ROLE OF CYCLIC NUCLEOTIDES IN CAROTID CHEMORECEPTION

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The role of cAMP as mediator between chemical stimulation and metabolic reactions of cel ls has been demonstrated for several different sensory systems [5]. Its decisive role has been shown in the reception of various alkaloids by cells of the taste apparatus [2]. A high concentration of cyclic nucleotides has been found in the carotid labyrinth of amphibians (cAMP 738 pmoles $\cdot g^{-1}$, cGMP 40 pmoles $\cdot g^{-1}$) [3, 4], and changes in the cAMP level have been found during the action of dopamine on mammalian chemoreceptors [7], yet the role of cyclic nucleotides in carotid chemoreception in mammals has not been studied in detail.

This paper describes an attempt to discover the role of cyclase systems in the mechanisms of perception of chemical substances of different types by the glomus cells.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 200-300 g under hexobarbital anesthesia. The carotid body of the rats was subjected to microscopic investigation, including a study of the dynamics of intensity of fluorescence of reduced forms of pyridine nucleotides (NADH) and of oxidized forms of flavoproteins (FP) by the method described previously [2-4]. In addition, the partial pressure of oxygen was determined polarographically in the parenchyma of the carotid body with the aid of an open platinum electrode with a tip 20-30 μ in diameter. The concentrations of the cyclic nucleotides were determined in the carotid body [8] with the aid of standard kits obtained from Amersham.

EXPERIMENTAL RESULTS

Under the influence alkaloids of the caffeine type, a definite decrease in the ratio between the intensities of fluorescence of FP and NADH on account of an increase in reduced forms of pyridine nucleotides took place in the carotid body cells. The "dose—effect" curve, reflecting dependence of the reactions studied on concentration of the stimulus, was approximated adequately by a logarithmic function. The trend of reactions of the oxidation—reduction systems of the carotid body cells under the influence of an acid stimulus was similar. To detect a functional connection between the processes observed and the act of reception, they were compared with the altered respiratory reflexes arising in response to perfusion of the carotid body by the same chemical substances. Changes in the amplitude of respiration were found also to be described by a logarithmic function The identity of the mathematical functions describing the respiratory reflexes from carotid chemoreceptors and metabolic reactions in the carotid body cells may be evidence of a direct involvement of the metabolic systems of the carotid body in the reception of acids and alkaloids.

Changes discovered in metabolism of the carotid body cells during chemical stimulation may be the result of activation of their cellular respiration with the formation of an excess of substrates for biological oxidation. According to the results of the polarographic investigation, in response to injection of caffeine and an acid stimulus, the oxygen consumption of the carotid body cells is increased in response to injection of caffeine and an acid stimulus into the perfusion system, as shown by reduction of the partial pressure of oxygen in the intercellular spaces. Evidently in the carotid body

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TABLE 1. Cyclic Nucleotide Concentrations (in pmoles·g^{-1}) in Rat Carotid Body during Action of different Types of Chemicals (M ± m)

Chemical stimuli	cAMP concentration	cGMP concentration
Caffeine, 20 mM	591±63	62±10*
Caffeine, 2 mM	393±22	61±3
Acid stimulus, pH 6.6	439±27	54±9*
Acid stimulus, pH 5.8	383±31	78±4
Acetylcholine, 10^{-10} g.liter^{-1}	245	—
Acetylcholine, 10^{-8} g.liter^{-1}	246±12	45±4
Acetylcholine, 10^{-6} g.liter^{-1}	250	34±6
Acetylcholine, 10^{-4} g.liter^{-1}	324±14*	28±1
NaCl, 1 M	241±7*	—
Sucrose, 1 M	224±31*	41±5
Physiological saline	214±26*	67

Legend. Asterisk indicates that differences with initial level are not significant ($p \geq 0.05$)

cells under these conditions activation mainly of catabolic reactions takes place, as a result of which an excess of incompletely oxidized metabolites is formed, and their utilization leads to an increase in the oxygen consumption of the carotid body cells.

After injection of acetylcholine, salts, and sucrose into the perfusion system, no such reactions could be observed in the carotid body cells.

Perfusion of the carotid body with dibutyryl membrane-penetrating preparations of cAMP (10^{-5} g·liter^{-1}) led to similar changes in the ratio of FP and NADH, which were observed under the influence of acids and alkaloids. Consequently, a regulatory role of cAMP in the reception of chemical substances can be postulated in the carotid chemoreceptors, more especially because this has been demonstrated in cells of the carotid labyrinth of frogs [3, 4] and the olfactory epithelium [10].

A high initial level of cyclic nucleotides in the cells of the rat carotid body has been demonstrated (cAMP 264 ± 25 pmoles·g^{-1}, cGMP 57 ± 1 pmoles·g^{-1}). Different chemical substances have different effects on the cyclic nucleotide concentrations in the carotid body (Table 1).

Caffeine and an acid stimulus, acting on the chemoreceptors, induced a significant increase in the cAMP concentration, whereas in response to acetylcholine, sucrose, and NaCl changes in the cAMP concentration were not significant It is worth recalling that the 2nd group of substances, when acting on chemoreceptor cells, did not induce any marked changes in the intensity of luminescence of the principal components of the respiratory chain.

Activation of the cAMP system in the chemoreceptor cells under the influence of alkaloids of the caffeine type is linked with inhibition of cAMP phosphodiesterase, one of the components of the cyclase system, by these alkaloids [3, 4]. Elevation of the cAMP level in cells of the carotid body leads to activation of protein kinases, which increase cell membrane permeability and lead to generation of a receptor potential on the plasmalemma of chemosensitive cells [9].

Thus synthesis of cAMP in the carotid body cells probably plays a role in reception of alkaloids of the caffeine type, which, by modifying activity of the enzymes of this system, lead to activation of glycolysis and lipolysis with an increase in the intensity of cell division. This restructuring of metabolism in cells of the carotid body may lie at the basis of reception of this class of chemical substances.

Acid stimuli caused a smaller increase in the cAMP concentration in the rat carotid body than caffeine, and in the chemoreceptors of amphibians, the concentration of this cyclic nucleotide was actually reduced in response to this kind of stimulation [4]. Analysis of the causes of these differences suggests the possibility of a secondary increase in the cAMP concentration during reception of an acid stimulus by the carotid body cells. Acids themselves, without the intervention of cAMP, can interfere with cell metabolism. We connect this action with carbonic anhydrase, found in the carotid body cells of mammals [4], to which a role is ascribed in the intracellular hydration of carbon dioxide, formed from the labile bicarbonate buffer during changes in pH of the blood and penetrating from it into the cells. Dissociation of the carbonic acid leads to an increase in the H^+ ion concentration inside the glomus cells, leading to changes in their metabolism and in the

cytosol Ca^{2+} pool, which in turn, can change activity of the cAMP system in the carotid body [4, 9]. This mechanism of an increase in the cAMP concentration in response to a change in the Ca^{2+} concentration has been demonstrated in cells of other organs also [1].

Reception of other chemical substances (NaCl, sucrose, acetylcholine) is probably effected through different molecular mechanisms, in which the degree of activation of metabolism, like a change in the cAMP concentration, is not significant. The reaction of the carotid receptors to acetylcholine is accompanied by lowering of the cGMP level, proportional to an increase in concentration of the stimulus. It can be tentatively suggested [6, 11] that exogenous acetylcholine evidently inhibits the secretion and synthesis of the endogenous transmitter, thereby reducing activity of the cGMP system in the glomus cells.

Thus in our experiments significant changes were found in the oxidation—reduction systems of carotid chemosensory cells of rats under the influence of alkaloids and acids. The intermediaries between the action of chemical stimuli and the metabolic systems of the carotid body cells are the universal cell regulators, one of which is the cAMP system, Meanwhile, reception of chemical stimuli of different types takes place in glomus cells by mechanisms that differ in their physicochemical nature, and which involve to different degrees their metabolism and energy production. The results of these investigations confirm one of the cardinal propositions of the hypothesis of heterogeneity of chemosensory systems [3, 4] in mammals: the hypothesis of nonhomogeneity of the biophysical and biochemical mechanisms on reception of chemical substances of different nature.

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