IDENTIFICATION AND PHYSIOLOGICAL ROLE OF NOREPINEPHRINE IN ADIPOSE TISSUE

R. Paoletti*, R.L. Smith**, R.P. Maickel, and B.B. Brodie
Laboratory of Chemical Pharmacology
National Heart Institute
National Institutes of Health
Bethesda 14, Maryland

Received July 2, 1961

Numerous studies suggest that the mobilization of free fatty acids (FFA) from adipose tissue is regulated in part by catecholamines. For example, the infusion of norepinephrine or epinephrine increases the plasma level of FFA in humans and dogs, the level returning to normal shortly after the infusion is stopped; pretreatment with an adrenergic blocking agent prevents this effect of the catecholamines (Havel and Goldfien, 1959). Again, the addition of epinephrine or norepinephrine to the epididymal fat pad of the rat *in vitro* stimulates the release of FFA (Gordon and Cherkes, 1958; White and Engel, 1958).

Other studies indicate that ACTH is also important in regulating the mobilization of FFA, through its action on the pituitary-adrenal system. For example, in rats exposed to cold or injected with ethanol there is an elevation in levels of plasma corticosterone, plasma FFA, and liver triglycerides; these responses are not elicited in hypophysectomized or adrenalectomized animals (Brodie *et al.*, 1961). Furthermore, levels of corticosterone and FFA are increased to the same extent by depot-ACTH as by cold-exposure or alcohol administration (Maickel and Paoletti, 1961).

* Riker Fellow. Present address: Institute of Pharmacology, University of Milan, Italy.

** Visiting Scientist. Present address: Department of Biochemistry, St. Mary's Hospital Medical School, London, England.

The present study describes the positive identification of catecholamines in adipose tissue of rats and rabbits, and shows that after depletion of adipose tissue norepinephrine by reserpine, the ACTH-induced mobilization of lipid is completely abolished. These results suggest that norepinephrine is an essential factor common to the action of ACTH and possibly other hormones in the mobilization of FFA.

MATERIALS AND METHODS

Reserpine (lyophilized phosphate salt, kindly supplied by Dr. A. Plummer, CIBA Labs., Summit, N.J.) was administered intravenously (1.5 mg/kg) twice in 30 hours. Depot ACTH (Acthar Gel, Armour Co.) was injected subcutaneously (20 USP units/rat). This dose of ACTH elicits a rise of plasma corticosterone in rats, persisting 6 to 10 hours (Maickel and Paoletti, 1961). The experiments were carried out with male Sprague-Dawley rats (190-200 g), fasted for 6 hours. Animals were stunned and then decapitated immediately. Blood was collected into beakers containing heparin, transferred to tubes, and centrifuged immediately. Plasma FFA were determined according to Dole (1956); liver triglycerides according to Butler et al. (1961). Brain and heart norepinephrine were assayed by the method of Shore and Olin (1958); norepinephrine in adipose tissues was assayed by a modification of this method (to be published).

RESULTS

Isolation and Identification of Norepinephrine in Adipose Tissue

Catecholamines in adipose tissue were extracted into n-butanol and returned to an aqueous phase as described by Shore and Olin (1958). About 10 grams of rabbit epididymal fat was homogenized in 2 volumes of 0.01 N HCl. The homogenate was transferred to a 500 ml glass-stoppered bottle containing 20 grams of solid NaCl and 300 ml of n-butanol (acid-washed and salt-saturated). After shaking for one hour, the mixture was centrifuged and 200 ml of the butanol extract was transferred to another bottle containing 350 ml of n-heptane and a small volume of 0.01 N HCl.

The bottle was shaken for 15 minutes and then centrifuged. One aliquot of the acid phase was adjusted to pH 3 and another to pH 5, and the catecholamines were oxidized to highly fluorescent trihydroxyindoles as described by Shore and Olin (1958).

The presence of catecholamines was indicated from the considerable fluorescence from oxidation at pH 5. The virtual absence of epinephrine was shown from the negligible fluorescence from oxidation at pH 3. The possibility that the substance extracted from fat was dopamine (3,4-dihydroxyphenylethylamine), which has activation and fluorescence spectra similar to those of norepinephrine, was tested by comparison of fluorescence intensities after oxidation with iodine and ferricyanide (Shore and Olin, 1958). The results showed that dopamine was not present in significant amounts.

Activation and fluorescence spectra of oxidized samples of authentic norepinephrine, determined with a spectrofluorimeter, were similar to those of the apparent norepinephrine (Fig. 1), with activation maxima at 410 mμ and fluorescence maxima at 510 mμ (uncorr.).

Norepinephrine Levels in Adipose Tissue

Table 1 shows the levels of norepinephrine in adipose tissue, brain, and heart of rat and rabbit.

Effect of Reserpine on Tissue Levels of Norepinephrine

Reserpine, an alkaloid which depletes catecholamine stores in various body tissues (Carlsson and Hillarp, 1956; Holzbauer and Vogt, 1956; Brodie et al., 1957), was administered to rats. As shown in Table 2, norepinephrine stores were depleted in epididymal fat as well as in brain and heart.

Effect of Norepinephrine Depletion on ACTH-Induced Lipid Mobilization

The administration of depot-ACTH to control rats almost tripled the levels of plasma FFA and liver triglycerides. However when administered 16 hours after reserpine treatment, ACTH no longer produced a

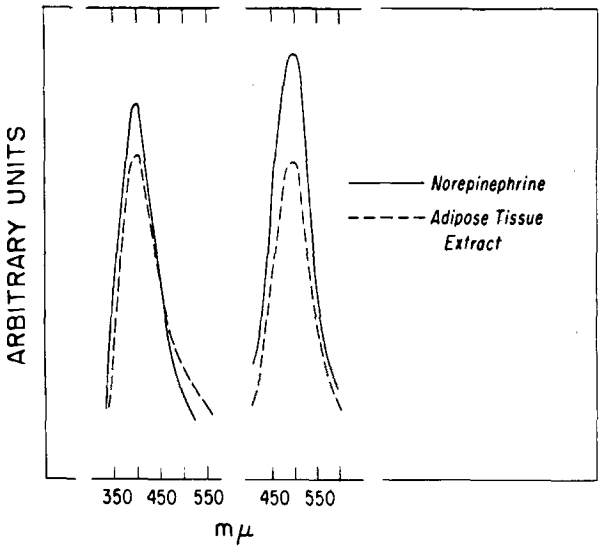

Figure 1: Activation spectra (left) and fluorescence spectra (right) of authentic norepinephrine, and apparent norepinephrine extracted from rabbit epididymal fat pad. To obtain the activation spectra, the fluorescence monochromator of an Aminco-Bowman Recording Spectrophotofluorometer was set at 510 mμ and the spectra from the activating monochromator were scanned. To obtain the fluorescence spectra, the activating monochromator was set at 410 mμ and the spectra from the fluorescence monochromator were scanned.

TABLE 1

NOREPINEPHRINE LEVELS IN ADIPOSE TISSUE OF RAT AND RABBIT

Each value is mean of 4 determinations. Tissues of 6 rats or 3 rabbits were pooled for each determination.

Tissue	Norepinephrine
	microgm/g ± S.E.
Rat epididymal fat	0.12 ± 0.02
Rat retroscapular fat	0.15 ± 0.02
Rabbit epididymal fat	0.15 ± 0.03
Rabbit retroscapular fat	0.19 ± 0.03
Rat brain	0.45 ± 0.05
Rat heart	1.05 ± 0.08

TABLE 2

NOREPINEPHRINE LEVEL IN VARIOUS RAT TISSUES 16 HOURS AFTER RESERPINE ADMINISTRATION

Each value is mean of 4 determinations. Tissues of 6 rats were pooled for each determination.

Treatment	Adipose Tissue	Brain	Heart
	microgm/g ± S.E.	microgm/g ± S.E.	microgm/g ± S.E.
Control	0.12 ± 0.02	0.45 ± 0.02	1.05 ± 0.04
Reserpine	$<$ 0.02	$<$ 0.07	$<$ 0.10

rise in plasma FFA or in liver triglycerides (Table 3). These responses were largely restored when norepinephrine (40 microgm/rat infused intravenously over 30 minutes) was administered 4 hours before the ACTH. In reserpine-treated animals, the norepinephrine infusion, followed in 4 hours by a saline injection, did not cause an increase in plasma FFA.

TABLE 3

EFFECT OF RESERPINE ON MOBILIZATION OF FFA BY ACTH

Each value is mean of 15 determinations. Two groups of rats were given ACTH (20 units/rat); one group was killed in 2 hours for assay of plasma FFA determinations, and the second group in 4 hours for assay of liver triglycerides.

Treatment	Plasma FFA	Liver Triglycerides
	microgm/g ± S.E.	microgm/g ± S.E.
Controls	0.45 ± 0.1	4.5 ± 0.5
ACTH	1.2 ± 0.2	13.0 ± 1.2
Reserpine + ACTH	0.4 ± 0.05	4.1 ± 0.6
Reserpine + ACTH + Norepinephrine	0.95 ± 0.1	9.8 ± 0.4

Conclusions

Norepinephrine has been identified as a normal constituent of adipose tissue, which contains significant amounts of this amine but

practically no epinephrine. In rats, depot-ACTH stimulates the release of free fatty acids and the deposition of liver triglycerides. These effects of ACTH are not produced when the adipose tissue is depleted of norepinephrine by pretreatment with reserpine. However, the responses are restored when norepinephrine is administered parenterally before ACTH. These results imply that norepinephrine in adipose tissue is essential for the mobilization of lipid by ACTH in vivo.

Preliminary data from this laboratory indicate that norepinephrine is also essential in the mobilization of lipid induced by ACTH in vitro: epididymal pads from reserpinized rats do not respond to ACTH in vitro unless norepinephrine is added to the incubation medium.

REFERENCES

Brodie, B.B., Olin, J.S., Kuntzman, R.G., and Shore, P.A., Science, 125, 1293 (1957).

Brodie, B.B., Maickel, R.P., and Westerman, E.O., in Regional Neuro-Chemistry, eds. S.S. Kety and J.Elkes (Pergamon Press, London, 1961) p. 351.

Butler, W.M., Maling, H.M., Horning, M.G., and Brodie, B.B., J. Lipid Research, 2, 95 (1961).

Carlsson, A. and Hillarp, N., Kgl. Fysiograf. Sällskap Lund Förh., 26, 8 (1956).

Dole, V.P., J. Clin. Invest., 35, 150 (1956).

Gordon, R.S., Jr. and Cherkes, A., Proc. Soc. Exptl. Biol. Med., 101, 624 (1959).

Havel, R.J. and Goldfien, A., J. Lipid Research, 1, 102 (1959).

Holzbauer, M. and Vogt, M., J. Neurochem., 1, 8 (1956).

Maickel, R.P. and Paoletti, R., Fed. Proc., 20, 276 (1961).

Shore, P.A. and Olin, J.S., J. Pharmacol. Exptl. Therap., 122, 295 (1958).

White, J.E. and Engel, F.L., Proc. Soc. Exptl. Biol. Med., 101, 624 (1958).