# THE POSTSYNAPTIC EFFECT OF AMPHETAMINE ON STRIATAL DOPAMINE-SENSITIVE NEURONES

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IT IS generally agreed that most of the behavioural effects of amphetamine involving the nigro-striatal dopaminergic pathway are related to a drug action at the presynaptic level (CARLSSON, 1970; UNGERSTEDT, 1971a; IVERSEN, 1971; TAYLOR and SNYDER, 1971; BOULU *et al.*, 1972). Amphetamine is known to cause release of dopamine from the striatal nerve terminals of dopamine-containing neurones of the substantia nigra (MCKENZIE and SZERB, 1968; CARR and MOORE, 1970; BESSON *et al.*, 1971). This same drug is also known to act on the inactivation of dopaminergic neurotransmission by inhibiting the reuptake of striatal dopamine (e.g. COYLE and SNYDER, 1969). However, these presynaptic effects of amphetamine, along with those ascribed to an inhibition of monoamine-oxidase (GLOWINSKI *et al.*, 1966; RUTLEDGE *et al.*, 1970) are not specific to dopaminergic pathways since noradrenergic systems are known to be similarly affected. Therefore many correlative behavioural and biochemical studies of amphetamine have been directed more towards an assessment of the relative involvement of dopamine and noradrenaline in a given behavioural pattern (CHRISTIE and CROW, 1971; COSTA *et al.*, 1972; CREESE and IVERSEN, 1972) rather than towards an elucidation of some possible direct effect of amphetamine on striatal neurones receiving a dopaminergic innervation.

Our interest in possible postsynaptic effects of amphetamine arose from electrophysiological experiments on dopamine sensitive neurones in the cat caudate nucleus. Amphetamine was chosen as an index of complete pharmacological disruption of the nigro-striatal dopaminergic pathway since the biochemical evidence clearly indicates that its effects *in vivo* are essentially presynaptic (e.g. JAVOY *et al.*, 1970; BUNNEY *et al.*, 1972). However we found and report herein that amphetamine mimics the effect of dopamine on neurones in the caudate nucleus even after the dopaminergic nigro-striatal pathway has been destroyed or functionally impaired by two different pharmacological techniques.

The dopaminergic nigro-striatal system was substantially destroyed in 17 cats by treatment with a series of bilateral intraventricular injections of 6-hydroxydopamine (6-OH-DA) given in 7 increasing doses over a period of 5 days (total dose 8–10 mg). Electrophysiological experiments lasting 12–24 hr were performed 2–25 days later and the extent of destruction of the dopaminergic system was assessed subsequent to the acute experiments using biochemical and histochemical techniques (FELTZ and DE CHAMPLAIN, 1972a).

In a second group of 9 cats with intact dopaminergic pathways, the endogenous storage pool of dopamine was severely depleted by the injection of reserpine (5 mg/kg, i.p.) 20 hr prior to the acute experiment. These experiments lasted for about 10 hr.

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During the acute experiments, 3 or 4 systemic injections of D-L-α-methyl-*p*-tyrosine methylester (α-M-*p*-T; 150–200 mg/kg) were given at intervals of 2–3 hr; this was assumed to inactivate the pool of newly synthetised dopamine.

A third group of 13 untreated cats served as controls.

The acute electrophysiological experiments were performed under general anaesthesia with diallylbarbituric acid (60 mg/kg) and urethane (240 mg/kg). The activity of caudate neurones was recorded with five-barreled micropipettes for simultaneous recordings and microiontophoretic injections of D-amphetamine (from 0·3 M solutions of the sulphate salt at pH 5·5–6·3, Sigma), L-amphetamine sulphate (0·3 M, pH 5·5–6, K and K Laboratories) and the following other substances: Na-glutamate and K-aspartate, as excitatory amino-acids; acetylcholine and γ-aminobutyric acid, for changing neuronal excitability; dopamine, noradrenaline and serotonine for full scanning of monoaminergic sensitivities; and papaverine and dimethoxyphenylethylamine (GONZALES VEGAS, 1972) as possible antagonists of dopaminergic effects. Before pharmacological testing, neurones were characterised as either anti- or orthodromically excited on stimulation of the substantia nigra (FELTZ and DE CHAMPLAIN, 1972a).

In the 13 control cats which had a mean endogenous dopamine content in the caudate nucleus of 11·6 ± 2·3 μg/g (SD), both isomers of amphetamine strongly depressed the neuronal firing evoked by excitatory amino-acids even when this firing was further enhanced by acetylcholine (Fig. 1A). This depressant action was seen on the 136 neurones tested but was effective in blocking the synaptically evoked responses of these neurones in 63 cases only. These results are consistent with those of previous experiments in which irrigation of the ventricular surface of the caudate nucleus with D-amphetamine ($10^{-6}$ M solutions in Ringer) inhibited firing of caudate neurones (FELTZ, 1970).

It was found that amphetamine retained full blocking potency in the cats treated with 6-OH-DA (Fig 1B). This was true for cells in areas of the caudate nucleus both partially and totally depleted of dopamine. At least 78 neurones tested for their sensitivity to amphetamine and dopamine were found to have been in the main zone of the caudate nucleus where the green fluorescence of dopamine-containing fibres was always shown to have totally disappeared (one third of the total volume of the nucleus). Another 157 neurones were recorded in sites obviously out of the region of maximal effectiveness of the 6-OH-DA treatment. These neurones were located within a neuronal network characterised by a marked reduction in the number and intensity of dopamine-containing terminals and by numerous swollen degenerating dopaminergic fibres. Total dopamine content in the caudate nucleus was reduced by between 42% and 87% (mean concentration and S.D. for the 17 cats: 4·2 ± 1·9 μg/g) with no systematic relation to the number of days of survival after the last 6-OH-DA injection. Although a substantial relation was found between biochemical estimates of dopamine depletion and the histochemical profile of the caudate nucleus from its ventricular surface down to its base, no clear correlation was found between the extent of 6-OH-DA induced damage and the doses of iontophoretic amphetamine (10–140 nA) required to block the firing. This was also true with respect to amphetamine induced blockage of nigro-caudate orthodromic and antidromic responses (26 cases out of 65 and 24 out of 32 respectively).

Although there was clearly a direct postsynaptic effect of amphetamine on the 78 caudate neurones studied in zones of total destruction of the dopaminergic terminals,

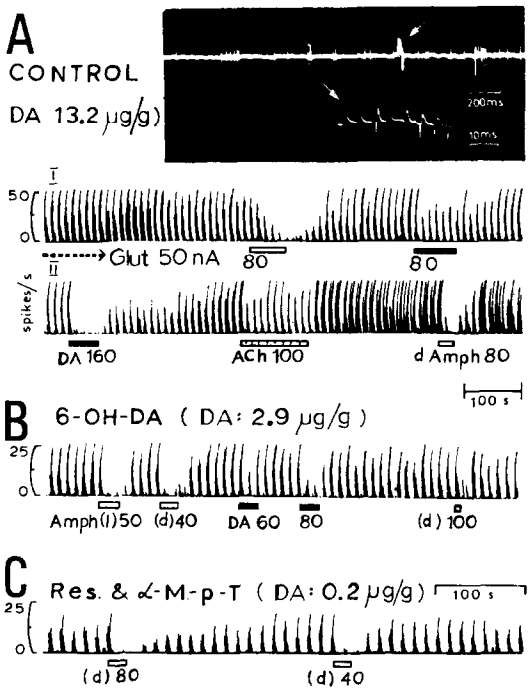


FIG. 1.—Responses to iontophoretically applied compounds of typical caudate neurones for the three groups of cats described in the text. Dopamine (DA) content in the caudate nucleus is given in each case. A: Normal cat. Photographic traces show excitatory response of caudate neuron to 4 shocks (arrows) delivered in substantia nigra. Penwriter recordings show periodic firing evoked by 50 nA of glutamate which was given in regular pulses throughout the recording period. I, responses to glutamate were blocked by D-amphetamine and dopamine (white and black bars respectively—numbers indicate iontophoretic current doses in nA; bars indicate periods of passage of current); II, enhanced firing following acetylcholine (Ach) is also depressed by amphetamine. B: 6-OH-DA treated cat; blocking effect seen with D- and L-amphetamine and dopamine. C: Reserpine and α-M-p-T treated cat; note prolonged effect of the highest dose of amphetamine. For comparisons of amphetamine and dopamine doses note that equal currents release at least 5 times more dopamine owing to different transport numbers.

at least one other explanation must be considered for the effects of amphetamine on the 157 neurones in zones of the caudate nucleus where some dopaminergic terminals remained. It is conceivable that the lack of change in the amphetamine induced depression of firing of these cells was the result of a release of dopamine from remaining undamaged terminals in conjunction with some form of real or apparent supersensitivity of postsynaptic neurones to extraneuronal dopamine (UNGERSTEDT, 1971b; FELTZ and DE CHAMPLAIN, 1972b). Although this implies a maintained significant contribution of presynaptic effects of amphetamine in spite of greatly reduced presynaptic innervation, it is difficult to imagine how it could account for the total absence of a physiological gradient of amphetamine sensitivity corresponding to the histochemical gradient observed within the caudate nucleus after treatment with 6-OH-DA. For this reason we suspected that amphetamine also has a major postsynaptic effect on cells in both the normal caudate nucleus and in that with a partially destroyed dopaminergic innervation.

The conclusion that amphetamine has a clear cut post-synaptic effect was further supported by the results obtained in the 9 cats with subtotal dopamine depletion (by 98 per cent) and inactivated synthesis of striatal dopamine (Fig. 1C). None of the 47 neurones tested in these cats showed a real change in sensitivity to either D- or L-amphetamine. In two cats we observed some trend towards a slight decrease in the number of neurones responding to amphetamine injected with iontophoretic currents below 80 nA, but this might have been related to the difficulties encountered in maintaining a reasonable blood pressure and satisfactory metabolic conditions.

The observed postsynaptic effects of amphetamine might well be specific to dopamine receptors. However, the possibility of a non-specific drug effect was investigated. The best comparison between the postsynaptic effects of dopamine and amphetamine could be made in the cats with destroyed dopaminergic terminals in which there could be no question of a presynaptic effect; in these animals both amphetamine and dopamine were found to 'hyperpolarize' the post-synaptic membrane (Fig. 2). If the similar action of amphetamine and dopamine can in fact be accounted for by an effect on the same receptor site, both actions should be blocked by the same antagonists. In some preliminary attempts with compounds free of any local anaesthetic effect (GONZALES VEGAS, 1971) we found that the effects of amphetamine were antagonized with much more difficulty than those of dopamine. However the data from these experiments by no means ruled out the possibility that amphetamine was acting on the dopamine receptors. There is some other indirect evidence available concerning the specificity of receptor sites for amphetamine.

It is possible to identify, using physiological criteria, several different kinds of neurones in the caudate nucleus. We have already referred to the two groups of caudate neurones which are antidromically and orthodromically excited by nigral

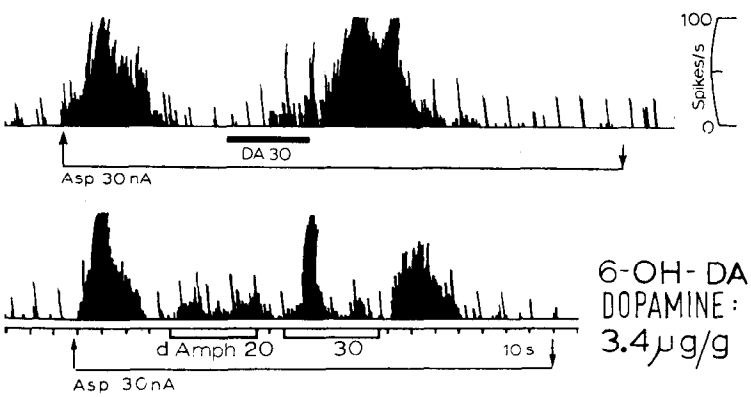


FIG. 2.—Evidence for a hyperpolarizing effect of both dopamine (DA) and amphetamine (Amph). The prolonged application of aspartate (between arrows) induced firing and then excessive depolarization manifested by the drop to zero of the firing frequency 30–40 s after beginning aspartate injection; firing was restored by either 30 nA of dopamine (above) or by 20 or 30 nA of amphetamine (below); note tendency of amphetamine to cause subsequent reduction of firing frequency when 30 nA application was maintained (antagonistic effect of depolarizing amino-acid and of potent hyperpolarizing action of amphetamine). In all tests, substantia nigra was stimulated by a train of 4 stimuli every 10 s to control further the amino-acid induced excessive depolarization characterised by a progressive decrease in spike amplitude and finally a disappearance of action potentials even in response to the nigral stimulus.

stimulation and inhibited by amphetamine; there is, for example, another group of caudate neurones, receiving a $\gamma$-aminobutyric mediated nigro-caudate inhibition without preceding excitation (FELTZ, 1972) which are similarly inhibited by amphetamine. The fact that these three and indeed other groups of caudate neurones are sensitive to amphetamine raises the question of the specificity of its action. However, it appears that the distribution of dopamine receptors (as shown in animals treated with 6-OH-DA) may be even wider within the caudate neuronal network than is reflected in estimates performed with an intact uptake mechanism (FELTZ and DE CHAMPLAIN, 1972b). Thus it appears not unlikely that the postsynaptic effects of amphetamine on striatal neurones reflect a direct interaction with dopaminergic receptor sites.

This raises the question of the appropriate interpretation of our observation that both D- and L-isomers proved to be equipotent. TAYLOR and SNYDER (1971) made a comparable observation on typical behaviours related to dopaminergic pathways (but see FERRIS *et al.*, 1972). Our data might well suggest a large contribution of postsynaptic effects of amphetamine when a given behaviour is equally affected by both isomers. Furthermore, this could well be an important feature of dopaminergic as opposed to noradrenergic mechanisms.

Such an assumption could explain the discrepancy between our findings of a postsynaptic effect of amphetamine in a specific dopaminergic system like the striatum and the demonstration by BOAKES *et al.* (1972) that the action of amphetamine in the noradrenergic system of the brain stem was entirely pre- rather than postsynaptic (but see HOFFER *et al.*, 1971). This may well be of some relevance for an understanding of some complex behavioural effects of amphetamine (COOLS, 1971) dependent on the assumption of a "dopamine-receptor-stimulating" drug action.

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