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EFFECT OF VARIOUS ANTIDEPRESSANT DRUGS ON THE SPONTANEOUS FIRING RATE OF LOCUS COERULEUS AND DORSAL RAPHE NEURONS OF THE RAT *

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The spontaneous firing rate of the noradrenergic neurons of the locus coeruleus and of the serotonergic neurons of the dorsal raphe was recorded with extracellular microelectrodes in chloral hydrate-anesthetized rats. A quantitative comparison of the effect of five tricyclic antidepressants, of tranylcypromine and of mianserin on the spontaneous activity of these two types of cells was performed. All drugs tested, except mianserin reduced the frequency of discharge of the noradrenergic neurons. Intravenous perfusion of the drugs allowed the doses required for inhibition of firing to 50% of the baseline rate (ID_{50}) to be determined. Secondary aminated antidepressants (desipramine and nortriptyline) were more potent inhibitors than their tertiary aminated analogues (imipramine, chlorimipramine and amitriptyline). All drugs tested, except desipramine decreased the rate of firing of the serotonergic cells. In this case, the tertiary aminated antidepressants were much more potent than their secondary analogues. Mianserin was only active at very high doses. These results are in good agreement with the relative potencies of the tricyclic antidepressants for blocking the uptake of noradrenaline and serotonin into central and peripheral neurons.

Tricyclic antidepressants Locus coeruleus Tranylcypromine Dorsal raphe Mianserin
Single unit activity

1. Introduction

Several factors are involved in the presynaptic regulation of monoamine transmitter function in the central nervous system: synthesis, compartmentation, intraneuronal metabolism and reuptake (Seiden et al., 1975; Green and Grahame-Smith, 1976). Antidepressant drugs are characterized by their ability to interact in one way or another with these regulatory mechanisms. For example tricyclic antidepressants are potent inhibitors of the neuronal uptake of noradrenaline (NA) and 5-hydroxytryptamine (5HT) (Hertting et al., 1961; Dengler et al., 1961; Axelrod et al., 1962; Glowinski and Axelrod, 1964; Ross and Renyi, 1967a,b). Tricyclic antidepressants with a secondary amine in the side chain (desipramine and nortriptyline) are more potent inhibitors of NA uptake than their tertiary aminated analogues (imipramine, chlorimipramine and amitriptyline) which in turn are more potent inhibitors of 5HT uptake (Carlsson et al., 1969ab; Carlsson, 1970, Shaskan and Snyder, 1970; Hamberger and Tuck, 1973; Ross and Renyi, 1969, 1975a,b). Since reuptake into nerve endings represents the major mechanism by which the action of NA and 5HT is terminated, tricyclic antidepressants increase the availability of these monoamines at postsynaptic sites. Monoamine oxidase inhibitors (IMAO) interfere

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with the enzymatic degradation of monoamines and markedly increase their concentration in the brain (Spector et al., 1958; Green and Erickson, 1960).

Electrophysiological techniques make it possible to study the influence of these interactions on the spontaneous firing of central monoaminergic neurons. In the present work, a quantitative comparison of the effect of five tricyclic antidepressants and one IMAO on single unit activity recorded from the locus coeruleus (L.C.) and dorsal raphe (D.R.) was performed. We also tested the influence of mianserin, a new antidepressant which seems to have a mode of action different from that of the classical antidepressants.

2. Materials and methods

2.1. Single unit recording

Male Wistar rats (200—300 g) were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic apparatus. The spontaneous firing of the noradrenergic cells of L.C. and of the serotonergic cells of D.R. was recorded by means of extracellular nickel-chrome microelectrodes implanted at the following coordinates : locus coeruleus : P. 2 mm, L. 1.2 mm from lambda and H. approximately 6 mm under the cerebellum surface; dorsal raphe: A. 0.6 mm, L. 0.0 mm from lambda, H. approximately 5 mm under the cortex surface (all extrapolated from König and Klippel, 1963). Before lowering the electrode, a small bone flap was removed and the transverse venous sinus (in the case of L.C.) or the longitudinal venous sinus (in the case of D.R.) was tied and cut. Single unit action potentials were passed through an impedance adapter and an amplifier into a Tektronix oscilloscope. Signals were also passed through an electronic amplitude discriminator and counted by a digital counter. The number of spikes was determined every 10 sec and the mean frequency per 10 sec was calculated every minute. The mean frequency/10 sec was plotted against time in the graphic representation of the results.

2.2. Method of drug comparison

After a base line firing rate had been established during at least 10 min, the drug tested was perfused into the jugular vein by means of a perfusion pump (flow 6 ml/h). Perfusion was stopped when the frequency of discharge had dropped under 50% of the control rate during 1 min. It was therefore possible to determine the total dose required for inhibition of firing to 50% of base line rate (ID_{50}) for each substance. The activity of the cell was still recorded during a third period as long as was possible. At the end of the experiment, the animal was perfused with a solution of formaldehyde 4% and the brain removed for histological examination. The drugs studied were, the dibenzazepine derivatives : desipramine, imipramine and chlorimipramine (Ciba-Geigy); the dibenzocycloheptadiene derivatives : nortriptyline (Lilly) and amitriptyline (Merck Sharp & Dohme); the IMAO, tranylcypromine (SKF) and the new antidepressant mianserin (Organon). Their chemical structures are presented in fig. 1.

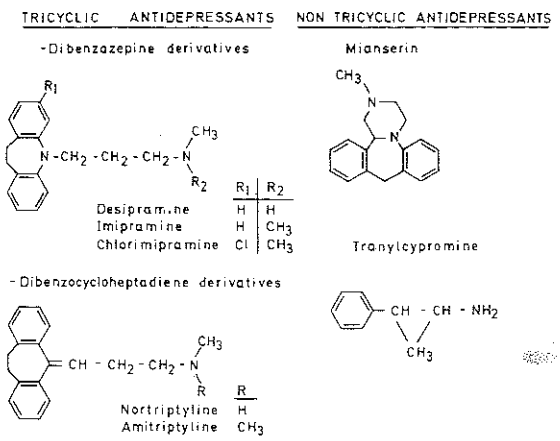

Fig. 1. Chemical structures of the various antidepressant drugs tested in this work.

3. Results

3.1. Recording characteristics of L.C. neurons

The locus coeruleus is the most homogeneous mass of noradrenergic cell bodies in the brain. It is located in the dorsal part of the pons, just under the cerebellum and between the fourth ventricle and the mesencephalic nucleus of the fifth nerve. These neurons have positive-negative action potentials and fire rather regularly at a rate of 0.5—5 spikes/sec. (fig. 2A) (Graham and Aghajanian, 1971, Korf et al., 1974). Just laterally to the L.C. the cells of the mesencephalic nucleus of the fifth nerve are characterized by bursts of spikes of high frequency.

3.2. Effect of antidepressants on L.C. firing rate

The spontaneous regular firing of locus coeruleus cells was recorded during a first control period of at least 10 min before administering the drug. Slow perfusion of the tricyclic antidepressants progressively decreased the spontaneous firing rate of the noradrenergic neurons of L.C., without modifying the amplitude (fig. 2B). The perfusion was stopped when the frequency had dropped to 50% of the control rate. The inhibitory effect of the drug persisted for quite a long time. Recovery to the control frequency seemed to be progressive and very slow. In a few cells which could be recorded for more than 50 min after perfusion, there was a slow increase in frequency and a tendency to recover towards the predrug firing rate.

As shown previously (Scuvée-Moreau et al., 1979) there was a great difference in potency between the five tricyclic antidepressants tested, the secondary aminated derivatives being more potent than their tertiary aminated analogues. For example desipramine was ten times more potent than chlorimipramine. The overall order of potency was: desipramine > nortriptyline > imipramine > amitriptyline > chlorimipramine. The IMAO, tranylcypromine, also caused prolonged depression of L.C. firing rate (fig. 3) but mianserin failed to alter the firing rate of L.C. cells. Detailed data are summarized in table 1.

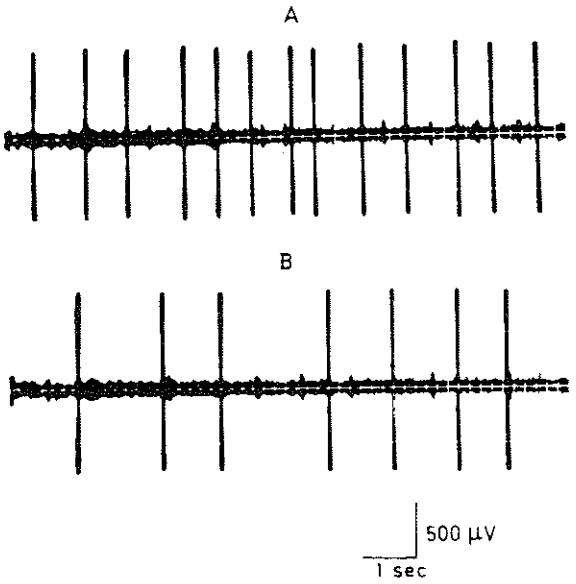

Fig. 2. (A) Spontaneous regular firing of a noradrenergic neuron of the locus coeruleus-Control rate. (B) Reduction of firing to 50% after perfusion with desipramine 0.05 mg/kg/min for 6 min.

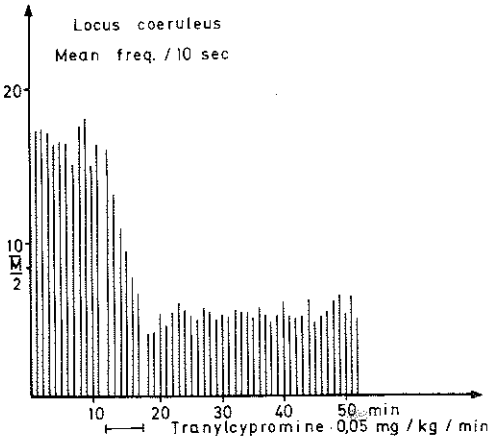

Fig. 3. Effect of an i.v. perfusion of tranylcypromine on the firing rate of a noradrenergic cell. Perfusion was stopped when the frequency was reduced to 50% of the control rate.

TABLE 1

Effect of various antidepressant drugs on the firing rate of locus coeruleus and dorsal raphe neurons. ID_{50} = mean doses required for inhibition of firing to 50% of the base line rate. n = number of animals.

Drug	ID_{50} (mg/kg ± S.E.)	
	Locus coeruleus (n)	Dorsal raphe (n)
Desipramine	0.29 ± 0.02 (6)	>12 (5)
Imipramine	1.30 ± 0.11 (6)	1.63 ± 0.39 (6)
Chlorimipramine	3.08 ± 0.30 (6)	0.34 ± 0.02 (6)
Nortriptyline	0.66 ± 0.06 (6)	9.16 ± 1.60 (5)
Amitriptyline	1.42 ± 0.24 (6)	1.78 ± 0.27 (6)
Tranylcypromine	0.80 ± 0.06 (6)	1.56 ± 0.21 (6)
Mianserin	>15 (5)	9.03 ± 1.03 (6)

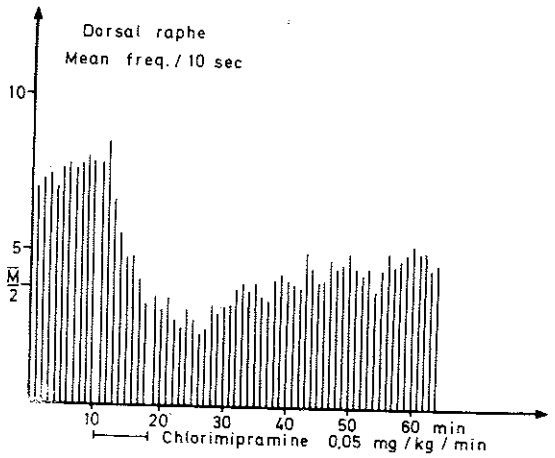

Fig. 4. Effect of an i.v. perfusion with chlorimipramine on the firing rate of a serotonergic cell.

3.3. Recording characteristics of D.R. neurons

The dorsal raphe nucleus of the midbrain has a large cluster of serotonin-containing neurons (Dahlström and Fuxe, 1965) but is less homogeneous than L.C. Serotonergic cells are slow (0.25—2.0 spikes/sec), regular, spontaneously active cells with an initial large positive spike of long duration (1—2 msec). Other cells can be encountered in the region of D.R.: these cells have relatively high rates of discharge (> 2 spikes/sec) and often show an irregular rhythm (Aghajanian et al., 1968; Bramwell, 1974; Afghajanian et al., 1978).

3.4. Effect of antidepressants on D.R. firing rate

The same experimental procedure as described above was also used to study the influence of antidepressants on the spontaneous activity of D.R. cells. All drugs tested, except desipramine decreased the frequency of discharge of the serotonergic cells of dorsal raphe. This inhibitory effect also persisted for several minutes (fig. 4). The order of activity of the tricyclic drugs was the reverse of that with L.C.; the tertiary aminated derivatives were much more potent than their secondary aminated analogues. The overall order of potency was chlorimipramine > imipramine > amitriptyline > nortriptyline > desipramine. The IMAO, tranylcypromine also inhibited D.R. neurons. Mianserin was also active but at rather high doses. The various ID_{50} are given in table 1.

4. Discussion

Our results show that the spontaneous activity of noradrenergic and serotonergic neurons may be influenced by several but not all of the antidepressant drugs used in therapeutics. There was a certain selectivity among the drugs tested: secondary aminated tricyclic antidepressants (desipramine and nortriptyline) which are more potent inhibitors of NA uptake were also very active inhibitors of locus coeruleus neurons and very weak inhibitors of dorsal raphe neurons. Conversely, tertiary aminated tricyclic antidepressants were more potent inhibitors of dorsal raphe neurons. This was particularly true for chlorimipramine which showed good selectivity for the serotonergic neurons. Chlorimipramine was ten times more potent on D.R. neurons whereas the ID_{50} for imipramine and amitriptyline on L.C. and D.R. neurons were very similar. It must be noted that the ID_{50}

determined with the perfusion technique differed from the classic ID_{50} calculated on the basis of a dose—response curve but such a procedure would have required a considerable multiplication of the experiments. Furthermore perfusion of the drug instead of single i.v. injections had the advantage of yielding more stable blood levels and having a progressive inhibitory effect. This procedure is somewhat comparable to the method for establishing a cumulative dose—response curve.

These experiments confirmed the results obtained by Nybäck et al., (1975) on locus coeruleus neurons. These authors found that tricyclic antidepressants depressed the rate of firing of these NA neurons. It was also shown that some tricyclic antidepressants could inhibit 5HT neurons firing (Sheard et al., 1972; Bramwell, 1974; Gallager and Aghajanian, 1975). Our results are in agreement with these experiments but we performed a more quantitative and systematic study. The order of activity of the tricyclic drugs to produce their inhibitory effect on L.C. and D.R. is also in good agreement with the in vitro experiments performed by Ross and Renyi (1975a) on the uptake of tritiated NA and 5HT by rat synaptosome preparations.

It is interesting to note that the tertiary aminated tricyclic antidepressants, which are more potent inhibitors of 5HT neurons firing are also more sedative clinically than their secondary aminated analogues.

Two different mechanisms may explain the depression of L.C. firing rate by the tricyclic antidepressants. It is generally admitted that inhibition of NA uptake leads to an accumulation of neurotransmitter at the synaptic level and thus to a stimulation of postsynaptic receptors which in turn may decrease the activity of the presynaptic neurons by a neuronal feedback loop (fig. 5 site 1). It is also possible that the effect observed was due to an action of NA on the α_2 autoreceptors situated on L.C. neurons. This effect could be mediated by inhibitory axonal collaterals (Cedarbaum and Aghajanian, 1977) (fig. 5 site 2). Furthermore, it must be noted that an additional mechanism may be implicated in the regulation of NA release. This would be mediated by the α_2 presynaptic receptors found on the noradrenergic nerve terminals (Starke and Montel 1973, Langer 1977) (fig. 5 site 3). These α_2 receptors mediate a local negative feedback mechanism which leads to inhibition of NA release during nerve stimulation. Thus three mechanisms are involved in the modulation of NA release, with or without influence on the neuronal firing. The same hypothesis can be considered to explain the inhibitory effect of tricyclic antidepressants on the activity of D.R. neurons (Aghajanian and Wang 1978).

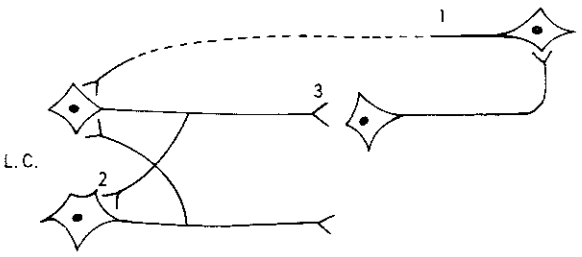

Fig. 5. Three different mechanisms may be implicated in the modulation of the noradrenergic neurons of the L.C. (1) a postsynaptic neuronal feedback loop; (2) a collateral autoregulatory mechanism (these two mechanisms affecting the firing rate); (3) a local feedback mechanism at presynaptic terminals (affecting only the release of NA).

Tranylcypromine, at low doses, depressed the firing of both noradrenergic and serotonergic neurons. This inhibitory effect of a monoamine oxidase inhibitor on D.R. neurons firing confirms the observations of Aghajanian et al. (1970) and of Bramwell (1974).

It is interesting to note that a drug practically devoid of effect on the uptake of monoamines, may also influence the firing of monoaminergic neurons. However, tranylcypromine also has amphetaminelike or catecholamine-releasing effects (Tedeschi et al., 1959; Lee et al., 1961) which may be partially responsible for the inhibitory effect observed.

Mianserin perfused at rather high doses, failed to produce any effect on L.C. firing

rate and was a very weak inhibitor of D.R. firing. Several authors have tried to elucidate the mode of action of mianserin. Unlike tricyclic antidepressants, mianserin does not antagonize reserpine-induced hypothermia (Van Riezen, 1972). Moreover, neurochemical experiments revealed that mianserin, in contrast to the tricyclic antidepressants, increases the turnover of noradrenaline in the rat brain (Kafoe and Leonard 1973). The effect of mianserin on the uptake of NA and 5HT was also investigated but the question remains controversial. Goodlet et al. (1977), found that although mianserin rivals imipramine and amitriptyline in blocking NA uptake in vitro, this does not hold in vivo. Furthermore the ability of mianserin to inhibit 5HT uptake in vitro is appreciably less than that of the tricyclic antidepressants studied. Mianserin is devoid of effect on rat brain 5HT uptake in vivo. However Baumann and Maître (1977) reported that mianserin inhibited NA uptake in vitro and in vivo but observed only a marginal inhibition of 5HT uptake. From studies on NA release, they concluded that mianserin increases the concentration of NA in the synaptic cleft by blocking the presynaptic α-receptors and inhibiting uptake. In experiments now in progress in our laboratory, we confirmed this inhibitory effect of mianserin on the uptake of NA both in vitro and in vivo and the lack of effect on 5HT uptake (N. Quinaux et al., in preparation).

Our electrophysiological results do not support the hypothesis of a selective α_2 presynaptic blocking effect which would be expected to produce an activation of L.C. neurons, unless the combined actions of mianserin on presynaptic receptors and uptake mechanisms neutralize each other. Further studies of this drug in combination with other substances will perhaps help to elucidate its mode of action.

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