The Actions of Drugs on Single Neurones in the Brain

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The technique of microiontophoresis, which enables pharmacologically active substances to be ejected from micropipettes in the vicinity of single neurones, can be used not only for studying the actions of putative transmitter substances in the CNS but also to examine the effects of centrally acting drugs at the neuronal level, and in particular, to determine whether such drugs mimic or interfere with the actions of synaptic transmitters. Using unanaesthetised decerebrate cats, we have studied the effects of iontophoretically applied acetylcholine (ACh), noradrenaline (NA) and 5-hydroxytryptamine (5-HT), as well as other substances, on the activity of single neurones in the brain stem reticular formation.

Neurones which respond to ACh showed either excitation (35.5%) or inhibition (22%) and the two responses can be differentiated pharmacologically, the excitatory response being both nicotinic and muscarinic, whilst inhibition was entirely muscarinic (Bradley *et al.*, 1966a). Furthermore, the size of both responses was dependent upon the current used to expel the ACh, and hence upon the amount released, since this has been shown to be linearly related to the current (Bradley and Candy, 1970).

The amines NA and 5-HT also caused excitation of some neurones and inhibition of others (Bradley and Wolstencroft, 1965) and many neurones responded to more than one substance, mixed effects often occurring (Bradley and Wolstencroft, 1965; Bradley, 1968). Figure 1A illustrates a neurone which showed excitatory responses to ACh, NA and 5-HT and in Fig. 1B is a neurone showing inhibitory responses to all 3 substances. However, various combinations of excitation and inhibition can also be observed.

The effects of 3 centrally acting drugs and their interactions with these 3 neurotransmitters have been studied. The phenothiazine tranquillizer chlorpromazine had been shown in earlier studies to have an action on the brain stem reticular formation (Bradley and Hance, 1957), and this depressant action appears to be selectively related to the afferent collateral input at the level of the reticular formation (Bradley and Key, 1958). In its peripheral actions, chlorpromazine is an antihistaminic. It is also anti-5-HT, a powerful antagonist to adrenaline and possesses some cholinolytic activity. In view of this wide spectrum of peripheral pharmacological actions it was thought worthwhile to examine the effects of this substance on single neurones in the brainstem and to determine whether there was any antagonism to the actions of ACh, NA or 5-HT. In fact, chlorpromazine was found to act mainly on neurones

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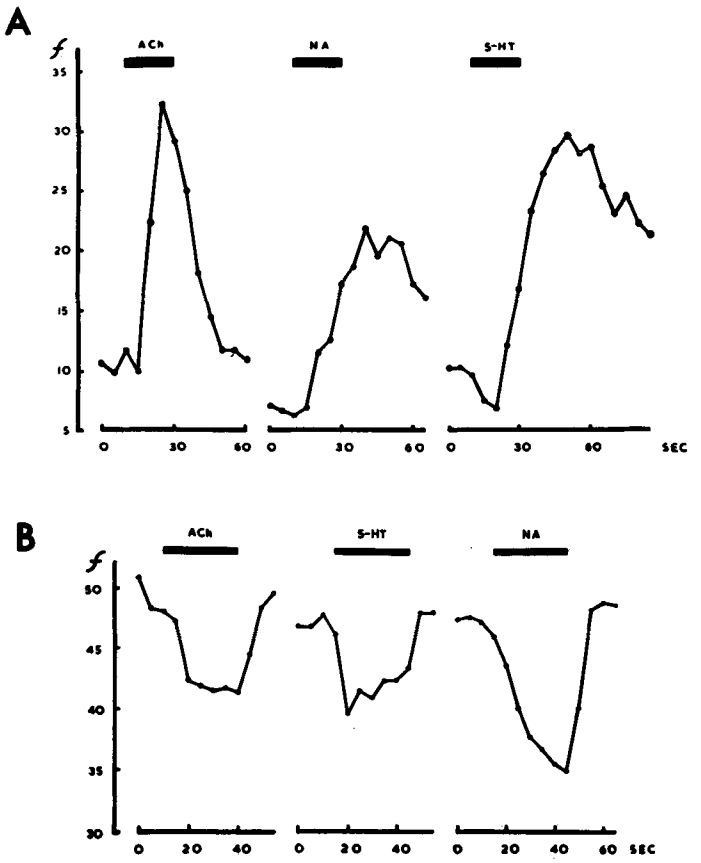

Fig. 1. The effects of acetylcholine (ACh), noradrenaline (NA) and 5-hydroxytryptamine (5-HT), applied iontophoretically to the same neurone in the brain stem of the unanaesthetised decerebrate cat. The frequency of discharge (f) is plotted against time and the black bars indicate the period of iontophoretic application (with currents of 50 nA) in each case.
A, A neurone which was excited by all 3 substances.
B, Another neurone which was inhibited.

which responded to NA, and it depressed their activity. It did not antagonise either the excitatory or inhibitory actions of ACh or 5-HT, nor did it antagonise inhibitory actions of NA but selectively blocked excitatory effects of NA. Thus, it appears that chlorpromazine may be a specific antagonist to NA in the brain stem at those sites at which the latter is excitatory. If NA is confirmed to be a synaptic transmitter in this region of the brain then it may be possible to explain the central effects of chlorpromazine in terms of an antagonism to NA at those synapses where it is excitatory (Bradley *et al.*, 1966b).

The second centrally acting drug to be studied was D-lysergic acid diethylamide (LSD-25). In 1953, Gaddum proposed that, since LSD-25 is a potent antagonist to 5-HT peripherally, a similar action in the central nervous system might explain the psychotomimetic effects of this substance. Against this suggestion was the finding

that Brom-LSD (BOL-148), which is without psychotomimetic actions, is just as potent as a 5-HT antagonist. Numerous investigations into the validity of Gaddum's original hypothesis have failed to produce conclusive results. The effects of iontophoretically applied LSD-25, as well as two related substances, BOL-148 and methysergide (UML), were therefore examined in relation to the actions of ACh, NA and 5-HT on brain stem neurones. Certain excitatory and inhibitory amino acids were also included in this study. LSD-25, applied iontophoretically for periods of up to 10 min, selectively antagonised excitatory actions of 5-HT but not its inhibitory actions; nor did it antagonise either excitation or inhibition produced by ACh and NA. It did not modify actions of any amino acids, with the exception of glutamate which was blocked when excitation by 5-HT was also blocked.

A similar antagonism was shown by UML, although it was less potent than LSD-25, but BOL-148 rarely exhibited antagonism. Thus, it has been concluded that, since there is a good correlation between hallucinogenic activity and potency as an antagonist to excitatory actions of 5-HT, such an action might explain the psychotomimetic effects of LSD-25 (Boakes *et al.*, 1970). The finding that the antagonism was also present with intravenous injections of LSD-25, in doses which produce characteristic changes in behaviour and the electrocorticogram (Bradley and Elkes, 1957; Bradley and Key, 1958; Key and Bradley, 1960), supports this hypothesis.

The third and last centrally acting drug whose effects I wish to describe is D-amphetamine. This substance has been shown to produce its central stimulant effects by an action on the brain stem reticular formation (Bradley and Elkes, 1957; Bradley and Key, 1958) and it has been suggested that it releases NA from presynaptic sites in the central nervous system as it does peripherally (Carlsson *et al.*, 1965). A comparison of the effects of iontophoretically applied NA and D-amphetamine on brain stem neurones showed a close correlation between the effects of these two substances (Bradley, 1968). Thus D-amphetamine closely mimicked the effects of iontophoretically applied NA in unanaesthetised cats and in halothane-anaesthetised rats, but had no effect on neurones unaffected by NA. However, in rats pretreated with reserpine D-amphetamine was without effect although NA was still effective and its excitatory actions were strong and prolonged. Since reserpine produces depletion of brain amines, including NA, the abolition of the actions of D-amphetamine after reserpine pretreatment supports the hypothesis that D-amphetamine acts by releasing endogenous noradrenaline from presynaptic structures (Boakes *et al.*, 1971).

These 3 examples illustrate how the actions of drugs at the neuronal level can be studied in the brain, and the possibility of interaction with synaptic transmitters, either presynaptically or postsynaptically, examined.

REFERENCES

BOAKES, R. J., BRADLEY, P. B., BRIGGS, I. AND DRAY, A. (1970) Antagonism of 5-hydroxytryptamine by LSD 25 in the central nervous system: a possible neuronal basis for the actions of LSD 25. *Brit. J. Pharmacol.*, **40**, 202–218.

BOAKES, R. J., BRADLEY, P. B. AND CANDY, J. M. (1971) Abolition of the response of brain stem neurones to iontophoretically applied D-amphetamine by reserpine. *Nature (Lond.)*, **229**, 496–498.

BRADLEY, P. B. (1968) Synaptic transmission in the central nervous system and its relevance for drug action. *Int. Rev. Neurobiol.*, **11**, 1–56.

BRADLEY, P. B. AND CANDY, J. M. (1970) Iontophoretic release of acetylcholine, noradrenaline, 5-hydroxytryptamine and D-lysergic acid diethylamide from micropipettes. *Brit. J. Pharmacol.*, **40**, 194–201.

BRADLEY, P. B., DHAWAN, B. N. AND WOLSTENCROFT, J. H. (1966a) Pharmacological properties of cholinoceptive neurones in the medulla and pons of the cat. *J. Physiol. (Lond.)*, **183**, 658–674.

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

BRADLEY, P. B. AND 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. AND 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.

BRADLEY, P. B. AND WOLSTENCROFT, J. H. (1965) Actions of drugs on single neurones in the brain-stem. *Brit. med. Bull.*, **21**, 15–18.

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

CARLSSON, A., LINDQVIST, M., DAHLSTRÖM, A., FUXE, K. AND MASUOKA, D. (1965) Effects of the amphetamine group on intraneuronal brain amines *in vivo* and *in vitro*. *J. Pharm. Pharmacol.*, **17**, 521.

GADDUM, J. H. (1953) Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. *J. Physiol. (Lond.)*, **121**, 15*P*.

KEY, B. J. AND BRADLEY, P. B. (1960) The effects of drugs on conditioning and habituation to arousal stimuli in animals. *Psychopharmacologia (Berl.)*, **1**, 450–462.

DISCUSSION

COHEN: When you apply a transmitter substance iontophoretically, is the type of reaction of the neurone (excitatory or inhibitory) independent of the dose or concentration applied?

BRADLEY: There is a dose-dependency with ACh but I think you are asking whether the effect is always in the same direction. The answer is that we have never seen a reversal of effect with dose or size of microelectrode tip or position of the micropipette. With regard to the concentration, in some of our earlier experiments we were probably using too high a concentration of the substance in the micropipette. Thus, with amphetamine we saw only inhibitory effects, but now that we are using more dilute solutions we are getting the results I presented here.

WOODRUFF: You have shown that the inhibitory actions of NA are not stereospecific. This suggests that the alcoholic hydroxyl group is not necessary for binding at the receptors and that the receptors might be classified as dopamine receptors. Have you tested the potency of dopamine on these neurones?

BRADLEY: Yes, we have tried dopamine and a variety of sympathomimetic amines. Dopamine only acted on neurones which responded to NA but there was not a close correlation between the effects, and on the whole dopamine was less effective than NA. In any case, I do not think there are supposed to be many dopaminergic neurones in the brain stem, so one would not expect dopamine to have independent effects.

WALKER: Reserpine pretreatment potentiated the NA response in the rat; did you observe similar potentiation in the ACh and 5-HT response after reserpine?

BRADLEY: I cannot answer that. We have only looked at actions of amphetamine and NA so far.

HOLMES: I noticed that all the neurones you studied had a resting frequency of firing. I wondered whether you had ever tried to test the response of a quiescent neurone. The neurone could be located perhaps by passing down some sodium chloride or by electrical stimulation. It would be interesting to see whether drugs produced different responses on these quiescent neurones. It worries me a bit that all the neurones you did your tests on were normally active.

BRADLEY: I'm sorry but it does not worry me. I am very happy that they are spontaneously active because I think that this is what the neurones would normally be doing in the brain, whereas in the work which has been done on anaesthetized animals the neurones have had to be activated by application of an excitant amino acid first. I am more worried about those responses myself.

HOLMES: I think the problem is that if the neurones are firing all the time it is not known which transmitter is making them fire and there may be a variety of neurotransmitters stimulating the neurones on which you are testing your drugs later on, *i.e.*, you do not exactly know where you are to begin with.

BRADLEY: I take your point. One does occasionally find a cell which is not spontaneously active but which appears, as it were, when ACh is injected. It is very difficult to study cells of this kind because you do not know whether it is still there when the iontophoresis is stopped. We can say, however, that the initial firing rate does not determine the type of response which is seen.