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An increase in sensitivity of rat cingulate cortical neurones to substance P occurs following withdrawal of chronic administration of antidepressant drugs

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1 The sensitivity of neurones in the cingulate cortex of the rat to iontophoretically applied substance P (SP) was tested one hour after a single dose of various antidepressant drugs and also 1 day following the termination of a chronic dosing schedule (14 once daily injections) of the same agents.

2 One hour after a single injection of desipramine (DMI), chlorimipramine (CMI), trimipramine (TMI) or zimelidine (ZIM) (all at 10 mg kg^{-1} i.p.) there was no change in the mean size of excitatory responses to SP compared to those before the injection. There was a tendency towards a decrease in the TMI group.

3 One day following the last of 14 consecutive daily injections (10 mg kg^{-1} i.p.) of the above agents there was a significant increase in the size of excitatory responses to SP compared to those in rats receiving daily saline injections. However, 14 days of treatment with DMI did not alter the responses to L-glutamate.

4 Similar chronic dosing schedules with either diazepam (5 mg kg^{-1}) or fluphenazine (5 mg kg^{-1}) did not affect the responses to SP.

5 Thus chronic but not acute administration of antidepressant drugs results in an increase in the sensitivity of neurones, in the cingulate cortex of the rat, to SP.

Introduction

The original monoamine hypotheses of the aetiology of depression (Schildkraut, 1965; Coppen, 1967) which were based on pharmacological observations, have led to an almost exclusive association of the action of antidepressant drugs with central neuronal systems which utilize noradrenaline (NA) or 5-hydroxytryptamine (5-HT) as neurotransmitters. However, this association has never adequately explained either the aetiology of the illness or the means by which drugs can ameliorate it (see Leonard, 1982). Little consideration has been given to the possible involvement of other central synaptic mediators or modulators in depression or the effects of antidepressant drugs. There are many other substances, both aminergic and non-aminergic, which are suspected of playing a role in central synaptic transmission and there seems to be no particular reason to dismiss a possible involvement of one or more of these substances in mediating the actions of antidepressant drugs.

Of the many peptides which are now listed as putative neurotransmitters in mammalian brain perhaps the most firmly established is the undecapeptide, substance P (SP) (see Porter & O'Connor, 1982). Recently, Fuxe *et al.* (1983) speculated that SP may be involved in the mechanism of action of antidepressant drugs. They propose that SP may be a modulator of 5-HT transmission in the CNS and that one of the actions of antidepressant drugs is to change the modulatory interaction between SP and 5-HT. In the present experiments we have investigated a possible involvement of SP in the action of antidepressant drugs by determining the effects of several of these agents on the responsiveness of neurones to SP following their acute and chronic administration. The neuronal population studied was in layers IV–V (approximately) of the anterior cingulate cortex. This region was selected for several reasons: it is easily accessible; we have previously shown that these neurones are strongly excited by SP and also its

C-terminal fragments with a similar structure activity profile to other systems (Jones & Olpe, 1982); the presence (albeit a sparse presence) of SP-containing terminals has been demonstrated here (Ljungdahl *et al.*, 1978; Inagaki *et al.*, 1982); autoradiographic studies show a moderate density of [^{3}H]-SP binding sites in the anterior cingulate cortex (O'Donohue *et al.*, 1983) and finally, in human brain at least, the concentration of SP in the anterior cingulate cortex is more than double that measured in any other cortical region (Crystal & Davies, 1982). We show here that following the chronic administration of 4 different antidepressant drugs the sensitivity of cingulate cortical units to SP is enhanced but no such effect is seen after a single acute dose. Some of these results have been previously presented in a preliminary form (Jones & Olpe, 1983a,b).

Methods

All experiments were conducted on male albino rats (RAIf, 300–400 g) anaesthetized with chloral hydrate (400 mg kg^{-1} i.p.). The neurones studied were located between 700 and 1200 μ from the brain pial surface in the anterior cingulate cortex (6–7.5 mm anterior to the interaural line; 0.5–1 mm lateral to the midline with the incisor bar set planar with the ear bars). Conventional techniques were used to record spontaneous action potentials extracellularly and to apply substances by iontophoresis using 3- or 4-barrelled micro-pipettes. The pipettes contained NaCl (4 M) for recording; NaCl (0.165 M) for balancing the iontophoretic currents; SP (0.001 M dissolved in 0.165 M NaCl, pH 5–6) and sometimes L-glutamate (0.1 M, pH 6–7).

In acute studies an electrode was introduced into the cortex and the responses of 7–10 neurones, to a standard application of SP (80 nA, 60 s), determined. A single injection (10 mg kg^{-1} i.p.) of an antidepressant drug was given and one hour later the same electrode was reintroduced into the same area and the responses to SP tested on the same number of neurones as before the drug was given. Each drug was tested in this way in 4 animals.

In chronic experiments drugs or the corresponding vehicle solution were administered (i.p.) once daily for 14 consecutive days between 09:00 and 10:00 a.m. A group of rats was also tested 1 day after 7 consecutive daily injections of desipramine (DMI). In addition, in the 14 day DMI group the neurones were also tested with a standard application of L-glutamate (40 nA, 20 s). Electrophysiological experiments were conducted between 24 and 36 h after the final injection. A single micropipette was used to study responses of 8 neurones to SP (80 nA, 60 s) in a vehicle treated rat and 8 neurones in a drug-treated rat on the same day. Each drug group comprised 4 such experimental pairs of rats. Thus each drug or vehicle group consisted of tests made on 32 cells. In both acute and chronic studies the investigator was unaware of the treatment each rat had received until the experiments were complete.

Responses to SP were quantified in the following way: Each response was divided into three 60 s periods; the 60 s period during the application of SP and two consecutive 60 s periods immediately following its termination. The number of spikes which occurred above the pre-SP baseline firing rate was determined in each period (designated S_1, S_2 and S_3) and this presumably represented the number of spikes produced in response to the peptide application. Comparison of the mean values of S_1, S_2 and S_3 in drug and control groups was by means of a Student's *t* test.

Drugs

Substance P (SP, Bachem); L-glutamate sodium salt (Sigma); desipramine HCl (DMI, Ciba-Geigy); chlorimipramine HCl (CMI, Ciba-Geigy); trimipramine methane sulphate (TMI, Servia); zimelidine HCl (ZIM, Astra); diazepam (Hoffman La Roche); fluphenazine HCl (Jannsen). In chronic studies antidepressant drugs were given at 10 mg kg^{-1} (i.p.) and diazepam and fluphenazine at 5 mg kg^{-1} (i.p.).

Results

These studies were conducted on a total of 704 neurones in 68 rats.

Table 1 Effect of antidepressants administered acutely on the responses of cortical neurones to substance P (SP)

	Spike number		
Treatment	S_1	S_2	S_3
Control	435 ± 41	480 ± 66	166 ± 51
After CMI (10 mg kg^{-1})	395 ± 47	508 ± 66	156 ± 44
Control	546 ± 73	580 ± 93	116 ± 41
After DMI (10 mg kg^{-1})	512 ± 68	580 ± 83	179 ± 48
Control	401 ± 44	421 ± 51	132 ± 35
After TMI (10 mg kg^{-1})	362 ± 49	385 ± 45	96 ± 40
Control	413 ± 62	417 ± 46	90 ± 34
After ZIM (10 mg kg^{-1})	445 ± 58	473 ± 62	88 ± 42

None of the drugs caused any significant alteration in responses to SP one hour after a single acute injection.

Table 2 Effect of the chronic administration of desipramine (DMI) on responses of cingulate neurones to substance P (SP) and glutamate

Treatment	*Duration*	*Spike number* S_1	S_2	S_3
		Responses to SP		
NaCl	7 days	523 ± 56	485 ± 63	132 ± 40
DMI (10 mg kg^{-1})	7 days	629 ± 90	667 ± 125	285 ± 85
NaCl	14 days	467 ± 73	449 ± 95	147 ± 69
DMI (10 mg kg^{-1})	14 days	688 ± 71[b]	762 ± 92[b]	329 ± 67[a]
		Responses to glutamate		
NaCl		503 ± 61	58 ± 54	
DMI (10 mg kg^{-1})	14 days	462 ± 93	5 ± 23	

Treatment for 7 days with DMI resulted in an increase in spike numbers one day later but this effect did not reach significance. One day following 14 days of treatment, the increase in all 3 periods was significant. Responses to L-glutamate (S_1 = 20 s during glutamate application, S_2 = succeeding 20 s) did not differ in control and drug treated rats.
[a] $P < 0.05$, [b] $P < 0.025$.

Acute studies

The acute administration of any of the antidepressant drugs produced no significant change in the mean firing rate of neurones, recorded 1–2 h after the injection or in the number of actions potentials evoked by SP measured in S_1, S_2 or S_3. The spike numbers before and after the acute administration of the antidepressant drugs are shown in Table 1. There was a tendency towards a decrease in the responses to SP in the TMI group and an increase in the ZIM group but neither effect reached significance.

Chronic studies

There was no significant difference in the baseline firing rate of cingulate cortical neurones from rats 1 day after receiving a course of treatment (7 or 14 days) with any of the drugs tested compared to their corresponding control groups (data not shown). Table 2 shows the effect of DMI (given for 7 or 14 days) on cortical neurone responses to SP and L-glutamate. There was an increase in spike number in S_1, S_2 and S_3, after 7 days of treatment with DMI, which did not reach significance. After 14 days of treatment the spike numbers for SP were significantly greater in all 3 response periods. In contrast spike numbers to glutamate were not changed. Table 3 shows that the effects of 14 days of treatment with CMI, TMI and ZIM were similar to those of DMI. Thus the spike numbers in S_1, S_2 and S_3 were all significantly increased. Therefore, all 4 antidepressants cause an increase in both the magnitude and duration of the responses to SP. In contrast to the effects of the antidepressants, chronic administration of either diazepam or fluphenazine did not alter responses of cingulate cortical neurones to SP (Table 4).

Table 3 Effect of chronically administered antidepressants on neuronal responses to substance P (SP)

Treatment	*Duration*	*Spike number* S_1	S_2	S_3
NaCl	14 days	461 ± 37	457 ± 41	142 ± 34
TMI (10 mg kg^{-1})	14 days	736 ± 105[b]	781 ± 97[c]	268 ± 47[a]
NaCl	14 days	422 ± 44	441 ± 38	141 ± 32
ZIM (10 mg kg^{-1})	14 days	630 ± 54[c]	669 ± 66[d]	308 ± 46[b]
NaCl	14 days	406 ± 31	442 ± 66	148 ± 42
CMI (10 mg kg^{-1})	14 days	734 ± 60[d]	839 ± 69[d]	344 ± 49[d]

Treatment with all 3 drugs caused an increase in spike number in all 3 periods one day following the final injection.
[a] $P < 0.025$, [b] $P < 0.01$, [c] $P < 0.005$, [d] $P < 0.001$.

Table 4 Effect of diazepam (DAZ) and fluphenazine (FLU) on cortical cell responses to substance P (SP)

		Spike number		
Treatment	*Duration*	S_1	S_2	S_3
NaCl	14 days	458±50	491±67	98±37
FLU (5 mg kg^{-1})	14 days	457±59	455±55	143±40
NaCl	14 days	458±50	491±67	98±37
DAZ (5 mg kg^{-1})	14 days	452±45	454±42	89±32

Neither drug evoked any change in response to SP measured one day following the final injection.

Discussion

Thus the chronic administration of all the antidepressant drugs tested gave rise to an increase in responsiveness of cingulate cortical neurones to SP tested one day after the final injection of each drug. This effect was specific in that a neuroleptic or an anxiolytic agent administered using the same dose schedule did not induce a similar increase. The increase in sensitivity to substance P is unlikely to be due to a generalized increase in postsynaptic excitability since the chronic administration of DMI did not result in a change in the responsiveness to L-glutamate. To our knowledge this is the first demonstration of a change in responsiveness of a population of neurones to a neuropeptide after administration of antidepressants. Scuvée-Moreau & Svensson (1982) found no change in the sensitivity of opiate receptors on locus coeruleus neurones, 1 day following 14 days administration of a number of antidepressants, to morphine, applied by iontophoresis. This latter result is supported by the binding data of Bürki *et al.* (1981) who found no change in the binding of [^{3}H]-naloxone in cerebral cortex 1 day following a 10 day course of treatment with DMI.

The mechanism by which the antidepressants exert their effects on the responses to SP is unknown at present and the known pharmacology of the drugs does not really give any clear indications. The most likely interpretation of the present results is that the drugs somehow increase the number or agonist-affinity of the cortical SP receptors. We are currently testing this hypothesis by looking at the binding of [^{3}H]-SP in the cingulate cortex after acute and chronic administration of antidepressants.

One other possibility which we are considering is that the change in responsiveness to SP arises secondarily to an adaptive change in the cortical monoamine systems. Chronic administration of antidepressant drugs has been shown to elicit adaptive changes in a number of parameters of central monoaminergic function (see Reisine, 1981; Sugrue, 1983 for recent reviews). Since SP-containing terminals as well as NA- and 5-HT-containing terminals exist in the cortex (Beaudet & Descarries, 1978; Ljungdall *et al.*, 1978; Inagaki *et al.*, 1982) it seems quite possible that the amines and peptides could functionally interact at the neuronal level and that a change in the amine system induced by the antidepressant could elicit changes in the responsiveness to the peptide. In the spinal cord, 5-HT has been shown to specifically reduce responses of dorsal horn neurones to SP (Davies & Roberts, 1981) and vice versa (Ward & Roberts, 1983). We have recently shown that responses to SP in the cingulate cortex can be reduced by both 5-HT or NA applied by iontophoresis, although the effects of the former appear less specific than those of the latter (Jones & Olpe, 1983c). It has been shown in this laboratory (Olpe & Schellenberg, 1980; Olpe *et al.*, 1981) that, in line with studies showing decreased binding to β-receptors in the cortex (see Sugrue, 1983), the sensitivity of cortical cells to the depressant effects of iontophoretically applied NA is reduced by chronic administration of antidepressants. If the NA system is functionally inhibitory on the SP system in the cortex then it is possible that a change in the former could increase responsiveness of the latter. The argument for a similar involvement of 5-HT is less strong since chronic administration of tricyclic antidepressants does not reduce the depressant effects of 5-HT on cortical neurones (Olpe & Schellenberg, 1981).

Fuxe *et al.* (1983) have recently proposed that a change in the interaction between 5-HT and SP may be involved in the mechanism of action of antidepressants. They have demonstrated that SP can increase the number of 5-HT-binding sites but decrease their affinity in the spinal cord. On this basis they hypothesize a regulatory role for SP on 5-HT-mediated transmission. They have speculated that a change in this regulatory interaction may underlie the mechanism of action of antidepressants (Fuxe *et al.*, 1983). How this relates to the present demonstration of an antidepressant-induced increase in responsiveness to SP is unclear.

Whatever the explanation of the present results it is unlikely that the blockade of monoamine reuptake is involved. The four drugs tested vary widely in their ability to inhibit NA and 5-HT uptake and TMI is virtually inactive on either system (Hyttel, 1983). It is clear however, that TMI is a clinically effective antidepressant (Settle & Ayd, 1980) as are the other 3 drugs tested and all 4 clearly increase responsiveness to SP, at least in the withdrawal period following their chronic administration. It is not possible to say on the basis of the present experiments whether this indicates a possible involvement

of SP in the aetiology of depression or the clinical effect of antidepressants. It would be extremely interesting to determine the concentrations of SP and/or its binding characteristics in post-mortem brain tissue from depressed patients.

Finally, several recently published abstracts have indicated an involvement of other peptides in the action of antidepressant drugs. Thyrotropin releasing hormone is decreased after repeated electroshock treatment but administration of antidepressants results in an elevation of this peptide in some regions of the CNS (Bennett *et al.*, 1983a,b). Conzelman *et al.* (1983) have described the ability of several antidepressant drugs to reduce the veratradine-induced release of cholecystokinin from slices of rat striatum. Also, Delbarre *et al.* (1983) have shown that both oxytocin and vasopressin are active in a test indicative of antidepressant activity. Thus the present study and others indicate that a role of neuropeptides in the aetiology of depression and the mode of action of antidepressants should be strongly considered.

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