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THE EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON CHOLINERGIC RESPONSES OF SINGLE CORTICAL NEURONES

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1 The technique of microelectrophoresis was used in order to study the effects of tricyclic antidepressants on responses of single cortical neurones to acetylcholine.

2 Both potentiation and antagonism of excitatory responses to acetylcholine could be observed after a brief application of imipramine or desipramine. A higher dose of the antidepressant was required to evoke antagonism than to evoke potentiation.

3 Responses to carbachol were affected by desipramine similarly, suggesting that inhibition of cholinesterase is not responsible for the potentiation of cholinergic responses.

4 A brief application of atropine also had a dual effect on responses to acetylcholine.

5 It is suggested that the potentiation of excitatory cholinergic responses by atropine and the antidepressants may be due to the blockade of masked inhibitory receptors.

Introduction

The anticholinergic effects of tricyclic antidepressant drugs in the periphery are well documented (Domenjoz & Theobald, 1959; Atkinson & Ladinsky, 1972), but there is only indirect information concerning a similar action in the brain (Gyermek, 1966). Although an effect on cholinergic systems has generally been regarded as irrelevant so far as the mood elevating effect of these drugs is concerned (Gyermek, 1966), it has been suggested recently that a central anticholinergic effect may also contribute to the therapeutic action of the antidepressants (Janowsky, Davis, El-Yousef & Sekerke, 1972).

We used the technique of microelectrophoresis in order to investigate how responses of single cortical neurones to acetylcholine and to carbachol can be modified by imipramine and desipramine. We also compared the effects of the antidepressants with the effects of atropine. Some of the results presented here have been communicated to the British Pharmacological Society (Bevan, Bradshaw, Roberts & Szabadi, 1973b).

This paper follows another report describing the effects of tricyclic antidepressants on neuronal responses to noradrenaline and 5-hydroxytryptamine (Bradshaw, Roberts & Szabadi, 1974).

Methods

Cats of either sex, weighing 2.0 to 3.5 kg were used. Our methods for the anaesthesia and preparation of animals are described elsewhere (Bradshaw *et al.*, 1974). An area of the anterior or posterior sigmoid gyrus was prepared for recording according to the method of Bradshaw & Szabadi (1972).

Five- or six-barrelled glass micropipettes were constructed and filled as described by Bradshaw, Roberts & Szabadi (1973). Two barrels of each micropipette contained 4 M NaCl, one barrel for recording action potentials, the other for use in current balancing. The remaining barrels contained drug solutions. The drug solutions used in these experiments were acetylcholine chloride (0.2 M, pH 3.6), carbamylcholine (carbachol) chloride (0.2 M, pH 6.9), imipramine hydrochloride (0.2 M, pH 7.4), desmethylimipramine hydrochloride (desipramine) (0.15 M, pH 7.5), and atropine sulphate (0.1 M, pH 5.9).

The techniques used for recording action potentials, and for the electrophoretic application of drugs, were as described by Roberts & Straughan (1967). A cumulative record of the total number of action potentials was obtained via a Grass UI-1 unit integrator.

All the neurones studied were spontaneously active. All the drugs were applied by microelectrophoresis. Repeated responses to acetylcholine (ACh) or carbamylcholine (carbachol) were obtained before and after a brief application of imipramine, desipramine or atropine. Our measure of the dose of the antidepressant was the electrophoretic charge passed (intensity of electro-

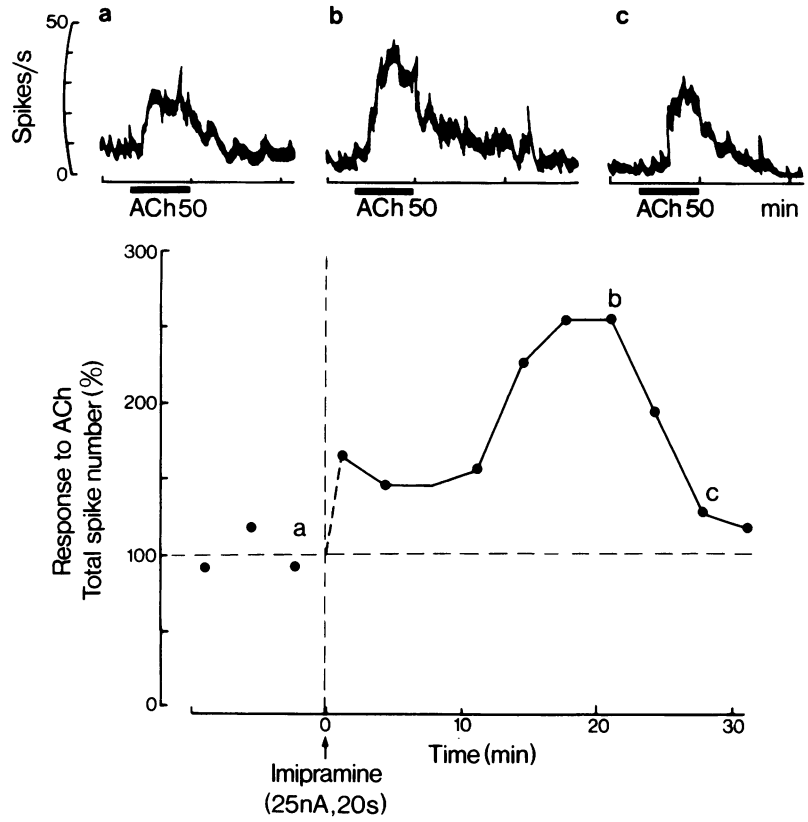

Figure 1 Potentiation of excitatory responses of a single cortical neurone to acetylcholine (ACh) by imipramine. Upper part of figure shows excerpts of the ratemeter recording of the firing rate of the neurone. Horizontal bars indicate applications of ACh; numbers refer to the intensity of the ejecting current (nA). (a) Control response to ACh. (b) Potentiated response to ACh 21 min after a brief application of imipramine (25 nA; 20 seconds). (c) Recovery of control response 28 min after the application of imipramine. Lower graph shows the time-course of the entire study. The sizes of the responses to ACh are expressed as a percentage of the mean of the control responses. Each point represents a single response. Letters above the graph indicate responses illustrated in the ratemeter tracings above.

phoretic current x time of passage of current). The sizes of excitatory responses to the agonists were expressed as the total number of spikes generated in response to each application of an agonist (total spike number) (Bevan, Bradshaw, Roberts & Szabadi, 1973a). The intervals between drug applications were kept constant. During these intervals a retaining current of −25 nA was passed.

Results

Effect of antidepressants on responses to acetylcholine

Imipramine. Drug-interaction studies were successfully completed on 28 neurones responding with a clear increase in firing rate to ACh. Both potentiation and antagonism of the response to ACh could be observed after a brief application (25-100 nA for 20-60 s) of imipramine.

Potentiation of the response was seen in 23 cells. (A response was regarded as potentiated if there was more than a 20% increase over the size of the average control response.) The potentiated response had a characteristic time-course compared to the control response: the peak of the response was higher, and the recovery time longer. The latency of the potentiated response could be either shorter or longer than that of the control response. In a few cells only one response showed potentiation; however, in the majority of cells several responses were potentiated, and the control

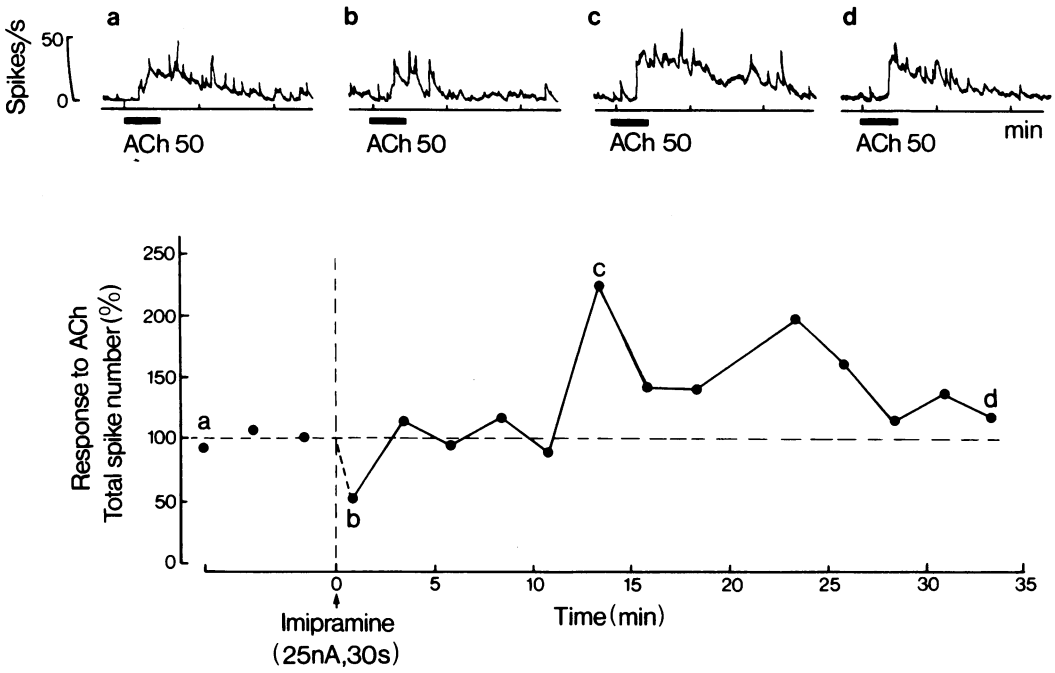

Figure 2 Antagonism and potentiation of excitatory responses of a single cortical neurone to acetylcholine (ACh) by imipramine. Upper part of figure shows excerpts of the ratemeter recording of the firing rate of the neurone (as in Figure 1). (a) Control response to ACh. (b) Antagonized response, 1 min after a brief application of imipramine (25 nA; 30 seconds). (c) Potentiated response, 13 min after the application of imipramine. (d) Recovery of control response, 33 min after the application of imipramine. Lower graph shows the time-course of the entire study (as in Figure 1).

response recovered only after a longer time (up to 90 minutes).

Antagonism of the response was seen in 12 cells. Antagonism appeared as a reduction in the total spike number compared to the control. This reduction in size varied between 20-100%.

The occurrence of potentiation and antagonism followed a well-defined time-course. The following patterns could be observed: (1) 'Early' potentiation. In this case the first response after imipramine showed the greatest degree of potentiation. (The first application of ACh after imipramine usually followed less than 1.5 min after the application of imipramine.) Subsequent responses became gradually smaller, until recovery of the control response could be seen. Recovery usually occurred 10-20 min after the antidepressant had been applied. Early potentiation was seen in 10 neurones. (2) 'Late' potentiation. In this case, the potentiation developed gradually, achieving a maximum 10-30 min after the application of imipramine. Recovery occurred 30-60 min after imipramine had been applied. Late potentiation was seen in seven cells. An example of late potentiation is shown in Figure 1. (3) Antagonism followed by potentiation. In this case, the first response after imipramine was reduced in size. This initial antagonism was later followed by potentiation, and finally by gradual recovery of the control response. This pattern of drug interaction was observed in six cells. An example is shown in Figure 2. (4) Antagonism only was seen in six cells. In this case, an initial antagonism of the response was followed by recovery. In some cells a number of studies were conducted using more than one dose of imipramine, and more than one pattern of drug interaction could be observed.

When both antagonism and potentiation were observed (pattern 3), antagonism invariably preceded potentiation (e.g. Figure 2), and the reverse was never seen. This suggested that antagonism appeared when the concentration of imipramine at the cell was likely to have been at its highest, that is immediately following the electrophoretic ejection of the antidepressant. In order to test this hypothesis, we investigated the correlation between the dose of imipramine applied, and the occurrence of antagonism or potentiation. The

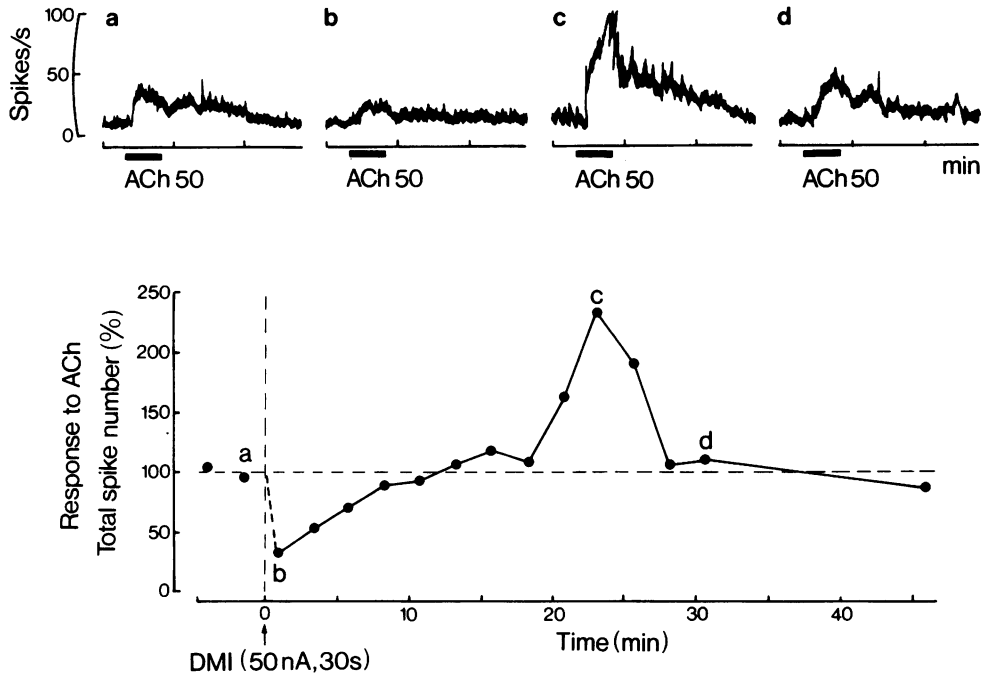

Figure 3 Antagonism and potentiation of excitatory responses of a single cortical neurone to acetylcholine (ACh) by desipramine (DMI). Upper part of figure shows excerpts of the ratemeter recording of the firing rate of the neurone (as in previous figures). (a) Control response to ACh. (b) Antagonized response, 1 min after a brief application of desipramine (50 nA; 30 seconds). (c) Potentiated response, 23 min after the application of desipramine. (d) Recovery of control response, 30 min after the application of desipramine. Lower graph shows the time-course of the entire study (as in previous figures).

results are summarized in Table 1. It is apparent that a lower dose of imipramine (0.4-2.0 μC) was more likely to cause potentiation only, whereas a higher dose ($\geqslant$2.0 μC) was more likely to result in antagonism as well (χ^2 test: $P < 0.01$).

Desipramine. The effect of desipramine was studied in 21 cells. Both potentiation and antagonism of the responses could be observed after a brief application of desipramine. The same patterns of drug-interaction were seen as with imipramine: (1) 'early' potentiation (four cells); (2) 'late' potentiation (four cells); (3) antagonism followed by potentiation (10 cells); (4) antagonism only (seven cells). An example of antagonism followed by potentiation is shown in Figure 3.

Effect of desipramine on responses to carbachol

In an attempt to test the hypothesis that potentia-

Table 1 Relationship between dose of imipramine applied and effect on responses to acetylcholine

	Number of cells	
Dose (charge)	*Potentiation only*	*Antagonism**
0.4-2.0 μC	17	4
$\geqslant$2.0 μC	2	5
$\chi^2 = 9.2034; P < 0.01$		

* Includes all cells in which antagonism was seen. In some of these cells the antagonism was later followed by potentiation.

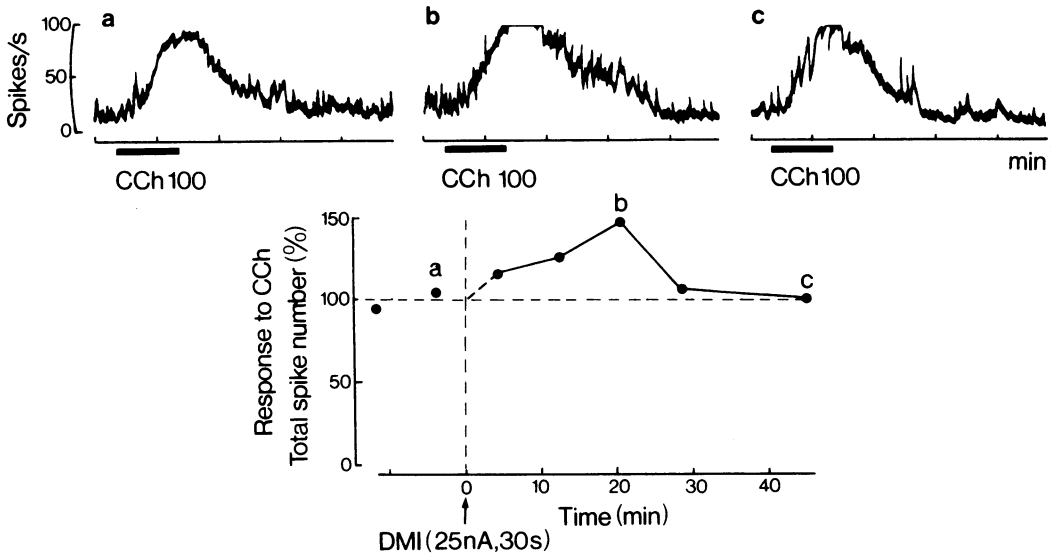

Figure 4 Potentiation of excitatory responses of a single cortical neurone to carbachol (CCh) by desipramine (DMI). Upper part of figure shows excerpts of the ratemeter recording of the firing rate of the neurone (as in previous figures). (a) Control response to carbachol. (b) Potentiated response 20 min after a brief application of desipramine (25 nA; 30 seconds). (c) Recovery of control response, 44 min after the application of desipramine. Lower graph shows the time-course of the entire study (as in previous figures).

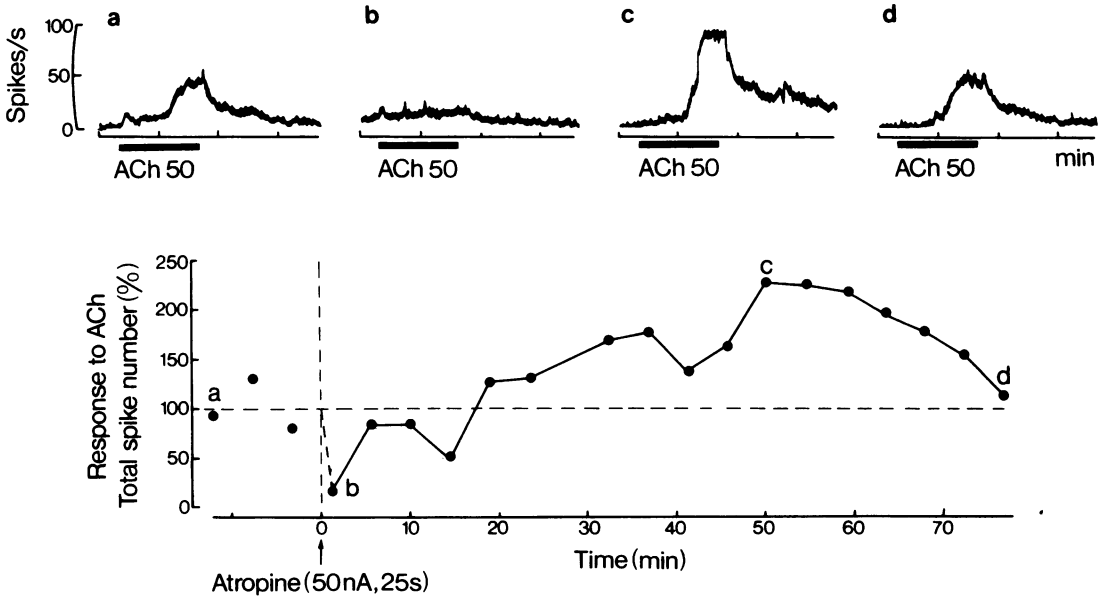

Figure 5 Antagonism and potentiation of excitatory responses of a single cortical neurone to acetylcholine (ACh) by atropine. Upper part of figure shows excerpts of the ratemeter recording of the firing rate of the neurone (as in previous figures). (a) Control response to ACh. (b) Antagonized response, 1 min after a brief application of atropine (50 nA; 25 seconds). (c) Potentiated response, 50 min after the application of atropine. (d) Recovery of control response, 76 min after the application of atropine. Lower graph shows the time-course of the entire study (as in previous figures).

tion of responses to ACh is due to the inhibition of the activity of cholinesterase (Osborne & Sigg, 1960), we have examined how responses to carbachol are influenced by desipramine. It is known that carbachol is not hydrolysed by cholinesterase (Goodman & Gilman, 1970).

The effect of desipramine on excitatory responses to carbachol was studied in 10 neurones. In six cells responses to carbachol were potentiated by desipramine; in three of these cells the potentiation was preceded by antagonism. In the remaining four cells, antagonism alone was seen. An example of potentiation is shown in Figure 4.

Effect of atropine on responses to acetylcholine

As the results with imipramine and desipramine suggested that a smaller dose of the antidepressant was needed to potentiate than to antagonize the response to ACh, we have examined the effect of a similar small dose of atropine on the responses to ACh in 14 cells. Potentiation was observed in 11 cells: in one cell potentiation alone was seen, whereas in 10 cells the potentiation was preceded by antagonism. In three cells antagonism only could be observed. An example of the dual effect of atropine is shown in Figure 5.

Discussion

The results show that imipramine and desipramine have a dual effect on responses of single cortical neurones to acetylcholine: both antagonism and potentiation can be observed. Moreover, this effect is dose-dependent: smaller doses potentiate and bigger doses antagonize the responses. This dual effect may reflect the operation of two independent mechanisms: a more sensitive 'potentiating' mechanism, and a less sensitive 'antagonizing' mechanism. According to this model, a lower concentration of the antidepressant at the cellular receptor sites would affect the more sensitive potentiating mechanism only, whereas a higher concentration would activate the antagonizing mechanism as well. The size of any particular response to ACh would be determined by the interaction between the two mechanisms, whereas the time-course of the drug-interaction patterns (e.g. antagonism followed by potentiation) would reflect a gradual decline in the concentration of the antidepressant at the neurone after the application of the antidepressant had been terminated (Bradshaw *et al.*, 1974).

The most plausible explanation for the antagonism is the blockade of muscarinic receptors by the antidepressant. There is a considerable amount of experimental information suggesting that the excitatory responses of cortical neurones to ACh may reflect the activation of muscarinic receptors (see Curtis & Crawford, 1969). Furthermore, it is well documented that tricyclic antidepressants can block muscarinic receptors in the periphery (Domenjoz & Theobald, 1959; Atkinson & Ladinsky, 1972).

It is more difficult to interpret the potentiation of responses to acetylcholine. Apart from one early report (Osborne & Sigg, 1960), this effect has not been studied in the periphery. Osborne & Sigg (1960) reported a dual effect: a smaller dose of imipramine potentiated, whereas a bigger dose antagonized some peripheral effects of acetylcholine. These authors suggested that the potentiating effect was due to the blockade of cholinesterase, since they could demonstrate an inhibitory effect of imipramine on serum cholinesterase. However, this is not a likely explanation for the potentiation seen in our experiments, since the activity of brain acetylcholinesterase is virtually unaffected by tricyclic antidepressants (Perkinson, Ruckart & DaVanzo, 1969). Moreover, our observation that responses to carbachol are also potentiated by desipramine confirms that potentiation is not due to the inhibition of cholinesterase activity, since cholinesterase does not play a role in the elimination of carbachol (Goodman & Gilman, 1970).

As cholinesterase blockade cannot explain potentiation in our system, we would like to propose an alternative explanation. It has been shown that both excitatory and inhibitory receptors to ACh can co-exist on neurones in molluscs (Kehoe, 1972). Furthermore, it has been suggested that a similar situation may occur on some mammalian neurones (Barker, Crayton & Nicoll, 1971). The occurrence of both excitatory and depressant responses of cortical neurones to ACh (see Curtis & Crawford, 1969) would suggest that both excitatory and inhibitory receptors to ACh may occur on these cells. Since in our experiments cortical neurones were invariably excited by ACh, the inhibitory receptors would seem to have been masked, their activation causing only a reduction in the size of the excitatory response. We propose that potentiation may be due to the selective blockade of masked inhibitory receptors by a smaller concentration of the antidepressant. A higher concentration of the antidepressant would block the dominant excitatory receptors as well, thus causing a reduction in the size of the observed response. Our observation of the dual action of atropine would be consistent with this hypothesis: a smaller concentration of atropine would block the masked inhibitory receptors selectively, thus causing potentiation of the response. This potentiating effect of a low

Table 2 Effect of antidepressants and antagonists on responses of single cortical neurones to potential neurotransmitters

	Noradrenaline	*5-Hydroxytryptamine*	*Acetylcholine*	*Glutamate*
Imipramine	P, A (1)	P, A (1)	P, A	O (1)
Desimpramine	P, A (1)	P, A (1)	P, A	O (1)
Sotalol	P, A (2)	P, A (2)	O (2)	O (4)
Methysergide	P, A (2)	P, A (2)	O (2)	O (3)
Atropine	O (4)		P, A	O (5)

P = potentiation, A = antagonism, O = not affected.
(1) Bradshaw, Roberts & Szabadi (1974). (2) Bevan, Bradshaw & Szabadi (1974). (3) Roberts & Straughan (1967). (4) Johnson, Roberts, Sobieszek & Straughan (1969). (5) Stone (1972).

dose of atropine has not been described before in single cell pharmacology in the central nervous system. It has been described in peripheral preparations, however, where a low concentration of atropine may cause 'paradoxical' potentiation of responses to ACh (Arunlakshana & Schild, 1959).

An alternative explanation for the potentiating effect of atropine on single neurones could be that it acts by blocking the uptake of ACh into pre-synaptic terminals. It has been reported that ACh is taken up actively into brain slices in the presence of irreversible cholinesterase inhibitors, and that this uptake is inhibited by atropine (Liang & Quastel, 1969). There is no evidence, however, that ACh uptake occurs *in vivo* when cholinesterase has not been inhibited (Katz & Chase, 1971).

A similar dual action of the tricyclic antidepressants has been described on neuronal responses to noradrenaline and 5-hydroxytryptamine (Bradshaw *et al.*, 1974). Moreover, it has been reported that some peripheral antagonists of noradrenaline and 5-hydroxytryptamine also have a dual action on responses to monoamines (Bevan, Bradshaw & Szabadi, 1974). The hypothesis of the two opposite receptors has been used to account for these observations (Szabadi & Bradshaw, 1974). The effects of antagonists and antidepressants on cortical neuronal responses to potential neurotransmitters are summarized in Table 2. It is apparent that while atropine seems to be a specific blocker of ACh receptors, and sotalol and methysergide are specific in blocking monoamine receptors, the tricyclic antidepressant can act at both acetylcholine and monoamine receptors.

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