Effects of Porcine β-Lipotropic Hormone on Lipid Metabolism

The so-called β- and γ-lipotropic hormones isolated by Li and co-workers from sheep and bovine pituitary[1–3] were chemically the most profoundly characteristic peptides of pituitary exhibiting specific lipid mobilizing effect. Recently, a polypeptide isolated from porcine pituitary by us was found to be an analogue of the β-lipotropic hormone (β-LPH) of sheep and bovine origin based on its behaviour in the course of the purification process, its molecular weight and electrophoretic mobility; in its amino acid composition, however, some slight species differences could be established[4].

The question arises whether the in vitro lipolytic activity of porcine β-LPH is also similarly dependent on the species characteristics of the target tissue as described for sheep and bovine β-LPH[3,5]. Furthermore, the in vivo activity of β-LPH in different species was studied as well, since relevant data are not available.

In vitro lipolytic activity was measured in the epididymal fat pads of rats and the retroperitoneale adipose tissue of rabbits fed a standard laboratory diet. 150–200 mg pieces of adipose tissue were shaken aerobically in Krebs-Henseleit phosphate buffer containing 3% albumin at 37 °C for 1 h in the presence of porcine β-LPH at concentrations ranging between 10^{-3} and 10^{2} μg/ml. After homogenization the free fatty acid content of the incubated fat pieces was determined according to Dole and Meinertz[6].

The changes in lipolysis in function of peptid concentration compared to controls are presented in Figure 1. As can be seen, β-LPH stimulated the lipolysis much more effectively in rabbit adipose tissue than in rat. The extrapolated minimal effective concentrations were 5×10^{-3} μg/ml and 2×10 μg/ml, respectively. Thus, the species characteristics of in vitro lipolytic activity of porcine β-LPH are similar to those of ovine and bovine β-LPH.

The species characteristics of β-LPH can also be demonstrated in vivo when comparing the effects on serum FFA in various species. The peptide was administered i.v. in doses of 3–1000 μg/kg and 1 h later the serum FFA levels[6] were determined. The highest increase of serum FFA level – in accordance with in vitro results – was observed in rabbits; in this species a significant effect could be obtained by doses as low as 3 μg/kg (Figure 2). In contrast, even a dose of 1000 μg/kg proved to be ineffective in dogs, mice and rats. In guinea-pigs a 100 μg/kg dose produced a significant serum FFA increasing effect. Porcine β-LPH was administered also for pigs. After a dose of 100 μg/kg in one pig the serum FFA level rose from 0.20 meqv/1000 ml to 0.72 meqv/1000 ml and in another the respective values were 0.11 and 0.65 meqv/1000 ml. In control pigs and in one which was given 10 μg/kg only, no changes of serum FFA levels could be observed.

The fact that the porcine β-LPH was found to have activity in pigs also, seems to be of interest since this species is relatively resistant to lipolytic effects[7].

The effects of porcine β-LPH on lipid metabolism were investigated in more detail in rabbits which presented the highest sensitivity. Various doses of β-LPH were administered i.v. and s.c. (Figure 2). Dose-response relationship was found after both ways of application; following i.v. administration the effect lasted for 3 h, while after s.c. injection it persisted for about 6 h. Generally, a higher dose resulted in a longer effect; however; after 18 h no change could be observed in serum FFA level at any dose. The blood sugar[8], serum triglyceride[9], total

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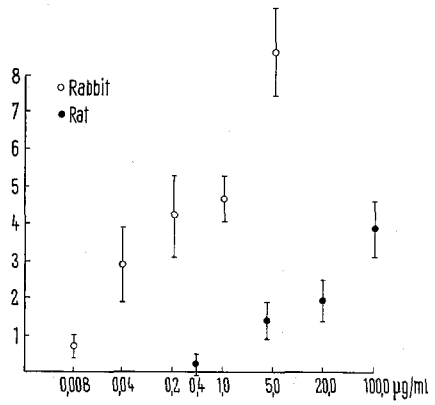

Fig. 1. Effect of porcine β-LPH on lipolysis in vitro. Abscisse: concentrations of β-LPH in μg/ml, ordinate: changes in rate of lipolysis in μeqv FFA/g adipose tissue · h. For further details see text.

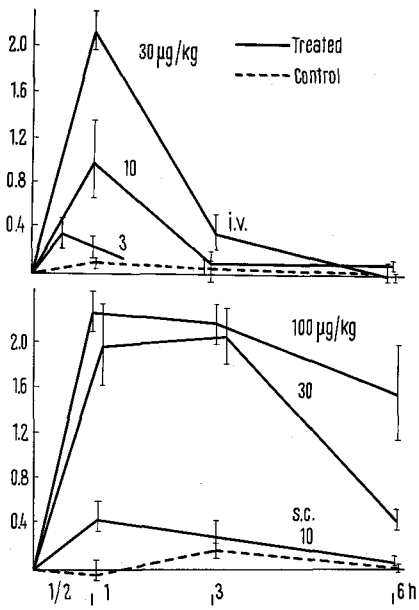

Fig. 2. Effect of porcine β-LPH on serum FFA level in rabbits after i.v. and s.c. administration. Abscisse: time in hours following administration, ordinate: changes of serum FFA level in meqv/1000 ml serum. For further details see text.

cholesterol[10] and phospholipid[11] levels did not show any significant change in a period of 18 h after the treatment with a 100 μg/kg s.c. dose. The blood total ketone bodies[12] increased from 0.20 ± 0.03 mg/100 ml pretreatment level to 1.00 ± 0.20 mg/100 ml 6 h after treatment. At this time the triglyceride contents of liver and heart muscle were elevated, as well, by 92 and 50% ($p < 0.01$), respectively, compared with controls. The cholesterol and phospholipid contents of these organs did not change significantly.

This in vivo effects of porcine β-LPH are closely similar to those of the previously isolated lipolytic fractions of porcine pituitary, the fraction H[13] and the peptid I and II[14]. These polypeptides, however, are unlike the porcine β-LPH regarding their molecular weight and other chemical characteristics. The apparent failure of β-LPH to exert a serum triglyceride enhancing effect as described for the above-mentioned peptides can be attributed to the low doses applied in our experiments[7].

Zusammenfassung. Es wird gezeigt, dass ein aus Schweinehypophysen gewonnenes lipotropes Hormon (β-LPH), in vitro und in vivo bei verschiedenen Tierarten untersucht, eine Erhöhung der Lipolyse herbeiführt. Die Wirkung war deutlich bei Kaninchen, Meerschweinchen und Schweinen, während bei Hunden, Ratten und Mäusen keine lipolytische Wirkung nachzuweisen war.

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Studies on DNA Methylase Activity in Mammalian Tissue

It has been previously demonstrated that the 5-methylcytosine in DNA of mammalian tissues is acquired by the enzymatic methylation of the preformed polynucleotide[1]. This concurs with the origin of the methylated bases (6-methyladenine and 5-methylcytosine) in the DNA of microbial origin[2,3]. The DNA methylase of mammalian tissues was shown to be localized in an insoluble nuclear subfraction in rat tissues[1] and in Krebs II ascites tumor[4]. It has also been identified as a nuclear enzyme in HeLa cells[5]. Recently, KALOUSEK and MORRIS[6] employed the exact procedure of SHEID et al.[1], and confirmed the observations of these authors with spleen. However, they found lower enzyme activity in rat liver. This may be attributed to the lability of the liver enzyme, and the greater difficulties in extracting and separating it from inhibitor(s) in liver nuclei[1].

A modification of the DNA methylase isolation and some properties of the enzyme in rat liver and Reuber minimal deviation hepatoma nuclei is reported in this communication.

Materials and methods. Salmon testis DNA was purchased from the Worthington Biochemical Corp. The active methyl donor (methyl ^{14}C)-S-adenosylmethionine, was obtained from the New England Nuclear Corporation. Holtzman and A × C male rats (120–150 g) were used in all of the experiments. The Reuber H-35 tumor was transplanted i.p. every month into the A × C rats. Radioactivity was determined in a Nuclear Chicago low background, end-window, gas-flow counter. Protein was assayed by the method of LOWRY et al.[7]. Deionized water was used throughout all of the experiments, and all manipulations of the rat tissues were performed at 4 °C.

Four grams of liver or hepatoma was homogenized by hand with a loosely fitting Ten Broeck homogenizer in 15 ml of 0.25 *M* sucrose-1×10^{-2} *M* EDTA. The nuclear fraction was isolated and washed in this media, then purified and subfractionated by a procedure described in an earlier paper[1]. The only modification in the subfractionation was the omission of magnesium from all of the extraction solutions. Enzyme assays were performed immediately after the nuclear extracts were prepared. The incubation mixture of 3–4 ml consisted of 2 ml of 0.02 *M* *Tris* (pH 8.9), 0.02 ml (10 μg) of RNase, 0.25 ml of freshly prepared glutathione (2 μmoles), 0.1–0.8 ml of enzyme extract (0.25–2 mg protein), and ammonium acetate where indicated. After 15 min of incubation at 38 °C, 0.02 ml (0.2 μC) of (methyl-^{14}C)-S-adenosylmethionine was added and the mixtures reincubated for 1 h at 38 °C. Saturation levels of ^{14}C incorporation into DNA, and chromatographic identification of the enzymatically synthesized methylated base was accomplished essentially by the procedures described by SHEID et al.[1].

Results. The Table depicts the effect of varying concentrations of EDTA used for the isolation of the nuclear fraction on DNA methylase activity, and the corresponding enzyme blank values which consists of enzyme extract plus radioactive methyl donor minus DNA. Nuclei isolated from solutions containing low amounts of EDTA have as much enzyme activity as nuclei isolated from solutions with higher quantities of EDTA. However, the enzyme blank values are markedly increased. EDTA added directly to the assay incubation mixture had no effect on the enzyme activity.

Figures 1A and 1B illustrate the effect of ammonium ions on DNA methylase activity in the normal Holtzman rat liver and Reuber hepatoma nuclear subfractions respectively. The addition of 0.15 *M* ammonium to the

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