Table 1 The effect of duration of the interval between stimuli upon the inhibition by clonidine (3 nM)

Interval between stimuli (sec)	*% Inhibition after clonidine (Mean ± s.e.mean)*
60	69.6 ± 4.7
30	65.7 ± 4.5
15	55.8 ± 4.7
7.5	32.0 ± 5.7
3	−10.3 ± 2.9

The results are the means ± s.e.mean of 5 experiments on electrically evoked contractions (5 pulses, 1 ms, 10 Hz) of rat anococcygeus.

ported by our observation that the clonidine-induced inhibition bears an inverse relationship with the train-length; contractions evoked by short trains of stimuli were inhibited to a greater extent than those produced in response to longer trains (% inhibition ± s.e.mean at 10 Hz: 84.40 ± 7.8 with 2 pulses; 1.9 ± 4 with 40 pulses). However, other findings do not support this explanation; niether NA (10^{-9}M–10^{-6}M) nor tyramine (10^{-7}M–10^{-5}M) inhibited the transmission and clonidine-induced inhibition persisted unimpaired after treatment with cocaine (3×10^{-6}M). An alternative explanation is that the intraneuronal calcium levels determine the extent of clonidine-induced inhibition and that NA does not exert a feed-back inhibition in this issue.

Experiments involving the effect of exogenous NA on transmitter release in the anococcygeus are in progress and will hopefully provide an unequivocal answer as to which of the two possibilities is the correct one.

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Modulation of noradrenergic transmission by the presynaptic α-inhibitory feedback process in the rat heart

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There is conflicting experimental evidence about the extent to which the presynaptic α-inhibitory process modulates noradrenergic transmission. The findings that phentolamine potentiated the tachycardia to cardiac nerve stimulation in guinea-pig atria (Langer, Adler-Graschinsky & Giorgi, 1977), pithed rats (Doxey, 1977) and anaesthetized dogs (Lokhandwala & Buckley, 1976) suggests that the α-inhibitory feedback process does modulate noradrenergic transmission. However, other investigators failed to demonstrate a potentiating effect of phentolamine in rat isolated atria (Idowa & Zar, 1977) or in anaesthetized dogs (Antonaccio, Halley & Kerwin, 1974; Cavero, Lefèvre & Roach, 1977). These differences might be attributable to the species used, the frequency and duration of stimulation, or the extent to which noradrenaline was inactivated by neuronal re-uptake. In an attempt to analyse the various factors involved, the effects of presynaptic α-adrenoceptor blockade on the tachycardia produced during short (15 s) or prolonged (2–4 min) periods of cardiac nerve stimulation have been re-evaluated in pithed rats, before and after inhibition of uptake$_1$.

The tachycardia produced during short periods of stimulation at 1 Hz was potentiated 10–20% by phentolamine (0.2 mg/kg i.v.) or by cocaine (5 mg/kg i.v.). Cocaine, but not phentolamine, also prolonged the duration of the response. The combination of phentolamine and cocaine produced no greater potentiation of responses at 1 Hz than did either drug alone.

Phentolamine (0.2 mg/kg i.v.) did not significantly enhance responses to prolonged periods of stimulation at 0.1 or 1 Hz; responses at 0.2 and 0.5 Hz were potentiated, but only by 21 and 12% respectively. Cocaine (5 mg/kg i.v.) potentiated responses at 0.1 Hz by 50%, but did not significantly enhance responses to stimulation at 0.2–1 Hz. The combination of phentolamine and cocaine potentiated responses to prolonged stimulation at 0.1 Hz by a further 10–20%, but did not enhance responses at 0.2–1.0 Hz. Similar results were obtained with a higher dose of phentolamine (2.0 mg/kg i.v.) used either alone or in combination with cocaine.

Phentolamine (0.2 mg/kg) reduced the positive chronotropic response to noradrenaline (0.5 μg/kg) and tyramine (50 μg/kg) by 21 and 15% respectively, which shows that the small potentiation by phentolamine of responses to cardiac nerve stimulation is not mediated postsynaptically.

It is concluded that in the rat heart the presynaptic α-adrenoceptor feedback process exerts some control over noradrenergic transmission, but the effect is small and does not seem to be limited by uptake$_1$.

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Presynaptic α-adrenoceptors and [^{3}H]-noradrenaline overflow from the mouse vas deferens

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The twitch response of the field stimulated mouse vas deferens is inhibited by clonidine, a selective presynaptic α-adrenoceptor agonist, and potentiated by yohimbine, a selective presynaptic α-adrenoceptor antagonist. (Marshall, Nasmyth, Nicholl & Shepperson, 1978).

We have now investigated the effects of these drugs upon the overflow of tritium, [^{3}H]-noradrenaline and its metabolites from the mouse vas deferens previously loaded with [^{3}H]-noradrenaline. Six vasa deferentia were suspended in a 1.0ml or 2.0ml organ bath containing magnesium free Krebs with 10mg/l EDTA, 20mg/l ascorbic acid, and 3.7μM oestradiol. Preparations were stimulated for 2 min at 1 Hz, 2 ms, 256 mA and the bath fluid was collected for 2 min periods before (basal), during and for 4 min after stimulation. Total tritium, and [^{3}H]-noradrenaline released upon stimulation (stimulated value minus basal), were expressed as a fraction of that remaining in the tissue at the time of stimulation (Dubocovich & Langer, 1976). Noradrenaline and its metabolites were separated on alumina and Dowex columns. (Graefe, Stefano & Langer, 1973).

Stjärne (1975) reported that clonidine (10 nM) did not alter the fractional release of tritium in the guinea pig vas deferens. In agreement with this, fractional release of tritium in the present experiments was not altered by 2.8 nM or 11.2 nM clonidine in the mouse vas deferens (t test, $P>0.05$).

The fractional release of [^{3}H]-noradrenaline (mean ± s.e. mean) in 3 successive stimulation periods was $5.94 \times 10^{-4} \pm 1.37$; $5.17 \times 10^{-4} \pm 1.75$; $3.99 \times 10^{-4} \pm 1.3$. This did not represent a significant fall off in [^{3}H]-noradrenaline over the 3 periods (t test $P>0.05$). Clonidine 2.8 nM was added 30 s before the second stimulation, and 11.2 nM 30 s before the third stimulation. Fractional release of [^{3}H]-noradrenaline in the control was $4.60 \times 10^{-4} \pm 0.8$, and clonidine 2.8 nM and 11.2 nM reduced this to $1.31 \times 10^{-4} \pm 0.34$ and $1.27 \times 10^{-4} \pm 0.31$ respectively. Unlike clonidine and in agreement with Starke, Borowski & Endo (1975) yohimbine increased tritium overflow from a control value of $3.80 \times 10^{-3} \pm 0.54$ to $5.59 \times 10^{-3} \pm 0.7$. When this is split into [^{3}H]-noradrenaline and metabolites, fractional [^{3}H]-noradrenaline increased from $4.05 \times 10^{-4} \pm 1.24$ to $10.11 \times 10^{-4} \pm 1.54$.

The regulation of twitch height by presynaptic α-adrenoceptors is potentiated by reducing the concentration of calcium in the Krebs from 2.54 to 1.27 mM (Marshall, Nasmyth & Shepperson, 1977). In low calcium, Krebs yohimbine potentiates the twitch but the overflow of tritium and [^{3}H]-noradrenaline was not increased above controls where no antagonist was present.

Low doses of selective presynaptic α-adrenoceptor agonists decrease the twitch height and decrease the overflow of [^{3}H]-noradrenaline although these were not dose related. Conversely a selective presynaptic α-adrenoceptor antagonist increases twitch height, tritium and [^{3}H]-noradrenaline overflow. This relationship is not observed with tissues in half calcium concentration Krebs.

N.B.S. is an MRC student.

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