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Effect of adrenergic blocking agents on the release of free fatty acids from rat adipose tissue*

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SUMMARY

Epididymal adipose tissue from epinephrine-treated rats release more fatty acids into the medium during *in vitro* incubation than do tissues from untreated rats. The effect of epinephrine was abolished when an adrenergic blocking agent was administered to the animals before epinephrine was injected. Experiments *in vitro* showed that increased release of free fatty acids from adipose tissue due to addition of ACTH, epinephrine, and norepinephrine was inhibited by addition of an adrenergic blocking agent to the medium. The results suggest that mobilization of fatty acids from fat depots is at least partly under the control of vasomotor nerves.

The literature implicating the nervous system in the regulation of fat metabolism prior to 1948 has been reviewed by Wertheimer and Shapiro (1). Recent evidence indicates that fatty acids are transported in plasma from adipose tissue to liver, heart, and other peripheral tissues as free fatty acids (FFA) bound to plasma albumin (2). Epinephrine (3, 4, 5) and norepinephrine (6, 7, 8) cause the release of FFA from adipose tissue and increase the plasma concentration of these acids. Further, treatment of the animals with adrenergic blocking agents prevented the increase in plasma FFA induced by epinephrine administration (7, 9).

Pharmacological evidence would suggest that the mode of action of the adrenergic blocking agent is to prevent the effect of epinephrine and norepinephrine at the peripheral receptor, but direct evidence is lacking. This study is concerned with two points. The first is to determine whether the prevention by adrenergic blocking agents of the increased FFA in plasma following epinephrine *in vivo* is due to depression of the release of FFA from adipose tissue. The experiments designed to confirm this point consisted of studying the release of FFA from excised adipose tissue of animals previously treated with epinephrine and blocking agents. The second point is whether the action of adrenergic blocking agents is exerted *in vitro* on adipose tissue. This was investigated by addition of the blocking agent to the medium containing adipose tissue and epinephrine.

METHODS

Male albino rats (Sprague-Dawley strain), 180 to 220 gm, previously fasted for 16 hours were used. Epinephrine,[1] 2 mg per kg, was injected subcutaneously and the animals killed by decapitation 15 minutes later. The adrenergic blocking agents were administered under light ether anesthesia 30 minutes prior to the time of death. Phentolamine, 15 mg per kg, was injected into the tail vein and simultaneously at the same dose subcutaneously; NDC, 40 mg per kg, or phenoxybenzamine, 8 mg per kg, was injected intravenously.

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[1] Abbreviations and trade names used are: epinephrine, Adrenalin® Chloride, Parke, Davis & Co., Detroit, Mich.; phentolamine, Regitine methanesulfonate, Ciba Pharmaceutical Products, Inc., Summit, N. J.; N,N-dibenzyl-beta-chloroethylamine (NDC), dibenamine hydrochloride, Smith, Kline and French Laboratories, Philadelphia, Pa.; phenoxybenzamine, Dibenzyline hydrochloride, Smith, Kline and French Laboratories, Chicago, Ill.; ACTH, ACTHAR, Armour Labs, Chicago, Ill.; norepinephrine, Levophed bitartrate, Winthrop-Stearns, New York, N. Y.

The epididymal fat tissue was removed, weighed, and incubated for 3 hours at 36° in 4 ml of Krebs-Ringer phosphate medium containing 5% bovine albumin[2] adjusted to pH 7.4. Additions to the incubation media of ACTH, epinephrine, norepinephrine, and phentolamine were made by diluting these compounds in Krebs-Ringer phosphate buffer (pH 7.4). An equal volume of Krebs-Ringer phosphate buffer was added to the incubation medium of the control flasks. The FFA were determined on aliquots of the medium before and after incubation (10).

RESULTS

Excised adipose tissue from the group of rats given epinephrine exhibited a 100% increase in the release of FFA into the medium in comparison to adipose tissue of untreated controls in three experiments (Table 1). In the group of animals which received both phentolamine and epinephrine (Table 1, Exp. I), release of FFA was decreased compared to the group which received epinephrine alone, but increased 100% in comparison to the group receiving phentolamine alone. Treatment of the animals with NDC (Table 1, Exp. II) or phenoxybenzamine (Table 1, Exp. III) completely inhibited the release of FFA normally elicited by epinephrine. In the animals treated with phentolamine alone (Table 1, Exp. I), or NDC alone (Table 1, Exp. II), release of FFA from adipose tissue was significantly lower than the corresponding controls. No significant difference was observed in the animals treated with phenoxybenzamine alone (Table 1, Exp. III) and corresponding untreated controls.

The results obtained *in vitro* with phentolamine alone show that its addition to the incubation medium in concentrations of 10^{-3}M and 5×10^{-4}M did not alter the FFA released from adipose tissue (Table 2, Exp. I). Addition of ACTH, epinephrine, or norepinephrine singly markedly increased the release of FFA in comparison to the controls (Table 2). When phentolamine was present with these hormones in the incubation medium, the release of FFA was partially, or completely, inhibited compared to the effect of the hormones alone. The low value for the control ob-

TABLE 1. EFFECT OF ADRENERGIC BLOCKING AGENTS ON FREE FATTY ACIDS RELEASED FROM ADIPOSE TISSUE OF RATS TREATED WITH EPINEPHRINE

Exp.	Treatment	No. Animals	FFA *	P †
I	None	5	6.34 ± 0.99	
	Epinephrine	6	14.59 ± 0.25	<0.001
	Phentolamine	6	3.21 ± 0.39	<0.05
	Phentolamine + epinephrine	6	8.34 ± 1.45	N.S.
II	None	5	6.78 ± 0.80	
	Epinephrine	5	13.39 ± 1.09	<0.005
	NDC	4	3.02 ± 0.61	<0.01
	NDC + epinephrine	4	3.67 ± 0.86	<0.05
III	None	6	7.20 ± 0.52	
	Epinephrine	6	14.10 ± 1.07	<0.001
	Phenoxybenzamine	6	6.41 ± 0.98	N.S.
	Phenoxybenzamine + epinephrine	6	7.71 ± 1.20	N.S.

* FFA released, μmoles/g of adipose tissue in 3 hours, mean ± standard error.

† Statistical significance of data determined by comparing mean values for experimental group with mean value of corresponding control group, in "student's" t-test.

TABLE 2. EFFECT OF PHENTOLAMINE *in Vitro* ON FREE FATTY ACIDS RELEASED BY ADIPOSE TISSUE

Exp.	Additions	No. Animals	FFA *	P †
I	None	6	9.27 ± 0.60	
	Phentolamine 10^{-3}M	6	9.41 ± 0.98	N.S.
	Phentolamine 5×10^{-4}M	5	9.21 ± 0.87	N.S.
	ACTH 25 M units	6	44.40 ± 3.22	<0.001
	ACTH + phentolamine 10^{-3}M	4	13.22 ± 1.43	<0.05
	ACTH + phentolamine 5×10^{-4}M	6	22.77 ± 1.84	<0.001
II	None	6	8.43 ± 1.04	
	Norepinephrine 3×10^{-7}M	6	26.26 ± 2.10	<0.001
	Norepinephrine + phentolamine 5×10^{-4}M	6	9.48 ± 0.81	N.S.
	Norepinephrine + phentolamine 1×10^{-4}M	6	13.34 ± 1.06	<0.01
III ‡	None	6	2.27 ± 0.22	
	Epinephrine 3×10^{-7}M	6	27.75 ± 4.27	<0.001
	Epinephrine + phentolamine 5×10^{-4}M	6	11.40 ± 1.75	<0.001
	Epinephrine + phentolamine 1×10^{-3}M	6	3.45 ± 0.46	<0.05

Conditions: ACTH, epinephrine, norepinephrine, and phentolamine were added at the start of the incubation in 0.1 ml to give a final concentration in the flask indicated above. All flasks were brought to a final volume of 4.2 ml by addition of Krebs-Ringer phosphate buffer.

* FFA released, μmoles/g of adipose tissue in 3 hours, mean ± standard error.

† Statistical significance of data determined by comparing mean values for experimental group with mean value of corresponding control group, in "student's" t-test.

‡ All animals given nembutal anesthesia before sacrifice.

[2] Bovine albumin, Fraction V, Nutritional Biochemical Corp., Cleveland, Ohio.

served in experiment III compared to that observed in experiments I and II may be due to the Nembutal these animals received prior to death. In all other experiments no anesthesia was given before sacrificing the animals.

DISCUSSION

The suggestion that the sympathetic nervous system plays a regulatory role in FFA metabolism is based on the observations that administration of epinephrine or norepinephrine increases the plasma FFA (6, 7), that their addition in physiological concentrations *in vitro* stimulates release of fatty acids from adipose tissue (5, 8), that administration of ganglion blocking agents decreases the plasma FFA (7), and that injection of adrenergic blocking agents inhibits the increase in plasma FFA produced by epinephrine or norepinephrine administration (7, 9). Goodman and Knobil (9) made the interesting observation that administration of adrenergic blocking agents did not prevent the rise in plasma FFA in starvation. This highlights the complexity of the mechanism involved in the regulation of the release of FFA from adipose tissue.

An indirect indication of the involvement of the autonomic nervous system is furnished by the observations that marked elevation of the plasma FFA was found in human subjects during acute emotional arousal (11, 12). This effect was inhibited by trimethaphan camphor sulfonate (Arfonad®), a rapidly metabolized ganglion blocking agent. Recently, orthostatic hypotension (60° upright tilt) was used to produce "stress" as a means of elevating plasma FFA in fasting persons (13). The response was present in a patient who had both adrenal glands removed but was greatly diminished in a patient with primary autonomic insufficiency. Administration of phentolamine diminished the response to tilt. In contrast, other workers (7, 14) found no elevation of plasma FFA in dogs treated with epinephrine following adrenalectomy or hypophysectomy. Since treatment with cortisone restored the response, they concluded that the adrenal cortex was essential for the response.

Our results show that following epinephrine injection, the subsequent release of FFA from excised adipose tissue did not occur if the animals were also previously injected with an adrenergic blocking agent. Also, addition of phentolamine *in vitro* inhibits the release of FFA from adipose tissue induced by epinephrine added to the incubation medium. Thus the effectiveness of the adrenergic blocking agents following epinephrine administration is due to inhibition of the release of FFA from adipose tissue. This conclusion is further supported by the work of Havel and Goldfien's (7) *in vitro* experiments with dogs.

It is of interest that phentolamine *in vitro* inhibited the release of FFA from adipose tissue due to epinephrine, norepinephrine, and ACTH. One interpretation is that epinephrine, norepinephrine, and ACTH act through a common receptor site. When phentolamine is present in the medium, it combines with this receptor and prevents the action of these hormones. A second interpretation is that these hormones act through different receptor sites, all of which are blocked by phentolamine.

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