EFFECT OF ATRIAL NATRIURETIC FACTOR ON THE PROLIFERATIVE RESPONSE AND NATURAL CYTOTOXICITY OF HUMAN LYMPHOCYTES

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UDC 616-005.4:612.017.4:612.015.3

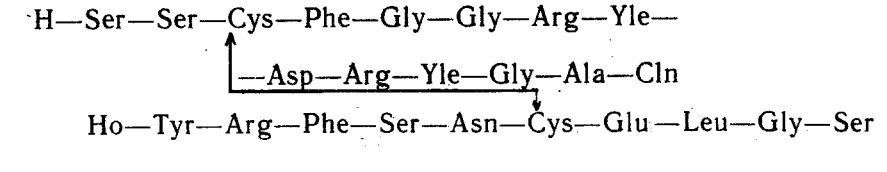
KEY WORDS: lymphocytes; proliferative response; natural cytotoxicity; atrial natriuretic peptide; low-density lipoproteins

Atrial natriuretic factor (ANF) is a polypeptide secreted in its active form by myocytes of the mammalian atria [12]. ANF causes relaxation of smooth-muscle cells and has a natriuretic and diuretic action [3]. Receptors for ANF are found in the kidneys, blood vessels, adrenals, and CNS [12]. The presence of specific high-affinity receptors for ANF on immunocompetent cells has recently been demonstrated [10], suggesting a role for ANF in regulation of the immune system.

The aim of this investigation was to study the action of synthetic analogs of ANF in vitro on the proliferative response and natural cytotoxicity (NCT) of human lymphocytes.

EXPERIMENTAL METHOD

Mononuclear cells were isolated from heparinized human venous blood in a Ficoll — Hypaque density gradient by the method in [4]. Adherent cells were removed by incubation on plastic Petri dishes for 1 h at 37°C [9]. Nonadherent cells were resuspended in complete culture medium RPMI 1640 ("Flow Laboratories") with 2 mM L-glutamine ("Merck"), 10% fetal calf serum and 10 mM HEPES ("Flow Laboratories"), and 50 μg/ml gentamicin, the cell concentration being adjusted to $1 \cdot 10^6$/ml. The proliferative response of the lymphocytes was estimated from incorporation of ^{3}H-thymidine [2]. Lymphocytes were added to the wells of flat-bottomed microplates ("Titertek") in a final concentration of $1 \cdot 10^6$ cells/ml medium, and stimulated by concanavalin A (con A, from "Flow Laboratories") in a suboptimal dose (1 μg/ml). The cells were incubated for 72 h at 37°C in a humid atmosphere with 5% CO_2. Three hour before the end of incubation 0.5 μCi ^{3}H-thymidine was added to the wells. After sedimentation of the lymphocytes on Millipore filters with the aid of an automatic "Titertek Cell Harvester 500" ("Flow Laboratories") the intensity of incorporation of ^{3}H-thymidine was determined on a "1215 RackBeta" liquid scintillation counter (LKB-Wallac). NCB of the lymphocytes was determined from release of ^{51}Cr from lysed K-562 target cells into the incubation medium (1-5) $\cdot 10^6$ target cells were incubated in 0.3 ml of complete medium RPMI 1640 with 100 μCi Na_2CrO_4 for 1 h at 37°C, washed three times, and resuspended in culture medium, adjusting the cell concentration to $1 \cdot 10^5$/ml. Lymphocyte suspension (0.1 ml) was added to each well of U-shaped microplates (Nunc) with labeled target cells in a ratio effector: target of 40:1. The cell mixture was incubated for 4 h at 37°C in a humid chamber with 5% CO_2, sedimented by centrifugation, and 0.1 ml of supernatant was taken from the wells to measure radioactivity on a Gamma counter (LKB). Synthetic analogs of ANF, namely ANF-III:



All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Sciences and of the Academy of Medical Sciences of the USSR E. I. Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 518-520, May, 1991. Original article submitted July 25, 1990.

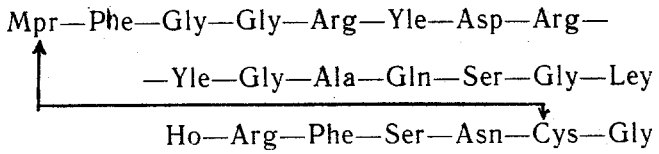
TABLE 1. Enhancement of Proliferative Response of Lymphocytes Stimulated by Con A under Influence of ANF-III and ANF-IV

Expt. No.	Increase in incorporation of ^{3}H-thymidine relative to control without factor, %					
	ANF-III		ANF-IV		IL-2	
	10^{-8} M	10^{-12} M	10^{-8} M	10^{-12} M	50 U/ml	100 U/ml
1	+15	+37	+40	+40	+34	+42
2	+36	+39	+17	+18	+49	+52
3	+19	+21	+18	+6	+28	+39
4	+14	+5	+17	+33	+35	+38
5	+19	+53	—	—	+42	+46
6	+20	+19	—	—	+31	+38

TABLE 2. Abolition by ANF-III, ANF-IV, and IL-2 of Inhibitory Action of LDL on Proliferative Response of Lymphocytes Stimulated by Con A

Expt. No.	IL-2, 100 U/ml	LDL, 100 U/ml	Incorporation of ^{3}H-thymidine, %				
			LDL + IL-2	LDL + ANF-III		LDL + ANF-IV	
			10^{-8}	10^{-12}	10^{-8}	10^{-12}	
1	+42	−38	+19	−2	+1	−8	−10
2	+52	−42	+28	+12	+5	+25	+12
3	+39	−29	+24	−7	−2	−14	−10
4	+38	−26	+29	+28	+11	+6	+3
5	+46	−44	+20	+7	+12	+5	+7

and its shortened analog ANF-IV:



(where Mpr denotes β-mercaptopropionic acid) were obtained by the classical methods of protein chemistry in solution, had the correct amino acid composition, were homogeneous on verification by methods of thin-layer and high-affinity liquid chromatography, and also were characterized by their H-NMR spectra. Peptides were obtained in the laboratory of peptide synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Dilutions of the peptides were prepared in culture medium within the concentration range 10^{-4}-10^{-6} M. Human recombinant interleukin-2 (IL-2) (from "Iskra") with activity of 10^7 units/mg protein was used in concentrations of 50 and 100 units/ml (U/ml). Low-density lipoproteins (LDL) were isolated from healthy human serum by differential centrifugation [7] and used in a concentration of 100 μg/ml. To carry out the blast transformation of lymphocytes reaction ANF-III, ANF-IV, IL-2, and LDL were added to the cell suspension 20 min before stimulation by con A. To study the action of ANF-III, ANF-IV, and IL-2 on NCT, the test substances were added to the culture of lymphocytes and target cells at the start of the cytotoxic test. All tests were carried out in three parallel determinations. The stimulating and inhibiting effect of the substances on the proliferative response and NCT of the lymphocytes was expressed as a percentage of the control (the level of cell function in the absence of the test substances).

EXPERIMENTAL RESULTS

Addition of ANF-III and ANF-IV to the cell culture in concentrations of 10^{-8} and 10^{-12} M enhanced blast transformation of healthy human lymphocytes induce by a suboptimal dose of con A (Table 1). In some cases, as Table 1 shows, ANF-III and ANF-IV stimulated the proliferative response of the lymphocytes to con A by the same degree as IL-2 in a dose of 50-100 U/ml. In another series of experiments ANF-III and ANF-IV were found to abolish the inhibitory

TABLE 3. Effect of ANF-III and ANF-IV on NCT of Lymphocytes from Normal Individuals and Patients with DCMP

Group of subjects	Serial No.	NCT without factor, %	Enhancement and weakening of NCT compared with control without factor, %		
			ANF-III, 10^{-8} M	ANF-IV, 10^{-12} M	IL-2, 50 U/ml
Normal blood donors	1	31,3	+16	−9	+39
	2	37,0	0	+8	+16
	3	61,0	+3	−16	+18
	4	52,1	+4	+5	+8
Patients with DCMP	1	38,7	+20	+18	+12
	2	43,5	−9	−10	+34
	3	60,3	−5	−30	+11
	4	10,7	−16	−22	+94

action of LDL on the proliferative response of con A-stimulated lymphocytes, although this effect of the peptides was weaker than that of IL-2 (Table 2). Table 3 gives data demonstrating the effect of ANF-III and ANF-IV on NCT of lymphocytes from healthy donors and from patients with dilatation cardiomyopathy (DCMP). The most effective concentration of ANF-III and ANF-IV for studying NVT was 10^{-8} M. These peptides acted in two directions on NCT: in some cases the presence of ANF-III and ANF-IV led to tendency for NCT to be strengthened, comparable with the effect of addition of IL-2 to the culture in a dose of 50 U/ml, but in others to weakening of NCT, when ANF-IV had this effect more often than ANF-III. In some cases natural killer (NK) cells did not react to the presence of ANF-III and ANF-IV in the culture. The character of the immunomodulating action of ANF-III and ANF-IV on NCT was independent of the initial level of cytolytic activity of the NK cells but it had definite differences in normal individuals and patients with DCMP. Reduction of NCT in response to ANF-III and ANF-IV was observed more often in the group of patients with DCMP and changes in NK-cell cytotoxicity induced by the peptides were more marked than those in normal individuals.

The results thus point to a complex effect of ANF-III and ANF-IV on human immunocompetent cells. They show that ANF-III and ANF-IV have a uniformly stimulating action on the proliferative response of lymphocytes induced by a suboptimal dose of con A, but they may act in opposite directions on NCT, enhancing it in some cases and weakening it in others.

Two opposite directions of immune activity are observed with many endogenous peptides and their analogs, including the synthetic opioid peptide analog dalargin [1]. It has been suggested that the majority of biological effects of ANF, including their effect on the immune system, is mediated through activation of guanylate cyclase and increased GMP production in the cells in response to interaction between ANF and specific cell membrane receptors [12, 13]. One of the mechanisms of the inhibitory effect of LDL on lymphocyte proliferation may be connected with suppression of cGMP accumulation in cells [8]. Meanwhile, according to data in [3], stimulation of cGMP formation requires higher ANF concentrations ($\geq$10 nM) than binding with cell receptors ($\leq$1 nM). This suggests that ANF-induced cGMP accumulation in cells is a secondary phenomenon, and indicates the presence of more complex mechanisms of realization of the immune properties of ANF, independent of cGMP. A report that ANF inhibit phosphorylation of protein kinase C and potentiate synthesis of prostaglandins E2 [11] is interesting in this connection. The possibility likewise cannot be ruled out that the action of ANF-III and ANF-IV on lymphocyte function in vitro depends to a certain degree on the level of endogenous atrial peptides, which may block receptors for ANF or compete with synthetic ANF analogs for binding sites on the cell membrane. Consequently, the differences which we found in the effects of ANF-III and ANF-IV on NCT of lymphocytes from healthy individuals and patients with DCMP may be linked with a raised plasma ANF concentration in congestive heart failure [12].

The general conclusion is that ANF may be involved in immunoregulation.

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