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METABOLISM OF CONNECTIVE TISSUE BIOPOLYMERS IN THE AORTA AFTER INTRAVENTRICULAR NEUROPEPTIDE INJECTION

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There is only limited information about the role of some physiologically active peptides in the metabolism of connective tissue biopolymers. In particular, it has been shown that repeated microinjections of angiotensin II and substance P into the mesencephalic reticular formation leads to collagen accumulation in the aorta and myocardium [4]. Neuropeptides are known to play an essential role in the realization of various bodily functions [5, 6, 11], including the regulation-of metabolism [3, 8].

The aim of this investigation was to study metabolism of connective tissue biopolymers in the aorta in response to repeated intraventricular injection of substance P, Leu-enkephalin, and beta-endorphin.

EXPERIMENTAL METHOD

Experiments were carried out on 40 adult Chinchilla rabbits weighing 3.0-3.5 kg. Taking coordinates from an atlas of the brain [1], and using a stereotaxic apparatus, cannulas were inserted into the left lateral ventricle of experimental and control animals (AP = −1, V = 1.5, S = 2.7). Substance P in a dose of 100 ng, Leu-enkephalin in a dose of 150 ng, and beta-endorphin in a dose of 150 ng in 10 μl of physiological saline were injected intraventricularly on alternate days for 30 days. Control animals received the same volume of physiological saline. Intact rabbits also were used as the control. Each experimental series and the control group consisted of eight animals, which were used in a chronic experiment 7-8 days after implantation of the cannulas. At the end of the experiment the animals were killed by air embolism under short-term ether anesthesia. The accuracy of location of the cannulas was verified histologically. Blood was taken in the course of the experiment on the 10th, 20th, and 30th days from the marginal vein of the ear 30 min after intraventricular injections of the neuropeptides, for determination of concentrations of glycosaminoglycans based on the hexuronic acid (HUA) level [10], sialic acids (SA) [9], and 11-hydroxycorticosteroids (11-HCS) [13] and urea, using a kit from "Lachema" (Czechoslovakia). Parameters of collagen metabolism – serum levels of free (FH), peptide-bound (PBH), and protein-bound (PrBH) hydroxyproline were analyzed by the aid of

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TABLE 1. Parameters of Metabolism of Connective Tissue Biopolymers in Blood after Intraventricular Injection of Physiological Saline (control) and Neuropeptides

Parameter	Experimental conditions	Days of blood sampling		
		10	20	30
Free hydroxyproline, μmoles/liter	Control	12,4±1,1	12,1±0,9	11,9±1,2
	Substance P	10,5±1,1	7,4±0,8*	7,2±0,7*
	Leu-enkephalin	12,7±1,2	10,8±0,9	8,4±0,7*
	Beta-endrophin	11,8±1,3	12,3±0,8	11,6±0,8
Peptide-bound hydroxyproline, μmoles/liter	Control	6,3±0,7	6,2±0,6	6,4±0,5
	Substance P	7,6±0,6	8,3±0,6*	8,7±0,5*
	Leu-enkephalin	7,1±0,6	7,5±0,5	8,3±0,6*
	Beta-endorphin	6,9±0,7	7,3±0,5	7,1±0,6
Hexuronic acids, mg/liter	Control	3,8±0,2	3,9±0,3	4,2±0,3
	Substance P	4,7±0,3*	3,7±0,3	3,1±0,3*
	Leu-enkephalin	5,9±0,4*	4,1±0,4	3,5±0,3
	Beta-endorphin	4,1±0,4	3,6±0,4	3,7±0,4
Sialic acids, mg/liter	Control	490±24	520±40	508±36
	Substance P	516±38	390±28*	346±22*
	Leu-enkephalin	540±34	470±26	382±19*
	Beta-endorphin	472±24	456±32	390±24*
Urea, mmoles/liter	Control	7,4±0,5	8,2±0,7	8,7±0,7
	Substance P	8,1±0,6	6,6±0,5	5,9±0,4*
	Leu-enkephalin	5,7±0,5*	5,6±0,6*	4,7±0,5*
	Beta-endorphin	7,0±0,6	7,1±0,7	6,3±0,5*
11-Hydroxycorticosteroids, μg/liter	Control	94±7,8	88±8,0	92±7,2
	Substance P	76±6,6	64±6,3*	66±5,8*
	Leu-enkephalin	112±12,0	102±9,8	76±6,0
	Beta-endorphin	97±10,7	86±7,0	78±7,4

Legend. *$p \leq 0.05$ indicates significance of differences between control and experiment.

TABLE 2. Parameters of Metabolism of Connective Tissue Biopolymers in Aorta ter Intraperitoneal Injection of Substance P, Leu-Enkephalin, and Beta-Endorphin

Parameter (per mass of defatted tissue)	Control	Injection of		
		substance P	leu-enkephalin	beta-endorphin
Free hydroxyproline, mmoles/kg mass of dry defatted tissue	1,38±0,10	0,61±0,11*	1,01±0,12*	1,20±0,14
Total collagen, mmoles/kg mass of dry defatted tissue	138±5,6	174±6,4*	168±5,8*	152±0,6
Sialic acids, g/kg mass of dry defatted tissue	3,60±0,12	6,16±0,36*	5,92±0,28*	4,46±0,30*
Hexuronic acids, g/kg mass of dry defatted tissue	4,70±0,30	6,84±0,40*	5,28±0,30	5,40±0,40
Collagenolytic activity, μmoles hydroxyproline/g protein/h	4,72±0,30	2,24±0,11*	2,94±0,20*	3,10±0,18*

Legend. *$p \leq 0.05$ indicates significance of differences between control and experiment.

p-dimethylaminobenzaldehyde [15]. Concentrations of glycosaminoglycans, reflected in the HUA level [10], SA [12], free hydroxyproline (FH), and total collagen (TC), as the hydroxoproline content [14], and collagenolytic activity (CA) [17] were determined in the aortic tissue.

EXPERIMENTAL RESULTS

As Table 1 shows, the serum FH level after intraventricular injection of substance P and Leu-enkephalin fell toward the end of the experiment by 39.5 and 29.4% respectively, whereas the PBH and PrBH levels rose significantly under these conditions, starting with the 20th day of the experiment. Meanwhile the SA, urea, and 11-HCS concentrations fell. The serum HUA level changed in phases: on the 10th day of intraventricular injection of substance P and Leu-enkephalin it rose by 23.7 and 55.3% respectively, whereas toward the end of the experiment, on the contrary, it fell by 26.2 and 16.7%. In the aortic tissue (Table 2) on the 30th day of microinjections of substance P and Leu-enkephalin a significant reduction was observed in the FH level (by 52.5 and 26.8% respectively), as well as

activity of collagen-degrading enzymes (by 52.5 and 37.7% respectively). At the same time, the levels of TC and SA in the tissue studied were increased. The HUA content in aortic tissue homogenate rose significantly, but only after intraventricular injection of substance P.

On injection of beta-endorphin changes in connective tissue biopolymer metabolism were less marked than after microinjections of substance P and Leu-enkephalin. Only the blood SA and urea levels were lowered, but in the aorta the SA content increased and CA decreased.

The blood and tissue FH levels reflect collagen breakdown [7], whereas the increase in the PBH reflects simultaneous acceleration of collagen breakdown and biosynthesis [7, 16]. Accordingly, it can be asserted that lowering of FH levels in the blood serum and aortic tissue, as well as the significant rise in the PBH level in the peripheral blood of the experimental animals during prolonged intraventricular injections of substance P and Leu-enkephalin are evidence in support of collagen accumulation in the aorta. This process is confirmed by the increase in TC and the simultaneous decrease in activity of collagen-degrading enzymes in the test tissue.

Collagen binds closely with other connective tissue biopolymers: glycosaminoglycans, glyproteins, and sialic acids [10]. Accordingly, data on intensification of collagen accumulation during prolonged intraventricular injections of substance P and Leu-enkephalin may be a sign of a general anabolic process in the connective tissue. This is confirmed by the results of our investigations (Tables 1 and 2): a fall of the blood SA and HUA levels and a rise of these parameters in aortic tissue after 30 days of a chronic experiment. Intensification of anabolism is indirectly supported by the significant decrease in the blood urea concentration.

The clear parallel between intensification of anabolism in collagen metabolism in the aorta and the significant fall of the peripheral blood 11-HCS concentration confirmed the abundant evidence of a catabolic action of glucocorticoids on connective tissue [2, 4]. Hence it is logical to suggest that under conditions of a chronic deficiency of corticosteroid hormones the processes of collagen catabolism are slowed and this protein accumulates in the aortic tissue.

Thus changes in central neurochemical processes caused by long-term intraventricular injection of neuropeptides, lead to weakening of adrenocortical function followed by intensification of metabolic processes in the connective tissue, characterized by accumulation of its biopolymers in the aorta. Injection of substance P is accompanied by more marked changes than injection of opioid peptides.

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