2. E. V. Golanov, Present State of the Problem of Endogenous Morphine-like Substances [in Russian], Moscow (1986).
3. A. D. Dmitriev, Opioid Peptides and Their Receptors [in Russian], Moscow (1982).
4. A. D. Dmitriev, A. V. Tennov, M. Ya. Tsutsul'kovskaya, et al., Vopr. Med. Khim., 87, No. 1, 88 (1987).
5. V. G. Smagin, V. A. Vinogradov, and S. A. Bulgakov, Ligands of Opiate Receptors [in Russian], Moscow (1983).
6. J. H. Eberwine and J. L. Roberts, J. Biol. Chem., 259, No. 4, 2166 (1984).
7. N. H. Kalin, S. C. Risch, R. M. Cohen, et al., Science, 209, No. 4458, 827 (1980).
8. B. A. Khalid, A. T. Lim, D. R. Fraillon, and J. W. Frunder, J. Clin. Invest., 70, No. 5, 443 (1982).
9. D. E. Krantz and W. A. Brown, Psychoneuroendocrinology, 10, No. 2, 211 (1985).
10. S. Nakanichi, A. Inoue, T. Kita, et al., Nature, 278, No. 5703, 423 (1979).
11. E. Nakao, I. Nakai, H. Jingami, et al., J. Clin. Endocrinol., 49, No. 6, 838 (1979).
12. E. Nakao, S. Oki, I. Tanaka, et al., J. Clin. Endocrinol., 51, No. 5, 1205 (1980).
13. M. E. Peterson, D. N. Orth, N. S. Halmi, et al., Endocrinology, 119, No. 2, 720 (1986).

CONCENTRATIONS OF CYCLIC NUCLEOTIDES IN ORGAN CULTURES OF NORMAL AND ATHEROSCLEROTIC HUMAN AORTA

V. V. Tertov, S. I. Tabagari, and A. N. Orekhov

UDC 616.132-004.6-07:[616.132-008.93:577.123.3]-092.4

KEY WORDS: human aorta; atherosclerosis; cyclic nucleotides

Disturbances of function of the cyclic nucleotide system are observed in various pathological states [5]. In particular, it has been shown that in experimental atherosclerosis the content of cyclic AMP and cyclic GMP in atherosclerotic arteries differs significantly from their levels in arteries of control animals [7-9]. However, there are no data in the literature on cyclic nucleotide levels in the human aorta in spontaneous atherosclerosis. In the present investigation, a short-living organ tissue culture was used to study cAMP and cGMP concentrations in atherosclerotic and unaffected areas of the human aorta.

EXPERIMENTAL METHOD

Autopsy material was taken from unhospitalized men aged 40-60 years dying suddenly from myocardial infarction, 0.5-1.5 h after death. After removal of the adventitia the aorta was washed with isotonic phosphate buffer (IPB) and tissue fragments were excised (1 × 1 cm) from three different areas: with no signs of atherosclerosis, from lipid streaks, and from atherosclerotic plaques. The intima and media were separated mechanically and each layer cultured separately in a dish 35 mm in diameter containing 2 ml of medium 199 (Gibeo, England) at a temperature of 37°C in an atmosphere of CO_2 and air in the ratio of 5:95, satured with water. At the end of incubation, tissue fragments were quickly washed with IPB solution and frozen in liquid nitrogen. Cyclic nucleotides were extracted by grinding the frozen tissue in a mortar with 2 ml of 96% ethanol. To estimate losses, 0.05 pmole of [^{3}H]cAMP (Amersham International, England) was added to the homogenate. The resulting homogenate was centrifuged (2500g, 30 min) and the residue washed twice in 4 ml of a mixture of 96% ethanol and water in the ratio of 7:1 (v/v). DNA was extracted from the residue [10] and assayed by Burton's method [4]. The pooled supernatant was applied to a column (0.6 × 2 cm) containing 0.5 g of dry alumina (Merck, West Germany). Nucleosides and purine bases were removed by washing the column with 6 ml of a mixture of ethanol and water (7:1, v/v), and the cyclic nucleotides were eluted with 2 ml of water-ethanol mixture (2:1, v/v). The eluate was evaporated at a temperature of 70°C under reduced pressure and dissolved

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 17, No. 5, pp. 575-577, May, 1989. Original article submitted June 28, 1988.

0007-4888/89/0005-0659$12.50

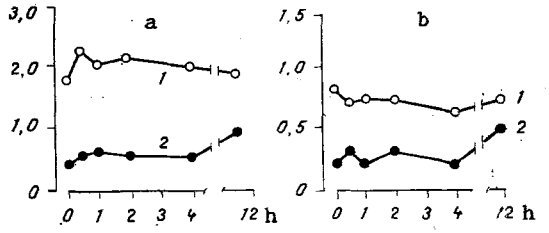

Fig. 1. cAMP (1) and cGMP (2) concentrations in organ culture of intima of atherosclerotic (a) and unaffected (b) areas of the human aorta. Abscissa, time of culture of cells; ordinate, cyclic nucleotide concentration, pmoles/μg DNA.

in 0.5-1 ml of 50 mM Tris-EDTA buffer, pH 7.2. The yield of CN after all stages was 93-95%. cAMP and cGMP were determined by radioimmunoassay and the radiosaturation method respectively, using kits from Amersham (England).

EXPERIMENTAL RESULTS

The study of the time course of changes in the cyclic nucleotide levels during culture (Fig. 1) showed that the cAMP content in the unaffected intima did not change significantly in the course of 24 h, but the cGMP level rose after 4 h. The content of both cyclic nucleotides showed no significant changes in the cultured atherosclerotic plaque, at least for 12 h, but the cGMP level fell a little toward the 12th hour of culture (Fig. 1). The same situation also was found in the medial layer of unaffected regions of the vessel (data not given). Thus the cyclic nucleotide content in the intima and media of unaffected and atherosclerotic regions of the aorta remained constant at least during 4 h in culture. This constancy of the cyclic nucleotide content during culture of the specimens, and also the fact that in a morphological study of specimens in culture no signs of tissue necrosis could be found at this time, suggest that the level of cyclic nucleotides measured in aortic tissue culture reflects their actual content.

Comparison of the cyclic nucleotide content in unaffected and atherosclerotic areas of the human aorta, cultured for 2 h, showed that the cAMP concentration in the atherosclerotic plaque is 5-7 times lower than in the unaffected intima (Table 1). The cAMP content of the lipid streak also is lower (by 4 times). The cAMP level in a culture of media obtained from unaffected areas is 2-4 times lower than in the intima. The cAMP concentration in media from under a lipid streak was the same as in normal media. Only in one of three cases was the cAMP level in the media from under an atherosclerotic plaque significantly lower was normally.

The cGMP concentration in the unaffected intima was 7-10 times lower than the cAMP concentration (Table 1). In regions of atherosclerosis the cGMP level was significantly higher than in the normal aorta. The cGMP concentration in the media under the region of an atherosclerotic plaque was the same as in unaffected areas.

In the region of the lipid streak and atherosclerotic plaque the cAMP content was several times lower than in the unaffected intima. These results are in agreement with data on a lowered cAMP level in the region of an atherosclerotic lesion of the aorta in animals with experimental atherosclerosis [7, 8]. On the other hand, we found no decrease in the concentration of the other cyclic nucleotide (cGMP) in the affected intima. Moreover, the cGMP content in the lipid streak and atherosclerotic plaque was higher than in the unaffected intima.

The mechanisms of the changes discovered are not yet clear. However, these data are evidence of dramatic disturbances in the cyclic nucleotide system in atherosclerosis. The cyclic nucleotide system controls many vitally important processes, including those whose normal course is disturbed in atherosclerosis. For instance, a rise of the intracellular cAMP level leads to suppression of proliferative activity of arterial cells [11], to more intensive hydrolysis of cellular lipids [6], to a fall in the rate of connective tissue synthesis [3], and so on. It can be tentatively suggested that one cause of the disturbance of these processes in atherosclerosis in man is a change in functional activity of the cyclic nucleotide system and, in particular, the fall of the cAMP level which we found. The writers

TABLE 1. cAMP and cGMP Concentrations in Cell Cultures of Intima and Media of Normal and Atherosclerotic Areas of Human Aorta

Aorta	Age, years	Type of lesion	cAMP and cGMP concentrations, pmoles/μgDNA			
			cAMP		cGMP	
			intima	media	intima	media
1	43	Normal	2,06±0,34	0,58±0,08	0,63±0,11	0,29±0,08
2	45	Normal	3,06±0,71	1,34±0,11	0,44±0,01	0,36±0,10
		Plaque	0,51±0,19*	0,49±0,06*	0,81±0,24*	0,36±0,10
3	58	Normal	3,21±0,46	0,73±0,04	0,28±0,02	0,19±0,06
		Lipid streak	0,70±0,17*	0,65±0,03	0,41±0,06*	0,18±0,04
		Plaque	0,47±0,04*	0,41±0,05	0,58±0,09*	0,25±0,06
4	52	Normal	2,45±0,36	0,63±0,05	0,24±0,02	0,16±0,01
		Lipid streak	1,36±0,08*	1,05±0,12	0,62±0,04*	0,21±0,02
		Plaque	1,23±0,11*	0,59±0,08	0,33±0,01*	0,21±0,01

Legend. Data shown in forms of average of three determinations ± standard deviation. Arrow $p < 0.05$ compared with normal.

showed previously [1, 2] that the dibutyryl derivative of cAMP depresses the proliferative activity and the concentration of cholesterol esters in cells of a primary culture of the human aorta when affected by atherosclerosis, confirming the possible role of the cyclic nucleotide system in the pathogenesis of atherosclerosis in man.

LITERATURE CITED

1. V. V. Tertov, A. N. Orekhov, V. S. Repin, and V. N. Smirnov, Byull. Éksp. Biol. Med., 95, 92 (1983).
2. V. V. Tertov, A. N. Orekhov, V. S. Repin, and V. N. Smirnov, Byull. Éksp. Biol. Med., 95, 108 (1983).
3. R. G. G. Andersson, H. J. Arnqvist, and L. Lundholm, Experientia (Basel), 32, 601 (1976).
4. K. Burton, Biochem. J., 62, 315 (1956).
5. B. Weiss (ed.), Cyclic Nucleotides in Disease, Baltimore (1975).
6. D. P. Hajjar, J. Clin. Invest., 70, 479 (1982).
7. Z. Jurukova and B. Boshkov, Prog. Biochem. Pharmacol., 13, 269 (1977).
8. F. Numano, H. Maezava, T. Shimamoto, and K. Adachi, Ann. N.Y. Acad. Sci., 275, 311 (1976).
9. F. Numano, T. Kuroiwa, K. Takeno, et al., Artery, 4, 322 (1978).
10. M. Ogur and G. Rosen, Arch. Biochem., 25, 262 (1950).
11. A. N. Orekhov, V. A. Kosykh, V. S. Repin, and V. N. Smirnov, Lab. Invest., 48, 395 (1983).