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ACTIONS OF THE HYPNOTIC ANAESTHETIC, DEXMEDETOMIDINE, ON NORADRENALINE RELEASE AND CELL FIRING IN RAT LOCUS COERULEUS SLICES

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SUMMARY

We have examined the effects of the highly selective alpha$_2$-adrenoceptor agonist, dexmedetomidine, on noradrenaline release and cell firing in isolated, superfused slices of rat locus coeruleus. Dexmedetomidine decreased both noradrenaline release and cell firing rate in a concentration-dependent fashion, with an EC_{50} of 3.97 (SEM 0.97) $\times 10^{-9}$ mol litre^{-1} for noradrenaline release and 0.92 (0.53) $\times 10^{-9}$ mol litre^{-1} for unit activity. Both effects were reversed completely by the selective alpha$_2$ antagonist, atipamezole 10^{-6} mol litre^{-1}. These results suggest that cell firing and noradrenaline release are under alpha$_2$ receptor control and that dexmedetomidine potently stimulates these receptors. We conclude that these effects are consistent with the locus coeruleus being a major site of action of the hypnotic anaesthetic alpha$_2$ agonists. (Br. J. Anaesth. 1993; **71**: 447–449)

KEY WORDS

Sympathetic nervous system, pharmacology: dexmedetomidine, atipamezole.

Over the past 2 years, there has been increasing interest in the potential of alpha$_2$ adrenoceptor agonists, particularly the highly selective drug, dexmedetomidine. It has been suggested that the locus coeruleus may be an anatomical site of action for these agents [1]. It is *the* major noradrenergic nucleus, has diffuse ascending and descending projections, and controls (*inter alia*) vigilance.

Our laboratory has recently [2] developed a method for simultaneously monitoring single cell activity and neurotransmitter release in brain slices using a single carbon fibre microelectrode (CFM). This combination makes it possible to investigate quantitatively the relationship between the noradrenaline release and cell excitation or inhibition.

Most previous experimental work on alpha$_2$ agonists has centred on the pharmacological modulation of post-anaesthetic sleep times. Our aim was to examine the effects of dexmedetomidine on *individual* locus coeruleus neurones and to attempt to relate, for the first time, actions on neuronal activity and release of noradrenaline.

METHODS AND RESULTS

Rat brain slices 350 μm thick were prepared and maintained as described previously [3]. The locus coeruleus was visualized as a translucent oval area on the ventrolateral border of the fourth ventricle. A CFM was then lowered into the slice until a spontaneously active single unit could be discriminated. Cell identity was further confirmed by the standard biphasic potentials and a firing rate of 0.2–5.0 Hz.

Quantitative, real-time release of noradrenaline was measured using fast cyclic voltammetry (FCV) as described previously [4]. An input voltage of 1.5 cycles of a triangular waveform (-1.0 to $+1.4$ V *vs* Ag/AgCl) at a scan rate of 480 V s^{-1} was applied to the potentiostat at a rate of 2 s^{-1}. A sample-and-hold circuit monitored the current at the oxidation potential for noradrenaline ($+600$ mV).

Between voltammetric scans, the CFM monitored the voltage at the microelectrode tip. This voltage was amplified and filtered, resulting in an electrophysiological signal with 50-ms gaps occurring at each voltammetric scan. Cells were counted with an integrating ratemeter. A dual-channel chart recorder was used to record continuously both firing rate and stimulated release of noradrenaline.

Trains of 20 pulses (10 mA, 0.2 ms, 200 Hz) were applied every 20 min. The rate of cell firing was measured for the 2 min immediately before stimulation. A pre-drug period of 1 h was used for each experiment; three measurements of noradrenaline release were made in this period. Drug effects on release of noradrenaline and cell firing were subsequently calculated relative to this initial period. This required that the cell would fire regularly for 60 min. Cells that did not maintain this were discarded. All experiments commenced within 3 h of the dissection. Control studies ($n = 8$) were performed in which no drug was added and firing rate and release monitored over a 200-min period.

Incremental concentrations of dexmedetomidine 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} and 10^{-7} mol litre^{-1} were added at 20-min intervals, immediately after the

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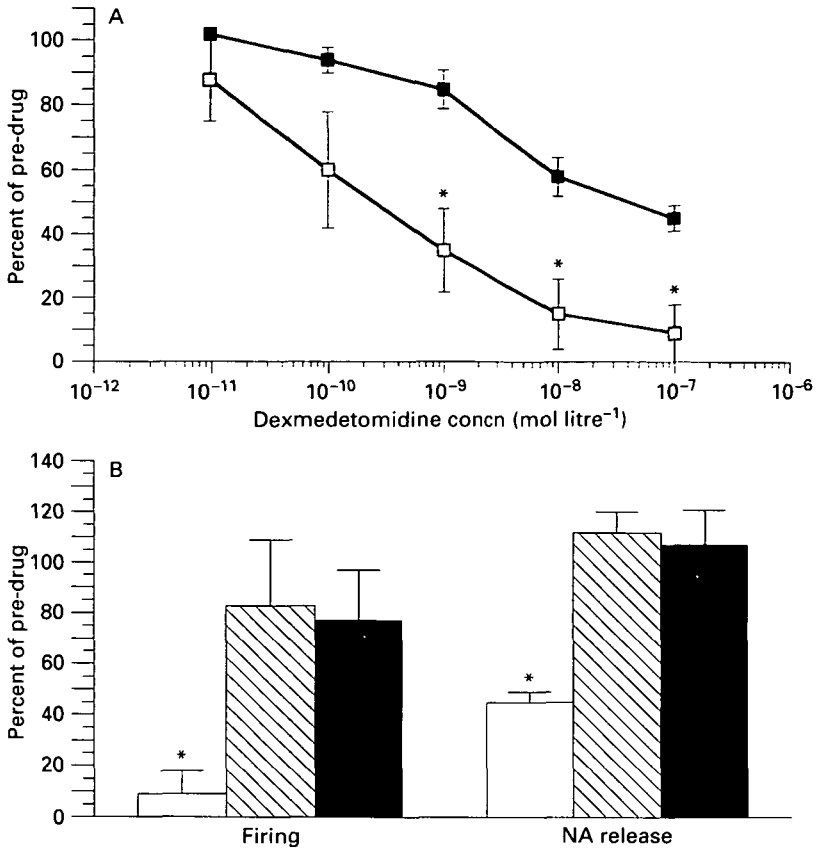

FIG. 1. A: Concentration–response curves for the reduction in cell firing rate (□) and release of noradrenaline (■) in the locus coeruleus caused by dexmedetomidine (mean, SEM; $n = 7$ slices). * $P < 0.05$: cell firing *vs* noradrenaline release (paired t test). B: Reversal of effect of dexmedetomidine 10^{-7} mol litre^{-1} by atipamezole 10^{-6} mol litre^{-1} (mean, SEM). □ = Dexmedetomidine ($n = 7$); ▨ = dexmedetomidine + atipamezole ($n = 7$) (■ = controls ($n = 8$)). * $P < 0.05$: dexmedetomidine *vs* all other groups (ANOVA). No other significant differences between groups.

stimulation that represented the effect of the previous concentration on noradrenaline release. After the effect of dexmedetomidine 10^{-7} mol litre^{-1} was recorded, atipamazole 10^{-6} mol litre^{-1} was added (to the dexmedetomidine) for a further 40 min to assess reversal. The electrodes were calibrated in noradrenaline 5×10^{-7} mol litre^{-1} at the end of the experiment.

The effects of dexmedetomidine on release of noradrenaline and cell firing were compared by paired t test. Comparison of control, dexmedetomidine and dexmedetomidine with atipamezole groups was by analysis of variance (ANOVA).

In the 15 slices examined, the mean cell firing rate was 1.7 Hz (range 0.64–4.30 Hz). Mean stimulated release of noradrenaline corresponded with a peak extracellular concentration of noradrenaline 2.1 (SEM 0.4) $\times 10^{-7}$ mol litre^{-1}. The response of the cells to the stimulations was variable, although the most common pattern was a brief excitation, followed by inhibition and then return to the baseline rate of firing. Release of noradrenaline and cell firing in control slices were generally stable over this quite long period of study (firing rate at 160 min being 86 (9)% of that in the initial 1 h, and noradrenaline release 110 (6)%).

Dexmedetomidine reduced significantly both cell firing and release of noradrenaline in a concentration-dependent manner (fig. 1A). The maximum decrease in release of noradrenaline was 55 (4)% whilst, for six of seven cells, dexmedetomidine completely abolished cell firing. EC_{50} for dexmedetomidine was 3.97 (0.97) $\times 10^{-9}$ mol litre^{-1} on release of noradrenaline and 0.92 (0.53) $\times 10^{-9}$ mol litre^{-1} on unit activity. Greater concentrations of dexmedetomidine induced a significantly ($P < 0.05$: paired t test) greater inhibition of cell firing than of release of noradrenaline.

The actions of dexmedetomidine 10^{-7} mol litre^{-1} on noradrenaline release and unit activity were reversed completely by simultaneous administration of atipamezole 10^{-6} mol litre^{-1} for 40 min, with no significant difference (ANOVA) from time-matched controls (fig. 1B).

COMMENT

Locus coeruleus neurones fire regular spontaneous action potentials in brain slice preparations for many hours [5]. The firing reflects a balance between a persistent inward sodium current active near the action potential threshold and a calcium-dependent potassium current activated after the action potential (causing an after-hyperpolarization). Known inhibitors include GABA, glycine and noradrenaline, while glutamate is excitatory. *In vivo*, the pattern of firing is more complex, as it is affected markedly by external stimuli, sleep etc.

The results of this study show that dexmedetomidine reduces both release of noradrenaline and the basal firing rate of locus coeruleus cells. The complete reversal of the effect by atipamezole, a highly selective alpha$_2$ antagonist, is strong evidence that both effects are mediated via alpha$_2$ receptors. The maximum response to alpha$_2$ receptor stimulation differs for the two indices: cell firing appears more sensitive, but also a more variable index of alpha$_2$ activity than release of noradrenaline.

It appears, on the basis of catecholamine depletion studies [6], that, in addition to the effects of the alpha$_2$ agonists on *presynaptic* alpha$_2$ receptors, some of the anaesthetic effects of dexmedetomidine result from action on *postsynaptic* alpha$_2$ receptors. The present study nevertheless shows that dexmedetomidine has potent actions in the locus coeruleus, where it acts presumably on dendritic (i.e. presynaptic) alpha$_2$ receptors, and supports the view that the locus coeruleus may be a major site of action of this drug. The apparent dissociation of the actions of the drug on cell firing and noradrenaline release may indicate different receptor subtypes or receptor reserves for these processes. This is currently under investigation.

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