 12.5° from the posterior and anterior sides. Two jeweller's screws were placed into the skull to firmly attach the cement. The diameter of the outer and inner cannulae were 0.65 and 0.30 mm respectively.
After surgery the animals were put into individual home cages. After a period of 2 weeks, in which the animals recovered from the operation and during which time they were accustomed to handling, the rats were put into the activity cages several times in order to adapt to the new environment.

Injections were given to the unanaesthetized animals by means of a 5 µl Hamilton syringe with a 31-gauge needle, which extended into the brain tissue 1.5–2.0 mm below the tip of the permanently embedded cannula. The rats were injected bilaterally and unilaterally. The injection volume was always 0.5 µl. Ergometrine maleate, dissolved in saline, was given in doses of 5.0, 2.0, 1.0, 0.5, 0.1 and 0.01 µg on each side. After replacement of the inner cannulae the animals were placed singly in semicircular cages to observe their behaviour or into a motor activity cage, where their locomotor activity was recorded for up to 6 h or longer if necessary. Following the injection the animals were left to recover for at least 2 days before they were used again.

In some experiments the animals were pretreated with neuroleptics before the intracerebral injection of ergometrine. For this purpose haloperidol, dissolved in propylene glycol, was given intraperitoneally in doses of 0.1 and 0.5 mg/kg 15 and 30 min respectively before the injection of ergometrine. Pimozide, dissolved in diluted (+)-tartaric acid, was injected intraperitoneally in a dose of 0.1 mg/kg 3 h before ergometrine.

The tyrosine hydroxylase inhibitor DL-a-methyl-p-tyrosine, emulsified with tragacanth, was administered orally in a dose of 200 mg/kg 8 h before the injection of ergometrine.

**Apparatus**

Locomotor activity was measured in activity cages, equipped with 3 light sources and opposite to them 3 photoelectric cells 2 cm above the floor. The dimensions of the cage were 36 cm × 24 cm × 25 cm. The light beams were arranged in such a manner, that one was directed perpendicular to the 2 others and the floor of the cage was divided into 6 squares of 12 cm × 12 cm. The cages were situated in a ventilated soundproof box and illuminated with a house light of constant intensity. The interruptions of the light beams were registered on a cumulative Ralph–Gerbrands recorder. After 500 interruptions the pen of the recorder returned automatically to the base line.

**Histological procedures**

After the end of the experiments the animals were perfused first with saline and then with 30 ml of a 10% formalin solution through the left cardiac ventricle under sodium pentobarbital anaesthesia. The brains were removed and kept in a 4% formalin solution for 7 days. After that time the brains were dehydrated and embedded in paraffin. Serial 15 µm thick frontal sections were cut and stained with haematoxylin–eosin. The position of the tips of the needle-tracks were marked on a diagram (see Fig. 1).
RESULTS

Intraperitoneal and intravenous injections

Ergometrine injected intraperitoneally (5.0 or 10.0 mg/kg) produced a characteristic response about 5–10 min after the injection. There was piloerection and the rats adopted a characteristic posture. The rats would crawl slowly round the perimeter of the cage with the belly touching the floor and the legs (especially the hind legs) extended. The movement was irregular and the rats would frequently stay motionless for several seconds before moving on again. When provoked by a sound stimulus the rats would temporarily adopt a normal running posture. The effect of ergometrine on the posture of the animal normally began to wear off about 60–90 min after the injection. In all cases the animals recovered and looked normal the following day.

A similar effect was observed after the intravenous injection of ergometrine (1.0, 2.0 or 5.0 mg/kg); in these experiments the effect came on within 1 min of the injection and lasted for about 20–60 min.

When animals treated with ergometrine intraperitoneally or intravenously were studied in an activity cage, there was no sign of a strong effect on locomotor activity (see Fig. 3B).

In some experiments, animals which had received ergometrine (5.0 or 10.0 mg/kg i.p.) were then injected after about 20 min with apomorphine (1.0 mg/kg i.p.). The effect of the ergometrine was reversed within 5 min and the animals now showed a strong behavioural response to apomorphine, including compulsive gnawing, arching of the back, Straub tail’s phenomenon and sometimes the bizarre behaviour described by Lammers and van Rossum. In some cases, especially with the higher doses of ergometrine, the combination of ergometrine followed by apomorphine was fatal; in
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Fig. 2. Motor activity of a rat (rat 1) as measured in the activity cage. After a period of adaptation to the cage, ergometrine 2.0 μg bilaterally is injected into the nucleus accumbens (black arrow). Following the injection there is a period of low activity. After about 1 h the motor activity increases and remains at a high constant level for several hours.

these animals there were circling movements, much tail-biting and convulsions with death, occurring about 30–60 min after the injection of apomorphine.

_Intracerebral injections_

_Bilateral injections._ After bilateral injection of different doses of ergometrine into the nucleus accumbens there was a biphasic response. Within a few minutes after the injection the animals showed ptosis and sedation. Further, the animals showed signs of shivering, abortive grooming, scratching the body and involuntary movements of the legs, the trunk and the head. In a few cases the characteristic crawling movements as seen after intraperitoneal or intravenous injection appeared after intracerebral injection. After a period of 30–60 min following the injection the animals began to run and showed a strong locomotor activity. This enhanced locomotor activity started in most cases very suddenly and lasted for up to 7 or 8 h after the injection of 5.0 μg. Fig. 2 shows the effect of injection of 2.0 μg bilaterally as measured in the activity cage. This phenomenon was observed in all 6 rats with cannulae embedded
Fig. 3. A: shows the time course of locomotor activity of a rat (rat 5) after injection of 2.0 μg bilaterally into the nucleus accumbens. In this and the following figures the values for motor activity, as calculated from the cumulative recording data, are averaged over 5 min periods and plotted. B: shows the motor activity of a rat following the injection of ergometrine 10 mg/kg intraperitoneally.

in the nucleus accumbens. The same rats injected with ergometrine 2 or more days later showed a similar locomotor stimulation. The injection of saline (0.5 μl bilaterally) had no effect on the motor activity (see Fig. 4A). When ergometrine was injected into the caudate nucleus the animals also showed ptosis and sedation, and the same signs of shivering, abortive grooming and so on were observed. However, relative to the effect seen after injection into the nucleus accumbens, there was only a slight
enhancement of motor activity starting about 90 min after the injection of 5.0 \( \mu \text{g} \) bilaterally and usually lasting for up to 3–4 h (see Fig. 4B).

Injection into the septum (2.0 \( \mu \text{g} \) bilaterally) had no stimulant effect on locomotor activity.

Ergometrine in lower doses (1.0 and 0.5 \( \mu \text{g} \) bilaterally) when injected into the nucleus accumbens again produced enhanced locomotor activity in all 6 animals. However, at these dose levels, although the motor activity, when measured and averaged over 5 min periods, was approximately as high as with a dose of 5.0 \( \mu \text{g} \) bilaterally, the duration of the effect was shorter. Thus after the administration of 1.0 \( \mu \text{g} \) the motor activity lasted for about 4–5 h. After the injection of 0.5 \( \mu \text{g} \), locomotor activity was increased for about 3–4 h. With even lower doses of ergometrine injected into the nucleus accumbens fewer animals responded. After doses of 0.1 \( \mu \text{g} \) bilaterally
there was locomotor stimulation in only 3 out of 6 animals. In the animals that did respond the locomotor stimulation lasted for about 2–3 h. Ergometrine 0.01 μg was only effective in 1 out of 6 animals which showed locomotor stimulation lasting for 2 h.

When injected into the caudate nucleus in doses of 1.0 μg or 0.5 μg bilaterally, ergometrine was without stimulant effect on locomotor activity.

Fig. 5. A: motor activity of a rat (rat 3) following the injection of ergometrine 1.0 μg bilaterally into the nucleus accumbens. B: motor activity of the same rat, pretreated with pimozide 0.1 mg/kg i.p., 3 h before the injection of ergometrine. The locomotor stimulation is strongly inhibited. About 3 h after the injection of ergometrine there is a slight stimulation of locomotor activity lasting for about 1 h.
In most cases the enhanced locomotor activity induced by injection of ergometrine into the nucleus accumbens started rather abruptly and remained continuous.

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**Fig. 6.** A: motor activity of a rat (rat 6) following the injection of 0.5 μg ergometrine bilaterally into the nucleus accumbens. B: motor activity of the same rat after pretreatment with haloperidol 0.1 mg/kg i.p. 15 min before the injection of ergometrine. The locomotor stimulation is completely inhibited.
ously high for long periods. The cessation of activity was rather sudden and was usually preceded by short pauses. It should be noted that during this period of strong locomotor activity the animals showed no sign of aggressiveness but, on the contrary, could be handled very easily without resistance. When they were handled, they stopped their activity and after they had been replaced in the observation cage they resumed their running. The motor activity consisted of coordinated movements. The animals continuously showed walking, running, rearing or climbing and could easily avoid obstacles. When the rats were placed on a table and reached the edge of it, they never fell off. During this period of activity very little or no grooming was observed.

Pimozide (0.1 mg/kg i.p.) given 3 h before the injection of ergometrine (1.0 μg bilaterally) into the nucleus accumbens completely blocked the locomotor stimulant effect in 3 out of 6 rats. In 2 other rats, pretreated with this dose of pimozide, ergo-

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**Fig. 7.** Motor activity of a rat (rat 2) following the injection of ergometrine 1.0 μg bilaterally into the nucleus accumbens after pretreatment with α-methyl-p-tyrosine 200 mg/kg orally 8 h before the injection of ergometrine.
metrine produced only a slight enhancement of locomotor activity which started after 3 and 3.5 h respectively and which lasted for only 1 h (see Fig. 5). In the remaining rat there was no sign of inhibition of the ergometrine effect by pimozide.

Haloperidol (0.1 mg/kg i.p.), when given 15 min before, completely antagonized the locomotor activity after bilateral injection of 0.5 μg ergometrine in 5 out of 6 rats (see Fig. 6). In the remaining rat locomotor stimulation was still produced but only after a delay of 100 min. The inhibition by haloperidol was overcome by higher doses of ergometrine. The effect of ergometrine, 5.0 μg bilaterally, was inhibited by 0.5 mg/kg i.p. given 30 min before in only 2 out of 6 rats.

Injection of the solvents propylene glycol or L(−)-tartaric acid solution had no effect on the enhanced activity.

α-Methyl-p-tyrosine (200 mg/kg orally), administered 8 h before the injection of ergometrine (1.0 μg bilaterally) into the nucleus accumbens, had no clear-cut effect on the locomotor stimulation in any of the 6 rats (see Fig. 7), although the rats were strongly sedated before they received ergometrine.

Unilateral injections. After unilateral injection of 5.0 μg ergometrine into the nucleus accumbens the animals showed the same phenomena as after bilateral injections. After a period of 30–60 min they became active. The duration of this activity was less than after bilateral injection, lasting for 3–4 h. Also the degree of activity was considerably less (see Fig. 8). When the animals started to run they often showed turning to the contralateral side (opposite to the side injected). In some animals there was only a slight preference for contralateral turning, in other animals this turning was very apparent and they turned 10–15 times/min. This contralateral turning was also observed, although to a lesser degree, after unilateral injection of 5.0 μg ergometrine into the caudate nucleus. In most cases this turning behavior was of shorter duration than the activity period.

Fig. 8. Motor activity of a rat (rat 6) following the injection of ergometrine 5.0 μg unilaterally (left side).
DISCUSSION

The crawling behaviour induced by intraperitoneal injection of ergometrine in rats is similar to the phenomenon in rabbits and cats reported by Brown and Dale, who concluded that this effect was mediated by a central mechanism.

A surprising finding in our study was the strong locomotor stimulation following the injection of small amounts of ergometrine into the nucleus accumbens. When injected intraperitoneally or intravenously ergometrine had no such effect. It is possible that ergometrine does not reach the nucleus accumbens in sufficient concentration following peripheral administration. Alternatively, it is likely that following intraperitoneal or intravenous injection any effect of ergometrine on the nucleus accumbens can be inhibited or modified by an action of ergometrine on other areas of the brain. Our results suggest that the nucleus accumbens might be important in controlling locomotor activity.

The nucleus accumbens contains a high density of dopamine nerve terminals. This nucleus takes part of the so called mesolimbic dopamine system. Axons of dopamine cell-bodies dorsocranial to the interpeduncular nucleus in the ventral mesencephalon (A10-group according to Dahlström and Fuxe) ascend together with axons of the nigrostriatal dopamine system, but follow a more medial route. The nerve terminals of these axons enter the nucleus accumbens, the nucleus interstitialis striae terminalis and the tuberculum olfactorium.

Little is known about the functional role of this dopamine system. Crow has shown that electrical self-stimulation can be elicited by electrodes in the A10-cell group. Also the nucleus accumbens is known to be a site for self-stimulation. In contrast to medial forebrain bundle self-stimulation, which influences the activity of many brain stem units and causes arousal, stimulation in the nucleus accumbens influences only very few brain stem units and causes hypoactivity. Therefore it is difficult to explain the strong locomotor activity after ergometrine injections into the nucleus accumbens by an influence on the brain stem reticular formation.

A remarkable fact is the long latency time with regard to the locomotor stimulation. Usually there is a delay of 30-60 min after the injections before the animals start to run. One possibility is that diffusion must take place to a site where the ergometrine is acting. However, the injection of ergometrine into surrounding structures such as the caudate nucleus and the septum had little or no effect on the locomotor activity. Moreover, diffusion is dependent on the concentration of the drug; it seemed, however, that different concentrations of ergometrine had the same latency time.

Another possibility is that a metabolite of ergometrine is the active component. Very little is known about the metabolism of ergometrine and other ergot-alkaloids. With regard to the rather abrupt start and finish of the locomotor activity and also to the fact that the activity remains at a constant high level independent of the concentration, it is most likely that some system must be 'triggered'. Which system this could be is unknown at present. In this respect it is worthy of mention that electrical stimulation of the dorsomedial posterior hypothalamus consistently produced running, rearing and other 'voluntary movements' throughout the stimulation period and that
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these movements were accompanied by rhythmical slow activity in the hippocampus as shown by Bland and Van der Wolf4.

Ergometrine is a substance, like other ergot-alkaloids, with a rather complex mechanism of action. It is known for instance that ergometrine will potentiate the action of noradrenaline on the guinea-pig vas deferens preparation. It is also known that ergometrine is a potent antagonist of 5-hydroxytryptamine8. However, methysergide, another antagonist of 5-hydroxytryptamine, was ineffective in producing locomotor stimulation (unpublished observation). As already mentioned in the introduction, ergometrine inhibits the dopamine response in the snail brain. In the rat, ergometrine injected intraperitoneally or intravenously was unable to inhibit the behavioural effects of apomorphine.

The locomotor stimulation was blocked by low doses of haloperidol and pimozide. Haloperidol is a potent antagonist of the stereotyped behaviour induced by amphetamine11,28 and also antagonizes the behavioural effects produced by injection of dopamine into the caudate nucleus of conscious cats6,7. Haloperidol and pimozide in low doses are believed to block dopaminergic receptors in mammals1,15,21,22. It is therefore quite possible that dopaminergic receptors are involved in this action of ergometrine. A presynaptic action is unlikely, because pretreatment with α-methyl-p-tyrosine, which causes a depletion of dopamine and noradrenaline in the brain19, had no influence. However, other mechanisms of action cannot be ruled out.

Further experiments to elucidate the mode by which ergometrine causes this strong and long-lasting motor activity are in progress.

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