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Effects of β-Adrenoceptor Antagonists on the Firing Rate of Noradrenergic Neurones in the Locus Coeruleus of the Rat

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Summary. Acute i.v. administration of the non-selective β-adrenoceptor antagonist *dl*-propranolol given in incremental doses (< 40 mg/kg) did not affect the firing rate of locus coeruleus (LC) neurones in the rat, as revealed by single cell recording techniques. Furthermore, no effect was seen 4 h after a single i.p. dose of this β-blocker (10 mg/kg). However, repeated treatment with *dl*-propranolol (1, 5, 10 or 20 mg/kg i.p., twice daily for 4 days) produced a significant, dose-dependent decrease of the average LC neuronal firing rate in comparison to controls. The dextro isomer of propranolol, which has negligible β-blocking activity but the same local anaesthetic potency as the racemate, had no corresponding effect. The non-selective β-adrenoceptor antagonist sotalol, which is one of the most hydrophilic β-blockers, had much less inhibitory effect on LC neurones than *dl*-propranolol. The β_1-selective antagonist metoprolol did not change the firing of noradrenergic neurones in the LC after similar treatment for 4 days. However, when the rats were subjected to oral treatment for 28 days, metoprolol was found to produce a slight inhibitory effect although much less than *dl*-propranolol.

In view of these findings we propose a stimulatory and mainly β_2-adrenoceptor-mediated control mechanism for the noradrenergic neurones in the LC. This mechanism seems to be characterized by a delayed responsiveness.

Key words: Locus coeruleus – Neuronal firing rate – Central β_1- and β_2-adrenoceptors

Introduction

Besides the undoubted peripheral actions of β-adrenoceptor antagonists these drugs evidently also affect the central nervous system (CNS). This view is supported inter alia by some of the psychotropic side-effects associated with β-adrenoceptor blocking therapy as well as the beneficial effect of these drugs in various states of hyperarousal (for review see Conway et al. 1978). Further evidence is provided by the demonstration of centrally located β-adrenoceptors by means of various techniques, including electrophysiological methods (Bloom et al. 1975), studies of c-AMP generating systems (see Daly 1977; Iversen 1977) and radioligand binding techniques (see Maguire et al. 1977; Minneman et al. 1979).

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Previous studies have shown that chronic treatment with *dl*-propranolol causes a reduction in preganglionic sympathetic nervous activity (Lewis and Haeusler 1975) indicating a central mode of action of this β-adrenoceptor antagonist. In addition biochemical studies have indicated chronic treatment with *dl*-propranolol to reduce the release of brain noradrenaline (Fludder and Leonard 1979). In view of the association between impulse activity and transmitter turnover in central noradrenergic pathways (c.f. Salzman and Roth 1979), this biochemical finding indirectly suggests that treatment with β-adrenoceptor antagonists may reduce the activity not only of the sympathetic nervous system but also of central noradrenergic neurones.

The largest central noradrenergic system originates in the pontine nucleus locus coeruleus (LC), which innervates almost the entire neuraxis via its wide-spread terminal distribution (Ungerstedt 1971; Lindvall and Björklund 1974; Nygren and Olsson 1977). These noradrenergic neurones have been suggested to be involved in e.g. control of behaviour such as maintenance of alertness and arousal reactions (Redmond et al. 1976) and may serve as an alarm system in the brain (Cedarbaum and Aghajanian 1978). The LC has also been implicated in the modulation of cardiovascular regulation. Thus, electrical stimulation of the LC elicits a pressor response (Przuntek and Philippu 1973; Ward and Gunn 1976a). Moreover, these noradrenergic neurones seem to be involved in various cardiovascular reflexes (Ward and Gunn 1976b; Svensson and Thorén 1979).

The noradrenergic neurones in the LC have been shown to be subject to an α_2-adrenoceptor mediated, tonically active inhibitory control mechanism (Svensson et al. 1975; Cedarbaum and Aghajanian 1976, 1977). So far, however, regulation of LC neurones by β-adrenoceptors has not been demonstrated.

In view of the above findings and considerations we have utilized single cell recording techniques to study in the rat the effect of acute and chronic treatment with various β-adrenoceptor blocking agents on the activity of central noradrenergic neurones in the LC.

Methods

General Procedure

All experiments were performed on male Sprangue-Dawley rats, weighing between 220–280 g, in the same way as has been described previously (Svensson and Usdin 1978). The animals were anaesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic apparatus. Additional injections of chloral hydrate were given as needed. The body temperature was kept at 37° C by means of a heating pad.

In the skull of the rat a 3 mm burr hole was drilld with its centre located 1.1 mm lateral to the midline and 1.1 mm postrior to lambda.

Single Cell Recording

Extracellular single cell recording was obtained via a micropipette with a tip diameter of about 1 μm filled with 2 N NaCl saturated with pontamine skyblue (impedance in vitro 3–7 MΩ measured at 135 Hz), which was lowered into the brain by means of a hydraulic microdrive. Each spike was discriminated and fed into an integrator which was reset every 10 s, displayed on an oscilloscope, an audiomonitor and finally represented in a rate histogram on an oscillographic recorder. After passing a zone of electrical silence (the 4th ventricle), the LC neurones were usually found 5.5–6.0 mm below the skull surface. A tentative LC neuron was localized by utilizing the neurophysiological characteristics of noradrenergic neurones in the LC, previously described. Thus, the cells of the LC were found to be firing spontaneously with a slow (1–5 spikes/s) and rather steady rhythm (Graham and Aghajanian 1971). Two general characteristics of LC and surrounding neurones were of great help in locating this tiny nucleus. Cells of the mesencephalic nucleus of the Vth nerve lie just lateral to the LC, and respond to proprioceptive stimulation of the facial region (e.g. jaw stretch) with brief bursts of firing (Cedarbaum and Aghajanian 1976). In addition, the LC neurones respond to noxious stimuli (e.g. compression of the contralateral hind paw) with a brief increase in firing followed by a quiescent interval (Korf et al. 1974). Each cell was recorded during stable baseline activity and held for a few minutes. In the acute experiments only one cell was recorded in each rat, whereas in the chronic experiments the average, spontaneous firing rate of randomly encountered single neurones throughout the LC were calculated (15–20 cells in each rat). The final recording sites were marked at the end of each experiment by iontophoretic ejection of dye. The rats were then perfused through the heart with a 10% formaldehyde solution and the brain was removed. Serial 50 μm frozen sections of the brain were cut, stained with cresylviolet and counterstained with neutral red. Only cells within the LC were included in this study.

Drug Treatment

Acute effects of the non-selective β-adrenoceptor antagonist *dl*-propranolol wer studied during a period of 10 min after the drug had been administered into a tail vein. Subacute effects of *dl*-propranolol were studied 4 h after i.p. injection.

Subchronic effects of different β-adrenoceptor antagonists were studied after the substances had been injected i.p. twice daily for 4 days. The recordings were made 2–3 h after the last injection. Chronic effects were studied after long-term, oral treatment with the selective β_1-antagonist metoprolol or *dl*-propranolol for 28 days. These rats were fed for 4 weeks with pelleted food containing the respective β-adrenoceptor antagonists.

Plasma and Tissue Analysis

In some experiments the concentrations of metoprolol and propranolol in plasma and brain tissue were determined by measurement of their trifluoroacetylderivatives using gas-chromatography and electron capture detection according to principles given by Ervik (1975). The samples were taken immediately after completion of the electrophysiological experiments.

Drugs

Metoprolol (Seloken, AB Hässle, Mölndal, Sweden), *dl*-propranolol (Inderal, ICI), sotalol (Sotacor, Mead Johnson). Stock solutions in saline were diluted immediately prior to administration.

Statistics

Statistical evaluation of the results was performed by means of Student's *t*-test. Differences were considered statistically significant when *P*-values were less than 0.05. Results are presented as means ±S.E.M.

Results

The firing rates of noradrenergic neurones within the LC of control rats varied between 1.5 and 4.5 spikes/s and the mean value was 2.98±0.09 spikes/s ($n = 11$, 198 cells). Acute administration of *dl*-propranolol (1–40 mg/kg i.v.) did not affect LC neuronal firing rates, nor was any affect obtained after subacute treatment with *dl*-propranolol (10 mg/kg i.p).

Subchronic treatment with various doses of *dl*-propranolol (1–20 mg/kg i.p., twice daily for 4 days) produced, however, a significant, dose-dependent reduction in the average firing rate of LC neurones, the maximal depression being 37% (to 1.87±0.13 spikes/s, $n = 6$, 94 cells, $P < 0.001$) in comparison to the average firing rate in the control group (Fig. 1).

Neither the dextro-isomer or propranolol (5 mg/kg i.p., twice daily for 4 days) nor metoprolol (5 and 10 mg/kg i.p., twice daily for 4 days) had any effect on LC neuronal firing rates (Figs. 1 and 2). The plasma concentrations of metoprolol and *dl*-propranolol after subchronic treatment (5 mg/kg i.p., twice daily for 4 days) were found to be 26 ±4 nmol/l ($n = 3$) and 27±13 nmol/l ($n = 3$), respectively, and in brain tissue 76±18 pmol/g wet weight ($n = 4$) and 160 ±70 pmol/g wet weight ($n = 4$), respectively.

Sotalol (50 mg/kg i.p., twice daily for 4 days) reduced the firing rate of LC neurones by 17% (to 2.46±0.12 spikes/s $n = 3$, 46 cells, $P < 0.02$) in comparison to control (Fig. 2). A lower dose of sotalol (5 mg/kg) had no significant effect on LC neuronal firing rates.

In one experiment the effect of withdrawal of the antagonist treatment was studied. Fourty-eight hours after withdrawal of *dl*-propranolol (5 mg/kg i.p., twice daily for 4 days) the average LC neuronal firing rate was 2.62±0.21 spikes/s ($n = 1$, 35 cells), which corresponds to a reduction of firing rate by 12% in comparison to control.

Long-term oral treatment with *dl*-propranolol (3 mg/g pelleted food for 28 days) reduced the average LC neuronal firing rate by 41% (to 1.76±0.07 spikes/s, $n = 13$, 167 cells, $P < 0.001$, Fig. 3) and corresponding treatment with metoprolol (6 mg/g pelleted food) caused a reduction of the firing rates by 19% (to 2.42±0.10 spikes/s, $n = 7$, 122 cells, $P < 0.001$). The weight gain of these rats tended to be less than that of the control rats. After the long-term treatment the plasma concentration of metoprolol was 500±110 nmol/l ($n = 5$).

Discussion

Whereas acute or subacute treatment with the non-selective β-adrenoceptor antagonist *dl*-propranolol even in very high doses (up to 40 mg/kg i.v.) did not affect the firing rate of noradrenergic neurones in the LC, subchronic treatment with *dl*-propranolol caused a significant depression of the neuronal activity. This effect of *dl*-propranolol was dose-dependent, although a maximal inhibition by 37% was obtained already at a dose of 5 mg/kg and no further reduction was seen with higher doses. Long-term oral treatment with *dl*-propranolol for 28 days did not cause any further reduction in LC neuronal firing rates in comparison to subchronic treatment. The dextro-isomer of propranolol, which possesses the same non-specific membrane stabilizing effect as the racemate but much less (<1%) β-adrenoceptor blocking activity (Barrett and Cullum 1968) had no effect on the average LC neuronal firing rate.

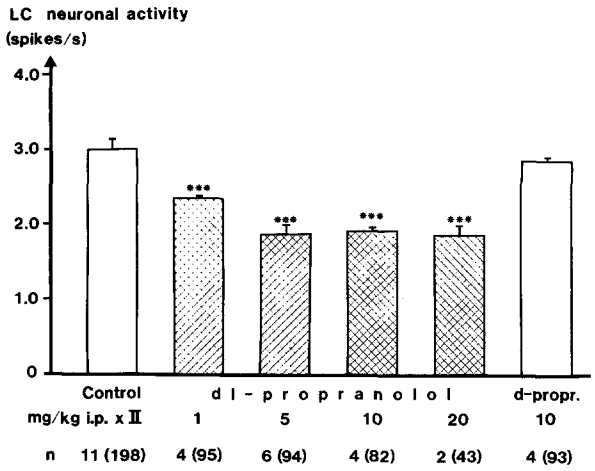

Fig. 1. Effects of subchronic treatment with *dl*- and *d*-propranolol on the average spontaneous firing rate of randomly encountered noradrenergic neurones in the locus coeruleus of the rat brain. The drugs were administered i.p. at various doses twice daily for 4 days. The figures within parenthesis indicate the number of cells recorded from, whereas the figures to the left indicate the number of animals. *** Indicate $P < 0.001$, when compared to the corresponding control values

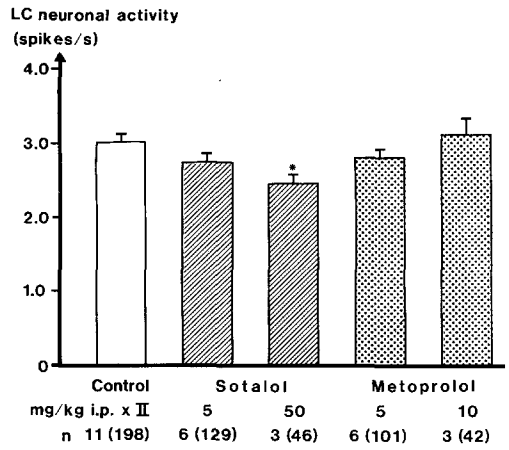

Fig. 2. Effects of subchronic treatment with sotalol and metoprolol on the average firing rate of locus coeruleus neurones of the rat. * Indicate $P < 0.05$, when compared to corresponding control values (for further explanation see Fig. 1)

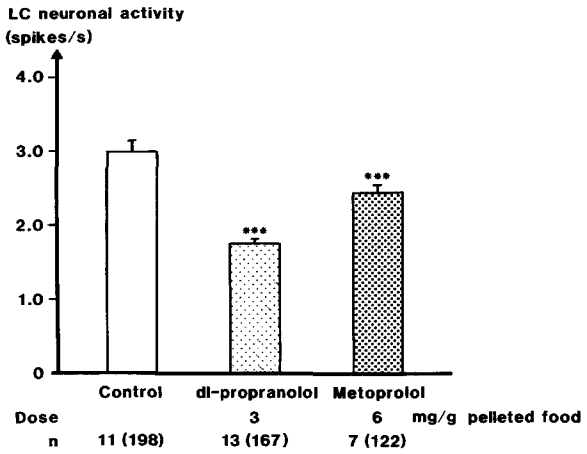

Fig. 3. Effects of long-term (chronic) treatment with *dl*-propranolol and metoprolol on the average firing rate of locus coeruleus neurones of the rat. The rats were fed for 4 weeks with pelleted food containing the respective substance. *** Indicate $P < 0.001$, when compared to corresponding control values (for further explanation see Fig. 1)

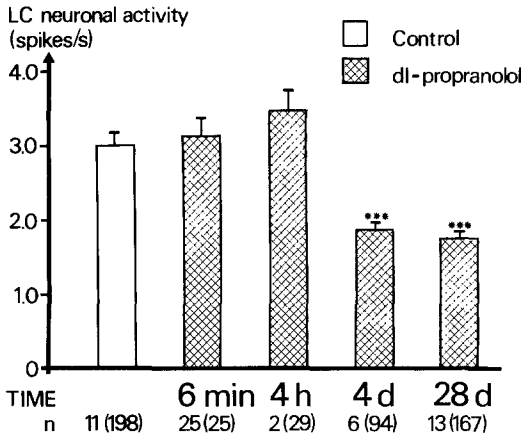

Fig. 4. Time-course of effect of *dl*-propranolol on the average firing rate of locus coeruleus of the rat. The neurones were recorded after acute (6 min after administration of *dl*-propranolol), subacute (4 h after a single injection), subchronic (4 days schedule) and chronic (28 days schedule) treatment. *** Indicate $P < 0.001$, when compared to corresponding control values (for further explanation see Figs. 1 and 3)

These findings indicate that the decrease in the firing rate of LC neurones produced by subchronic treatment with *dl*-propranolol is due to inhibition of β-adrenoceptors and not due to a local anaesthetic action of the substance. By inference, the findings indicate the existence of a stimulatory β-adrenoceptor mediated control mechanism for the firing rate of noradrenergic neurones in the LC. It is noteworthy, that the inhibitory effects of *dl*-propranolol required repeated administration of the drug. Furthermore, it appears that the depressant effect of *dl*-propranolol on the neuronal activity is still present 48 h after withdrawal of the blocker. Thus, both the onset and termination of the effects of *dl*-propranolol on central noradrenergic neurones were of a delayed nature.

With respect to their potency in inhibiting the cardiac chronotropic and peripheral vasodilator response to isoprenaline, about equipotent doses of sotalol and *dl*-propranolol were used in the present experiments (Åblad et al. 1973; B. Lundgren, personal communication). Yet the two blockers did not produce the same effect centrally, although a very high dose of sotalol (50 mg/kg) produced a slight decrease of the neuronal activity. One can therefore conclude that the depressant action of *dl*-propranolol on central noradrenergic neuronal activity is not due to a peripheral effect of the drug, but to a central β-blocking effect. The lack of activity of the hydrophilic blocker sotalol is in all probability due to its poor penetration through the blood brain barrier.

Subchronic treatment with the β_1-selective antagonist metoprolol did not produce any effect on the average LC neuronal firing rate, although the plasma and brain tissue contents of metoprolol and propranolol (5 g/kg i.p., respectively) were found to be essentially the same.

It has previously been shown, that *dl*-propranolol is about equipotent to metoprolol as regards β_1-adrenoceptor blockade but much more (50 times) potent as regards blockade of

β_2-adrenoceptor mediated actions (Åblad et al. 1973). More recently, it was shown that i.v. administration of metoprolol or *dl*-propranolol in a dose of 0.5 mg/kg caused an equipotent inhibition (about 95%) of cardiac chronotropic response to sympathetic nerve stimulation in anaesthetized dogs (Åblad et al. 1980). The plasma "peak" concentration of each drug associated with this dose is approximately 800 nmol/l, both in dog and rat. At this plasma level metroprolol did not significantly affect the vasodilator response to adrenaline in the skeletal muscle, while this response was totally inhibited by *dl*-propranolol (Åblad et al. 1980). Concequently, the lack of effect of metoprolol in the present study, suggests that the inhibitory action of *dl*-propranolol on the spontaneous firing rate of LC neurones was due to blockade of mainly β_2-adrenoceptors.

In the present study, long-term oral treatment with metoprolol had a slight depressant effect on the average firing rate of noradrenergic neurones in the LC. The plasma concentration of metoprolol was much higher after this long-term treatment than after subchronic treatment (500 and 26 nmol/l, respectively). Since the plasma samples were taken in the morning, it can be assumed that the concentration of metoprolol was 3–4 times higher during the night, which is the preferred eating period of the rat. A plasma metoprolol level of this magnitude might be sufficient to produce a blockade of central β_2-adrenoceptors, which hence could explain the weak inhibitory effect of long-term metoprolol treatment on LC neuronal activity. Alternatively, the firing rate of central noradrenergic neurones may be stimulated by β-adrenoceptors of both subtypes, which would be in accordance with the presynaptic β-adrenoceptor mediated regulation of adrenergic transmitter release in the periphery (Dahlöf et al. 1980). If this were so, the minor depression by metoprolol suggests a predominance of β_2-adrenoceptors over β_1-adrenoceptors in this control mechanism.

Taken together, the present findings suggest that the noradrenergic neurones in the LC, in addition to their inhibitory α_2-adrenoceptor mediated regulation (cf. introduction), are stimulated by a mainly β_2-adrenoceptor mediated, central mechanism. Whether the discussed effects of β-antagonists are mediated through pre- or postsynaptic β-adrenoceptors is an open question. Additional studies are required to elucidate the nature of the proposed stimulation of noradrenergic brain neurones mediated by central β-adrenoceptors as well as the localization of these receptors. The inhibition of noradrenergic neurones in the brain by β-adrenoceptor antagonists may explain some of the clinically observed central effects of these drugs.

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