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RESPONSES OF NEURONES IN THE CEREBRAL CORTEX AND CAUDATE NUCLEUS TO AMANTADINE, AMPHETAMINE AND DOPAMINE

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1 Dopamine, amantadine and amphetamine have been applied directly by microiontophoresis to single neurones in the caudate nucleus and cerebral cortex of rats anaesthetized with urethane.

2 The predominant response to all three agents was a depression of neuronal firing rate. The responses to dopamine and amantadine could be antagonized by the dopamine receptor blocking agent, chlorpromazine.

3 Amantadine did not cause any potentiation of dopamine responses, suggesting that inhibition of amine uptake was not responsible for its effects.

4 The responses of pyramidal tract cells in the cerebral cortex to dopamine, amphetamine and amantadine were compared in control groups of rats and rats pretreated with reserpine (10 mg/kg i.p.) or α-methyl-*p*-tyrosine methyl ester (200 mg/kg i.p.). The reduction of cortical catecholamine concentrations was confirmed by a direct fluorimetric assay method.

5 Responses to dopamine were unaltered in the amine-depleted animals compared with controls. Responses to amantadine and amphetamine were reduced but not abolished.

6 It is concluded that amantadine acts partly by releasing catecholamines from neuronal stores. The residual responses to amantadine and amphetamine may be the result of a direct postsynaptic receptor stimulation.

Introduction

Amantadine hydrochloride (1-amino-adamantan; Symmetrel) is being used increasingly in the treatment of Parkinson's disease (Birdwood, Gilder & Wink, 1971). This use has developed from the observations of Schwab, England, Poskanzer & Young (1969) that amantadine, which was for several years used as an antiviral agent, could substantially reduce Parkinsonian symptoms.

The mechanism of amantadine's anti-Parkinsonian action is not completely understood. The behavioural effects of amantadine, such as motor hyperactivity (Lassen, 1973; Maj, Sowińska & Baran, 1973), stereotypy (Strömberg & Svensson, 1971; Cox & Tha, 1973), and turning in unilaterally striatotomized rats (Strömberg & Svensson, 1971) suggest a stimulation of central catecholamine receptors. The turning behaviour in particular indicates that amantadine can activate the nigrostriatal dopaminergic neurone system (Andén, Dahlström, Fuxe & Larsson, 1966; Arbuthnott & Crow, 1971; Crow, 1971). Since it is a disturbance of this system which appears to be a crucial factor in the aetiology of Parkinsonism (Hornykiewicz, 1966; Calne, 1970) it has been assumed that it is amantadine's ability to act on this system which underlies its anti-Parkinsonian properties.

The literature contains evidence that amantadine may act by directly stimulating post-synaptic receptors (Cashin & Sutton, 1973; Lassen, 1973; Papeschi, 1974), by releasing catecholamines (Grelak, Clark, Stump & Vernier, 1970; Scatton, Cheramy, Besson & Glowinski, 1970; Farnebo, Fuxe, Goldstein, Hamberger & Ungerstedt, 1971; Spilker & Dhasmana, 1974), or by inhibiting the neuronal uptake of catecholamines (Fletcher & Redfern, 1970; Baldessarini, Lipinski & Chace, 1972; Heimans, Rand & Fennessy, 1972).

The present experiments were designed to investigate the effects of amantadine on neurones in the caudate nucleus and cerebral cortex of rats when applied by microiontophoresis.

The effects of amantadine were compared with responses to dopamine itself and to amphetamine, which is thought to stimulate central catecholamine receptors indirectly by releasing the amines from neuronal storage sites (Moore, 1963; Carlsson, Lindqvist, Dahlström, Fuxe & Masuoka, 1965; Carlsson, Lindqvist, Fuxe & Hamberger, 1966;

Christie & Crow, 1971; Boakes, Bradley & Candy, 1972). Chlorpromazine was used as a dopamine receptor blocking agent (van Rossum, 1966; York, 1972) to examine the specificity of amantadine's effects on dopamine receptors.

Neuronal responses were also examined in rats pretreated with reserpine or the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine methyl ester, both of which deplete the central nervous system of catecholamines.

Some of these results have been communicated to the Pharmacological Society (Stone, 1975).

Methods

Forty male hooded Wistar rats weighing 250–300 g were used in this study. Fourteen of these were used for the qualitative examination of cortical and caudate neurone responses, and 26 were divided into control and test groups for the later amine-depletion experiments. For all acute experiments, animals were anaesthetized with urethane 1–1.25 g/kg intraperitoneally. The lower doses were needed for amine-depleted rats, which died within 1 h if given the normal dose of 1.25 g/kg. The anaesthetic was sufficient to produce full surgical anaesthesia in all animals.

The left fronto-parietal cerebral cortex was exposed. The animals' rectal temperatures were automatically maintained at 37°–38°C by means of a heating pad and rectal thermistor probe (Krnjević & Mitchell, 1961).

A stereotaxic frame was used which allowed orientation of the head suitable for using the stereotaxic atlas of Fifková & Marsala (1967). Penetrations into the caudate nucleus were made at co-ordinates AP 0, L 3.0, V 5.0.

Five-barrelled micropipettes were used for microiontophoresis, each barrel having a resistance of 2–12 MΩ when filled with 3 M KCl. The pipettes contained one or more of the following solutions: acetylcholine chloride 200 mM, pH 4.5 (Koch-Light); dopamine hydrochloride, 200 mM, pH 4.0 (Koch-Light); (+)-amphetamine sulphate 200 mM, pH 4.5 (S.K. & F.); amantadine hydrochloride 200 mM, pH 4.5 (Geigy); sodium L-glutamate 200 mM, pH 6.5 (B.D.H.); 5-hydroxytryptamine creatinine sulphate 50 mM, pH 4.5 (Koch-Light); chlorpromazine hydrochloride 200 mM, pH 4.5 (May & Baker).

Micropipettes also contained 200 mM sodium chloride in one barrel so that current balancing and current testing was possible (Stone, 1972a). Unit activity was recorded through a single microelectrode containing molar potassium acetate or chloride, fixed alongside the iontophoretic complex (Stone, 1973b). This allowed more satisfactory recording of small spike activity, and allowed all five barrels of the multi-barrel assembly to be used for microiontophoresis. Spikes were amplified by a Fenlow AD 55 preamplifier and either displayed directly on Telequipment oscilloscopes, or passed through a capacitance coupled circuit to filter out slow waves, then through a spike height discriminator and an Ecko Ratemeter. A continuous reading of neuronal firing rate was obtained on a Servoscribe pen recorder.

Depletion of central catecholamines

To examine the effects of amantadine and amphetamine in animals whose brains were depleted of catecholamines, four groups of animals were studied. One group (8 rats) was injected 24 h before the acute experiment with reserpine (10 mg/kg i.p.) suspended in Tween 80. A control group (8 rats) received Tween 80 (1 ml/kg i.p.). A third group (4 rats) was treated 24 h before iontophoresis with α-methyl-p-tyrosine methyl ester hydrochloride (AMPT) (200 mg/kg i.p.) in 0.9% w/v NaCl solution (saline) and the corresponding control animals (6 rats) received saline (1 ml/kg i.p.).

Estimation of catecholamine concentrations

To confirm that reserpine and AMPT did reduce central catecholamine concentrations, the brains of most of the experimental animals were perfused with saline via a carotid artery at the end of the acute experiment and the frontal and parietal cerebral cortices were removed and stored at −28°C. The cortices were subsequently homogenized and the noradrenaline and dopamine concentrations estimated by the method of Cox & Perhach (1973). The method involved conversion of the catecholamines to fluorescent derivatives, the concentrations of which were measured in an Aminco-Bowman SPF 125 spectrofluorimeter. The excitation and emission wavelengths used were 320 nm and 370 nm (dopamine) and 385 nm and 485 nm (noradrenaline).

Comparison of neuronal responses

To try and reduce the variation of neuronal responses normally found with microiontophoresis, the cells used for comparing amine responses in normal and amine-depleted animals in the present study were all spontaneously active, identified pyramidal tract cells. The identification of these cells has been described previously (Stone, 1972b).

To enable any differences to be detected in the neuronal responses of normal and amine-depleted animals it was necessary to measure various parameters of the neuronal response, which could then be subjected to statistical analysis. Three parameters were measured: h, the maximum change of firing rate produced; t, the time between beginning a drug ejection and h; d, the total duration of response. All

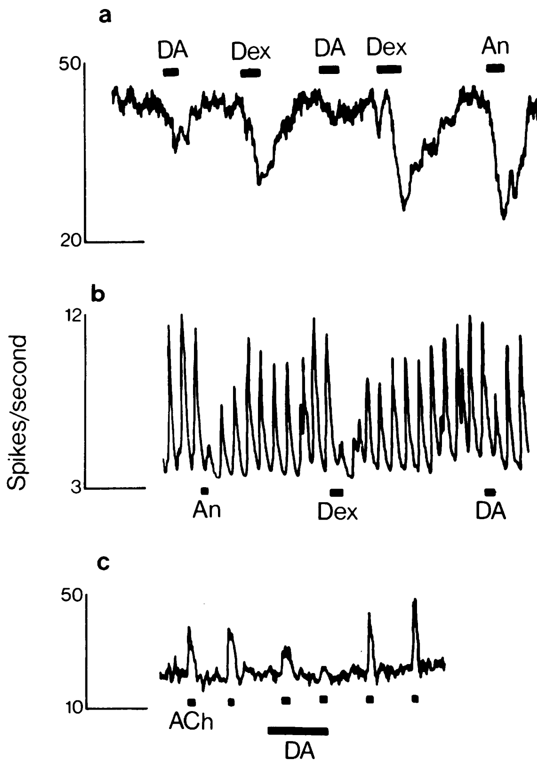

Figure 1 Records of the firing rates of neurones showing: (a) a cortical unit which is depressed by the iontophoresis of dopamine, 60 nA (DA), amphetamine, 60 nA (Dex), and amantadine, 60 nA (An). Note the apparently greater potency of the latter two drugs. (b) A neurone in the caudate nucleus which showed little spontaneous activity and which was therefore excited by applications of glutamate (60 nA for 5 s every 30 s). Depression of the cell excitability is thus revealed as a reduction in the height of the glutamate response. (c) A neurone in the caudate nucleus which was spontaneously active, but which was further excited by acetylcholine, 60 nA (ACh) applied as indicated by bars beneath record. Depression of acetylcholine responses by dopamine shows that dopamine-induced depression of glutamate was not a specific antagonism of glutamate. Dopamine, 60 nA (DA). Time in all traces 2 minutes.

three measurements were made with respect to a line drawn through the basal firing rate existing before the drug ejection. Also, to facilitate comparison of the results, all the agonist compounds were ejected with currents of 60 nA for 15 seconds.

Results

All the units studied in the cerebral cortex and 50% of those in the caudate nucleus were spontaneously active. The remaining 50% of caudate units were excited artificially by iontophoretic pulses of glutamate (Connor, 1970) (60 nA for 5 s every 30 s) or acetylcholine (60 nA for 15 s periods).

Qualitative examination of responses of caudate and cortical units

In the initial experiments the three agonists, dopamine, amantadine and amphetamine were each ejected with outward currents of 60 nA. A summary of the results obtained, including number of cells tested, the type of response and the changes of firing rate produced is presented in Table 1.

Examples of records of neuronal firing rates are shown in Figure 1. Figure 1a is taken from a spontaneously active cortical neurone. Figure 1b and 1c, showing firing rates in the caudate nucleus, illustrates that depression by the agonists could be seen when cells were excited with glutamate (Figure 1b) or acetylcholine (Figure 1c). Nevertheless if a cell was depressed by any of the agonists, the firing

Table 1 Neuronal responses to dopamine, amantadine and amphetamine

Drug	*Area*	*No. of cells*	*Responses No. (%)* +	−	*O*	*Depression of firing rate (spikes/s)**
Dopamine	Cortex	142	7 (5)	128 (90)	7 (5)	8.4±0.64
	Caudate	32	2 (6)	23 (72)	7 (22)	9.0±0.82
Amantadine	Cortex	129	5 (4)	108 (84)	16 (12)	15.2±1.08
	Caudate	32	1 (3)	26 (81)	5 (16)	13.0±1.05
Amphetamine	Cortex	83	7 (8)	70 (85)	6 (7)	14.8±1.15
	Caudate	12	0	12 (100)	0	15.5±1.46

* Mean ± s.e. of mean.
Responses are shown as: + = excitation; − = depression; *O* = no effect.

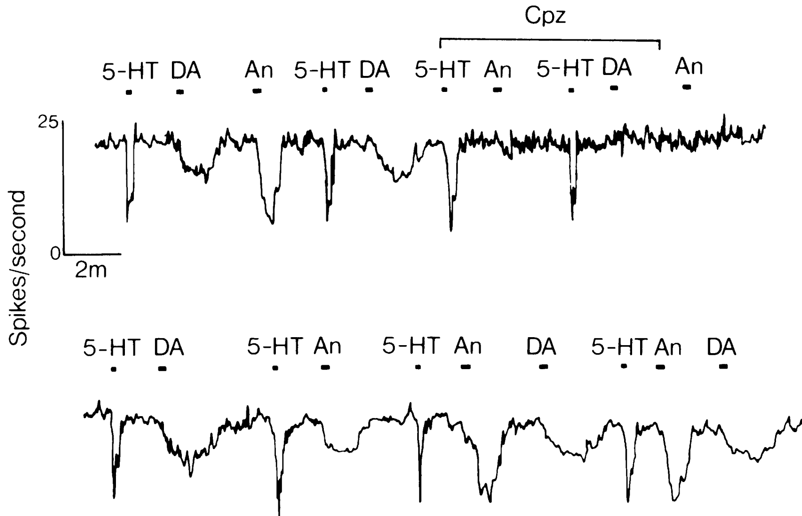

Figure 2 A recording of the firing rate of a cortical neurone, showing responses to 5-hydroxytryptamine, 60 nA (5-HT), dopamine, 60 nA (DA) and amantadine, 60 nA (An). The iontophoresis of chlorpromazine, 30 nA (Cpz), produces a reversible antagonism of dopamine and amantadine responses. The two parts of the record are consecutive. Time 2 minutes.

rate could be increased by increasing the ejection of glutamate or acetylcholine. This fact, and the absence of any change of spike height as monitored on the oscilloscopes, indicated that depression was not due to movement of the electrode, local anaesthetic effects on the cell membrane, or over-depolarization.

The latencies of dopamine responses were in the range 6–20 s, whereas latencies for amphetamine and amantadine responses were in the range 1–12 seconds.

Interactions of amantadine and dopamine

On a number of cells in both cerebral cortex and caudate nucleus regularly spaced, reproducible responses to dopamine were obtained and an ejection of amantadine was then made to determine whether the dopamine response could be changed by the presence of amantadine. In no case has a potentiation of dopamine resulted from this sequence of applications.

Dopamine receptor blocking agents

Chlorpromazine was applied to a total of 23 units (15 cortical) with currents of 30 nA. Larger current applications often led to direct neuronal depression and reduction of spike height, presumably as a result of chlorpromazine's local anaesthetic properties. Chlorpromazine blocked the depressant action of dopamine on 17 of the 23 cells (74%) and blocked amantadine depressions on 8 of 11 cells (72%) tested. No reduction of responses to 5-hydroxytryptamine were seen on any of 23 cells. Part of the record of the firing rate of a unit showing blockade of dopamine and amantadine whilst 5-hydroxytryptamine responses were unaffected, is shown in Figure 2.

Table 2 Concentrations of noradrenaline and dopamine in rat cerebral cortex

Pretreatment (i.p., 24 h previously)	*Noradrenaline (ng/g tissue)*	*Dopamine (ng/g tissue)*
Tween 80 1 ml/kg	283.1 ± 30.4* (n=8)	183.0 ± 25.5 (8)
Reserpine 10 mg/kg in tween 80	22.64 ± 4.8 (7)	14.62 ± 2.8 (7)
Saline 1 ml/kg	261.6 ± 42.2 (6)	168.1 ± 26.0 (6)
AMPT 200 mg/kg in saline	129.8 ± 20.8 (4)	69.5 ± 8.7 (4)

* mean ± s.e. of mean.

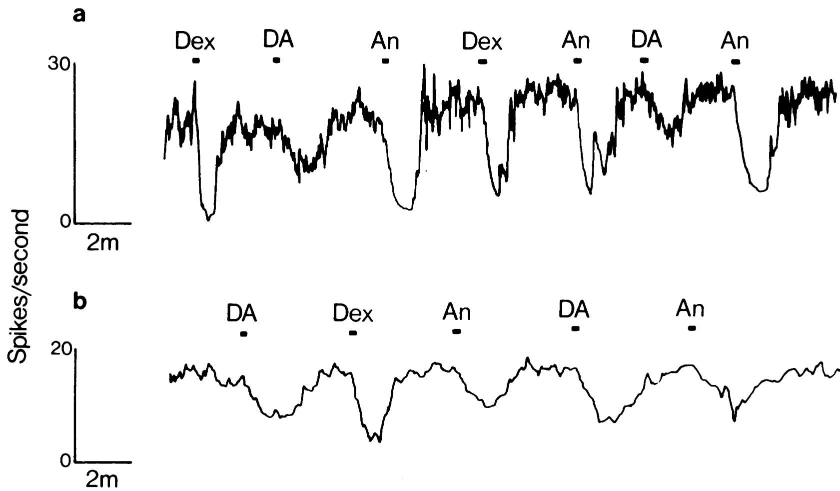

Figure 3 Records of the firing rates of two pyramidal tract neurones: (a) in a control rat pretreated with Tween 80 1 ml/kg 24 h before the experiment, (b) in a rat pretreated with reserpine 10 mg/kg in Tween 80, 24 h before the experiment. Responses to the three drugs are present after reserpine-treatment. The bars indicate the iontophoresis of amphetamine, 60 nA (Dex); dopamine, 60 nA (DA) and amantadine, 60 nA (An). Time 2 minutes.

Comparison of control and amine-depleted animals

Catecholamine concentrations. The results of the fluorimetric estimation of catecholamines in the cerebral cortex are shown in Table 2. After reserpine pretreatment noradrenaline and dopamine concentrations were both reduced by 92% compared with the Tween 80 controls. After AMPT injections, the amine levels were reduced by 50% and 59% respectively, compared with the saline controls.

Unit responses to iontophoresis. In this part of the experimental series the cells studied were cortical pyramidal tract cells. All iontophoretic ejections were

Table 3 Responses of pyramidal tract neurones to dopamine,amantadine and amphetamine in control and amine-depleted rats

Pretreatment*	Iontophoretic drug	No. of cells	Responses No. (%) +	−	0
Tween 80	Dopamine	52	3 (6)	44 (85)	5 (9)
	Amantadine	50	1 (2)	40 (80)	9 (18)
	Amphetamine	44	0	40 (91)	4 (9)
Reserpine	Dopamine	40	1 (2)	33 (83)	6 (15)
	Amantadine	46	3 (6)	39 (85)	4 (9)
	Amphetamine	46	1 (2)	38 (83)	7 (15)
Saline	Dopamine	48	3 (6)	37 (77)	8 (17)
	Amantadine	36	0	29 (81)	7 (19)
	Amphetamine	30	0	27 (90)	3 (10)
α-methyl-*p*-tyrosine	Dopamine	30	3 (10)	24 (80)	3 (10)
	Amantadine	30	1 (3)	24 (80)	5 (17)
	Amphetamine	22	0	20 (91)	2 (9)

* Doses as in Table 2.
+ = excitation; − = depression; *0* = no effect.

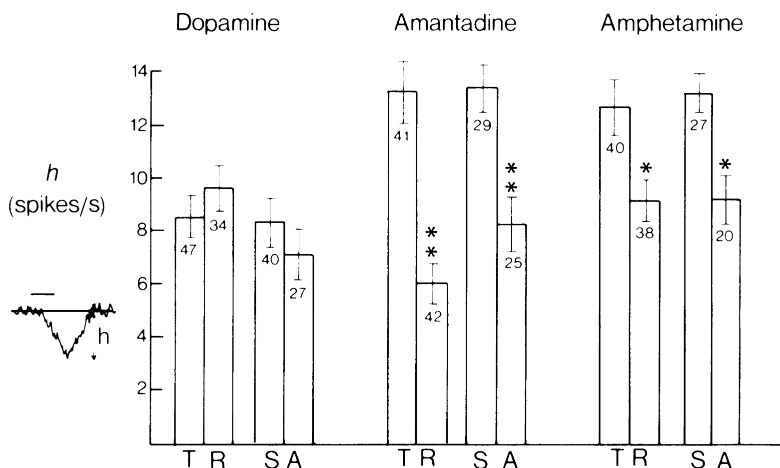

Figure 4 Histograms showing the values obtained for *h*, the maximum change of firing rate produced, resulting from the iontophoresis of dopamine, amantadine and amphetamine. The height of each column indicates the mean value, the vertical bar shows ± s.e. of the mean, and the number within each column indicates the number of cells used in the calculations. T = Tween 80 pretreatment; R = reserpine pretreatment; S = saline pretreatment; A = α-methyl-p-tyrosine pretreatment.
* $P < 0.02$; ** $P < 0.001$.

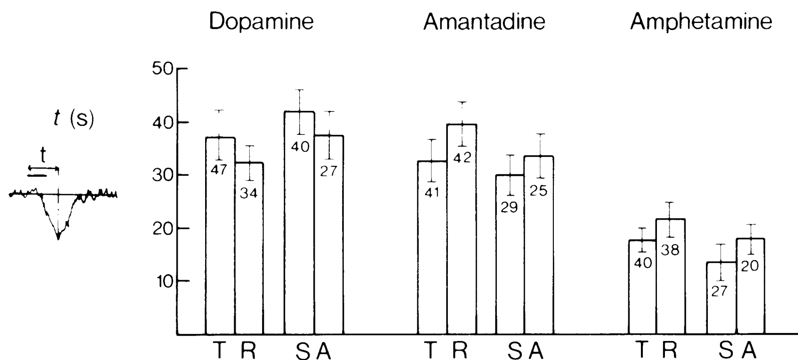

Figure 5 Histograms showing the values obtained for *t*, the time between beginning a drug ejection and the peak change of firing produced. Details as for Figure 4.

effected with currents of 60 nA for 15 seconds. The numbers of units tested and their responses to the various agonists are presented in Table 3. The percentage figures are similar to those for the normal untreated animals discussed above (Table 1).

Figure 3 shows records of the firing rates of two units, one in a control animal and one in a reserpine-treated animal, to illustrate the absence of any marked difference in the responses.

The values obtained for the parameters *h*, *t* and *d* are most conveniently summarized in histogram form in Figures 4, 5 and 6 respectively. There was a significant reduction in the maximum responses (*h*) to amantadine ($P < 0.001$) and amphetamine ($P < 0.02$) in the amine-depleted compared with control rats (Figure 4). There was no statistically significant change in the parameters *t* (Figure 5) and *d* (Figure 6) for amphetamine and amantadine, and no significant change was apparent for any of the three parameters in the case of dopamine.

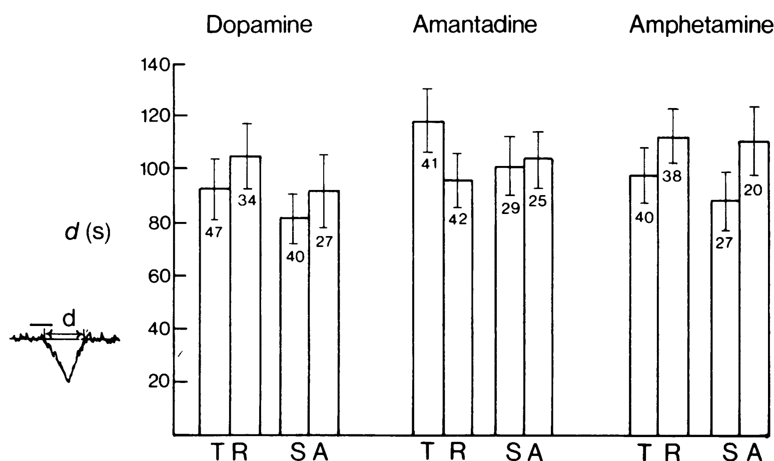

Figure 6 Histograms showing the values obtained for *d*, the total duration of drug response. Details as for Figure 4.

Discussion

Most of the available evidence on the mechanism of action of amantadine indicates an action primarily on the dopaminergic systems of the brain (Strömberg & Svensson, 1971; Von Voigtlander & Moore, 1973). The present results support this idea by demonstrating that iontophoretically applied amantadine had the same effect as dopamine and amphetamine on neurones in the cerebral cortex and caudate nucleus. The usual effect of these substances was depression of neuronal firing rate. Since amphetamine and amantadine can act on noradrenergic as well as dopaminergic neurones (Glowinski, 1970; Sinclair, 1973; Papeschi, 1974) this similarity of action is particularly significant in the caudate nucleus where dopamine is the predominant catecholamine, and only minute quantities of noradrenaline are present (Vogt, 1954; Bertler & Rosengren, 1959).

The similarity of action is more difficult to interpret in the cerebral cortex since there are both noradrenaline-containing and dopamine-containing nerve terminals present (Fuxe, Hamberger & Hökfelt, 1968; Lindvall, Björklund, Moore & Stenevi, 1974). Nevertheless the similarity of action of dopamine and amantadine can be considered meaningful in the light of the additional observation that responses to both substances could be blocked by chlorpromazine at a time when responses to 5-hydroxytryptamine were unaltered. Chlorpromazine has been shown previously to block dopamine depressions of unit firing in the striatum (York, 1972) and cerebral cortex (Stone, 1974), but has little effect on noradrenaline depressions in the cortex (Stone, 1973a).

Although cholinergic nerve endings are present in the cortex and striatum, amantadine's effects are not likely to be due to an effect on such terminals, since amantadine has been shown to have very little effect on peripheral cholinergic systems (Vernier, Harmon, tump, Lynes, Marvel & Smith, 1969; Grelak *et al.*, 1970; Bianchi & Tomasi, 1973).

The primarily depressant action of dopamine on striatal neurones is consistent with the idea that most nigrostriatal dopaminergic neurones are inhibitory to caudate cells (McLennan & York, 1967; Connor, 1970). The results are also in agreement with previous studies of the effects of iontophoretically applied dopamine. McLennan & York (1967), for example, found that most caudate units (60%) were depressed by dopamine and only 9% were excited. Connor (1970) found that 68% of caudate units were depressed by dopamine. Many cortical units have also been shown to be depressed by dopamine (Krnjević & Phillis, 1963).

The significance of the excitatory responses to the three agonists is not clear. They were observed too rarely for attempts at antagonism or the assessment of changes after amine depletion to be meaningful. It is not likely that the excitatory responses indicate an excitatory dopaminergic nigrostriatal pathway. The possible existence of such a pathway was mentioned by Feltz (1971) following his demonstration of a monosynaptic excitatory nigrostriatal pathway which could be blocked by haloperidol. Feltz & de Champlain (1972a) later showed that the transmitter mediating this excitation was probably not a catecholamine since the excitation persisted after the degeneration of catecholamine containing neurones induced by 6-hydroxydopamine.

The most likely explanation of the excitations seen

in the present experiments is that they are the result of an indirect action of the drugs causing depression of a nearby neurone which has an inhibitory influence on the unit being recorded.

It is generally accepted that neuronal uptake processes are responsible for the removal of transmitter catecholamines from the synaptic cleft (Iversen, 1967). Hence drugs which inhibit the uptake process can usually be shown to potentiate the actions of the catecholamines (Bradshaw, Roberts & Szabadi, 1971). In the present experiments, amantadine, in doses sufficient to cause some neuronal depression, did not produce any apparent potentiation of dopamine responses. This suggests that amantadine has very little, if any, ability to inhibit the neuronal uptake of dopamine. This conclusion has been reached by most other groups in direct biochemical experiments (Fletcher & Redfern, 1970; Strömberg & Svensson, 1971; Baldessarini *et al.,* 1972; Heikkila & Cohen, 1972).

Studies of amine-depleted rats

As a comparison of Tables 1 and 3 shows, the proportions of cortical cells responding to dopamine, amantadine and amphetamine were similar in the various groups of pretreated rats (Table 3) and in the original sample of normal untreated animals (Table 1). This may be considered to indicate that none of the pretreatment injections caused a gross disturbance in the pattern of neuronal sensitivity.

Dopamine responses appeared to be unchanged by amine depletion. Certainly no reduction in this response would be expected since dopamine is assumed to act postsynaptically and its effects should therefore be independent of presynaptic amine stores. Ungerstedt (1971) has shown that following a single intraperitoneal injection of reserpine 10 mg/kg, there is a marked supersensitivity of dopamine receptors after 24 hours. Although in the present experiments there is a slight increase in the value of h for dopamine in reserpine-treated animals compared with controls, the increase is not statistically significant. However, the supersensitivity described by Ungerstedt (1971) was seen as turning behaviour of unilaterally striatotomized rats after the injection of normally subthreshold doses of apomorphine (0.25 mg/kg). The supersensitivity therefore was of striatal dopamine receptors. It is possible that receptors in the cerebral cortex developed supersensitivity with a slower time course.

The significant reduction of neuronal responses to amantadine and amphetamine probably indicates a substantial contribution of an indirect amine-releasing effect to their mechanism of action. Such a conclusion would be consistent with previous studies of the action of both amantadine (Grelak *et al.,* 1970; Strömberg, Svensson & Waldeck, 1970; Farnebo *et al.,* 1971) and amphetamine (Carlsson *et al.,* 1965, 1966; Fuxe & Ungerstedt, 1968; Boakes *et al.,* 1972).

The residual effects of these substances after amine depletion suggest that they may also exert a direct action on postsynaptic receptors. A direct action of amantadine has been postulated by several workers who found that the drug still had anticataleptic properties after central catecholamine depletion by AMPT or reserpine (Cashin & Sutton, 1973; Maj, Sowińska & Baran, 1973; Papeschi, 1974). Similarly, from an examination of the locomotor activity of mice or rats it has been concluded that amphetamine can directly stimulate catecholamine receptors (Smith, 1963; Van Rossum & Hurkmans, 1964).

An alternative explanation of the residual responses to amantadine and amphetamine is that the small amount of catecholamine remaining after reserpine is sufficient to maintain a store of amine in a form which can be released by amantadine and amphetamine.

However, several other groups have found no difference between neuronal responses to amphetamine in control rats and rats pretreated with reserpine or 6-hydroxydopamine (Hoffer, Siggins & Bloom, 1971; Feltz & de Champlain, 1972b; Kostopoulos & Yarbrough, 1974). 6-Hydroxydopamine produces a profound loss of catecholamines and aminergic terminals from treated areas of the brain, so that these observations support the possibility that amphetamine can act directly on postsynaptic receptors. The results obtained in the present study that amantadine can produce neuronal responses after reserpine-treatment, which are comparable to those produced by amphetamine provides suggestive evidence that amantadine can also act in this way.

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