Stimulation of lipolysis in adipose tissue in vitro by inhibitors of lipid mobilization

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ABSTRACT 5-Methylpyrazole-3-carboxylic acid (U-19425) and nicotinic acid, which apparently inhibit lipolysis in vivo as indicated by low plasma FFA and glycerol concentrations, stimulate lipolysis in vitro in adipose tissue removed from fasted rats 30–90 min after treatment. This stimulation is not the result of low initial levels of FFA in adipose tissue. An increased rate of lipolysis is not induced in vitro by preincubating tissue of untreated rats with U-19425. The increase can be blocked in incubated adipose tissue of U-19425-treated animals if U-19425 is added to the incubation medium. The β-adrenergic blocking agent propranolol, at a concentration which nhibits epinephrine-stimulated lipolysis, does not affect the increase produced by administration of U-19425.

SUPPLEMENTARY KEY WORDS 5-methylpyrazole-3-carboxylic acid (U-19425) · nicotinic acid · plasma and tissue FFA · glycerol · epinephrine · propranolol

5-METHYLPYRAZOLE-3-CARBOXYLIC ACID (U-19425) is a potent oral hypoglycemic agent in alloxan-diabetic and fasted glucose-primed rats (1). It inhibits basal and epinephrine-stimulated FFA release from adipose tissue and lowers plasma FFA concentrations of fed and fasted rats (1, 2). In addition, it blocks glycerol release from adipose tissue of fasted-refed rats and depresses plasma FFA and blood glucose concentrations in rats made acutely diabetic with anti-insulin serum (3). The response of plasma FFA to U-19425 in man is biphasic; a depression is followed by an elevation above normal concentrations (4). Recently it was shown that in rats pretreated for 4 days with U-19425 the inhibition of lipolysis in vivo is of shorter duration (tachyphylaxis) than in control animals (5) and that in vitro epinephrine-stimulated lipolysis is enhanced (6). The tachyphylaxis is not due to altered metabolism of the drug since the metabolism and excretion of U-19425-^{14}C is the same in pretreated and control rats (7). In this report it is shown that lipolysis in vitro in adipose tissue of the rat is stimulated after a single dose of U-19425 or nicotinic acid and that stimulation occurs at a time when plasma and adipose tissue FFA concentrations are depressed. A preliminary report of our work has been presented (8).

MATERIALS AND METHODS

Male Spartan rats (Spartan Research, Haslett, Mich.), each weighing 200–250 g before an overnight fast, were used. Drugs were administered orally or by intraperitoneal injection, as indicated. Animals were lightly anesthetized with ether and bled from the abdominal aorta into syringes coated with 1% heparin solution. Fragments of epididymal adipose tissue (50–60 mg) were incubated in duplicate in 1 ml of Krebs–Ringer bicarbonate medium containing 3% crystalline bovine albumin (Armour) and 0.9 mg of glucose. The incubations were in Potter–Elvehjem homogenizer tubes at 37°C in an atmosphere of 5% CO_2 and 95% air in a Dubnoff metabolic shaker, oscillating at 60–70 cycle/min. After incubation, the adipose tissue was homogenized in the medium. Aliquots of homogenate and of plasma were analyzed for free fatty acid concentration by the procedure of Dole (9) as modified by Ko and Royer (10). Glycerol concentration of adipose tissue and plasma was measured by the fluorometric method of Ko and Royer (11).

RESULTS

The data in Table 1 show that treatment of fasted rats with U-19425 or nicotinic acid reduces the FFA concen-

Abbreviations: FFA, free fatty acid(s); cyclic AMP, adenosine 3′,5′-monophosphate.

TABLE 1 EFFECT OF U-19425 OR NICOTINIC ACID ON PLASMA AND ADIPOSE TISSUE FFA CONCENTRATION AND ON ADIPOSE TISSUE GLYCEROL CONCENTRATION OF FASTED RATS

Experiment Number and Treatment	Plasma FFA	Zero-Time Concentration	
		FFA	Glycerol
	μeq/liter	*μeq/g*	*μmoles/g*
1 Control	354 ± 76	3.6 ±,0.7	0.78 ± 0.08
U-19425, 5 mg/kg	116 ± 10*	1.9 ± 0.2†	0.41 ± 0.07†
U-19425, 25 mg/kg	105 ± 10*	1.6 ± 0.2*	0.37 ± 0.05†
2 Control	354 ± 40	3.3 ± 0.4	0.77 ± 0.10
U-19425, 5 mg/kg	103 ± 20*	1.3 ± 0.1*	0.43 ± 0.04*
3 Control	491 ± 26	3.9 ± 0.3	1.02 ± 0.04
Nicotinic acid, 25 mg/kg	163 ± 16*	1.5 ± 0.3*	0.54 ± 0.10*

Rats were sacrificed 90 min after U-19425 was administered and 75 min after administration of nitocinic acid. In experiments 1 and 3, the drug was given by intraperitoneal injection; in experiment 2 it was given orally.

Results are expressed as mean ±SEM of duplicate measurements in six animals.

* $P < 0.001$ vs. control.

† $P < 0.005$ vs. control.

A maximal effect is produced by 5 mg/kg of U-19425, and epinephrine-stimulated lipolysis is not increased in tissue of treated animals.

Subsequent experiments were carried out in attempts to elucidate the mechanism of this unexpected enhancement of lipolysis. Table 3 shows that lipolysis in vitro is stimulated as early as 30 min after a small dose of U-19425.

The effect on lipolysis of adding U-19425 or the β-adrenergic blocking agent, propranolol, to the incubation medium is shown in Table 4. U-19425 in the medium inhibits basal lipolysis and completely blocks the stimulation in adipose tissue from animals pretreated with U-19425. In contrast, propranolol neither inhibits basal lipolysis nor blocks stimulation of lipolysis in tissue from rats administered U-19425, but as expected, it is effective in reducing epinephrine-stimulated lipolysis.

To determine whether the stimulus for increased lipolysis is present at the time that adipose tissue is excised or whether it is induced during incubation, pieces

TABLE 2 EFFECT OF TREATMENT WITH U-19425 OR NICOTINIC ACID ON LIPOLYSIS IN VITRO IN ADIPOSE TISSUE OF FASTED RATS

Experiment Number and Treatment	Basal Lipolysis		Epinephrine-Stimulated Lipolysis			
			5.5×10^{-8} M		5.5×10^{-7} M	
	FFA	Glycerol	FFA	Glycerol	FFA	Glycerol
	μeq/g	*μmoles/g*	*μeq/g*	*μmoles/g*	*μeq/g*	*μmoles/g*
1 Control	0.4 ± 0.1	2.5 ± 0.2	—	—	16.8 ± 1.2	9.2 ± 0.4
U-19425, 5 mg/kg	6.5 ± 1.0*	4.9 ± 0.6†	—	—	14.5 ± 1.4	8.8 ± 0.9
U-19425, 25 mg/kg	4.5 ± 1.0†	4.1 ± 0.4‡	—	—	18.2 ± 1.8	10.0 ± 0.9
2 Control	2.8 ± 0.1	3.7 ± 0.3	7.0 ± 0.9	3.2 ± 0.3	16.6 ± 2.1	8.7 ± 0.6
U-19425, 5 mg/kg	7.2 ± 0.7†	4.5 ± 0.3‡	7.2 ± 0.9	3.2 ± 0.4	15.2 ± 1.9	6.9 ± 0.6
3 Control	2.1 ± 0.1	2.8 ± 0.3	—	—	—	—
Nicotinic acid, 25 mg/kg	5.8 ± 0.5†	4.2 ± 0.3†	—	—	—	—

Animals used in these experiments were the same as used for measurements in the corresponding experiments of Table 1.

Lipolysis shows the amount of FFA and glycerol released from tissue triglycerides during a 2 hr incubation. Results are expressed as mean ±SEM of duplicate measurements in six animals.

* $P < 0.001$ vs. control.

† $P < 0.005$ vs. control.

‡ $P < 0.025$ vs. control.

trations in plasma to approximately one-third of control values.

The results are in accord with reduced lipolysis (as opposed to increased turnover of FFA) in vivo which has been demonstrated for U-19425 (1) and for nicotinic acid (12, 13). Depressed lipolysis is indicated also by the reduced glycerol and FFA concentration in excised adipose tissue and is consistent with the antilipolytic activity in vitro of both compounds (1, 13).

Table 2 shows that lipolysis in vitro is stimulated in adipose tissue taken from the drug-treated animals used in Table 1. Basal lipolysis, whether measured by glycerol or FFA release, is greatly increased in adipose tissue removed from rats treated with U-19425 or nicotinic acid.

TABLE 3 EFFECT OF U-19425 ON PLASMA AND ADIPOSE TISSUE FFA AND ON LIPOLYSIS IN VITRO 30 MIN AFTER TREATMENT

Treatment	Plasma FFA	Zero-Time FFA Concentration	Lipolysis FFA
	μeq/liter	*μeq/g*	*μeq/g*
Control	706 ± 30	6.60 ± 0.3	3.27 ± 1.1
1 mg/kg U-19425	201 ± 9*	2.37 ± 0.2*	8.75 ± 1.0†

U-19425 was given orally 30 min before the animals were sacrificed.

Each number is the mean ±SEM of duplicate measurements in five animals. Lipolysis indicates the amount of FFA released from adipose tissue triglycerides during a 2 hr incubation.

* $P < 0.001$ vs. controls.

† $P < 0.005$ vs. controls.

TABLE 4 EFFECT OF ADDITION OF U-19425 OR PROPRANOLOL TO THE INCUBATION MEDIUM ON LIPOLYSIS IN VITRO IN ADIPOSE TISSUE FROM RATS TREATED WITH U-19425

Experiment Number and Treatment	Glycerol Released				
	Basal Lipolysis			Epinephrine-Stimulated Lipolysis, 5.5×10^{-7} M	
	No Addition	U-19425, 10^{-5} M	Propranolol, 10^{-4} M	(−)Propranolol	(+)Propranolol, 10^{-4} M
	μmoles/g	*μmoles/g*	*μmoles/g*	*μmoles/g*	*μmoles/g*
1 Control	2.63 ± 0.44	0.85 ± 0.19	2.62 ± 0.40	—	—
5 mg/kg	5.16 ± 0.30	0.96 ± 0.13*	5.34 ± 0.40	—	—
2 Control	2.95 ± 0.17	1.19 ± 0.09	2.96 ± 0.34	5.61 ± 0.49	−0.57 ± 0.17
5 mg/kg	5.24 ± 0.41	0.97 ± 0.16*	5.21 ± 0.33	4.72 ± 0.52*	−1.13 ± 0.20*

U-19425 was given by intraperitoneal injection 90 min before the animals were sacrificed. Where indicated, U-19425 or propranolol was added to the medium during incubation of tissue. The results show the amount of glycerol released from tissue triglycerides during a 2 hr incubation. Each number is the mean ±SEM of duplicate measurements in six animals. Negative numbers indicate that the amount of glycerol released was less than the amount released under basal conditions.

* These are not significantly different from control values. All others are significantly different ($P < 0.001$).

TABLE 5 EFFECT OF PREINCUBATING ADIPOSE TISSUE WITH U-19425 ON SUBSEQUENT LIPOLYSIS

Conditions	FFA Found	FFA Released
	μeq/g	
Nonincubated tissue	4.02 ± 0.28	—
Incubated tissue + medium	4.80 ± 0.41	0.78
Preincubated tissue + medium	3.22 ± 0.38	(−)0.80

Three pieces of adipose tissue taken from each of four fasted rats were preincubated 30 min in medium containing U-19425 (10^{-5} M). They were rinsed, transferred to fresh medium, and incubated for 90 min. FFA were measured in preincubation medium and in tissue and incubation medium. Control pieces were incubated 2 hr.

of tissue were incubated for varying periods of time. Fig. 1 shows that the lipolytic rate is greater in tissue of treated animals than in controls at all times during the 2 hr incubation period. It is obvious that the rate of lipolysis is not linear after 1 hr. Nevertheless, the 2 hr incubation period which was used in this work does not alter the conclusions since the data of Fig. 1 demonstrate that the amount of FFA liberated from adipose tissue of treated animals is already different from control values during the linear-response period.

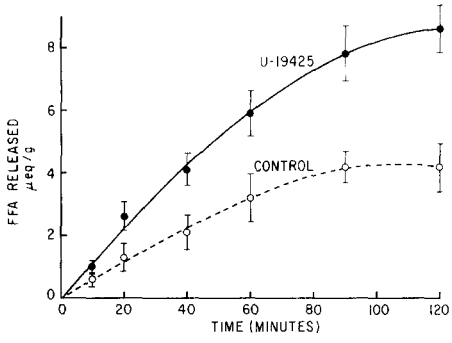

FIG. 1. Release of free fatty acids from adipose tissue as a function of incubation time. The concentration of FFA in tissue and medium (expressed as μeq/g of tissue) for treated and control rats, respectively, was: 0 min, 1.4 and 2.3; 10 min, 2.4 and 2.9; 20 min, 4.0 and 3.6; 40 min, 5.5 and 4.4; 60 min, 7.3 and 5.5; 90 min, 9.2 and 6.5; 120 min, 10.0 and 6.5. Vertical bars = ±SEM. Each point represents mean of determinations in tissue from 10 rats in treated group and nine in control group.

Subjecting adipose tissue to U-19425 in vitro does not stimulate lipolysis during a subsequent period of incubation in drug-free medium. Table 5 shows that there is no increased release of FFA from incubated adipose tissue if lipolysis in vitro is inhibited by preincubating with U-19425. There is, in fact, a decrease in FFA in tissue and medium, probably due to reesterification or oxidation of the FFA present in adipose tissue at the time of its excision.

DISCUSSION

The demonstrated enhancement of lipolysis induced by two antilipolytic agents in adipose tissue appears to be a paradox. It is known that lipid mobilizing hormones, for example ACTH (14) and the catecholamines (15), elevate plasma FFA concentrations and stimulate lipolysis in incubated adipose tissue removed from treated animals. Thus the antilipolytic agents studied here would have been mistaken for lipid mobilizing agents if FFA release from adipose tissue in vitro were taken as an index of activity.

Both nicotinic acid and U-19425 cause an initial depression of plasma FFA which is followed by a rebound above normal levels (4). The early onset of increased lipolysis in vitro (Table 4) suggests that the increase is not due to increased synthesis of triglyceride lipase in adipose tissue.

A low initial concentration of FFA in adipose tissue cells increases lipolysis (16). That the low initial concentration of FFA is not responsible for the increased lipolysis reported here is shown by Fig. 1. The data demonstrate that the rate of lipolysis in tissue from treated animals is greater than that in tissue from control animals throughout the incubation period. If concentration of

FFA in tissue were the determining factor, the rate of lipolysis should be equal in treated and control tissue after the concentration of FFA in treated tissue becomes equal to FFA concentration in control tissue (about 20 min in Fig. 1). Furthermore it is shown that lowering of FFA in tissue by inhibiting lipolysis in vitro during a preincubation period fails to increase FFA release after the tissue is removed from medium containing the inhibitor (Table 5). Thus it is clear that lipolysis can not be stimulated by inhibiting lipolysis for a brief period in vitro.

Since propranolol, a β-adrenergic blocking agent, failed to reduce the lipolysis in vitro induced by administration of U-19425 to rats, but inhibited epinephrine-stimulated lipolysis, it seems unlikely that catecholamine concentration is increased in tissue and is responsible for the effect. However, it is possible that a lipolytic hormone which is not blocked by propranolol begins to exert its influence once the tissue is removed from the animal.

Increased tissue concentration of cyclic AMP at the time that the animal is sacrificed would appear to be ruled out, since neither nicotinic acid (17) nor U-19425 (2) blocks the lipolytic activity of dibutyryl cyclic AMP. Yet the increased lipolysis shown here is blocked when U-19425 is added in vitro. It is possible, however, that nicotinic acid and U-19425 do not block dibutyryl cyclic AMP-stimulated lipolysis because the phosphodiesterase which hydrolyzes cyclic AMP splits the substituted nucleotide very poorly (18). We have confirmed this observation in unpublished experiments. Thus, recent evidence indicating that phosphodiesterase is stimulated by nicotinic acid in incubated adipose tissue (19) could help explain the results reported here. It is conceivable that stimulation of phosphodiesterase in vivo inhibits lipolysis. A compensatory increase in cyclic AMP would then stimulate lipolysis only after the tissue is removed from the animal and from the influence of U-19425 or nicotinic acid. The failure of propranolol to inhibit increased lipolysis in vitro would be expected under these conditions since β-adrenergic blocking agents inhibit formation of cyclic AMP but do not block its activity. Inhibition of enhanced lipolysis in incubating tissue by U-19425 would be consistent with stimulation of phosphodiesterase.

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References

1. Gerritsen, G. C., and W. E. Dulin. 1965. *J. Pharmacol. Exp. Ther.* **150:** 491.
2. Kupiecki, F. P., and N. B. Marshall. 1968. *J. Pharmacol. Exp. Ther.* **160:** 166.
3. Froesch, E. R., M. Waldvogel, U. A. Meyer, A. Jakob, and A. Labhart. 1967. *Mol. Pharmacol.* **3:** 442.
4. Gundersen, K., and H. V. Demmisianos. 1969. *In* Advances in Experimental Medicine and Biology. Plenum Publishing Corporation, New York.
5. Gerritsen, G. C., and W. E. Dulin. 1967. *Proc. Soc. Exp. Biol. Med.* **126:** 524.
6. Bizzi, A., A. M. Codegoni, A. Lietti, and S. Garattini. 1967. *J. Pharm. Pharmacol.* **19:** 549.
7. Smith, D. L., J. G. Wagner, and G. C. Gerritsen. 1967. *J. Pharm. Sci.* **56:** 1150.
8. Kupiecki, F. P., and D. I. Schneider. 1968. *In* Abstracts: Third International Congress of Endocrinology. C. Gual, editor. Excerpta Medica Foundation, New York. **157:** 173.
9. Dole, V. P. 1956. *J. Clin. Invest.* **35:** 150.
10. Ko, H., and M. E. Royer. 1967. *Anal. Biochem.* **20:** 205.
11. Ko, H., and M. E. Royer. 1968. *Anal. Biochem.* **26:** 18.
12. Carlson, L. A., and L. Orö. 1962. *Acta Med. Scand.* **172:** 641.
13. Carlson, L. A. 1963. *Acta Med. Scand.* **173:** 719.
14. Lebovitz, H. E., and F. L. Engel. 1965. Handbook of Physiology: Adipose Tissue. A. E. Renold and G. F. Cahill, Jr., editors. Waverly Press, Inc., Baltimore, Md. **V:** 541.
15. Triner, L., and G. G. Nahas. 1966. *J. Pharmacol. Exp. Ther.* **153:** 569.
16. Rodbell, M. 1965. *Ann. N.Y. Acad. Sci.* **131:** 302.
17. Peterson, M. J., C. Patterson, and J. Ashmore. 1968. *Life Sci.* **7:** 551.
18. Butcher, R. W., R. J. Ho, H. C. Meng, and E. W. Sutherland. 1965. *J. Biol. Chem.* **240:** 4515.
19. Schwandt, P., and T. Hartmann. 1968. *Z. klin. Chem. klin. Biochem.* **6:** 497.