Lipolytic Effect of Insulin and Other Hormones in vitro in Aortic Tissue of Experimental Animals

In adipose tissue which is considered to be the most important depot of triglycerides, lipolysis is widely controlled by a number of hormones. Catecholamines, ACTH, growth hormone, TSH, glucagon and other hormones are known to stimulate adipose tissue triglyceride lipase[1-3], some of them (especially catecholamines) probably through the elevation of cyclic 3'5'-AMP level[4]. Besides these lipolytic hormones, insulin was the only hormone found in adipose tissue to decrease the hormone-sensitive lipase activity and to decrease the lipolytic effect of other hormones[5]. Although lipolysis represents an important metabolic process also in other tissues, its regulation there remains to be studied.

In this paper we centred our attention on aortic tissue where lipases are considered to preserve aortic wall against lipid deposition and might therefore play an important role in pathogenesis of atherosclerosis[6,7].

Materials and methods. The experiments were carried out in descendent thoracic aorta free of adventitia in rats, guinea-pigs, rabbits and pigs. The material was worked up immediately after killing the animals. Procedure A: the samples of intact tissue were incubated for 30 min at 37 °C in 2.5 ml of Krebs phosphate buffer (pH 7.4) with albumin 25 mg/ml medium in the presence or in the absence of hormone. Glycerol released from the endogenous substrate to the medium was estimated after the end of incubation colorimetrically[8]. Procedure B: the samples of tissue homogenate (supernatant obtained after homogenization in distilled water and centrifugation at 2000 *g* for 5 min) were preincubated at 37 °C for 10 min in 2 ml of buffered medium (pH 7.4) containing 80 μEq. KCl with or without hormone. After 10 min preincubation, 5 ml of tristearin emulsion ($10^{-3}M$) in buffered medium (pH 7.4) was added and incubated with tissue homogenate for 30 min at 37 °C. Glycerol released from tristearin was estimated colorimetrically[8]. Hormones added to the media were: ACTH (Synacthen Ciba), epinephrine (Adrenalin Spofa), norepinephrine (Noradrenalin Spofa), glucagon (Eli Lilly Co.) and insulin (Insulin Spofa). Besides Insulin Spofa also Insulin Novo free of glucagon was used and the results in both cases did not differ. The results were expressed in nmoles of glycerol released/mg of wet tissue (mean ± S.E.), and were statistically evaluated by means of *t*-test.

Results and discussion. The results of all experiments showed the presence of hormone-sensitive lipase in rat aortic tissue as well as in aortic tissue from pigs (Table), guinea-pigs and rabbits. We have found analogical results when lipolysis was measured indirectly by glycerol released from endogenous substrate as well as when lipase activity was measured by glycerol released from exogenous substrate tristearin (Table). In aortic tissue of adult animals there is apparently only a very small amount of glycerokinase, if any. It is supported by indirect evidence obtained in experiments where 5-AMP, an inhibitor of glycerokinase[9], was added to the medium with intact tissue and caused in $10^{-3}M$ concentration no significant increase of glycerol estimated in the medium. Therefore, similarly to adipose tissue, glycerol released from endogenous or exogenous substrate was proportional to lipase activity.

In guinea-pig, pig, rat and rabbit aortic tissue the results were analogical. However, the basic activity of hormone-sensitive lipase in rat aortic tissue was significantly higher than in other species studied ($p < 0.01$). Whether the significant differences in hormone-sensitive lipase activities of aortic tissue of rat, animal resistant to experimental atherosclerosis and other species which are sensitive, are in causal connection to pathogenesis of atherosclerosis, however, is not possible to decide from these in vitro experiments.

The stimulatory effects of ACTH, epinephrine, norepinephrine and glucagon on aortic tissue lipase activity were analogous to those in adipose tissue[1-3]. The effect of insulin, on the other hand, was completely different. In aortic tissue of all species studied insulin considerably stimulated lipase activity when it was added to intact

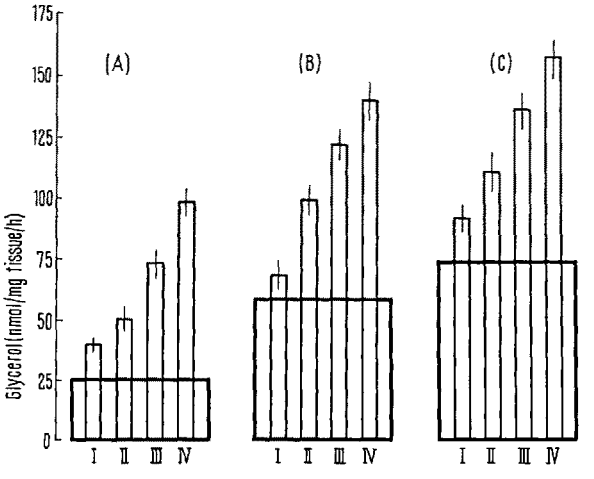

Fig. 1. Effect of insulin on rat aortic tissue lipase. Procedure B (homogenate, substrate tristearin). White wide columns (A) control medium, (B) + ACTH 0.01 μg/ml, (C) + norepinephrine 10 μg/ml. Narrow columns: + insulin (I) 1 μU/ml, (II) 10 μU/ml, (III) 100 μU/ml, (IV) 1000 μU/ml.

Effect of hormones on the release of glycerol from endogenous and exogenous substrates (glycerol nmol/mg tissue per h)

	Procedure A (intact tissue, endogenous substrate)	Procedure B (homogenate, substrate tristearin)
Control medium	2.1 ± 0.03	11.2 ± 0.3
+ epinephrine 10 μg/ml	4.9 ± 0.7[a]	26.7 ± 3.8[a]
+ norepinephrine 10 μg/ml	5.1 ± 0.8[a]	33.1 ± 4.0[b]
+ ACTH 0.01 μg/ml	4.1 ± 0.5[a]	27.4 ± 4.2[a]
+ glucagon 10 μg/ml	6.7 ± 0.8[b]	49.5 ± 5.4[b]
+ insulin 1 mU/ml	5.5 ± 0.4[b]	42.6 ± 4.9[b]

[a] $p < 0.01$, [b] $p < 0.001$.

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tissue as well as to tissue homogenate (Table). Moreover, insulin also potentiated the stimulatory effect of other lipolytic hormones (Figure 1). The stimulation was proportional to insulin concentration in the medium and was demonstrated even in the minutest doses of insulin (1 μU/ml).

This first evidence of lipolytic effect of insulin supposes in aortic tissue quite different properties of tissue receptor. The difference of insulin effect in adipose tissue[5] and in aortic tissue can consist either in different structure of enzyme molecule itself or in its different activation system or combination of both. To characterize hormone-sensitive lipase in aortic tissue we have used some substances which are known to inhibit hormone-sensitive lipase in rat adipose tissue[3], especially monoiodacetic acid and 2-propanol, in our experiments with rat aortic tissue. Figure 2 shows the marked difference of aortic tissue hormone-sensitive lipase: 2-propanol as well as monoiodacetic acid did not inhibit but activated the studied lipase. Inhibition with NaF differed this enzyme from lipoprotein lipase. It seems therefore that in aortic tissue we are dealing with different hormone-sensitive lipase isoenzyme. The mode of insulin action on this lipase in aortic tissue, however, cannot be evaluated from these experiments, and will be analyzed in another paper.

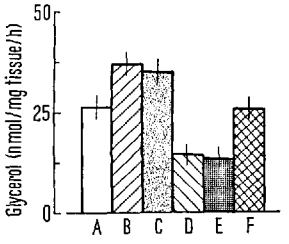

Fig. 2. Effect of inhibitors on rat aortic tissue lipase. Procedure B (homogenate, substrate tristearin). (A) control medium, (B) + monoiodacetic acid $10^{-4}M$, (C) + 2-propanol 1%, (D) + NaF $2 \times 10^{-2}M$, (E) + diethyl *p*-nitrophenyl phosphate $10^{-5}M$, (F) + eserine $10^{-5}M$.

From the theoretical point of view it is important that aortic tissue is sensitive to a number of hormones including insulin, although URRUTIA, BEAVAN and CAHILL[10] and MULCAHY and WINEGRAD[11] concluded that aorta is not sensitive to insulin as they found no potentiation of glucose uptake and metabolism by insulin. The stimulatory effect of insulin on aortic tissue lipase represents apparently another primary effect of insulin[12].

Zusammenfassung. In der Aortenwand von Ratten, Meerschweinchen, Kaninchen und Schweinen wird eine hormonempfindliche Lipase beschrieben, die in vitro durch Katecholamine, ACTH und Glucagon beeinflusst wird. Glucagonfreies Insulin wies eine deutliche lipolytische Wirkung auf und unterschied sich dadurch von der Wirkung im Fettgewebe.

M. CHMELAŘ and M. CHMELAŘOVÁ

First and Second Departments of Medical Chemistry, Charles University, Praha (Czechoslovakia), 27 May 1968.

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Polyamines and Nucleic Acids in the Growing Yeast

Spermine and spermidine are widely distributed in animal tissues and their level seems to be particularly high in those organs where protein synthesis is more active[1]. It has also been observed that, at least in the chick embryo, the developmental pattern of nucleic acids is similar to that of polyamines: these appear at the beginning of the incubation and rapidly increase in the first period of differentiation, when the greatest morphological changes are observed[2].

This similarity suggested the existence of a correlation between polyamines and nucleic acids, and recent observations seem to confirm this possibility[3].

Polyamines are also present in a variety of microorganisms; for some of them, such as *H. parainfluenzae*, they represent an essential growth factor[4], for others (lactobacilli) they are factors of cellular stimulation[5].

The presence of spermine and spermidine has been demonstrated also in yeast. However, it is interesting to note that spermine can inhibit the growth of various types of yeast, when present at adequate concentrations in the culture media[6].

All these observations prompted us to study the level of natural polyamines and of nucleic acids in yeast, at various stages of cellular growth.

The experiments were performed with cells of *S. cerevisiae* (strain Fleishman ATCC 7754), cultured in liquid broth according to KLEIN[7], in a 30-l glass shake-fermentor. Air flow rate was 3 l/min.

Samples containing an identical number of cells were taken after different time periods, cooled at 0 °C, centrifuged at 18,000 rpm and lyophylized.

The dry powder was extracted with 0.1 *N* HCl and then with *N*-butanol. The butanol extract was dried and dissolved in 0.1 *N* HCl, and the polyamines were separated by electrophoresis, as described by RAINA[8], using a citric acid-sodium hydroxide buffer at pH 3.5. Spermine and spermidine were stained with ninhydrin and the optical density of eluates was measured at 505 nm.

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