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A PRESYNAPTIC SITE OF ACTION WITHIN THE MESENCEPHALIC RETICULAR FORMATION FOR (+)-AMPHETAMINE-INDUCED ELECTROCORTICAL DESYNCHRONIZATION

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1 Changes induced in the electrocorticogram by the bilateral perfusion of (+)-amphetamine into the mesencephalic reticular formation (MRF) have been studied in cat encéphale isolé preparations.

2 (+)-Amphetamine, applied for 5 min in the MRF, mimicked the electrocortical desynchronization induced by the perfusion of (–)-noradrenaline (NA) or (–)-α-methylnoradrenaline (AMNA) into the same sites.

3 Perfusion of 6-hydroxydopamine (6-OHDA) also induced desynchronization but, over the 1 h perfusion period, slow wave activity gradually returned to the electrical record.

4 Following the application of 6-OHDA the effect of (+)-amphetamine was abolished or significantly attenuated, whereas the effect of NA or AMNA was not affected.

5 The electrocortical desynchronization induced by (+)-amphetamine could be restored if its application was preceded by perfusion with NA or AMNA.

6 Fluorescence studies using AMNA indicated that 6-OHDA depleted noradrenergic nerve terminals near the cannulae tips. However, the terminals were still capable of taking up exogenously applied AMNA.

7 These results suggest that (+)-amphetamine has a presynaptic action on noradrenergic nerve terminals within the MRF.

Introduction

Early studies investigating the alerting action of amphetamine have emphasized the role played by neural mechanisms situated in the brainstem reticular formation. In the intact cat, or animals with spinal sections at the cervical level, amphetamine produces prolonged electrocortical desynchronization (Bradley & Elkes, 1957). This effect is not influenced by total brainstem transections which interrupt ascending projections from the caudal pons and medulla, but is markedly attenuated when the rostral pons is also eliminated (Hiebel, Bonvallet, Huvé & Dell, 1954). In contrast, brainstem lesions which effectively sever all connections between mesencephalic and telencephalic structures completely abolish both the behavioural and electrocortical signs of arousal induced by this drug (Bradleh & Elkes, 1957). On the basis of these studies it would appear that neural mechanisms located in the rostral pons and midbrain region play a major role in the electrocortical alerting effects of amphetamine.

At the neuronal level several theories have been formulated to account for the effect of amphetamine. The direct action of the drug on postsynaptic receptors has been suggested (Rossum, Schoot & Hurkmans, 1962; Smith, 1963) and more recently proposed to account for the effect of amphetamine on single neurones following iontophoretic application in the cerebellum, hippocampus and caudate nucleus (Hoffer, Siggins & Bloom, 1971; Feltz & De Champlain, 1972; Segal & Bloom, 1974). Other studies would indicate that the mechanism of action of amphetamine is indirect and related to the release of endogenous catecholamines (Moore, 1963; Stein, 1964; Carr & Moore, 1970; Boakes, Bradley & Candy, 1972). This latter hypothesis is interesting in view of the observation that noradrenaline-containing nerve terminals are present within the brainstem reticular formation (Fuxe, 1965). Moreover, direct perfusion of (–)-noradrenaline (NA) into the mesencephalic or rostro-pontine reticular formation will induce behavioural alerting and electrocortical desynchronization similar to that seen after intravenous injection of small doses of amphetamine (Key, 1975).

This similarity between the effect of systemically

administered amphetamine and locally applied noradrenaline prompted the following study in which the action of (+)-amphetamine when perfused directly into caudal areas of the mesencephalic reticular formation (MRF) is investigated. By the use of the selective catecholamine depletor, 6-hydroxydopamine (6-OHDA), attempts have also been made to relate the changes induced in the pattern of electrocortical activity to the presence or absence of endogenous noradrenaline within this area. A preliminary account of the work has been given to the British Pharmacological Society (Candy & Key, 1976).

Methods

Experiments were carried out on a total of 32 cat encéphale isolé preparations, of which 11 animals were also used for catecholamine fluorescence studies. The operative procedure, carried out under halothane/oxygen anaesthesia, the type of cortical recording electrode, the concentric, two-tube cannulae and the implantation techniques have been described previously (Bradley & Elkes, 1957; Bradley & Key, 1958; Key, 1975). The cannulae were placed stereotactically into the caudal mesencephalic reticular formation at co-ordinates A 1.0, Tr 2.0, H−2.0 (Snider & Niemer, 1961). The area of perfusion at the tip of each cannula was 0.53 mm^2. Drugs were dissolved in artificial cerebro-spinal fluid (CSF) and perfused at a rate of 120 µl/min with a 20-channel Watson-Marlow H.R. Flow Inducer. The artificial CSF, slightly acidified in each experiment to match the pH of the drug solutions (pH 5.9–6.8), was perfused continuously and the drug solutions switched in for 5 min periods. All solutions were warmed to 38°C before application to the brain by passage through a heat exchanger situated close to the head of the animal.

Systemic blood pressure was monitored by means of a mercury manometer connected to the femoral artery. Upon completion of the operative procedures, and subsequently at intervals throughout the experiment, wound edges and pressure points were infiltrated with 0.5% w/v xylocaine hydrochloride solution. The animals were then released from the stereotactic apparatus and a period of 1 h allowed for recovery from the effects of the anaesthetic. Control recordings of electrocortical activity were taken over a further 1 h before continuous perfusion of CSF was started.

The electrocorticogram (ECoG) was monitored throughout the experiment with an 8-channel Mingograph EEG and for descriptive purposes reference has been made to four basic patterns of activity representing the alert, relaxed, drowsy and sleeping behavioural states according to the schema described by Bradley & Elkes (1957). The rapid eye movement (paradoxical) sleep stage was not observed in these preparations. For a quantitative assessment of the changes induced in the ECoG by drug perfusion, integration of the electrocortical waveform was carried out using the bipolar recording from the association area of the middle suprasylvian gyrus. The output from the integrator was in the form of pulses representing fixed increments of electrical energy. These pulses were printed on the electrical record coincident with the appropriate electrocortical activity (Figure 1). The data were further quantified by counting the number of pulses for each successive 20 s period and expressing these cumulative integrals graphically to reflect the changing levels of synchrony or desynchrony in the ECoG (Figure 1).

Upon completion of the experiment the brain was removed, the brainstem dissected out and fixed in 10% formol saline solution and later examined histologically to determine the position of the tips of the cannulae.

Fluorescence studies

In this series of experiments the experimental procedure was identical to that already described. However, (−)-α-methylnoradrenaline (AMNA) was perfused in place of noradrenaline. This compound is equipotent with NA in eliciting electrocortical desynchronization (Key, 1975) and yields a strong fluorophore (Jonsson, 1971), but has the added advantage of being more resistant to monoamine oxidase (Blashko, Richter & Schlossmann, 1937). At the end of each experiment the drug solutions were removed from the cannulae by passing air through the system and the animal immediately killed with an intravenous overdose of sodium pentobarbitone. The mesencephalon was removed from the brain within 11 min, quenched in isopentane and then transferred to liquid nitrogen. Due to the size of the tissue it was necessary to freeze-dry it for 12 days at −38°C before gassing with 70% relative humidity paraformaldehyde at 80°C for 1 h and then embedding in paraffin wax (54°C m.p.). Ten micron serial sections were cut and examined with a Zeiss Universal microscope under incident illumination from a HBO 200 mercury vapour lamp. The filters used were primary BG 38 and BG 12 with a secondary cut-off filter at 500 nm. Photographs of the distribution of the fluorescence were taken on Agfa Gaevert RP1 film.

In some cases microspectrofluorometric analyses were carried out. The Zeiss Universal microscope was equipped with an HBO 100 W mercury vapour lamp and an EMI 6256B photomultiplier. Fluorescence emission spectra were recorded using a UG1 primary filter and a continuous interference filter monochromator (400–700 nm), the diameter of the areas from which microspectrofluorometric measurements were made was 12.6 µm.

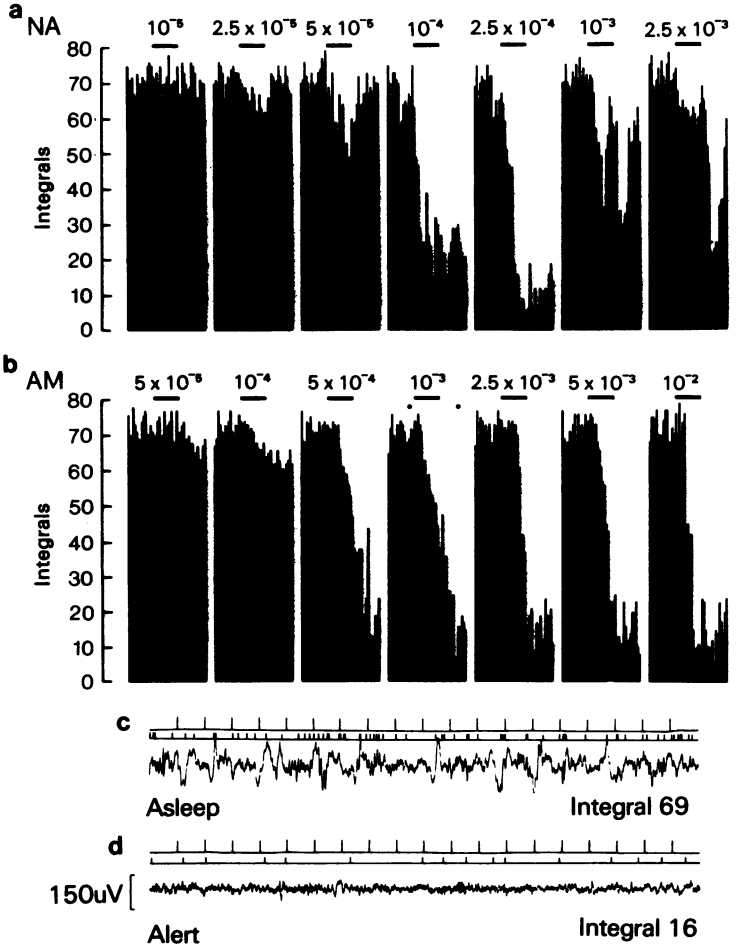

Figure 1 Effect of increasing perfusate concentrations of (–)-noradrenaline (NA) or (+)-amphetamine (Am) on the electrocortical activity in the same animal. Molar concentrations are shown. Electrical activity, recorded from the left middle suprasylvian gyrus, was integrated and the histograms represent the counts recorded over successive 20 s periods. The black bars represent a 5 min bilateral perfusion into the mesencephalic reticular formation. In this Figure and Figure 2, a decrease in the integral count indicates an increase in the degree of desynchronization of the ECoG. (a) NA produced dose-related changes in the ECoG. Perfusion of NA 5×10^{-5} M produced phasic electrocortical responses while NA 10^{-4} M elicited tonic desynchronization with behavioural alerting. Concentrations of NA in excess of 10^{-3} M produced a complex pattern of response consisting of desynchronization, synchronization and then a further period of desynchronization on withdrawal of the drug. (b) (+)-Amphetamine produced similar dose-related responses except that higher concentrations were required and using a 5 min perfusion period the complex biphasic responses were not observed with the higher concentrations. Filled circles show the position from which the sample electrocortical records (c) and (d) (asleep and alert) were taken; also shown are the pulse integration counts and a 1 s time marker.

Drugs

The drugs used were (–)-noradrenaline hydrochloride, pH 6.2–6.4 (Sigma), dopamine hydrochloride, pH 6.2–6.4 (Sigma), (–)-α-methylnoradrenaline hydrochloride, pH 6.2–6.4 (Hoechst), (+)-amphetamine sulphate, pH 6.4–6.8 (SKF), fluoxetine hydrochloride (Lilly) pH 6.6–6.8 and 6-hydroxydopamine hydrochloride, pH 5.9–6.0 (Lab Kemi AB). Concentrations of the drugs used are expressed in terms of molarity in terms of the salt. The 6-OHDA was dissolved before use in cold, de-gassed artificial CSF to which 0.07 mg/ml ascorbic acid had been added to prevent oxidation. Perfusion of the solution

was started immediately and continued for 1 or 1.5 hours. The reservoir containing the drug was kept cold during this time by means of an ice jacket.

Results

Electrocortical changes induced by the perfusion of catecholamines and (+)-amphetamine

The bilateral perfusion of NA into the dorso-medial aspect of the mesencephalic reticular formation within the concentration range of 10^{-5} to 10^{-2} M resulted in electrocortical desynchronization and behavioural alerting as previously reported (Key, 1975). The degree of electrical change appeared to be dose-related in that following perfusion of 1 to 2.5×10^{-5} M NA the effect was limited to a reduction in the amplitude of the slow wave activity and the introduction of slightly faster frequencies. In some cases, this change was hardly discernible on visual inspection of the electrical record but was apparent from the integration data by the small, but consistent reduction in the count during the perfusion period (Figure 1). Perfusion of 5×10^{-5} M NA usually resulted in the appearance of phasic, 1–5 s periods of desynchronized activity, 1 to 2 min after application. It was only when higher concentrations, in excess of 10^{-4} M, were employed that tonic electrocortical desynchronization and behavioural alerting occurred (Figure 1). The effect appeared within 1 to 3 min and in all of the experiments outlasted the period of perfusion by 1 to 15 minutes. Application of 1 to 5×10^{-3} M NA resulted in the progressive attenuation of the initial period of tonic desynchronization and slow wave activity returned to the ECoG even while the NA was still perfused (Figure 1). However, a further period of desynchronization usually occurred following withdrawal of the drug and, indeed, when higher doses of NA were used it became the dominant feature of the response (Figure 1).

The changes in electrocortical activity induced by the perfusion of (+)-amphetamine were comparable to those induced by NA. (+)-Amphetamine always produced phasic or tonic electrocortical desynchronization at the same sites as NA. However, basic differences in the responses, were apparent. When (+)-amphetamine was used, the latency to the onset of the response was longer (2 to 5 min) and in the lower concentration range (5×10^{-5} to 10^{-4} M) changes in electrocortical activity were only observed after the 5 min perfusion of the drug had ceased (Figure 1). An interesting difference in the responses induced by NA and (+)-amphetamine was the inability of (+)-amphetamine, even in high concentrations (5×10^{-3} to 10^{-2} M), to induce during the 5 min perfusion period the two stage response seen with 10^{-3} or 2.5×10^{-3} M solutions of NA. Once electrocortical desynchronization and behavioural alerting had been produced by (+)-amphetamine it continued throughout the perfusion period and persisted a further 5 to 60 minutes.

As in previous studies (Key, 1975) it was noticed that during perfusion of NA the animal appeared to be more responsive to sensory stimulation and in the majority of cases the short periods of desynchronization or phasic responses could be correlated with the incidence of ambient auditory stimuli to which the animal had previously been unresponsive. Indeed, it was possible by reduction or elimination of auditory stimuli to delay the appearance of phasic and especially tonic responses, to NA. This correlation between response and environmental conditions was even more apparent with (+)-amphetamine, in that the latency of the phasic responses could be doubled and tonic responses, at least using the lower concentrations of the drug ($< 5 \times 10^{-3}$ M), blocked by the drastic reduction of sensory stimulation.

Dopamine was also perfused at the same sites as (+)-amphetamine and NA but even though concentrations of up to 10^{-3} M were used the results were not consistent. In 6 of the 12 experiments a reduction in the cumulative integral count was noted when high concentrations of dopamine were employed (10^{-4} to 10^{-3}M), the effect being comparable to that induced by a concentration of 2.5×10^{-5} M NA. The latency of the response however was 1 to 3 min longer than that of NA and did not persist on withdrawal of the drug.

Electrocortical changes induced by the perfusion of 6-hydroxydopamine

The electrocortical response to the perfusion of 6-OHDA was initially examined in 6 preparations. In this series of experiments NA (10^{-4} M) and (+)-amphetamine (10^{-4} M) were applied at the same sites in concentrations which had been shown in the initial experiments to produce tonic desynchronization of the ECoG under normal laboratory conditions within the 5 min perfusion period (Figure 2). Application of 6-OHDA in the sleeping animal invariably produced tonic desynchronization of the ECoG and behavioural alerting within 7 to 10 min from the start of the perfusion. The effect was relatively long lasting and persisted for 5 to 20 minutes. However, even though the 6-OHDA was perfused continuously for 1 or 1.5 h, periods of slow wave activity gradually returned to the electrical record, such that after 40 to 45 min the normal sleep/walking cycle of the encéphale isolé preparation was again apparent (Figure 2). Indeed, the responsiveness of the animal to sensory stimulation did not appear to be changed at the end of the perfusion. A short auditory stimulus (Figure 2) was still capable of producing an alerting response little different from that seen in the control record before application of the 6-OHDA. Similarly, the electro-

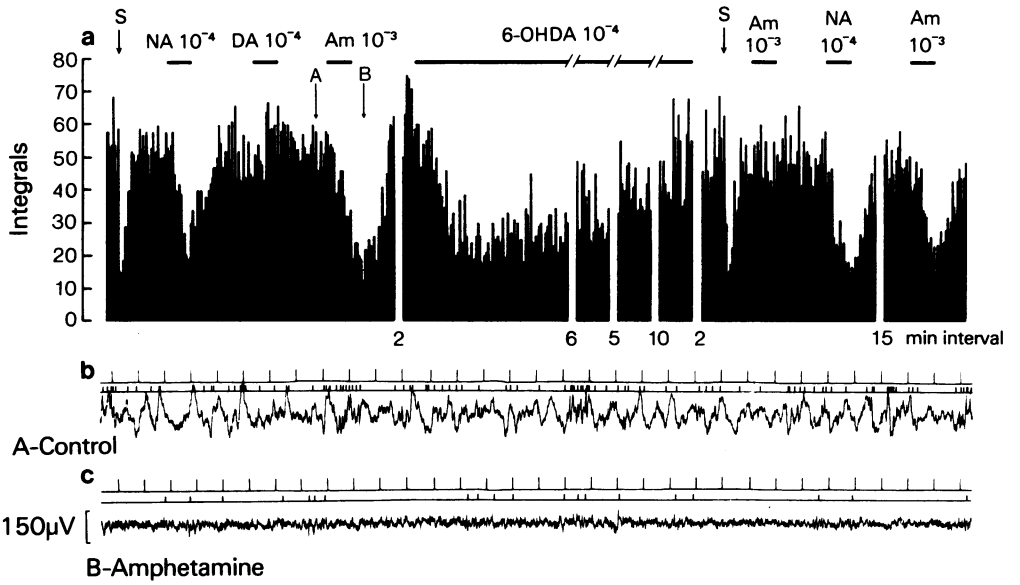

Figure 2 (a) Effect of the perfusion of 6-hydroxydopamine (6-OHDA) on the responses to (–)-noradrenaline (NA) and (+)-amphetamine (Am). Integration data as in Figure 1. Molar concentrations are shown. The position of the sample electrocortical records (b) and (c) is indicated by A and B. NA and (+)-amphetamine both produced tonic desynchronization of the ECoG, while dopamine (DA) was without effect. The perfusion of 6-hydroxydopamine (6-OHDA) over a period of 72 min initially produced tonic desynchronization but slow wave activity returned to the electrical record even while the 6-OHDA was still being perfused. Following the 6-OHDA the responsiveness of the animal to an auditory stimulus (S) appeared unchanged and the effect of NA was also unchanged. The response to (+)-amphetamine was abolished when tested prior to the perfusion of NA but subsequently reappeared after the NA perfusion.

cortical response to the perfusion of NA was not altered, whereas that produced by (+)-amphetamine was either greatly reduced (1 expt) or, as in Figure 2, abolished completely (5 expts). This lack of effect of (+)-amphetamine however, was conditional upon the order of perfusion. If NA was the first drug to be tested following application of 6-OHDA, or (+)-amphetamine was retested for a second time after NA, then a period of electrocortical desynchronization could be elicited. The degree of desynchronization and the duration of the response were both reduced but nevertheless an effect was quite apparent (Figure 2).

In 6 experiments AMNA was substituted for NA in an equimolar concentration (10^{-4} M). The response produced by AMNA was similar to that of NA. Pretreatment with 6-OHDA did not affect this response. However, as in the experiments with NA, the response to (+)-amphetamine was greatly reduced (2 expts) or abolished (4 expts) but again could be partially restored by a prior 5 min perfusion of AMNA.

Fluorescence studies

A 5 min perfusion of AMNA at 10^{-4} M in 2 cats resulted in the appearance of green catecholamine fluorescence within nerve terminals up to a distance of 750 μm from the cannulae tips (Figure 3a). The uncorrected fluorescence emission spectra exhibited a peak of emission between 480 and 500 nm consistent with the presence of a catecholamine (Jonsson, 1971). In these animals the background tissue fluorescence was not appreciably enhanced in the immediate vicinity of the cannulae tips nor was fluorescence visible in the endothelium or lumen of local blood vessels. In one cat both cannulae were close to the dorsal noradrenergic pathway ascending from the locus coeruleus. As a result, catecholamine fluorescence appeared in the fibres of this bundle, which are normally non-fluorescent.

The selective 5-hydroxytryptamine uptake inhibitor, fluoxetine (Wong, Horng, Bymaster, Hauser & Molloy, 1974) was perfused for 10 min at 10^{-5} M (2 cats) and then immediately followed by a perfusion of a mixture of AMNA (10^{-4} M) and fluoxetine for a further 5 minutes. Following this procedure uptake of AMNA did not appear to be suppressed since fluorescence was still visible in the nerve terminals at the cannulae tips (Figure 3b).

Perfusion of AMNA (10^{-4} M) for 5 min followed by perfusion of artificial CSF for 1 h in 3 cats did not lead to the disappearance of catecholamine fluorescence

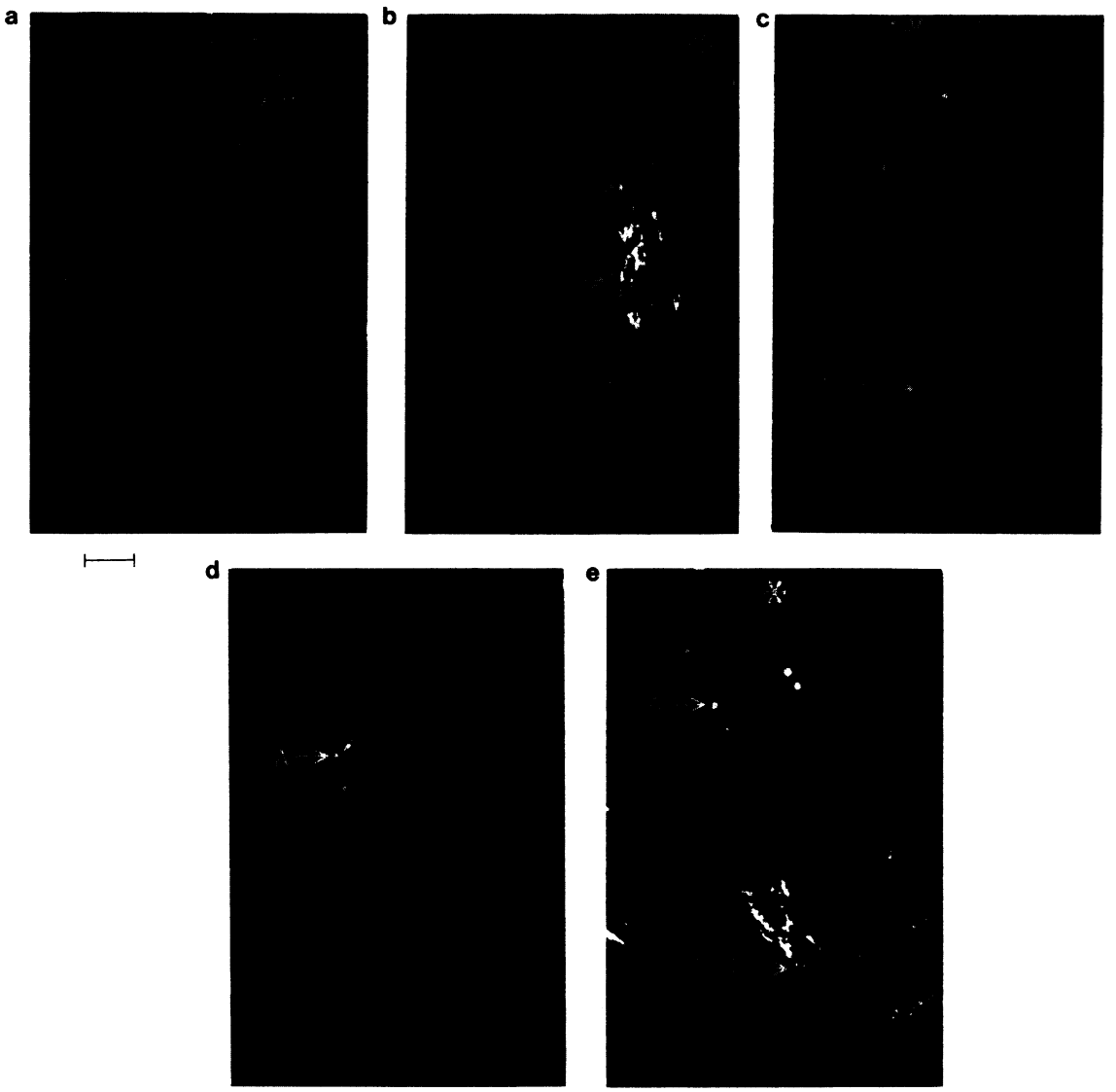

Figure 3 Fluorescence micrographs showing the presence or absence of catecholamine fluorescence in nerve terminals (T) and axons (A) in the immediate vicinity of the cannulae tip (*) in different animals. (a) Fluorescence induced in terminals after a 5 min perfusion of α-methylnoradrenaline (AMNA) at 10^{-4} M. Note also the presence of fluorescence in axons of the dorsal noradrenergic pathway. (b) Fluorescence induced in terminals by AMNA (10^{-4} M) in the presence of the specific 5-hydroxytryptamine uptake inhibitor, fluoxetine (10^{-5}M). Fluorescence in axons of the dorsal noradrenergic pathway is again present. (c) Fluorescence in terminals and axons still present 1 h after a 5 min perfusion of AMNA (10^{-4} M). (d) Lack of terminal fluorescence after perfusion of AMNA (10^{-4} M), followed by a 1 h perfusion of 6-hydroxydopamine (10^{-4} M). Note that the 6-hydroxydopamine has not depleted the fluorescence from axons in the dorsal noradrenergic pathway. (e) Reappearance of fluorescence in terminals after initial depletion by 6-hydroxydopamine (10^{-4} M) followed by perfusion of AMNA (10^{-4} M). Fluorescence in axons is again present. Calibration bar is 100 μm.

from the nerve terminals (Figure 3c) or from the fibres of the dorsal noradrenergic pathway. In contrast, after perfusion of AMNA (10^{-4} M) for 5 min followed by perfusion of 6-OHDA (10^{-4} M) for 1 h (3 cats) no catecholamine fluorescence could be observed in terminals near the cannulae tips (Figure 3d).

The initial experiments had shown that after pretreatment with 6-OHDA, (+)-amphetamine was ineffective in producing changes in the ECoG unless preceded by a 5 min application of NA or AMNA. To assess the viability of uptake mechanisms 6-OHDA was perfused in 2 experiments and immediately followed by a 5 min application of AMNA. Weak catecholamine fluorescence was again visible in the terminals within the immediate vicinity of the cannulae tips (Figure 3e).

Discussion

The results show that (+)-amphetamine is able to mimic both the phasic and tonic electrocortical desynchrony induced by NA or AMNA when perfused at the same sites in the dorsal aspect of the MRF. On the basis of the fluorescence data, catecholamine-containing nerve terminals would appear to be present at these sites. AMNA was taken up within the immediate vicinity of the cannulae tips and this uptake was not blocked by the highly selective 5-hydroxytryptamine uptake inhibitor, fluoxetine. The finding that dopamine did not induce any consistent change in the ECoG would further suggest that the terminals in the MRF are NA rather than dopamine-containing.

Perfusion of the selective neurotoxic agent, 6-OHDA, invariably elicited an initial period of electrocortical desynchronization. Since this drug does not possess a catecholamine agonist action (Furness, Campbell, Gillard, Malmfors, Cobb & Burnstock, 1970) the effect may be attributed to the release of endogenous amines, similar to that reported in the peripheral sympathetic nervous system (Furness *et al.*, 1970; Haeusler, 1971; Jonsson & Sachs, 1972). Indeed, following a 1 h perfusion of 6-OHDA, depletion in the immediate vicinity of the cannulae tips appeared to be complete and no terminal fluorescence could be detected in this area. It is unlikely that a simple 'washing out' process had occurred over the perfusion period for control experiments demonstrated that fluorescent nerve terminals were still in evidence after perfusion of AMNA followed by a 1 h perfusion of artificial CSF.

The perfusion of 10^{-4} M AMNA for 5 min induced fluorescence in nerve terminals for a distance of up to 750 μm from the cannulae tips. It is obvious therefore that to affect depletion the 6-OHDA must have diffused to at least this distance and may be expected, due to the longer perfusion period, to shown an even wider dissemination. However, the effect of 6-OHDA still appeared to be exerted on a relatively circumscribed area, leaving the major portion of the alerting mechanism unaffected. Thus the perfusion of 6-OHDA produced no permanent changes in arousal function since within 40 to 45 min, even while the drug was still being applied, the normal sleep/waking pattern of the encéphale isolé was re-established with little difference in the responsiveness of the animal to sensory stimulation. It would appear that the arousal system was capable of compensating for that portion which had been functionally impaired by the application of 6-OHDA.

Immediately following catecholamine depletion by 6-OHDA, AMNA or NA were still capable of producing electrocortical desynchronization and behavioural alerting similar to that induced before pretreatment. In contrast, the effect of (+)-amphetamine was abolished or greatly attenuated. Thus the inability of (+)-amphetamine to produce electrocortical changes, taken together with the lack of catecholamine fluorescence in nerve terminals after perfusion of 6-OHDA, indicates that (+)-amphetamine has a predominantly, if not exclusively, presynaptic action within the MRF. Data from *in vitro* investigations on the effect of (+)-amphetamine at NA terminals would indicate that blockade of reuptake rather than a direct releasing action is responsible for the apparent efflux of NA produced by (+)-amphetamine (Raiteri, Bertollini, Angelini & Levi, 1975).

One interesting finding which tends to support the presynaptic nature of the (+)-amphetamine effect, was that even after catecholamine depletion by 6-OHDA (+)-amphetamine was able to induce a short period of electrocortical desynchronization, providing it was given after a perfusion of AMNA or NA. It has been shown that the amine pump in the membranes of adrenergic nerve terminals in the peripheral sympathetic nervous system is affected early in the degenerative processes induced by 6-OHDA (Haeusler, 1971; Jonsson & Sachs, 1972; Hökfelt, Jonsson & Sachs, 1972) and within the CNS degenerative changes following local application of 6-OHDA have been observed after only 15 min (Ungerstedt, 1971). Even so, the secondary, short alerting effect of (+)-amphetamine would suggest that the catecholamine containing nerve terminals, although depleted after the 1 h perfusion of 6-OHDA, had not degenerated to the degree that they were incapable of taking up, or of releasing, exogenously applied NA or AMNA. This assumption is supported by the finding that application of AMNA after 6-OHDA consistently resulted in the appearance of some fluorescence within terminals around the cannulae tips. Presumably this AMNA could be made available for release by (+)-amphetamine or by afferent neuronal activity with subsequent potentiation by amphetamine due to blockade of re-uptake.

In summary, the results of the present study show that (+)-amphetamine produces electrocortical changes when perfused locally into the MRF. The depletion studies with 6-OHDA indicate that this effect is related to NA containing terminals and suggest that the action of (+)-amphetamine within the MRF is presynaptic in nature.

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References

BLASHKO, H., RICHTER, D. & SCHLOSSMAN, H. (1937). The oxidation of adrenaline and other amines. *Biochem. J.*, **31**, 2187–2196.

BOAKES, R.J., BRADLEY, P.B. & CANDY, J.M. (1972). A neuronal basis for the alerting action of (+)-amphetamine. *Br. J. Pharmac.*, **45**, 391–403.

BRADLEY, P.B. & ELKES, J. (1957). The effects of some drugs on the electrical activity of the brain. *Brain*, **80**, 77–117.

BRADLEY, P.B. & KEY, B.J. (1958). The effect of drugs on arousal responses produced by electrical stimulation of the reticular formation of the brain. *Electroenceph. clin. Neurophysiol.*, **10**, 97–110.

CANDY, J.M. & KEY, B.J. (1976). Blockade of the electrocortical changes induced by perfusion of amphetamine in the brain stem reticular formation. *Br. J. Pharmac.*, **56**, 374P.

CARR, L.A. & MOORE, K.E. (1970). Effects of amphetamine on the contents of norepinephrine and its metabolites in the effluent of perfused cerebral ventricles of the cat. *Biochem. Pharmac.*, **19**, 2361–2374.

FELTZ, P. & DE CHAMPLAIN, J. (1972). Enhanced sensitivity of caudate neurones to microiontophoretic injections of dopamine in 6-hydroxydopamine treated cats. *Brain Res.*, **43**, 601–605.

FURNESS, J.B., CAMPBELL, G.R., GILLARD, S.M., MALMFORS, T., COBB, J.L.S. & BURNSTOCK, G. (1970). Cellular studies of sympathetic denervation produced by 6-hydroxydopamine in the vas deferens. *J. Pharmac. exp. Ther.*, **174**, 111–122.

FUXE, K. (1965). Evidence for the existence of monoamine-containing neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta. physiol. scand.*, **64**, Suppl. 247.

HAEUSLER, G. (1971). Early pre- and postjunctional effects of 6-hydroxydopamine. *J. Pharmac. exp. Ther.*, **178**, 49–62.

HIEBEL, G., BONVALLET, M., HUVÉ, P. & DELL, P. (1954). Analyse neurophysiologique de l'action centrale de la d-amphétamine (Maxiton). *Semaines des Hopitaux de Paris*, **30**, 1880–1887.

HOFFER, B.J., SIGGINS, G.R. & BLOOM, F.E. (1971). Studies on norepinephrine-containing afferents to Purkinje cells of rat cerebellum. II. Sensitivity of Purkinje cells to norepinephrine and related substances administered by microiontophoresis. *Brain Res.*, **25**, 523–534.

HÖKFELT, T., JONSSON, G. & SACHS, C. (1972). Fine structure and fluorescence morphology of adrenergic nerves after 6-hydroxy-dopamine *in vivo* and *in vitro*. *Z. Zellforsch.*, **131**, 529–543.

JONSSON, G. (1971). Quantitation of fluorescence of biogenic monoamines. *Progr. Histochem Cytochem.*, **2**, 299–334.

JONSSON, G. & SACHS, C. (1972). Degenerative and nondegenerative effects of 6-hydroxydopamine on adrenergic nerves. *J. Pharmac. exp. Ther.*, **180**, 625–635.

KEY, B.J. (1975). Electrocortical changes induced by perfusion of catecholamines into the brainstem reticular formation. *Neuropharmacology*, **14**, 41–51.

MOORE, K.E. (1963). Toxicity and catecholamine releasing actions of d- and l-amphetamine in isolated and aggregated mice. *J. Pharmac. exp. Ther.*, **142**, 6–12.

RAITERI, M., BERTOLLINI, A., ANGELINI, F. & LEVI, G. (1975). d-Amphetamine as a releaser or reuptake inhibitor of biogenic amines in synaptosomes. *Eur. J. Pharmac.*, **34**, 189–195.

ROSSUM, J.M. VAN, SCHOOT, J.B. VAN DER & HURKMANS, J.A.Th.M. (1962). Mechanism of action of cocaine and amphetamine in the brain. *Experientia*, **18**, 229–231.

SEGAL, M. & BLOOM, F.E. (1974). The action of norepinephrine in the rat hippocampus. I. Iontophoretic studies. *Brain Research*, **72**, 79–97.

SMITH, C.B. (1963). Enhancement by reserpine and α-methyl DOPA of the effects of d-amphetamine upon the locomotor activity of mice. *J. Pharm. exp. Ther.*, **142**, 343–350.

SNIDER, R.S. & NIEMER, W.T. (1961). *A Stereotaxic Atlas of the Cat Brain*. Chicago: University of Chicago Press.

STEIN, L. (1964). Self-stimulation of the brain and the central stimulant action of amphetamine. *Fedn Proc.*, **23**, 836–850.

UNGERSTEDT, U. (1971). Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxy dopamine on monoamine neurons in the rat brain. In *6-Hydroxydopamine and Catecholamine Neurons*, ed. Mamfors, T. & Thoenen, H., pp. 101–127. Amsterdam: North-Holland.

WONG, D.T., HORNG, J.S., BYMASTER, F.P., HAUSER, K.L. & MOLLOY, B.B. (1974). A selective inhibitor of serotonin uptake, Lilly 110140, 3-(*p*-trifluoromethyl-phenoxy)-N-methyl-3-phenylpropylamine. *Life Sci.*, **15**, 471–479.

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