EFFECT OF ATRIAL NATRIURETIC PEPTIDE AP II ON EPITHELIAL TISSUE PROLIFERATION IN ALBINO RATS

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It has been shown that besides natriuretic and vasodilator activity and a broad spectrum of neuroendocrine effects [8, 9, 11, 15], atrial natriuretic peptides (ANP) affect proliferative processes in cultures of smooth-muscle, mesangial, and erythroid cells [4, 5, 7, 13]. No information on the effect of ANP on proliferative processes in vivo could be found in the accessible literature.

The aim of this investigation was to study the character of the effect of ANP on proliferative processes in epithelial tissue in the whole organism.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 120-190 g. The 23-amino acid peptide atriopeptin (AP II), which possesses the basic biological properties and structural characteristics of ANP, was used as a representative of this peptide family [1]. AP II, synthesized in the Laboratory of Peptide Chemistry, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, was injected intraperitoneally in the experiments of series I in a single dose of 10 μg/kg [1]. In the experiments of series II a larger dose (100 μg/kg) was injected. Animals into which an equal volume of isotonic sodium chloride solution was injected intraperitoneally served as the control. Proliferative processes in the epithelia of the skin, cornea, tongue, and duodenum were studied 4 and 24 h after injection of the substance. The character of the proliferative processes was judged by the mitotic index (MI), following administration of colchicine (MI_{col}, in promille) and the index of labeled nuclei (ILN, in per cent) and the labeling intensity (LI; the mean number of grains of silver above the nucleus) during autoradiography with ^{3}H-thymidine. Histological sections and autoradiographs were prepared by the methods described in [2]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Changes in proliferative processes in the epithelial tissue of the various organs after injection of AP II in a dose of 10 μg/kg differed in character (Table 1). In the epithelium of the cornea and skin reduction of MI_{col} was observed 24 h after injection of the peptide, probably indicating premitotic delay. When this effect is analyzed, a temporary inhibition of DNA synthesis, which escaped recording, and leading to a subsequent decrease of MI, cannot be ruled out. In the epithelium of the tongue and duodenum injection of AP II led to activation of proliferation. Although in the duodenal epithelium we observed no changes in MI, activation of DNA synthesis took place at both times of investigation. In the epithelium of

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TABLE 1. Effect of AP II in a Dose of 10 μg/kg on Proliferative Processes in Epithelial Tissue of Albino Rats 4 and 24 h after Injection

Experimental conditions	MI_{col}, ‰	ILN, %	MI
	Cornea		
Control	9,2±1,0	7,7±1,0	13,8±1,3
4 h	10,5±1,0	7,7±0,8	10,1±0,9
24 h	3,2±0,3**	9,4±0,7	17,5±1,8
	Epithelium of skin		
Control	6,8±1,2	1,8±0,2	9,4±0,2
4 h	4,1±0,8	1,6±0,2	8,7±0,5
24 h	3,3±0,7*	1,9±0,3	10,8±0,7
	Tongue		
Control	57,4±6,2	12,6±0,4	18,3±1,2
4 h	91,6±7,2*	11,2±0,7	24,6±0,4**
24 h	73,6±13,6	18,8±0,9**	27,8±2,4*
	Duodenum		
Control	142,4±6,8	27,4±1,0	16,6±0,9
4 h	142,3±2,3	30,8±1,0*	23,6±1,2**
24 h	146,0±4,9	37,9±1,3**	27,6±1,3**

Legend. Here and in Table 2 *$p < 0.05$ compared with control, **$p < 0.001$ compared with control.

TABLE 2. Effect of AP II in a Dose of 100 μg/kg on Mitotic Activity of Epithelial Tissue of Albino Rats 4 and 24 h after Injection

Experimental condition	MI_{col}, ‰			
	cornea	epithelium of skin	tongue	duodenum
Control	18,1±1,2	5,3±1,4	35,7±5,1	170,8±5,2
4 h	24,2±2,3*	7,3±1,5	57,7±4,6*	206,6±10,1*
24 h	26,4±3,4*	13,1±2,4*	62,3±5,4*	240,5±8,1**

the tongue a significant rise of mitotic activity was recorded 4 h after injection of AP II. At the same time a significant increase in the intensity of labeling was observed, evidence of more rapid DNA replication under these conditions. After 24 h the increase in the labeling intensity was combined with an increase in ILN by 1.7 times.

Thus injection of AP II in a dose of 10 μg/kg led to activation of proliferative processes in the epithelium of the tongue and duodenum and to inhibition of mitotic activity of the epithelium of the cornea and skin. The data indicate that AP II may affect cell division. One of the points of application of its action may perhaps be the premitotic period. Another important result of AP II administration is an increase in the number of cells entering the S period. This phenomenon was observed in the epithelium of the tongue and duodenum. The third effect of AP II is acceleration of the passage of the cells through the S-period. Evidence of this is given by a significant increase in the labeling intensity in the epithelium of the tongue and duodenum.

Differences in reception of ANP, associated in particular with the number of receptors for ANP in the tissues and the relative numbers of their subpopulations in the existing pool, may be the cause of the differential response of the epithelium of the different organs to atriopeptin. Both these parameters, according to data in the literature, can vary significantly in different organs and tissues [10, 14], but we found no information on the representation of receptors for ANP in epithelial tissue. One of the subtypes of ANP receptors, accounting for about 5-10% of the total pool, is coupled with guanylate cyclase, and its activation leads to elevation of the intracellular level of cGMP [12], which transmits the mitogenic signal. According to data in the literature, opposite responses to ANP also were observed in experiments on different cell cultures [4, 5, 7, 13]. Another possible cause of differences in the character of response to AP II is the pattern of the blood supply: in the cornea there is no direct blood supply, in the epithelium of the skin it is at a lower level than

in the epithelium of the tongue and duodenum. A low blood flow, just like a small number of receptors for ANP in the tissues, may impair the bioaccessibility of atriopeptin to the epithelial cells of the cornea and skin.

The results of a study of cell division in response to injection of AP II in a dose of 100 μg/kg (Table 2) may serve as confirmation of the correctness of this hypothesis that the character of the effect of AP II on proliferation is linked with accessibility of the substance to the epithelium. In this dose the peptide increased the mitotic activity of the epithelium in all the organs studied. However, these results may also be evidence of a differential response of proliferative processes to different doses of atriopeptin.

One possible way in which AP II may be involved in the regulation of proliferative processes is indirect action through the neuroendocrine system [15]. The direct action of the peptide on cell division, both independent and through interaction with growth factors, likewise cannot be ruled out. In experiments on cell cultures competitive relations were found between ANP and growth factors [4, 5, 7]. There is evidence of homology of individual regions in the structure of the precursor of epidermal growth factor and the primary structure of the ANP precursor [6], which can be interpreted as evidence of their phylogenetic kinship [3].

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