J. Lipid Research, October, 1962
Volume 3, Number 4

The lipolytic activity of rat kidney cortex and medulla*

C. H. Hollenberg and I. Horowitz
University Medical Clinic,
The Montreal General Hospital,
Montreal 25, Canada

[Received for publication May 10, 1962]

SUMMARY

Slices of rat kidney medulla and cortex were incubated for 30 min in a triglyceride medium with and without heparin; the slices were then removed and the lipolytic activity of the medium measured over the following 30 min. Heparin markedly increased the activity in the medium when medullary slices were used; it had a much smaller effect with cortical preparations. Protamine sulphate and 1 M sodium chloride inhibited the activity released by medullary slices. Homogenates of rat kidney medulla hydrolyzed activated triglyceride to a greater extent than nonactivated substrate. The activity of medullary homogenates was enhanced by heparin and inhibited by protamine and strong salt solutions. Cortical homogenates hydrolyzed activated substrate to only a slightly greater extent than nonactivated substrate, and the activity of these homogenates was not affected by heparin or by protamine sulphate. The results suggest that lipoprotein lipase is present in rat kidney medulla and that a lipase differing from this enzyme in a number of respects is present in rat kidney cortex.

Recent studies suggest that the substrates used by the kidney as energy sources are predominantly noncarbohydrate in nature (1). As lipoprotein lipase has been implicated in the metabolism of circulating triglyceride, it was considered of interest to determine whether this enzyme could be demonstrated in either the cortical or medullary portions of rat kidney. Earlier observations by Korn suggested that acetone-dried extracts of rat kidney might contain lipolytic activity similar to lipoprotein lipase, since these extracts hydrolyzed chylomicron triglyceride to a greater extent than triglyceride alone (2).

METHODS

Male Wistar rats weighing 150–180 g were anesthetized with sodium pentobarbital and the kidneys were quickly excised. Following decapsulation and removal of pelvic fat, transverse sections of the organs 1- to 2-mm thick were prepared. Separation of cortex and medulla was carried out under transillumination, and adequate separation was confirmed by histological examination. Studies were performed using either tissue slices or homogenates; in all experiments the tissue was chilled from time of removal from the animal to the start of incubation.

The initial studies were designed to determine the effect of heparin on the release of lipase from tissue slices. Two groups of medullary slices, each weighing approximately 50 mg, and two groups of cortical slices, each weighing about 100 mg, were prepared from each animal and incubated in four flasks. The time from removal of the kidneys to the start of incubation rarely exceeded 10 min. To one of each pair of flasks was added 125 μg of heparin per ml (Liquaemin, Organon, 10 mg/ml). The medium (unless otherwise specified) consisted of 5.5 parts of Krebs-Ringer phosphate buffer, 1.5 parts of 0.4 M Tris (tris(hydroxymethyl)aminomethane) buffer, 2.5 parts of 20% human albumin pretreated to remove bound fatty acid (3), and 0.5 parts of 20% coconut oil (Ediol-Schenlabs) in a total volume of 3 ml. The coconut oil was used either in an activated form, prepared by incubating 40% triglyceride with an equal volume of fresh human serum for 15 min at 37°, or as nonactivated triglyceride, prepared by diluting 40% coconut oil with an equal volume of phosphate buffer. The pH of the medium was adjusted to 8.0 prior to addition of the slices.

Incubation was carried out in a Dubnoff shaker at 37° under air. At the end of 30 min, the tissue was

* Supported by Grant MA-872 from the Medical Research Council of Canada, and by PHS Research Grant H-5282 from the National Institutes of Health, U.S. Public Health Service.

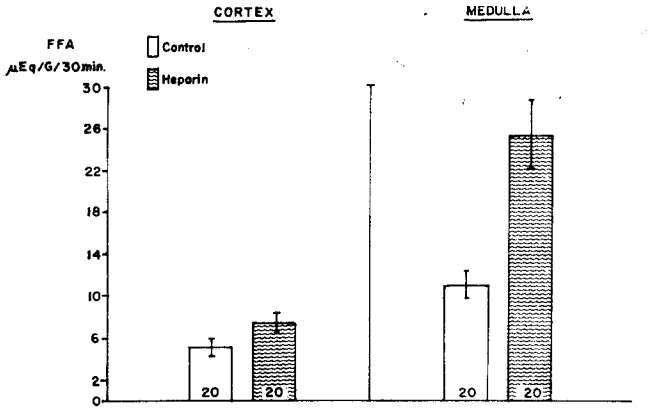

FIG. 1. The Release of Lipolytic Activity from Slices of Rat Kidney Cortex and Medulla. In this and succeeding figures, the brackets designate the standard error, and the number of experiments are indicated at the foot of each column. Statistical analysis: cortex control vs. cortex with heparin, $0.02 < p < 0.05$; medulla control vs. medulla with heparin, $p < 0.001$.

removed and the free fatty acid (FFA) content of a sample of the medium was determined by the method of Dole (4). The medium was incubated for another 30 min, at which time a second sample was removed. The lipase activity released into the medium was estimated by the change in the FFA content of the medium over the second 30 min of incubation and was expressed as microequivalents of FFA produced per gram of tissue per 30 min.

In experiments in which homogenates were used, approximately 100 mg of cortex or medulla was ground in a glass homogenizer containing 1.2 ml of Krebs-Ringer phosphate buffer. An aliquot of each homogenate was added to a medium that had a final composition of 1 part homogenate, 2 parts phosphate buffer, 1.5 parts 0.4 M Tris buffer, 5 parts 20% human albumin, and 0.5 parts 20% coconut oil. The medium was adjusted to pH 8.0 prior to the addition of homogenate, and the volume of the final mixture was 3.5 ml.

Incubation was carried out for 20 min under the conditions described above, and the extent of lipolysis was determined by measuring the change in the FFA content of the mixture over the 20-min period. This was expressed as microequivalents of fatty acid produced per gram of tissue per 20 min. Virtually no change in FFA concentration occurred when coconut oil was omitted from the homogenate–substrate mixture.

TABLE 1. INHIBITION OF LIPOLYTIC ACTIVITY RELEASED FROM RAT KIDNEY MEDULLA*

Inhibitor	Lipolytic Activity as a Percentage of Control
1 M NaCl (4)†	16 (0–45)‡
Protamine sulphate (4)	15 (0–33)

* In the studies with 1 M NaCl, two groups of medullary slices were incubated separately in 3 ml of medium containing 125 γ/ml of heparin. One flask contained 0.176 g of NaCl dissolved in the medium. Incubation and sampling were performed as previously described. In the studies with protamine sulphate, two groups of medullary slices were each incubated for 30 min in a medium (pH 8.0) of 0.75 ml 20% human albumin, 0.45 ml 0.4 M Tris buffer, 1.65 ml phosphate buffer, and 125 γ/ml heparin. The tissue was then removed, 0.3 ml of protamine sulphate (Lilly, 10 mg/ml) was added to one flask, and 0.3 ml of phosphate buffer to the other; incubation continued for 10 min. To both flasks was then added 0.155 ml of activated substrate (pH 8.0), and lipolysis was measured over a final 30-min period of incubation.

† Number of experiments shown in parentheses.

‡ Mean and range shown in parentheses.

TABLE 2. LIPOLYSIS OF ACTIVATED AND NONACTIVATED SUBSTRATE BY LIPASE RELEASED FROM RAT KIDNEY MEDULLA*

	Lipolysis *μEq/g/30 min*
Activated substrate (5)†	29 ± 5‡
Nonactivated substrate (5)	13 ± 1

* Two groups of medullary slices were each incubated in 3 ml of medium containing 125 γ/ml of heparin. Incubation and sampling procedures and composition of the medium have been previously described.

† Number of experiments shown in parentheses.

‡ Mean ±SE. P value of difference between means <0.01.

RESULTS AND DISCUSSION

The Release of Lipolytic Activity from Cortical and Medullary Slices of Rat Kidney. Previous studies have shown that heparin augments release of lipoprotein lipase from slices of rat heart, diaphragm, and adipose tissue (3, 5, 6, 7). Figure 1 illustrates the results of similar studies with slices of rat kidney cortex and medulla. In the absence of heparin, the activity found in the medium following incubation of medullary slices was significantly greater than that found with cortical preparations. Although heparin increased the activity in the medium when added in the presence of either medullary or cortical slices, this effect was much greater with medullary preparations.

Activity released from slices of kidney medulla was substantially inhibited by 1 M sodium chloride and protamine sulphate (Table 1), and hydrolyzed a serum-triglyceride substrate to a greater extent than triglyceride alone (Table 2). The characteristics of this activity are thus similar to those of lipoprotein lipase (2, 8). As heparin is known to activate partially purified preparations of lipoprotein lipase (2), the increase in medium activity after incubation with

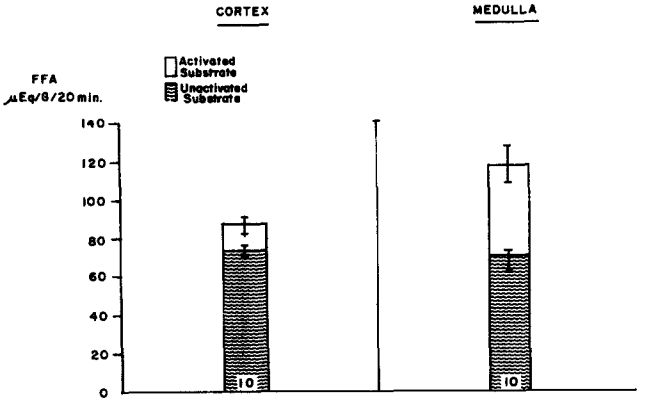

FIG. 2. Lipolysis of Activated and Nonactivated Triglyceride by Homogenates of Rat Kidney Cortex and Medulla. The height of the entire bar represents lipolysis of activated substrate, while that of the hatched area indicates lipolysis of nonactivated triglyceride. Statistical analysis: cortex, activated substrate vs. nonactivated substrate, N. S.; medulla, activated substrate vs. nonactivated substrate, $p < 0.001$.

heparin may have been due to a combination of enhanced release of enzyme from tissue sites and activation of enzyme in tissue and/or medium. The major effect of heparin, however, was probably on the release phenomenon, for addition of heparin to medium in which medullary slices had been previously incubated resulted in only a small increase in lipolytic activity (control, 6 μEq/g; heparin, 8 μEq/g). Furthermore, homogenates of medullary slices incubated with heparin prior to grinding actually had slightly less lipolytic activity than did control preparations (control, 87 μEq/g; heparin, 83 μEq/g).

Lipolytic Activity of Homogenates of Rat Kidney Cortex and Medulla. The lipolytic activity of homogenates of rat kidney cortex and medulla on activated and unactivated substrate is shown in Figure 2, and the effect on the activity of these homogenates of heparin, protamine sulphate, and 1 M NaCl is demonstrated in Table 3.

In keeping with the results of the experiments on medullary slices, the characteristics of the lipolytic activity of medullary homogenates were found to be similar to those of lipoprotein lipase. Homogenates of medulla hydrolyzed activated substrate to a greater extent than they hydrolyzed nonactivated triglyceride, and the activity of these preparations was augmented by heparin and inhibited by protamine and strong salt solutions.

The lipolytic activity of cortical homogenates differed from that of medulla in several respects. Cortical homogenates hydrolyzed activated substrate to only a slightly greater extent than nonactivated triglyceride,

TABLE 3. EFFECT OF HEPARIN, PROTAMINE SULPHATE, AND 1 M NaCl ON THE LIPOLYTIC ACTIVITY OF HOMOGENATES OF RAT KIDNEY CORTEX AND MEDULLA*

Agent	Lipolytic Activity as a Percentage of Control	
	Cortex	Medulla
Heparin (3)†	105 (101–111)‡	130 (121–135)
Protamine sulphate (3)	95 (80–111)	39 (37–43)
1 M NaCl (2)	55 (51–58)	25 (18–32)

* In the studies with heparin, two aliquots of both cortical and medullary homogenates were added to medium containing activated substrate. Heparin (50 γ/ml) was added to one of each pair of flasks. Incubation and sampling were performed as previously described. The same procedure was followed in the experiments with NaCl, except that in this instance no heparin was added, and one of each pair of flasks contained 0.205 g of NaCl in 3.5 ml of homogenate-substrate mixture. In the studies with protamine sulphate, two aliquots of both cortical and medullary homogenates were added to medium containing all ingredients except coconut oil. Protamine (250 γ/ml) was present in one of each pair of flasks. This mixture was incubated for 10 min, activated substrate was added, and lipolysis was measured over the ensuing 20 min.

† No. of experiments shown in parentheses.

‡ Mean and range.

and the activity of these homogenates was little affected by heparin or protamine sulphate when these agents were used in concentrations sufficient to produce definite effects in medullary tissue. These results suggest that, although an active lipase exists in rat kidney cortex, this lipase is not similar to lipoprotein lipase.

The role that lipoprotein lipase may play in the metabolism of rat kidney medulla remains to be clarified. It has been suggested that this enzyme hydrolyzes circulating triglyceride, liberating, in the various tissues that contain this enzyme, fatty acids which are then available for oxidation or esterification (3). Hence the presence of lipoprotein lipase in the medullary portion of the kidney may enable it to use serum triglyceride efficiently as an energy source.

Miss Heather McLeod provided valuable technical assistance during this study.

REFERENCES

1. Chinard, F. P., W. R. Taylor, M. F. Nolan, and T. Enns. *Am. J. Physiol.* **196**: 535, 1959.
2. Korn, E. D. *J. Biol. Chem.* **215**: 1, 1955.
3. Hollenberg, C. H. *Am. J. Physiol.* **197**: 667, 1959.
4. Dole, V. P. *J. Clin. Invest.* **35**: 150, 1956.
5. Hollenberg, C. H. *J. Clin. Invest.* **39**: 1282, 1960.
6. Cherkes, A., and R. S. Gordon, Jr. *J. Lipid Research* **1**: 97, 1959.
7. Robinson, D. S. *J. Lipid Research* **1**: 332, 1960.
8. Korn, E. D. *J. Biol. Chem.* **215**: 15, 1955.