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IONIC MECHANISMS FOR THE TRANSDUCTION OF ACIDIC STIMULI IN RABBIT CAROTID BODY GLOMUS CELLS

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SUMMARY

1. The release of [^{3}H]dopamine (DA) in response to inhibition of the Na^+ pump or to intracellular acid load was studied in rabbit carotid bodies (CB) previously incubated with the precursor [^{3}H]tyrosine. The ionic requirements of the release response and the involvement of specific ion transport systems were investigated.

2. Inhibition of the Na^+ pump, by incubating the CB with ouabain or in K^+-free medium, evokes a DA release response which requires the presence of Na^+ and Ca^{2+} in the medium and is insensitive to nisoldipine. This suggests that the response is triggered by entry of external Ca^{2+} through Na^+–Ca^{2+} exchange, a consequence of the increase in intracellular Na^+ resulting from inhibition of the pump.

3. Incubation of the CB in medium equilibrated with 20% CO_2 at pH 6·6, or in medium containing the protonophore dinitrophenol (DNP) or the weak acid propionate, elicits a DA release response which requires also the presence of Na^+ and Ca^{2+} in the medium and is insensitive to dihydropyridines.

4. Ethylisopropylamiloride (EIPA), an inhibitor of the Na^+–H^+ exchanger, markedly decreases the release response elicited by DNP or propionate in bicarbonate-free medium, but has not any effect in bicarbonate-buffered medium. In the latter condition, the EIPA-insensitive release of DA is inhibited by reducing the HCO_3^- concentration in the medium to 2 mM or by removal of Cl^-, suggesting that in bicarbonate-buffered medium a Na^+-dependent HCO_3^-–Cl^- exchanger is involved in the release response.

5. It is concluded that the release of DA by the chemoreceptor cells in response to acidic stimulation is triggered by entry of external Ca^{2+} through Na^+–Ca^{2+} exchange. This exchange is promoted by the increase of intracellular Na^+ that results from the operation of Na^+-coupled H^+-extruding mechanisms activated by the acid load.

INTRODUCTION

The mammalian carotid body (CB) is a chemoreceptor organ which is stimulated by decreases in the arterial pressure of O_2 (P_{O_2}) and pH and by increases in the arterial pressure of CO_2 (P_{CO_2}) (Heymans, Bouckaert & Dautrebande, 1930). It is widely accepted that these stimuli cause glomus, or type I, cells to release neurotransmitters which activate the closely apposed afferent nerve terminals (Fidone & Gonzalez,

1986; Biscoe & Duchen, 1989). Among the putative neurotransmitters that are present in type I cells, dopamine (DA) is abundant in all species studied (Fidone & Gonzalez, 1986), and close correlations have been described in CB preparations *in vitro* between DA release, intensity of stimulation, and electric activity in the afferent nerve fibres in response to a variety of stimuli (Fidone, Gonzalez & Yoshizaki, 1982; Obeso, Almaraz & Gonzalez, 1986, 1989; Rigual, López-López & Gonzalez, 1991). Therefore, DA release seems to be an appropriate indicator to assess the activation of type I cells by different stimuli.

Both in cat and in rabbit CB the release of DA induced by low P_{O_2} is totally dependent on the presence of Ca^{2+} in the medium and is inhibited by blockers of voltage-operated Ca^{2+} channels, such as nitrendipine and nisoldipine (Obeso, Fidone & Gonzalez, 1987; also in preparation). A release of DA of similar properties is elicited when the CB is incubated in high-K^+ medium, a manoeuvre that presumably depolarizes the cells (Almaraz, Gonzalez & Obeso, 1986). Direct electrophysiological evidence has been obtained recently showing that type I cells have voltage-dependent Na^+, Ca^{2+} and K^+ channels (Duchen, Caddy, Kirby, Patterson, Ponte & Biscoe, 1988; Hescheler, Delpiano, Acker & Pietruschka, 1989; Ureña, López-López, Gonzalez & López-Barneo, 1989), and that the K^+ channel activity of resting cells is reversibly inhibited by low P_{O_2} (López-Barneo, López-López, Ureña & Gonzalez, 1988; Delpiano & Hescheler, 1989; López-López, Gonzalez, Ureña & López-Barneo, 1989). These observations make very plausible the hypothesis that the release of neurotransmitters elicited by hypoxia in type I cells is mediated by depolarization of the cell membrane and subsequent entry of Ca^{2+} through voltage-dependent channels (Obeso *et al.* 1987; López-Barneo *et al.* 1988).

The mechanisms involved in the transduction of the acidic stimuli (high P_{CO_2}/low pH) by glomus cells seem to be different. As shown in the present paper, although the release of DA in this case is also highly dependent on external Ca^{2+} (80% inhibition in Ca^{2+}-free medium) it is not modified by either nisoldipine or Bay K 8644, a blocker and activator, respectively, of voltage-operated Ca^{2+} channels, which suggests that under acidic stimulation these channels are not involved and that Ca^{2+} entry occurs through a different pathway. We present evidence in this paper suggesting that this alternative pathway might be the Na^+–Ca^{2+} exchanger, a mechanism that seems to operate in type I cells (Biscoe, Duchen, Eisner, O'Neill & Valdeolmillos, 1989). It is known in other systems that this antiporter can mediate net Ca^{2+} entry when the concentration of intracellular Na^+ (Na_i^+) increases (Mullins, 1984; Blaustein, 1988; Bountra & Vaughan-Jones, 1989). We show that type I cells release DA in a Ca^{2+}-dependent and nisoldipine-insensitive mode in response to manoeuvres that should increase their Na_i^+. On the other hand, we find that the release of DA elicited by intracellular acidification is dependent on the presence of external Na^+ and is inhibited by drugs or experimental manipulations that hinder the operation of Na^+-dependent H^+-extruding systems involved in cell pH recovery. These observations suggest that acidic stimuli increase Na_i^+ by activating Na^+-coupled H^+ extrusion mechanisms, and that the increased Na_i^+ promotes Ca^{2+} influx through the Na^+–Ca^{2+} exchanger.

METHODS

Experiments were performed with CBs removed from adult rabbits (New Zealand White, 1·5–2·5 kg). The animals were anaesthetized with sodium pentobarbitone (30–40 mg/kg, I.V.), and their carotid artery bifurcations were excised and placed in a lucite chamber containing ice-cold Tyrode solution equilibrated with 100% O_2; here the CBs were identified and cleaned of surrounding tissues under a dissecting microscope.

In order to label the catecholamine deposits, the isolated CBs were incubated in a shaking bath for 2 h, at 37 °C, in 0·5 ml of 100% O_2-equilibrated Tyrode solution containing 20 μM-[3,5-^{3}H]tyrosine (specific activity 20 Ci/mmol; Amersham), 1 mM-ascorbic acid, and 100 μM-DL-6-methyl-5,6,7,8-tetrahydropterine (Sigma). Each CB was then incubated for another 2 h in 4 ml of medium without [^{3}H]tyrosine, in order to wash out the non-incorporated precursor and the rapidly releasable pools of labelled catecholamine (Almaraz *et al.* 1986); during this period the medium was replaced by pre-warmed fresh solution every 30 min and the samples were discarded. Thereafter, the release of labelled catecholamines by the CB was followed by analysing them in successively collected samples of incubation medium, which was now replaced every 10 min, intercalating the appropriate test solutions as required for each experiment. In order to lower pH and prevent catecholamine oxidation, 50 μl of a glacial acetic acid: water solution (1:1) containing 50 mM-ascorbic acid were added to each 4 ml sample.

The analysis of labelled catecholamines in the collected samples was carried out by adsorption to alumina (100 mg per sample; BioRad) at pH 8·6, followed by extensive washing of the alumina with distilled water and elution with 1 M-HCl. The eluates (1 ml) were counted in 10 ml of scintillation cocktail. In selected experiments the [^{3}H]catechols were identified by thin-layer chromatography, as previously described (Gonzalez, Rigual, Fidone & Gonzalez, 1987). In all the cases, about 90% of the radioactivity present in the alumina eluates was accounted for by [^{3}H]DA plus its catabolite [^{3}H]2,4-dihydroxyphenylacetic acid ([^{3}H]DOPAC), the remainder being [^{3}H]noradrenaline plus [^{3}H]dihydroxymandelic acid. Therefore, we refer in the text only to DA release.

The composition of the standard Tyrode solution was (mM): NaCl, 140; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1·1; glucose, 5; *N*-2-hydroxethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES), 10. The solution was adjusted to pH 7·4 with NaOH and equilibrated throughout with 100% O_2. In Na^+-free medium, NaCl was replaced by equimolar amounts of choline chloride and the pH adjusted with Tris base. In Ca^{2+}-free medium, $CaCl_2$ was omitted. Bicarbonate-buffered media consisted of the standard Tyrode solution in which a part of NaCl was replaced by equimolar amounts of sodium bicarbonate as required (24 mM-$NaHCO_3$ for solutions equilibrated with 5% CO_2–95% O_2 at pH 7·4; 14 mM for 20% CO_2–80% O_2 at pH 6·6). In bicarbonate-buffered and Na^+-free medium both NaCl and $NaHCO_3$ were replaced by the corresponding choline salts.

The data on DA release by the CB is presented either as the [^{3}H]DA (c.p.m.) released to the medium in successive 10 min periods, or as the total release evoked by a stimulus application. The last is calculated by adding up the c.p.m. found in the successive (10 min) medium samples and subtracting the basal (or control) release corresponding to the same time period. This total evoked release is expressed as a multiple of the basal release. Mean values are quoted ± standard error of mean.

RESULTS

Release of DA elicited by inhibition of the Na^+ pump

The aim of these experiments was to test whether chemoreceptor cells possess a Na^+–Ca^{2+} exchanger capable of promoting Ca^{2+} influx and triggering off neurotransmitters secretion in response to increased Na_i^+. The experimental manoeuvre used to increase Na_i^+ was the inhibition of the Na^+ pump.

Figure 1 shows that the presence of ouabain in the incubation medium elicits an increase in the rate of DA release by the CB. The effect of ouabain is both dose dependent and time dependent (Fig. 1*A*), the evoked release increasing gradually along the period of incubation with the drug and also returning gradually to the basal

level after its removal. Figure 1*B* shows that the effect of ouabain depends on the presence of Na^+ in the medium. The release of DA decreases as the Na^+ concentration is reduced, so that the evoked release in medium with 50 mM-Na^+ is only 25% of that obtained in standard 140 mM-Na^+ medium, and there is practically no evoked release

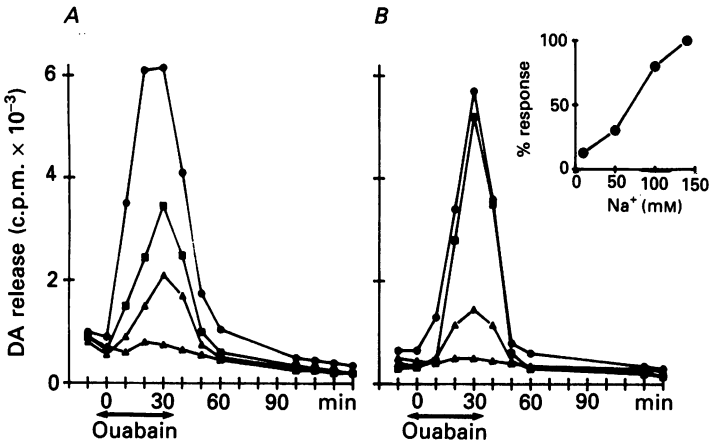

Fig. 1. Effect of ouabain on the release of DA by the CB. *A*, effects of different concentrations of ouabain in HEPES-buffered Tyrode solution. In each case the drug was added to the medium at the time marked zero and removed 30 min later, as indicated by the horizonal bar. Each point represents the amount of DA released to the medium in the preceding 10 min period. The drug concentrations used were (μM): ▲, 1; △, 5; ■, 10; ●, 50. *B*, Na^+ dependence of the effect of ouabain on DA release. The CBs were incubated in media containing different Na^+ concentrations (mM): ●, 150; ■, 100; △, 50; ▲, 10. Ouabain (50 μM) was applied for 30 min (horizontal bar). The inset shows the whole evoked release, expressed as the percentage of the response in standard medium, at the different Na^+ concentrations. All the data are means of two experiments.

in medium with 10 mM-Na^+. Both the dependence on external Na^+ and the time course of the response to ouabain suggest that this response is a consequence of the increase in Na_i^+ resulting from the inhibition of the Na^+ pump by the drug (Catterall & Nirenberg, 1973; Grinstein & Furuya, 1986), so that the increase in the rate of DA release parallels the increase in Na_i^+ in the chemoreceptor cells.

The release of DA induced by ouabain requires the presence of extracellular Ca^{2+}. Figure 2*A* shows that incubation of the CB in Ca^{2+}-free medium completely prevents the secretory response elicited by the presence of ouabain. The reintroduction of Ca^{2+}-containing medium just at the end of the incubation with the drug causes a fast and transient release response, which reaches its maximal rate within the initial 10 min period after reintroduction of Ca^{2+}. These results indicate that the release of DA induced by ouabain depends on entry of Ca^{2+} into the cells from the extracellular medium. On the other hand, the previously shown Na^+ dependence and time course of the response suggest that the entry of Ca^{2+} occurs through the operation of a Na^+–Ca^{2+} exchanger driven by the increase in the concentration of Na_i^+ (Eisner, Lederer & Vaughan-Jones, 1984; Biscoe *et al.* 1989; Bountra & Vaughan-Jones, 1989). The fast and transient release response observed on returning to Ca^{2+}-containing medium (Fig. 2*A*), which appears even at the lowest drug concentration

used (compare with the effects in Fig. 1*A*), is consistent also with the involvement of a Na^+–Ca^{2+} exchanger; in this situation, a fast entry of extracellular Ca^{2+} should be expected in exchange for the Na^+ accumulated intracellularly during the previous incubation with the drug.

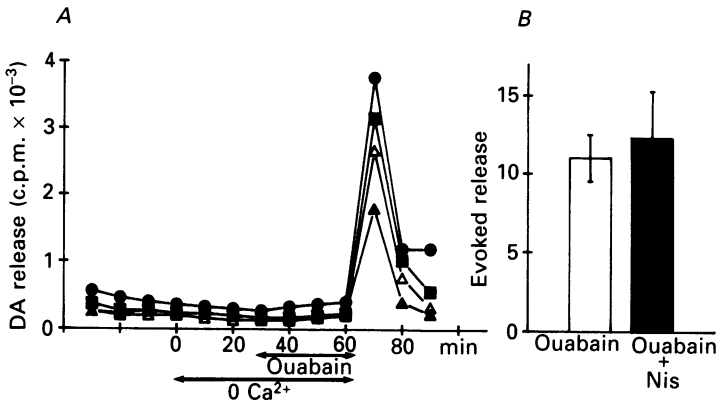

Fig. 2. *A*, Ca^{2+} dependence of the release of DA elicited by ouabain. The CBs were incubated in Ca^{2+}-free medium for 60 min, starting at the time marked zero, as indicated by the horizontal bar labelled 0 Ca^{2+}; ouabain was present in the medium during the last half of this period (indicated by the horizontal bar labelled ouabain), and then removed simultaneously with the reintroduction of Ca^{2+}-containing medium. The drug concentrations and symbols as in Fig. 1*A*. All the data are means of two experiments. *B*, sensitivity to nisoldipine of the release of DA elicited by ouabain. The response to ouabain was studied in paired CBs in the presence and in the absence of nisoldipine (Nis). Ouabain (10 μM) was applied for 30 min. Nisoldipine (125 nM) was added 10 min prior to the addition of ouabain, and the two drugs were removed simultaneously. The evoked release is expressed as a multiple of the basal release. The data are means ± S.E.M. for six experiments.

Figure 2*B* shows that the release of DA evoked by ouabain is insensitive to nisoldipine, a very effective blocker of voltage-dependent Ca^{2+} channels, which inhibits DA release by type I cells in response to hypoxia or to high-K^+ medium (Obeso *et al.* 1987; A. Obeso, A. Rocher, S. J. Fidone & C. Gonzalez, unpublished). Thus, this result suggests that these channels do not participate in the response to ouabain and lends further support for the involvement of the Na^+–Ca^{2+} exchanger.

The incubation of the CB in K^+-free medium, an alternative procedure to inhibit the Na^+ pump, also causes a gradual increase of the rate of DA release by the organ (Fig. 3), this response requiring extracellular Ca^{2+} and being insensitive to nisoldipine too. In this case there is also a fast and transient release response on reintroduction of Ca^{2+} after a period of incubation in medium lacking both K^+ and Ca^{2+}.

DA release elicited by intracellular acidification is dependent on extracellular Ca^{2+} and insensitive to dihydropyridines

Since previous work suggests that the effective acidic stimulus for chemoreceptor cells is an increase in the intracellular H^+ concentration (Rigual *et al.* 1991), in the present experiments the CBs were challenged either with the natural high P_{CO_2}–low pH stimulus or by incubating them in normal pH medium containing the

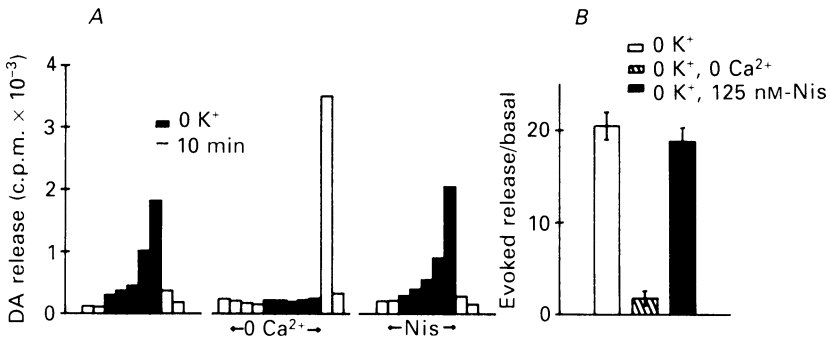

Fig. 3. Effects of incubation in K^+-free medium on the release of DA by the CB. *A*, results of a typical experiment showing the time course of DA release in Ca^{2+}-containing medium (left), in Ca^{2+}-free medium (middle), and in the presence of nisoldipine (125 nM; right). *B*, mean results from four similar experiments. The evoked release is expressed as a multiple of the basal release. Data are means ± S.E.M.

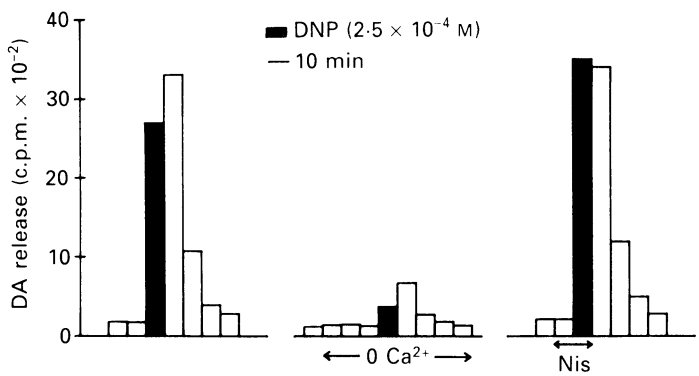

Fig. 4. Effects of DNP on the release of DA by the CB. Results of a typical experiment showing the time course of DA release in Ca^{2+}-containing medium (left), in Ca^{2+}-free medium (middle), and in the presence of nisoldipine (125 nM; right). DNP ($2{\cdot}5 \times 10^{-4}$ M) was applied for 10 min (filled bars).

protonophore dinitrophenol (DNP) or the weak acid propionate. DNP and other uncouplers produce intense intracellular acidification by bringing H^+ ions to electrochemical equilibrium across the plasma membrane (see Grinstein & Cohen, 1987), and they elicit a potent release of DA by the CB (see Fig. 4) at concentrations that do not modify the ATP content of the whole organ (Obeso *et al.* 1989). Weak acids, on the other hand, diffuse through the plasma membrane in their protonized form and dissociate to form H^+ ions inside the cell (Thomas, 1984).

Figure 4 shows the results of a typical experiment of stimulation of the CB with DNP either in standard medium, in Ca^{2+}-free medium, or in standard medium containing nisoldipine. An identical protocol was followed to study the response to stimulation with propionate or with high P_{CO_2}–low pH; however, since the release response with these stimuli is much smaller than with DNP, the sensitivity to dihydropyridines was tested using Bay K 8644, a derivative that enhances the activity of Ca^{2+} channels (Garcia, Sala, Reig, Viniegra, Frias, Fonteriz & Gandia,

1984), and increases the release of DA in type I cells elicited by low P_{O_2} or high external K^+ (A. Obeso, A. Rocher, S. J. Fidone & G. Gonzalez, unpublished). As shown in Fig. 4 and in Table 1, the release of DA elicited either by DNP ($2{\cdot}5 \times 10^{-4}$ M), 20% CO_2–pH 6·6, or propionate (15 mM), is strongly inhibited in Ca^{2+}-free medium, indicating that in the three cases the evoked release depends on entry

TABLE 1. Ca^{2+} dependence and dihydropyridine sensitivity of the release of dopamine elicited by different acidic stimuli*

Stimuli	Ca^{2+}-containing media†	Ca^{2+}-free media	Ca^{2+}-containing media + dihydropyridine‡
20% CO_2 (pH 6·6)	1·6 ± 0·17 (8)	0·3 ± 0·14 (4)	1·5 ± 0·21 (4)
DNP ($2{\cdot}5 \times 10^{-4}$ M)	48·0 ± 7·0 (8)	11·0 ± 1·5 (4)	47·4 ± 8·5 (4)
Propionate (15 mM)	1·0 ± 0·16 (8)	0·2 ± 0·3 (4)	1·1 ± 0·17 (4)

* The stimuli were applied for 10 min, according to the protocol indicated in Fig. 4. The evoked release was calculated as the difference between the release observed after application of the stimulus (S) and that for an identical period under control condition (C), and it is expressed as times the control release: $(S-C)/C$. The data are means ± S.E.M. for the number of carotid bodies given in parentheses.

† In the DNP and propionate experiments, the incubation medium was the standard HEPES-buffered Tyrode solution. In the 20% CO_2 (pH 6·6) experiments, part of NaCl in this solution was replaced by $NaHCO_3$: 24 mM when the medium was equilibrated with 5% CO_2–95% O_2 at pH 7·4 (control condition), and 14 mM for equilibration with 20% CO_2–80% O_2 at pH 6·6 (stimulus).

‡ The dihydropyridine added was nisoldipine (125 nM) in the DNP experiments, and Bay K 8644 (1 μM) in the other two cases.

of Ca^{2+} into the cells from the extracellular medium. It can be seen that in spite of great differences in the intensity of the response, the percentage of inhibition in Ca^{2+}-free medium is pratically the same for the three stimuli, about 80%, a result suggesting that in the three cases the chemoreceptor cells are activated by an identical mechanism. On the other hand, Fig. 4 and Table 1 show that nisoldipine does not inhibit the effect of DNP and neither does Bay K 8644 enhance the effect of high P_{CO_2}–low pH or propionate, which suggests that dihydropyridine-sensitive Ca^{2+} channels are not involved in the entry of Ca^{2+}.

DA release elicited by intracellular acidification is dependent on extracellular Na^+

The data presented in Table 1 pose the question of which is the pathway for the entry of Ca^{2+} under acidic stimulation. A plausible alternative for the dihydropyridine-sensitive Ca^{2+} channels is the Na^+–Ca^{2+} exchanger, a mediation that, as suggested by the effects of Na^+ pump inhibition (see before), could promote Ca^{2+} entry into the chemoreceptor cells when the concentration of Na_i^+ increases. On the other hand, it is conceivable that acidic stimuli cause an increase in the concentration of Na_i^+ as a result of the operation of Na^+-coupled H^+ extruding mechanisms activated by the intracellular acid load (Grinstein & Rothstein, 1986; Bountra & Vaughan-Jones, 1989; Grinstein, Rotin & Mason, 1989). As a test for this hypothesis we studied first the Na^+ dependence of the release response induced by intracellular acidification.

As shown in Fig. 5, the release of DA elicited by DNP ($2{\cdot}5 \times 10^{-4}$ M) is decreased by about 90% when the CB is incubated in Na^+-free medium. This result suggests

that the effect of DNP depends on entry of Na^+ into the cells and, therefore, it is consistent with the proposal that acidic stimulation increases Na_i^+ and causes entry of Ca^{2+} through the Na^+–Ca^{2+} exchanger. It is interesting to note that, as Fig. 5 shows, the mere incubation of the CB in Na^+-free medium elicits a moderate

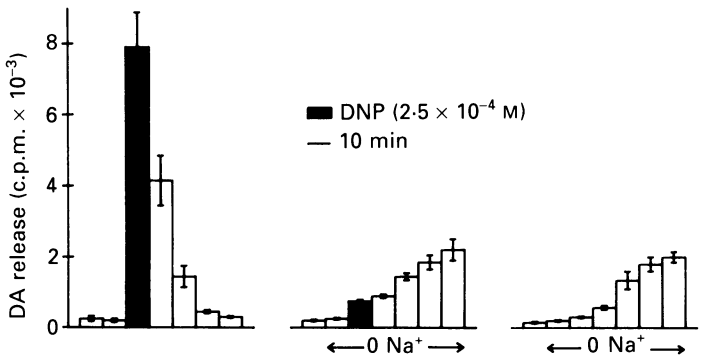

Fig. 5. Dependence on extracellular Na^+ of the release of DA elicited by DNP in HEPES-buffered medium. The figure shows the time course of DA release elicited in Na^+-containing (left) and in Na^+-free (middle and right) medium. DNP ($2{\cdot}5 \times 10^{-4}$ M) was applied for 10 min (filled bars). Data are means ± S.E.M. from four experiments.

secretory response. That response was to be expected if the chemoreceptor cells have a functional Na^+–Ca^{2+} exchanger; thus, on removal of extracellular Na^+, the imposed inversion of the Na^+ concentration gradient across the cell membrane might reverse the operation of the exchanger and cause entry of Ca^{2+} into the cells (Biscoe *et al.* 1989; see Carafoli, 1987). Nevertheless, the release of DA elicited by DNP in Na^+-containing medium is very much greater than that elicited by merely removing the extracellular Na^+, and it is this difference that practically disappears when DNP is tested in Na^+-free medium. Results showing the same trend as those of Fig. 5 were also obtained when the CB was challenged with high P_{CO_2}–low pH or with propionate (data not shown).

Involvement of Na^+-coupled H^+ extrusion systems in the release of DA elicited by acidic stimuli

As shown in Fig. 6, if the CB is incubated in standard HEPES-buffered medium, the presence of the amiloride analogue ethylisopropylamiloride (EIPA), an inhibitor of the Na^+–H^+ exchanger, markedly decreases the effect of DNP on DA release. Table 2 presents the results obtained in similar experiments carried out with two different concentrations of DNP and with propionate, showing that in each case the release of DA observed in presence of EIPA (10 μM) is only about 40% of the response observed in its absence. Increasing the concentration of EIPA to 40 μM does not increase the extent of the inhibition (data not shown). Since about 90% of the release response elicited by DNP in HEPES-buffered medium is dependent on external Na^+ (see Fig. 5), the incomplete inhibition observed with EIPA suggests either that this drug does not block totally the Na^+–H^+ exchanger or that other systems, insensitive to EIPA, take part also in the entry of Na^+ into the cells after intracellular acidification.

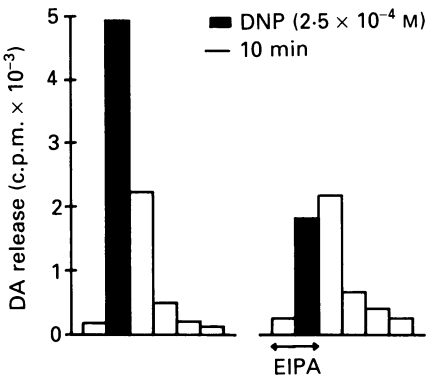

Fig. 6. Effects of ethylisopropylamiloride (EIPA) on the release of DA elicited by DNP in HEPES-buffered medium. Results of a typical experiment showing the time course of DA release in the absence (left) and in the presence (right) of EIPA (10 μM). DNP ($2{\cdot}5 \times 10^{-4}$ M) was applied for 10 min (filled bars) and EIPA 10 min prior to and during the incubation with DNP.

TABLE 2. Effects of ethylisopropylamiloride (EIPA) on the release of dopamine elicited by acidic stimuli*

Stimuli	Without EIPA	With EIPA (10^{-5} M)
In HCO_3^--free medium†		
DNP ($7{\cdot}5 \times 10^{-5}$ M)	2·7 ± 0·3 (6)	1·2 ± 0·11 (6)
DNP ($2{\cdot}5 \times 10^{-4}$ M)	42·0 ± 2·5 (6)	18·4 ± 1·75 (6)
Propionate (15 mM)	1·0 ± 0·14 (6)	0·4 ± 0·11 (6)
In HCO_3^--containing medium†		
20% CO_2 (pH 6·6)	2·4 ± 0·32 (4)	3·1 ± 0·36 (4)
DNP ($7{\cdot}5 \times 10^{-5}$ M)	2·5 ± 0·2 (4)	2·8 ± 0·44 (4)

* The stimuli were applied for 10 min, according to the protocol indicated in Fig. 6. The evoked release was calculated and expressed as in Table 1. The data are means ± S.E.M. for the number of carotid bodies given in parentheses.

† The HCO_3^--free medium was the standard HEPES-buffered Tyrode solution, equilibrated with 100% O_2. The HCO_3^--containing medium was the same solution with 24 mM-$NaHCO_3$ replacing an equimolar amount of NaCl, and it was equilibrated with 5% CO_2–95% O_2 at pH 7·4. During stimulation with 20% CO_2 (pH 6·6), the HCO_3^- concentration was reduced to 14 mM and the solution equilibrated with 20% CO_2–80% O_2 at pH 6·6.

Table 2 shows that EIPA does not inhibit at all the release of DA elicited either by 20% CO_2–pH 6·6 or by DNP in HCO_3^--buffered medium, even though in both situations the response remains highly dependent on Na^+, as is shown for DNP in Fig. 7. These observations suggest that chemoreceptor cells possess other Na^+-coupled H^+-extruding systems, besides the Na^+–H^+ exchanger, which are HCO_3^- dependent and account for most of the entry of Na^+ in response to an acid load when the CB is incubated in medium containing HCO_3^-. Figure 8 shows that if the concentration of HCO_3^- is reduced to 2 mM, EIPA is able to inhibit moderately (about 20%), although consistently, the release of DA elicited by DNP, suggesting that in this situation the operation of the HCO_3^--dependent systems becomes limited by the availability of HCO_3^-, and then the participation of the Na^+–H^+ exchanger begins to be significant.

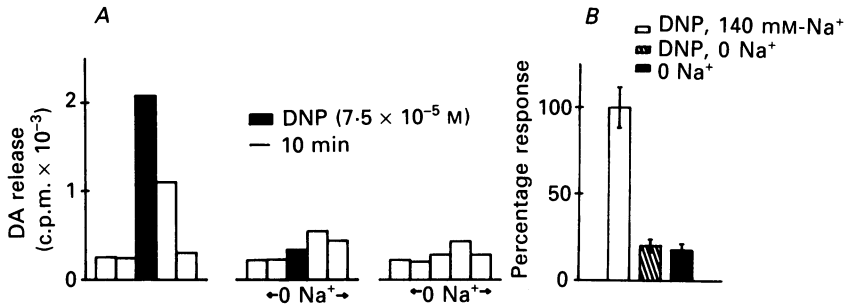

Fig. 7. Dependence on extracellular Na^+ of the release of DA elicited by DNP in bicarbonate-buffered medium. *A*, results of a typical experiment showing the time course of DA release elicited by DNP in Na^+-containing (left) and in Na^+-free (middle) medium; right panel shows the effects of Na^+ removal alone. The medium contained 24 mM-$NaHCO_3$ and was equilibrated with 5% CO_2–95% O_2 at pH 7·4. DNP ($7{\cdot}2 \times 10^{-5}$ M) was applied for 10 min (filled bars). *B*, results (means ± S.E.M.) for four similar experiments; the evoked DA release is expressed as percentage of that observed in Na^+-containing medium.

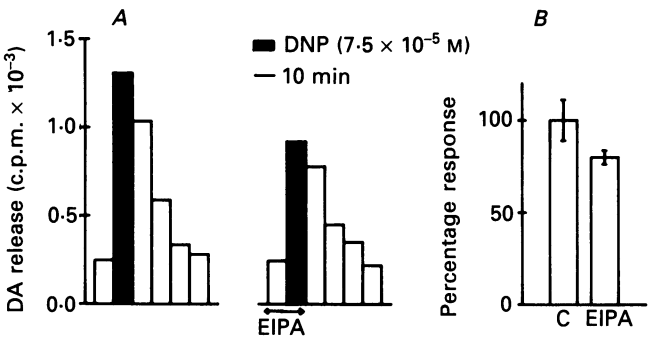

Fig. 8. Sensitivity to ethylisopropylamiloride (EIPA) of the release of DA elicited by DNP in medium with low HCO_3^- concentration. *A*, results of a typical experiment showing the time course of the release of DA in the absence (left) and in the presence (right) of EIPA (10 μM). For these experiments, HEPES was removed from the incubating solution (substituted by NaCl) and the HCO_3^- concentration was maintained at about 2 mM by continuous equilibration with 0·4% CO_2–99·6% O_2 at pH 7·4. DNP ($7{\cdot}5 \times 10^{-5}$ M) was applied during 10 min (filled bars), and EIPA 10 min prior to and during the incubation with DNP. *B*, results (means ± S.E.M.) from eight similar experiments; the evoked DA release is expressed as percentage of that observed in the absence of EIPA ($P < 0{\cdot}05$).

Two different Na^+-coupled and HCO_3^--dependent mechanisms are known which might be involved in pH_i recovery after an intracellular acid load: an electrogenic Na^+–$(HCO_3^-)_n$ symport, which has been described only in some epithelial cells and mediates the co-transport of 1 Na^+ with 2 or 3 HCO_3^-, and an electroneutral Na^+-dependent HCO_3^-–Cl^- exchanger which appears widely distributed and carries out the exchange of 1 Na^+ and 1 HCO_3^- for 1 Cl^- and 1 H^+ (see Boron, 1986). Both systems are inhibited by stilbene derivatives, such as 4,4′-diisothiocyanostilbene-2,2′-disulphonate (DIDS) or 4-acetamide-4′-isothiocyanostilbene-2,2′-disulphonate (SITS), and the Na^+-dependent HCO_3^-–Cl^- exchanger can also be inhibited by Cl^- removal. Attempts to assess the involvement of these systems in the response of the

CB to acidic stimulation by inhibiting them with DIDS or SITS were unsuccessful, because the incubation of the CB with these compounds results in an intense and sustained release of DA, without any externally imposed acid load and insensitive to EIPA, probably reflecting non-specific effects of these agents as general protein

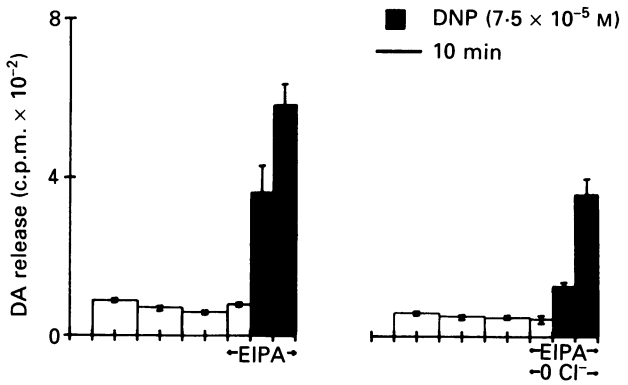

Fig. 9. Effect of Cl^- removal on EIPA-insensitive DA release elicited by DNP in low-HCO_3^- medium. The CBs were incubated in the same low-HCO_3^- solution described in the legend of Fig. 8. DNP ($7{\cdot}5 \times 10^{-5}$ M) was applied for 10 min (filled bars) either in Cl^--containing (left) or in Cl^--free (substituted by gluconate; right) medium. In both cases, EIPA (10 μM) was present 5 min prior to and during the DNP application. The Cl^--free medium (0 Cl^-) was also introduced 5 min prior to the DNP application. Data are means ± S.E.M. from four experiments.

reactants. The removal of Cl^- (substituted by gluconate) gives place also to a secretory response by the CB, which is slow in onset but increases quickly thereafter (not shown), so that the prolonged incubations required to remove most of the intracellular Cl^- were not feasible. Nevertheless, Fig. 9 shows that, even within the first 15 min after Cl^- substitution, in medium containing only 2 mM-HCO_3^-, the EIPA-insensitive release of DA elicited by DNP is reduced by about 55% in Cl^--free medium. This result indicates that the operation of the HCO_3^--dependent systems is severely hindered by Cl^- removal, suggesting that the system that is present in the chemoreceptor cells is the Na^+-dependent HCO_3^-–Cl^- exchanger.

DISCUSSION

The aim of the present work was to elucidate the ionic mechanisms involved in the activation of the chemoreceptor cells by acidic stimuli, by studying the evoked release of DA as the indicator of this activation and analysing its ionic requirements and its sensitivity to specific ion-transport inhibitors.

The observed effect of Na^+ pump inhibition in the CB (i.e. an increase in the rate of DA release that depends on external Ca^{2+} and Na^+ and is insensitive to nisoldipine) is consistent with the presence in chemoreceptor cells of a functional Na^+–Ca^{2+} exchanger (Biscoe *et al.* 1989), and strongly suggests that influx of external Ca^{2+} mediated by this antiporter maintains a DA release response that compares in magnitude to those elicited by a variety of stimuli. The Na^+–Ca^{2+} exchanger, known

to be present in a great variety of tissues, normally extrudes Ca^{2+} in exchange for external Na^+. However, it also can promote Ca^{2+} influx, working in the reverse mode, whenever the electrochemical potential gradient of Na^+ is reduced, a situation that physiologically might occur either through an increase in the intracellular Na^+ concentration or by depolarization of the cell membrane (Mullins, 1984; Carafoli, 1987; Blaustein, 1988). In our experiments, inhibition of the Na^+ pump would result in a raised level of internal Na^+ in the chemoreceptor cells (Catterall & Nirenberg, 1973; Grinstein & Furuya, 1986) and, thus, in entry of Ca^{2+} through the Na^+–Ca^{2+} exchanger. It could be argued that cell membrane depolarization might occur after Na^+ pump inhibition, and that this might also activate voltage-dependent Ca^{2+} channels. In our experiments, however, the involvement of these channels in the entry of Ca^{2+} seems very unlikely because the evoked release response on inhibition of the Na^+ pump was completely insensitive to nisoldipine, a Ca^{2+} channel blocker which inhibits DA release in response to hypoxia or to high-K^+ medium (Obeso *et al.* 1987; A. Obeso, A. Rocher, S. J. Fidone & C. Gonzalez, unpublished).

The dependence on external Ca^{2+} and Na^+ and the insensitivity to either nisoldipine or Bay K 8644 of the release of DA elicited by high P_{CO_2}–low pH, propionate, or DNP, suggest that the activation of chemoreceptor cells by acidic stimuli involves the influx of Ca^{2+} through the Na^+–Ca^{2+} exchanger and, at the same time, rule out the participation of voltage-dependent Ca^{2+} channels as a pathway for Ca^{2+} entry. The fact that the release response is inhibited by drugs or experimental manipulations that hinder the operation of the Na^+-coupled H^+-extruding systems involved in cell pH regulation, suggests that these systems also take part in the activation process, presumably by producing the increase in Na_i^+ required to reverse the Na^+–Ca^{2+} exchanger. It is well established in a great variety of cells that an acidic load is followed by activation of these systems, which promote H^+ efflux coupled to Na^+ influx and, as a consequence, the concentration of intracellular Na^+ increases (Grinstein & Rothsein, 1986; Bountra & Vaughan-Jones, 1989; Grinstein *et al.* 1989). Thus, to account for our observations, we propose a minimum model for the transduction of acidic stimuli by the CB chemoreceptor cells (Fig. 10) in which: (1) the effective activating signal is the increase in intracellular H^+ concentration after stimulus application, (2) the drop in cell pH stimulates the operation of Na^+-coupled H^+-extruding systems that bring Na^+ into the cell, and (3) the increase in intracellular Na^+ reverses 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.

On challenging the CB with different agents for causing intracellular acidification or with different intensities of the same stimulus, only about 80% of the DA release evoked in each situation can be abolished by removal of Ca^{2+} from the medium (Table 1). It seems, therefore, that about 20% of the response to acidic stimuli depends on Ca^{2+} released from internal stores. Although other possibilities exist, the fact that most of the response still is dependent on external Na^+ points to the mitochondrial Na^+–Ca^{2+} exchanger as a plausible mechanism providing this internal release of Ca^{2+} (Carafoli, 1987).

Our results are consistent with the existence in the CB chemoreceptor cells of at least two different Na^+-coupled H^+-extruding systems involved in pH recovery after intracellular acidification. The inhibition of DA release by EIPA in HEPES-buffered

medium suggests the presence of a Na^+–H^+ exchanger, whereas the lack of effects of EIPA in HCO_3^--buffered medium and the progressive sensitivity to this drug as the HCO_3^- and Cl^- concentrations are reduced suggest the existence of a Na^+-dependent HCO_3^-–Cl^- exchanger, which seems, on the other hand, the only operative system or

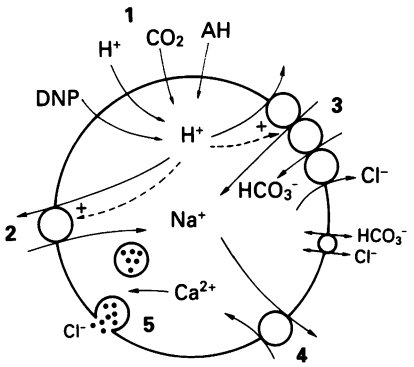

Fig. 10. Proposed model for the transduction of acidic stimuli in the carotid body chemoreceptor cells. The scheme represents several possible ways of increasing the intracellular H^+ concentration (1), such as by diffusion of protonated weak acids (AH), CO_2 or hydrogen ions (H^+), or by the operation of protonophores (DNP). The increase in intracellular H^+ stimulates (dashed arrows) the operation of Na^+-coupled H^+-extruding systems that bring Na^+ into the cell; two of these systems are represented, the Na^+–H^+ exchanger (2) and a Na^+-dependent HCO_3^-–Cl^- exchanger (3). The increase in intracellular Na^+ drives the entry of Ca^{2+} through the Na^+–Ca^{2+} exchanger (4), and that results in the activation of the exocytotic machinery (5).

the predominant one when the CB is incubated in HCO_3^--buffered media. Buckler, Nye, Peers & Vaughan-Jones (1990*a*) have presented preliminary evidence, obtained in isolated cells, indicating that type I cells possess both H^+-extruding systems. It is known that both systems act in concert in controlling the intracellular pH in various cell types (Thomas, 1977; Roos & Boron, 1981; L'Allemain, Paris & Pouysségur, 1985; Bierman, Cragoe, Laat & Moolenaar, 1988; Ganz, Boyarsky, Sterzel & Boron, 1989), and that they are activated by a drop in cell pH but stay inactive as long as the pH remains above a critical value or 'set point' (Boron, 1986; Grinstein *et al.* 1989). Since in most of these cells the steady-state intracellular pH is higher in the presence of bicarbonate than in HCO_3^--free media, it has been suggested that the set point of the Na^+-dependent HCO_3^-–Cl^- exchanger is higher than that of the Na^+–H^+ exchanger, so that the former system is still operative at cell pH levels above the set point of the Na^+–H^+ exchanger, i.e. pH levels at which this last antiporter remains quiescent (Bierman *et al.* 1988; Grinstein *et al.* 1989). If the Na^+-dependent HCO_3^-–Cl^- exchanger of the chemoreceptor cells also maintains a steady-state pH at a level beyond the operating range of the Na^+–H^+ exchanger, it would explain the predominance of that system in HCO_3^--buffered media and the lack of effect of EIPA on the response to acidic stimuli in this situation.

According to our model (Fig. 10), the complete inhibition of the Na^+–H^+ exchanger in the absence of HCO_3^- should abolish all the evoked release of DA that is Na^+ dependent. Under these conditions, however, EIPA inhibits only about 60% of the

response. Incomplete inhibition of the exchanger, a first possibility explaining this inconsistency, seems unlikely because increasing the concentration of EIPA from 10 to 40 μM does not increase the inhibition. Another possibility is that the more intense and sustained intracellular acidification resulting from the inhibition of the Na^+–H^+ exchanger produces a greater release of internal Ca^{2+} or an increased sensitivity of the exocytotic machinery to it. That also seems unlikely because the percentage of inhibition by EIPA does not change with the intensity of the stimulus. It seems more probable that a considerable part of the response observed in the presence of EIPA is related to residual activity of the Na^+-dependent HCO_3^-–Cl^- exchanger, due to the presence of traces of HCO_3^- derived from metabolic CO_2. For instance, in isolated barnacle muscle fibres, it has been estimated that with the HCO_3^- derived from metabolism, trapped in the superficial unstirred layer, the Na^+-dependent HCO_3–Cl^- exchanger could operate at about 10% of its maximal rate (Boron, McCormick & Roos, 1981). Since the affinity of this system for HCO_3^- also seems very high in the CB, judging from the response observed with only 2 mM-HCO_3^-, and the concentration of metabolism-derived HCO_3^- in the tissue interstitial space may be considerably higher than in the superficial unstirred layers, a greater residual activity of the Na^+-dependent HCO_3^-–Cl^- exchanger should be expected in our preparation in the nominal absence of bicarbonate. It is, finally, possible that the inhibitory effect of EIPA on the Na^+ pump (Renner, Lake, Cragoe & Scharschmidt, 1988), although small at the concentration used, could also contribute to increasing Na_i^+ and enlarging the residual response.

A more general question raised by our model concerns its specificity in the chemoreceptor cell. In other words, if both the Na^+-coupled H^+-extruding systems and the Na^+–Ca^{2+} exchanger are widely distributed, being considered ordinary housekeeping instruments of most animal cells, we may ask about the characteristics of type I cells that, with the same instruments, make them specifically responsive to acidic stimulation. An adequate answer to this question must await the confirmation of the model by analysing all its implications in isolated type I cells. We can only guess that the specific responsiveness of these cells to acidic stimulation may be the consequence of (1) a special capacity to transform the external high P_{CO_2}–low pH signal into a drop in intracellular pH, and (2) a generous equipment of the cell with Na^+-coupled H^+-extruding systems and Na^+–Ca^{2+} exchangers. Concerning the first point, the abundance of intracellular carbonic anhydrase (Rigual, Iñiguez, Carreres & Gonzalez, 1985) certainly facilitates the almost instantaneous conversion of the high P_{CO_2} into an internal pH signal. On the other hand, it has been reported recently that type I cells display by far the steepest dependence of internal pH on external pH of any mammalian cell type thus far examined (Buckler, Nye, Peers & Vaughan-Jones, 1990*b*). Finally, a high capacity for Na^+–Ca^{2+} exchange at the plasma membrane of type I cells may be anticipated from their neuroectodermal origin and their recently discovered condition of being electrically excitable cells.

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REFERENCES

ALMARAZ, L., GONZALEZ, C. & OBESO, A. (1986). Effects of high potassium on the release of [^{3}H]dopamine from the cat carotid body *in vitro*. *Journal of Physiology* **379**, 293–307.

BIERMAN, A. J., CRAGOE, E. J. JR, LAAT, S. W. & MOOLENAAR, W. H. (1988). Bicarbonate determines cytoplasmic pH and suppresses mitogen-induced alkalinization in fibroblastic cells. *Journal of Biological Chemistry* **263**, 15253–15256.

BISCOE, T. J. & DUCHEN, M. R. (1989). Electrophysiological responses of dissociated type I cells of the rabbit carotid body to cyanide. *Journal of Physiology* **413**, 447–468.

BISCOE, T. J., DUCHEN, M. R., EISNER, D. A., O'NEILL, S. C. & VALDEOLMILLOS, M. (1989). Measurements of intracellular Ca^{2+} in dissociated type I cells of the rabbit carotid body. *Journal of Physiology* **416**, 421–434.

BLAUSTEIN, M. P. (1988). Calcium transport and buffering in neurones. *Trends in Neurosciences* **11**, 438–443.

BORON, W. F. (1986). Intracellular pH regulation. In *Physiology of Membrane Disorders*, ed. ANDREOLI, T. E., HOFFMAN, J. F., FANESTIL, D. D. & SCHULTZ, S. G., pp. 423–435. Plenum Press, New York.

BORON, W. F., MCCORMICK, W. C. & ROOS, A. (1981). pH regulation in barnacle muscle fibers: dependence on extracellular sodium and bicarbonate. *American Journal of Physiology* **240**, C80–89.

BOUNTRA, C. & VAUGHAN-JONES, R. D. (1989). Effect of intracellular and extracellular pH on contraction in isolated, mammalian cardiac muscle. *Journal of Physiology* **418**, 163–187.

BUCKLER, K. J., NYE, P. C. G., PEERS, C. & VAUGHAN-JONES, R. D. (1990*a*). Regulation of intracellular pH in single type I carotid body cells isolated from the neonatal rat. *Journal of Physiology* **423**, 65*P*.

BUCKLER, K. J., NYE, P. C. G., PEERS, C. & VAUGHAN-JONES, R. D. (1990*b*). Effects of simulated respiratory and metabolic acidosis/alkalosis on pH_i in isolated type I carotid body cells from the neonatal rat. *Journal of Physiology* **426**, 66*P*.

CARAFOLI, E. (1987). Intracellular calcium homeostasis. *Annual Reviews of Biochemistry* **56**, 395–433.

CATTERALL, W. A. & NIRENBERG, M. (1973). Sodium uptake associated with activation of action potential ionophores of cultured neuroblastoma and muscle cells. *Proceedings of the National Academy of Sciences of the USA* **70**, 3759–3763.

DELPIANO, M. A. & HESCHELER, J. (1989). Evidence for a P_{O_2}-sensitive K^+ channel in the type-I cell of the rabbit carotid body. *FEBS Letters* **249**, 195–198.

DUCHEN, M. R., CADDY, K. W. T., KIRBY, G. C., PATTERSON, D. L., PONTE, J. & BISCOE, T. J. (1988). Biophysical studies of the cellular elements of the rabbit carotid body. *Neuroscience* **26**, 291–311.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1984). The quantitative relationship between twitch tension and intracellular sodium activity in sheep cardiac Purkinje fibres. *Journal of Physiology* **355**, 251–266.

FIDONE, S. J. & GONZALEZ, C. (1986). Initiation and control of chemoreceptor activity in the carotid body. In *Handbook of Physiology. The Respiratory System II*, ed. FISHMAN, A. P., pp. 247–312. American Physiological Society, Bethesda, MD, USA.

FIDONE, S. J., GONZALEZ, C. & YOSHIZAKI, K. (1982). Effects of low oxygen on the release of dopamine from the rabbit carotid body *in vitro*. *Journal of Physiology* **333**, 93–110.

GANZ, M. B., BOYARSKY, G., STERZEL, R. D. & BORON, W. F. (1989). Arginine vasopressin enhances pH_i regulation in the presence of HCO_3^- by stimulating three acid–base transport systems. *Nature* **337**, 648–651.

GARCIA, A. G., SALA, F., REIG, J. A., VINIEGRA, S., FRIAS, J., FONTERIZ, R. & GANDIA, L. (1984). Dihydropyridine Bay K 8644 chromaffin cell calcium channels. *Nature* **309**, 69–71.

GONZALEZ, E., RIGUAL, R., FIDONE, S. J. & GONZALEZ, C. (1987). Mechanism for termination of the action of dopamine in carotid body chemoreceptors. *Journal of the Autonomic Nervous System* **18**, 249–259.

GRINSTEIN, S. & COHEN, S. (1987). Cytoplasmic [Ca^{2+}] and intracellular pH in lymphocytes. Role of membrane potential and volume-activated Na^+/H^+ exchange. *Journal of General Physiology* **89**, 185–213.

GRINSTEIN, S. & FURUYA, W. (1986). Characterization of the amiloride-sensitive Na^+–H^+ antiport of human neutrophils. *American Journal of Physiology* **250**, C283–291.

GRINSTEIN, S. & ROTHSTEIN, A. (1986). Mechanisms of regulation of the Na^+/H^+ exchanger. *Journal of Membrane Biology* **90**, 1–12.

GRINSTEIN, S., ROTIN, D. & MASON, M. J. (1989). Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. *Biochimica et Biophysica Acta* **988**, 73–97.

HESCHELER, J., DELPIANO, M. A., ACKER, H. & PIETRUSCHKA, F. (1989). Ionic currents on type-I cells of the rabbit carotid body measured by voltage-clamp experiments and the effect of hypoxia. *Brain Research* **486**, 79–88.

HEYMANS, C., BOUCKAERT, J. J. & DAUTREBANDE, L. (1930). Sinus carotidien et réflexes respiratoires. II. Influences respiratoires réflexes de l'acidose, de l'alcalose, de l'anhydride carbonique, de l'ion hydrogène et de l'anoxémie. Sinus carotidiens et échanges respiratoires dans le poumons et au dela des poumons. *Archives Internationales de Pharmacodynamie et Therapie* **39**, 400–408.

L'ALLEMAIN, G., PARIS, S. & POUYSSÉGUR, J. (1985). Role of a Na^+-dependent Cl^-/HCO_3^- exchange in regulation of intracellular pH in fibroblasts. *Journal of Biological Chemistry* **260**, 4877–4883.

LÓPEZ-BARNEO, J., LÓPEZ-LÓPEZ, J. R., UREÑA, J. & GONZALEZ, C. (1988). Chemotransduction in the carotid body: K^+ current modulated by P_{O_2} in type I chemoreceptor cells. *Science* **241**, 580–582.

LÓPEZ-LÓPEZ, J. R., GONZALEZ, C., UREÑA, J. & LÓPEZ-BARNEO, J. (1989). Low P_{O_2} selectively inhibits K^+ channel activity in chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology* **93**, 1001–1014.

MULLINS, L. J. (1984). An electrogenic saga: consequences of sodium–calcium exchange in cardiac muscle. In *Electrogenic Transport: Fundamental Principles and Physiological Implications*, ed. BLAUSTEIN, M. P. & LIEBERMAN, M., pp. 161–179. Raven Press, New York.

OBESO, A., ALMARAZ, L. & GONZALEZ, C. (1986). Effects of 2-deoxy-D-glucose on in vitro cat carotid body. *Brain Research* **371**, 25–36.

OBESO, A., ALMARAZ, L. & GONZALEZ, C. (1989). Effects of cyanide and uncouplers on chemoreceptor activity and ATP content of the cat carotid body. *Brain Research* **481**, 250–257.

OBESO, A., FIDONE, S. J. & GONZALEZ, C. (1987). Pathways for calcium entry into type I cells: significance for the secretory response. In *Chemoreceptors in Respiratory Control*, ed. RIBEIRO, J. A. & PALLOT, D. J., pp. 91–98. Croom Helm, London.

RENNER, E. L., LAKE, J. R., CRAGOE, E. J. JR & SCHARSCHMIDT, B. F. (1988). Amiloride and amiloride analogs inhibit Na^+/K^+-transporting ATPase and Na^+-coupled alanine transport in rat hepatocytes. *Biochimica et Biophysica Acta*, **938**, 386–394.

RIGUAL, R., IÑIGUEZ, C., CARRERES, J. & GONZALEZ, C. (1985). Localization of carbonic anhydrase in the cat carotid body. *Histochemistry* **82**, 577–580.

RIGUAL, R., LÓPEZ-LÓPEZ, J. R. & GONZALEZ, C. (1991). Release of dopamine and chemoreceptor discharge induced by low pH and high P_{CO_2} stimulation of the cat carotid body. *Journal of Physiology* **433**, 519–531.

ROOS, A. & BORON, W. F. (1981). Intracellular pH. *Physiological Reviews* **61**, 296–434.

THOMAS, R. C. (1977). The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. *Journal of Physiology* **273**, 317–338.

THOMAS, R. C. (1984). Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. *Journal of Physiology* **354**, 3–22*P*.

UREÑA, J., LÓPEZ-LÓPEZ, J. R., GONZALEZ, C. & LÓPEZ-BARNEO, J. (1989). Ionic currents in dispersed chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology* **93**, 979–1001.