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SOME MECHANISMS OF INVOLVEMENT OF OPIOID PEPTIDES IN THE REGULATION OF CARBOHYDRATE METABOLISM

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Endogenous opioid peptides (OP) can influence many functions of the body. It has been reported that enkephalins and endorphins can prevent glycogenolysis and hyperglycemia, induced by adrenalin and parathyroid hormone [4, 5, 12]. OP also significantly alter secretion of catecholamines, cortisol, parathyroid hormone, and also several other hormones which influence carbohydrate metabolism [1, 7, 5], a fact which also suggests that they may be involved in the regulation of carbohydrate metabolism.

Nevertheless, the mechanisms of these effects of OP and, in particular, the role of different types of opiate receptors, in the realization of the action of enkephalins and endorphins on the parameters of carbohydrate metabolism still remain unexplained. For this reason research in this direction using selective agonists of mu- and delta-opiate receptors is of definite interest. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 141 male Wistar rats weighing 200-250 g. The OP for study and the adrenalin hydrochloride were injected intraperitoneally in doses of 0.5 mg/kg, dissolved in 0.5 ml of isotonic NaCl solution. Rats of the control group received an injection of the same volume of NaCl solution. The following reagents were used in the work: leucine-enkephalin (LE) was obtained from "Fluka" (Switzerland), the enkephalin analogs Tyr-D-Ala-Gly-Phe-D-Leu (DADLE), Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAGO), and Tyr-D-Ala-Gly-Phe-Leu-Arg (dalargin) were obtained from the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR; Tyr-D-Arg-Gly-Phe-OEt (ee[D-Arg^2,des-Leu^5]enkephalin (the ethyl ester of [D-Arg^2,des-Leu^5]-enkephalin) was synthesized in the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR. The animals were killed by decapitation under superficial ether anesthesia 30 min after injection of the compounds. The glucose concentration in the blood plasma

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

Preparation injected	n	Glucose, mM	Glycogen, g/kg
Isotonic NaCl solution	30	5,47±0,18	14,2±0,8
Dalargin	10	5,29±0,27	15,9±1,7
DAGO	8	5,03±0,22	19,7±2,8
DADLE	8	5,11±0,19	17,6±2,5
LE	10	5,78±0,53	11,6±1,5
ee[D-Arg^2, des-Leu^5]-enkephalin	9	5,24±0,21	16,1±1,7
Adrenalin	19	9,16±0,55**	5,7±0,7**
Dalargin + adrenalin	11	6,30±0,44*	12,1±1,3*
DAGO + adrenalin	8	9,11±0,69**	6,3±0,8**
DADLE + adrenalin	8	6,24±0,53*	11,2±1,6*
LE + adrenalin	11	7,61±0,45**	9,4±0,8**
ee[D-Arg^2, des-Leu^5]-enkephalin + adrenalin	9	8,44±0,43**	6,7±0,8**

Legend. *$p < 0.05$ significant difference compared with rats receiving adrenalin, **$p < 0.05$ compared with control (NaCl).

was determined by the "Bio-La-Test" kit of reagents (Czechoslovakia). The glycogen concentration was determined in samples of liver tissue [9]. Optical density was recorded on the "SF-46" spectrophotometer. The insulin concentration in the blood plasma was determined by radioimmunoassay using the INS-PG-^{125}I kits of Soviet manufacture. The radioactivity of the samples was recorded on a "Gamma-12" gamma-spectrometer.

The effect of the peptides on opiate receptors was determined by their ability to inhibit contractions of a segment of the longitudinal muscle of the guinea pig ileum (GPI) and the isolated mouse vas deferens (MVD). Contractions were recorded under isometric conditions using a TB-611T transducer, connected to a "Nihon Kohden" polygraph (Japan). Activity of the substances was expressed as IC_{50} (the concentration inhibiting contractions by 50%). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Injection of OP into intact rats did not cause significant changes in the blood glucose or liver glycogen concentrations; however, under the influence of DAGO there was a clear tendency for the latter to rise (Table 1). Injection of adrenalin led to a decrease in the liver glycogen and an increase in the blood glucose concentrations. Preliminary injection of LE, DADLE, and dalargin prevented the hyperglycemia and the fall of the liver glycogen content caused by adrenalin, whereas DAGO and ee[D-Arg^2,des-Leu^5]enkephalin had no such action. Under the influence of dalargin a decrease in the blood insulin concentration of the rats was observed from 9.09 ± 1.11 to 6.00 ± 0.82 μU/ml ($p < 0.05$). Injection of DAGO, on the other hand, led to a rise of the blood insulin level to 14.27 ± 1.24 μU/ml ($p < 0.05$).

Determination of the degree of affinity of ee[D-Arq^2,des-Leu^5]-enkephalin for opiate receptors showed that it had low activity on preparations of both GPI and MVD, values of IC_{50} being $(4.05 \pm 0.99) \cdot 10^{-6}$ M and $(2.33 \pm 0.55) \cdot 10^{-6}$ M respectively. For comparison, experiments were carried out using LE: the value of IC_{50} on preparations of GPI was $(2.0 \pm 0.1) \cdot 10^{-8}$, and on MVD $(2.85 \pm 0.78) \cdot 10^{-7}$ M, in agreement with data in the literature [2, 6].

Activity of dalargin, DAO, and DADLE on preparations of GPI and MVD was not studied, for the characteristics of affinity of these compounds for mu- and delta-receptors are well known and described in the literature [6, 8, 11]. Dalargin interacts sufficiently effectively with both types of opiate receptors, DADLE is an agonist predominantly of delta-receptors, and DAGO – of mu-receptors [6, 11].

As will be seen from the results described above, analogs of enkephalins of varied structure have different effects on the parameters of carbohydrate metabolism and of pancreatic beta-cell function studied. In particular, LE, dalargin, and DADLE prevented the hyperglycemic and glycogenolytic action of adrenalin, whereas DAGO and ee[D-Arg^2,des-Leu^5]-enkephalin had no such action.

These differences may be due to differences in the degree of affinity of the peptides for opiate receptors. For instance, LE, DADLE, and dalargin, by contrast with DAGO, interact effectively with delta-receptors, but have much weaker affinity than the latter for mu-receptors [8, 11].

As the results of our experiments show, ee[D-Arg^2,des-Leu^5]-enkephalin possesses extremely low affinity for both mu- and delta-receptors. Its marked opiatelike analgesic action [3] is evidently due to the similarity of the structure of this peptide with that of kyotorphin [3], an endogenous dipeptide Tyr-Arg, which stimulates activation of the endogenous opioid system [10]. Thus the ability to prevent the hyperglycemic and glycogenolytic action of adrenalin is a feature of peptides which interact effectively with delta-receptors, independently of their affinity for mu-receptors. The concrete mechanisms of involvement of OP in the regulation of carbohydrate metabolism have now been assessed in sufficient detail. A definite role is evidently played by the ability of opioids to inhibit adenylate cyclase activity and to prevent elevation of the liver cAMP level induced by catecholamines [5]. As was shown previously, enkephalins similarly inhibit the glycogenolytic and hyperglycemic action of parathyroid hormone [4, 5], the effects of which are known to be mediated by a cAMP-dependent mechanism [13]. The character of affinity for receptors is evidently important also in realization of the action of enkephalins on secretory activity of the pancreatic beta-cells. In particular, the mu-agonist DAGR led to a rise, whereas dalargin, on the contrary, led to a fall of the blood insulin level. This last observation is in agreement with the results of studies of the effect of selective mu- and delta-agonists on pancreatic cell function in vitro [14].

Thus the correlation between affinity of OP for opiate mu- and delta-receptors, on the one hand, and their effect on the mechanisms of regulation of the blood glucose and liver glycogen levels, on the other hand, is perfectly evident. Natural OP, namely enkephalins, beta-endorphin, and neoendorphins, possess different degrees of affinity for the various opiate receptors [10], a fact which evidently suggests that the endogenous opioid system has a modulating influence on the regulation of carbohydrate metabolism.

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