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ROLE OF CALCIUM IONS IN CAROTID CHEMORECEPTION

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UDC 612:285.1:535:37

KEY WORDS: carotid chemoreceptors; membrane-bound calcium

Data relating to the direct involvement of the metabolic systems of the amphibian carotid labyrinth in the reception of certain chemical substances, which are among the most important arguments supporting the hypothesis of heterogeneity of chemoreception [5], have been confirmed also by experiments on mammals [6, 7]. In addition to this hypothesis there is also a "calcium theory" of carotid chemoreception. Its authors link the perception of chemical stimuli with changes in the "calcium homeostasis" of the glomus cells [14]. The important role of Ca^{2+} in carotid chemoreception has been demonstrated by experiments in which liposomes containing Ca^{2+} were injected into the glomus cells [9]. On the basis of experimental data showing differences in the time course of spike activity in fibers innervating the glomus cells under the influence of various modulators of calcium metabolism it has been suggested that the Ca^{2+} content in the glomus cells is dependent on the partial pressure of oxygen in the glomus tissue, and a determinant role of this cation in the mechanisms of excitation of chemoreceptors has been postulated [9, 14].

We have analyzed the role of Ca^{2+} in carotid chemoreception in mammals and have compared our results with the factual evidence in support of the hypothesis of heterogeneity of carotid chemoreception [5]. We set out from the modern view of the determinant role of Ca^{2+}, bound with membranes of organoids, in the regulation of cellular metabolism [1, 2].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 200-300 g, anesthetized with hexobarbital. Chemical stimulation of the carotid chemoreceptors was carried out with solutions of caffeine sodium benzoate (20 mM) and phosphate buffer, pH 6.2, which induced marked reflex responses of external respiration, and also changes in metabolic activity of the carotid body [6]. Effector influences on glomus cells were simulated by electrical stimulation of the peripheral end of the divided carotid sinus nerve by square pulses with a frequency of 20 Hz, for it was during similar stimulation at this same frequency that the most significant changes in cellular respiration of the glomus were recorded previously in rats [7].

Changes in the concentration of membrane-bound Ca^{2+} in cells of the carotid glomus were recorded by the aid of the fluorescent probe chlortetracycline (CTC), in a concentration of 25 μM. The time course of the intensity of fluorescence of the Ca^{2+} CTC – biomembrane (BM) complex, reflecting the kinetics of Ca^{2+} release from cell membranes [1, 11], was studied in the LYUMAM-IUF-1 luminescence microscope. The intensity of luminescence was recorded at a wavelength of 530 nm and excitation wavelength of 390 nm by means of an ÉM-1 electrometer and KSP-4 self-recording potentiometer.

S. M. Kirov Military Medical Academy, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 10, pp. 368-371, October, 1991. Original article submitted October 7, 1990.

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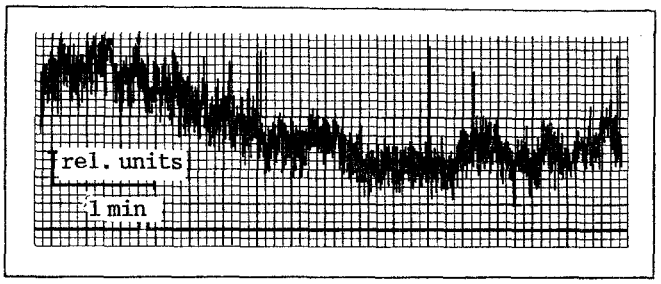

Fig. 1. Time course of fluorescence of membrane CTC–Ca^{2+} complex in cells of rat carotid body under the influence of an acid stimulus, pH 6.2. Here and in Fig. 2: abscissa, time (in min); ordinate, intensity of fluorescence (in relative units). Tape winding speed 30 mm/min. Arrow indicates time of injection of stimulus.

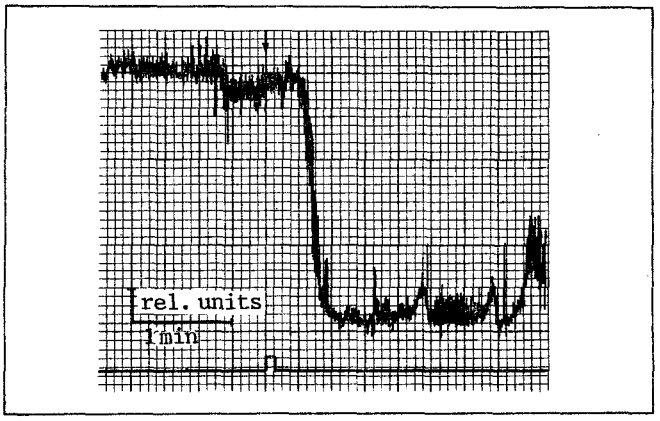

Fig. 2. Time course of fluorescence of membrane CTC–Ca^{2+} complex in cells of rat carotid body under the influence of caffeine (20 mM).

The type of membranes responding to stimulation by a change in Ca-accumulating power was determined by introducing a solution of EGTA (2 mM), which chelates calcium ions adsorbed on the outer surface of the plasmalemma of cells [2, 3], into the perfusion fluid. In other experiments a solution of 2 mM $CdCl_2$, which displaces calcium ions into the cytosol from areas of their adsorption predominantly on intracellular membranes, was added to the probe.

EXPERIMENTAL RESULTS

Introduction of chemical stimulants (caffeine and an acid stimulus) into the perfused carotid body caused a sharp decrease in the intensity of fluorescence of the Ca^{2+}–CTC–BM complex in cells of the carotid glomus (Figs. 1 and 2). Table 1 shows that the decrease in the intensity of fluorescence was particularly well marked for acids. This indicates a decrease in the Ca-accumulating capacity of the membranes of the glomus cells and release of Ca^{2+} from the membranes. Against the background of perfusion of the carotid glomus with CTC and EGTA, a marked decrease in its fluorescence also took place. Comparison of the time course of the intensity of luminescence in both groups of cell responses to chemical stimuli revealed no significant differences between them. Some decrease in the intensity of fluorescence of glomus cells treated with EGTA was observed, and was probably due to binding (chelation) by EGTA of that fraction of calcium ions which was located on the outer surface of the plasma membranes. The absence of any significant differences in the responses of intact and EGTA-treated carotid bodies to chemical stimulation is evidence, in our view, in support of the hypothesis of a change in Ca-accumulating ability mainly of the intracellular membranes of the glomus cells during reception of chemicals. This is confirmed by data showing that more than half of the total CTC-fluorescence of calcium is bound with intracellular membranes of the cell organoids, which are inaccessible to the action of EGTA [10].

TABLE 1. Fall in Level of Fluorescence (in %) of Membrane-Bound Calcium in Cells of Rat Carotid Body under the Influence of Various Stimuli (M ± m)

Stimuli	Composition of perfusion solution, %		
	CTC	CTC + EGTA	CTC + $CdCl_2$
Acid stimulus (pH 6.2)	—32,6±9,6	—25,4±13,8	—13,8±14,6
Caffeine, 20 mM	—18,0±11,5	—16,5±5,9	—8,8±8,9
Square pulses, 20 Hz	—23,8±13,0	—17,8±9,6	—14,5±12,2

In connection with the hypothesis mentioned above we also studied the action of alkaloids and acids on the carotid body, perfused simultaneously with CTC and Cd^{2+} — a competitive blocker of the Ca^{2+}-channels of the Plasmalemma. In these experiments CTC-fluorescence of the glomus cells was unchanged under the influence of chemical stimuli. Thus experiments with EGTA and Cd^{2+} suggest that in our experiments the acid stimulus and caffeine induced weakening of the Ca-accumulating ability of the intracellular membranes of the carotid body.

The action of electrical pulses with a frequency of 20 Hz on the peripheral end of the divided carotid sinus nerve also led to a decrease in the intensity of CTC-fluorescence of the carotid body cells, evidence of weakening of the Ca-accumulating capacity of their membranes. Consequently, membrane-bound calcium takes part not only in the receptor function of the carotid glomus, but also in effector regulation of the chemoreceptors. The dynamics of fluorescence of the Ca^{2+} CTC–BM complex on the addition of EGTA and $CdCl_2$ did not differ from changes in CTC-fluorescence in response to chemical stimulation of the chemoreceptors. It can be postulated that release of Ca^{2+} from organoids (chiefly mitochondria and the endoplasmic reticulum) of the glomus cell leads to an increase in the concentration of this ion in the cytosol.

The increase in the Ca^{2+} concentration in the cytosol, which evidently takes place during stimulation of the carotid body, may alter the intensity of metabolic reactions of the glomus cells due to the formation of an NADH–Ca^{2+} complex, with considerable intensification of oxidation of pyridine nucleotides and with an increase in the intensity of natural fluorescence of NADH [8]. The level of fluorescence of the Ca^{2+}–CTC–BM complex in the mitochondria may be lowered, on the one hand, by release of Ca^{2+} from the mitochondrial membranes, and on the other hand, by its binding with NADH in the intermembranous space of the mitochondria. This may lead to more rapid transfer of electrons along the respiratory chain with an increase in the intensity of natural fluorescence of the pyridine nucleotides. It is striking that the same changes in cell respiration were recorded by the present writers previously by microspectrofluorometry when the glomus cells were acted upon by alkaloids and acids [6]. Meanwhile the action of electrical pulses led to directly opposite changes in metabolism of the glomus cells [7], whereas the time course of calcium metabolism during chemical stimulation of the glomus cells and during exposure to effector influences was similar.

Correlation between changes in fluorescence of the Ca^{2+}–CTC–BM complex evidently reflects interaction of Ca^{2+} with the metabolic systems of the carotid body cells. Together with the cyclic nucleotide system, calcium ions belong to the secondary mediator class, coupling the action of extracellular stimuli with the functional and metabolic activity of the cells [5, 13]. This may lead to coupling of metabolic reactions with transmitter relay during effector influences on the glomus cells, which probably take place with the aid of the cGMP system [5, 7], and also with the action of alkaloids of caffeine type, which activate predominantly the cAMP system in the glomus [5, 6]. Metabolic reactions connected with the action of an acid stimulus may induce a change in the intracellular Ca^{2+} concentration and, besides the cyclic nucleotide system, they may activate the working of the $Ca^{2+}/2H^{+}$ antiport [12].

Calcium ions, released from intracellular membranes, may probably intensify exocytosis of catecholamines contained in chromaffin granules of the glomus cells [14]. One result of this process may be stimulation of spike activity of the sinus nerve [9].

The experiments suggest the existence of mechanisms of different physicochemical nature, for reception of different types of chemical substances, thereby confirming for mammals one of the key postulates of the hypothesis of heterogeneity of chemosensory systems [4, 5]. The "calcium theory" is not the antithesis to the hypothesis of heterogeneity of chemoreception, but it can be regarded as a fragment of it.

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