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INHIBITION OF ADIPOSE TISSUE LIPASE ACTIVITY FOLLOWING ADMINISTRATION OF VASOPRESSIN

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Our previous studies[1,2] demonstrated that the injection of vasopressin into rats caused a marked decrease in plasma free fatty acid (FFA) levels and that pretreatment of rats with vasopressin inhibited the FFA-mobilizing effect of norepinephrine. Suppressive effects of vasopressin on the body temperature and metabolic rate[3,4] were thus attributed, at least in part, to the reduced plasma concentration of FFA. However, *in vitro* release of FFA by epididymal fat pad of the rat was found not to be influenced by the addition of vasopressin into incubation medium. Moreover, *in vitro* effect of norepinephrine on the adipose tissue was not affected by vasopressin[1]. Hereupon, we have attempted to examine the *in vivo* effect of vasopressin injection on adipose tissue lipase activity in the rat.

METHODS

Male Wistar rats, weighing 200 to 300 g, were used throughout the study. They were housed at an ambient temperature of 20±2°C and fed rat biscuit *ad libitum*. Lysine vasopressin (Sandoz) and norepinephrine (Sankyo) were injected subcutaneously. The animals were in the fed state at the time of sacrifice. Epididymal fat pads and interscapular brown adipose tissue were removed as rapidly as possible.

The first series of experiments were made to observe FFA and glycerol release by the adipose tissues. After removal, 100 to 200 mg of white adipose tissue or 30 to 60 mg of brown adipose tissue slices were placed in a solution of Krebs-Ringer phosphate buffer at pH 7.4 which contained bovine albumin (Armour Fraction V) in a final concentration of 2 percent. Incubation was made at 37°C for 60 minutes. In experiments of glycerol determination, bovin albumin was omitted in the incubation medium, since albumin was found to interfere with the colorimetric determination of glycerol. FFA was determined by the method of Duncombe as described by Itaya and Ui[5] and glycerol by the modification of the method of Lambert and Neish described by Korn[6].

Hormone-sensitive lipase activity of the adipose tissues was measured as described by Rizack[7]. The fat pads were homogenized in 3 ml of 0.25 M sucrose per g of tissue. The homogenate was centrifuged at 12,000×g for 10 minutes. A fat layer which floated to the top was discarded. Remaining supernatant fluids were assayed to deter-

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mine their lipolytic activity by incubating them at 37°C after adding olive oil emulsion (Sigma) as substrate, bovine albumin in a final concentration of 5 percent, phosphate buffer at pH 6.8 and distilled water to make a final volume of 2.0 ml. After 20 minutes of incubation the reaction was stopped by the addition of organic solvents used to extract fatty acids. FFA released during the incubation period was determined and the results were expressed in terms of μEq FFA per g wet tissue per hour.

Lipoprotein lipase activity was measured by the method of CHARKES and GORDON[8] with minor modifications. Heparin (Novo) was added in a concentration of 6 units USP and olive oil emulsion activated by rat serum was used as substrate. In 2 ml of incubation medium, 100 to 200 mg white adipose tissue or 30 to 60 mg brown adipose tissue slices were incubated at 37°C for 60 minutes. FFA content of the assay mixture was determined before and after incubation.

RESULTS

1. *Effect of vasopressin on the release of FFA and glycerol by adipose tissues.* Rats were subcutaneously injected with lysine vasopressin in a dose of 100 mU per 100 g body weight 30 minutes before sacrifice. As shown in TABLE 1,

TABLE 1.
Effect of vasopressin injection on lipolytic activity of adipose tissues.

	No. of expt.	Incubation period		
		30 min	60 min	120 min
White adipose tissue				
FFA release (μEq/g)				
Saline control	9	4.71± .30	6.81± .45	
LVP*	7	3.02± .58	4.48± .78	
		P<0.01	P<0.01	
Glycerol release (μM/g)				
Saline control	12	1.05± .08	1.27± .08	1.66± .11
LVP	7	0.72± .02	0.84± .02	1.14± .14
		P<0.01	P<0.01	P<0.05
Brown adipose tissue				
FFA release (μEq/g)				
Saline control	5		14.86± .87	
LVP	6		12.58±2.14	
			NS	
Glycerol release (μM/g)				
Saline control	12		3.71± .19	
LVP	7		3.35± .16	
			NS	

* LVP: Subcutaneous injection of lysine vasopressin in a dose of 100 mU per 100 g body weight 30 minutes before sacrifice. Values are the mean± S.E. NS: not significant.

epididymal fat pads of rats treated with vasopressin released significantly less FFA and glycerol into incubation medium than the tissue of control rats. On the other hand, the release of FFA and glycerol by brown adipose tissue was not affected by vasopressin injection.

FIG. 1 shows the effect of norepinephrine injection (200 μg/100 g, s. c.) on the release of FFA and glycerol, and the influence of vasopressin treatment on it. Although norepinephrine markedly stimulated the release of FFA, vasopressin effectively inhibited the stimulating effect of norepinephrine on the adipose tissue. Similar results were also obtained in the release of glycerol.

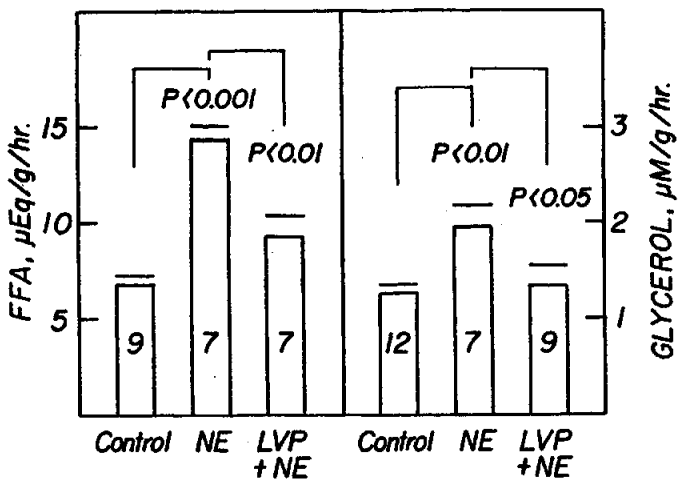

FIG. 1. Effect of norepinephrine (NE) and lysine vasopressin (LVP) on the release of FFA (left) and glycerol (right) by rat epididymal fat pads. Bars at the top of each column represent standard error of the mean. Numbers in the column indicate the number of experiments.

2. *Effect of vasopressin on hormone-sensitive lipase activity in adipose tissues.* Vasopressin was shown to be a potent inhibitor of hormone-sensitive lipase activity in epididymal fat pads (TABLE 2). Subcutaneous injection of lysine vasopressin in a dose of 40 mU per 100 g caused maximal inhibition, and further reduction of the activity was not observed even if the dose was elevated to 400 mU/100 g. The inhibitory effect was still demonstrable in tissues obtained 2 hours after vasopressin injection (400 mU/100 g, s. c.), although there observed a tendency of restoring toward the preinjection level.

When norepinephrine (200 μg/100 g, s. c.) was given, the lipase activity of white adipose tissue increased markedly 15 minutes after the injection. However, in rats treated with vasopressin (100 mU/100 g, s. c.) did not respond to norepinephrine at all (TABLE 3). The result indicates apparently that

TABLE 2.
Effect of vasopressin injection on hormone-sensitive lipase activity in adipose tissues.

	White adipose tissue			Brown adipose tissue		
	No. of expt.	FFA released μEq/g/hr	P	No. of expt.	FFA released μEq/g/hr	P
Saline control	14	10.98± .44		6	35.58±5.27	
Lysine vasopressin						
40 mU, 30 min	7	5.62± .37	<0.01	5	41.40±4.68	NS
100 mU, 30 min	6	6.38±1.06	<0.01	6	37.04±3.40	NS
400 mU, 30 min	6	5.86± .41	<0.01			
400 mU, 60 min	6	7.44± .26	<0.01			
400 mU, 120 min	6	8.67±1.25	<0.05			

Values are the mean±S.E. Pvs saline control.
NS: not significant.

TABLE 3.
Effect of subcutaneous injection of norepinephrine (200 μg/100 g) and lysine vasopressin (100 mU/100 g) on hormone-sensitive lipase activity of adipose tissues.

	White adipose tissue		Brown adipose tissue	
	No. of expt.	FFA released μEq/g/hr	No. of expt.	FFA released μEq/g/hr
Saline control	8	11.68±1.28	7	98.87±21.89
Norepinephrine	9	20.99±1.66	8	125.56±17.76
P		<0.001		NS
Saline control	9	14.05±1.70	8	108.75±15.40
Lysine vasopressin plus norepinephrine	9	14.14±1.27	8	135.87±26.36
P		NS		NS

Values are the mean±S.E. NS: not significant.

vasopressin is capable of inhibiting the lipolytic action of norepinephrine. On the other hand, the effect of vasopressin on the lipase activity of brown adipose tissue was rather equivocal, as seen in TABLE 2 and 3*.

* It is known that the rate of lipase activity depends on conditions of substrate oil emulsion. It was noticed that the rate of reaction was not the same when different batches of the substrate were employed and that physical conditions of oil emulsion changed during storage. These facts are possibly the reason that a considerable difference in the lipase activity occurred between the first series of experiment (TABLE 2) and the second one (TABLE 3). In the present study, therefore, parallel measurements of treated and control samples were always made in each experiment.

3. *Effect of vasopressin on lipoprotein lipase activity in adipose tissues.* The injection of lysine vasopressin (100 mU/100 g, s. c.) was found not to cause any significant change in the lipoprotein lipase activity of epididymal adipose tissue as well as interscapular brown adipose tissue (TABLE 4). The injection of norepinephrine showed a slight increase in the activity, but the value was not significantly different from the level of controls.

TABLE 4.
Effect of subcutaneous injection of lysine vasopressin (100 mU/100 g) and norepinephrine (200 μg/100 g) on lipoprotein lipase activity of adipose tissues.

	White adipose tissue		Brown adipose tissue	
	No. of expt.	FFA released μEq/g/hr	No. of expt.	FFA released μEq/g/hr
Saline control	9	9.37± .79	7	18.51±1.29
Lysine vasopressin	7	7.72±1.29	7	18.40±3.80
Norepinephrine	7	11.63±2.06	7	21.09±5.39

Values are the mean±S.E.

DISCUSSION

We have previously observed a marked decrease in the plasma concentration of FFA, but neither vasopressin nor oxytocin had any effect on epididymal fat pad *in vitro* in stimulating or inhibiting the release of FFA at all. Furthermore, vasopressin was found not to influence the lipolytic action of norepinephrine *in vitro*[1,2]. Hereupon, the present study was carried out under the same conditions as used in the previous investigations. Adipose tissues removed from rats which had been injected with vasopressin were assayed for their lipase activities. Results obtained indicated a marked inhibition of hormone-sensitive lipase activity in epididymal fat pads of rats treated with vasopressin. Catecholamines are known to activate the adipose tissue lipase[7,9,10,11]. It was shown in this study that pretreatment with vasopressin blocked the stimulatory effect of norepinephrine on lipolytic activity of the adipose tissue. This observation is in well accord with the finding that rats treated with vasopressin could not respond to norepinephrine in elevating plasma FFA levels. The fact that vasopressin was ineffective in affecting lipolysis *in vitro*, but inhibited the lipase activity when the hormone was given *in vivo*, suggests that the action of vasopressin on the adipose tissue is not direct, but the hormone exerts its inhibitory action through some other mechanism(s).

Since adipose tissue contains two types of lipases, it seemed of necessary to observe the effect of vasopressin not only on hormone-sensitive lipase but

also on lipoprotein lipase activity. The present study indicated that neither vasopressin nor norepinephrine significantly affected lipoprotein lipase activity in rat epididymal fat pads. Failure to demonstrate any effect of norepinephrine on lipoprotein lipase activity is in accord with observations by others. It was reported that epinephrine did not affect lipoprotein lipase activity in perfused or incubated rat epididymal fat pads[12,13] and that single injection of epinephrine had no influence on cardiac lipoprotein lipase activity in the rat[14].

Lipase activities of brown adipose tissue were, different from those of white adipose tissue, scarcely influenced by vasopressin treatment of animals. Brown adipose tissue is known as one of the major sites of heat production in cold-adapted homeotherms as well as in hibernators undergoing arousal[15,16]. In brown adipose tissue heat is produced by the local oxidation of triglycerides, while the main function of white adipose tissue is the synthesis and storage of triglycerides and the release of FFA into the blood stream. In view of the functional differences between the two types of adipose tissues, poor response of brown adipose tissue to vasopressin is not hard to understand.

From the results in the present as well as previous investigations, it is concluded that the injection of vasopressin into rats caused a marked inhibition of hormone-sensitive lipase activity in white adipose tissue and consequent decrease in the plasma concentration of FFA. The lowered plasma FFA level is considered to be a cause of decreasing metabolic rate which in turn to induce a lowering of the body temperatre. However, the action of vasopressin on the adipose tissue is not direct, but through other mechanism(s). This point should be elucidated by further experimentations.

SUMMARY

Epididymal fat pads of rats treated with lysine vasopressin released significantly less FFA and glycerol than the tissue of control rats. Vasopressin treatment effectively blocked the stimulatory effect of norepinephrine on adipose tissue lipolysis. Vasopressin, when given *in vivo*, was shown to be a potent inhibitor of hormone-sensitive lipase activity in white adipose tissue. On the other hand, appreciable change was not observed in the lipase activity of brown adipose tissue following vasopressin treatment.

Lipoprotein lipase activity of epididymal white fat as well as interscapular brown fat was not affected by both vasopressin and norepinephrine injections.

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