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# STUDIES ON THE BEHAVIOURAL PHARMACOLOGY OF A CYCLIC ANALOGUE OF DOPAMINE FOLLOWING ITS INJECTION INTO THE BRAINS OF CONSCIOUS RATS

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1 The cyclic analogue of dopamine, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) was injected into the lateral ventricle or bilaterally into the nucleus accumbens or caudate nucleus of conscious rats and its effect on locomotor activity was investigated.

2 When given intraventricularly, ADTN produced some stereotyped responses which were followed by a strong and long lasting stimulation of locomotor activity. When administered bilaterally into the nucleus accumbens a similar stimulation of locomotor activity was observed. ADTN had no effect on locomotor activity when injected bilaterally into the caudate nucleus.

3 The ADTN-induced locomotor stimulation following its intraventricular injection was completely abolished by a low dose of pimozide (0.01 mg/kg, i.p.) or haloperidol (0.5 mg/kg, i.p.). Pimozide (1 mg/kg, i.p.) given 30 min before ADTN injected bilaterally into the nucleus accumbens completely blocked locomotor stimulation.

4 Unilateral injections of ADTN (5 $\mu$g) into the nucleus accumbens caused locomotor stimulation but no turning.

5 Bilateral injections into the nucleus accumbens of 2-amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene or 0.9% w/v NaCl solution had no effect on locomotor activity.

6 It is concluded that the central stimulant action of ADTN is due to an effect on the dopamine receptors in the nucleus accumbens.

## Introduction

The functional role of dopamine in different aspects of animal behaviour has been reviewed by Hornykiewicz (1971) and Van Rossum (1970). It has been suggested that an increased locomotor activity can be produced by the stimulation of central dopamine receptors (Van Rossum & Hurkmans, 1964; Van Rossum, 1970). Dopamine may also have a major role in mediating turning behaviour in rats with unilateral lesions of the nigro-striatal dopamine tract (Ungerstedt, 1971).

The presence of dopamine in highly localized areas within the extrapyramidal system led to the suggestion that it may be involved in several physiological processes (Hornykiewicz, 1971), and in some extrapyramidal disorders (Papeschi, 1972; Klawans, 1973). The long dopamine-containing fibres arise from cell bodies in the substantia nigra (A9) or from cells caudal and dorsal to the substantia nigra (A8 and A10). These fibres terminate without crossing the midline in the striatum, the nucleus accumbens and the tuberculum olfactorium (see Dahlström & Fuxe, 1964). Microinjections of dopamine or ergometrine into the nucleus accumbens produce strong stimulation of locomotor activity in rats (Pijnenburg & Van Rossum, 1973; Pijnenburg, Woodruff & Van Rossum, 1973).

The tetralin derivative, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) contains the dopamine skeleton in a rigid molecule and might be expected to stimulate dopamine receptors (Woodruff, 1971). Recently it has been shown that ADTN causes strong and long-lasting stimulation of locomotor activity following its intraventricular administration into conscious mice and there is evidence that this action of ADTN is due to dopamine receptor activation (Woodruff, Elkhawad & Pinder, 1974b; Munday, Poat & Woodruff, 1974; Woodruff, Elkhawad, Crossman & Walker, 1974a).

In the experiments described in this paper we have investigated the site of the stimulant action of ADTN by injecting the compound into two areas of the rat brain where dopamine is highly

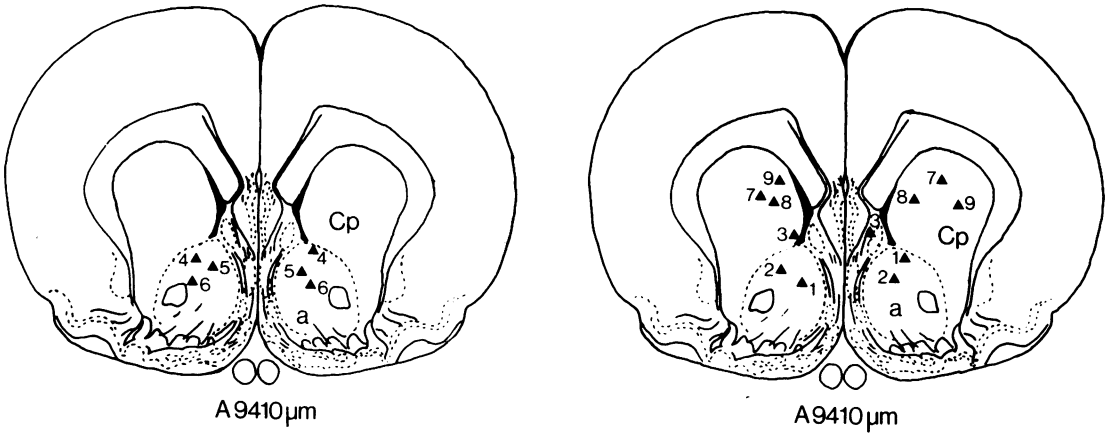


**Figure 1** Frontal sections of the rat brain according to the atlas of König & Klippel (1963) in which the injection site is indicated by ▲. The numbers 1-6 refer to the rats with cannulae in the nucleus accumbens; the numbers 7-9 to rats with cannulae in the caudate nucleus. Abbreviations: a: nucleus accumbens; Cp: caudate nucleus putamen.

localized. Since we have used rats for the experiments involving direct intracerebral injections it was first necessary to study the neuropharmacological actions of intraventricular injections of ADTN in conscious rats. The ability of some dopamine receptor antagonists, essentially neuroleptic drugs, to modify the behavioural actions of ADTN was also examined.

## Methods

### *Intraventricular injections*

Male rats (180-200 g) were lightly anaesthetized with a mixture of halothane, nitrous oxide and oxygen. Injections were made into the lateral ventricles by the method of Noble, Wurtman & Axelrod (1967). All drugs were dissolved in 0.9% w/v NaCl solution (saline) and injected unilaterally in a volume of 5 µl. The needle was allowed to stay in place for about one minute and then withdrawn. After recovery the animals were transferred to activity cages.

### *Intracerebral injections*

Stainless steel cannulae were implanted bilaterally into the nucleus accumbens or caudate nucleus of male Wistar rats (200 g) under sodium pentobarbitone anaesthesia (50 mg/kg, i.p.). Coordinates for the caudate nucleus were A 9.4, L 2.0, H 1.0, (3 rats), the nucleus accumbens A 9.4, L 1.5, H−0.6, (6 rats) according to the atlas of König & Klippel (1963). The cannulae were fixed onto the skull with acrylic dental cement. Two stainless steel jeweller's screws were placed into the skull to firmly attach the cement. After recovery from the operations the animals were housed individually. The animals were allowed to recover for at least two weeks during which they were accustomed to handling and to the activity cages.

Injections were given by a 1 µl Hamilton syringe fitted within a 31 gauge needle. The tip of the needle was allowed to extend into the brain tissue 0.1 mm below the tip of the cannula. All drugs were dissolved in saline and injected in a volume of 0.5 µl. After the injection the animals were transferred to the activity cages and the behaviour observed for several hours. Motor activity of the rats was measured for a period of 24 h starting at 14 h 00 min each day. Animals had free access to food and water. The rats were allowed a period of at least 48 h before they were used again.

### *Measurement of locomotor activity*

Locomotor activity was measured in conventional activity cages of the light beam type or in an Animex DSE activity meter. The former cages were equipped with three light sources and three photoelectric cells. The cages were circular, 36 cm in diameter and 25 cm high. The light sources and the photoelectric cells were equally distributed around the activity cage. The interruptions of the light beam were recorded on a Harvard cumulative recorder (Ralph Gerbrands). When using the Animex meter the animals were put singly in a perspex cage (38 x 26 x 25 cm) which was mounted on top of the activity meter. The apparatus was tuned in such a way that only large movements were recorded (sensitivity 10).

*Histology*

The animals were anaesthetized with sodium pentobarbitone (50 mg/kg, i.p.). They were perfused first with 30 ml of saline and then with 50 ml of Heidenhain's 'Susa' solution. The cannulae were carefully removed and the brains kept in Susa solution for a maximum period of 24 hours. Frontal sections (15 $\mu$m thick) were cut and stained with haematoxylin-eosin. The needle tracks were located and their tips marked on a frontal diagram of the rat brain (Figure 1).

*Drugs*

The following drugs were used: 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (ADTN), 2-amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene hydrochloride. Both were dissolved in saline. Haloperidol and pimozide (Janssen Pharmaceutica) were both dissolved in 2% tartaric acid.

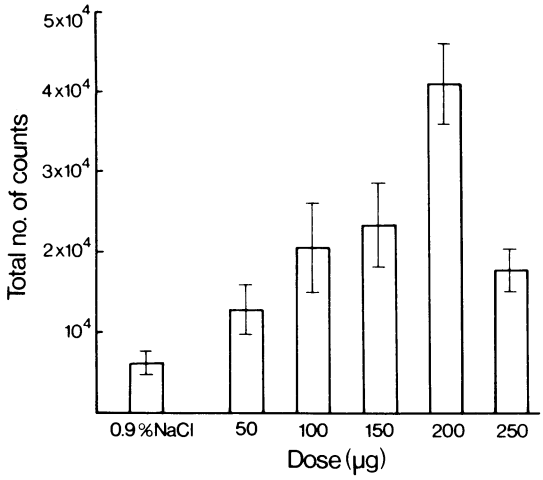


**Figure 2** Dose-total counts relationship of the different doses of ADTN injected intraventricularly on one side. Activity was measured in the Animex activity meter. Maximum response was obtained at 200 $\mu$g. Each column is mean result from 6 rats. Vertical bars show s.e. mean.

## Results

*Intraventricular injections*

Following the unilateral intraventricular injection of ADTN a biphasic effect was observed. In the first 60 min there was backward walking, piloerection, stereotyped head movements and turning away from the site of injection. In some rats the circling was accompanied by a twisted body position. Jerky and uncoordinated movements were also observed. Those responses were followed by increased sensitivity to sound and by rearing. The rearing gradually increased in intensity. The rats showed strong locomotor stimulation which lasted for several hours. The rats often made quick rushes across the cage. The effect of intraventricular injection of different doses of ADTN is shown in Figure 2, which shows a dose-total count relationship. The counts represent the large movements of the animal which consisted of forward walking, rearing and running activity. Maximum stimulation was obtained after a dose of 200 $\mu$g of ADTN. The activity of animals injected intraventricularly with saline was not significantly different from untreated animals during a period of 20 h after injection. Intraventricular injections of 2-amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene had no stimulant effect on motor activity.

The stimulation of locomotor activity produced by ADTN was not apparent until 1-2 h after the injection and then lasted between 12 and 18 h depending on the dose injected (Figure 3). The animals showed normal activity 24 h after the injection.

The ADTN-induced locomotor stimulation could be completely abolished by pimozide (0.01 mg/kg, i.p.) injected 30 min before ADTN (Figure 3). Haloperidol (0.5 mg/kg, i.p.) was similarly effective (Figure 3).

*Injections into nucleus accumbens*

Bilateral injections of ADTN into the nucleus accumbens, 5-25 $\mu$g on each side, produced initial sedation followed by strong central stimulation. During the first 15-60 min after injection shivering was observed. In some rats a flat posture was observed for a few minutes; these animals showed no signs of ataxia. The animals then became hyperactive with predominant rearing behaviour at first. Locomotor stimulation was strong and the animals moved in all directions in the cage. The running started suddenly and lasted for a very long period of time as shown in Figure 4a. During this period of enhanced activity the animals responded to handling, without undue signs of fear.

ADTN was effective in all 6 rats with cannulae in the nucleus accumbens. The tip of the cannula of rat number 3 (see Figure 1) was slightly above the nucleus accumbens, but the syringe needle track was detected inside the nucleus.

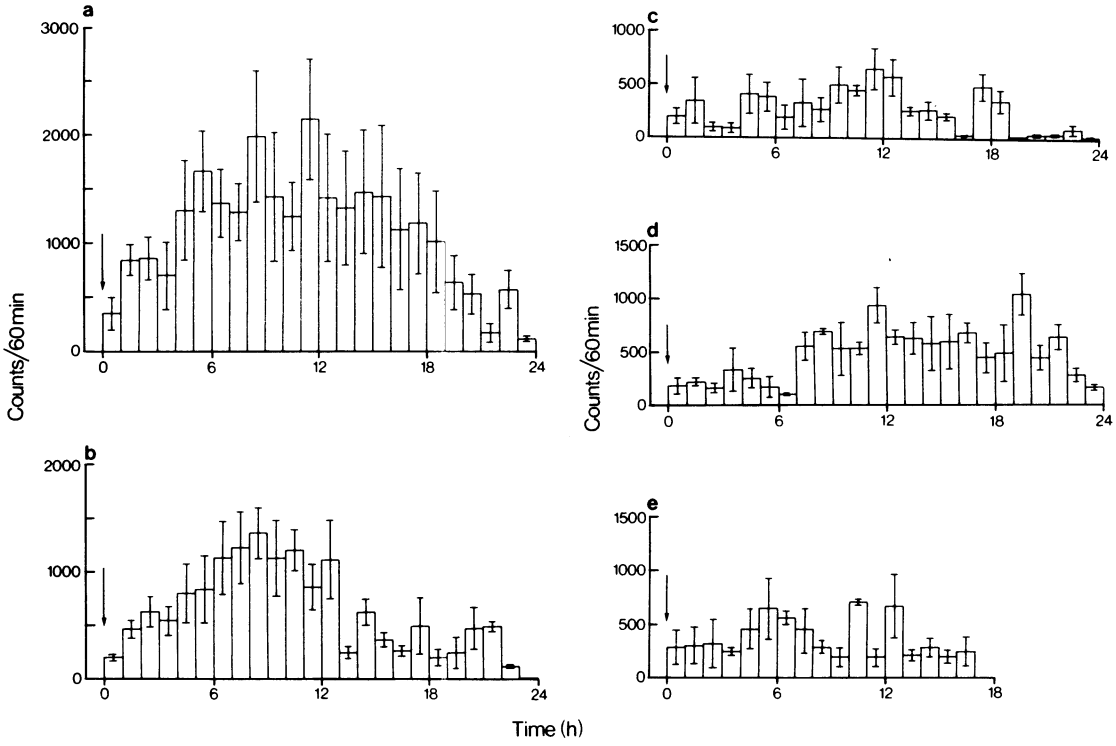


**Figure 3** Mean motor activity of rats measured in the Animex activity meter. Vertical bars show standard errors of mean. Times of intraventricular injections are indicated by the arrows. (a) Motor activity (mean of 6 rats) after the intraventricular injection of 150 μg of ADTN unilaterally. (b) Motor activity (mean of 3 rats) following the injection of ADTN 150 μg given unilaterally into the lateral ventricle 30 min after haloperidol (0.1 mg/kg, i.p.). The effect of ADTN was attenuated. (c) Motor activity (mean of 3 rats) following the injection of 5 μl saline unilaterally into the lateral ventricle. (d) Motor activity (mean of 4 rats) following the injection of ADTN 150 μg given unilaterally into the lateral ventricle 30 min after pretreatment with pimozide (0.01 mg/kg, i.p.). The effect of ADTN was abolished. (e) Motor activity (mean of 3 rats) following the injection of ADTN 150 μg 30 min after pretreatment with haloperidol (0.5 mg/kg, i.p.).

Enhanced locomotor activity was also produced by lower doses of ADTN (0.5, 2 or 5 μg on each side). All 6 rats responded to the bilateral injection of 5 μg of ADTN (Figure 5a). The onset of action was slower and the duration shorter than with the higher dose. After 2 μg of ADTN, two out of three rats tested showed increased activity, and one rat responded to the lower dose of 0.5 μg.

Unilateral injections of ADTN (5 μg) into the nucleus accumbens also caused enhanced locomotor activity but there was no turning. The onset of action was delayed but the duration of action seemed to be unchanged compared with that after bilateral injections (Figure 5).

Pimozide (1 mg/kg, i.p.) injected 30 min before ADTN completely abolished the locomotor stimulation produced by the bilateral injection of 5 μg ADTN (Figure 5). The effects of the same dose of ADTN were greatly attenuated by pimozide (0.4 mg/kg) (Figure 5). With lower doses of pimozide (0.1 or 0.2 mg/kg) the effect of ADTN was not blocked although the onset of action was delayed.

The injection of 0.5 μl of saline or 25 μg 2-amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene bilaterally into the nucleus accumbens had no effect on locomotor activity as shown in Figure 4.

*Injections into caudate nucleus*

When ADTN (25 μg) was injected bilaterally into the caudate nucleus it had no effect on locomotor activity (Figure 4), although some stereotyped sniffing and licking were observed. Piloerection and flat posture were shown by some rats.

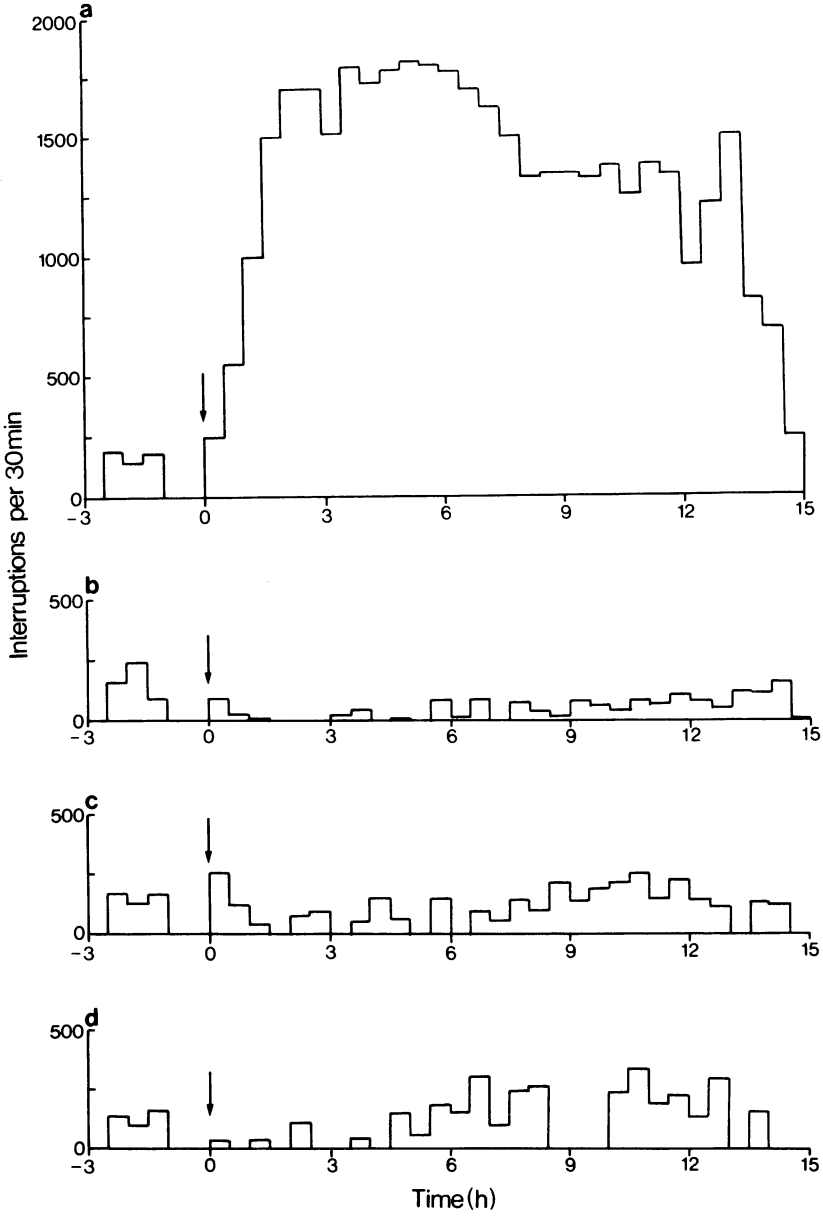


**Figure 4** Motor activity of rats as measured in the light beam activity cages. Times of intracerebral injections are indicated by the arrow. (a) Motor activity of rat No. 1 following the injection of 25 μg of ADTN bilaterally on each side, into the nucleus accumbens. (b) Motor activity of the same rat after injection of 2-amino-6, 7-dimethoxy-1,2,3,4-tetrahydronaphthalene, 25 μg bilaterally on each side into the nucleus accumbens. (c) As a control the motor activity of the same rat is shown following the injection of 0.5 μl saline bilaterally into the nucleus accumbens. (d) Motor activity of rat No. 8 after the injection of ADTN 25 μg bilaterally into the caudate nucleus.

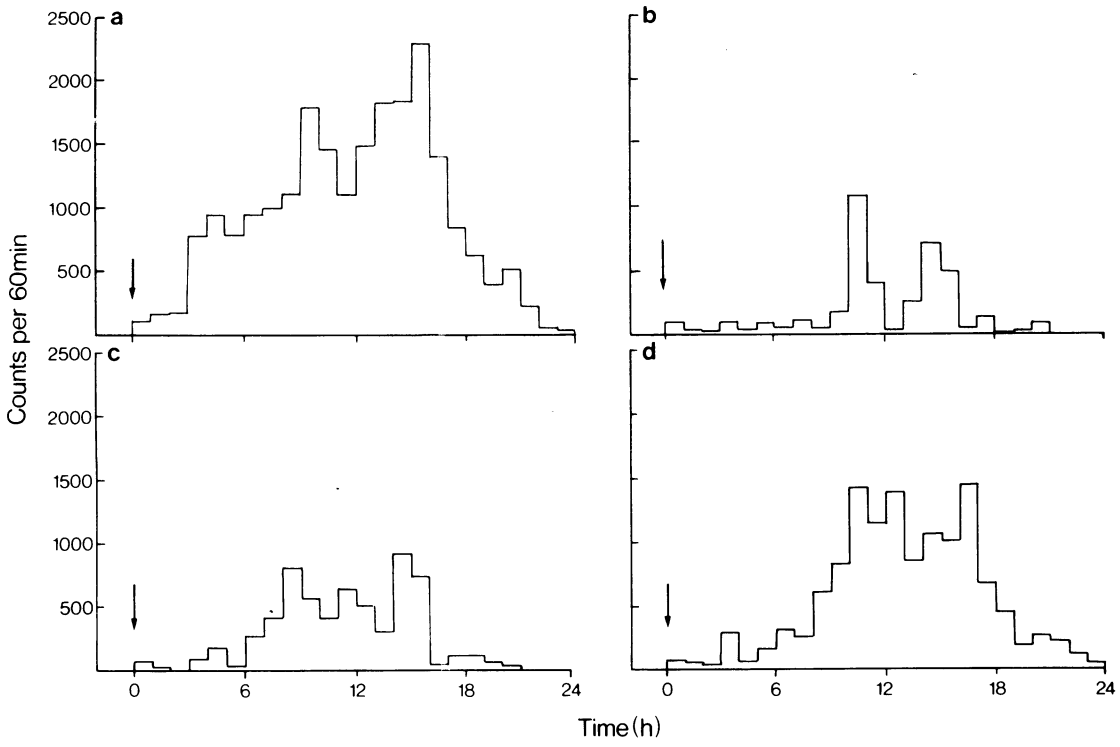


**Figure 5** Motor activity of rats measured in the Animex activity meter. Times of intracerebral injections indicated by the arrows. (a) Motor activity of rat No. 6 after an injection of ADTN 5 μg bilaterally into the nucleus accumbens. (b) Motor activity of the same rat after pretreatment with pimozide (1 mg/kg, i.p.) 30 min before the injection of ADTN 5 μg bilaterally into the nucleus accumbens. Activity is completely inhibited. (c) Motor activity of the same rat after pretreatment with pimozide (0.4 mg/kg, i.p.) 30 min before the injection of ADTN 5 μg bilaterally into the nucleus accumbens. The effect is greatly attenuated. (d) Motor activity of rat No. 4 following the injection of ADTN 5 μg unilaterally (left side) into the nucleus accumbens.

## Discussion

There is a large body of evidence to suggest that many of the actions of dopamine, both peripheral and central, are mediated by specific dopamine receptors (Hornykiewicz, 1971; Woodruff, 1971; Goldberg, 1972). The discovery by Ehringer & Hornykiewicz (1960) that there is a deficiency of dopamine in the basal ganglia of parkinsonian patients led to the introduction of L-DOPA as a drug for its treatment (Cotzias, Van Woert & Schiffer, 1967). Drugs which stimulate dopamine receptors could be of great value in the therapy of parkinsonism and also as a research tool in the study of the physiological significance of dopamine.

In our present study we have shown that ADTN causes a strong and long-lasting stimulation of locomotor activity in rats. The effect is probably mediated by an action on dopamine receptors. This is supported by the fact that low doses of haloperidol and pimozide antagonized the effect of ADTN. We have shown in a previous experiment that the effect of ADTN on locomotor activity in mice is not affected by the α-adrenoceptor blocking agent phenoxybenzamine (unpublished observation) nor by pretreatment with the inhibitor of dopamine synthesis, α-methyl-*p*-tyrosine (Woodruff *et al.*, 1974b). There is good evidence from other experiments that ADTN stimulates mammalian dopamine receptors. Thus, ADTN has been shown to stimulate the production of cyclic adenosine 3′,5′-monophosphate in the rat striatum (Munday *et al.*, 1974; Miller, Horn, Iversen & Pinder, 1974). In addition it has been shown that ADTN induces

turning behaviour in rats with unilateral lesions of the nigro-striatal tract which is consistent with a direct action of this compound on dopamine reeptors, an effect which was also reported by Woodruff *et al.* (1974a). Furthermore, ADTN was shown to exhibit inhibitory actions on dopamine-sensitive neurones in the caudate nucleus when applied iontophoretically (Woodruff *et al.*, 1974a). In the peripheral vascular system it has been shown that ADTN had a vasodilator activity on the renal arteries of the dog similar to that of dopamine (Goldberg & Crumly, personal communication), whereas it had no effect on the femoral arteries. ADTN also has a dopamine-like action on the guinea-pig blood pressure (Woodruff *et al.*, 1974b).

The dimethyl ether of ADTN, 2-amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene has little or no effect on striatal dopamine receptors (unpublished observation). In the present study we have shown that this compound is similarly inactive behaviourally.

Thus our results support the concept that dopamine receptors are involved in locomotor stimulation of drugs. The evidence that the actions of ADTN are not affected by the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine, (Woodruff *et al.*, 1974b), and its effect on turning behaviour strongly supports the concept that ADTN acts by a direct action on dopamine receptors.

A remarkable feature of the actions of ADTN has been the prolonged effects in mice and rats, following intraventricular injections and injections into the nucleus accumbens. The long lasting effects of ADTN might be due to its resistance to enzymatic action in the brain, or its removal from the receptor vicinity at a very slow rate. However, recently it has been shown (Horn, 1974) that ADTN is a potent inhibitor of dopamine uptake into rat striatal homogenates; these results could mean that ADTN is itself taken up presynaptically. Other possible explanations for the long duration of action of ADTN, such as the formation of a stable complex with the dopamine receptor, cannot be ruled out.

Evidence presented by Rolls (1971) suggests that the nucleus accumbens may be a site of self stimulation. Our experiments suggest that the nucleus accumbens may be involved in the site of action of ADTN. This is supported by the finding that ADTN produces strong stimulation of locomotor activity following its injection into the nucleus accumbens but is inactive when injected into the caudate nucleus. The nucleus accumbens contains high dopamine levels and a dopamine-sensitive adenyl cyclase (Horn, Cuello & Miller, 1974). Thus the nucleus accumbens may be involved in the mediation of locomotor stimulant activity of drugs. Recently, it has been shown that injections of dopamine or the dopamine receptor stimulant, ergometrine, into the nucleus accumbens produce stimulation of locomotor activity in rats (Pijnenburg & Van Rossum, 1973; Pijnenburg *et al.*, 1973).

Intraperitoneal injections of ADTN produce no obvious behavioural changes, probably because the molecule does not readily penetrate the blood-brain barrier. The study of more lipid soluble analogues of ADTN might produce active compounds that are capable of passing from the blood into the brain.

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