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The action of norepinephrine on the transport of fatty acids and triglycerides by the isolated perfused rat liver

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IN RECENT years it has become apparent that the catecholamines, epinephrine and norepinephrine, stimulate release of nonesterified fatty acids (NEFA) from fat depots[1, 2] and raise the levels of the plasma NEFA.[3–6] Furthermore, stimulation of the nerve supply to adipose tissue was observed to increase the release of NEFA,[7] whereas denervation increased the lipid content of the depot.[8] The nervous system was further implicated as a regulator of NEFA transport since it was reported that psychological and physical stimulation increase plasma levels of NEFA in man.[9, 10] It has been tacitly assumed that the effects of the catecholamines on plasma NEFA levels have been primarily a result of stimulation of lipolysis of adipose tissue triglycerides. In order to ascertain whether the transport and metabolism of lipids by the liver was affected by catecholamines, we investigated the action of norepinephrine on the uptake of fatty acids and the release of triglycerides by the isolated, perfused rat liver. We observed that both fatty acid uptake and triglyceride release were significantly inhibited by the addition *in vitro* of norepinephrine to the perfusion medium.

EXPERIMENTAL

Male rats,* weighing 250 to 400 g, maintained on a balanced ration and water *ad libitum*, were used as liver donors. Details of the perfusion procedure, apparatus, and medium have been reported previously.[11, 12] The liver was removed from the donor and placed in the perfusion apparatus.[11] A constant infusion of *l*-norepinephrine bitartrate in 0·9 per cent NaCl was started after maximum flow rates were obtained (20 to 30 min). The norepinephrine had a marked vasconstrictor effect on the liver, and flow of perfusate through the liver was sharply reduced. This vascular effect of norepinephrine, however, was inhibited by microgram quantities of phenoxybenzamine: 500μg phenoxybenzamine HCl in 0·9 per cent NaCl, injected directly into the portal vein cannula, were sufficient to maintain the normal maximum flow rates through the liver during the constant infusion of norepinephrine. The phenoxybenzamine, moreover, appeared to have no effect on the metabolic actions of norepinephrine. The phenoxybenzamine was injected routinely within a few minutes after the start of the norepinephrine infusion; 20 mg of palmitic acid, as the fatty acid-serum complex,[12] were added to the perfusate 10 min after the phenoxybenzamine addition. At this time, maximal flow rates had been reattained. After an additional 3 min, samples were taken for initial analytical measurements. The triglyceride and glucose data represent net changes in perfusate concentration during the following 3 hr. The fatty acid uptake, recorded as disappearance from the medium, was measured 10 min after the initial sample was taken. The rate of uptake was linear during this period. The concentration

* Obtained from the Holtzman Co., Madison, Wis.

of palmitate used in these experiments was removed almost completely from the perfusate by normal livers within 30 min, and within 60 min by the liver receiving the norepinephrine infusion. Fatty acids were estimated by the Trout modification [13] of the Dole procedure,[4] triglycerides by the method of Van Handel and Zilversmit[14] after adsorption of phospholipids on silicic acid, and glucose by the Nelson procedure.[15]

The results in Table I demonstrate the inhibition of both triglyceride release and the rate of fatty

TABLE I. EFFECT OF NOREPINEPHRINE ON HEPATIC LIPID TRANSPORT

Additions to medium	Uptake of NEFA/g liver (%)	Triglyceride release (μmole/g liver)	Glucose release (mg/g liver)
(A) None	4·6 ± 1·6 (8)	0·83 ± 0·51 (7)	14·6 (3)
(B) Phenoxybenzamine	5·3 ± 0·8 (5)	0·99 ± 0·49 (5)	12·7 ± 3·2 (5)
(C) Phenoxybenzamine + norepinephrine	2·7 ± 0·4 (6)	0·04 ± 0·35 (6)	43·4 ± 5·9 (6)
(D) Phenoxybenzamine + norepinephrine	3·0 ± 0·4 (4)	0·28 ± 0·43 (4)	35·8 (2)

Values are means ± standard deviation. Figures in parentheses refer to number of experiments. In (C) the infusion rate was 0·1 μg of *l*-norepinephrine free base/ml perfusate/min (preparation from K and K Labs.). With an initial perfusate vol = 100 ml, and assuming no hepatic catabolism of norepinephrine, this rate of infusion at the end of 1 hr would give a free base concentration of $3{\cdot}5 \times 10^{-5}$ M. In (D) the infusion rate was 0·5 μg/ml/min (preparation from Mann Labs.). Phenoxybenzamine HCl (Dibenzyline) was obtained from the Smith, Kline and French Laboratories.

Statistical Analysis

I. Uptake of NEFA
A vs. B, $t_{11} = 1{\cdot}02$, NS
B vs. C, $t_9 = 6{\cdot}89$, $P < 0{\cdot}001$*
B vs. D, $t_7 = 5{\cdot}02$, $P = 0{\cdot}001$*

II. Triglyceride release
A vs. B, $t_{10} = 0{\cdot}54$, NS
B vs. C, $t_9 = 3{\cdot}76$, $P < 0{\cdot}005$*
B vs. D, $t_7 = 2{\cdot}25$, $P < 0{\cdot}05$*

III. Glucose release
B vs. C, $t_9 = 10{\cdot}33$, $P < 0{\cdot}001$*

* Calculated on the basis of a one-tailed test.

acid uptake induced by norepinephrine. It does not appear reasonable that the inhibition of net outward transport of triglyceride resulted only from a decreased rate of fatty acid sequestration by the liver since, in these experiments, the total fatty acid uptake was identical in the presence or absence of the hormone. Norepinephrine may well inhibit triglyceride synthesis, accelerate oxidation, or do both. The rise in plasma NEFA levels after administration of norepinephrine to the intact animal may result not only from increased release of NEFA from adipose tissue stores, but also from decreased uptake by the liver. The increased levels of plasma NEFA would thus become more available as an immediate energy source for muscle. The observed stimulation by norepinephrine of glucose release from the liver confirms the glycogenolytic effect of this hormone reported previously by Sokal and co-workers.[16] The requirement for an adrenergic blocking agent to inhibit the vascular effect of norepinephrine may be a reflection of the relative sensitivity of the hepatic vascular bed and parenchymal cells to exogenously administered norepinephrine. Stimulation of the adrenergic nerves to the liver may deliver small concentrations of norepinephrine to intracellular sites of action without the production of any significant vasoconstriction. Inhibition by phenoxybenzamine of the vasoconstriction without any effect on the metabolic action of norepinephrine suggests a significant physiological role for this neurohormone in hepatic lipid and carbohydrate metabolism.

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The effect of *bis*-(2,2,2-trifluoroethyl)ether on brain electrolytes and water distribution in the rat

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IN OUR studies with the anesthetic, trifluoroethyl vinyl ether, we observed that a closely related fluorinated ether $CF_2{\cdot}CH_2{-}O{-}CH_2{\cdot}CF_3$, *bis*-(2,2,2-trifluorethyl)ether (BTE) evoked marked convulsive seizures upon inhalation in laboratory animals.[1] This observation prompted us to use the agent as a substitute for electroshock in the treatment of mentally depressed patients.[2] In studies of the mechanism of action of BTE, Ling *et al.*[3] showed that the convulsive seizure in rats was accompanied by a mobilization of brain acetylcholine from its storage sites. Woodbury[4] observed that intracellular brain sodium was increased by electroshock in rats.

These studies describe the effect of BTE-evoked convulsive seizures on the brain electrolytes Na^+ and K^+, and water distribution.

The BTE used in these studies was Indoklon®, used in human convulsive therapy. Male albino rats (150 to 200 g) were convulsed with BTE (0·5 ml in a 3·4-liter chamber). During the convulsion each rat was decapitated, and the brain *in toto* was removed. Adequate blood samples were collected for serum electrolyte determinations. The brains were dried to constant weight (Yannet and Darrow[5]) and the content of brain water was determined. Each whole brain was homogenized in deionized-distilled water, deproteinized with 10% trichloroacetic acid, and diluted with deionized-distilled water. After centrifugation, supernatant samples of the brain preparation were taken and analysed for brain sodium and potassium by means of the Baird-Atomic model KY-1 flame photometer with lithium as the internal standard. Standard curves were constructed with each series of determinations.