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POTENTIATION OF RESPONSES TO MONOAMINES BY ANTIDEPRESSANTS AFTER DESTRUCTION OF MONOAMINE AFFERENTS

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1 Stereotaxic lesioning and microiontophoretic techniques were used to study the effects of lesions of the medial forebrain bundle (MFB) on the potentiation by antidepressant drugs of responses to monoamines of cortical neurones.

2 Active uptake of noradrenaline (NA) and 5 hydroxytryptamine (5-HT) by synaptosomes from the motor and somatosensory cortex was reduced to approximately 20%, 10 to 14 days following lesion of the MFB in rats.

3 Unilateral lesions of the MFB caused changes in responsiveness of neurones to NA and 5-HT, applied by iontophoresis, in the cortex ipsilateral to the lesion. Excitatory responses to both amines were observed less frequently and depression was the predominant response. Excitatory responses on the lesioned side were significantly smaller than on the unlesioned side, but the size of depressant responses was unaltered.

4 Viloxazine strongly potentiated responses of cortical neurones to NA and 5-HT on both sides of the brain of MFB-lesioned rats. There were no significant differences in the potentiation of responses to monoamines on the lesioned or unlesioned sides of the brain.

5 Desipramine potentiated responses to NA of neurones in the cortex ipsilateral to MFB lesions.

6 Chlorimipramine potentiated responses to 5-HT of neurones in the cortex ipsilateral to MFB lesions.

7 It is concluded that antidepressants can potentiate responses to monoamines despite a profound reduction in presynaptic terminals. The potentiation is unlikely to be the result of blockade of monoamine uptake into presynaptic terminals, and is probably a postsynaptic effect of the antidepressant drugs.

Introduction

The postsynaptic actions of noradrenaline (NA) and 5 hydroxytryptamine (5-HT) are terminated primarily by active reuptake into the presynaptic terminals (Coyle & Snyder, 1969, Iversen, 1974). Tricyclic antidepressant drugs are potent inhibitors of monoamine uptake in brain tissue (Ross & Renyi, 1967; 1969). It has therefore been suggested that the clinical actions of tricyclics may result from potentiation of monoamine transmission in the central nervous system due to blockade of uptake (see Schildkraut, 1965; Coppen, 1972).

The technique of iontophoresis has been used to investigate the actions of tricyclic antidepressant drugs on single cells (Bradshaw, Roberts & Szabadi, 1974). The tricyclics potentiated responses of single neurones in the neocortex to NA and 5-HT applied by iontophoresis. Responses to acetylcholine (ACh) were also potentiated. There is little evidence to suggest that ACh is inactivated by reuptake in the CNS (Katz & Chase, 1971). Further experiments showed that iprindole, a tricyclic with little effect on NA uptake (Gluckman & Baum, 1969), could potentiate neuronal responses to this amine (Bevan, Bradshaw & Szabadi, 1975a).

Viloxazine, a non-tricyclic antidepressant drug, also potentiates neuronal responses to NA and 5-HT, and yet it has little effect on the uptake of either amine in the brain (Jones & Roberts, 1977; 1978). On the basis of these, and other experiments (Bevan, Bradshaw & Szabadi, 1974; 1975b, c; 1976) it has been suggested that potentiation of neuronal responses to monoamines may be a postsynaptic effect of the antidepressants (Bradshaw *et al.*, 1974; Bevan *et al.*, 1975a, b; Jones & Roberts, 1978).

The majority of iontophoresis experiments have been done on single neurones in the cerebral cortex of rats and cats. It is probable that both NA and 5-HT act as neurotransmitters in the cerebral cortex (Anden, Dahlstrom, Fuxe, Larrson, Olson & Unger-

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stedt, 1966). Cell bodies containing NA and 5-HT in the hindbrain and midbrain send axons to the cortex via the medial forebrain bundle (MFB) (Ungerstedt, 1971; Fuxe & Jonsson, 1974).

Stimulation of the cell bodies situated in the locus coeruleus and raphe nuclei results in increased turnover and release of the amines in the cortex (Aghajanian, Rosecrans & Sheard, 1967; Arbuthnott, Crow, Fuxe, Olson & Ungerstedt, 1970; Shields & Eccleston, 1972).

It is likely therefore that cortical neurones possess subsynaptic receptors for NA and 5-HT and presynaptic terminals which release these amines. Cortical neurones seem to be appropriate cells for the investigation of the interactions between antidepressants and monoamines.

If the antidepressants potentiate responses to monoamines by a postsynaptic mechanism, then potentiation will occur in the absence of presynaptic terminals. In the present experiments, lesions of the medial forebrain bundle reduced the ability of the cortex to take up NA or 5-HT. This was presumably due to the degeneration of monoamine terminals following section of the ascending axons. However, there was no change in the potentiating actions of the antidepressant drugs. It is concluded that potentiation of responses by the antidepressants cannot be explained by an action on presynaptic terminals.

Methods

Stereotaxic lesions

Male albino Wistar rats (200 to 250 g) were anaesthetized with sodium pentobarbitone (55 mg/kg i.p.). A bipolar stainless steel electrode was stereotaxically positioned in the MFB of the left side of the brain (co-ordinates: A.P., +3.6.; lateral, 1.6; vertical, −2.8; König & Klippel, 1963). The lesion was made by passing a direct current of 2 mA for 15 s. The electrode was removed and the animal allowed to recover for 2 h before being returned to normal housing; 10 to 14 days after lesioning, animals were used either for iontophoresis experiments or for uptake studies.

Histology

The brains of all animals used in uptake and iontophoresis experiments were stored in formal saline for 1 week. Sections, 50 μm thick, were cut on a freezing microtome, mounted and stained with thionin to verify the position and extent of the lesion. Data from animals with less than total destruction of the MFB were discarded.

Noradrenaline and 5-hydroxytryptamine uptake studies

Neocortical tissue was removed from the motor and somatosensory areas to a depth of approximately 2 mm. Suspensions rich in synaptosomes were prepared from this tissue by the method of Kurokawa, Sakamoto & Kato (1965) with the modification described by Collard (1978).

A discontinuous density gradient consisting of 2.5% Ficoll in sucrose, layered on 13% Ficoll in sucrose was used to isolate synaptosomes from crude cortical homogenates. The synaptosomal suspension was diluted with Krebs-bicarbonate buffer gassed with 95% O_2 and 5% CO_2. Two samples of synaptosomes from each side of the brain were taken, one incubated at 4°C, and the other at 37°C for 10 min. [^{14}C]-5-HT (0.05 μM, specific activity 54 mCi/mmol or [^{14}C]-NA (0.1 μM, specific activity 64 mCi/mmol) was then added to all four samples and the incubations terminated by placing them in an ice bath after a further 10 min.

Following centrifugation the synaptosomal pellet was resuspended in 0.4N perchloric acid to release the labelled amine and precipitate protein. The suspension was centrifuged again, the supernatant transferred to a scintillation counting vial for determination of the labelled amine. The pellet was dissolved in 0.5N NaOH and protein determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

The uptake of amine was related to the protein content of the suspension and expressed as pmol amine taken up per mg of protein in 10 min (pmol mg^{-1} 10 min^{-1}). Active uptake was determined by subtraction of the uptake at 4°C from the uptake at 37°C. Uptake on the lesioned side was compared to the intact side with Student's t test.

Iontophoresis experiments

Rats weighed 250 to 300 g at the time of iontophoresis experiments. They were anaesthetized with halothane (4% in O_2). On completion of tracheal cannulation the halothane was reduced to 0.6% and maintained at this level. Systemic blood pressure, respiratory rate and electrocardiogram were monitored routinely. Methods for the preparation of animals and exposure of the cortical surface have been described elsewhere (Bradshaw *et al.*, 1974).

Five-barrelled microelectrodes were used to record from, and apply drugs to, single neurones in the motor and somato-sensory cortex. Two barrels were filled with 3M NaCl, one for recording action potentials and the other for balancing the iontophoretic currents. The remaining barrels contained a combination of the following drugs: noradrenaline bitartrate (0.2M, pH 4.0); 5-hydroxytryptamine bimaleinate (0.2M, pH 4.0); viloxazine hydrochloride (0.2M, pH

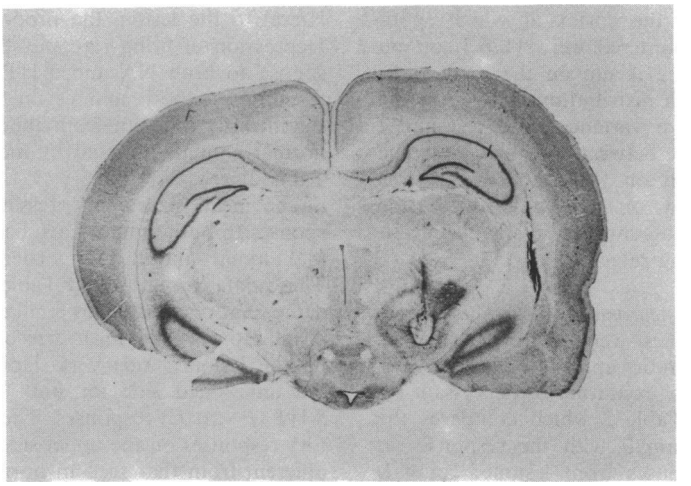

Figure 1 Photomicrograph of a 50 μm thick section of the brain of a lesioned rat. The section is taken from an A.P. position of approximately 3.6 mm (König & Klippel, 1963). Part of the electrode track and the electrolytic damage to the medial forebrain bundle can be seen on the right of the section.

6.0); desipramine hydrochloride (0.1M, pH 7.5); chlorimipramine hydrochloride (0.2M, pH 7.0).

Only spontaneously active neurones were studied. Action potentials were counted by means of a pulse height selector and ratemeter. The ratemeter output was fed to a polygraph which provided an on-line record of cell firing rate. A sequential timing device controlled the application of drugs. When a neurone was located, NA or 5-HT was applied with a current of 100 nA for 30 s. This was repeated at regular intervals, usually of 4 min. When constant agonist responses were obtained a single brief application (25 to 100 nA, 15 to 50 s) of an antidepressant was made, without interrupting the sequence of agonist applications. Retaining currents of −20nA were passed between agonist applications. Responses to the agonists were quantified by measuring the total spike number (Bradshaw *et al.*, 1974) from the polygraph records. The total spike number of a response is the number of action potentials generated during the response minus the number generated during an equivalent period of baseline firing. Alternate electrode tracks were usually made on the lesioned and intact sides of the brain.

Results

Position of lesions

A typical lesion of the MFB is shown in Figure 1. The lesion was at the level of the lateral hypothalamus and normally sectioned only the MFB; 5 animals were excluded from analysis due to less than complete destruction of the MFB. Occasionally some damage to other areas occurred, including the internal capsule, the caudate putamen, the zona incerta, the subthalamic nucleus, the ventral thalamus and the substantia nigra. Data from these animals were included in the analysis.

Uptake experiments

Uptake studies were conducted on 9 rats with MFB lesions. On the lesioned side of the brain the mean uptake of 5-HT by cortical synaptosomes was 23.9% of that on the intact side ($n = 5$). NA uptake was similarly reduced to 20.9% on the lesioned side ($n = 4$). The reduction of active uptake of both amines was highly significant (t test, $P < 0.001$). There was no significant difference between the uptake of NA and 5-HT at 4°C and at 37°C on the lesioned side of the brain (t test). The results of the uptake experiments are summarized in Table 1.

Iontophoresis experiments

Effect of the lesion on neuronal activity. On the lesioned side of the brain 144 spontaneously active neurones were encountered in 76 vertical penetrations of the cortex whilst 112 cells were found in 35 tracks on the intact side. Thus an average of 1.9 ± 0.2 cells were found per track on the lesioned side, compared with 3.2 ± 0.4 cells per track on the intact side ($P < 0.002$).

The mean depths in the cortex at which spontaneous cells were encountered were 1176.3 μm on the lesioned side and 1121.1 μm on the intact side. Comparison of the depth distribution of cells on the two sides by means of a variance ratio test and t test showed no difference between the two sides. The mean firing rate of cells on the lesioned side was 13.6 ± 0.7 spikes/s and on the unlesioned side 14.7 ± 0.9 spikes/s. A t test showed no significant difference between the firing rates.

Effect of the lesion on neuronal responses to monoamines. Cortical neurones were either excited or depressed by iontophoretic application of NA or 5-HT. The proportions of excitatory or depressant responses are shown in Table 2 which compares the responses in normal animals with the responses in lesioned animals. Responses from lesioned animals are subdivided into those on the unlesioned side and those from the lesioned side of the brain. The predominant response to both amines in normal animals and on the intact side is one of excitation with depressions occurring less often. However, in the cortex ipsilateral to the lesion, the proportions were reversed. Depression of firing rate was the more common response to both NA and 5-HT. The proportions of responses to both amines on the lesioned side are significantly different from the intact side and from normal animals assessed by means of a χ^2 test (see Table 2).

The mean sizes of depressant and excitatory responses to both amines have been determined in normal animals and in both cortices of lesioned animals. These data are shown in Table 3. The mean size of depressant responses was similar on both sides of the brain. However the mean size of excitatory responses on the lesioned side was significantly less than on the unlesioned side for both NA ($P < 0.001$) and 5-HT ($P < 0.02$) responses. The mean size of excitatory responses on the intact side was not significantly different from that seen in normal animals. The size of excitatory responses to ACh was unaffected by MFB lesions. Responses of similar size were seen on both sides of the brain of lesioned animals. These were not significantly different from ACh responses in normal animals (Table 3).

Table 1 Uptake of monoamines following medial forebrain bundle (MFB) lesions

	Active uptake (pmol mg^{-1} protein 10 min^{-1})	
	Noradrenaline	*5-Hydroxytryptamine*
Unlesioned side	4.01 ± 0.42 (4)	5.96 ± 0.71 (5)
Lesioned side	0.84 ± 0.14 (4)	1.43 ± 0.54 (5)
P	<0.001	<0.001

The figures are the mean uptake ± s.e. mean. The figures in parentheses are the number of rats used. Mean uptake of noradrenaline on the lesioned side was 20.9% of the unlesioned side whilst the uptake of 5-hydroxytryptamine was 23.9% on the lesioned side of the unlesioned side

Table 2 Effect of medial forebrain bundle (MFB) lesions on proportions of cortical cells excited or depressed by monoamines

	% of cells responding							
	5-Hydroxytryptamine				*Noradrenaline*			
	+	−	0	n	+	−	0	n
Normal	55.2	31.0	13.8	(58)	51.9	40.3	7.8	(77)
Intact	51.8	38.8	9.4	(54)	54.8	35.5	9.7	(31)
Lesion	26.9	59.6	13.5	(52)	20.0	69.1	10.9	(55)

+ = Excitatory response, − = depressant response, 0 = no effect. 'Normal' refers to animals in which no lesion was made. 'Intact' and 'lesion' refer to the cortex contralateral and ipsilateral to a lesion in the MFB respectively. In the cortex of normal animals and in the intact cortex there is a predominance of cells excited by the amines and depressant responses are less common. In the cortex ipsilateral to the lesion these proportions are reversed, with depression of firing becoming the predominant response to both amines and excitations seen far less often. There was a significant difference between the distribution of responses to 5-HT on the intact and lesioned sides ($\chi^2 = 6.4$, $P < 0.02$), and between the lesioned side and the cortex of normal animals ($\chi^2 = 10.3$, $P < 0.01$). The distribution of responses to NA was also significantly different between the intact and lesioned ($\chi^2 = 11.2$, $P < 0.001$) and normal and lesioned cortex ($\chi^2 = 13.5$, $P < 0.001$). There was no significant difference in the response distribution to either amine in the cortex of normal animals and the intact cortex of lesioned animals.

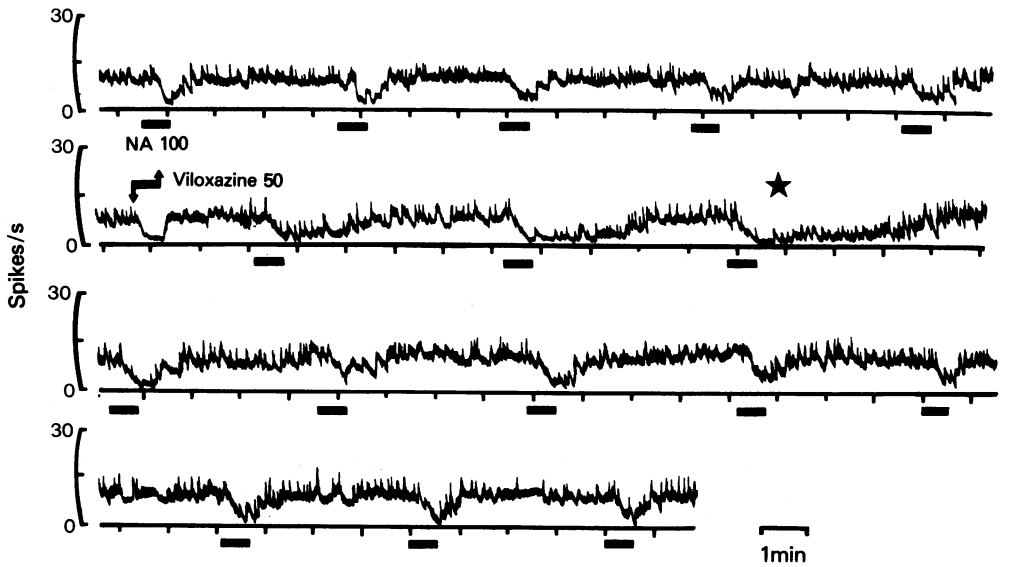

Figure 2 Potentiation of depressant responses to noradrenaline (NA) of a neurone in the denervated cortex by viloxazine. The entire ratemeter record of the study taken from the polygraph is shown. The black bars beneath the trace indicate the NA applications and the figures refer to the intensity (nanoamps) of the ejecting current. The black bar above the trace indicates the viloxazine application. The star above the trace indicates the response showing the maximum potentiation following viloxazine: 10 min after viloxazine application the NA response was potentiated to 354% of mean control. Responses gradually returned to baseline values after 42 minutes.

Effect of viloxazine on neuronal responses to noradrenaline and 5-hydroxytryptamine. The effect of viloxazine on responses to NA has been investigated on 5 cells depressed and one excited by NA in the denervated cortex. With the exception of one cell, viloxazine markedly potentiated the responses in all studies. An example of potentiation of a depressant response is shown in Figure 2. On this occasion the response was potentiated to 354% of mean control within 10 min of the viloxazine application. The responses returned to control levels after 42 minutes.

Potentiation was also seen in the one study where NA caused excitatory responses of the cell. A maximum potentiation of 208% of mean control occurred after 11 minutes. The mean maximum potentiation, mean time to reach maximum potentiation and mean

Table 3 Effect of medial forebrain bundle (MFB) lesions on the size of neuronal responses to monoamines and acetylcholine in the cortex

	Response size (total spike number)				
	5-Hydroxytryptamine		*Noradrenaline*		*Acetylcholine*
	+	−	+	−	+
Normal	2343 ± 219 (11)	1374 ± 141 (16)	2945 ± 546 (11)	1471 ± 200 (19)	1452 ± 103 (21)
Intact	2369 ± 217 (28)	1782 ± 221 (21)	2679 ± 292 (17)	1983 ± 342 (11)	1646 ± 148 (8)
Lesion	1435 ± 220 (14)	1625 ± 163 (31)	1238 ± 100 (11)	1583 ± 136 (37)	1607 ± 183 (13)

+ = Excitatory response; − = depressant response. The figures are the mean total spike numbers (see text) ± s.e. mean. The figures in parentheses are the number of cells. 'Normal' refers to animals in which no lesion was made. 'Intact' and 'lesion' refer to the cortex contralateral and ipsilateral to the MFB lesion. The mean size of excitatory responses to 5-HT in the cortex on the lesioned side of the brain was significantly smaller than in normal ($P < 0.01$) or intact ($P < 0.02$) cortex, assessed by a Student's t test. Similarly NA excitatory responses were significantly smaller on the lesioned side than in normal ($P < 0.01$) or intact ($P < 0.001$) cortex. There were no significant differences in the size of depressant responses. Excitatory responses to ACh were similar on both sides in lesioned animals and in the cortex of normal animals.

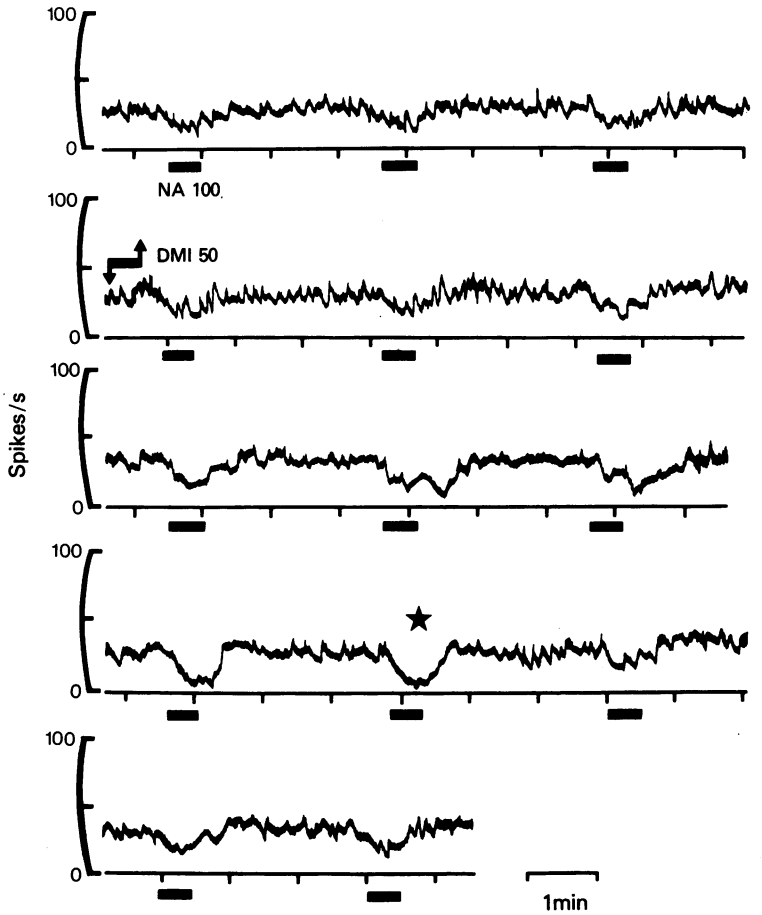

Figure 3 Potentiation of responses to noradrenaline (NA) of a neurone in the denervated cortex, by desipramine (DMI). Details as in Figure 2. Following the application of DMI the depressant responses to NA gradually increased in size. The largest response, indicated by the star, was 206% of the main control response and occurred after 22 min.

Table 4 Comparison of maximum potentiation, time to reach maximum and time to recovery of baseline for viloxazine-monoamine interaction studies in intact and denervated cortex

	5-Hydroxytryptamine				*Noradrenaline*			
	Maximum potentiation (%)	*Time to maximum* (*min*)	*Time to recovery* (*min*)	*n*	*Maximum potentiation* (%)	*Time to maximum* (*min*)	*Time to recovery* (*min*)	*n*
Intact	189 ± 17.5	16.2 ± 1.8	31.2 ± 2.4	13	174 ± 6.9	18.2 ± 1.5	34.3 ± 2.0	12
Lesion	181 ± 9.2	14.0 ± 2.6	24.2 ± 2.4	9	210 ± 33.7	12.8 ± 3.0	30.0 ± 4.4	5
P	NS	NS	<0.1 (NS)		NS	<0.1 (NS)	NS	

The figures are mean ± s.e. mean. *n* gives the number of studies in which potentiation of responses by viloxazine was seen. The maximum potentiation is the maximum increase in response size after viloxazine. The time to maximum is the time after the viloxazine at which the greatest increase in response size was seen. The time to recovery is the time after viloxazine at which the amine response returned to within 20% of the mean control response before viloxazine. Student's *t* test showed no significant differences between these parameters for studies in intact or denervated cortex.

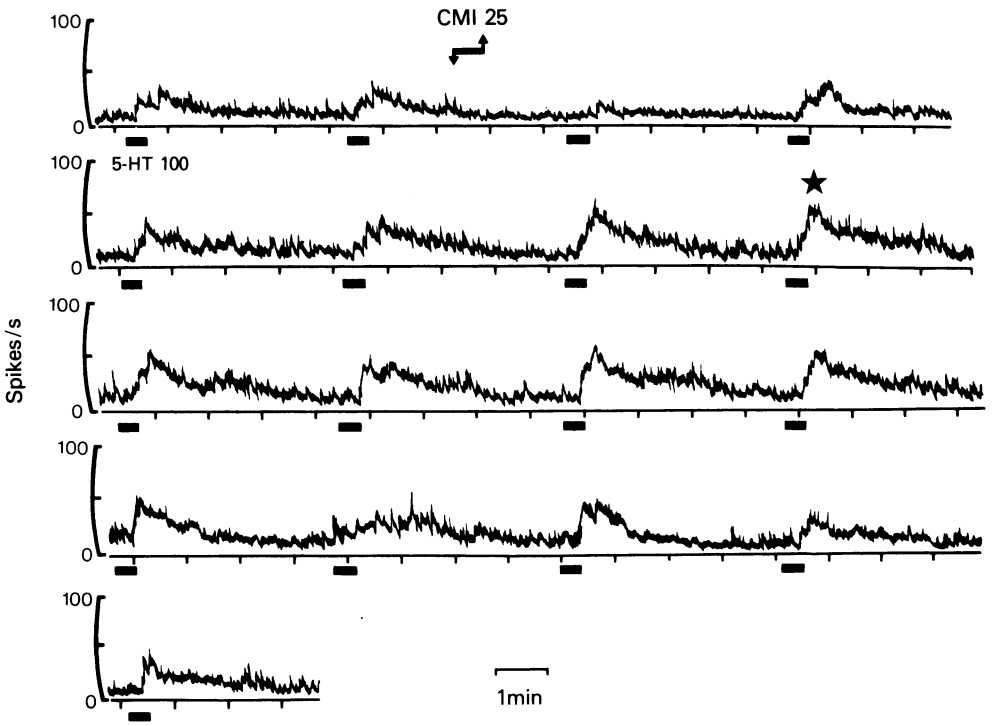

Figure 4 Potentiation of excitatory responses to 5-hydroxytryptamine (5-HT) of a neurone in the denervated cortex, by chlorimipramine (CMI). Details as in Figure 2. Following the application of CMI the next response to 5-HT was almost abolished. Thereafter the responses became profoundly potentiated. A maximum potentiation to 268% of mean control was seen after 17 min, the response indicated by the star. Responses returned to control levels 54 min after CMI.

time to recovery were determined for studies with NA on the lesioned side. Student's *t* test showed no significant differences in these parameters from studies on the unlesioned side or from normal animals. (See Table 4).

The effects of viloxazine on responses to 5-HT were studied on 9 cells in the denervated cortex. All 9 cells were depressed by 5-HT. Potentiation of responses occurred in all studies with a mean maximum potentiation of 181% of mean control. Again, the mean maximum potentiation, time to reach maximum and mean recovery time were not significantly different for studies in intact and denervated cortex. (See Table 4.)

There was no difference in 'dose' of viloxazine applied in studies in the intact or denervated cortices. Viloxazine was invariably applied with a current of 50 nA. The duration of the application was in most cases 50 s but this was occasionally varied slightly (range 30 to 50 s).

Effects of desipramine on neuronal responses to noradrenaline. Interactions between NA and desipramine have been studied on 9 cells depressed by NA in the denervated cortex. Potentiation of responses was seen in all but one study. Figure 3 shows the entire ratemeter record of one study; 22 min after the application of desipramine the response was increased to 206% of mean control. Potentiation of excitatory responses to NA by desipramine was also seen in one study on the lesioned side.

Effects of chlorimipramine on neuronal responses to 5-hydroxytryptamine. Figure 4 shows the entire ratemeter record of an interaction study between 5-HT and chlorimipramine in the denervated cortex. On this occasion 5-HT excited the cell. The response immediately following chlorimipramine was almost abolished. Thereafter, potentiation occurred to a maximum of 268% of mean control 17 min later. Responses returned to control levels after 41 min. Potentiation, by chlorimipramine, of excitatory responses to 5-HT also occurred in another study in the denervated cortex and potentiation of depressant responses was seen in 3 further studies.

Discussion

It is likely that a high affinity uptake system for a substance will be associated with synapses where the substance is a transmitter (for review see Kuhar, 1973). Ten to 14 days following MFB lesions there was a marked reduction of active uptake of NA and 5-HT by synaptosomes from the cortex ipsilateral to the lesion. It is likely that the reduction in uptake represents the degree of degeneration of presynaptic axon terminals containing monoamines in the cortex. The uptake of amines on the lesioned side at 37°C was only slightly higher than, and did not differ significantly from the uptake at 4°C. It is likely that the residual uptake after MFB lesions is the result of passive diffusion of amine into the tissue rather than active accumulation. It would be of interest to determine if the residual uptake is sensitive to inhibition by antidepressants. Following similar lesions to those employed in these experiments, there is an almost complete disappearance of fluorescence due to 5-HT and NA in the cortex of rats (Ungerstedt, 1971). It is probable therefore that cortical neurones ipsilateral to the MFB lesion have a large reduction in the number of synaptic contacts from monoamine terminals.

In several systems, lesioning afferent pathways causes an increase in sensitivity of the postsynaptic structures to iontophoretically applied transmitters (Feltz & DeChamplain, 1972; Bird & Aghajanian, 1975; Wright & Roberts, 1978). No such increase in sensitivity of cortical neurones to either NA or 5-HT was seen following MFB lesions. On the contrary, the mean size of excitatory responses to both amines was much reduced on the lesioned side. However, excitatory responses to ACh were unaffected by the lesion so it is unlikely that the effect is non-specific. The size of depressant responses to NA and 5-HT were also unaffected by the lesion. In normal animals and in the intact cortex of lesioned animals, excitatory responses were more frequent than depressant responses. However, on the lesioned side the situation was reversed. It is unlikely that the results indicate any denervation supersensitivity of cortical neurones to the depressant effects of the amines, because there was no change in the 'dose' of amine applied or any change in the size of depressant responses. Haas & Wolf (1978) have reported supersensitivity of cortical neurones to the depressant effects of NA following MFB lesions in guinea-pigs. However, these authors did not observe excitatory responses on the lesioned or unlesioned sides so it is difficult to make comparisons between their study and the present one.

There was a reduction in the number of spontaneously active cells encountered in the denervated cortex. It is possible therefore that cells which would normally be active due to the release of endogenous monoamines may not be spontaneously active following the lesion. This could account for a decreased frequency of excitatory responses. The depths of the neurones studied on the two sides were not significantly different so it is unlikely that different populations were sampled. Again, no difference in firing rates of cells were found on the two sides so the change in responsiveness of the cells was not the result of a change in baseline firing rate. It is difficult to find a satisfactory explanation for the altered responsiveness at present, but it is apparent that the excitatory responses seem to be more affected by degeneration of terminals than the depressant responses.

The results of the uptake studies suggest a large reduction in presynaptic monoamine terminals in the cortex following lesions of the MFB. The altered responsiveness of the postsynaptic cells to the monoamines indirectly supports this suggestion. Despite the reduction in presynaptic terminals we have found that the ability of antidepressant drugs to potentiate monoamine responses is apparently undiminished. Although viloxazine has little effect on the uptake of NA or 5-HT by brain tissue (Lippmann & Pugsley, 1976) it can strongly potentiate responses of cortical neurones to NA and 5-HT (Jones & Roberts 1977; 1978). The present experiments confirm that blockade of uptake into presynaptic terminals cannot account for the amine potentiating actions of viloxazine.

Desipramine can potentiate cortical neurone responses to NA and 5-HT (Bradshaw *et al.*, 1974). It can also potentiate responses of caudate neurones to monoamines despite having little effect on uptake mechanisms in this nucleus (Bevan *et al.*, 1975c). Responses of cortical neurones to mescaline are potentiated by desipramine although uptake of mescaline by cortical synaptosomes is not inhibited by the antidepressant (Bevan *et al.*, 1976). The demonstration that desipramine can potentiate neuronal responses to NA in the denervated cortex confirms that potentiation of responses to NA occurs by a mechanism other than blockade of reuptake. Chlorimipramine potentiated both excitatory and depressant responses to 5-HT in the denervated cortex. Again, the potent 5-HT uptake blocking action of this antidepressant (Ross & Renyi, 1969) is unlikely to explain the potentiation of responses.

In several studies the antidepressants caused a reduction of the response size before the potentiation occurred. This effect has been reported previously in normal animals and probably represents postsynaptic receptor blockade by the antidepressants (Bradshaw *et al.*, 1974; Bevan *et al.*, 1975a, b, c; Jones & Roberts, 1978).

The mechanism of the postsynaptic potentiating action is unknown. A theory has been proposed which explains the potentiation in terms of blockade of postsynaptic membrane receptors (Bradshaw *et al.*,

1974). An alternative mechanism may be by intracellular enhancement of transmitter-stimulated cyclic nucleotide formation at the postsynaptic membrane (Jones & Roberts, 1978). *In vitro* studies have shown that NA stimulated formation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in rat cerebral cortex can be enhanced by low concentrations of antidepressants (Jones, 1978). Also, preliminary experiments in this laboratory have shown that desipramine can potentiate responses of cortical neurones to iontophoretically applied cyclic AMP (unpublished observations). Further experiments are being carried out to investigate this possibility.

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