A LIPID MOBILIZING SUBSTANCE FROM THE CALF MID-BRAIN

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Received February 5, 1968

Many naturally occurring substances stimulate the mammalian fat cell to convert stored triglycerides into fatty acids and glycerol, which are transported to other organs. These adipokinetic, or lipid mobilizing, substances are thought to stimulate a lipolytic enzyme within the fat cell. Such substances include at least seven pituitary hormones (Rudman, et al 1965), the catecholamines (Rudman, et al 1965) and a number of peptides which are related, either directly or indirectly, to the pituitary gland (Chalmers et al 1960, Rudman et al 1962). Lipid mobilizing activity has been found in extracts of the hypothalamus and of the posterior pituitary of cadavers (Kadas and Nagy 1965).

This report describes the isolation and properties of a lipid mobilizing factor (LMF) from the calf mid-brain. Since the hypothalamus and certain other areas of the mid-brain are particularly rich in norepinephrine (1966), comparative studies of the catecholamines and the mid-brain extract were performed. Evidence is presented to show that the LMF from the mid-brain is not a catecholamine.

Materials and Methods

Preparation of Mid-brain Extract. Fresh calf mid-brains (free of the pituitary gland and the pituitary stalk) were excised at the

slaughter house and immediately placed in cold acetone:HCl (80:20, vol), pH 4.0. A 3 per cent homogenate of the tissue was made with the acetone-HCl mixture. After standing at 4C for 16 to 18 hours, the homogenate was centrifuged at 10,000 rev/min in a refrigerated centrifuge. The clear supernatant was collected and concentrated on a rotary evaporator at 30C. The concentrate was treated with methanol and filtered; methanol was removed from the filtrate under vacuum. The extract was a brownish yellow color and very viscous; the pH was 5.6 to 5.9. A batch of mid-brains, weighing 17 lbs, after the above process, was concentrated to a volume of 56 ml. For use, the crude extract was diluted 1:60 with water; 0.10 ml of the diluted extract was used in the assay system.

Method of Assay. Lipid mobilizing activity was determined by the ability of the mid-brain extract to (a) increase the free fatty acid (FFA) content of adipose tissue and (b) to release FFA from adipose tissue into a medium containing albumin as fatty acid acceptor.

Tissue Preparation and Incubation. Male Sprague-Dawley rats, weighing between 200-250 g were used in all experiments. Epididymmal fat pads were removed under Nembutal and rinsed in 3 changes of saline at room temperature. Pieces of fat pad (about 50 mg) were placed in 10 ml erlenmeyer flasks containing 0.90 ml of 0.06M phosphate buffer, pH 7.4 and 0.10 ml of the diluted extract, with and without albumin; the final volume was always 1.0 ml. Albumin when used, was at a concentration of 2.5 per cent in buffer. Incubation was at 37C in a Dubnoff water bath, with gentle agitation. The gas phase was 95 per cent oxygen and 5 per cent carbon dioxide. After 30 minutes, the tissues were removed, rinsed in saline and homogenized in 5.0 ml of chloroform: to measure the FFA content of

the medium a 0.10 ml sample was used.

Epinephrine HCl (Epi) and Norepinephrine Bi-tartrate (NE) were made up in phosphate buffer, and unless otherwise stated, the concentration used in the assay system was 1 µg.

Free Fatty Acids (FFA) were measured by the colorimetric method described by Duncombe (1963), with a minor modification (Itaya and Ui, 1965). Results are expressed as mMoles FFA released/100 mg adipose tissue/30 minutes.

Protein Nitrogen was determined by the method of Lowry et al (1951).

Results and Discussion

Comparison of Lipid Mobilizing Activities of Mid-brain Extract and Epinephrine. Initial studies were conducted to compare the lipid mobilizing activity of the mid-brain extract with

TABLE I

A Comparison of Lipid Mobilizing Activities of Mid-brain Extract and Epinephrine

Experimental Conditions	mMoles FFA/100 mg/30 minutes			
	Tissue		Medium	
With albumin:				
Control	0.36 ± 0.05*	(6)	0.21 ± 0.02	(8)
Mid-brain Extract	1.30 ± 0.09	(6)	0.58 ± 0.08	(8)
Epinephrine	1.36 ± 0.07	(3)	0.52 ± 0.05	(3)
Without albumin:				
Control	0.37 ± 0.02	(7)	None	
Epinephrine	1.40 ± 0.08	(12)	"	
Control	0.33 ± 0.03	(17)	"	
Mid-brain Extract	1.38 ± 0.10	(19)	"	

* ± S.E. of Mean. The figures in parentheses represent the number of experiments. The Controls contained either buffer + albumin, or buffer alone.

that of Epi and to observe (a) the effect on FFA release to the medium, with albumin as fatty acid acceptor, and (b) the accumulation of FFA within the adipose tissue, with and without albumin in the incubation medium. The results are shown in Table 1. The crude mid-brain extract, in the concentration used, was equivalent in lipid mobilizing activity to 1 ug of Epi, both with respect to FFA release to the medium, when a fatty acid acceptor was present, and to the accumulation of FFA in the tissue, with and without albumin.

The accumulation of FFA within the tissue in the absence of albumin was adjudged to be a measure of lipolytic activity within the fat cell; therefore, subsequent experiments were conducted without albumin, under these conditions there was no release of FFA to the medium.

Method of Purification. The procedure developed for the purification of the mid-brain extract (Table 2) was gel filtration on Sephadex G-25 and elution with 0.006M phosphate buffer, pH 7.4, followed by chromatography of the active fraction on carboxymethyl cellulose and elution with 0.5N NH_4Ac, pH 6.0. A

TABLE 2

Method of Purification

Fraction of Mid-brain Extract	Total Protein	Total Activity	Percent Recovery	*Specific Activity
	mg			
Crude Extract	1481.25	8295.00	100	5
Sephadex G-25	661.06	7840.17	94	11
Carboxymethyl cellulose	2.69	2709.21	33	1007

*mMoles FFA/mg protein

purification of 200-fold was achieved with a recovery of 33 per cent of the activity.

Lipid Mobilizing Activity under a Variety of Conditions. The lipid mobilizing activity of the partially purified LMF (Sephadex Fraction) and NE was compared under a variety of experimental conditions. The results are shown in Table 3.

The two substances differed in their responses to proteolytic enzymes, under acid, neutral and alkaline conditions; both were stable during heating 2 hours at pH 2.6, but not at pH 5.0. LMF and NE were inhibited by dichloroisoproterenol (a beta adrenergic blocking agent), and activated by theophylline (1.3 dimethyl-xanthine). The inhibition by DCI was reversed by theophylline. It is probable that LMF and NE stimulate lipolysis by a similar mechanism.

TABLE 3

Experimental Conditions	mMoles FFA/100 mg/30 minutes	
	Mid-brain Extract	Norepinephrine
No treatment	1.38 (6)*	1.46 (6)
2 hrs. at 100 C, pH 2.6	1.38 (6)	1.36 (6)
2 hrs. at 100 C, pH 5.0	1.32 (6)	0.43 (6)
Pepsin, 5 mg/ml	0.77 (5)	1.06 (5)
Papain, 5 mg/ml	1.22 (5)	0.94 (5)
Trypsin, 84 units/ml, pH 8.5	0.68 (6)	0.44 (6)
Exposure to pH 8.5	1.24 (6)	0.46 (6)
Dichloroisoproterenol, 0.64 μg	0.70 (4)	0.51 (4)
Theophylline, 1 x 10^{-2}M	2.42 (4)	1.70 (4)

*Figures in parentheses represent the number of experiments.

Ultraviolet Absorption. A major difference between the lipid mobilizing factor from the mid-brain and the catecholamines was

the ultraviolet absorption spectrum. The results are shown in Table 4.

TABLE 4

Compounds	Absorption Maxima Wavelength mμ
Crude Mid-brain Extract	250
Sephadex Fraction	248
Carboxymethyl Cellulose Fraction	250
Epinephrine	278
Norepinephrine	278
Adrenochrome	294
Purified LMF + Epinephrine	240,270

Co-chromatography of the Mid-brain Extract and the Catecholamines. The catecholamines may be made to fluoresce in u.v. light on paper chromatograms stained with 0.66% potassium ferricyanide and 40% formaline, 9:1 (1959). Chromatograms were prepared with 1 μg each of Epi and NE and the equivalent in lipid mobilizing activity of the purified extracts, separately and in combination. The catecholamines showed characteristic fluorescence in u.v. light, Epi, a yellow-green (sensitivity 0.4 μg), and NE, turquoise (sensitivity 0.2 μg); the purified LMF exhibited no fluorescence and had no component corresponding to the catecholamines.

Experiments are in progress to identify the substance from the mid-brain.

Acknowledgements. The author wishes to acknowledge the technical assistance of Mr. Settle W. Townsend and Mr. Littleton H. Wade.

This investigation was supported, in part, by a grant-in-

aid from the American Heart Association, and in part by U.S. Public Health Service research grant 09235-03 MET and by a Public Health Service research career program award (1-K3-HE-25, 392-03) from the National Institutes of Health.

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