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Pre- and postsynaptic α-adrenoceptor antagonism by indoramin in isolated tissues of the rat

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There is considerable evidence that adrenergic nerve terminals carry α-adrenoceptors which play an inhibitory role in transmitter release. It has been suggested that the presynaptic and postsynaptic α-adrenoceptors differ and that certain antagonists show specificity for one or other type of receptor (Starke, Borowski & Endo, 1975; Borowski, Ehrl & Starke, 1976).

We have compared indoramin hydrochloride, an antihypertensive agent with α-adrenoceptor antagonist activity (Alps, Hill & others, 1972) with phentolamine mesylate (Ciba) and thymoxamine hydrochloride (Warner) for presynaptic effects on the rat field-stimulated vas deferens preparation using a modification of the method of Drew (1977).

Desheathed vasa deferentia from sexually mature rats were suspended in a 6 ml organ bath in Krebs solution at 37° and bubbled with 5% CO_2 in oxygen. Platinum ring electrodes were positioned above and below the tissue for field stimulation, the stimulus parameters being 0·1 Hz, 1 ms pulse width at supramaximal voltage (Square wave stimulator, Scientific and Research Instruments Ltd.). Twitch responses were recorded isotonically (Harvard Apparatus Smooth Muscle Transducer) with a 0·5 g loading. Clonidine hydrochloride was used as the α-adrenoceptor agonist and cumulative concentration-response curves were constructed for the inhibition of twitch obtained with clonidine in the range 0·125 to 4 ng ml^{-1}. After washing out clonidine, the twitch response quickly recovered and an antagonist was then introduced into the Krebs reservoir. Clonidine concentration-response curves were repeated 90 min after introduction of the antagonist. The concentrations of clonidine producing 50% inhibition of twitch before and after introduction of antagonist were obtained and the dose-ratio for clonidine was calculated. Molar concentrations of antagonists used were: indoramin 10^{-6}, 10^{-5} and 5×10^{-5}, thymoxamine 10^{-5}, $2{\cdot}239 \times 10^{-5}$ and 5×10^{-5} and phentolamine 10^{-7}, $2{\cdot}239 \times 10^{-7}$, 5×10^{-7} and 10^{-6}. At least four preparations were used at each concentration.

None of the antagonists inhibited the twitch response. Both phentolamine and thymoxamine produced concentration-dependent antagonism of the clonidine response.

These results were plotted in the manner described by Arunlakshana & Schild (1959) and the values of pA_2 and slope were calculated. Indoramin produced no antagonism at 10^{-6} M, and a mean pA_2 of 5·13 ($n = 5$) at 10^{-5} M (pA_2 values calculated from individual dose-ratios assuming a plot slope of unity). Preparations showed spontaneous contractions when exposed to 5×10^{-5} M indoramin and the effects of clonidine could not be assessed. A summary of results is shown in Table 1. This table also includes data previously published from this laboratory (Collis & Alps, 1973) showing the activity of these antagonists against noradrenaline at postsynaptic α-adrenoceptors in the isolated perfused rat mesenteric vasculature preparation.

These results suggest that indoramin possesses little antagonist activity at presynaptic α-adrenoceptors in contrast to its marked competitive antagonism at the postsynaptic site. Thymoxamine resembles indoramin in having some selectivity for postsynaptic receptors but the slope of the Schild plot is not that expected for a simple competitive antagonism at presynaptic receptors.

Table 1. *Plot slopes and pA_2 values for α-adrenoceptor antagonists on the field-stimulated rat vas deferens and on the perfused rat mesenteric vasculature preparations derived according to Arunlakshana & Schild* (1959).

	Rat vas deferens		Rat mesenteric vasculature	
Antagonist	pA_2	Slope	pA_2	Slope
Indoramin	5·13*	—	8·05	−1·06
Phentolamine	7·90	−1·07	7·84	−0·91
Thymoxamine	4·85	−1·67	6·47	−1·00

* Mean value calculated from dose-ratios obtained at 10^{-5} M indoramin assuming a plot slope of unity ($n = 5$).

* Correspondence.

Phentolamine is a competitive antagonist with no apparent selectivity for pre- or postsynaptic sites.

Rand, McCulloch & Storey (1975) have suggested that the ineffectiveness of α-adrenoceptor antagonists such as phentolamine as antihypertensive agents could be related to blockade of presynaptic receptors. This would result in a loss of the inhibitory feedback loop and enhanced release of noradrenaline from sympathetic nerves. The increased noradrenaline concentration could overcome competitive antagonism at α-adrenoceptors on vascular smooth muscle. It is possible that indoramin, an antagonist with specificity for the postsynaptic α-adrenoceptor, might prove more effective in lowering blood pressure in man.

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Effects of 4-aminopyridine on the isolated parasympathetically-innervated oesophagus of the domestic fowl chick

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4-Aminopyridine facilitates neuromuscular transmission in skeletal muscle by an action on the motor nerve endings through which acetylcholine release is increased (Molgo, Lemeignan & Lechat, 1975; Foldes, Agoston & others, 1976; Bowman, Harvey & Marshall, 1977; Lundh & Thesleff, 1977); it has been used as an anti-curare agent in anaesthetic practice (Stoyanov, Vulchev & others, 1976). The compound also facilitates adrenergic transmission in the rabbit isolated vas deferens (Johns, Golko & others, 1976), the rat isolated portal vein (Leander, Arner & Johansson, 1977), and the cat spleen (Kirpekar, Kirpekar & Prat, 1978).

In the experiments described here, the effects of 4-aminopyridine were studied on the isolated parasympathetically-innervated upper oesophagus preparation of domestic fowl chicks (White Leghorn, aged 3–8 days). The muscle in this preparation is entirely smooth muscle. The preparation, with its right parasympathetic nerve attached, was suspended in Krebs solution at 32°, exactly as described by Bowman & Everett (1964). The nerve was stimulated with rectangular pulses of 0·5 ms duration and of twice the strength required to produce a maximal contraction when stimulated at a frequency of 1 Hz. Contractions were recorded with an isometric (Grass model FTO3) or an isotonic (Washington, type T11) transducer, the latter loaded with 1 g, on a Servoscribe (model RE520.20) or a Washington (model 400 MD/2) pen recorder. Generally, the preparations responded more consistently when contracting isotonically, but similar results were obtained with both methods.

4-Aminopyridine in concentrations of 10^{-5} to 10^{-4} M increased the contractions produced by stimulation of the nerve when the applied frequency was below that which produced maximal responses, but maximal responses to nerve stimulation or those produced by acetylcholine, carbachol, or methacholine (2×10^{-7} to 10^{-6} M) were usually not affected. These results indicate that neuroeffector transmission was facilitated by these concentrations of 4-aminopyridine but that contractility of the smooth muscle was unaffected. Fig. 1 illustrates an experiment in which trains of 50 stimuli were applied to the nerve at different frequencies, and responses to methacholine were produced, before and in the presence of two concentrations (5×10^{-5} and 10^{-4} M) of 4-aminopyridine. With the higher concentration, a previously ineffective control frequency of stimulation now produced a substantial response, and the previous threshold frequency produced about the maximal response. With lower concentrations of 4-aminopyridine (10^{-5} M), the facilitatory action on transmission took about 20 min to reach its maximum. The effect developed more quickly with higher concentrations. In a concentration of 10^{-4} M, 4-aminopyridine restored contractions to nerve stimulation (1 Hz for 10 s) that had been blocked by atropine (5×10^{-7} M).

High concentrations of 4-aminopyridine (5×10^{-4} to 10^{-3} M) produced oscillating contractions of the muscle and a slowly developing increase in tone in the absence of nerve stimulation. The increase in tone was aug-

* Correspondence.