Inhibition of catecholamine Uptake$_2$ by steroids in the isolated rat heart

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Various steroids (17-β-oestradiol, corticosterone, deoxycorticosterone, progesterone, testosterone and androsterone) produced a dose-dependent inhibition of the uptake of ^{3}H-noradrenaline by the Uptake$_2$ mechanism in the isolated perfused heart. It is suggested that these results may explain the potentiating effects of such steroids on the responses of vascular smooth muscle to catecholamines.

Steroid hormones have been shown to enhance the responses of vascular smooth muscle to catecholamines (Zweifach, Shorr & Black, 1953; Schayer, 1964; Kalsner, 1969a, b) and to decrease their rate of inactivation by these tissues (Kalsner, 1969a, b). In vascular smooth muscle, which generally possesses a relatively sparse sympathetic innervation, extraneuronal uptake and metabolism may play an important role in inactivating catecholamines which reach it both via the blood stream and via release from its sympathetic nerve supply (Lightman & Iversen, 1969). The present experiments were designed to test the effects of steroids on the extraneuronal uptake process for catecholamines in the rat heart (Uptake$_2$) (Iversen, 1965).

Methods.—Male albino Wistar rats weighing 150–200 g were injected intraperitoneally with sodium pentobarbitone, 60 mg/kg, and heparin 1,000 units. Five minutes later the hearts were removed and perfused by the Langendorff technique, as previously described (Iversen, 1963). After perfusion for 5 min with amine-free solution the hearts were perfused for a further 4 min with Krebs solution containing (±)-^{3}H-noradrenaline (NA) (Radiochemical Centre, Amersham, 7·0 Ci/mmol) 125 nCi/ml, diluted with non-radioactive (±)-NA to a final concentration of 5 μg/ml. The steroids and the stilboestrol were dissolved in a small volume of propylene glycol and added to this medium. Hearts were removed, blotted and homogenized in 2 ml 1% EDTA and 10 ml of acid ethanol. The radioactivity in 1 ml aliquots of the supernatant was measured in a Packard Tri-Carb liquid scintillation spectrometer using 0·4% Butyl PBD (CIBA) in toluene as the scintillator. Efficiency of counting was determined by the use of external standards. The content of labelled NA and metabolites was calculated and the results were corrected for the presence of ^{3}H-NA in the extracellular space of the tissue, assuming this to be 33% of the wet weight (Iversen, 1963). Lightman & Iversen (1969) showed that at a perfusion concentration of 5 μg/ml, unchanged ^{3}H-NA accounted for more than 70% of the total radioactivity content of the heart, and in preliminary experiments we have found that steroids do not alter the proportion of ^{3}H-NA metabolites formed during the course of such experiments. The extraneuronal uptake of NA was calculated after correcting for a predicted intraneuronal uptake of ^{3}H-NA of 0·8 μg/g per 4 min (Lightman & Iversen, 1969). The concentration of steroid causing 50% inhibition of Uptake$_2$ (IC50) was calculated by a graphical method (Fig. 1).

Results.—All of the steroids tested produced a concentration-dependent inhibition of Uptake$_2$. Figure 1 shows the results obtained with some of the steroids tested and also with diethylstilboestrol, a synthetic non-steroidal compound with strong oestrogenic activity. 17-β-oestradiol, corticosterone and deoxycorticosterone appear to be potent inhibitors of Uptake$_2$, with IC50 values comparable with the most active inhibitors hitherto studied (Lightman & Iversen, 1969).

Preliminary experiments using female albino Wistar rats indicated that the potency of the steroids tested as inhibitors of Uptake$_2$ did not differ significantly from their activity in the males. Testosterone and androsterone are of comparable potency with 17-β-oestradiol in both sexes.

It is of interest that whereas corticosterone, the naturally secreted glucocorticoid in the rat, was a potent inhibitor of Uptake$_2$, hydrocortisone—which is not secreted in the rat—had little or no activity; the latter at a concentration of 10 μg/ml produced only 6% inhibition compared to the 95% inhibition caused by the same concentration of corticosterone.

In experiments in which ^{3}H-NA and labelled metabolites were separated by ion

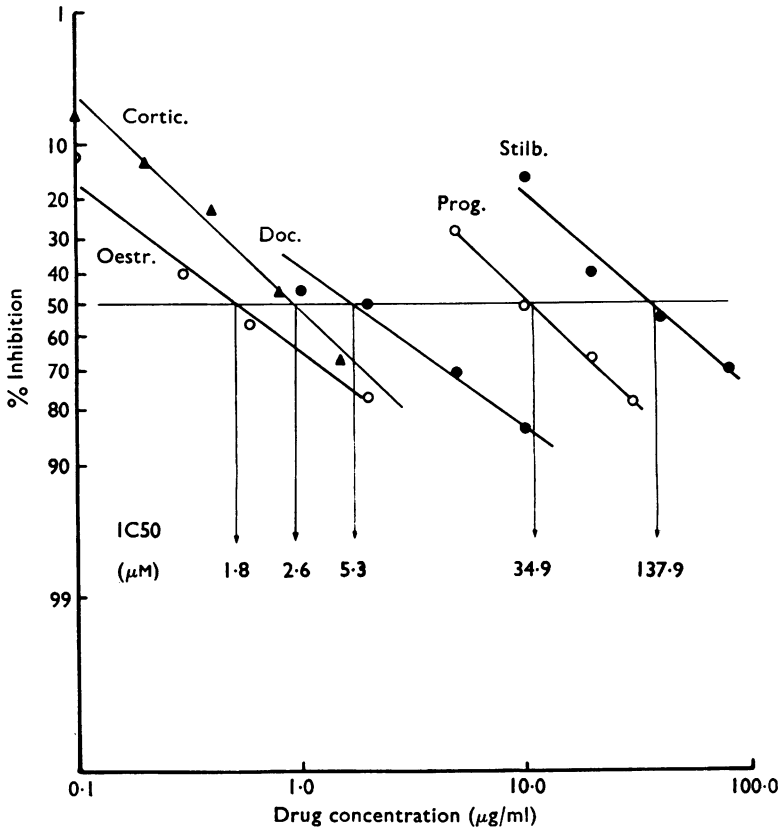

FIG. 1. Determination of Uptake$_2$ IC50 values for steroids. Inhibition of Uptake$_2$ in the rat heart by the addition of steroids to the perfusion medium. Percentage inhibition of Uptake$_2$ (probability scale) is plotted against steroid concentration (log scale) to determine IC50 steroid concentration producing 50% inhibition. Each point is the mean value for six hearts. Oestr., 17-β-oestradiol; Cortic., corticosterone; Doc., deoxycorticosterone; Prog., progesterone; Stilb., diethylstilboestrol.

exchange chromatography it was found that the accumulation of both unchanged ^{3}H-NA and its metabolites was reduced by the steroids indicating that the primary action of the steroids is on Uptake$_2$ rather than on the subsequent metabolism of catecholamines by catechol-O-methyl transferase (COMT) as previously suggested by Kalsner (1969a). This conclusion is also supported by the finding that steroids inhibited the accumulation of ^{3}H-NA even when the metabolism of the catecholamine was prevented by inhibition of monoamine oxidase and COMT.

Some of the steroids were also tested as inhibitors of the uptake of ^{3}H-NA by the neuronal uptake system in the sympathetic innervation of the rat heart (Uptake$_1$) (Iversen, 1967). Corticosterone, even at a concentration of 10 μg/ml had no effect on Uptake$_1$, but oestradiol was found to be weakly active as an inhibitor of Uptake$_1$.

Discussion.—The present results indicate that various steroids inhibit the Uptake$_2$ mechanism in the rat heart with potencies comparable with the most active inhibitors previously described. It seems probable that the steroids may also inhibit uptake of NA into vascular smooth muscle, and this may account for their potentiation of the actions of exogenous NA in such tissues (Kalsner, 1969a, b). There is, in general, a good agreement between our present results regarding the rank order of potencies of steroids as Uptake$_2$ inhibitors and the results reported for the same compounds by Kalsner (1969b) concerning the potentiation of the effects of adrenaline in the rabbit aortic strip preparation.

The concentrations of free corticosterone in the plasma of the rat are normally at least ten times lower than the IC50 value for $Uptake_2$. In certain conditions, however, plasma concentrations may rise towards levels at which an effective block of $Uptake_2$ could occur, for instance during stress. In such circumstances the physiological effects of circulating catecholamines might be enhanced.

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