Inhibition of accumulation of adrenaline and noradrenaline in arterial smooth muscle by steroids

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Steroid hormones potentiate the response of arterial smooth muscle to catecholamines (Kalsner, 1969a, b). It is possible that in arteries, where few smooth muscle cells are in close proximity to adrenergic nerves, extraneuronal accumulation and metabolism of noradrenaline may serve as an important route of inactivation (Lightman & Iversen, 1969). A steroid-induced inhibition of $Uptake_2$ in the rat heart has been demonstrated (Iversen & Salt, 1970). The present experiments were undertaken to assess directly the effect of steroids on the accumulation of adrenaline and noradrenaline in arterial smooth muscle.

Dutch rabbits of either sex (1·5–2·5 kg) were killed by a lethal intravenous dose of sodium pentobarbitone. The central ear arteries were both cannulated, removed and perfused with Krebs bicarbonate solution (de la Lande & Harvey, 1965). The steroids, dissolved in propylene glycol, were added to the perfusate. Noradrenaline and adrenaline were perfused at a concentration of 5×10^{-4} g/ml. After the perfusions, short lengths were cut from the end of the arteries and prepared for fluorescence microscopy using the technique of Falck and Hillarp. Transverse sections were cut at 7 μm and immersed in liquid paraffin on a microscope slide where the fluorescence brightness of the smooth muscle cells was measured photometrically. In this way, the increase in fluorescence brightness of the smooth muscle could be easily distinguished from that of the adrenergic nerves and connective tissue, following perfusion with adrenaline or noradrenaline.

17β-Oestradiol, corticosterone and the non-steroidal oestrogen, diethylstilboestrol all exhibited a dose-dependent inhibition of the smooth muscle accumulation of adrenaline and noradrenaline in the rabbit ear artery. The catecholamine binding ability of collagen was unaffected. 17β-Oestradiol was the most potent inhibitor of noradrenaline accumulation (ID50 $1 \cdot 6 \times 10^{-7}$ M). ID50 values for corticosterone and diethylstilboestrol were $2 \cdot 0 \times 10^{-7}$ M and $1 \cdot 8 \times 10^{-6}$ M respectively. The rank order of potency for these inhibitors is the same as that found by Iversen & Salt (1970) for $Uptake_2$ in the rat heart. In our hands, normetanephrine, a potent inhibitor of $Uptake_2$ in the rat heart, gave an ID50 of $6 \cdot 0 \times 10^{-5}$ M. The smooth muscle accumulation of adrenaline was also inhibited although higher concentrations of steroids were necessary, for example the ID50 of 17β-oestradiol for inhibition of noradrenaline accumulation was $2 \cdot 4 \times 10^{-6}$ M.

The perfused artery preparation had the advantage that the steroid-induced potentiation of the vasoconstrictor response could also be followed. A further effect was that the prevention of smooth muscle accumulation of adrenaline and noradrenaline greatly reduced the prolongation of the vasoconstrictor response to these amines which normally follows their uptake into and subsequent release from arterial smooth muscle (Avakian & Gillespie, 1968). The high plasma concentrations of corticosteroids encountered during stress might, by such a mechanism, prevent an undue prolongation of the peripheral vascular effects of adrenaline.

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Is thymoxamine a specific α-adrenoceptor blocking agent?

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Thymoxamine is a competitive α-adrenoceptor blocking agent with fewer unwanted actions than other α-adrenoceptor blocking drugs. It has some antihistaminic activity (Birmingham & Szolcsanyi, 1965) but is otherwise considered to be specific. In this study using the guinea-pig isolated vas deferens-hypogastric nerve preparation, it is shown that thymoxamine has activity which appears to be independent of α-adrenoceptor blockade.

The guinea-pig vas deferens was stimulated via the nerve at a rate of 20 Hz for 7 s every 2 min, using supramaximal voltage and a pulse width of 0·5 ms. The preparation was bathed in McEwen's Ringer (1956) maintained at 31±1° C and aerated with a mixture of 95% O_2 and 5% CO_2. Figure 1 shows the effects of thymoxamine in these conditions. Appreciable reduction in the size of response was produced in concentrations of 5–125 ng/ml. A similar situation existed when the transmurally stimulated vas was used (Birmingham & Wilson, 1963). In these concentrations,

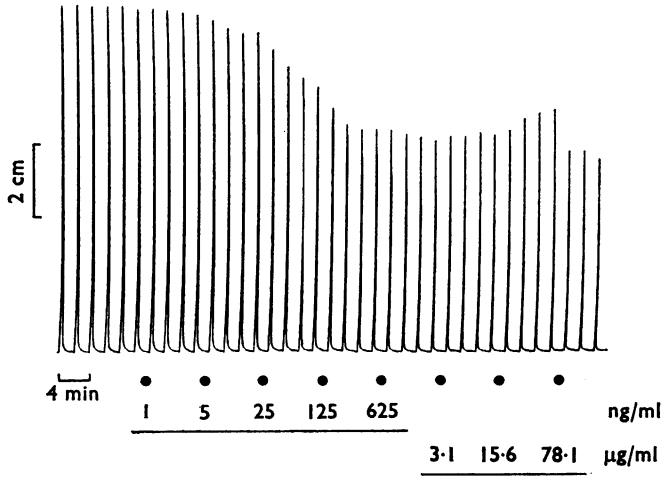

FIG. 1. Cumulative concentration-effect curve for thymoxamine on the guinea-pig isolated vas deferens stimulated via the hypogastric nerve.