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A note on the subcellular distribution of adipose tissue noradrenaline

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The distribution of noradrenaline between the residue and high-speed supernatant of adipose tissue homogenates in rats, guinea-pigs and cats has been examined. Most of the noradrenaline is in the supernatant fraction; little is in the 100,000 *g* fraction.

THE presence of noradrenaline in the adipose tissue has been demonstrated by fluorimetric (Paoletti, Smith & others, 1961; Sidman, Perkins & Weiner, 1962) and histochemical methods (Dawkins, Duckett & Pearse, 1966). We have shown that the pattern of distribution of noradrenaline in adipose tissue differs among various animal species (Spano, Vargiu & others, 1967). In rats and in guinea-pigs, interscapular brown fat bodies are the richest in this amine, while in cats and dogs noradrenaline is concentrated in perirenal fat. We have now examined the subcellular localization of endogenous noradrenaline in adipose tissue in rats, guinea-pigs and cats.

Experimental

MATERIAL AND METHODS

Male Long-Evans rats (150–160 g) and guinea-pigs (350–450 g) were killed by neck fracture. Cats were killed with pentobarbitone. Soon after death, hearts, brains and interscapular adipose tissues were removed. When needed, organs from more than one animal were pooled and 1 g of each tissue was homogenized for 2 min with a Kontes glass homogenizer in 10 volumes of ice-cold 0·3M sucrose containing 100 μg/ml ascorbic acid and 1 mg/ml EDTA. All operations were on ice in a room refrigerated to 4°. The homogenates were centrifuged in a Spinco refrigerated ultracentrifuge at 100,000 *g* for 1 hr. After centrifugation each adipose tissue homogenate presented a particulate fraction, a fluid supernatant and, at the surface, a 1–2 mm thick film of fat.

Concentrated perchloric acid was added to the supernatant fluids to obtain a final normality of 0·4N. The particulate fractions of each tissue and the fatty films were taken up, by homogenization, in 0·4N perchloric acid. The noradrenaline from each fraction was extracted by a modification (Spano & others, 1967) of the method described by Brodie, Comer & others (1966) and assayed fluorimetrically according to the method of Chang (1964).

Results and discussion

Adipose tissue noradrenaline has a subcellular distribution different from that of other organs so far examined (Table 1). Most of the

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TABLE 1. SUBCELLULAR DISTRIBUTION OF ADIPOSE TISSUE NORADRENALINE IN COMPARATION WITH THAT OF HEART AND BRAIN

Species	Organ	% total noradrenaline			Total noradrenaline μg/g of tissue
		100,000 × g fraction	Fatty film	Supernatant	
Rat	Interscapular adipose tissue	16·2 ± 2·3	14·2 ± 1·0	69·5 ± 3·1	0·279 ± 0·02
,, ...	Heart	65·7 ± 4·2	—	34·3 ± 3·8	1·120 ± 0·12
,, ...	Brain	58·0 ± 3·0	—	42·0 ± 4·1	0·630 ± 0·13
Guinea-pig ..	Interscapular adipose tissue	6·7 ± 1·1	18·3 ± 2·4	74·0 ± 3·1	0·251 ± 0·09
,, ..	Brain	57·6 ± 7·1	—	42·0 ± 5·8	0·530 ± 0·19
Cat	Perirenal adipose tissue	5·2 ± 3·1	15·1 ± 2·8	79·7 ± 7·3	0·710 ± 0·17

Values for rats and guinea-pigs are means (± s.e.) from at least 10 experiments; values for cats are means (± s.e.) from 5 experiments.

amine occurs in the supernatant fractions (liquid supernatant and fatty layer).

A similar subcellular distribution of catecholamines, but less markedly in favour of the supernatant fractions, was described by Wurtman, Axelrod & Potter (1964) for rat uterus. However, unlike in the uterus, adipose tissue noradrenaline is contained in nerve terminals (Weiner, Perkins & Sidman, 1962).

There are several possible explanations of our finding. It was possible that adipose tissue contained synaptic vesicles with sedimentation characteristics differing from those of other adrenergic structures: this seemed not to be so, since the same results have been obtained varying homogenization media (0·25M sucrose containing 5×10^{-3} phosphate buffer, pH 7·4, and 10^{-3}M $MgCl_2$), times of centrifugation (up to 4 hr) and of homogenization (1 min). It is also possible that adipose tissue might contain some substance (U-factor?) (Lehninger & Remmert, 1959) which releases noradrenaline from the vesicles during the preparation of the samples. To check this, portions of adipose tissue and brain of the same weight were homogenized together, and the supernatant-particulate distribution of noradrenaline was assayed in the mixture. The results of these experiments showed that the noradrenaline distribution for one tissue is not influenced by the presence of the other.

TABLE 2. EFFECT OF TYRAMINE, GUANETHIDINE AND L-METARAMINOL ON NORADRENALINE CONTENT OF INTERSCAPULAR ADIPOSE TISSUE, IN RATS

Treatment mg/kg	Interval between treatment and death	Noradrenaline μg/g
—	—	0·279 ± 0·02
Tyramine 20 i.m.	4 hr	0·080 (0·008–0·092)
Guanethidine 15 i.p. ..	14 hr	0·000
(−)-Metaraminol 2·5 i.p. ..	4 hr	0·002 (0·000–0·004)

It is interesting (Table 2) that noradrenaline levels of adipose tissue are depleted by drugs such as tyramine, guanethidine and metaraminol,

which are considered to release the amine by interfering with its uptake into granulated vesicles (Giachetti & Shore, 1966).

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