ROLE OF LEU-ENKEPHALIN IN THE REGULATION OF CARBOHYDRATE METABOLISM

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It has been shown that endorphins and enkephalins influence food behavior and certain metabolic processes [5, 8], and modulate the activity of the endocrine system, including that of the beta-cells of the pancreas [4]. On the basis of available data, a role of endogenous opioids can be postulated in the regulation of carbohydrate metabolism. However, there is no direct evidence in support of this view in the literature.

The aim of this investigation was to study the possible role of Leuenkephalin (LE) in the regulation of carbohydrate metabolism.

EXPERIMENTAL METHOD

Experiments were carried out on 166 male albino rats weighing 180-220 g. Opioid peptides, namely LE (from Fluka, Switzerland), D-Ala^2-Leu^5-Arg^6-enkephalin (DLE), and d-Ala^2-D-Leu^5-D-Arg^6-enkephalin (DDLE), synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Director, Dr. Chem. Sci. M. I. Titov), were injected intraperitoneally in a dose of 500 μg/kg, naloxone (from Endo Laboratories, USA), a blocker of opiate receptors, was injected in a dose of 100 μg/kg, and the pharmacopoeial preparations Parathyroidin (an aqueous solution of parathyroid hormones) in a dose of 10 U/kg and adrenalin hydrochloride in a dose of 500 μg/kg. Animals of the control group were given injections of 0.2 ml of physiological saline. The rats were decapitated under superficial ether anesthesia 1 h after injection of the drugs.

Plasma glucose levels were determined by an enzymatic methods using kits from Lachema (Czechoslovakia), insulin levels were determined by radio-immunoassary using RIO-INS-PG kits (USSR), and C-peptide by means of kits from Behrigwerke (West Germany). Radioactivity was counted on a Gamma-spectrometer (Tractor, USA).

Samples of liver tissue were rapidly frozen in liquid nitrogen. The glycogen concentration in the samples was determined spectrophotometrically [3], and the cAMP concentration by radioimmunoassay using kits from Amersham International (England), and radioactivity was counted on a Mark III scintillation counter (USA).

The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results in Table 1 show that injection of natural LE and its synthetic analogs into the animals did not cause any statistically significant change in the blood glucose concentration or the liver glycogen concentration. Marked hyperglycemia and an almost threefold reduction in the liver glycogen concentration were observed 1 h after injection of adrenalin. Parathormone also caused a moderate decrease in the liver glycogen concentration and an increase in the blood glucose level. The latter is in agreement with data in the literature on the glycogenolytic action of parathormone [6]. All the opioid peptides tested effectively prevented the rise in the blood glucose level and the fall in the liver glycogen level due to adrenalin. Under these circumstances, whereas natural LE merely weakened the effect of adrenalin, its stable synthetic analogs completely abolished the action of the catecholamine. Preliminary (5 min beforehand) injection of naloxone, a blocker of opiate receptors, into the animals receiving LE with adrenalin, however, did not change the ability of LE to pre-

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TABLE 1. Effect of Enkephalins, Adrenalin, and Parathormone on Liver Glycogen and Blood Glucose Concentrations in Intact Rats (M ± m)

Experimental conditions	Number of animals	Glucose, mmoles/liter	Liver glycogen, g/kg
Control	30	5,47±0,18	14,15±0,75
DLE	10	5,29±0,27	15,90±1,71
DDLE	10	5,49±0,32	12,3±1,29
Adrenalin	19	9,16±0,55*	5,57±0,67*
Parathormone	9	6,75±0,41*	10,97±0,94*
DLE + adrenalin	11	6,30±0,44	12,10±1,34
DLE + parathormone	10	5,96±0,38	15,00±1,37
DDLE + adrenalin	9	6,19±0,44	13,58±0,97
DDLE + parathormone	10	5,53±0,31	15,30±1,41
LE	10	5,78±0,53	11,60±1,51
LE + adrenalin	11	7,61±0,45*	9,43±0,77*
Naloxone	10	6,74±0,45*	8,72±0,72*
LE + adrenalin + naloxone	11	8,38±0,83*	10,41±1,32*

TABLE 2. Effect of Enkephalins on Blood Glucose and C-Peptide Levels of Rats Receiving and Not Receiving Glucose Loading (M ± m)

Experimental conditions	Insulin, μU/ml	C-peptide, ng/ml
Control	9,09±1,11	1,03±0,12
Glucose (400 mg internally after 1 h)	13,99±1,97*	1,77±0,14*
Glucose + DLE	7,98±2,25	1,48±0,20
DLE	6,00±0,82*	—
DDLE	4,58±1,01*	—

Legend. Here and in Table 2, asterisk indicates $p < 0.05$ compared with control.

vent adrenalin-induced glycogenolysis. However, injection of naloxone alone caused a statistically significant fall in the liver glycogen concentration and rise of the blood glucose level. The effect of parathormone on the blood glucose and liver glycogen concentrations also was prevented by injection of LE analogs (Table 1).

The influence of enkephalins on the effects of parathormone and adrenalin could be connected with the ability of the opioids to inhibit adenylate cyclase activity and to prevent an increase in the intracellular cAMP concentration [1, 2]. In the present experiments injection of adrenalin increased the cAMP concentration in the liver from 1124 ± 80 to 4290 ± 875 pmoles/g tissue ($p < 0.01$). Preliminary injection of LE into the animals lowered the cAMP level to 1326 ± 175 pmoles/g tissue ($p > 0.05$). Since the glycogenolytic action of parathormone also is mediated through an increase in the cAMP concentration in the hepatocytes [7], it can be postulated that prevention of the effect of parathormone on carbohydrate metabolism also was connected with inhibition of adenylate cyclase activity by the opioids.

The ability of LE and its synthetic analogs to inhibit the glycogenolytic action of adrenalin and parathormone, and of naloxone to raise the blood glucose level and lower the liver glycogen concentration suggests that endogenous opioid peptides *in vivo* possess synergism for insulin in relation to carbohydrate metabolism. Nevertheless, injection of the opioids in the present experiments did not cause the blood glucose concentration to fall or the liver glycogen concentration to rise, whereas after injection of natural LE and of DDLE there was actually a tendency (although not statistically significant) for the liver glycogen concentration to fall.

A study of the effect of opioid peptides on pancreatic β-cell function showed that the basal blood insulin concentration is lowered by stable LE analogs (Table 2). The next experiments showed that injection of DLE lowers not only the basal, but also the glucose-stimulated blood insulin level. The parallel between the time course of the insulin and C-peptide blood levels (Table 2) is evidence of a change in insulin secretion and not in the rate of its metabolism under the influence of enkephalins. Depression of pancreatic β-bell function after injection of enkephalins may perhaps also be connected with inhibition of adenylate cyclase, because cAMP plays an exceptionally important role in the mechanisms of insulin secretion [9].

In the literature in recent years the role of modulating factors has been ascribed frequently to opioids [4]. The results now obtained also suggest that the action of opioids on carbohydrate metabolism is modulating in character. In particular, LE and its synthetic analogs exhibit antagonism to endogenous hyperglycemic factors, realizing their action through an increase in the cAMP concentration in the hepatocytes. From this point of view opioid peptides can be regarded as synergists of insulin.

Meanwhile, enkephalins inhibit the secretory activity of pancreatic β-cells. The possibility cannot be ruled out that the physiological response of the parameters of carbohydrate

metabolism to a rise or fall of the functional activity of the endogenous opioid system may depend on the functional state of the body. Nevertheless, the mechanisms of the modulating action of enkephalins on the various components of regulation of carbohydrate metabolism at the cellular level are evidently universal and consists of inhibition of adenylate cyclase activity and lowering of the intracellular cAMP concentration.

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NEUROGENIC MUSCARINIC VASODILATATION OF THE FELINE CAUDAL FEMORAL ARTERY *IN VITRO*

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The adrenergic (vascoconstrictor) innervation of the blood vessels of the cardiovascular system of most animals has been sufficiently well studied [3, 16], whereas there are few data in the literature on their neurogenic dilatation. The neurogenic cholinergic innervation of the lungs [8], heart [7], uterus, and kidneys [2] has been demonstrated. Most investigators consider that these cholinergic influences are mediated by fibers belonging to the parasympathetic system [3]. However, there are physiological data on the existence of cholinergic dilator fibers of sympathetic nature. They innervate blood vessels of skeletal muscles of some species of mammals [14]. It has been shown that cholinergic influences of sympathetic nature are realized in certain behavioral responses [1]. Morphological data on the localization of fibers of this type are few in number and are based on determination of cholinesterase, an enzyme which is found in other types of fibers also [6]. The mechanism of realization of sympathetic cholinergic influences in blood vessels of skeletal muscles remains unclear and requires further investigation.

The aim of this investigation was to study whether neurogenic cholinergic dilatation can be demonstrated in a vascular preparation isolated from feline skeletal muscles.

EXPERIMENTAL METHOD

The caudal femoral artery, which runs between the medial and lateral heads of the gastrocnemius muscle of the hind limb, was dissected in cats weighing 2.5-3 kg, anesthetized with urethane (0.9 g/kg, intervenously). A segment (internal diameter 0.6-0.4 mm) 8-10 mm long was excised, freed from fat and connective-tissue membranes, and incubated for 40 min at 8-10°C in modified Krebs' solution: NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.52 mM, $MgSO_4$ 1.64 mM, $NaHCO_3$ 24.88 mM, KH_2PO_4 1.18 mM, and glucose 10 mM [4]. The vessel was then placed in a

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