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METABOLISM OF ADRENALINE IN THE ISOLATED PERFUSED LIVER OF THE RAT

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Abstract—The liver has a high concentration of the enzymes necessary for the metabolism of catecholamines. Experiments with the perfused liver were designed to investigate the rate of hepatic uptake of adrenaline from blood and its subsequent metabolism. Adrenaline was rapidly removed from the perfusion medium, but did not accumulate in the liver. Metabolites of adrenaline, however, appeared in the liver and were soon found at a concentration exceeding that of the adrenaline in the medium. The concentration of adrenaline metabolites was directly proportional to the duration of the perfusion and the concentration of adrenaline in the medium. The time course of accumulation of adrenaline metabolites resembled that of their subsequent disappearance from the liver in "washout" experiments (half-time about 35 min). The accumulation of adrenaline metabolites in the liver and the removal of adrenaline from the perfusion medium were markedly reduced by the presence of corticosterone (10 μg/ml) in the medium.

IN MAMMALS, circulating catecholamines are rapidly removed from the bloodstream.[1,2] Although the normal circulating levels of catecholamines are very low, they can rapidly increase during times of stress when the rate of their removal from the bloodstream will determine their duration of action. The first stage in the clearance of catecholamines from blood involves their uptake into tissues. In the heart, for example, two transport mechanisms have been characterized.[3] One of these, designated uptake-1, involves uptake into sympathetic nerve endings, while the other, uptake-2, transports the amines into extra-neuronal tissue. Blockade of uptake-2 results in inhibition of the metabolism of noradrenaline.[4]

The liver has been implicated in the clearance of catecholamines from the blood.[1,5] It contains high concentrations of Monoamine oxidase (MAO) and Catechol-*O*-methyl transferase (COMT),[6,7] the major enzymes of catecholamine metabolism. However, the hepatic uptake of circulating catecholamines has not been systematically studied. In this paper results are presented of a study of adrenaline uptake and metabolism in the isolated perfused rat liver.

METHODS

The livers of well-fed Sprague–Dawley albino rats, weighing 200–260 g, were perfused essentially according to Hems *et al.*[8] with a bicarbonate saline, pH 7·4, containing 2·5% (w/v) bovine serum albumin (Fraction V; Armour Pharmaceutical Co., Eastbourne). The gas phase was 95% oxygen, plus 5% carbon dioxide. Before use, the albumin was dialysed against several changes of bicarbonate–saline, as it was

found that the adrenaline was not stable in undialysed albumin solutions. No red cells were added to the medium; provision of oxygen was not critical, since substitution of nitrogen for oxygen in the perfusion chamber decreased the rate of production of adrenaline metabolites by only about 30 per cent. The perfusions were carried out in a cabinet at a temperature of 37°.

The perfusion medium contained L-adrenaline (I.C.I. Ltd.), usually at a concentration of 1 μg/ml and ^{3}H-DL adrenaline (Radiochemical Centre, Amersham, 5·3 Ci/m-mole) so that the radioactivity was 33 nCi/ml. Ascorbic acid, glucose and EDTA were also included in the medium.[9] Extracts of liver and perfusion medium were assayed by liquid scintillation counting following cation exchange chromatography on Amberlite CG-120 resin,[4] which separated adrenaline from its metabolites. Products included de-aminated and *O*-methylated derivatives; the latter comprised more than 50 per cent of the total ^{14}C in metabolites in all experiments. Such separation of liver extracts into adrenaline and metabolites[4] allows distinction between processes of catecholamine uptake and storage, and those of enzymic attack.

During perfusion of the liver the flow rate was 8–10 ml/min; the medium was not recirculated. Liver samples were obtained after tying off the base of the lobes with thread, immediately dropping them into liquid nitrogen, and grinding to a powder in a mortar. ^{3}H-Adrenaline and its metabolic products were extracted from the liver and perfusion medium with perchloric acid.[4]

Corticosterone was dissolved in about 1 ml ethanol, for addition to 300 ml perfusion medium. In some experiments MAO inhibition was achieved by the administration of pargyline (Abbott Labs. Ltd., 65 mg/kg, 18 hr before perfusion); COMT inhibition was achieved by the addition of B-thujaplicin (Koch-Light Ltd.), to the perfusion medium (final concentration 0·25 mM).

RESULTS

Accumulation of adrenaline in the liver. There was negligible accumulation of adrenaline in the liver during perfusion. Even after perfusions for 2 hr, with adrenaline concentrations of 10–50 μg/ml, the adrenaline concentration in the liver was only 0·1–0·3 μg/g. The presence of inhibitors of MAO and COMT increased the accumulation of adrenaline in the liver, but even then the concentration in the liver was less than 10 per cent of that in the perfusing medium.

Accumulation of metabolites of adrenaline in the liver. During perfusion of the liver with adrenaline (1 μg/ml), there was a gradual accumulation of metabolites of adrenaline in the tissue (Fig. 1). Their concentration soon exceeded the concentration of adrenaline in the input medium. The proportion of adrenaline which was removed and converted to metabolic products (Table 1) attained a steady level within a few minutes. Thus the proportion extracted at 28–30 min (Table 1) was unchanged throughout 5–28 min perfusion. In a typical 30-min perfusion, 300 μg of adrenaline was passed through the liver, and about 40–50 μg of metabolites accumulated in the liver, while approximately 120 μg of metabolites emerged in the effluent medium.

The rate of accumulation of metabolites in the liver was dependent on the concentration of adrenaline in the medium (Fig. 2). In these experiments there was no discernible alteration of the vascular resistance of the liver if the adrenaline concentration was less than 20 μg/ml. At higher concentrations, however, increasing vascoconstriction developed.

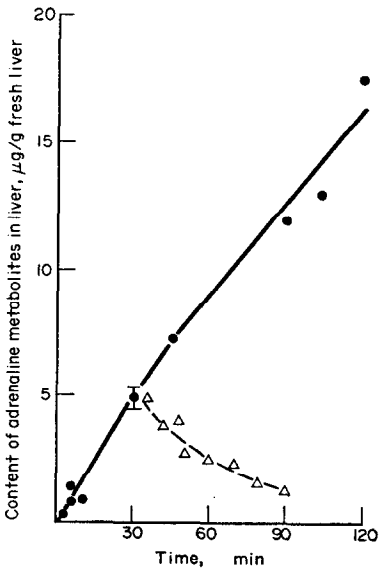

FIG. 1. Time course of changes in hepatic content of adrenaline metabolites. Livers were perfused with medium containing ^{3}H-adrenaline (1 μg/ml) for various periods (●; results from five perfusions). In three perfusions (△), the medium was substituted after 30 min by a medium which contained adrenaline (1 μg/ml) but no ^{3}H-adrenaline. Other details are given in the text. Apart from the 30 min point (see Table 1), each point represents a single analysis; between two and four liver samples were taken during each perfusion.

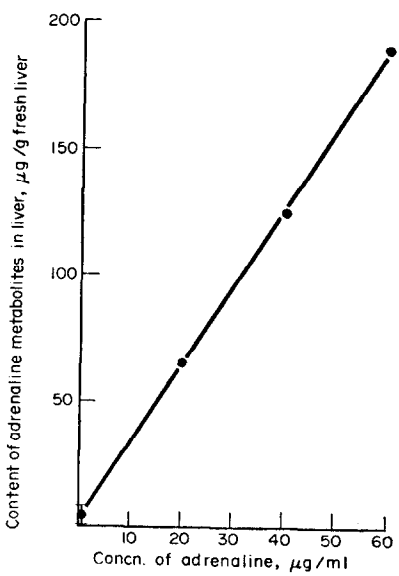

FIG. 2. Dependence on adrenaline concentration of accumulation of products in the liver. Livers were perfused for 30 min with medium containing ^{3}H-adrenaline. Results are the mean of five perfusions (where the adrenaline concentration was 1 μg/ml: see Table 1) or are from single perfusions.

TABLE 1. UPTAKE AND METABOLISM OF ADRENALINE BY THE PERFUSED LIVER

Additions to medium	Content of adrenaline metabolites in liver (μg/g liver)	Adrenaline removed from input medium (%)
None	5·0 ± 0·2 (5)	55 ± 7 (3)
Ethanol	4·5 ± 0·3 (5)	46 ± 2 (5)
Ethanol plus corticosterone (1 μg/ml)	4·2 ± 0·4 (5)	43 ± 2 (5)
Ethanol plus corticosterone (10 μg/ml)	3·2 ± 0·2 (10)	35 ± 2 (10)

Livers were perfused with adrenaline (1 μg/ml) for 30 min. Corticosterone was dissolved in ethanol for addition to the medium to achieve the indicated concentration. The perfusion medium was analysed after 28 min perfusion. Other details are given in the text. Results are means ± S.E.M.; the number of measurements are in parentheses.

The rate of disappearance or "washout" of metabolites from the liver was measured after 30-min perfusion with ^{3}H-adrenaline, by substituting a medium which contained adrenaline (1 μg/ml) but no ^{3}H-adrenaline. The time course of disappearance of ^{3}H (in metabolites) from the liver resembled that of their accumulation (Fig. 1). About 35 min was required for half of the accumulated metabolites to disappear.

Effect of corticosterone on the hepatic metabolism of adrenaline. In the presence of corticosterone (10 μg/ml) there was a diminished accumulation of adrenaline metabolites in the liver, and a reduced proportion of the input adrenaline was extracted from the perfusion medium (Table 1).

DISCUSSION

Effect of corticosterone on the liver. When present in the perfusion medium, corticosterone inhibited the removal of adrenaline from the medium, and also diminished the accumulation of adrenaline metabolites in the liver. In this situation, there was no detectable accumulation of adrenaline in the liver, which suggests that corticosterone was inhibiting the entry of the catecholamine into the liver, rather than the enzymes involved in the metabolism of adrenaline. A similar action of corticosterone and other steroids has been reported in the isolated perfused rat heart.[10]

Although a relatively high concentration of corticosterone was required to achieve significant inhibition of adrenaline uptake, the concentration of adrenaline in the medium was also considerably greater than that which is encountered *in vivo*; hence it remains possible that *in vivo* glucocorticoids can inhibit the uptake of adrenaline by the liver. Such an action of glucocorticoids could be significant during "stress" reactions of the type in which circulating concentrations of both glucocorticoids and adrenaline are increased, as it would prolong the period of action of circulating adrenaline. Steroids have been shown to have this effect in intact animals.[11,12] Such an interaction between glucocorticoids and catecholamines at the extramedullary tissues would complement the action of the adrenal corticoids in stimulating adrenal medullary secretion.[13]

Uptake of adrenaline by the liver. The negligible accumulation of adrenaline in the liver corresponds to observations in the intact rat with noradrenaline.[14] The fact that

the present experiments were performed under conditions where active uptake has been observed in the perfused heart[4] suggests that the adrenaline uptake processes "1" and "2", if present in liver, do not have properties which resemble those in the excitable tissues. Nevertheless, penetration of adrenaline into liver was rapid, as shown by the removal of about half of the adrenaline from the input medium, confirming that the liver may exert a major role in clearing catecholamines from blood. This entry of adrenaline into liver may correspond to the transport component in the "uptake-2" process, which appears to involve non-neural cells,[15,16] and which is inhibited by adrenal steroids.[10]

The gradual accumulation of products of adrenaline in liver occurred despite the fact that the medium was not recirculated during perfusion. This suggests that metabolic products derived from adrenaline in the liver do not all diffuse rapidly out of liver cells. This was confirmed in the "washout" experiments, which showed that the accumulated metabolites required about 35 min perfusion before they decreased to half their initial concentration in the liver. However, the major proportion of the products formed from adrenaline were detected in the effluent medium, presumably corresponding to the metabolites which are excreted in the intact animal.

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