THE INVOLVEMENT OF CATECHOLAMINE IN SCOPOLAMINE-INDUCED LOCOMOTOR ACTIVATION AND ROTATIONAL BEHAVIOUR IN MICE

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Accepted January 20, 1978

Abstract—Scopolamine-induced locomotor activation was studied in comparison with the responses to apomorphine and methamphetamine in mice. The responses to scopolamine and methamphetamine were markedly depressed by the pretreatment with the catecholamine synthesis inhibitor, \( \alpha \)-methyl-p-tyrosine, while the activation response to apomorphine was not affected. \( p \)-Chlorophenylalanine did not affect the response to scopolamine. Phenoxybenzamine reduced the responses to scopolamine and methamphetamine, but did not affect the apomorphine response. Propranolol did not affect the responses to the three agonists, scopolamine, apomorphine and methamphetamine. Antipsychotic drugs haloperidol and pimozide reduced the responses to the three agonists. Haloperidol was especially effective in this regard. These results suggest the involvement of catecholamine in the locomotor activation produced by scopolamine. In the rotational behaviour model which is sensitive to dopamine receptor stimulating agents, effects of the three agonists were studied. Scopolamine produced the ipsilateral rotation in mice with unilateral striatal 6-hydroxydopamine-induced lesions. Methamphetamine induced the ipsilateral rotation, while apomorphine produced the contralateral rotation. The rotations induced by three agonists were suppressed by pimozide. The results indicate the participation of dopamine in the scopolamine-induced rotational behaviour in mice.

Anticholinergic drugs produce very little effects on the central nervous system (CNS) in man with usual therapeutic dosages, while symptoms which are ascribed to central effects, such as drowsiness, restlessness, excitation, muscular incoordination and hallucination, are observed with increasing the dose of anticholinergic drugs (1). Anticholinergic agents, such as scopolamine and atropine, were reported to increase locomotor activity, orientational hypermotility and exploratory activity in open-field in mice and rats (2, 3, 4, 5, 6). These excitatory effects of anticholinergics have been investigated in connection with changes in brain acetylcholine (ACh) contents or the release of ACh from cerebral cortex in small animals (7, 8, 9).

We have found that scopolamine-induced locomotor activation was reduced by the pretreatment with \( \alpha \)-methyl-p-tyrosine (\( \alpha \)-MPT) or antipsychotic drugs. To determine whether or not brain catecholamine (CA) plays a role in the scopolamine-induced response, we tested various drugs with specific or nonspecific actions on CA for their ability to modify the locomotor activation induced by scopolamine as well as apomorphine and methamphetamine. We also studied the rotational behaviour following administration of scopolamine to mice with unilateral striatal 6-hydroxydopamine (6-OHDA) lesions, since rotational behaviour in animals with unilateral lesions in nigro-striatal dopaminergic pathway has been...
reported to be a sensitive model for dopaminergic stimulating agents (10, 11).

MATERIALS AND METHODS

Adult male albino mice (ddY strain) weighing between 20 and 30 g housed under uniform humidity, temperature, feeding and lighting conditions (on 0700, off 1900), were used in all experiments.

Measurement of locomotor activity

Locomotor activity was measured in the activity cage equipped with 2 light sources and opposite to them, 2 photoelectric cells 1 cm above a floor. The dimensions of the plastic cage were 30 × 20 × 13 cm. The interruptions of the light beams were registered on a counter. Only one animal at a time was placed in the cage. All experiments were performed between 0900 and 1800. After 20 min for adaptation to the activity cage, locomotor activity was measured for at least one hour following the administration of a drug.

Injection of 6-OHDA into the unilateral striatum

Under pentobarbital anesthesia the head of the mouse was positioned on the stereotaxic apparatus and injection of 6-OHDA into the unilateral striatum was performed through a stainless steel pipe with 0.2 mm in the outer diameter connected to Gilmont micrometer syringe. The pipe was processed with a micromanipulator. 6-OHDA-HBr 16 μg dissolved in sterile saline contained ascorbic acid (0.2 mg/ml) was injected in a volume of 4 μl over 2 min according to the method of Voigtlander and Moore (11). The stereotaxic coordinates of the injection site were A: 4.0, L: 2.0, H: +2.5 according to the stereotaxic atlas of Montemurro and Dukelow (12).

Measurement of rotational behaviour

A mouse was placed in the plastic cage with dimensions of 24 × 17 × 12 cm. A drug was given i.p. after 10 min of adaptation to the cage. Rotational behaviour was directly observed and 360° turn was taken for one rotation for at least 1 hr following the administration of a drug.

Drugs

Scopolamine hydrobromide, scopolamine methylbromide, apomorphine hydrochloride, methamphetamine hydrochloride, phenoxybenzamine hydrochloride, propranolol hydrochloride and p-chlorophenylalanine methylester hydrochloride (PCPA) (Sigma) were dissolved in physiological saline. Pimozide (Janssen) and haloperidol (McNeil) were mixed with tartaric acid and dissolved in hot water. The doses of drugs were expressed as salts except for PCPA.

RESULTS

Effects of scopolamine, scopolamine methylbromide, apomorphine and methamphetamine on spontaneous locomotor activity

Scopolamine at the doses of 2.5, 5 and 10 mg/kg, s.c., increased locomotor activity for 30 min after the administration. The time course of the locomotor activation and the total
count, which increased dose-dependently, for 1 hr after the administration are shown in Fig. 1. Scopolamine methylbromide 10 mg/kg, s.c., had no effect on the locomotor activity, while 20 mg/kg increased locomotor activity in two of ten mice, as did scopolamine. Apomorphine at the doses of 0.5 and 1.25 mg/kg, s.c., increased locomotor activity in a dose-dependent manner, while stereotyped behaviour appeared when a dose of over 4 mg/kg was given. The time course of locomotor activity and the total count for 1 hr are shown in Fig. 2. After the administration of methamphetamine 2.5 mg/kg, s.c., the locomotor activation appeared at 10 min, reached a maximum at 50 min, and lasted over 2 hr. Me-

**Fig. 1.** Locomotor activity counts following scopolamine (above) and methylscopolamine (below). Activity at each time expressed as the mean of ten minute counts (left) and total counts accumulated for 1 hr (right) from ten mice. Vertical bars indicate standard errors of the means.

**Fig. 2.** Locomotor activity counts following apomorphine (above) and methamphetamine (below). Activity at each time and total counts are the same as in Fig. 1.
thamphetamine at the doses of 1.25 and 2.5 mg/kg increased the total count for 1 hr dose-
dependently (Fig. 2). In the following experiments the responses to scopolamine 10 mg/kg, apomorphine 2.5 mg/kg and methamphetamine 2.5 mg/kg were examined after the pretreatment with monoamine synthesis inhibitors and antagonists.

Effects of various drugs on locomotor activation responses induced by scopolamine, apomorphine and methamphetamine

The responses to scopolamine and methamphetamine were reduced after the treatment with α-MPT 100 mg/kg, i.p., three times at 24, 16 and 3 hr before the drugs, while the effect of apomorphine was not modified. The treatment with PCPA 300 mg/kg, i.p., slightly increased the locomotor activation responses to apomorphine and methamphetamine, but did not affect the response to scopolamine.

The α-adrenergic blocking agent phenoxybenzamine 10 mg/kg, s.c., impaired the responses to scopolamine and methamphetamine, but not to apomorphine. The β-adrenergic blocking agent propranolol 10 mg/kg, s.c., did not affect the responses to the three agonists, i.e. scopolamine, apomorphine and methamphetamine.

Haloperidol 0.2 mg/kg, s.c., which blocks both noradrenergic and dopaminergic receptors in the CNS (13), suppressed the responses to three agonists. When pimozide, which has only a dopaminergic receptor blocking effect (13), was given at the dose of 0.2 mg/kg, s.c., one hour before the three agonists, it prevented the responses to apomorphine and methamphetamine. The activation response to scopolamine was reduced by pimozide 0.4 mg/kg, s.c. These results are summarized in Table 1 as changes in the total counts for one hour after administration of the three agonists.

| Table 1. Effects of pretreatment with monoamine-related drugs on the locomotor activation responses to scopolamine, apomorphine and methamphetamine |
|---|---|---|---|
| Treatment | Drugs | Scopolamine 10 mg/kg, s.c. | Apomorphine 2.5 mg/kg, s.c. | Methamphetamine 2.5 mg/kg, s.c. |
| No treatment | 418±61 | 507±108 | 777±114 |
| α-Methyl-p-tyrosine 100 mg/kg, i.p. × 3 | 112±19** | 613±67 | 61±17** |
| p-Chlorophenylalanine 300 mg/kg, i.p. | 476±50 | 749±52 | 1007±100 |
| Phenoxybenzamine 10 mg/kg, s.c. | 250±35** | 553±70 | 401±74** |
| Propranolol 10 mg/kg, s.c. | 411±82 | 489±82 | 710±121 |
| Haloperidol 0.2 mg/kg, s.c. | 61±15** | 98±27* | 37±10** |
| Pimozide | 87±8** | 94±31** | 71±20** |

Activity is expressed as the mean ± standard error of the mean of total counts for one hour from ten mice. Total counts for one hour in the saline group were 27±3. α-Methyl-p-tyrosine was given 24, 16 and 3 hr, p-chlorophenylalanine 24 hr, phenoxybenzamine, propranolol and haloperidol 20 min and pimozide 1 hr before the agonists.

a) Pimozide 0.4 mg/kg was given for the scopolamine group. **P<0.01 compared with the respective control.
Rotational behaviour in mice following apomorphine, methamphetamine and scopolamine

Mice were given apomorphine 1.25 mg/kg, i.p., 10 days after the injection of 6-OHDA into the right striatum. Different doses of apomorphine, scopolamine or methamphetamine were given to the mice that had showed marked rotational behaviour after apomorphine 1.25 mg/kg. As shown in Fig. 3, apomorphine 0.625, 1.25 and 2.5 mg/kg induced contralateral rotation, which appeared in 2–3 min after the injection and disappeared within one hour, in a dose-dependent manner with respect to total rotation for one hour. Methamphetamine 2.5 and 5 mg/kg, i.p., caused an ipsilateral rotation, which reached a maximum at 30 to 40 min after the injection and lasted over 2 hr. Total rotation for 2 hr was also shown in Fig. 3. As shown in Fig. 4, scopolamine 2.5 and 5 mg/kg produced an ipsilateral rotation, which was maximum at 10 min and thereafter decreased rapidly. Total rotation by scopolamine was less than that of methamphetamine. The rotational behaviour induced by apomorphine was prevented by pimozide 0.8 mg/kg and those of methamphetamine and scopolamine reduced after pimozide 0.4 mg/kg given one hour before.

Fig. 3. Rotational behaviour following methamphetamine (M.A.P.) and apomorphine (Apo.) in mice with right striatal 6-hydroxydopamine lesions. Rotational behaviour is expressed as the mean of five minute counts from ten mice. Positive y-value shows an ipsilateral rotation and negative y-value the contralateral rotation. Total counts for rotation accumulated for 1 hr for apomorphine and for 2 hr for methamphetamine.

Fig. 4. Rotational behaviour following scopolamine 2.5 and 5 mg/kg in mice with right striatal 6-hydroxydopamine lesions. Rotational behaviour (left) is expressed in the same manner as in Fig. 3 and total counts (right) indicate the values for one hour (10 mice).
DISCUSSION

White et al. (14) studied actions of atropine and scopolamine on the CNS phylogenetically in a number of animal species. Both atropine and scopolamine exerted similar central effects, i.e. excitation and depression, in dogs. On the other hand, the increase in locomotor activity and the exploratory hypermotility in mice and rats were produced by scopolamine and atropine (2–6). In the present study scopolamine produced locomotor activation, while scopolamine methylbromide did not induce the response as the quarternary salt of scopolamine was less permeable to the CNS. Both apomorphine and methamphetamine produced an increase in locomotor activity at the low doses, while they produced the stereotyped behaviour, i.e. sniffing, gnawing and licking, at the high doses. These results coincided with the results reported by Tseng and Loh (15) that d-amphetamine (1–10 mg/kg) and apomorphine (1 and 10 mg/kg, i.p.) produced the increase in locomotor activity in mice.

\( \alpha \)-MPT, a tyrosine hydroxylase inhibitor, has been used as a pharmacological tool to reduce brain CA content which prevents drug actions mediated by endogenous CA. In the present study the locomotor activation response to scopolamine was markedly suppressed by \( \alpha \)-MPT. Sanger and Steinberg (16) reported that pretreatment with \( \alpha \)-MPT (3 x 60 mg/kg) depressed the increase in exploratory behaviour of rats as induced by scopolamine. These results suggest the involvement of brain CA in the locomotor activation and exploratory behaviour induced by scopolamine. If responses to scopolamine are exerted through brain CA, such would be antagonized by catecholaminergic receptor blockades. The locomotor activation produced by scopolamine was antagonized by the pretreatment with phenoxybenzamine. Phenoxybenzamine, an adrenergic \( \alpha \)-blocking agent, blocked noradrenergic but not dopaminergic receptors in the CNS (13). It has been reported that propranolol, an adrenergic \( \beta \)-blocker, antagonized the methamphetamine-induced excitation in mice (17). In the present study, however, propranolol affected neither the response to methamphetamine nor those to scopolamine and apomorphine. The reason for the discrepancy is not clear at present. Haloperidol and pimozide which have blocking effects on dopaminergic receptors in the CNS (13) prevented the responses to the three agonists. Taken together with above results, these findings strongly suggest that scopolamine-induced locomotor activation may be mediated by brain CA.

The excitation and the increase in locomotor activity following amphetamine were prevented by \( \alpha \)-MPT in mice (14, 16). Amphetamine-induced locomotor activation has been reported to be antagonized by phenoxybenzamine 25 mg/kg (18). These results suggested the participation of brain CA in the motor excitation with the agent (15, 19). Apomorphine-induced stereotypy in rats was not suppressed by the pretreatment with \( \alpha \)-MPT (20), and such was explained by the suggestion that apomorphine induced the response by the direct stimulation of dopamine receptors (20, 21).

In the rotational behaviour model of rats with unilateral degeneration of the nigrostriatal dopamine system by intracerebral injection of 6-OHDA, the direction of the rotation has been found to reflect the degree of dopamine receptor stimulation in the denervated or innervated striatum (22). In the present study, scopolamine produced an ipsilateral rotation
as did methamphetamine in the model of mice with 6-OHDA-induced lesions in the unilateral striatum. Amphetamine as well as methamphetamine produced an ipsilateral rotation due to the release of dopamine from the presynaptic site in the normal side of the striatum (23). Apomorphine produced the contralateral rotation due to denervation supersensitivity to the dopamine receptor stimulation in the denervated striatum in mice as well as rats (11, 22). These results suggest that scopolamine induces stimulation of dopamine receptors through an indirect mechanism. Ungerstedt et al. (10) observed an ipsilateral rotation in the rat that had been given 6-OHDA into the unilateral substantia nigra following the administration of scopolamine. They explained that removal of an antagonistic cholinergic influence on the dopamine transmission in the normal striatum produced such an effect. It is well known that certain anticholinergic agents have a therapeutic effect on the extrapyramidal syndromes in Parkinson’s disease. These clinical experiences led to the supposition that functional balance between dopaminergic and cholinergic nerves plays an important role in the extrapyramidal system.

Neurochemical evidence presented by previous workers indicated the release of ACh (9) but not of dopamine following anticholinergic agents, and these agents were reported to have an inhibitory effect on dopamine metabolism (24–26). On the other hand, Anden and Bedard found both the rate of disappearance of brain noradrenaline after α-MPT and the haloperidol-induced acceleration of noradrenaline utilization to be increased after scopolamine (26). The results seem to favour the noradrenergic but not dopaminergic participation in the locomotor activation effect of scopolamine. In conclusion, we found that the scopolamine-induced locomotor activation is probably mediated by brain CA. Dopamine participation in the striatum was evidenced with application of the mouse rotational model used herein. The participation of brain CA may be related to the anticholinergic action of scopolamine.

REFERENCES

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