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Effects of clonidine and yohimbine on a C-fibre-evoked blood pressure reflex in the rat

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1 The involvement of α_2-adrenoceptors in a capsaicin-induced depressor reflex in the rat has been investigated.

2 The reflex fall in blood pressure following an intra-arterial injection of capsaicin was partially blocked by an intrathecal infusion of clonidine (1 μg) at the spinal cord level L4–L5, an effect which was abolished by the prior intrathecal infusion of yohimbine (5 μg).

3 Clonidine (1 μg) given intracisternally blocked the depressor reflex almost completely; however, it also produced a gradual, prolonged fall in mean carotid pressure.

4 Yohimbine (20 μg) given intracisternally did not change mean blood pressure but had a dual effect on the depressor reflex in response to the capsaicin injection: a short period of enhanced reflex response was followed by a long lasting inhibition of the response.

5 It was concluded that α_2-adrenoceptors in the spinal cord inhibit the capsaicin-evoked depressor reflex and that pre- and postsynaptic α_2-adrenoceptors in the brain stem modulate this reflex.

Introduction

The intra-arterial (i.a.) injection of capsaicin into the rat hind paw is followed by a reflex fall in blood pressure (Donnerer & Lembeck, 1982). The afferent limb of this reflex consists of capsaicin-sensitive C-fibres, the reflex centre is located in the brain stem and the efferent pathway consists of a loss of the sympathetic vasoconstrictor tone (somato-sympathetic reflex, Donnerer & Lembeck, 1983). Capsaicin is far more specific in stimulating C-type primary afferents than electrical nerve stimulation (Buck & Burks, 1986).

There is evidence that, like opiates, α_2-adrenoceptor agonists, such as noradrenaline and clonidine, exert an inhibitory effect on the afferent transmission of nociceptive signals in the spinal cord (Hylden & Wilcox, 1983; Probst *et al.*, 1985; Sullivan *et al.*, 1987; Howe *et al.*, 1987). On brain stem and medullary cardiovascular centres clonidine is mainly suppressive while the α_2-antagonist yohimbine has been shown to release tonic inhibition (for reviews see Goldberg & Robertson, 1983; Kobinger, 1986).

The present work investigated whether the capsaicin-induced fall in blood pressure can be affected by clonidine and yohimbine applied locally onto the dorsal spinal cord (intrathecally) and onto the brain stem (intracisternally).

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Methods

Sprague-Dawley rats of either sex (Himberg, Austria) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.). The trachea was cannulated to ensure unobstructed respiration and the carotid artery was cannulated for blood pressure recording. A superficial epigastric artery was cannulated retrogradely for infusion of capsaicin into the hind leg as described by Donnerer & Lembeck (1982). To evoke the blood pressure reflex, 30 ng of capsaicin dissolved in 30 μl 0.9% w/v NaCl solution (saline) was infused for 30 s. The capsaicin infusions were repeated at 10 min intervals.

For the intrathecal (i.t.) application of drugs the spinal cord was exposed at the lumbar level by laminectomy and a PE 10 cannula was placed subdural with the open tip at level L4–L5 (afferent input from the sciatic and saphenous nerve). Drugs infused intrathecally were dissolved in 10 μl of saline and infused over a period of 3 min.

For intracisternal (i.cist.) drug applications the membrane covering the cisterna cerebellomedullaris

was exposed and pierced with a 35 gauge needle connected to a PE 10 cannula; 10 μl of saline or of drug dissolved in saline were infused over a period of 3 min.

The depressor responses to the capsaicin injections were calculated as decrease in blood pressure (mmHg) from the immediately preceding levels. If necessary, the duration of the depressor reflex was assessed by measuring the time it took for the blood pressure to return to the baseline after the injection (within a range of $\pm 5\%$). Differences in the depressor response during the application of various drugs were analysed by use of one way analysis of variance and Scheffe's comparisons test.

Substances

Capsaicin (Merck, Darmstadt, FRG) and yohimbine hydrochloride (Sigma, St Louis, U.S.A.) were used. Clonidine was a gift from Boehringer (Ingelheim, FRG).

Results

Each i.a. infusion of capsaicin resulted in a short-lived drop in blood pressure (depressor reflex), a response which remained constant over a period of 50 min (see Figures 1 and 2). The i.t. application of 10 μl saline did not change these responses. From Figure 1 it can be seen that clonidine, 1 μg i.t., reduced the capsaicin effect without significantly changing the mean basal blood pressure. Yohimbine, 5 μg i.t., given before clonidine abolished the inhibitory effect of clonidine on the blood pressure response to capsaicin. Intrathecal yohimbine itself had no effect on basal blood pressure or on the capsaicin elicited reflex ($n = 4$, not shown in the figure).

As shown in Figure 2, the intracisternal application of 1 μg clonidine markedly reduced the capsaicin-induced blood pressure reflex. However, clonidine lowered the basal blood pressure by 40 mmHg within 20 min. Yohimbine (20 μg i.cist.) had a dual effect: The first blood pressure response to capsaicin following intracisternal yohimbine was enlarged in magnitude and duration; whereas after an intracisternal infusion of saline the reflex lasted 1.4 ± 0.1 min, after yohimbine it lasted 3.2 ± 0.3 min ($n = 6$, $P < 0.05$). The following responses to capsaicin were gradually reduced. Despite these effects the drug did not change basal blood pressure.

When after i.cist. yohimbine one or two capsaicin infusions were omitted in the standard schedule, the next response correlated with the second or third response after i.cist. yohimbine shown in Figure 2c ($n = 4$). It was not possible to shorten the time interval between the capsaicin infusions to less than 8 min to test whether several enlarged responses could occur after yohimbine. This shows a clear time-dependence of the observed dual effects after i.cist. yohimbine.

Discussion

To overcome the systemic effects of clonidine and yohimbine on blood pressure, the drugs were applied locally either to the brain stem in the form of an intracisternal infusion or intrathecally to the lumbar spinal cord. It is unlikely that the dose of clonidine (1 μg) infused i.t. produced any systemic effects (see Marwaka *et al.*, 1983) or caused a change in spinal cord blood flow (Gordh *et al.*, 1986).

The reversibility of the effect of intrathecal clonidine by yohimbine confirmed the involvement of α_2-adrenoceptors. A high density of α_2-binding sites using [^{3}H]-*para*-aminoclonidine (Unnerstall *et al.*, 1984) or [^{3}H]-rauwolscine and [^{3}H]-yohimbine (Sullivan *et al.*, 1987) has been described in the substantia gelatinosa of the dorsal horn.

The synaptic location of α_2-binding sites in association with afferent sensory pathways has not yet been fully clarified. Observations in the mouse indicated a postsynaptic location (Wickberg & Hajos, 1987), whereas Howe *et al.* (1987) showed that in the cat lumbar spinal cord approximately 20% of the α_2-adrenoceptors are located presynaptically on the axons or terminals of primary sensory afferents. Kuraishi *et al.* (1985) and Pang & Vasko (1986) found that in the spinal cord the release of substance P, a representative of C-fibre neuropeptides, was inhibited by noradrenaline and that this effect was mediated by α_2-adrenoceptor activation. This would point to a partial involvement of presynaptic receptors.

The interpretation of the data obtained after the intracisternal injection of clonidine is complicated by the fact that, at this site, clonidine causes a decrease in sympathetic outflow and consequently a drop in mean blood pressure (Kobinger & Pichler, 1976; Baum & Becker, 1983). However, from previous experiments, it is known that the final step in the capsaicin-induced depressor reflex is also a loss in sympathetic tone (Donnerer & Lembeck, 1982). Thus, it was not surprising that in the rats injected with clonidine i.cist. there occurred a prolonged fall in blood pressure together with a loss of the capsaicin-induced depressor reflex. It is possible that the same neuronal circuit in the brain stem is involved in both phenomena. In earlier experiments it was shown that the centre of the capsaicin-depressor reflex lies in the brain stem (Donnerer & Lembeck, 1983). The excitation of a noradrenergic interneurone appears to be involved. Afferent impulses could cause the release of noradrenaline

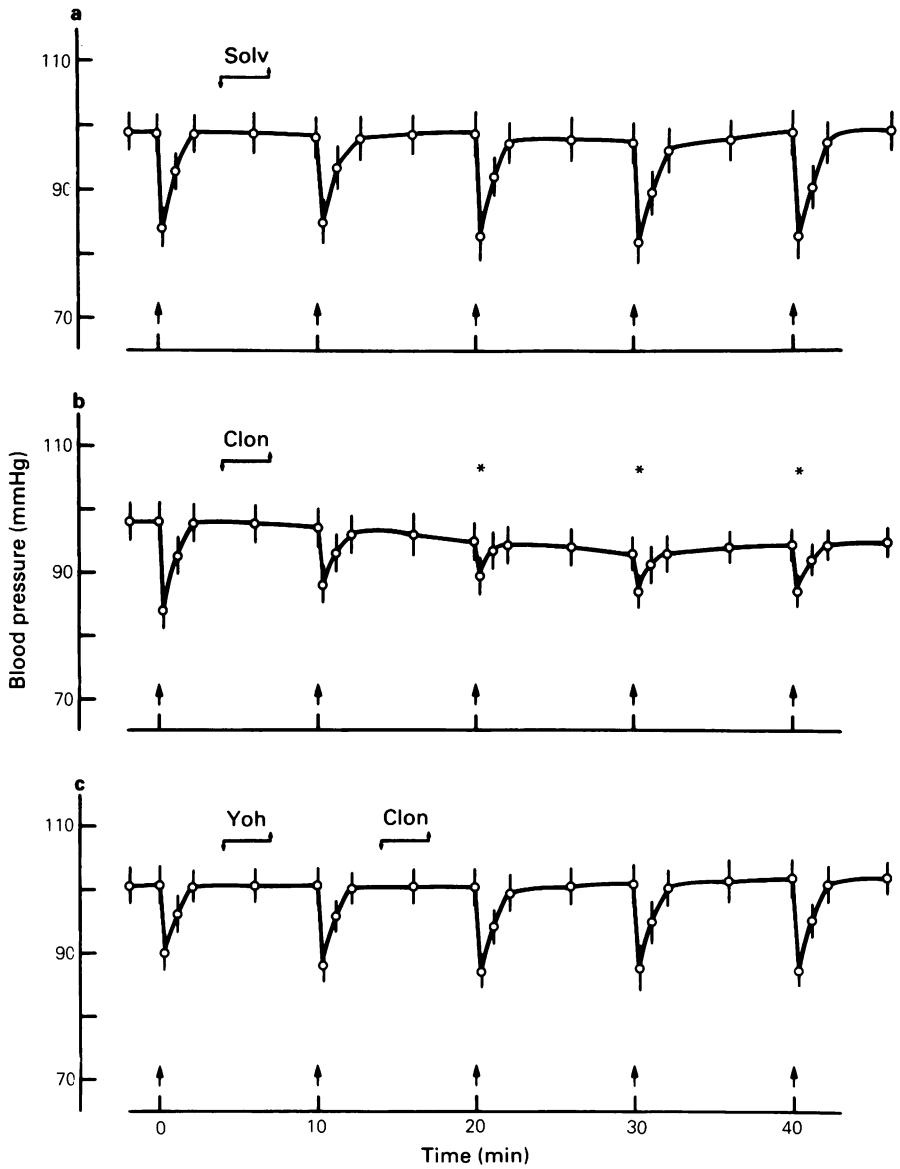

Figure 1 Mean blood pressure values in the rat carotid artery. Effect of intrathecal infusion at spinal cord level L4–L5 of (a) saline (Solv, 10 μl), (b and c) clonidine (Clon, 1 μg in 10 μl saline) and (c) yohimbine (Yoh, 5 μg in 10 μl saline) on the capsaicin (30 ng in 30 μl saline, infused i.a. over 30 s at arrows on abscissa scale)-evoked decrease in blood pressure. Mean values are shown, $n = 6$; vertical lines indicate s.e.mean. Significance of difference from 'Solv' values: $^*P < 0.05$ (Scheffe's comparison test).

within the brain stem which, in turn, would activate the same postsynaptic receptors as those activated by clonidine.

The observations made after i.cist. injections of yohimbine would fit this concept. Yohimbine, an α_2-adrenoceptor antagonist, significantly augmented the first capsaicin-induced drop in blood pressure. This effect was time-dependent, since after extending the interval to the first capsaicin infusion after i.cist. yohimbine there was no longer an enlarged response.

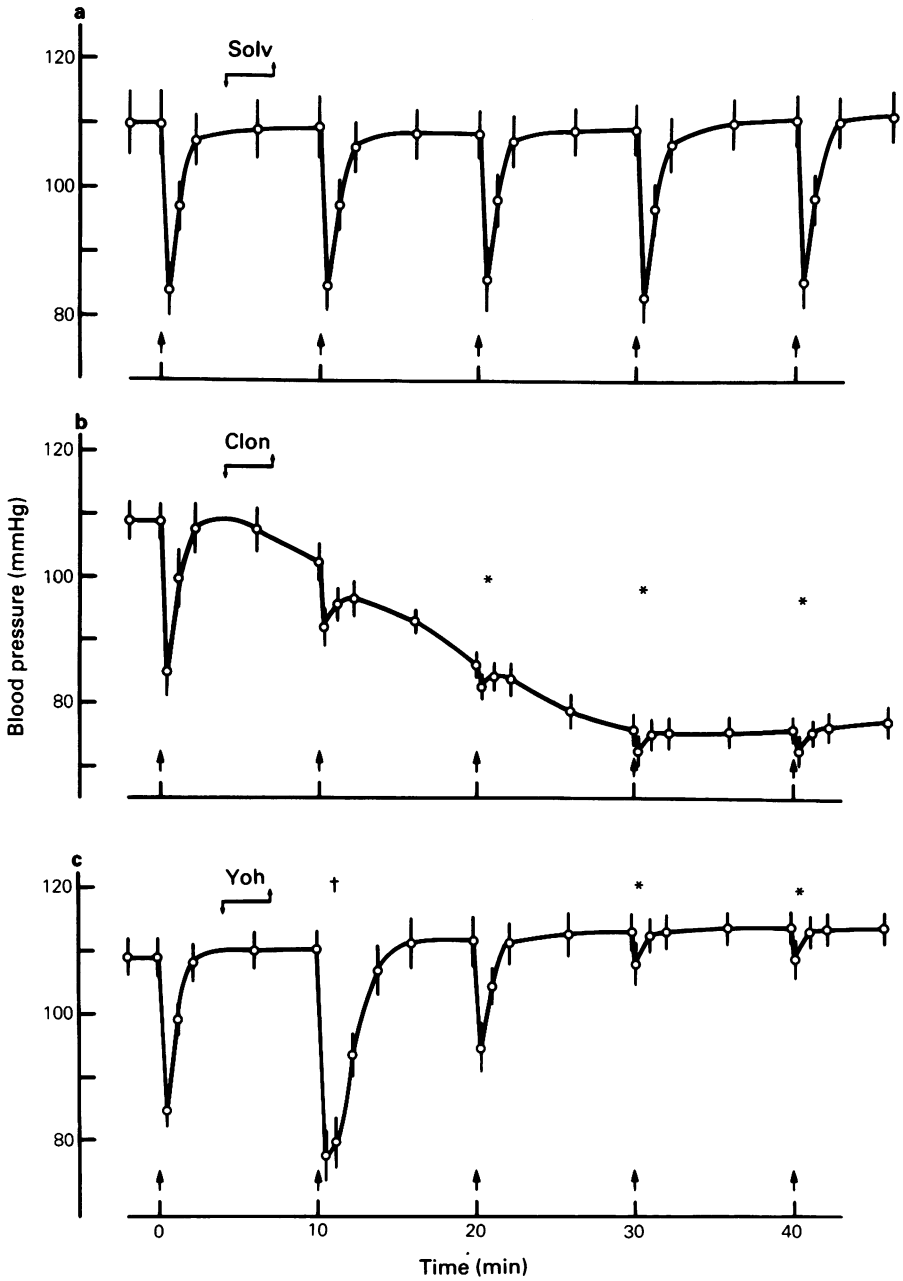

Figure 2 Mean blood pressure values in the rat carotid artery. Effect of intracisternal infusion of (a) saline (Solv, 10 μl), (b) clonidine (Clon, 1 μg in 10 μl saline) and (c) yohimbine (Yoh, 20 μg in 10 μl saline) on the capsaicin (30 ng in 30 μl saline infused i.a. over 30 s at arrows on abscissa scale)-evoked decrease in blood pressure. Mean values are shown, $n = 6$; vertical lines indicate s.e.mean. Significance of difference from 'Solv' values: $^{*}P < 0.05$; from pre-'Yoh' depressor effect: $\dagger P < 0.05$ (Scheffe's comparison test).

This could be explained if certain central α_2-adrenoceptors inhibit tonically the release of noradrenaline and blockade of these receptors resulted in an overshoot of noradrenaline release (Starke, 1987; Goldberg & Robertson, 1983). This increased noradrenaline release might initially overcome a slow onset of postsynaptic α_2-adrenoceptor blockade which, later on, is responsible for the diminished reflex response.

In summary, the results of the present experiments suggest that α_2-adrenoceptors play an important role in the initiation of somatosympathetic reflexes within the brain stem and in their modulation at a spinal level. The experiments with clonidine provide evidence that α-adrenoceptors, located in the brain stem and in the lumbar spinal cord are involved in the reflex fall in blood pressure elicited by stimulation of afferent C-fibres.

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