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Effects of chronic administration of amitriptyline or mianserin on rat cardiac and central adrenoceptors

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1 Rats were administered either amitriptyline (20 mg kg^{-1}, i.p.) or mianserin (10 mg kg^{-1}, i.p.) for 21 consecutive days and the α- and β-adrenoceptor characteristics of cardiac ventricles, cerebral cortex and hippocampus examined by ligand-binding procedure.

2 Chronic administration of amitriptyline significantly reduced the maximum density of β-receptors in the cerebral cortex without significantly altering cardiac α- or β-receptors; mianserin treatment had no significant effect on any of the receptors studied.

Introduction

Clinical use of the tricyclic antidepressants, including amitriptyline, has been associated with a variety of serious cardiac disorders (Jefferson *et al.*, 1975; Langou *et al.*, 1980; Marshall & Forker, 1982). The adverse effects of acute overdosage with amitriptyline include slowing of conduction, increase in ventricular rate, cardiac arrhythmias and bundle branch block (Petit *et al.*, 1977). Clinical experience of the effects of therapeutic dosage with amitriptyline shows that while the major cardiovascular complication is an orthostatic hypotension, patients with pre-existing bundle block may suffer heart block; conversely, patients with pre-existing arrhythmias may be improved (Glassman, 1984).

Few studies have been made of the detailed electrophysiological and mechanical aspects of the effects of tricyclic drugs on cardiac function. Amitriptyline administered orally at therapeutic dosage elicited an immediate and sustained increase in heart rate in healthy human volunteers (Ikeda *et al.*, 1982). Electrocardiagraphic studies have shown that therapeutic dosage slows cardiac conduction (Ziegler *et al.*, 1977). Animal studies have indicated similar effects of amitriptyline on cardiac conduction and heart rate in dogs (Lindenfield & Horwitz, 1981; Nattel *et al.*, 1984). While some of the autonomic and cardiovascular effects of tricyclics in general may be due to anticholinoceptor actions, the effects on the heart may involve adrenoceptor-mechanisms. Tricyclic antidepressants are known to cause down-regulation of cerebral cortical β-receptors when chronically administered to rats at dosages that correspond to those used therapeutically in man (Sellinger-Barnette *et al.*, 1980). In view of the importance of ventricular β-adrenoceptors in cardiac function it was of interest to determine whether chronic administration of amitriptyline led to any alterations in the characteristics of these receptors.

In the present investigation rats were given amitriptyline or the tetracyclic antidepressant, mianserin, daily for 21 days and the adrenoceptor characteristics of both myocardium and brain then examined. Mianserin has not been implicated in cardiac disorders in man and does not cause down-regulation of cerebral cortical β-adrenoceptors as monitored by ligand-binding measurements (Mishra *et al.*, 1980). Cardiac and cerebral cortical β-adrenoceptors may be assessed using the radio-ligand [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) (Alexander *et al.*, 1975; Bylund & Snyder, 1976). Adrenoceptors of the α_1 subtype may also be monitored by ligand-binding techniques. Amitriptyline treatment for 21 days has been reported to lead to a two fold increase in apparent density of α_1 binding sites in mouse hippocampus (Rehavi *et al.*, 1980). In this investigation the characteristics of α_1 receptors were determined by use of the ligand [^{3}H]-prazosin which has been shown to be suitable for the determination of these adrenoceptors in both myocardium (Karliner *et al.*, 1979) and brain (Rainbow & Biegon, 1983).

Methods

Male Wistar rats (200–250 g) were used. Amitriptyline hydrochloride was administered intraperitoneally in two divided doses daily of 20 mg kg^{-1} for 21 consecutive days, and mianserin hydrochloride was

given intraperitoneally at 10 mg kg^{-1} daily in two divided doses for the same period. Controls were given isotonic saline in similar protocol. At the end of the treatment periods, drugs were withheld for 12 h before the animals were killed for measurement of receptor characteristics.

Rats were killed by rapid stunning followed by decapitation. In the case of the hearts the extraction and binding assays were performed on fresh tissue. Hearts were rapidly removed and trimmed to yield ventricles, minced and homogenized in 15 ml of ice-cold homogenizing medium (0.3 M sucrose, 10 mM $MgCl_2$, pH 7.40) using a Polytron PT10 homogenizer at setting 7 for 15 s. The crude homogenate was spun at 500 *g* for 10 min and the resulting supernatant centrifuged at 49,000 *g* for 15 min at 5°C; this spin was repeated and the washed pellet resuspended to ca. 0.6 mg protein ml^{-1} of incubation medium (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.50) for measurement of receptor binding. Brains were removed and dissected on ice to yield cerebral cortex or hippocampus. Brain tissue was deep-frozen and subsequently thawed and homogenized (Potter homogeniser) to yield crude homogenates which were subsequently processed as described for heart tissue above.

Binding of [^{3}H]-DHA was determined by the method of Bylund & Snyder (1976) and that of [^{3}H]-prazosin by the method of Karliner *et al.* (1979) with minor modifications. Isoprenaline hydrochloride at 10 μM was employed as competitor with [^{3}H]-DHA, and phentolamine hydrochloride at 10 μM as competitor with [^{3}H]-prazosin. Incubations were carried out over a range of 6 concentrations of labelled ligands (0.5–15 nM) with duplicate incubations at each concentration. Binding was terminated by rapid dilution and washing of incubated extracts with ice-cold incubation medium (3 × 5 ml) by filtration on Whatman GF/B filters under vacuum. Filters were dried and added to 10 ml of toluene: Triton-based scintillator and counted by liquid scintillation spectrometry. Binding data were analysed to yield maximum density B_{max} and binding affinity K_d by the method of Scatchard (1949). Protein was estimated by the method of Lowry *et al.* (1951). Effects of treatment were assessed by unpaired Student's *t* test.

Results

The results are shown in summary in Table 1. Cardiac ventricular binding of [^{3}H]-prazosin was somewhat greater and of significantly higher affinity than ventricular binding of [^{3}H]-DHA, in keeping with observations of other authors on rat myocardial adrenoceptors (Williams *et al.*, 1983). Neither the affinity nor the density of binding sites for either ligand was significantly altered by treatment with amitriptyline or mianserin.

Cerebral cortical binding sites for [^{3}H]-DHA were more abundant but of lower affinity than the myocardial binding sites in the untreated animals. Rats given amitriptyline showed a significant reduction in B_{max} for this ligand in cortical extracts without any alteration in the apparent affinity for binding; neither was there any change in non-specific binding of [^{3}H]-DHA associated with the amitriptyline effect. Administration of mianserin did not lead to any significant effect on cerebral cortical [^{3}H]-DHA binding characteristics. In the hippocampal extracts, neither amitriptyline nor mianserin appeared to have any significant effect on binding characteristics for [^{3}H]-prazosin.

Table 1 Effects of chronic amitriptyline or mianserin on adrenoceptor characteristics of rat cardiac ventricle, cerebral cortex and hippocampus

		Control	*Amitriptyline*	*Mianserin*
Cardiac ventricle				
[^{3}H]-DHA	$^1B_{max}$	23.5 ± 3.1	23.0 ± 5.3	25.3 ± 3.6
	^2K_d	3.1 ± 0.7	3.3 ± 0.9	2.8 ± 0.7
[^{3}H]-prazosin	B_{max}	41.0 ± 4.0	38.9 ± 2.9	45.5 ± 5.9
	K_d	1.9 ± 0.3	1.7 ± 0.3	1.7 ± 0.4
Cerebral cortex				
[^{3}H]-DHA	B_{max}	96.8 ± 11.1	64.5 ± 6.5*	90.5 ± 10.3
	K_d	7.9 ± 1.9	8.1 ± 2.0	8.0 ± 2.2
Hippocampus				
[^{3}H]-prazosin	B_{max}	34.0 ± 2.7	38.5 ± 3.5	29.5 ± 3.2
	K_d	2.0 ± 0.3	2.2 ± 0.3	1.8 ± 0.2

Figures are mean ± s.e.mean, for 8 animals in each group.
$^1B_{max}$ is in fmol mg^{-1}; ^2K_d, nM.
*$P < 0.01$ (Student's *t* test).

Discussion

These results show rat cardiac adrenoceptors to be unaffected by amitriptyline after chronic drug treatment which causes down-regulation of cerebral cortical β-adrenoceptors. This finding implies that the effects of amitriptyline on cardiac functions do not involve receptor-mediated alterations in adrenoceptor-mediated mechanisms. The reduction in density of cerebral cortical β-adrenoceptors may be attributed to an increased agonist availability brought about by inhibition of neurotransmitter re-uptake or other mechanisms; amitriptyline treatment for 14 days leads also to subsensitivity of presynaptic α_2-receptors which could also contribute to such an effect (Crews & Smith, 1980; Smith *et al.*, 1981). Despite the lack of an effect of chronic treatment with amitriptyline it does nevertheless appear that adrenoceptor mechanisms are involved in the acute cardiac responses to the drug (Lindenfield & Horwitz, 1981).

Cerebral cortical β-adrenoceptors were reduced in density by about one-third following chronic amitriptyline; although there has not been unanimity in the literature on this subject, the balance of evidence is in favour of down-regulation of these receptors by antidepressants (Pandey & Davis, 1983). Mianserin was found not to have this effect, confirming an earlier investigation (Mishra *et al.*, 1980). The binding of the α_1-receptor-specific ligand [^{3}H]-prazosin was not affected by chronic treatment with amitriptyline. Using a somewhat higher drug dosage (30 mg kg^{-1}) in mice, Rehavi *et al.* (1980) found that after 21 days of amitriptyline treatment there were significant increases in the density of hippocampal sites for α- and the muscarinic receptor-specific ligands. The absence of any corresponding effect in the present study may be attributable to the different radioligand ([^{3}H]-WB 4101) used for the α_1-receptor as well as the species and dosage differences involved.

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