The relationship between pre-synaptic α-adrenoceptors, stimulation frequency and calcium

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The pre-synaptic α-adrenoceptor feedback mechanism controlling noradrenaline (NA) release shows frequency dependence and is maximally effective at low rates of stimulation (Starke, Endo & Taube, 1975; Dubocovich & Langer, 1976). Various drugs, which we have previously suggested to act via this mechanism, have now been studied to see if they exhibit frequency dependence.

Responses of the mouse vas deferens were elicited by field stimulation using pulses of 2.0 ms, 64 V and frequencies of 0.2, 1.0, 5.0, 10 and 16 Hz. Agonists which stimulate pre-synaptic α-adrenoceptors such as clonidine and noradrenaline (Marshall, Nasmyth, Nicholl & Shepperson, 1977) or tyramine which acts by releasing endogenous noradrenaline (Axelrod, Gordon, Hertting, Kopin & Potter, 1962) all inhibit the twitch and this is inversely proportional to the rate of stimulation. Clonidine (5.6 nM), noradrenaline (3 μM), and tyramine (20 μM) inhibited the twitch at 0.2 Hz by 78%, 79% and 68% respectively and this was reduced at 16 Hz to only 2%, 18% and 2% respectively.

Antagonists at the pre-synaptic α-adrenoceptor, yohimbine (128 nM), phenoxybenzamine (15 μM) and phentolamine (10 μM) potentiated the twitch and this was greatest at low rates of stimulation. However with all the antagonists the maximal effect occurred at 1.0 Hz and not at 0.2 Hz as with the agonists.

The pre-synaptic regulation of NA output may be inversely proportional to the influx of calcium ions. Therefore, at high rates of stimulation the influx of calcium is assumed to be so large that pre-synaptic α-adrenoceptor regulation is ineffective (Starke, Taube & Borowski, 1977; Langer, 1977). To test this possibility the effect of halving the calcium concentration in the Krebs (from 2.5 mM to 1.25 mM) on the twitch and on the effects of clonidine and yohimbine at different rates of stimulation have been studied.

The lowering of the calcium decreased the twitch tension developed at all rates of stimulation ($P < 0.05$). If the regulatory effect of pre-synaptic α-adrenoceptor agonists is reduced by the accumulation of calcium at high rates of stimulation, then reducing the concentration of this ion should increase the inhibitory effect of clonidine at all rates of stimulation. This it did most markedly at 10 Hz and 16 Hz where clonidine (5.6 nM) now inhibited the twitch by 53% and 34% respectively compared to 25% and 2% in Krebs containing 2.5 mM calcium. There was also a change in the potentiation of the twitch produced by yohimbine. Halving the calcium concentration shifted the yohimbine (128 nM) frequency-effect curve to the right with the maximum increase in twitch height now occurring at 5 Hz instead of 1 Hz.

All the agonists and antagonists showed frequency-dependence supporting earlier evidence suggesting a pre-synaptic mode of action. The hypothesis that high calcium influx reduces the effectiveness of this regulatory mechanism was supported by experiments in which halving of the calcium concentration increased the effectiveness of clonidine and yohimbine at high rates of stimulation.

NBS is an MRC student.

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