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Editors

Arterial Chemoreception

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7 Ionic Mechanisms of the Chemoreception Process in Type I Cells of the Carotid Body

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Introduction

The receptor complex in the carotid body (CB) is formed by clusters of type I cells that are connected synaptically to the endings of the chemosensory fibers of the carotid sinus nerve (CSN), partially covered by type II cells, and surrounded by a dense net of fenestrated capillaries (1).

Some aspects of CB chemoreceptor physiology such as the identity of adequate stimulus, the characteristics of the receptor response to the different stimuli, and the reflex responses elicited upon CB stimulation are well known. Contrary to this, the basic mechanisms operating in this receptor are not completely understood (2). It has been proposed that low P_{O_2} will decrease the ATP levels in the chemoreceptor or type I cells and that this decrease in ATP will trigger the release of neurotransmitters capable of activating the sensory nerve endings. However, there is no proposal on how the decrease in ATP levels can activate the release process. It has been proposed also that high P_{CO_2} and/or low pH in blood will increase the intracellular H^+ concentration and that it will result in increased intracellular Ca^{2+} in type I cells and in the release of transmitters. Once again, there is no proposal on the mechanisms by which the increase in H^+ can produce the increased Ca^{2+} concentration in type I cells (2,3).

The present work addresses some of these issues. Our interest is directed to identify some of the events taking place in the chemoreceptor cells from the arrival of the stimulus to the release of neurotransmitters. By the use of neurochemical techniques, we obtained evidence indicating that the release of neurotransmitters, when induced by low P_{O_2}, involves the activation of voltage-dependent Ca^{2+} channels. Using whole-cell clamp recordings, we demonstrated that chemoreceptor cells possess Na^+, Ca^{2+}, and K^+ channels and that a subset of K^+ channels are blocked reversibly by low P_{O_2}, providing a plausible mechanism by which type I cells can be depolarized and Ca^{2+} channels activated. We have obtained also evidence suggesting the participation of Na^+ channels in the response to hypoxia. Finally, we have obtained support for the notion that a functional coupling between Na^+-dependent proton-extruding mechanisms and a Na^+-Ca^{2+} exchange brings into type I cells the Ca^{2+} required to release neurotrans-

mitters in response to high P_{CO_2}/low pH. From this information gained in the CB and from known data in other composite receptors, it may be stated that the sensory transduction processes in the receptor cells are not on the whole dissimilar to the stimulus–secretion coupling in endocrine cells.

Methods

In the present work we used CBs excised from New Zealand rabbits (1.5–2.5 kg). Experimental procedures for the neurochemical experiments, including anesthesia, surgery, and labeling of catecholamine deposits by incubating with natural precursor [^{3}H] tyrosine, have been described before (4–6). The release of labeled catecholamines to the medium was studied by incubating the CBs in a shaking bath at 37°C in vials containing 4 ml of the appropriate test solutions which were collected for analysis and replaced by prewarmed fresh aliquots every 10 min. The solutions were bubbled continuously with the correspondent gas mixture and, in selected experiments, P_{O_2} was monitored with an O_2 electrode. In these conditions we found that the threshold hypoxia to evoke release of dopamine (DA) was in the vicinity of 75 mm Hg (i.e., a saline equilibrated with 10% O_2/90% N_2). This threshold is completely different than that obtained in the drop superfusion chamber used in previous experiments (4,5). The standard incubation medium used in the present experiments was a modified Tyrode (in m*M*: NaCl, 140; KCl, 4.7; $CaCl_2$, 2; $MgCl_2$, 1.1; HEPES 10; glucose, 5.5; adjusted at pH 7.4 with NaOH) all ionic substitutions were made maintaining the solutions isoosmotical and any organic solvent used as carrier of the drugs was tested for possible nonspecific effects. The analytical procedures for released labeled catecholamines have been described before (7).

For the electrophysiological experiments the CBs were dissociated by sequential incubation, first in a mixture of collagenase (2 mg/ml), trypsin (2 mg/ml), and DNase (0.5 mg/ml) for 20 min, and then in collagenase (4 mg/ml) and DNase (0.5 mg/ml) for another 20 min. The incubation medium was a Ca^{2+} and Mg^{2+}-free Tyrode containing 0.5% albumin. After three washings to remove the enzymes, the dissociated cells were plated on glass coverslips coated with polylysine and incubated in Minimum Essential Medium supplemented with 5% calf serum, 2 m*M* glutamine and penicillin–streptomycin. Recording techniques and data acquisition and analysis are described in detail elsewhere (8).

Results

Low P_{O_2} Activates Voltage-Dependent Ca^{2+} Channels

The chemoreceptor cells of the CB contain a great variety of putative neurotransmitters, including different biogenic amines and neuropeptides (3). Direct measurements of the release of these neurotransmitters only exists for catecholamines and it has been found that low P_{O_2} stimulation

induces the release of dopamine from type I cells parallel with the intensity of stimulation (4,5). In these studies it was found also that the removal of Ca^{2+} from the superfusion media abolished the low Po_2-induced release.

A logical extension of these initial observations was to search for the pathways involved in the entry of Ca^{2+} during low Po_2 stimulation. Because of the availability of selective blockers and activators of Ca^{2+} channels, first we explored the participation of these channels in the secretory response. Figure 7.1 shows the response to a moderate hypoxic stimulus (7% O_2 in N_2) in control conditions (left), in the presence of the dihydropyridine Ca^{2+} antagonist nisoldipine (625 n*M*; middle) and in the presence of the Ca^{2+} agonist BAY K8644 (1 μ*M*; right). In these experiments nisoldipine inhibited the release of DA induced by low Po_2 by 79 ± 7% and BAY K8644 stimulated it by nearly 600%. In parallel experiments, similar effects were found for both pharmacological agents on the release of DA induced by high extracellular K^+. These observations clearly indicate that type I cells possess Ca^{2+} channels that pharmacologically can be classified as L-type (9), and that the release of DA induced by low Po_2, as well as by high K^+, is mediated by the entry of Ca^{2+} via these channels.

Given the voltage dependence of the dihydropyridine-sensitive Ca^{2+} channels, it may be reasonably concluded that low Po_2 depolarizes type I

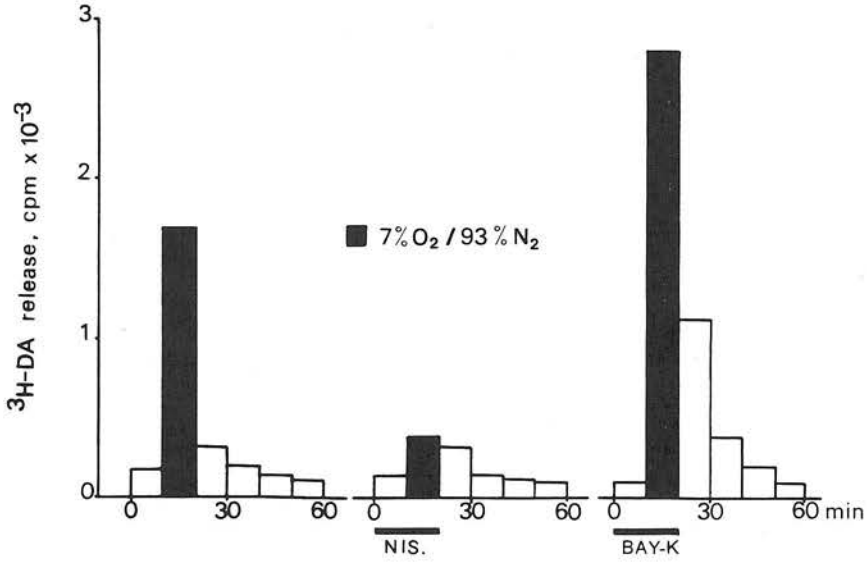

FIGURE 7.1. Effects of agonists and antagonists of Ca^{2+} channels on the release of DA elicited by mild hypoxic stimulation. The results of a typical experiment are shown. The carotid bodies were exposed to a Po_2 of 52 torr during 10 min (black bars). Left, control; Middle, in the presence of the Ca^{2+} antagonist nisoldipine (Nis; 625 n*M*); Right, in the presence of the Ca^{2+} agonist BAY K8644 (Bay K; 1 μ*M*).

cells. The observations made under high K^+ stimulation reinforce that conclusion. Stronger hypoxic stimulation (incubation with a media equilibrated with 2% O_2) applied also during 10 min evoked a much greater release of DA, which was also totally Ca^{2+}-dependent. In this case, however, nisoldipine at a concentration of 625 nM inhibited only 20–25% of the evoked release. If the duration of the stimulus was shortened to 3 min, the inhibitory effect of nisoldipine reached 80% again. These observations indicate that on prolonged severe hypoxia other pathways for Ca^{2+} entry in addition to the Ca^{2+} channels enter into play. As the work of Delpiano and Acker suggested (10), it is conceivable that intracellular acidosis develops as a result of the activation of glycolysis during stimulation with strong and prolonged hypoxia. Therefore, it should be expected that in this situation pathways for Ca^{2+} entry activated by intracellular acidification enter also into play (see pages 51–52 of the Results section).

Electrophysiological Demonstration of Na^+, Ca^{2+}, and K^+ Channels in Type I Cells: A New Type of K^+ Channel Sensitive to PO_2

The observations presented in the preceding section indicate that membrane depolarization of type I cells plays a part in the response of type I cells to hypoxia. Therefore, we decided to study electrophysiologically the major ionic currents present in type I cells and the effects of hypoxia on these currents. We choose patch-clamp recording techniques in our study because the small size of the chemoreceptor cells (about 12 μm in diameter) makes extremely difficult conventional intracellular recordings (11).

Over 150 chemoreceptor cells were patch-clamped in the whole-cell configuration and studied under voltage-clamp conditions. The cells had an average capacitance of about 7 pF, and the major current components recorded in response to an 8 msec depolarizing pulse to +20 mV from a holding potential of −70 mV are shown in Figure 7.2. Trace A was obtained from a cell bathed with standard solution equilibrated at a PO_2 of 150 torr and with a standard high K^+ solution in the pipette. In this trace it is evident that the pulse depolarization elicited a fast inward current followed by a slower outwardly directed current. At the end of the pulse a large inward tail current was recorded. When K^+ in the recording pipette was replaced by Cs^+, the outward current disappeared and a fast and sustained inward current followed by an inward tail were recorded in response to the depolarizing pulse (trace B). This indicates that the outward current is carried mostly by K^+ ions. Trace C was obtained in the same recording conditions but in the presence of 1 μ*M* tetrodotoxin (TTX), a selective blocker of voltage-operated Na^+ channels. In these conditions the fast component of the inward current disappeared, indicating that it is produced by the flow of Na^+ via voltage-operated TTX-sensitive Na^+ channels. The slow inward current and the tail inward current were unaffected by TTX; in a new group of experiments it has been demonstrated that these two components are due to the activation of Ca^{2+} channels during depolari-

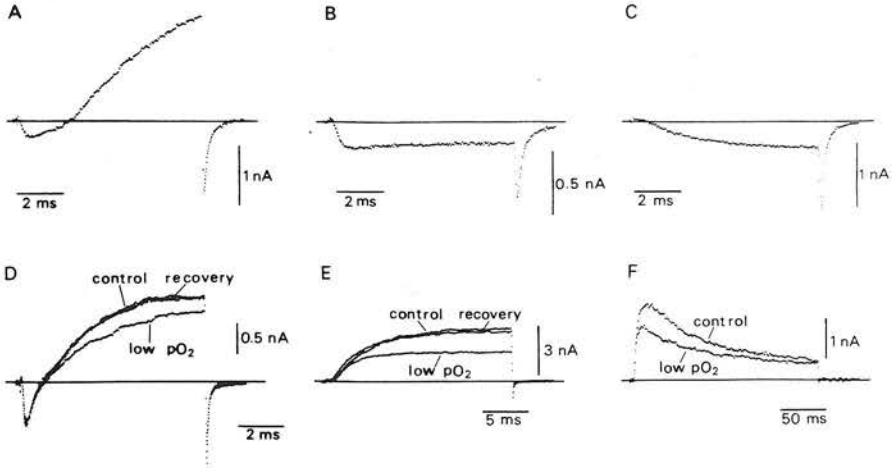

FIGURE 7.2. Voltage-dependent ionic currents in type I cells. The currents were elicited by pulse-depolarization to +20 mV from a holding potential of −70 or −80 mV. Standard recording solutions were: NaCl 140 m*M*; $CaCl_2$ 2.7 m*M*; KCl 10 m*M*; HEPES 10 m*M* (pH 7.3); and Po_2 150 torr (bathing solution); potassium glutamate 80 m*M*; KCl 30 m*M*; KFl 20 m*M*; $MgCl_2$ 2 m*M*; HEPES 10 m*M* (pH 7.3); and EGTA 1 m*M* (pipette solution). Trace A, inward and outward currents recorded under standard conditions. Trace B, inward currents recorded after blockade of K^+ currents by internal Cs^+. Trace C, record of Ca^{2+} current in isolation after blockade of K^+ currents with internal Cs^+ and Na^+ current with external TTX. Trace D, effects of low Po_2 in the bathing solution on the major currents of type I cells—the superimposed records were obtained from the same cell at a Po_2 of 150 torr (control and recovery) and at a Po_2 of 50 torr (low Po_2)—the pipette solution contained 0.5 μ*M* Ca^{2+} and 3 m*M* ATP. Trace E, effects of low Po_2 in the bathing solution on K currents in isolation—the records were obtained in the presence of TTX and after most of the Ca^{2+} channels were washed out—Po_2 in bathing media as in trace D. Trace F, effects of low Po_2 on K^+ current in response to a prolonged depolarization.

zation because they disappear when 0.5 m*M* Cd^{2+} was added to the bathing medium and also when bathing Ca^{2+} was replaced by Mg^{2+} (8,12). The tail component is produced by the flow of Ca^{2+} through Ca^{2+} channels, which due to their slow closing kinetics, remain open after the instantaneous return to the holding potential. These results indicate that the chemoreceptor cells of the CB possess Na^+, Ca^{2+}, and K^+ channels. The basic characterization of these channels will be published elsewhere (8).

Trace D of Figure 7.2 shows the effects of lowering Po_2 in the bathing medium on the whole-currents of type I cell. The recording conditions and internal and external solutions were identical to those in trace A, except for the variable Po_2 in the bathing medium (150 torr, control; 50 torr, low Po_2; 150 torr, recovery) and the presence in the internal solution of 3 m*M*

ATP. It is evident from the superimposed traces that lowering the oxygen tension in the superfusion fluid did not modify the fast inward current carried by Na^+ ions nor the fast tail current. On the contrary, the outwardly directed current was reduced reversibly, indicating a sensitivity of the K^+ currents to oxygen tension. In additional experiments where each individual current was recorded in isolation by adequate manipulation of internal and external solutions, the exclusive sensitivity of the K^+ currents to O_2 tension was confirmed. Thus, neither the Na^+, nor Ca^{2+} current showed any modification in amplitude or kinetics upon changing P_{O_2} in the bathing medium, whereas a reduction in the amplitude of K^+ current upon lowering P_{O_2} was observed consistently, this effect being completely reversible (trace E). The effect of low P_{O_2} on K^+ currents could be recorded both in cells immediately after whole-cell clamping with ATP and Ca^{2+} in the pipette, and after several minutes of clamping without ATP and Ca^{2+} ($<10^{-9}$ *M*) in the recording pipette. In the last condition (trace E), the Ca^{2+} channels have been washed out (compare tail currents in D and E); thus, since there is no Ca^{2+} entry into the cell and, moreover, Ca^{2+} is absent in the recording pipette, it indicates that the K^+ current, which is sensitive to P_{O_2}, is not Ca^{2+} dependent. The degree of inhibition of the K^+ current was, on the average, no different in the presence than in the absence of ATP in the recording pipette, suggesting that ATP does not modulate the O_2-sensitive K^+ channel (13) and also that it is not a critical parameter in the sensitivity to hypoxia as the metabolic hypothesis proposed. Finally, trace F shows that the low P_{O_2}-sensitive K^+ current is greatly inactivated during prolonged depolarizing pulses. The inactivation properties of this current render it suitable for a pacemaker function (8).

Evidence for the Participation of Na^+ Channels in the Release of DA Evoked by Low P_{O_2} in Type I Cells

We have reported recently that pharmacological activation of Na^+ channels elicits a strong release of DA from type I cells, which is both Na^+ and Ca^{2+} dependent and sensitive to TTX (14). Also, we have found that type I cells produce single spikes or trains of spikes when they are impaled in the whole-cell configuration at a holding potential of -70 or -80 mV and then the recording mode is switched to current clamp (8). The action potentials are preceded by a pacemaker potential, the slope of which increases during low P_{O_2} stimulation and leads to an increase in firing frequency (15). These two sets of data suggest that Na^+ channels may play a physiological role in the transduction of the hypoxic stimulus into a secretory response by producing a fast depolarization and the recruitment of more Ca_2^+ channels.

To test this possibility directly, the release of DA induced by low P_{O_2} was studied in two groups of CBs, one in the absence of and the other in the presence of TTX (Fig. 7.3). When the hypoxic stimulation was of intermediate intensity (5% O_2-equilibrated media), there was no meas-

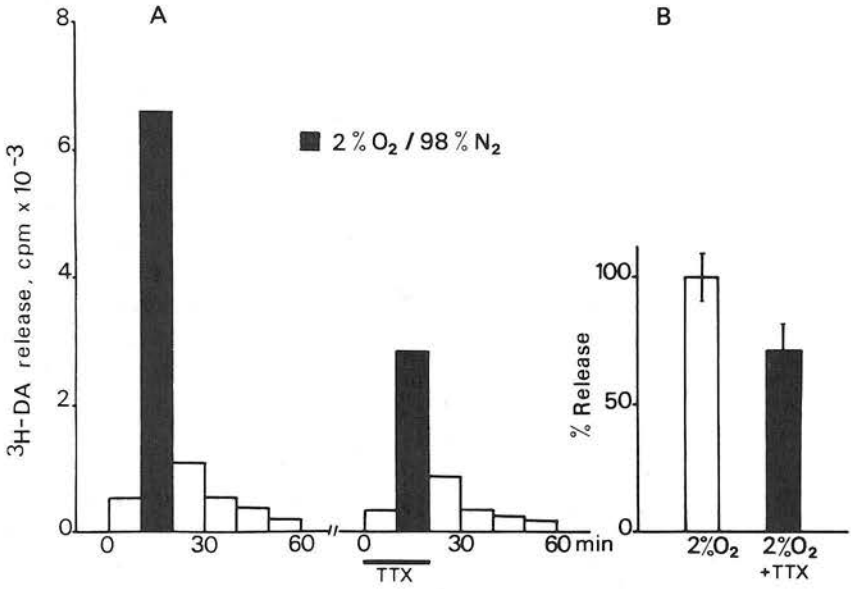

FIGURE 7.3. Effects of TTX on the release of DA elicited by strong hypoxic stimulation. (A) Release of [^{3}H]dopamine in a typical experiment. The carotid bodies were exposed to a P_{O_2} of 15 torr during 10 min (black bars); Left, control; Right, in the presence of 1 μM tetrodotoxin (TTX). (B) Mean inhibitory effect of TTX in 10 pairs of carotid bodies.

urable difference in the secretory response of control and TTX-treated CBs (not shown). Under intense hypoxic stimulation, however, TTX reduced significantly the release of DA. In ten pairs of CBs, the mean inhibitory effect was 30 ± 9% during stimulation with 2% O_2. The same effect was observed with N_2-equilibrated media (not shown). As a whole, the data obtained in these experiments indicate that in the intact CB, intense hypoxic stimulation activates Na^+ channels and that this activation plays a significant part in setting the level of DA release.

Ionic Mechanisms in the Transduction of the Acidic Stimulus

Contrary to previous proposals (16), the work of Hanson et al. (17) strongly suggested that the effective stimulus during high P_{CO_2} stimulation was an increase in intracellular H^+ concentration in the glomic structure that possesses carbonic anhydrase. Rigual et al. (18) showed histochemically that type I cells were the only CB structures positive to this enzyme, defining then the identity of the H^+ sensor in Torrance terminology (16,17). Confirmation of this proposal has been obtained in our laboratory in different ways: (1) The release of DA elicited by the superfusion of the CB with a solution equilibrated with 20% CO_2/80% O_2 at pH 6.8 is practically

identical to that obtained during superfusion with a solution equilibrated with the same gas mixture, but at a pH of 7.4; (2) The release evoked by high P_{CO_2} at a normal pH is reduced by >50% in the presence of acetazolamide, an inhibitor of carbonic anhydrase; (3) Perfusion of the CB with control solutions (100% O_2-equilibrated, pH 7.4) containing different weak acids (10–15 m*M*) results also in the release of DA from type I cells; and (4) Uncouplers elicit a potent release of DA at concentrations that do not modify the ATP content measured in the whole organ (6). Uncouplers bring H^+ concentrations across membranes to their electrochemical equilibrium, producing intense intracellular acidifications without affecting membrane potential (19). In type I cells with a membrane potential in the range of -50 to -60 mV (20) and a bathing medium pH of 7.4, a maximum pH drop to 6.4 can be expected.

The release evoked with the stimuli described above was inhibited by about 80% in nominally Ca^{2+}-free solutions, indicating that there was an entry of Ca^{2+} coupled to the increase in intracellular H^+ concentrations. The participation of Ca^{2+} channels as a pathway for Ca^{2+} entry appeared unlikely, at least in the case of uncouplers that do not change the membrane potential (19). In fact, it was found that neither the Ca^{2+} agonist nor the antagonist, which were effective in high K^+ and low P_{O_2} induced-release, modified the secretory response elicited by high P_{CO_2}/low pH or dinitrophenol (DNP). Therefore, we focused our attention towards the Na^+–Ca^{2+} exchanger as the mechanism by which Ca^{2+} enters into the cell during acidic stimulation. The exchanger can work bidirectionally and is electrogenic, moving three Na^+ by each Ca^{2+} (21). The direction of the net Ca^{2+} flux depends on the electrochemical gradients for both Na^+ and Ca^{2+} across the plasma membrane in the different functional moments of the cells (22,23). With the above stoichiometry, the equilibrium situation (no net flux) is given by the equation: $3(E_m - E_{Na}) = 2(E_m - E_{Ca})$, in which E_m is the membrane potential and E_{Na} and E_{Ca} are the Nernst potential for Na^+ and Ca^{2+}, respectively. Using typical resting concentrations for Na_i (10^{-2} *M*), Na_e (1.4×10^{-1} *M*), Ca_i (10^{-7} *M*), and Ca_e (2×10^{-3} *M*, the concentration used in our experiments), it can be calculated that the equilibrium E_m is -53 mV. This E_m is, by definition, the reversal potential of the exchanger; at E_m more negative than -53 mV, the electrochemical gradient for Na^+ will drive Ca^{2+} out of the cell, whereas at E_m less negative than -53 mV, Ca^{2+} enters the cell and Na^+ exits the cell. Therefore, a way of changing the direction for the net Ca^{2+} flux is to change the actual potential of the cell membrane. Another way of achieving this is to change the reversal potential of the exchanger, which varies with the internal concentration of Na^+ (assuming that the external one is constant); for example, when Na_i^+ increases from 10 m*M* to 12 m*M*, the reversal potential drops to -67 mV (i.e., at E_m less negative than -67 mV Ca^{2+} will be moved in and Na^+ will be moved out).

Since neither uncouplers nor high P_{CO_2}/low pH appeared to modify the

membrane potential, the only mean to reverse the direction of the Na^+–Ca^{2+} exchanger in order to produce Ca^{2+} entry is to increase the intracellular Na^+ concentration. It is well established in many systems (24) that an acidic load is followed by a dramatic activation of the Na^+–H^+ exchanger that moves Na^+ in and H^+ out of the cell and, as a consequence, the concentration of intracellular Na^+ increases. Our working hypothesis was that this increase was responsible for the reversal of the Na^+–Ca^{2+} exchanger and the consequent entry of Ca^{2+} during acidic stimulation. If the hypothesis is correct, blocking either the Na^+–Ca^{2+} exchanger or the Na^+–H^+ exchanger should result in an inhibition of the release of DA evoked by the acidic stimuli. Good inhibitors of the Na^+–Ca^{2+} exchanger are not available (25); therefore, we performed release experiments with selective blockers of the Na^+–H^+ exchanger using uncouplers and weak acids as type I cell stimulants. As shown in Figure 7.4, ethylisopropylamiloride (EIPA), at micromolar concentrations, inhibits the DNP-induced release by about 55%. The same inhibition was obtained using 15 m*M* of propionate as a stimulant. However, the expectancy for these experiments was to block about 80% of the release, the part known to be dependent on external Ca^{2+}. Therefore, there was a mismatching of about 25% between the expectations and the experimental findings (see Discussion). We found also that EIPA potently inhibits the release of DA induced by strong hypoxic stimulation. This finding indicates that during intense hypoxia intracellular acidosis develops, explaining the low sensitivity of the release process to dihydropyridines under these circumstances.

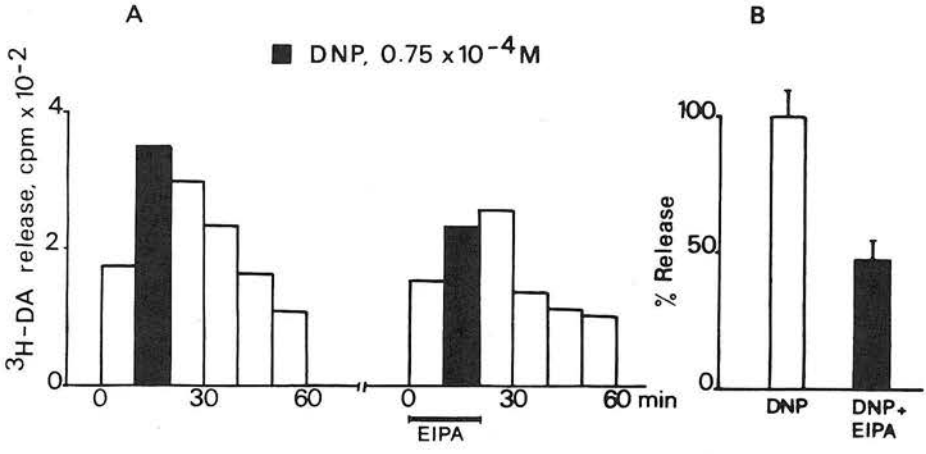

FIGURE 7.4. Effects of EIPA on the release of DA elicited by DNP. (A) Release of [^{3}H]dopamine from a single carotid body stimulated with DNP (7.5×10^{-5} *M*, 10 min; black bars); Left, control; Right, in the presence of EIPA (10^{-5} *M*); (B) Mean inhibitory effect of EIPA in 6 pairs of carotid bodies.

Discussion

The aim of the present work was to define the ionic mechanisms involved in the chemoreceptor cell function. The presented results indicate that type I cells are excitable cells, with voltage-dependent Na^+, Ca^{2+}, and K^+ channels and capable of generating action potentials similar to those generated by neurons. Our results indicate also that low Po_2 inhibits K^+ channels and that during hypoxic stimulation Na^+ and Ca^{2+} channels are activated. Finally, our results indicate that the effective acidic stimulus for CB chemoreceptors is the increase in intracellular H^+ concentration in type I cells and that the response depends on the function of a Na^+-H^+ antiporter that extrudes H^+ and brings Na^+ into the cells. The increased intracellular Na^+ produced by the activation of the Na^+-H^+ exchanger will reverse the direction of the basal operation of the Na^+-Ca^{2+} exchanger with the result of Ca^{2+} entry into the cell and activation of the exocytotic machinery. Intense hypoxic stimulation also causes a glycolysis-dependent intracellular acidification (10) and, therefore, the release of DA in this circumstance depends on Ca^{2+} entering the cell both via Ca^{2+} channels and via the Na^+-Ca^{2+} exchanger. A confluence of hypoxic and acidic stimuli, as suggested by Torrance (16), occurs during intense hypoxic stimulation.

As a whole, these findings trace very plausibly the sequence of events that define the sensory transduction in type I cells for the hypoxic and acidic stimuli. Nonetheless, it should be acknowledged that confirmation of some of our data and more direct verification of others must be achieved before the emerging model of transduction can be established firmly. For example, while we found that the O_2-sensitive K^+ current is not Ca^{2+} dependent, results were presented at this symposium by Acker's group indicating the opposite. From the standpoint of the transduction process, this difference is important: while our data pose the K^+ current inhibition as previous to the increase in intracellular Ca^{2+}, the findings of the German group (Acker et al., Chapter 3) will require that Ca^{2+} increases before the K^+ current is affected by low Po_2. Somehow related to this problem is the intracellular regulation of the ionic currents. For example, the question can be posed whether low O_2 is the direct regulator of the K^+ current or, on the contrary, if low O_2 is detected elsewhere, generating an intracellular mediator that regulates the transducing current, as is the case in other sensory systems (26–28). Another question is, how are the Ca^{2+} channels regulated in type I cells (9)?

Another finding that requires more experimental work is the apparent capacity of type I cells to generate action potentials. On the one hand, the direct recording of action potentials in dissociated cells by us and others using patch-clamp techniques seems to leave out of question the excitability of type I cells. But, on the other hand, with classical intracellular recording techniques the occurrence of action potentials in type I cells has never been reported, despite the fact that in some of them an E_m of -60 mV

has been obtained (11). The fact that TTX partially inhibits low Po_2-induced release of DA from the intact CB, strongly advocates for the existence of Na^+ action potentials in type I cells unbiased by the dissociation or culture techniques. An open question, however, is the role of these action potentials in the chemoreceptor cells. It appears that in type I cells, as in the embryologically parented adrenomedullary cells, Na^+ action potentials have a limited importance in the stimulus–secretion coupling, perhaps recruiting upper threshold Ca^{2+} channels during intense stimulation (29). In fact, I-V curves for Ca^{2+} show their maximum at about +20 mV (8).

The transduction of the acidic stimulus appears to rest not on ion channels but on ion exchangers. The lack of effect of Ca^{2+} agonists and antagonists on the acidic stimuli-induced release suggests that no significant depolarization occurs in type I cells. Although we have found that low pH bathing solutions reduced reversibly the O_2-sensitive K^+ current (15)—a finding favoring depolarization of type I cells by acid—experiments in progress show that at any acidic extracellular pH, Na^+ currents are reduced equally or more intensely than K^+ currents. We lack information on the Ca^{2+} channels of type I cells, but it has been found in other cells that Ca^{2+} action potentials are blocked when external pH is lowered to 6.5 (30). This argues also against a participation of Ca^{2+} channels in the acidic response of type I cells.

The Ca^{2+} dependence of the release of DA is very close to 80% with different acidic stimuli (defined as experimental maneuvers that increase intracellular H^+). This implies that about 20% of the DA release process is triggered by Ca^{2+} released from intracellular deposits. We have no indication either on the intracellular deposit(s) involved or on the mechanism(s) of Ca^{2+} release, about which different possibilities exist (31). A different problem is posed by the interpretation of our data: if the entry of Ca^{2+} into the cells during acidic stimulation occurs as a consequence of the activation of the Na^+-H^+ exchanger, why does the inhibition of this exchanger not block completely the part of the secretory response that is dependent on extracellular Ca^{2+}? A first possibility, incomplete inhibition of the exchanger, seems unlikely because increasing EIPA concentration from 10 to 40 μM did not increase the inhibition of the secretory response. A second possibility was that the more intense and sustained intracellular acidification resulting from the inhibition of Na^+–H^+ exchange will alter the sensitivity of the secretory response to internally released Ca^{2+}. This possibility was ruled out because the percentage of inhibition by EIPA was independent of the intensity of the stimulus. A final possibility is that type I cells possess an additional Na^+-dependent H^+ extruding mechanism that is not sensitive to amiloride derivatives. Such a mechanism could be a Na^+-dependent Cl^-–HCO_3^- exchanger (32), which exchanges external Na^+ and HCO_3^- for internal Cl^- and H^+. This exchanger has an absolute requirement for external Na^+ and HCO_3^- and a very high affinity for HCO_3^-. This high affinity would permit the exchanger to work at about

20–30% of its maximal rate with only the HCO_3^- derived from the cellular metabolism (33). Type I cells appear to have this H^+-extruding mechanism because in CO_2/HCO_3^--buffered media, the acidic stimuli evoked the release of DA by a mechanism, which being Na^+-dependent, is insensitive to EIPA. Experiments are currently in progress to incorporate this Na^+-dependent anion exchanger into the model of the transduction of the acidic stimuli.

Conclusions

1. Type I cells in short term culture have Na^+, Ca^{2+}, and K^+ voltage-sensitive channels and when released of the voltage clamp (−70 mV) generate action potentials.
2. A Ca^{2+} independent K^+ current of type I cells is reversibly blocked by low Po_2. Na^+ and Ca^{2+} currents are unaffected by low Po_2.
3. Ca^{2+} channels constitute the main pathway for Ca^{2+} entry into type I cells during moderate low Po_2 stimulation. With increasing intensity of hypoxic-stimulation, a dihydropyridine-insensitive pathway, probably a Na^+-Ca^{2+} exchanger, acquires progressive importance for the entry of Ca^{2+}.
4. Tetrodotoxin significantly reduces the secretory response induced by strong hypoxic stimulation.
5. The influx of Na^+ coupled to the efflux of H^+ that occurs during acidic stimulation of the carotid body is followed by the reversal of the direction of the Na^+-Ca^{2+} exchanger. Influx of Ca^{2+} and release of neurotransmitters from type I cells ensue.

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