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CYCLIC NUCLEOTIDES (cAMP AND cGMP) IN THE BLOOD PLASMA OF DOGS DURING EXTRACORPOREAL CHARCOAL HEMOPERFUSION

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Until recently no satisfactory explanation was forthcoming for the fact that cyclic nucleotides (CN), which are intracellular mediators of the action of hormones and other biologically active substances, are constantly present also in extracellular fluids — in lymph, saliva, urine, and blood plasma. It has been suggested that the outflow of CN into the extracellular fluid regulates their content in the cells, i.e., extracellular CN were regarded as ballast substances [6]. In fact, stimulators of intracellular cAMP formation such as isoproterenol cause an increase in the concentration of the nucleotide in blood plasma and urine [12]. The cGMP concentration is similarly increased by cholinergic agents [8]. However, it was shown more recently that an increase in the CN concentration in cells is not always accompanied by their outflow into the surrounding medium. For instance,the CN phosphodiesterase inhibitor papaverine, which raises the intracellular cAMP level, does not change the rate of its liberation from erythrocytes [7]. CN transport from cells likewise is not a passive, but an active, energy-dependent process, which requires the presence of ATP and is inhibited by inhibitors of oxidative phosphorylation [5].

The view also is held that CN synthesis takes place not only inside, but also outside the cell, on the outer side of the membrane [7].

Metabolism of CN in blood plasma may follow several pathways. Some CN is excreted unchanged with urine, some is hydrolyzed by plasma phosphodiesterase [3] and also by the phosphodiesterase of the liver and kidneys [4]. Meanwhile the CN concentration in blood plasma is very constant under normal conditions and long-lasting changes in it are observed only in pathological states [11].

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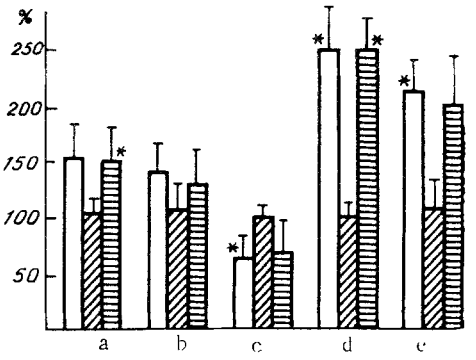

Fig. 1. Concentration of CN and cAMP/cGMP ratio in blood plasma of dogs during extracorporeal charcoal hemoperfusion: a, d) charcoal hemoperfusion, b, e) perfusion by a system without absorbent, c) injection of thiopental sodium and heparin into control animals. Unshaded colums represent cAMP, obliquely shaded columns cGMP, horizontally shaded columns cAMP/cGMP ratio. Ordinate, concentrations of cAMP and cGMP and cAMP/cGMP ratio: a, b, c) in percent of normal (i.e., of values of the corresponding parameters before the procedure); d, e) in percent of level of these parameters produced by injection of thiopental sodium and heparin. *) Differences significant ($P < 0.05$).

It can be expected that procedures not affecting the intracellular CN level in the body, but causing changes in the extracellular CN concentration, ought to be compensated in order to maintain normal homeostasis. To test this hypothesis, CN were removed from dogs' blood plasma by an artificial method, using extracorporeal charcoal hemoperfusion.

EXPERIMENTAL METHOD

Charcoal hemoperfusion was carried out on male dogs weighing 16-24 kg, which received no food for 18-20 h before the beginning of the experiment. Before the procedure the animals were anesthetized with thiopental sodium (1 g/20 g body weight) and were given an intravenous injection of heparin in a dose of 500 units/kg body weight. After exposure of the femoral vessels the artery and vein were cannulated and connected to an extracorporeal circulation system, with or without a column packed with synthetic SKN-2M charcoal absorbent. The blood was circulated through the system by means of a roller pump (from "Nikisso"; the perfusion rate was 70-80 ml/min and the duration of the procedure 1 h).

Blood for determination of CN was withdrawn from the vein or artery before the beginning of the experiment, after injection of thiopental sodium and heparin, and again 30 and 60 min after the beginning of the procedure. Blood samples were taken during charcoal hemoperfusion before the blood reached the charcoal and after it had passed through the absorbent.

Blood samples (5 ml in volume) were collected in siliconized test tubes, to each of which 50 µl of 0.5 M EDTA solution, pH 7.5 (phosphodiesterase inhibitor) and heparin solution (5.085 mg/ml) were added beforehand.

The tubes with blood were quickly placed in ice and all subsequent procedures were carried out in the cold. The blood was centrifuged (1200g) for 30 min, 1 ml plasma was withdrawn for analysis, 2 ml of 96% ethanol was added, and the mixture was stirred and centrifuged (14,000g) for 30 min to separate the denatured proteins. The supernatant was decanted into a test tube and the residue washed with 1 ml of a mixture of 96% ethanol and water (2:1 by volume). The supernatant fractions were pooled and evaporated to dryness on a water bath at 55° C under a current of argon. The dried samples were dissolved in 0.5 ml of 0.05 M Tris-HCl, pH 7.5. cAMP and cGMP in the resulting solution were determined radioimmunologically, using kits of reagents from the Radiochemical Centre, Amersham, England. Radioactivity was counted in dioxane scintillator on a "Delta" counter (the Netherlands). The results were subjected to statistical analysis by Student's t test. From five to nine animals were used at each experimental point.

EXPERIMENTAL RESULTS

During investigation of blood plasma taken immediately before the charcoal column and immediately after passing through the absorbent, significant differences were found. Blood which had passed through the column with charcoal completely lost the cAMP and cGMP which it contained. The concentrations of CN in the blood samples taken before passage through the charcoal column, and also 30 min after the beginning of charcoal hemoperfusion and after 60 min (toward the end of the procedure), moreover, were identical.

A considerable increase (by 50%) in the cAMP level compared with normal was found in plasma obtained from blood before passage through the charcoal column. The cGMP concentration under these circumstances remained within the former limits. The cAMP/cGMP ratio, reflecting relative activity of the adrenergic and cholinergic system [10], also increased correspondingly and significantly (Fig. 1a). Consequently, during charcoal hemoperfusion the blood in the body was constantly saturated with CN, despite their unreplaced loss by absorption on the charcoal; the cAMP concentration under these circumstances was in fact higher than normally.

Since the procedure of charcoal hemoperfusion incorporates not only interaction between blood and the absorbent, but also such factors as the extracorporeal circulation of the blood and the action of a combination of thiopental sodium and heparin, it was essential to estimate the contribution of each of these factors to the final levels of the CN concentration in the plasma. Experiments showed that both extracorporeal circulation of blood and injection of thiopental with heparin modifify the plasma CN concentration in a definite manner. For instance, during circulation of the blood through the system without a charcoal column, a tendency was observed for the cAMP level and the cAMP/cGMP ratio to rise (Fig. 1b).

A decrease in the cAMP concentration in the plasma by 33% was observed 60 min after injection of thiopental sodium with heparin; the cGMP concentration showed no significant change (Fig. 1c).

Considering the unique character of the response of the animal to thiopental and heparin, the CN concentration in the plasma during charcoal hemoperfusion was determined with allowance for the values obtained after injection of these substances alone. In this case, during charcoal hemoperfusion the cAMP concentration and cAMP/cGMP ratio in the plasma were more than doubled (Fig. 1d). A rather smaller increase in these parameters was observed when blood was perfused with a system without charcoal (Fig. 1e).

This investigation showed that irreversible removal of CN from blood plasma not only does not lower their concentration, but may even stimulate their synthesis and entry into the blood stream. When a charcoal absorbent was used, secretion on CN into the plasma was on a particularly large scale, for as calculations show, during the 60 min of the procedure three times the total quantity of CN in the plasma was adsorbed irreversibly on the charcoal. cAMP was produced particularly intensively: Its concentration in the plasma was higher during charcoal hemoperfusion than under normal conditions.

It can be tentatively suggested that the unusual circulatory conditions and mobilization of blood from the depots are accompanied by increased secretion of various biologically active substances (hormones, biogenic amines, etc.) into the blood stream, stimulating CN production. This evidently explains the increased cAMP concentration in experiments with an extracorporeal circulation with a system without the charcoal column. When charcoal was used, an additional factor raising the plasma cAMP level was the artificial reduction of the concentration of this nucleotide by absorption, causing compensatory stimulation of cAMP formation and its entry into the blood stream. To maintain the normal cGMP concentration during charcoal hemoperfusion, its synthesis and secretion must also be increased.

Compensatory stimulation of CN formation and of their entry into the blood stream can evidently also be achieved by increased elimination of CN by the kidneys, which to some extent can be regarded as simulating the charcoal hemoperfusion procedure. At the same time, the possibility cannot be ruled out that the charcoal absorbed certain compounds which inhibit liberation of CN from the cells.

The results confirm the importance of the presence of Cn in the plasma for the normal course of metabolism. This fact may perhaps be explained by the independent role of plasma CN as hormone-like intercellular regulators [2], which have special receptors on the outer side of the cell membrane [1]. Such a role of cAMP has been demonstrated for primitive eukaryotes [9].

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ENZYMIC DETOXICATION OF THE SUPEROXIDE ANION-RADICAL AND OF LIPID PEROXIDES IN THE INTIMA AND MEDIA OF THE ATHEROSCLEROTIC AORTA

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The hypothesis of the important role of free-radical lipid peroxidation (LPO) in the formation of atherosclerotic lesions of the vascular wall [11, 15] has recently received increasing experimental confirmation [2, 4, 6, 8]. It has been shown, in particular, that aliphatic lipid hydroperoxides [10] and endoperoxides [12] can cause substantial injury to the vascular endothelium. The level of lipid peroxides in the tissues is regulated with the participation of protective enzyme system: superoxide dismutase (SOD) and glutathione peroxidase (GP), which reduce superoxide $O_2^{\cdot}$ anion-radicals and lipid peroxides respectively [3].

For the reasons given above, enzymic utilization of active forms of oxygen and lipid peroxides in the intima and media of the aorta with various types of atherosclerotic lesion was investigated.

EXPERIMENTAL METHOD

Specimens of the thoracic aorta were taken from men aged 40-60 years 1-4 h after sudden death: The principal diagnosis made at autopsy was ischemic heart disease (IHD) with coronary atherosclerosis and cardiosclerosis. Pieces of aorta without visually detectable atherosclerotic changes and also regions of lipid stains and large fibrous plaques were isolated. The intima and media were separated mechanically as described previously [14]. In separate experiments, isolated cells were separated from the samples of intima and media with different types of atherosclerotic lesions by hydrolysis of the connective-tissue matrix with collagenase and elastase [1]. The number of cells isolated was counted in a Goryaev's chamber and the viability of the cells (as shown by vital staining with trypan blue) was not less

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