

Inhibition of catecholamine Uptake₂ by steroids in the isolated rat heart

L. L. IVERSEN AND P. J. SALT

*Department of Pharmacology,
University of Cambridge*

Various steroids (17- β -oestradiol, corticosterone, deoxycorticosterone, progesterone, testosterone and androsterone) produced a dose-dependent inhibition of the uptake of ³H-noradrenaline by the Uptake₂ 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 (Uptake₂) (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 (\pm)-³H-noradrenaline (NA) (Radiochemical Centre, Amersham, 7.0 Ci/mmol) 125 nCi/ml, diluted with non-radioactive (\pm)-NA to a final concentration of 5 μ g/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 ³H-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 μ g/ml, unchanged ³H-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 ³H-NA metabolites formed during the course of such experiments. The extraneuronal uptake of NA was calculated after correcting for a predicted intraneuronal uptake of ³H-NA of 0.8 μ g/g per 4 min (Lightman & Iversen, 1969). The concentration of steroid causing 50% inhibition of Uptake₂ (IC50) was calculated by a graphical method (Fig. 1).

Results.—All of the steroids tested produced a concentration-dependent inhibition of Uptake₂. 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 Uptake₂, 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 Uptake₂ 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 Uptake₂, hydrocortisone—which is not secreted in the rat—had little or no activity; the latter at a concentration of 10 μ g/ml produced only 6% inhibition compared to the 95% inhibition caused by the same concentration of corticosterone.

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

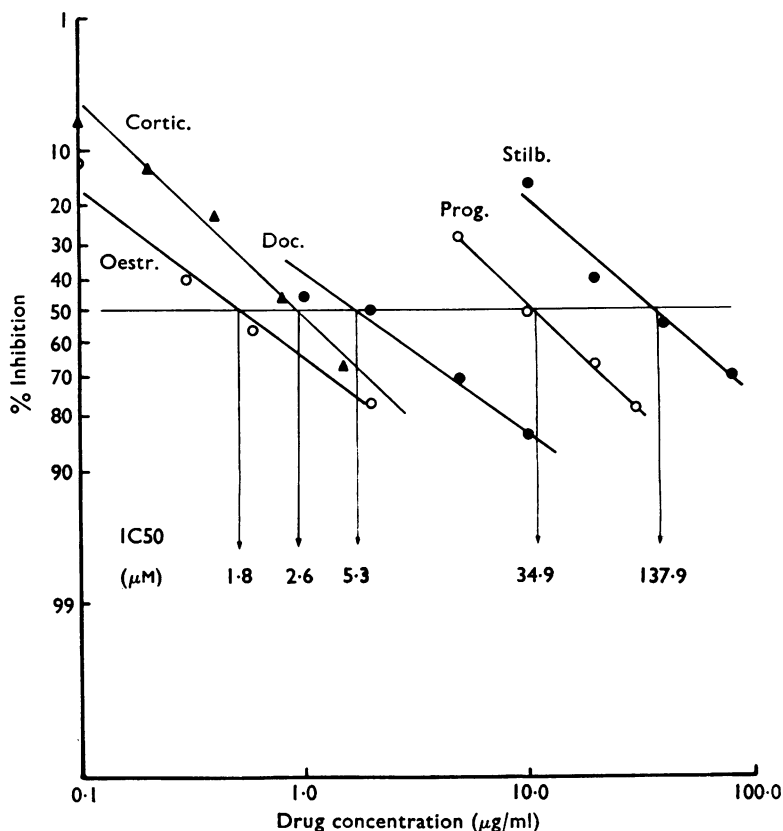


FIG. 1. Determination of Uptake₂ IC₅₀ values for steroids. Inhibition of Uptake₂ in the rat heart by the addition of steroids to the perfusion medium. Percentage inhibition of Uptake₂ (probability scale) is plotted against steroid concentration (log scale) to determine IC₅₀ steroid concentration producing 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 ³H-NA and its metabolites was reduced by the steroids indicating that the primary action of the steroids is on Uptake₂ 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 ³H-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 ³H-NA by the neuronal uptake system in the sympathetic innervation of the rat heart (Uptake₁) (Iversen, 1967). Corticosterone, even at a concentration of 10 μ g/ml had no effect

on Uptake₁, but oestradiol was found to be weakly active as an inhibitor of Uptake₁.

Discussion.—The present results indicate that various steroids inhibit the Uptake₂ 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 Uptake₂ 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 Uptake₂. In certain conditions, however, plasma concentrations may rise towards levels at which an effective block of Uptake₂ could occur, for instance during stress. In such circumstances the physiological effects of circulating catecholamines might be enhanced.

This study was supported in part by a grant to L. L. I. by the British Heart Foundation. P. J. S. is in receipt of a Science Research Council Studentship for training in research methods.

REFERENCES

- IVERSEN, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.*, **21**, 523-537.
- IVERSEN, L. L. (1965). The uptake of catecholamines at high perfusion concentrations in the isolated heart: a novel catecholamine uptake process. *Br. J. Pharmac. Chemother.*, **25**, 18-33.
- IVERSEN, L. L. (1967). *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*. London: Cambridge University Press.
- KALSNER, S. (1969a). Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. *Circulation Res.*, **24**, 383-395.
- KALSNER, S. (1969b). Steroid potentiation of responses to sympathomimetic amines in aortic strips. *Br. J. Pharmac.*, **36**, 582-593.
- LIGHTMAN, S. L. & IVERSEN, L. L. (1969). The role of Uptake₂ in the extraneuronal metabolism of catecholamines in the isolated rat heart. *Br. J. Pharmac.*, **37**, 638-649.
- SCHAYER, R. W. (1964). A unified theory of glucocorticoid action. *Perspectives in Biol. Med.*, **8**, 71.
- ZWEIFACH, B. W., SHORR, E. & BLACK, M. M. (1953). Influence of the adrenal cortex on behaviour of terminal vascular bed. *Ann. N.Y. Acad. Sci.*, **56**, 626.

(Received August 27, 1970)