The disposition of L-[^{3}H]-noradrenaline by the isolated epididymal fat pad of the rat and the effect of antilipolytic agents

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The *in vitro* lipolytic effect of catecholamines is paradoxically enhanced (this increase has been termed hypersensitivity, Bizzi, Codegoni & Garattini, 1969) when the rate of lipolysis is estimated in epididymal adipose tissue removed from rats pretreated with antilipolytic agents like carboxy methyl pyrazole (5C3MP) or nicotinic acid. We wanted to ascertain whether such action depends on a drug altered disposition of the catecholamines by the preparation.

L-[^{3}H]-noradrenaline ([^{3}H]-NA) within the range (25 or 250 ng/ml) producing concentration dependent lipolytic responses (Bizzi *et al.*, 1969) was measured in tissue and medium at different time intervals (Figure 1). The specificity of these results was verified by comparison with:

(1) Those obtained with [^{14}C]-sorbitol which reached saturation levels in tissue (about 21% of the medium concentration) within 5 to 10 min incubation;

(2) Those relative to epididymal fat obtained from animals pretreated with either of the reference drugs desipramine, pargyline (10 mg/kg, i.p., 1 h prior to sacrifice) or reserpine (2.5 mg/kg, i.p., 12 h prior to sacrifice).

After 60 min incubation desipramine and reserpine reduced both total radioactivity (noradrenaline plus metabolites) and [^{3}H]-NA in tissue when the catecholamine concentration in the medium was 25 ng/ml; only [^{3}H]-NA at 250 ng/ml. Pargyline decreased total radioactivity in tissue, either at 25 or at 250 ng catecholamine/ml medium, while increased [^{3}H]-NA at 250 ng/ml.

Pretreatment of the animals with either 5C3MP or nicotinic acid (15 and 50 mg/kg respectively, i.p. 1 h prior to sacrifice) failed to alter the disposition of noradrenaline as evaluated under the

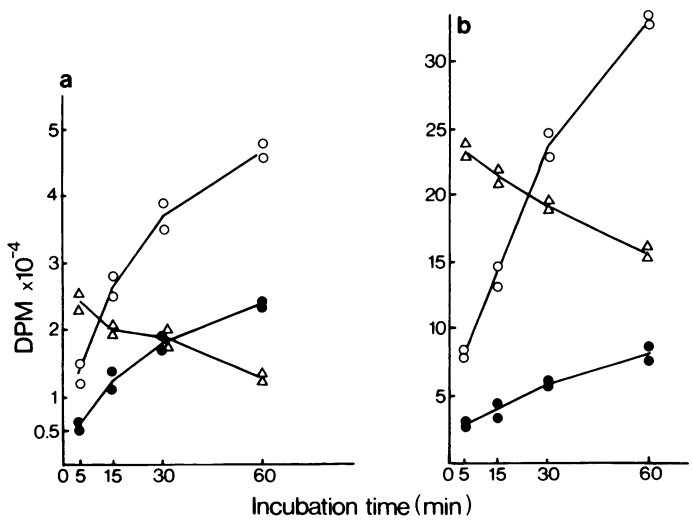

Fig. 1 The disposition of L-[^{3}H]-noradrenaline ([^{3}H]-NA) by the isolated epididymal fat pad of the rat. Fasted male Sprague-Dawley rats were used for these experiments and the adipose tissue incubated as described previously (Bizzi, Codegoni & Garattini, 1969) except for the use of l-noradrenaline 7-^{3}H (NEN, Dreieichenhain) diluted to a specific activity of 1.11 x 10^3 DPM/ng. The initial concentration of [^{3}H]-NA in the medium was 25 or 250 ng/ml (A and B respectively). Liquid scintillation radioassay of $HClO_4$ extracts, before and after Al_2O_3 chromatography was used for an estimate of total radioactivity (noradrenaline plus metabolites) and [^{3}H]-NA respectively: (○) total radioactivity, tissue; (●) [^{3}H]-NA tissue; (x) [^{3}H]-NA medium. The points refer to individual samples and the relative figures are d min^{-1} g^{-1} (tissue) or ml (medium). After 60 min incubation ^{3}HNA in the medium still accounted for over 80% of total radioactivity.

conditions reported in Figure 1. Washout experiments (after 60 min preincubation with the labelled compound tissues were transferred to fresh medium) showed a relatively slow rate of loss of either total radioactivity and [^{3}H]-NA (the latter declined linearly with a $T_{½}$ of about 4 h from tissue preincubated with the catecholamine at 25 ng/ml), while 95% of the radioactivity was lost within 5 min from tissues preincubated with [^{14}C]-sorbitol. The effect of 5C3MP pretreatment in washout experiments was of questionable significance.

It is concluded that hypersensitivity following pretreatment with antilipolytic agents does not depend on major changes in the disposition of noradrenaline by the isolated fat pad.

Reference

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The effect of cyproterone acetate on enzymic steps in the biosynthesis of steroid hormones

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Testosterone exerts its action upon the testis, on the male secondary sex characteristics, on the trophism of the male accessory sexual glands, prostate and seminal vesicles, and by a negative feedback on the hypothalamo-pituitary axis. These androgenic effects have been shown experimentally to be antagonized by the administration of cyproterone acetate (CA) to the intact animal.

Goslar, Mehring & Neumann (1970) reported that CA enlarges the interstitial cell complex of the testis and increases 3β-hydroxysteroid dehydrogenase activity in the rat, suggesting increased androgen production. However, decreased testosterone formation from rat testicular incubates containing CA was reported by Sommerville, Bottiglioni, Collins & Neumann (1969). Similarly, experiments by Engel & Karsznia (1971) showed that, when rat testicular tissue was incubated in the presence of CA, the production rate of testosterone from pregnenolone was lowered. Recent work in our laboratories (Grants & Stitch, 1972) has shown that CA may mediate at least some of its anti-androgenic effects by blockage of enzymatic steps in the biosynthesis of testosterone.

Samples for analysis were taken every 20 min during a 3 h incubation of tritium-labelled androgen precursors of high specific activity with testicular minces from adult rabbits. Intermediates in the biosynthesis of androgen were characterized after the addition of ^{14}C-labelled compounds and purification to constant isotopic ratios. These experiments demonstrated that CA impairs the Δ^4 pathway of androgen biosynthesis in the rabbit testis. The transformation of [^{3}H]-pregnenolone to [^{3}H]-testosterone and [^{3}H]-androstenedione is almost completely blocked in the presence of CA (25 μg/100 ml incubation medium). The cumulative yield of testosterone formed from pregnenolone at 130 min reached a maximum in the presence of CA of 0.005% of the precursor, whereas in control incubations without CA the conversion amounted to 0.035%. Further examination of the Δ^4 pathway of steroid biosynthesis has shown that CA at a concentration of 0.1 μg/100 ml of incubation medium markedly impairs 3β-ol dehydrogenase activity required for the transformation of [^{3}H]-pregnenolone to [^{3}H]-progesterone. The conversion of [^{3}H]-pregnenolone to [^{3}H]-17-α-hydroxyprogesterone and the side-chain cleavage of 17-α-hydroxyprogesterone to androstenedione appear to be inhibited in the presence of CA. When [^{3}H]-pregnenolone is incubated with rabbit testicular minces in the presence of CA, the formation of dehydroepiandrosterone is enhanced compared with that obtained from control incubations without CA. This would suggest that CA acts by impairing the conversion of pregnenolone to progesterone on the Δ^4 pathway of steroid biosynthesis, causing a shunt to 17-α-hydroxypregnenolone and dehydroepiandrosterone on the Δ^5 pathway of steroid biosynthesis. This result would suggest further that CA does not impair side-chain cleavage in the Δ^5 pathway of steroid biosynthesis to the same extent that it does in the Δ^4 pathway.

References

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