ELECTROPHYSIOLOGICAL EFFECTS OF AMPHETAMINE ON DOPAMINERGIC NEURONS

BENJAMIN S. BUNNEY and GEORGE K. AGHAJANIAN
Departments of Psychiatry and Pharmacology, Yale University School of Medicine, and the Connecticut Mental Health Center New Haven, Conn. 06508, U.S.A.

INTRODUCTION

D-AMPHETAMINE (*d*-AMP) has many interesting CNS effects, including the ability to induce paranoid psychosis in man and stereotyped behaviour in animals (SNYDER, 1972). Over the years a large body of indirect biochemical and behavioural evidence (WEISSMAN and KOE, 1965; ERNST, 1967; HANSON, 1967) has accumulated suggesting that *d*-AMP exerts its central action through its effect on the catecholamine (CA) systems of the brain. In low doses *d*-AMP has been shown to increase dopamine (DA) turnover and preferentially release newly synthesized DA from dopaminergic terminals in the neostriatum (BESSON *et al.*, 1969a, b). *d*-AMP also blocks DA uptake (SNYDER and COYLE, 1969). CORRODI *et al.* (1967) have hypothesized that the release of CA by *d*-AMP results in increased postsynaptic CA concentrations, leading to an inhibition of catecholaminergic cell activity via a neuronal feedback circuit.

This paper presents direct evidence supporting this hypothesis based on extracellular recordings from single DA neurons in the substantia nigra zona compacta (A9) and adjacent ventral tegmental areas (A10). DA containing cells in these areas were first demonstrated anatomically by Dahlström and Fuxe using fluorescence histochemical techniques (DAHLSTRÖM and FUXE, 1964). Recently we have reported (BUNNEY *et al.*, 1973b), based on combined neurophysiological and fluorescence histochemical methods, that the firing pattern of DA neurons is distinctive and easily recognizable. This identification of DA neurons on the basis of histochemical and neurophysiological characteristics has made it possible to determine the effect of drugs on the firing rate of these units.

EFFECT OF AMPHETAMINE AND ANTAGONISTS ON ACTIVITY OF DOPAMINERGIC NEURONS

In previous single unit recording studies (BUNNEY *et al.*, 1973b), using albino rats (Charles River) we have shown that intravenously administered *d*-AMP stops or markedly slows A9 and A10 cell activity in low doses (Fig. 1A). This depressant action was highly specific in that non-DA cells in the midbrain did not decrease their rate. In doses as low as 0·25 mg/kg *d*-AMP stopped 20 per cent of DA cells. The mean dose of *d*-AMP for inhibition of DA cells to 50 per cent of baseline rate was 1·6 mg/kg ($\pm$ 0·35 S.E.M.). All DA cells ceased firing by the time a dose of 6·4 mg/kg had been reached. This sensitivity of DA cells to *d*-AMP is in marked contrast to their response to *l*-AMP. Recently (BUNNEY, KUHAR and AGHAJANIAN, unpublished data) we have found that over 50 per cent of DA cells are relatively unresponsive to *l*-AMP in that even when intravenous doses as high as 25 mg/kg are given 50 per cent inhibition is not achieved. For those DA units that are responsive it appears to take 6–10 times as much *l*-AMP as *d*-AMP to produce 50 per cent inhibition.

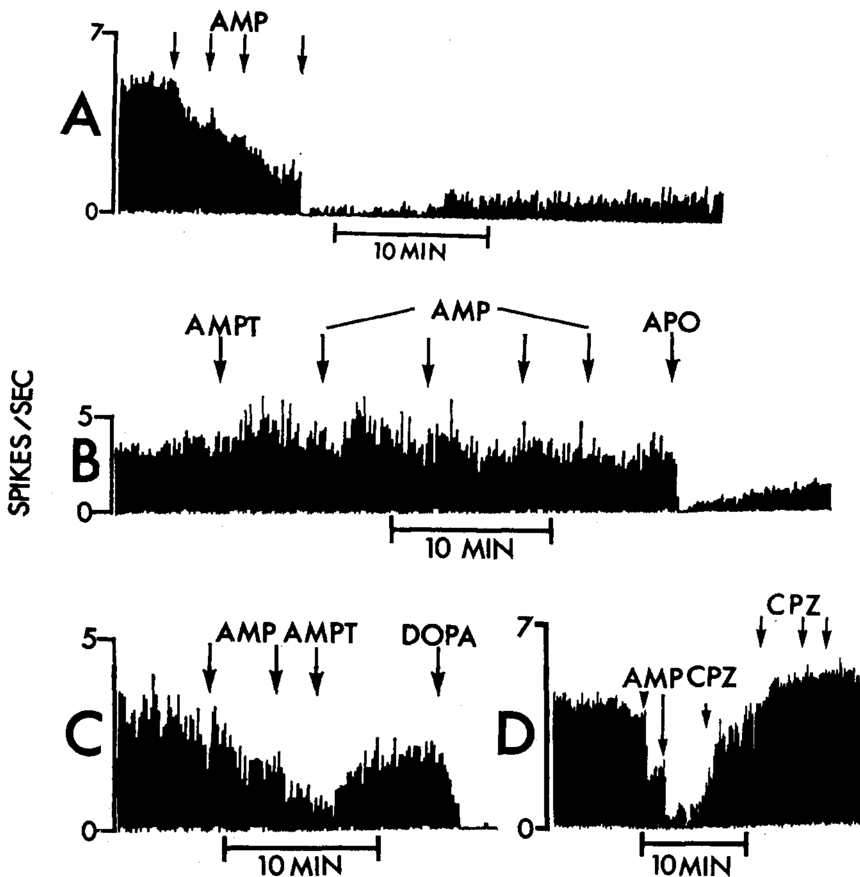

FIG. 1.—A. Typical effect of *d*-amphetamine (*d*-AMP) on the firing rate of a dopaminergic cell. Serial injections of *d*-AMP (0·25, 0·25, 0·5, 1·0 mg/kg) progressively depressed unit activity until firing ceased. Recovery was slow—25 per cent in ½ hr.

B. Prevention by α-methyl-*p*-tyrosine (AMPT) of *d*-AMP induced slowing of dopaminergic cell activity. AMPT (50 mg/kg) completely prevented any significant decrease in firing rate in ZC and VT cells. In the example given, *d*-AMP was administered in total dose of 3·5 mg/kg (0·50 mg/kg followed by 1·0 mg/kg × 3). AMPT, however, had no effect on the usual depressant response of these cells to apomorphine (APO)—0·10 mg/kg (BUNNEY *et al.*, 1973a).

C. AMPT reversal of *d*-AMP induced slowing of dopaminergic cell activity with a subsequent decrease and cessation of activity after administration of *l*-dihydroxyphenylalanine (DOPA). AMP in consecutive doses of 0·5 mg/kg each, slowed this DA cell markedly. AMPT (50 mg/kg) rapidly reversed the *d*-AMP effect in this and all other DA cells tested. Subsequent *l*-DOPA (50 mg/kg) overcame the blockade of *d*-AMP depression by AMPT and inhibited the cell (BUNNEY *et al.*, 1973a).

D. Reversal of *d*-AMP induced depression of a dopaminergic cell by chlorpromazine (CPZ). Two injections of *d*-AMP (0·25, 0·50 mg/kg) resulted in a 75 per cent decrease in the activity of a DA containing cell. CPZ (0·25 mg/kg) rapidly reversed the *d*-AMP induced depression and returned DA unit activity almost to baseline rate. CPZ (0·5 mg/kg) increased firing rate beyond baseline rate. Two further doses of CPZ (0·5 mg/kg each) had little additional effect on unit activity.

All drugs were administered intravenously. Methods as described in BUNNEY *et al.* (1973a, b).

Antipsychotic phenothiazines and haloperidol (drugs thought to be DA receptor blockers) were shown to reverse (Fig. 1D) and block the depressant effect of *d*-AMP (BUNNEY *et al.*, 1973b). In addition, we found that blockade of DA synthesis by the intraperitoneal administration of 50 mg/kg of the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (AMPT) (SPECTOR *et al.*, 1965) prevented and reversed the slowing of DA cells induced by intravenous *d*-AMP (BUNNEY *et al.*, 1973a) (Fig. 1B, 1C). When the blockade of DA synthesis by AMPT was bypassed by the intravenous administration of the immediate precursor of DA*l* dihydroxyphenylalanine (*l*-dopa), the cell firing rate again decreased (Fig. 1C). Taken together, these results suggest that ongoing DA synthesis is necessary for *d*-AMP to exert its effect and that DA receptors may be involved in *d*-AMP's ability to depress DA neurons. Since AMPT prevented the *d*-AMP depression of DA unit activity a significant *direct* action on postsynaptic neurons by *d*-AMP seems unlikely. How then is *d*-AMP affecting the firing of DA cells? Neuronal feedback inhibition remains a possibility. Although as yet unidentified anatomically, two forms of this pathway are conceivable: a postsynaptic striatal-nigral pathway or a direct recurrent collateral system consisting of dopaminergic terminals with synapses on DA cell bodies. In the latter case iontophoresis of *d*-AMP into the vicinity of the DA cell body should cause a depression of firing rate.

INHIBITION OF DOPAMINERGIC NEURONS BY AMPHETAMINE: EVIDENCE FOR A NEURONAL FEEDBACK PATHWAY

To test this hypothesis *d*-AMP was iontophoresed onto DA cells in the midbrain of rats through multibarreled micropipettes. *d*-AMP was found either to have no effect on DA unit activity (Fig. 2A) or to cause a minimal slowing the first time ejected. This effect did not increase significantly with increasing ejection currents (Fig. 2B). Multiple application rapidly led to an attenuation of the depressant response (Fig. 2B). However, *d*-AMP administered intravenously in a dose of 0·5 mg/kg had its usual marked inhibitory effect (Fig. 2A). In many cases *d*-AMP produced a local anesthetic action as indicated by a decreasing spike amplitude in association with DA cell slowing. DA iontophoresed onto DA cells had a greater depressant effect than *d*-AMP but demonstrated "tachyphylaxis" to the same degree (AGHAJANIAN and BUNNEY, this volume). No local anesthetic effect was observed with DA. These iontophoretic results suggest that a DA receptor may be present on the dendrites and/or cell bodies of DA neurons. However, it is doubtful that the depressant effect seen with iontophoretic *d*-AMP has any physiological significance as the response was small even at concentrations many times that which would reach these cells when small doses of *d*-AMP are administered intravenously. In addition, the attenuation of response seen after repeated iontophoretic administrations of *d*-AMP is never seen when *d*-AMP is given systemically, suggesting further that the small direct effect of iontophoretically applied *d*-AMP on DA cell activity has no physiological significance.

In contrast to the minimal response of DA cells to iontophoretic *d*-AMP we found postsynaptic cells in the caudate nucleus, accumbens nucleus and olfactory tubercles that were markedly depressed by low ejection currents of *d*-AMP and DA (Fig. 2C). No attenuation of response could be demonstrated. In cells responsive to both DA and *d*-AMP local anesthetic effects of *d*-AMP were seen only at higher ejection currents ($>$ 20 nA). Interestingly, adenosine 3′, 5′-monophosphate applied iontophoretically also markedly depressed many of these cells (Fig. 2C).

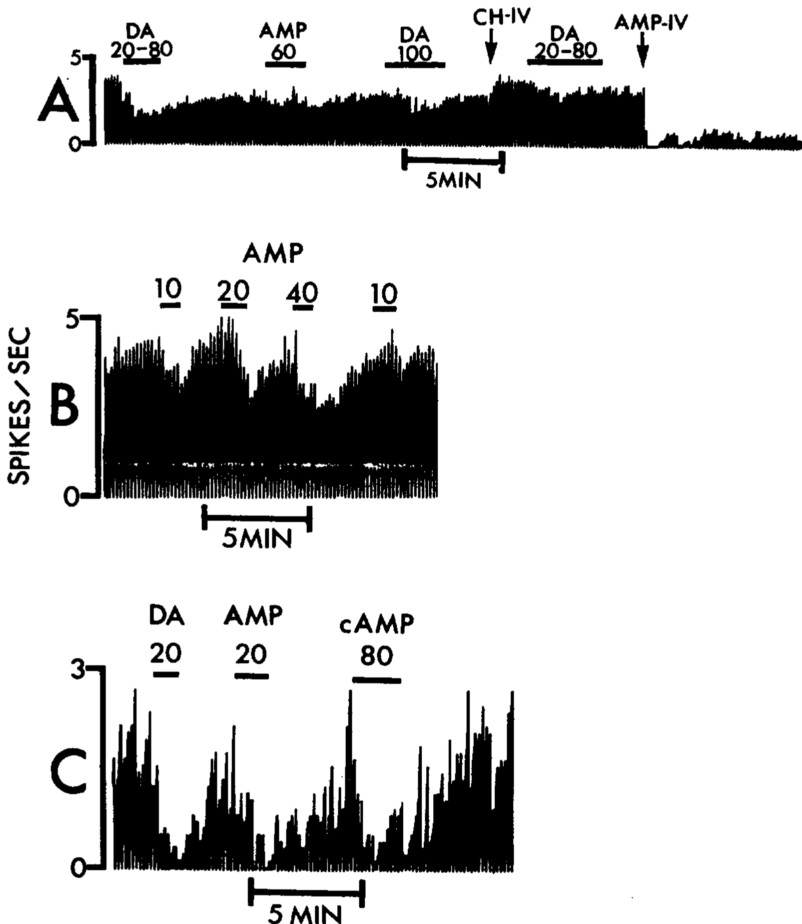

FIG. 2.—A. Effects of microiontophoretic and intravenous *d*-AMP on the firing rate of a dopaminergic neuron. Using a five barrel micropipette DA (0·2 M, pH 4) and *d*-AMP (0·2 M, pH 4) were ejected onto this DA cell in the substantia nigra zona compacta ($n = 16$). DA ejected with currents ranging from 20 to 80 nA had only a minimal depressant effect. Subsequently when applied to the same cell DA had less and less of an effect. *d*-AMP (60 nA) had no significant effect on this cell's firing rate. However, when given intravenously *d*-AMP (0·5 mg/kg) produced the usual marked inhibition of neuronal activity. Also shown in this figure is the typical increase in baseline rate of DA neurons seen after intravenous chloral hydrate (CH-I.V.) (40 mg/kg).

B. Effect of serial applications of microiontophoretically ejected *d*-AMP on the firing rate of a dopaminergic neuron. *d*-AMP ejected with a 10 nA current produced a moderate depressant effect on cell activity. Subsequent ejections of *d*-AMP at higher currents (20, 40 nA) failed to significantly further depress cell activity. A repeat application of *d*-AMP ejected at 10 nA produced no change in firing rate. This figure demonstrates the typical diminution in DA cell response seen after repeated iontophoretic applications of *d*-AMP.

C. Effect of DA, *d*-AMP and adenosine 3′, 5′-monophosphate (cAMP) on the firing rate of a cell in the accumbens nucleus. DA and *d*-AMP, in contrast to their effect on DA cells (see A), caused this postsynaptic cell to markedly decrease in rate. Typical of the postsynaptic cells tested this cell showed no diminution of response after repeated ejections of DA and *d*-AMP (not shown in this figure; $n = 20$). cAMP (0·2 M, pH 8) also depressed the firing rate of this cell.

Each tracing shows the activity of a single cell. Each vertical line represents the integrated rate of firing expressed as spikes/sec. The duration of each microiontophoretic drug application is depicted by length of bar above tracing. The number

Thus it would appear from the relative lack of response of DA cells to microiontophoretically ejected *d*-AMP that recurrent collaterals are not involved in the marked slowing of these cells by low doses of systemically administered *d*-AMP. However, these results do not provide *positive* proof for a postsynaptic non-dopaminergic feedback pathway. If such a pathway exists one should be able to abolish the inhibitory effect of systemically administered *d*-AMP on DA cells by destroying the pathway with a lesion. Accordingly, under chloral hydrate anesthesia, using a stereotoxically placed fine retractable knife the rat brain was transected at a level just in front of the most anterior edge of the substantia nigra zona compact (A2970-A3290μ, KÖNIG and KLIPPEL, 1970) ($n = 8$). Following this lesion almost all DA cells were firing at abnormally high rates ($\sim$10/sec). Intravenous administration of *d*-AMP in doses up to 14 mg/kg either had no effect or caused only a slight decrease in activity below baseline rate. Thus doses of *d*-AMP twice that needed to stop all DA cells in unlesioned animals produced only a minimal slowing.

DISCUSSION

Our results show that *d*-AMP in small systemic doses has a marked depressant effect on the activity of dopaminergic neurons. However, DA cells appear to be relatively insensitive to *l*-AMP. This finding directly parallels the report by COSTA *et al.* (1972) that 0·3 mg/kg of *d*-AMP increases striatal DA turnover while 1·0 mg/kg of *l*-AMP has no effect. Our results are also in accord with the recent report that *d*-AMP administered intravenously is 3 to 4 times more potent than *l*-AMP in increasing striatal ^{3}H–DA release (VON VOIGTLANDER and MOORE, 1973). On the other hand, in studies of DA uptake in synaptosomes little difference was found between the blocking effects of *d*- and *l*-AMP (COYLE and SNYDER, 1969). It is not clear how the latter finding relates to our own due to the difference in the preparation used.

Inhibition of DA synthesis by AMPT blocked the slowing of DA cells induced by *d*-AMP. Until recently this finding would have been interpreted as conclusive proof that ongoing synthesis is necessary for *d*-AMP to exert its effect. However, ENNA *et al.* (1973) have reported that in addition to inhibition of tyrosine hydroxylase AMPT inhibits *d*-AMP induced efflux of exogenous ^{3}H-norepinephrine (^{3}H–NE) from brain slices. They suggest that AMPT interferes with movement of NE to an AMP releasable site. We do not know yet whether AMPT has a similar action in the DA system or whether this action is present *in vivo* as well as *in vitro*. In addition, the concentration of *d*-AMP used by Enna *et al.* is higher than could have been achieved intracerebrally when low doses of *d*-AMP are administered intravenously. A hypothesis which might explain these various results is that release of NE by *d*-AMP is synthesis coupled, i.e. NE release may be dependent upon the availability of newly synthesized NE to replace it. It this were true then synthesis inhibition by AMPT would lead to the decreased efflux of ^{3}H–NE from brain slices reported by Enna *et al.* Final resolution of this question, however, will have to await further studies.

The discovery of this new action of AMPT calls into question the validity of using AMPT inhibition of the depressant effect of *d*-AMP on DA cell activity as the only evidence that ongoing DA synthesis is necessary for *d*-AMP to exert its action. We have therefore inhibited DA synthesis at the next step in its synthetic pathway and again examined the effect of *d*-AMP on DA cell activity. N^{1}-(DL-seryl)-N^{2}-(2,3,4-trihydroxybenzyl) hydrazine (R04-4602) completely blocked the depressant effect of

d-AMP on these cells when given intraperitoneally in a dose of 800 mg/kg. At this high dose R04-4602 has been shown to be an effective dopa-decarboxylase inhibitor in the CNS (WEISS *et al.*, 1972).

SUMMARY

(1) *d*-AMP administered intravenously in low doses markedly depresses the firing rate of dopaminergic neurons. This effect of *d*-AMP appears dependent upon the presence of ongoing DA synthesis as it is abolished by AMPT (a tyrosine hydroxylase inhibitor) and by high doses of R04-4602 (a decarboxylase inhibitor).

(2) We have confirmed the previously proposed hypothesis (CORRODI *et al.*, 1967) that *d*-AMP depresses DA cell firing rate indirectly via a postsynaptic non-dopaminergic feedback pathway. This conclusion is based on the following findings:

(a) As predicted, *d*-AMP selectively depresses the firing rate of dopaminergic neurons in the substantia nigra zona compacta (A9) and adjacent ventral tegmental (A10).

(b) The direct effect of *d*-AMP on DA cell activity when applied iontophoretically, even with high ejection currents, is weak and transient as compared to its marked depressant effect when applied with low currents in the vicinity of postsynaptic neurons (i.e. neurons receiving a dopaminergic input).

(c) Transection between the substantia nigra and striatum abolishes the depressant effect on DA cell firing rate of systemically administered *d*-AMP.

The latter result strongly suggests the existence of a neuronal feedback pathway although its precise anatomical location has yet to be determined.

Acknowledgement—This research was supported by NIMH Grant MH-17871, USPHS Research Scientist Development Award MH-14459 (to G.K.A.), and the State of Connecticut.

REFERENCES

BESSON M. J., CHERAMY A., FELTZ P. and GLOWINSKI J. (1969a). *Proc. Nat. Acad. Sci.* **62**, 741–748.
BESSON M. J., CHERAMY A. and GLOWINSKI J. (1969b). *Europ. J. Pharmacol.* **7**, 111–114.
BUNNEY B. S., AGHAJANIAN G. K. and ROTH R. H. (1973a). *Nature*, in press.
BUNNEY B. S., WALTERS J. R., ROTH R. H. and AGHAJANIAN G. K. (1973b). *J. Pharmacol. Exp. Ther.*, 185, 560–571.
CORRODI H., FUXE K. and HÖKFELT T. (1967). *Europ. J. Pharmacol.* **1**, 363–368.
COSTA E., GROPPETTI A. and NAIMZADA (1972). *Brit. J. Pharmacol.* **44**, 742–751.
DAHLSTRÖM A. and FUXE K. (1964). *Acta Physiol. Scand.* **62**, Suppl. 232, 1–55.
ENNA S. J., DORRIS R. L. and SHORE P. A. (1973). *J. Pharmacol. Exp. Ther.* **184**, 576–582.
ERNST A. M. (1967). *Psychopharmacologia* **10**, 316–323.
HAIGLER H. J. and AGHAJANIAN G. K. (1973). *Europ. J. Pharmacol.* **21**, 53–60.
HANSEN L. C. F. (1967). *Psychopharmacologia* **10**, 289–297.
KÖNIG J. F. R. and KLIPPEL R. A. (1970). *The Rat Brain: A Stereotaxic Atlas.* Robert E. Krieger.
SNYDER S. H. (1972). *Archs Gen. Psychiat.* **27**, 169–179.
SNYDER S. H. and COYLE J. T. (1969). *J. Pharmacol. Exp. Ther.* **165**, 78–86.
SPECTOR S., SJOERDSMA A. and UDENFRIEND S. (1965). *J. Pharmacol. Exp. Ther.* **147**, 86–95.
VON VOIGTLANDER P. F. and MOORE K. E. (1973). *J. Pharmacol. Exp. Ther.* **184**, 542–552.
WEISS B. F., MUNROE L. A., ORDONEZ R. J. and WURTMAN R. J. (1972). *Science* **177**, 613–616.
WEISSMAN A. and KOE B. K. (1965). *Life Sci.* **4**, 1037–1048.