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Modification of cortical neuron responses to acetylcholine by viloxazine

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Viloxazine, an antidepressant with no peripheral antiacetylcholine activity, was capable of reducing responses of single cortical neurons to acetylcholine. Acetylcholine responses could also be potentiated by viloxazine. Both potentiation and reduction by viloxazine were often seen in the same study, reduction of responses invariably preceding potentiation. These results suggest that viloxazine may have selective effects on central cholinergic receptors. Responses of cortical neurons to monoamines could also be potentiated by viloxazine although it has little effect on monoamine uptake. These results are compatible with the idea that potentiation of monoamine responses may occur by a postsynaptic mechanism.

Tricyclic antidepressants inhibit the active uptake of monoamines into monoamine nerve terminals (Ross & Renyi 1967, 1969) and this has been widely accepted as the basis of their clinical effectiveness (Schildkraut 1965; Davis 1970). There is a possibility however that central cholinergic and aminergic mechanisms are imbalanced in clinical depression (Janowsky et al 1972). The potent antiacetylcholine activity of tricyclics (Domenjoz & Theobald 1959; Atkinson & Ladinsky 1972) may be implicated, therefore, in their clinical action.

Iontophoresis studies have shown that low concentrations of tricyclics can potentiate not only the effects of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) on single cells in the c.n.s. (Bradshaw et al 1974) but also the effects of acetylcholine (ACh) (Bevan et al 1975a). Reduction of monoamine and ACh responses occurred with high concentrations of tricyclics. A similar concentration-dependent effect on ACh responses of single cortical cells was seen following the iontophoretic application of atropine (Bevan et al 1974; 1975a). This led to the suggestion that potentiation of cortical neuron responses to ACh may be due to blockade of postsynaptic 'masked' inhibitory receptors.

Viloxazine hydrochloride is a clinically active antidepressant (Peet 1973; Pichot 1975) structurally dissimilar from the tricyclics. It is devoid of peripheral antiacetylcholine activity, having no effect on lacrimation and salivation induced by oxotremorine in mice (Lippmann & Pugsley 1976). It does not inhibit ACh responses of the guinea-pig ileum (Greenwood 1975). Either viloxazine has different effects on central and peripheral ACh receptors or an antiacetylcholine action is not essential for antidepressant activity. We have investigated the actions of viloxazine on cholinergic responses of single cortical neurons in the rat.

Bradshaw et al (1974) have suggested potentiation of NA and 5-HT as well as ACh responses of cortical neurons may be due to blockade of 'masked' receptors. Iprindole, a tricyclic with little effect on NA uptake (Rosloff & Davis 1974) can potentiate NA responses of cortical neurons (Bevan et al 1975b) and postsynaptic antagonists of NA and 5-HT can potentiate cortical cell responses to these amines (Bevan et al 1974).

Viloxazine, like iprindole, has little effect on monoamine uptake by brain tissue (Blackburn et al 1978) so we have also studied its effects on cortical cell responses to NA and 5-HT.

MATERIALS AND METHODS

Male albino wistar rats (250–400 g) were used. Anaesthesia was induced with halothane (4% in O_2) and maintained with halothane (0·5–0·8%). Animals respired spontaneously through a tracheal cannula. Systemic blood pressure, e.c.g. and respiration were continuously monitored. Rectal temperature was maintained at 37·5 ± 0·5 °C.

5-barrelled microelectrodes were introduced into the cortex through a small burr hole in the skull over the somatosensory cortex. Glass microelectrodes were constructed and filled as described by Bradshaw et al (1973). Two barrels of each electrode contained 3 M NaCl, one for recording action potentials and the other for current balancing purposes. The remaining barrels were used for the iontophoretic application of drugs from the following solutions: noradrenaline bitartrate (0·2 M, pH 4·0), 5-HT bimaleinate (0·2 M, pH 4·0), acetyl-

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choline HCl (0·2 M, pH 5·0), L-glutamic acid sodium salt (0·2 M, pH 7·0), viloxazine HCl (0·2 M, pH 6·0).

Standard techniques for recording action potentials and applying drugs were used. All neurons studied were spontaneously active. Action potentials were counted by means of a variable voltage gate and a ratemeter, the output of which was displayed on a pen recorder.

On finding a spontaneously firing neuron, the baseline firing rate was recorded for 5–10 min. Standard pulses of an agonist drug (25–100 nA, 15–60 s) were then applied at regular intervals (usually every 3 or 4 min) by means of a sequential timing device. When constant repeatable responses of the cell were obtained a single brief application of viloxazine was made (20–100 nA, 20–50 s) and the regular applications of agonist continued. The time course of the effects of the antidepressant application on the agonist responses was thus followed. A retaining current of 20 nA was passed through the drug barrels between ejecting pulses.

The magnitude of agonist drug responses was measured by calculating the difference between the number of spikes generated during the response and the number generated during an equivalent period of baseline firing. This is called the total spike number (Bradshaw et al 1974).

RESULTS

The iontophoretic application of ACh to cortical neurons invariably caused an increase in cell firing rate. L-glutamate, occasionally used as an agonist, was also only excitatory. Both excitatory and inhibitory responses to NA and 5-HT were recorded. Most cells tested (69%) were not directly affected by the application of viloxazine but occasionally a short lasting increase (13%) or decrease (18%) in firing rate was observed, not lasting more than 60 s.

Effects of viloxazine on ACh responses

The interaction of viloxazine with ACh was studied on 17 cortical cells. The variance of responses to ACh before viloxazine administration was compared with the variance after viloxazine. The post-viloxazine variance was significantly different from control ($f = 30·6$, d.f. 56, 190, $P = 0·01$). The qualitative effects of viloxazine on ACh responses were very similar to those reported for tricyclics (Bevan et al 1975 a,b). The responses of 12 cells were reduced following the application of viloxazine. In 3 of these studies a reduction of the response size was the only effect seen. More commonly (9 cells), the ACh responses were first reduced in size and then potentiated before recovery of the baseline response. An example of a study in which reduction alone was seen is shown in Fig. 1. The response of this cell was reduced to 44% of the mean control value, 14·5 min after the viloxazine application. 29 min after the viloxazine the response had returned to mean control level.

Another study is shown in Fig. 2. On this occasion the response was reduced to 61% of mean control and then potentiated to 180% of mean control after 26 min, with recovery after 35 min.

In 5 studies, viloxazine potentiated the responses to ACh with no prior reduction of the responses.

It was possible in two studies to apply viloxazine twice to the same cell, once with a low ejecting current and following recovery of baseline responses, again with a higher ejecting current. On both occasions the lower ejecting current resulted in potentiation of ACh responses and the higher current resulted in reduction followed by potentiation.

Three cells excited by ACh showed no change in response following viloxazine. Viloxazine also had no detectable effect on responses of 4 cells excited by L-glutamate.

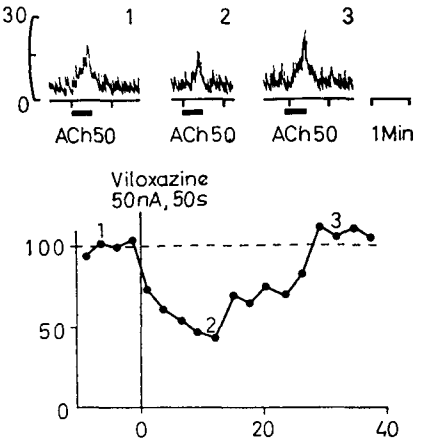

FIG. 1. Reduction of excitatory responses of a cortical neuron to ACh by viloxazine. Excerpts from the ratemeter write-out of the pen recorder are shown (upper figure; ordinate: spikes s^{-1}). Bars indicate the applications of ACh. Numbers refer to the intensity (nA) of the ejecting current. The graph shows the time course of the study. Each point represents a single drug response. Responses are measured as total spike numbers (see text) and all responses are expressed as % of the mean control response (ordinate). Numbers on the curve show the position in the time course from which the illustrated responses have been taken. Abscissa: min.

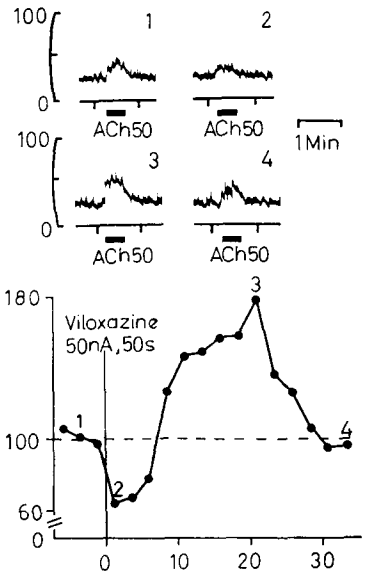

FIG. 2. Reduction and potentiation of excitatory responses of a cortical neuron to ACh by viloxazine. Excerpts from the ratemeter write-out and time course of the study are as in Fig. 1. Ordinate: response to ACh expressed as % of the mean control response.

Effect on monoamine responses

Responses of neurons to NA and 5-HT before and after viloxazine have been compared by a variance ratio test. The variance of post-viloxazine responses was significantly greater than control responses ($P = 0{\cdot}01$). Viloxazine showed a similar pattern of effect on 5-HT and NA responses to that described for ACh. Thus three effects were discernible. Reduction of response size, potentiation of responses alone and reduction followed by potentiation. In one study of 5-HT depressant responses and one study of NA depressant responses two applications of viloxazine were made to the same cell. Again the lower ejecting current caused potentiation of responses and the higher current, reduction followed by potentiation.

The effects of viloxazine on 5-HT, NA and ACh responses are summarized in Fig. 3. It is obvious from the mean peak potentiation and reduction percentages that the effects of viloxazine on responses to the three agonists are quantitatively similar.

DISCUSSION

Tricyclic antidepressants have been shown to have a concentration-dependent effect on 5-HT, NA and ACh responses of cortical neurons (Bradshaw et al 1974; Bevan et al 1975 a,b). Low iontophoretic ejecting currents of tricyclics caused potentiation of responses whilst high currents resulted in reduction of responses. It is likely that the effects of viloxazine on ACh, 5-HT and NA responses seen in these experiments are similarly dependent on concentration. This is supported by four studies (2 ACh, 1 NA, 1 5-HT) in which an initial application of viloxazine potentiated responses and a second, larger application caused reduction followed by potentiation. Also, in studies where both potentiation and reduction of responses occurred the reduction always preceded the potentiation. The concentration of viloxazine in the vicinity of the cell is probably at a peak immediately following its application, when reduction occurs. It is likely that as the concentration of viloxazine declines with time then potentiation occurs.

It is possible that reduction of agonist responses by viloxazine is due to a non-selective action resulting from high concentrations of the drug. A similar interpretation could be applied to the reduction of agonist responses by tricyclic antidepressants. The transport number of imipramine in microelectrodes has been determined as being 0·046 (Zieglgänsberger et al 1974) suggesting that its concentrations at electrode tips would not be excessively high. However, it is not possible to calculate finite concentrations of drug at the receptor

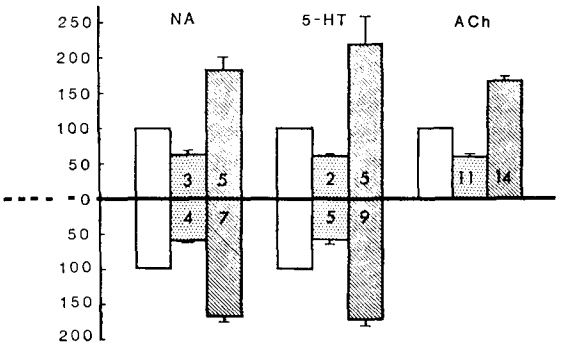

FIG. 3. Summary of the effects of viloxazine on cortical neuron responses to NA, 5-HT and ACh. The upward columns represent excitatory and the downward columns depressant responses (ordinate). Reduction of response size (possibly at high concentrations—see text) occurred in some studies and potentiation in others (low concentrations) following the application of viloxazine. Numbers in the columns are the number of studies in which these effects occurred. Open columns represent control responses to agonists before viloxazine. These were averaged and represented as a percentage of the mean control. They did not normally vary by more than ± 10%. Dotted columns represent the mean maximum reduction of agonist responses following viloxazine. Hatched columns are the mean maximum potentiations.

sites following iontophoretic applications (Curtis 1964). Preliminary observations of the effects of intravenous viloxazine on cortical neuron-responses have been made. In one study 5-HT responses were potentiated by 2 mg kg^{-1} viloxazine and in another, ACh responses were reduced by 4 mg kg^{-1} of viloxazine (T. J. Hendra, unpublished). These results would argue against a non-specific action of high concentrations of viloxazine.

The reduction and potentiation of ACh responses in these experiments were unexpected effects. The antiacetylcholine effect of viloxazine assessed on the guinea-pig ileum is negligible compared with the effect of tricyclics (Greenwood 1975). That responses of cortical cells can be reduced by viloxazine may suggest that cholinergic receptors in the c.n.s. differ from those in peripheral tissues and are more susceptible to blockade by viloxazine. Weinstock & Cohen (1976) have shown that cholinergic responses in the cat superior cervical ganglion can be reduced by similar doses of viloxazine and tricyclics. They suggest that cholinergic receptors in neuronal tissue are different from those in non-neuronal tissue.

Patients treated with viloxazine show a much reduced incidence of peripheral antiacetylcholine side effects compared with patients receiving tricyclics (Ekdawi 1975). However if viloxazine can preferentially block central cholinergic receptors, then this may still suggest the involvement of central cholinergic systems in depression (Janowsky et al 1972).

As it seems likely from the present findings that viloxazine can block central ACh receptors, then potentiation of ACh responses by blockade of 'masked' receptors (Bevan et al 1975a) may be a valid explanation. Possible alternative explanations for this effect could be the inhibition of cholinesterase or blockade of an active uptake for ACh proposed by Schubeth & Sundwall (1967). There are no data to support or deny such actions of viloxazine.

Viloxazine has little effect on NA or 5-HT uptake by brain tissue (Blackburn et al 1978) and the potentiation of monoamine responses is unlikely to result from an increase of the monoamines at the receptor sites due to uptake blockade. However, the monoamine receptor blocking activity of viloxazine may make blockade of masked receptors a feasible explanation.

An alternative explanation for the potentiation of 5-HT, NA and ACh responses would be in terms of an action of viloxazine and tricyclics on cyclic nucleotide systems. The actions of 5-HT and NA may be mediated postsynaptically by cAMP in the c.n.s. (Pagel et al 1976; Stone & Taylor 1977) and those of ACh by cGMP (Stone & Taylor 1977). Tricyclic antidepressants are known to have effects on various components of brain cyclic nucleotide systems (Janiec et al 1974; Palmer 1976). It may be that the effects of tricyclics and viloxazine on cortical neuron responses arise from an effect on the formation and/or degradation of cyclic nucleotides in the postsynaptic membrane.

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