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INTERACTIONS OF (+)-AMPHETAMINE AND CHLORPROMAZINE ON NEURONES IN THE LOWER BRAIN STEM OF THE RAT

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1 The ability of chlorpromazine to antagonize the effects of iontophoretic application of (+)-amphetamine to single neurones in the medulla and lower pons of anaesthetized rats has been studied.

2 Chlorpromazine, administered systemically or iontophoretically, consistently and specifically antagonized the excitatory actions of (+)-amphetamine, but not those of noradrenaline on the same neurone.

3 It is concluded that chlorpromazine reduces the effect of (+)-amphetamine by a presynaptic mechanism.

4 (+)-Amphetamine did not mimic the prolonged inhibitory response of some neurones to noradrenaline but often excited these neurones and chlorpromazine blocked these excitatory responses to (+)-amphetamine.

Introduction

The stimulant effect of amphetamine is generally thought to involve catecholamines in the CNS. Rossum, Schoot & Hurkmans (1962) suggested that amphetamine acts directly on catecholamine receptors and iontophoretic studies in the cerebellar cortex, hippocampus and caudate nucleus have supported this (Hoffer, Siggins & Bloom, 1971; Feltz & de Champlain, 1972; Segal & Bloom, 1974). However, an indirect mechanism of amphetamine action involving release of endogenous catecholamines has also been proposed (Weissman, Koe & Tenen, 1966) and experiments in this laboratory (Boakes, Bradley & Candy, 1972) demonstrated that the effects of iontophoretically applied (+)-amphetamine on neurones in the brain stem of anaesthetized rats were dependent on the presence of endogenous noradrenaline. It has been suggested that amphetamine produces electrocortical desynchrony and behavioural alerting by an action on the reticular formation of the brain stem (Bonvallet, Dell & Hiebel, 1954; Bradley & Elkes, 1957).

The actions of amphetamine on the electrocorticogram and behaviour are antagonized by chlorpromazine (Hiebel, Bonvallet & Dell, 1954; Bradley & Hance, 1957), which also has been found to interact with noradrenaline receptors in the brain stem reticular formation (Bradley, Wolstencroft, Hösli & Avanzino, 1966). Experiments have, therefore, been carried out to determine whether (+)-amphetamine and chlorpromazine interact when applied by iontophoresis to neurones in the brain stem. Noradrenaline, acetylcholine, 5-hydroxytryptamine and glutamate have been used as control agonists to determine the specificity of the effects of chlorpromazine since this drug has a wide range of pharmacological actions. A preliminary account of some of this work has been published (Boakes, Bradley & Candy, 1973).

Methods

Experiments were performed on 28 male albino rats (300 to 400 g) anaesthetized either with halothane (1.0 to 1.5%) or with urethane (1.6 to 1.8 g/kg i.p. after induction with halothane). Body temperature was maintained at 36° to 38°C. After removal of the cerebellum, 5-barrelled micropipettes were inserted into the medulla and lower pons for iontophoretic studies of spontaneously active neurones. The drugs used were: (+)-amphetamine sulphate (K & K Laboratories, 54 mM, pH 5.0 to 6.0); (−)-noradrenaline hydrochloride (Sigma, 243 mM, pH 5.0 to 6.0); serotonin (5-hydroxytryptamine) bimaleinate (Koch-Light, 171 mM, pH 5.0 to 6.0); chlorpromazine hydrochloride (May & Baker, 14 mM, or 56 mM, acidified to pH 4.0 to 5.0 with HCl); sodium glutamate (Koch-Light, 267 mM, pH adjusted to 8.0 to 9.0 with NaOH); acetylcholine hydrochloride (Sigma, 275 mM, pH 5.0 to 6.0); dopamine hydrochloride (Sigma, 263 mM, pH 5.0 to 6.0).

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Systemic injections of chlorpromazine hydrochloride were made either intraperitoneally or intravenously into the saphenous vein. The interactions of (+)-amphetamine and systemically administered chlorpromazine were only examined on one neurone in each animal studied. During iontophoretic ejections of chlorpromazine, care was taken to ensure that the spike height did not decrease, and the ejecting currents were adjusted for each neurone. As far as possible, care was taken to maintain a regular cycle of agonist applications to reduce possible variations in response size (Bradshaw, Szabadi & Roberts, 1973; Candy, Boakes, Key & Worton, 1974). A response was considered to be antagonized if its vertical height was reduced by 50% or more. Current effects were monitored by passing appropriate currents through a barrel of the electrode containing Pontamine Sky Blue (Boakes, Bramwell, Briggs, Candy & Tempesta, 1974). Penetrations into the brain stem were made with reference to the obex, and were directed at the following reticular nuclei: parvocellularis, paramedianus, gigantocellularis and lateralis. The positions of some neurones on which systemic effects of chlorpromazine were studied were marked with Pontamine Sky Blue (Godfraind, 1969; Boakes *et al.*, 1974).

Results

Comparison of the effects of (+)-amphetamine and noradrenaline applied to the same neurones

A total of 76 neurones was studied. The actions of iontophoretically applied (−)-noradrenaline on these neurones were qualitatively similar to those described in previous reports of its effects in the cat and rat (Boakes, Bradley, Brookes, Candy & Wolstencroft, 1971; Boakes *et al.*, 1972): excitatory, inhibitory and mixed effects were observed. (+)-Amphetamine sometimes caused depression of spike height during ejection and neurones showing marked spike depression have not been included. (+)-Amphetamine excited 33 out of 43 neurones excited by noradrenaline (Table 1). The excitatory response to (+)-amphetamine was usually shorter in duration (Figure 1a) or smaller in amplitude than that to noradrenaline, despite the fact that (+)-amphetamine was usually applied for a longer time. The latency of the excitatory response to (+)-amphetamine was usually similar to that for noradrenaline (Figure 1a). On 2 neurones noradrenaline elicited a mixed response: this consisted of an excitatory phase followed by a prolonged inhibitory phase. (+)-Amphetamine mimicked the excitatory but not the inhibitory phase on both neurones. Noradrenaline produced a prolonged inhibitory response on 23 neurones; (+)-amphetamine excited 10 of these neurones (Figure 1b) and had no effect on the remaining 13 neurones (Figure 1c). Only 2 neurones were observed where noradrenaline elicited a short-lasting inhibitory response that was clearly distinguishable from the effects of current; (+)-amphetamine produced a short-lasting inhibitory response on 1 of these neurones. (+)-Amphetamine had no effect on 3 out of 6 neurones unaffected by noradrenaline but excited the remaining 3 neurones.

Interactions of iontophoretically applied chlorpromazine with (+)-amphetamine

(+)-Amphetamine excitations The interactions of iontophoretic chlorpromazine and excitatory responses to (+)-amphetamine were studied on 29 neurones. On some cells, more than one control agonist was used. The results are summarized in Table 2. Chlorpromazine antagonized 26 (+)-amphetamine excitations: expelling currents ranged from 0 to 50 nA and the duration of the ejections were from 50 s to 30 min. None of the effects of the other compounds on these neurones were blocked, viz. acetylcholine excitations 5/5 (Figure 2); glutamate excitations 7/7; 5-hydroxytryptamine excitations 2/2 and 1 inhibition not blocked. On 2 neurones the excitatory response to (+)-amphetamine was blocked only when the response to a control agonist was reduced. Only 1 of the (+)-amphetamine excitations could not be blocked by chlorpromazine. Noradrenaline was used as a control agonist on 22 of the 29 neurones on which (+)-amphetamine excitation was studied. Of those, 16 were excited by noradrenaline, 5 gave a long-lasting inhibitory response

Table 1 Comparison of the effects of (−)-noradrenaline and (+)-amphetamine on brain stem neurones

		(+)-Amphetamine			Total No. of
		Excitation	Inhibition	No effect	neurones
(−)-Noradrenaline	Excitation	33	1	9	43
	Mixed effect	2	0	0	2
	Long inhibition	10	0	13	23
	Short inhibition	0	1	1	2
	No effect	3	0	3	6

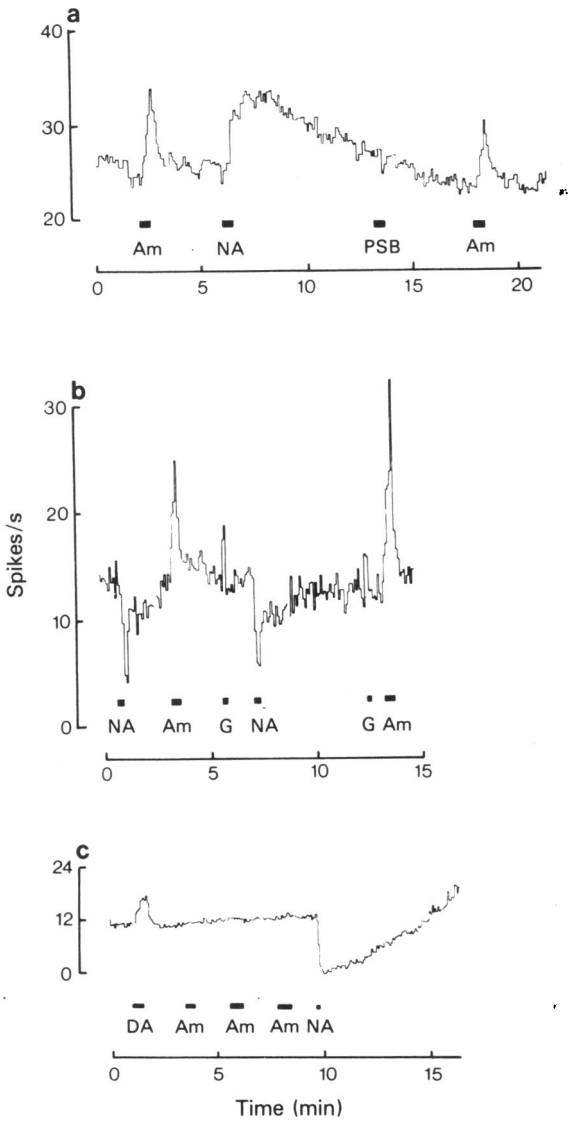

Figure 1 Effects of noradrenaline (NA) and (+)-amphetamine (Am) on the spontaneous firing rates of 3 neurones. The mean firing rate f in spikes/s in successive 5 s epochs are plotted against time in minutes. Iontophoretic applications are shown by the horizontal bars. (a) A neurone excited by NA, 50 nA and Am, 50 nA. The same current passed through Pontamine Sky Blue (PSB) had no effect. (b) A neurone where NA, 50 nA and Am, 50 nA elicited opposite effects; NA produced a long-lasting inhibitory response while Am elicited excitation. Glutamate (G) 20 nA did not have a marked excitatory action on this neurone. (c) Am, 50 nA had no effect on this neurone that gave a long-lasting inhibitory response to NA, 50 nA and was excited by dopamine (DA) 50 nA.

and 1 showed a mixed response (excitation followed by inhibition). Twelve out of the 16 noradrenaline excitations were not reduced by chlorpromazine (Figure 3) while all the noradrenaline inhibitions were blocked.

(+)-Amphetamine inhibitions In only 2 cases could (+)-amphetamine inhibition be clearly distinguished from the effects of current. One of these responses was blocked by iontophoretically applied chlorpromazine (Table 2).

Systemic chlorpromazine and (+)-amphetamine

The interactions between systemically applied chlorpromazine and the excitatory effects of iontophoretically applied (+)-amphetamine were studied on 10 neurones (Table 2), in 7 cases after intraperitoneal injection and in the other 3 cases after intravenous injection. Chlorpromazine selectively antagonized the excitatory action of (+)-amphetamine on 6 out of the 7 neurones (Figure 4) where it was injected intraperitoneally in doses ranging from 0.15 mg/kg to 1.5 mg/kg. In one experiment, even after a dose of 5.5 mg/kg, the effect of the control agonist was unaltered. The positions of 4 neurones, on which actions of intraperitoneal chlorpromazine were determined, were confirmed histologically: 3 were in reticular nuclei: (1 each in r.n. gigantocellularis, parvocellularis, and paramedianus); 1 neurone was on the border between parvocellularis and the median vestibular nucleus. The 3 intravenous injections of chlorpromazine (0.1, 1.0 and 2.0 mg/kg) resulted in a block of the effect of (+)-amphetamine but not of the effect of the control agonist in one experiment, and in two experiments (+)-amphetamine excitation and the effects of control agonists were reduced together. The control agonists used were acetylcholine (Figure 4, 7 neurones) and glutamate (3 neurones). Noradrenaline was also used as a control agonist on 8 neurones, 7 of which were excited and one showed a mixed effect. The excitatory effects of noradrenaline were not reduced by chlorpromazine (Figure 4) while the inhibitory phase of the mixed response was blocked.

(+)-Amphetamine excitation and dopamine

In the present study 23 neurones showed a long-lasting inhibitory response to noradrenaline and 10 of these were excited by (+)-amphetamine. The possibility that these excitatory responses to (+)-amphetamine were mediated by dopamine was investigated by determining the responses to dopamine of nine of these neurones. Dopamine mimicked 5 noradrenaline inhibitions, had no effect on one neurone and excited 3 neurones: none of the 3 neurones excited by dopamine were affected by (+)-amphetamine.

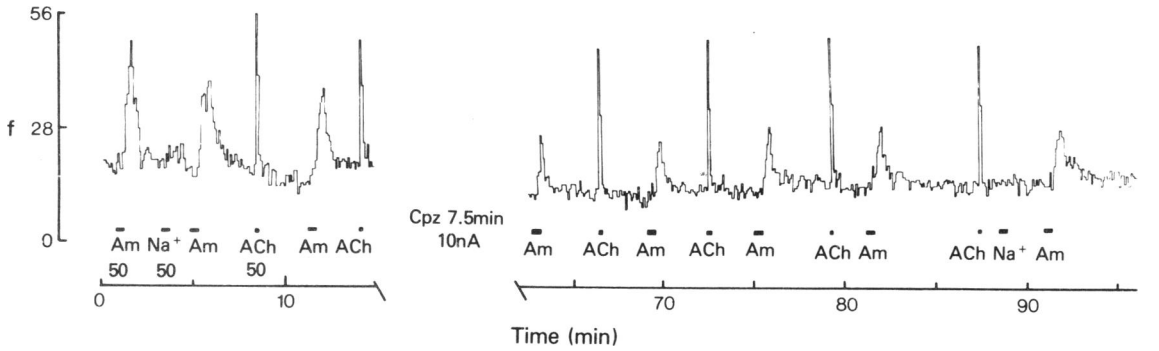

Figure 2 The effect of iontophoretically applied chlorpromazine (Cpz) on excitatory responses to (+)-amphetamine (Am) and acetylcholine (ACh). Cpz, applied for 7.5 min at 10 nA initially blocked both the excitatory responses to Am and to ACh, (not shown), but 45 min after the Cpz application the response to Am was still markedly reduced while the ACh response had recovered. Recovery of the response to Am was not observed with this neurone. Axes as in Figure 1.

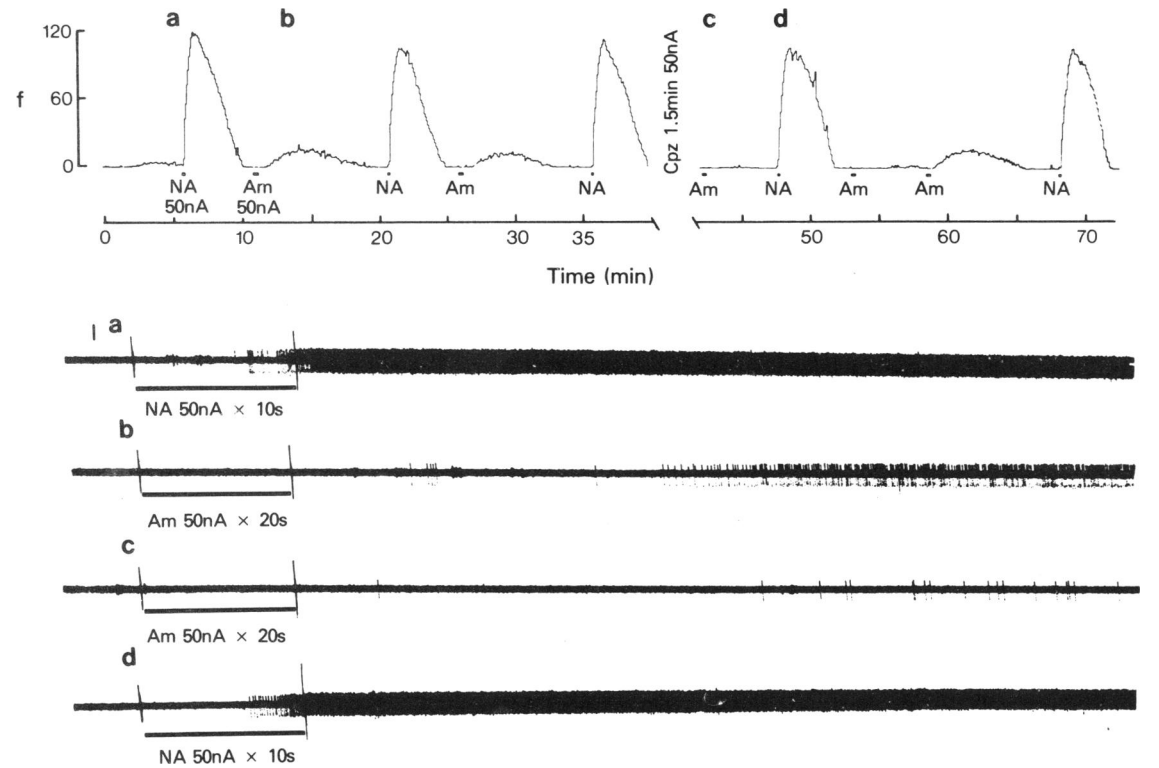

Figure 3 Upper trace: the effect of iontophoretically applied chlorpromazine (Cpz) on the excitatory response to (+)-amphetamine (Am) and (−)-noradrenaline (NA). Am and NA consistently elicited prolonged excitatory responses from this neurone, which was otherwise almost silent. A prior application of NA for 15 s, which is not shown, elicited a response which was greater and longer than those shown, indicating that the responses shown were not supramaximal. Cpz was applied for 1.5 min at 50 nA and completely abolished the response to (+)-amphetamine 45 s after the Cpz application while the response to NA was unaffected. Recovery of the response to (+)-amphetamine was seen 17 min after the Cpz application. Axes as in Figure 1. Lower record: oscillograms of the spike activity of the neurone plotted in the upper trace. Sections (a), (b), (c) and (d) correspond to oscillograms (a), (b), (c) and (d). Note that the film speed was halved in oscillograms (b) and (c) because of the long latency of the (+)-amphetamine response. Calibration bar = 200 μV.

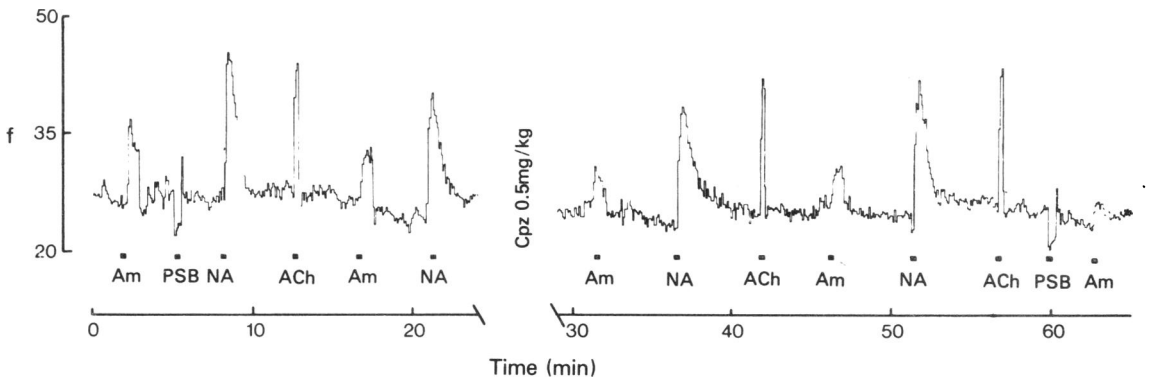

Figure 4 Specific antagonism of the excitatory action of (+)-amphetamine (Am) by systemic chlorpromazine (Cpz). Acetylcholine (ACh) 50 nA, Am 50 nA, and (−)-noradrenaline (NA) 50 nA consistently elicited strong excitatory responses. Cpz 0.5 mg/kg administered intraperitoneally reduced the response to Am 6 min after injection of Cpz and almost completely abolished the response 32 min later without affecting the response to either NA or ACh. Axes as in Figure 1.

Discussion

The observations presented in this paper extend those of Boakes *et al.* (1972) in which a correlation was found between the actions of (−)-noradrenaline and of (+)-amphetamine on brain stem neurones. In the present study, 77% of neurones excited by (−)-noradrenaline were also excited by (+)-amphetamine: three neurones unaffected by (−)-noradrenaline were excited by (+)-amphetamine but this is a small sample. The failure of (+)-amphetamine to mimic the prolonged inhibitory responses to (−)-noradrenaline, reported by Boakes *et al.* (1972), has been confirmed and it has also been found that (+)-amphetamine excites over half the neurones which show long inhibitory responses to (−)-noradrenaline. It appears unlikely that these (+)-amphetamine excitations are related to dopamine as dopamine excited few neurones which were inhibited by noradrenaline and none of those were excited by (+)-amphetamine. A possible explanation is that there are both excitatory and inhibitory receptors for noradrenaline on the same neurone (Boakes *et al.*, 1971) but the involvement of another neurotransmitter, e.g. 5-hydroxytryptamine, in these responses cannot be excluded.

Twenty-six out of twenty-nine (+)-amphetamine excitations studied were blocked or reduced by iontophoretically applied chlorpromazine. This antagonism was selective since the effects of a range of other compounds were not altered by chlorpromazine. Although in one case iontophoretically applied chlorpromazine blocked (+)-amphetamine inhibition it is difficult to draw any conclusions as this inhibition was only rarely distinguishable from the effect of current. The results obtained with systemic administration of chlorpromazine are in good agreement with the results obtained with iontophoretic applications. Intraperitoneal injections of chlorpromazine produced blockade of (+)-amphetamine excitation on all except one neurone, whereas blockade of control agonist was only seen after intravenous injections. In the only other study of (+)-amphetamine/chlorpromazine interactions at the neuronal level, Graham & Aghajanian (1971) found that intravenous injection of (+)-amphetamine inhibited locus coeruleus neurones and that this effect was reversed by intravenous chlor-

Table 2 Comparison of the number of (+)-amphetamine effects blocked by iontophoretic and systemic chlorpromazine (Cpz)

		Number of neurones			
(+)-Amphetamine effect	*Cpz route*	*Blocked*	*Not blocked*	*Non-specific block*	*Total*
Excitation	Iontophoretic	26	1	2	29
	Systemic	7	1	2	10
Inhibition	Iontophoretic	1	1	0	2

promazine. The systemic doses of chlorpromazine used were similar to those described here, but it is not clear whether the antagonism observed by Graham & Aghajanian (1971) was due to direct effects on the locus coeruleus, or whether it was specific.

In view of the fact that (+)-amphetamine excitations were blocked while noradrenaline excitations remained largely unaffected, it seems unlikely that chlorpromazine blocks the effects of (+)-amphetamine by an action at the postsynaptic receptors which mediate the excitatory actions of noradrenaline. Thus it appears that the chlorpromazine/(+)-amphetamine antagonism which was observed may be due to a presynaptic action of chlorpromazine. There is evidence that neuroleptics can affect presynaptic processes in other brain regions. Suppression of spike activity in dopaminergic terminals has been proposed as a mechanism for the action of neuroleptics (Seeman, Staiman & Chau-Wong, 1974) and Iversen, Miller & Rogawski (1975) found that butyrophenones reduced protoveratrine-induced dopamine release from striatal synaptosomes. Thus, it is possible that chlorpromazine blocks (+)-amphetamine by preventing noradrenaline release from noradrenaline-containing terminals. Another possibility is that chlorpromazine blocks the uptake of (+)-amphetamine into presynaptic terminals: chlorpromazine blocks noradrenaline uptake (Iversen, 1967) but it is not known whether (+)-amphetamine has to enter the presynaptic terminals to exert its releasing actions.

The results presented in this paper demonstrate a direct antagonism between (+)-amphetamine and chlorpromazine, probably involving actions of both of these substances on presynaptic noradrenergic sites in the brain stem. The participation of an adrenergic component in the brain stem arousal system has been suggested from a number of studies (Cordeau, Moreau, Beaulnes & Laurin, 1963).

According to Key (1975) the electroencephalographic alerting caused by stimulation of noradrenaline receptors in the brain stem is dependent on the level of ambient sensory stimulation. Antagonism of this noradrenergic 'sensitization to environmental stimuli' (Key, 1975) by chlorpromazine may be significant in the selective depression by chlorpromazine of the effects of sensory stimulation on the electrocorticogram and behaviour.

References

BOAKES, R.J., BRADLEY, P.B., BROOKES, N., CANDY, J.M. & WOLSTENCROFT, J.H. (1971). Actions of noradrenaline, other sympathomimetic amines and antagonists on neurones in the brain stem of the cat. *Br. J. Pharmac.*, **41**, 462–479.

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.

BOAKES, R.J., BRADLEY, P.B. & CANDY, J.M. (1973). Antagonism of the effects of iontophoretically applied (+)-amphetamine by chlorpromazine on single neurones. *Br. J. Pharmac.*, **49**, 175–176P.

BOAKES, R.J., BRAMWELL, G.I., BRIGGS, I., CANDY, J.M. & TEMPESTA, E. (1974). Localization with Pontamine Sky Blue of neurones in the brainstem responding to microiontophoretically applied compounds. *Neuropharmacology*, **13**, 475–479.

BONVALLET, M., DELL, P. & HIEBEL, G. (1954). Tonus sympathique et activite electrique corticale. *Electroenceph. clin. Neurophysiol.*, **6**, 119–144.

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. & HANCE, A.J. (1957). The effect of chlorpromazine and methopromazine on the electrical activity of the brain in the cat. *Electroenceph. clin. Neurophysiol.*, **9**, 191–215.

BRADLEY, P.B., WOLSTENCROFT, J.H., HOSLI, L. & AVANZINO, G.L. (1966). Neuronal basis for the central action of chlorpromazine. *Nature*, **212**, 1425–1427.

BRADSHAW, C.M., SZABADI, E. & ROBERTS, M.H.T. (1973). The reflection of ejecting and retaining currents in the time-course of neuronal responses to microelectrophoretically applied drugs. *J. Pharm. Pharmac.*, **25**, 513–520.

CANDY, J.M., BOAKES, R.J., KEY, B.J. & WORTON, E. (1974). Correlation of the release of amines and antagonists with their effects. *Neuropharmacology*, **13**, 423–430.

CORDEAU, J.P., MOREAU, A., BEAULNES, A. & LAURIN, C. (1963). EEG and behavioural changes following microinjections of acetylcholine and adrenaline in the brain stem of cats. *Archs. ital. Biol.*, **101**, 30–47.

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

GODFRAIND, J.-M. (1969). Localisation de l'extremité de microelectrodes de verre dans le système nerveux central par electrophorese de pontamine. *J. Physiol. (Paris)*, **61**, Suppl. 2, 436–437.

GRAHAM, A.W. & AGHAJANIAN, G.K. (1971). Effects of amphetamine on single cell activity in a catecholamine nucleus, the locus coeruleus. *Nature*, **234**, 100–102.

HIEBEL, G., BONVALLET, M. & DELL, P. (1954). Sympathetic tonus, central electrical activity and waking state. Effects of Chlorpromazine (Largactil) and of a dextrorotatory benzedrine. *Electroenceph. clin. Neurophysiol.*, **6**, 160.

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

IVERSEN, L.L. (1967). *The Uptake and Storage of Nor-*

adrenaline in Sympathetic Nerves. p. 168. Cambridge University Press.

IVERSEN, L.L., MILLER, R.J. & ROGAWSKI, M. (1975). Pre- and postsynaptic actions of neuroleptic drugs. *Br. J. Pharmac.*, **55**, 297–298P.

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

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

SEEMAN, P., STAIMAN, A. & CHAU-WONG, M. (1974). The nerve impulse-blocking action of tranquillizers and the binding of neuroleptics to synaptosome membranes. *J. Pharmac. exp. Ther.*, **190**, 123–130.

SEGAL, M. & BLOOM, F.E. (1974). The action of norepinephrine in the rat hippocampus. 1. Iontophoretic studies. *Brain Res.*, **72**, 99–114.

WEISSMAN, H., KOE, B.K. & TENEN, S.S. (1966). Antiamphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **151**, 339–352.

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