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SYNTHETIC ENKEPHALIN ANALOGS AS ANTIATHEROGENIC AGENTS

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Definite progress in the search for new pathogenetically based methods of treatment of atherosclerosis has been made by the study of endogenous protective systems and their metabolites, which prevent development of the atherosclerotic process. In particular, according to one hypothesis [13], endogenous opioid peptides can be regarded as antiatherogenic factors, and this view is confirmed by data on the hypolipidemic action of enkephalins and endorphins [11].

In the investigation described below, the possibility of using synthetic analogs of opioid peptides as antiatherogenic agents was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male Wistar rats weighing 200-250 g. Hypercholesterolemia was produced by administration of 5% cholesterol solution in a dose of 500 mg/kg body weight by the gastric route through a PVC tube [2]. The animals were decapitated under superficial ether anesthesia 6 h after a single dose and 24 h after the last of 20 daily doses of cholesterol indicated above. The substances for testing were injected intraperitoneally in a dose of 0.1 mg/kg. In acute experiments the preparations were given once, 3 h before the animals were taken from the experiment; in experiments with chronic cholesterol feeding the preparations were injected in the above dose on alternate days. Arginine-containing analogs of Leu-enkephalin (dalargin and DFEN) were obtained from the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Head of Laboratory Professor M. I. Titov). Altogether 52 patients (men aged 42-56 years) with obliterative atherosclerosis (OA) of the lower limbs in stage II-III, with concomitant arterial hypertension, were investigated. All the patients had received the traditional treatment [1, 3]; 24 patients had received dalargin in a dose of 2 mg intravenously daily for 5 days as a hypotensive agent, and the patients of the comparison group (28) had received papaverine and dibazol (2-benzylbenzimidazole hydrochloride). The control group consisted of 25 clinically healthy persons aged 37-50 years. Blood samples were taken in the morning before breakfast, before and 7 days after the beginning of treatment. Plasma parathormone (PTH) levels were determined by radioimmunoassay using kits from "Byk-Mallinckrodt" (West Germany). Radioactivity was counted on a "Tracor" gamma-spectrometer (USA). The blood cholesterol concentration was determined by kits from "Bio-La-Chema" (Czechoslovakia). Serum lipoprotein fractions were separated by electrophoresis in gel, using kits of reagents from "Miles" (USA) and relative percentages of high- (HDL), low- (LDL), and very low- (VLDL) density lipoproteins were calculated. The blood lactate concentration was determined by an enzymic method using kits from

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TABLE 1. Effect of Leu-Enkephalin Analogs on Cholesterol Concentration and Relative Percentages of Lipoprotein Fractions in Rats' Blood Serum (M ± m)

Experimental conditions	Parameter		
	cholesterol, mmoles/liter	HDL, %	LDL + VLDL, %
Intact rats	2,39±10	66±10	34±5
Receiving cholesterol	3,26±0,29 $p<0,05$	47±7 $p>0,05$	53±7 $p<0,05$
Receiving cholesterol and dalargin	2,33±0,21* $p>0,05$	56±6 $p>0,05$	44±5 $p>0,05$
Receiving cholesterol and DFEN	2,29±0,23* $p>0,05$	70±9 $p>0,05$	30±5* $p>0,05$

Legend. *p < 0.05 compared with corresponding value in rats receiving cholesterol.

"Boehringer" (West Germany). The results were subjected to statistical analysis with determination of M ± m, and by the tied pairs method ($M_1 - M_2 \pm m$).

EXPERIMENTAL RESULTS

It will be clear from the data in Table 1 that 6 h after enteral administration of cholesterol its blood level in the rats rose and the relative percentages of LDL and VLDL in the blood increased. A single injection of both dalargin and DFEN into animals with acute hypercholesterolemia effectively prevented the rise in the animals' blood cholesterol level.

DFEN also restored the normal ratio between HDL and LDL + VLDL; administration of dalargin was not followed by a statistically significant change in the relative percentages of the blood lipoprotein fractions in the rats compared with the corresponding values in animals receiving physiological saline.

In animals receiving cholesterol solution for 20 days, the blood levels rose from 2.39 ± 0.10 to 2.91 ± 0.19 mmoles/liter ($p < 0.05$). Administration of dalargin to rats receiving cholesterol lowered the blood cholesterol level to 2.64 ± 0.15 mmoles/liter ($p > 0.05$ compared with intact rats).

Leu-enkephalin analogs thus possess a distinct hypocholesterolemic action. DFEN is more effective than the pharmacologic preparation dalargin, for unlike the latter it reduced the relative percentage of the LDL + VLDL fraction, i.e., it has a hypocholesterolemic action by lowering the level of cholesterol associated with atherogenic lipoprotein fractions.

The possibility that endogenous opioids can influence the mechanisms of atherogenesis also is suggested by the character of their relations with the neuroendocrine system. Opioid peptides inhibit secretion and peripheral effects of catecholamines [10, 18], which in turn, potentiate the development of atherosclerosis [17]. The writers showed previously that enkephalins and their synthetic analogs have a stimulating action of calcitonin secretion and depress parathyroid activity [9]. The results of the present experiments are confirmed by a morphologic study of the C-cells of the thyroid glands of animals receiving enkephalins [15]. These changes in function of the calcium-regulating glands, according to the authors of [16], prevent the development of atherosclerosis. This view is in full agreement with known data on the potentiating effect of PTH and calcium ions on the mechanisms of atherogenesis [12, 16], and also with the observed intensification of parathyroid function in patients with OA [8]. The properties of opioid peptides mentioned above, together with their hypocholesterolemic action may have an inhibitory effect on the mechanisms of development of atherosclerosis.

TABLE 2. Blood Cholesterol, Lactate, and PTH Levels in Patients with OA of the Lower Limbs ($M_1 - M_2 \pm m$)

Parameter	Patients receiving traditional treatment				Patients receiving dalargin			
	M_1	M_2	m	p	M_1	M_2	m	p
Cholesterol, mmoles/liter	5,87	5,69	0,38	>0,05	5,83	4,87	0,23	<0,01
PTH, ng/ml	0,62	0,75	0,08	>0,05	0,54	0,34	0,06	<0,01
Lactate, mmoles/liter	2,04	1,94	0,07	>0,05	1,91	1,67	0,04	<0,01

Legend. M_1) Before beginning of treatment, M_2) 7 days after beginning of treatment.

To confirm the results of the animal experiments, an investigation was carried out on patients with OA. Before the beginning of treatment the patients showed changes in the parameters chosen for study characteristic of OA. The blood cholesterol level was raised from 3.72 ± 0.22 to 5.85 ± 0.31 mmoles/liter ($p < 0.01$), PTH was raised from 0.36 ± 0.04 to 0.59 ± 0.09 ng/ml ($p < 0.05$), and the lactate concentration in venous blood from the affected limb was raised from 1.61 ± 0.08 to 1.97 ± 0.08 mmole/liter ($p < 0.05$).

After the end of the course of dalargin treatment the patients' blood levels of cholesterol and PTH were lowered, whereas in patients of the comparison group, these blood levels showed no significant change at the same times of observation (Table 2). Patients receiving dalargin showed a clear tendency for the lactate level in venous blood from the affected limb to fall. This fall of the raised blood lactate level was evidently not linked directly with a fall in the blood levels of cholesterol and PTH, but was evidently due to the ability of dalargin to reduce manifestations of regional ischemia and tissue hypoxia. The anti-ischemic action of opioid peptides in various types of circulatory failure has been reported [4, 6, 10]. This effect is due, on the one hand, to lowering of the tone of the resistive vessels and the total peripheral vascular resistance with improvement of the peripheral hemodynamics [5, 14], and on the other hand, to weakening of the catabolic reaction under the influence of opioids and elevation of the tissue energy potential [7, 11].

The combination of properties possessed by opioid peptides suggests that enkephalins may influence several different stages of the pathogenesis of atherosclerosis. Whereas the effect of opioids on function of the endocrine system and on the blood cholesterol concentration is aimed directly at the mechanisms of atherogensis, lowering of the level of regional hyperlactatemia characterizes abolition of the ischemic syndrome, which is essentially the dominant feature in the pathogenetic and clinical picture of OA [3].

The results given above are evidence that synthetic Leu-enkephalin analogs constitute a promising group of biologically active substances from the point of view of the study and creation of new pharmacologic preparations for the treatment of atherosclerosis.

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