EFFECT OF ACYCLIC ANALOGS OF ATRIAL NATRIURETIC FACTOR ON PROLIFERATION IN ALBINO RAT EPITHELIAL TISSUE

E. N. Goncharova, S. S. Timoshin, Zh. D. Bespalova, and S. G. Vyborov

UDC 612.125.018:576.355

KEY WORDS: atrial natriuretic factor; mitosis; DNA synthesis

In previous investigations we showed that injection of atriopeptide AP II in a dose of 10 μg/kg leads to inhibition of mitotic activity of the corneal and cutaneous epithelium of albino rats, accompanied by stable parameters of DNA synthesis. An increase in the mitotic index and activation of DNA synthesis were observed in the epithelium of the duodenum and tongue under these circumstances. In a dose of 100 μg/kg the natriuretic peptide increased mitotic activity in the epithelium of all organs tested. Because of the prospects for creating pharmacologic preparations based on atrial natriuretic factor (ANF), shortened analogs of the atriopeptide, containing the hypothetical active center of the ANF molecule in their structure, have been obtained in the laboratory of peptide synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR.

The aims of the investigation described below included a study of the character of the possible effect of acyclic shortened analogs of ANF on proliferative processes in the intact organism.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 120-190 g. The ANF analogs: AP-H-6-OH (Arg-Ile-Asp-Arg-Ile-Gly) and its formyl derivative AP-For-6-OH (HCONH-Arg-Ile-Asp-Arg-Ile-Gly) (obtained in the laboratory of peptide synthesis, All-Union Cardiological Scientific Center, Academy of Medical Sciences of the USSR) were injected intraperitoneally in single doses of 10 and 100 μg/kg. Animals of the control group were given an intraperitoneal injection of an equal volume (0.2 ml) of sterile isotonic sodium chloride solution. Proliferative processes in the epithelium of the cornea, the skin of the ear, and the duodenum and large intestine were studied 4 and 24 h after injection of the peptides. Colchicine was injected intraperitoneally in a dose of 1 mg/kg 2 h, and ^{3}H-thymidine (molar activity 1570 TBq/mole) in a dose of 0.6 μCi/g 1 h before sacrifice. Corneas for autoradiographic study were incubated with ^{3}H-thymidine (2 μCi/ml) at 37.0°C for 1 h. The course of proliferative processes was judged from the mitotic index, after exposure to the action of colchicine (MI_{col} ‰) and parameters of DNA synthesis: the index of labeled nuclei (ILN, %) and the labeling intensity (the mean number of grains of silver above the nucleus – LI) during autoradiography. Histological sections and autoradiographs were prepared by methods described previously [1]. the numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

After injection of the test peptides in a dose of 10 μg/kg the trend of the changes in proliferative processes, just as after injection of AP V, depended on the location of the epithelium.

Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Russian Academy of Medical Sciences Yu. A. Romanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 647-649, June, 1992. Original article submitted November 20, 1991.

 0007-4888/92/0006-0870$12.50

TABLE 1. Effect of Acyclic Analogs of ANF in a Dose of 10 μg/kg on Proliferation in Corneal and Cutaneous Epithelium of Albino Rats 4 and 24 h after Injection

Experimental conditions	AP-H-6-OH			AP-For-6-OH		
	MI_{col}, ‰	ILN, %	LI	MI_{col}, ‰	ILN, %	LI
			Corneal epithelium			
Control	14,5±1,7	8,0±0,8	14,1±1,6	14,5±1,7	8,0±0,8	14,1±1,6
4 h	14,4±2,0	5,8±0,3*	11,9±1,3	14,6±1,6	7,0±1,0	9,9±0,7*
24 h	7,8±0,9*	9,0±1,0	18,6±1,6	15,3±1,8	10,3±0,9	23,9±4,8
			Cutaneous epithelium			
Control	6,0±0,5	1,8±0,2	9,4±0,4	6,0±0,5	1,8±0,2	9,4±0,4
4 h	4,8±0,9	1,2±0,2	8,3±0,5	5,7±0,9	1,9±0,3	8,8±0,5
24 h	5,0±0,8	2,0±0,2	9,5±0,5	3,4±0,5*	2,4±0,5	12,6±1,3*

Legend. Here and in Tables 2 and 3: *$p < 0.05$, **$p < 0.001$ compared with control.

TABLE 2. Effect of Acyclic ANF Analogs in a Dose of 10 μg/kg on Proliferation in Intestinal Epithelium of Albino Rats 4 and 24 h after Injection

Experimental conditions	AP-H-6-OH			AP-For-6-OH		
	MI_{col}, ‰	ILN, %	LI	MI_{col}, ‰	ILN, %	LI
			Duodenal epithelium			
Control	142,2±6,8	27,4±1,0	16,6±1,0	142,2±6,8	27,4±1,0	16,6±1,0
4 h	141,4±4,1	29,0±1,1	18,9±1,5	128,6±2,8	28,5±1,3	19,6±1,9
24 h	154,7±5,4	34,6±1,7*	25,0±1,7**	131,7±5,3	32,7±0,9*	17,2±1,9
			Epithelium of large intestine			
Control	71,6±9,2	11,9±0,8	16,7±0,7	71,6±9,2	11,9±0,8	16,7±0,7
4 h	81,3±3,4	13,3±1,5	19,1±1,2	69,8±8,5	—	—
24 h	80,8±7,5	14,4±1,1	22,3±1,2*	92,5±5,9	15,1±0,9*	19,6±1,6

TABLE 3. Effect of Acyclic ANF Analogs in a Dose of 100 μg/kg on Mitotic Index (MI_{col}) of Epithelium of Albino Rats 4 and 24 h after Injection

Experimental conditions	AP-H-6-OH	AP-For-6-OH
	Corneal epithelium	
Control	18,1±1,2	18,1±1,2
4 h	29,3±4,7*	23,9±2,0*
24 h	18,6±1,9	22,7±3,0
	Cutaneous epithelium	
Control	5,3±1,4	5,3±1,4
4 h	7,3±1,4	10,4±1,4*
24 h	8,7±1,0	4,5±0,6
	Duodenal epithelium	
Control	178,2±5,2	178,2±5,2
4h	212,5±10,5*	212,9±6,5*
24 h	214,7±5,0**	225,8±7,4**
	Epithelium of large intestine	
Control	61,0±5,4	61,0±5,4
4 h	69,6±9,8	120,5±11,9**
24h	115,7±4,4**	108,3±6,4**

In the corneal epithelium (Table 1) the two ANF analogs caused inhibition of DNA synthesis 4 h after injection, accompanied by stable mitotic activity. After 24 h, AP-For-6-OH had no significant effect on proliferative processes in the corneal epithelium, and AP-H-6-OH depressed the mitotic index by 1.9 times. The sequence of development of the effects (early inhibition of DNA synthesis and a delayed depression of mitotic activity) may indicate an effect of the test peptides on the presynaptic phase of the cell cycle in the corneal epithelium.

In the cutaneous epithelium (Table 1) no significant changes were observed in the test parameters 4 h after injection of the ANF analogs. After 24 h AP-For-6-OH caused the mitotic index to fall by 1.8 times. The accompanying increase in labeling intensity indicates acceleration of DNA replication, and evidently reflects the beginning of a compensatory rise of proliferative activity.

In the epithelium of the duodenum and large intestine (Table 2) the ANF analogs did not affect the parameters of cell division studied 4 h after injection, but activated DNA synthesis after 24 h. No significant changes could be recorded in the mitotic index in the intestinal epithelium at the times of testing.

Injection of the peptides in a dose of 100 μg/kg (Table 3) caused stimulation of mitotic activity in the epithelium of all organs studied. In the duodenal epithelium AP-H-6-OH and AP-For-6-OH increased the mitotic index at both times of testing. In the epithelium of the large intestine AP-For-6-OH had a similar effect, whereas AP-H-6-OH caused delayed (by 24 h) stimulation of mitotic activity. In the corneal epithelium we observed a significant increase in the mitotic index 4 h after injection of the peptides in a dose of 100 μg/kg. At the same time we recorded an increase in the number of mitoses in the cutaneous epithelium in response to injection of AP-For-6-OH. The "earlier" change in mitotic activity of the corneal and cutaneous epithelium in response to injection of the ANF analogs in a dose of 100 μg/kg is indirect evidence that this dose of the peptides acts on the premitotic phase of the cell cycle.

The linear ANF analogs AP-H-6-OH and AP-For-6-OH are thus generally similar in their effect on proliferative processes in epithelial tissue. The only exception is the cutaneous epithelium: AP-H-6-OH, in both doses tested, had no effect on physiological regeneration of the epidermis, whereas injection of AP-For-6-OH led to significant (in opposite directions to different doses) changes in proliferative processes in the cutaneous epithelium. Addition of a formic acid residue to the amino acid chain may perhaps affect the pharmacokinetics of the peptide, without substantially affecting its biological activity in relation to cell division.

The effect of shortened acyclic ANF analogs on proliferation in epithelial tissue, in doses of both 10 and 100 μg/kg, was thus virtually identical with effects of the 23 amino acid atriopeptide AP II, which we observed in previous investigations.

According to data in the literature there are two types of specific binding sites of atriopeptides: B- and C-receptors [5]. B-receptors are associated with membrane guanylate cyclase and, it is suggested, they mediate biological activity of atrial peptides [5, 6]. C-receptors are not associated with any known system of secondary messengers and they evidently perform a clearance function [2, 7]. Native cyclic atriopeptides bind with high affinity with both types of receptors, whereas the acyclic ANF analogs are selective ligands for the C-type [4, 9, 10] and, according to data in the literature, they do not possess any biological activity of their own [3, 8]. Linear analogs, when introduced into a living organism, by occupying the clearance system can bring about a raised endogenous ANF level [7] which, in turn, directly or indirectly affects proliferative processes in the epithelium. The possibility of an independent action of acyclic ANF analogs on physiological regeneration of epithelial tissue likewise cannot be ruled out.

Our experimental results are evidence that linear ANF analogs, as well as atriopeptides with a cyclic structure, can influence proliferative processes in the intact organism.

LITERATURE CITED

1. G. M. Kalivetskaya, S. S. Timoshin, A. L. Lykov, et al., Byull. Éksp. Biol. Med., No. 4, 92 (1982).
2. F. A. Almeida, Muneya Suzuki, R. M. Scarborough, et al., Am. J. Physiol., **256**, R469 (1989).
3. L. Chartier, E. Schiffrin, and G. Thibault, Biochem. Biophys. Res. Commun., **122**, 171 (1984).
4. J. DiMaio, J. Jaramillo, D. Wernic, et al., J. Med. Chem., **33**, 661 (1990).
5. D. C. Leitman and F. Murad, Endocr. Metab. Clin. N. Am., **16**, 79 (1987).
6. D. G. Lowe, Ming-Shi Chang, R. Hellmiss, et al., Eur. Med. Biochem. Org. J., **8**, 1377 (1989).

7. T. Maack, M. Suzuki, F. A. Almeida, et al., Science, **238**, 675 (1987).
8. K. S. Misono, H. Fukumi, R. D. Grammer, and T. Inagami, Biochem. Biophys. Res. Commun., **119**, 524 (1984).
9. K. L. Spear, M. S. Brown, G. M. Olins, and D. R. Patton, J. Med. Chem., **32**, No. 5, 1094 (1989).
10. G. P. Vlasuk, K. E. Arcuri, T. M. Ciccarone, and R. F. Nutt, FEBS Lett., **228**, 290 (1988).

RAPID PROTEIN TRANSPORT BY SPINAL MOTONEURONAL AXONS OF RATS ADAPTED AND UNADAPTED BEFOREHAND TO PHYSICAL EXERCISE

A. G. Mustafin and V. N. Yarygin

UDC 616.832-018.1-008.939.6-02:612.766.1/-07

KEY WORDS: axonal protein transport; spinal motoneurons of rats; adaptation to physical exercise

Nerve cells in an active state are distinguished by several features of a morphological character, by intensification of synthesis and accumulation of RNA and protein, increased oxygen uptake, activation of respiratory enzymes, and so on [1-3, 6, 9]. The writers showed previously that anterograde protein transport in nerve fibers depends on the functional state of the neurons and their processes [5]. According to data in the literature, the resistance of cells, organs, and systems to changes taking place during exercise is increased during adaptation [4].

In the investigation described below the effect of physical exercise, consisting of swimming for different periods of time and of different intensity, on the fast component of axonal transport (AT) of proteins was studied in motor fibers of the sciatic nerve of rats adapted and unadapted to physical exercise by swimming.

EXPERIMENTAL METHOD

Male albino rats weighing 280-320 g were used. The animals were compelled to swim daily in water at a temperature of 33-35°C for 3 h unloaded and 10-15 min carrying a load, equivalent to 1/11 of the animal's body

TABLE 1. Transport of Rapid Component of Labeled Protein along Motor Fibers of Rats Unadapted and Adapted to Physical Exercise

Experimental conditions	Swimming without load for 12±2 h		Swimming w. load of 1/11 of body wt. for 60 ± 10 min	
	velocity of AT (mm/day)	level of radioactivity of transp. mat. (con. units)	velocity of AT (mm/day)	level of radioactivity of transp. mat. (con. units)
Control	392,42±10,23	3,93±0,29	392,42±10,23	3,93±0,29
Swimming by unadapted rats	322,0±8,71*	1,92±0,2*	431,51±9,58*	8,08±0,41*
Training (swim. daily for 3 h without load or 10-15 min. w. load of 1/11 of body wt.)				
5 days	351,63±10,11*	2,23±0,16*	426,0±10,71*	6,79±0,49*
10 days	360,51±19,35	2,41±0,11*	432,0±12,05*	7,55±0,52*
20 days	387,62±11,05	3,16±0,21	391,62±19,72	3,69±0,22

Legend. *$p < 0.05$: differences significant compared with group of control rats; each value obtained by investigation of 10-12 animals.

Department of Biology, N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 649-651, June, 1992. Original article submitted October 11, 1991.

0007-4888/92/0006-0873$12.50