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RELEASE OF DOPAMINE AND CHEMORECEPTOR DISCHARGE INDUCED BY LOW pH AND HIGH P_{CO_2} STIMULATION OF THE CAT CAROTID BODY

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SUMMARY

1. Cat carotid bodies were incubated with the precursor [^{3}H]tyrosine to label the catecholamine deposits and then mounted in a superfusion chamber which allowed simultaneous collection of the released [^{3}H]dopamine (DA) and recording of action potentials from the carotid sinus nerve.

2. Low pH (7·2–6·6) superfusion of the carotid bodies for periods of 10 min produced a parallel increase in the release of [^{3}H]DA and chemoreceptor discharge.

3. Carotid sinus nerve denervation of the carotid body 12–15 days prior to the experiments did not modify the release of [^{3}H]DA elicited by low pH.

4. Superfusion of the carotid bodies with Ca^{2+}-free, high-Mg^{2+} (1·6 mM) media reduced basal release of [^{3}H]DA and chemoreceptor discharge by about 30%. Release evoked by low pH was reduced by 82%. Peak and average chemoreceptor discharge recorded in response to low pH were reduced by 28%.

5. Solutions containing weak acids (sodium acetate, 10 mM), adjusted at pH 7·4, elicited release of [^{3}H]DA and increased chemoreceptor discharge.

6. With HCO_3–CO_2-buffered superfusion media, a reduction of bicarbonate to 5·6 mM (pH 6·8), an increase in CO_2 to 20% (pH 6·8), or a simultaneous increase in CO_2 to 20% and bicarbonate to 90 mM (pH 7·4), resulted in all cases in a corresponding increase in [^{3}H]DA release and chemoreceptor discharge. The most effective stimulus was 20% CO_2–pH 6·8 and the least effective 5% CO_2–5·6 mM-HCO_3–pH 6·8.

7. Inhibition of carbonic anhydrase with acetazolamide while perfusing the carotid bodies with a 20% CO_2-equilibrated (pH 7·4) solution resulted in comparable reductions in the release of [^{3}H]DA and chemoreceptor discharge.

8. It is concluded that the effective acidic stimulus at the carotid body chemoreceptors is an increase in hydrogen ion concentration in type I cells. It is also concluded that DA plays a critical role in the genesis of carotid sinus nerve discharges.

INTRODUCTION

The carotid body (CB) is a chemoreceptor organ located in the vicinity of the carotid bifurcation. This organ detects changes in plasma P_{O_2}, P_{CO_2} and $[H^+]$. It is

composed of clusters of cells of two types, type I or chemoreceptor and type II or sustentacular cells, penetrated by the sensory nerve endings of the carotid sinus nerve (CSN). These cellular clusters are separated from each other by thin walls of connective tissue densely populated by fenestrated capillaries which bring the natural stimuli to the immediate vicinity of the chemoreceptor cells (De Castro, 1928; Torrance, 1968; McDonald, 1981). Functionally, it is nowadays accepted that chemoreceptor cells are the sensing structures in the CB, which, by releasing neurotransmitters, drive the sensory nerve endings of the CSN (López-Barneo, López-López, Ureña & Gonzalez, 1988; Biscoe & Duchen, 1989; Fidone, Gonzalez, Obeso, Gómez-Niño & Dinger, 1990).

In addition to the mechanisms responsible for stimulus detection (see Belmonte & Gonzalez, 1983 and Fidone & Gonzalez, 1986 for reviews), the other main open question in the chemoreception process in the CB is the identification of the transmitter(s) in the sensory synapses formed between type I cells and sensory nerve endings. Amongst the neurotransmitters identified in type I cells (catecholamines (CAs), acetylcholine and different neuropeptides), CAs have received most attention in recent years. In pharmacological studies, many authors have found that dopamine (DA) and noradrenaline (NA) are capable of modulating the basal and stimulus-induced electrical activity in the CSN. It is most commonly found that DA injected into the animal inhibits the on-going CSN activity while NE causes, most frequently, an increase in the action potential frequency of the CNS; the mechanism of action of these biogenic amines remains to be elucidated (see Fidone *et al.* 1990 for a recent review on this topic). In contrast, it has been reported that, in various animals, DA and in some instances also NA levels decrease in the CB after episodes of acute hypoxia (Hanbauer & Hellstrom, 1978; Fitzgerald, Garger, Hauer, Raff & Fechter, 1983; Starlinger & Acker, 1986), suggesting that hypoxia releases CA from type I cells. Moreover, *in vitro* CBs of both rabbit and cat, previously loaded with [^{3}H]tyrosine, release [^{3}H]DA in direct proportion to the intensity of the hypoxic stimulus, showing also a close relationship between the amount of [^{3}H]DA released and the simultaneously recorded electrical activity in the CSN (Fidone, Gonzalez & Yoshizaki, 1982; Rigual, Gonzalez, Gonzalez & Fidone, 1986). However, few studies have explored changes in CA metabolism during acidic/hypercapnic stimulation. Fitzgerald *et al.* (1983) did not find any change in CA content of the CB after an episode of hypercapnic stimulation *in vivo*, whereas Rigual, Gonzalez, Fidone & Gonzalez (1984), in a preliminary study, reported that low pH incubation or superfusion of the CB resulted in an increase in synthesis and release of [^{3}H]CA.

The present work was undertaken to characterize further the metabolism of CA in the CB during acidic stimulation *in vitro*. Three specific aims have been pursued: (1) to define the relationship between the pH of the superfusion saline, the release of [^{3}H]CA from type I cells and action potential frequency in the CSN; (2) to define the relationship between the release of [^{3}H]CA and action potential frequency during CB superfusion with solutions containing weak acids or equilibrated with high P_{CO_2}; (3) to study the same relationship during superfusion of the CBs with high P_{CO_2}-equilibrated media in the presence of acetazolamide, an inhibitor of carbonic anhydrase. As previously shown for hypoxic stimulation (Fidone *et al.* 1982; Rigual *et al.* 1986), we found a direct relationship between intensity of acidic stimulation,

release of [^{3}H]DA by type I cells and action potential frequency in the CSN, which strengthens the notion of a crucial role for DA in the genesis of propagated chemoreceptor activity. Additionally the data indicate that intracellular acidification at a normal pH in the media (pH_o 7·40) constitutes an effective stimulus for the CB chemoreceptors, and that inhibition of carbonic anhydrase decreases and slows down the CB responses to high P_{CO_2} stimuli.

METHODS

All the experiments were performed with CBs from adult cats (2–3·5 kg). The animals were anaesthetized with sodium pentobarbitone (30–40 mg, I.P.; Sigma), tracheostomized and artificially ventilated. After exposing the carotid bifurcations, the CBs with their CSN attached were removed and placed in a lucite chamber (filled with 100% O_2-equilibrated ice-cold modified Tyrode solution at pH 7·40; Table 1) and cleaned of surrounding connective tissue under a dissecting microscope. The CSN was preserved and the perineurium removed to record electrical activity. In chronic denervation experiments CSNs from one side of the animals were removed under aseptic conditions 12–15 days prior to the experiments; the contralateral CBs served as controls.

TABLE 1. Composition of superfusing Tyrode solutions (in mM)

Equilibrating gas phase	NaCl	Sodium glutamate	$NaHCO_3$	KCl	$CaCl_2$	$MgCl_2$	HEPES	Glucose
100% O_2*	111·2	34·3	—	4·7	2·2	1·1	5	5·5
5% CO_2, pH 7·4	111·2	17·7	22·5	4·7	2·2	1·1	5	5·5
5% CO_2, pH 6·8	128·1	17·7	5·6	4·7	2·2	1·1	5	5·5
20% CO_2, pH 7·4	62·6	—	90	4·7	2·2	1·1	5	5·5
20% CO_2, pH 6·8	111·2	17·7	22·5	4·7	2·2	1·1	5	5·5

* The 100% O_2-equilibrated Tyrode solution was adjusted to the desired pH with 1 M-NaOH.

In order to label the deposits of CA, the CBs were incubated for 3 h in a metabolic shaker at 37 °C in 1 ml of 100% O_2-equilibrated Tyrode solution (see Table 1), containing 20 μM [^{3}H]tyrosine of high specific activity (20–40 Ci/mmol, Amersham), 100 μM-DL-6-methyl-5,6,7,8-tetrahydropterine, and 1 mM-ascorbic acid (Sigma). At the end of this incubation period, the organs were mounted in a superfusion chamber which allowed the collection of the superfusates containing the released material and the recording of CSN activity simultaneously (Fidone *et al.* 1982). The different superfusing solutions used in this work are presented in Table 1. Since in this preparation the threshold for hypoxic activation of chemoreceptors is about 50% O_2 (Fidone *et al.* 1982; Rigual *et al.* 1986), the superfusing solutions were equilibrated with 80–100% O_2 (0–20% CO_2, as indicated in Table 1) so that the effects of acidic stimulation could be studied free of possible interactions with the hypoxic stimulus. The collection of the superfusates was grouped in stimulation cycles; one stimulation cycle consisted of a period (5 or 10 min) of superfusion with the stimulus (test) solution, preceded by a similar period and followed by several post-stimulus periods of superfusion with standard solution to determine basal responses and to allow the recovery of the preparation, respectively (see Fig. 1). The superfusates were collected in vials containing 50 μl of glacial acetic acid: water solution (1:1) containing 50 mM-ascorbic acid to prevent CA oxidation. The samples were analysed by adsorption in alumina at pH = 8·6 and, after a thorough washing with distilled water, the adsorbed [^{3}H]catechols and their metabolites were eluted with 1 M-HCl (Weil-Malherbe, 1971). In selected experiments the 1 M-HCl eluates were dried in a vacuum concentrator (Savant) and the [^{3}H]catechols identified by thin-layer chromatography. More than 85% of the radioactivity present in the alumina eluates was [^{3}H]DA + [^{3}H]2,4-dihydroxyphenylacetic acid ([^{3}H]DOPAC) (Almaraz, Gonzalez & Obeso, 1986; Rigual *et al.* 1986) and the remainder was [^{3}H]NA- + [^{3}H]dihydroxymandelic acid; therefore we refer in the text to DA release. Since the response of the CB exceeds the time of stimulus application, the evoked release of DA was estimated as the amount of [^{3}H]DA released above the basal level during the stimulus and post-stimulus periods, until the release returned again to the basal level. In some instances the evoked release is expressed

as a multiple of the basal release. The electrical activity recorded from the CSN was conveniently filtered and amplified for display in an oscilloscope (Tektronix) and also fed to a window discriminator. Outputs of the window were connected to a counter printer to obtain the frequency of action potentials in periods of time previously determined (1 or 10 s) and to a chart recorder. The stimulus-induced electrical activity was also calculated in relation to the basal activity recorded immediately prior to the stimulus application.

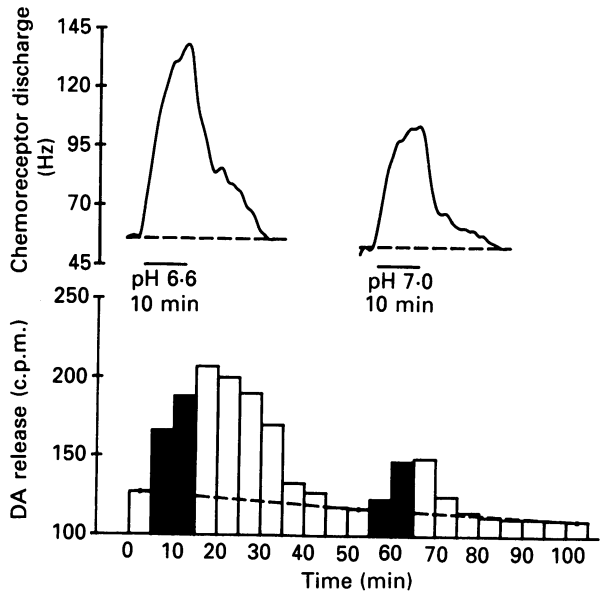

Fig. 1. Effects of carotid body superfusion with 100% O_2-equilibrated Tyrode solution adjusted to low pH. The results of a representative experiment with two stimulation cycles are shown. Top, chart records of the chemoreceptor discharge in response to 10 min superfusion with HEPES-buffered solution at pH 6·6 (left) and 7·0 (right). Bottom, histograms of the [^{3}H]dopamine present in the superfusate (5 min samples). Filled bars correspond to the periods of perfusion at the indicated low pH. Dashed lines indicate basal chemoreceptor discharge and [^{3}H]dopamine release.

RESULTS

Effects of extracellular acidosis on DA release and CSN discharge

Preliminary experiments (Rigual *et al.* 1984) showed an increase of DA release on superfusing the CBs with low pH solutions. Figure 1 represents a typical release experiment in which the CB was superfused with a modified Tyrode solution equilibrated with 100% O_2, adjusted to pH 7·4 in basal and post-stimulus periods and to pH 6·6 or 7·0 during the stimulus periods. The application of the low pH solutions induced a slow but maintained increase in the DA release. The electrical response recorded in the CSN is also slow in onset and persists for some minutes after termination of the low pH_o stimulus. The close correlation between stimulus-evoked release of DA and electrical activity in the CSN is more evident when both evoked responses are expressed as multiples of the basal response, as shown in Fig. 2.

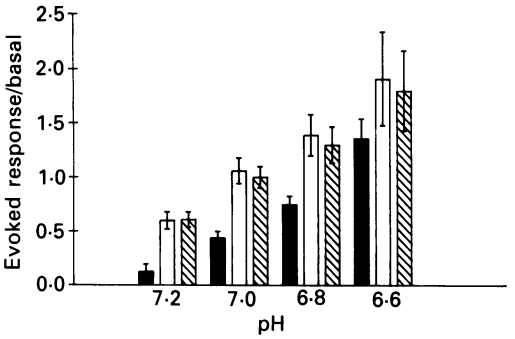

Fig. 2. Relationship between evoked [^{3}H]dopamine release (■) and evoked chemoreceptor discharge during different intensities of low pH stimulation. The evoked responses are expressed as a multiple of the basal (evoked response/basal). Mean discharge bars (□) are equal to or higher than peak discharge bars (▨) because the increased activity in the carotid sinus nerve exceeds stimulus application (see Fig. 1) and all the evoked response is computed as generated during the 10 min of stimulus application. Results are means ± S.E.M. from at least six stimulation cycles for each intensity.

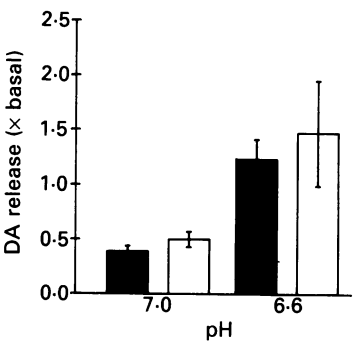

Fig. 3. Effect of carotid sinus nerve (CSN) denervation on the release of [^{3}H]dopamine by the carotid body in response to acidic stimulation with 100% O_2-equilibrated HEPES-buffered media. ■, control carotid bodies. □, contralateral denervated carotid bodies 12–15 days prior to the experiments. Results are means ± S.E.M. from eight stimulation cycles obtained from six different pairs of carotid bodies.

Prior denervation of the CB (12–15 days in advance) does not modify appreciably the low-pH-induced release of DA (Fig. 3). This finding indicates on the one hand that type I cells are capable of transducing a physiological stimulus (low pH_o) into a secretory response (the release of DA), and on the other hand that the sensory endings of the CSN do not exert a significant control on the chemosensitivity of type I cells to an acidic stimulus.

The Ca^{2+} dependence of the responses induced by low pH is presented in Fig. 4. The figure summarizes the results of four experiments with four stimulation cycles each: after the first stimulation cycle carried out with Ca^{2+} containing 100% O_2-equilibrated Tyrode solution, the CBs were superfused with a Ca^{2+}-free solution (containing 1·6 mM-Mg^{2+} in order to stabilize the CSN discharges; see Fidone *et al.*

1982) and two stimulation cycles were performed; the experiments ended with the reintroduction of normal Tyrode solution and a new cycle of stimulation. In all cases the stimulus was 10 min superfusion with solution adjusted to pH 6·8. This experimental design allowed us to compare directly the effects of 0 Ca^{2+} on the basal

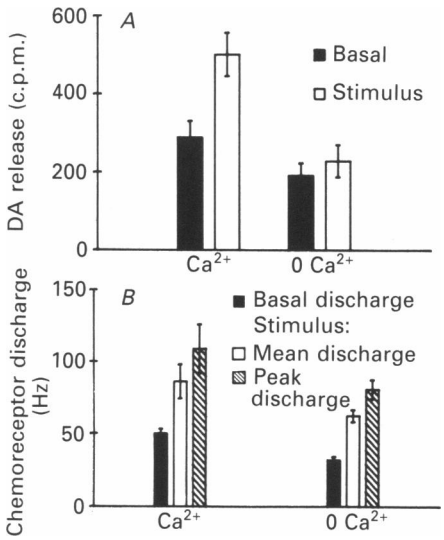

Fig. 4. Effects of Ca^{2+} removal from the superfusion media 100% O_2-equilibrated (HEPES-buffered Tyrode solution) on the release of [^{3}H]dopamine from the carotid body and on the electrical activity in the carotid sinus nerve. Filled bars, basal (pH 7·4) release (*A*) and electrical activity (*B*). Open and hatched bars, release of [^{3}H]dopamine (*A*) and chemoreceptor discharge (*B*) during superfusion with low pH solutions (pH 6·8). Data are means ± S.E.M. for eight stimulation cycles from four carotid bodies.

and evoked responses in the same CB eliminating any time-dependent effect (Fidone *et al.* 1982). In the Ca^{2+}-free (Mg^{2+}-enriched) media the spontaneous basal release of DA and the electrical activity were reduced by about 30%. The evoked release of DA (stimulus minus basal) showed a marked Ca^{2+} dependence, with a reduction of about 80%. Both peak and average action potential frequency in the CSN were also reduced by 28% in Ca^{2+}-free media, but evoked electrical activity (stimulus minus basal) showed no change (Fig. 4).

CB responses to superfusion with media containing weak acids

Different hypotheses have been advanced in which the effective parameter at the CB chemoreceptors during acidic stimulation is the increase in [H^+] either in the extracellular medium or intracellularly in some glomic structure (Winder, 1937; Torrance, 1974, 1977; Hanson, Nye & Torrance, 1981; see Fidone & Gonzalez, 1986). In the experiments presented in Fig. 5 we show that superfusion of the CBs with saline containing a weak acid (10 mM-sodium acetate) and adjusted to pH 7·4, evokes a simultaneous increase in DA release and in CSN electrical activity; butyrate and propionate elicited similar responses. Since weak acids acidify intracellularly (see Thomas, 1984), the most simple interpretation of these results is that the effective

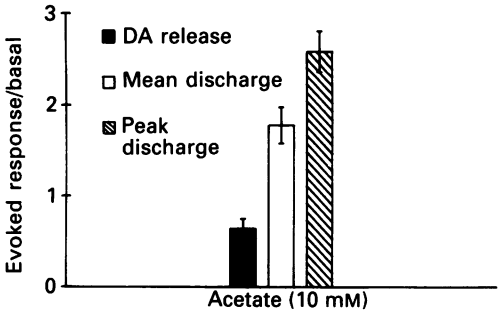

Fig. 5. Effects of carotid body superfusion with a HEPES-buffered solution containing 10 mM-sodium acetate at a pH of 7·4. The evoked responses, [^{3}H]dopamine release and chemoreceptor discharge, are expressed as multiples of the basal responses. Means ± S.E.M. for ten stimulation cycles from four carotid bodies.

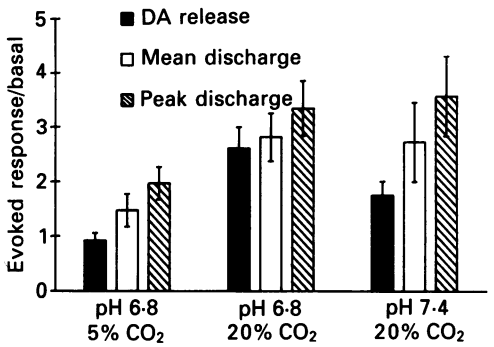

Fig. 6. Carotid body responses to the superfusion with HCO_3^-–CO_2 buffered solutions. Data are expressed as the ratios of evoked to basal response. Media composition for basal conditions (Tyrode solution equilibrated with 95% O_2–5% CO_2; pH = 7·4) and the different types of stimulation are given in Table 1. Data are means ± S.E.M. for eight to ten stimulation cycles.

stimulus for the CB chemoreceptors is an increase in intracellular hydrogen ion concentration. The involvement of external H^+ receptors in the response (Torrance, 1974) would require in this case that a rapid efflux of the intracellularly generated H^+ creates a localized proton concentration jump on the external surface of the cell. This possibility, however, is not supported by our results on stimulation with CO_2 (see below) nor by the analysis given in the accompanying paper on the ionic mechanisms involved in the response of type I cells (Rocher, Obeso, Gonzalez & Herreros, 1991).

CO_2 freely crosses cellular membranes following pressure gradients and hydrates to $HCO_3^- + H^+$, behaving also as a weak acid. Therefore it is possible to acidify intracellularly while keeping extracellular pH constant by simultaneously increasing P_{CO_2} and HCO_3^- in the perfusing solution (Thomas, 1976, 1984; Roos & Boron, 1981). Figure 6 compares average CB responses following pulse superfusions with solutions equilibrated with 5% CO_2 at pH 6·8, 20% CO_2 at pH 6·8, and 20% CO_2 at pH 7·4 (see Table 1 for media composition). As in the case of superfusion with acetate-containing media, 20% CO_2-equilibrated media at external pH of 7·4 promoted a clear increase

in the rate of release of DA and in the activity of the CSN, indicating that intracellular acidification is effective in triggering the release of DA. When the 20% CO_2-equilibrated media was adjusted to pH 6·8 by reducing the bicarbonate, the release of DA was significantly greater than that observed with 20% CO_2 at pH 7·4,

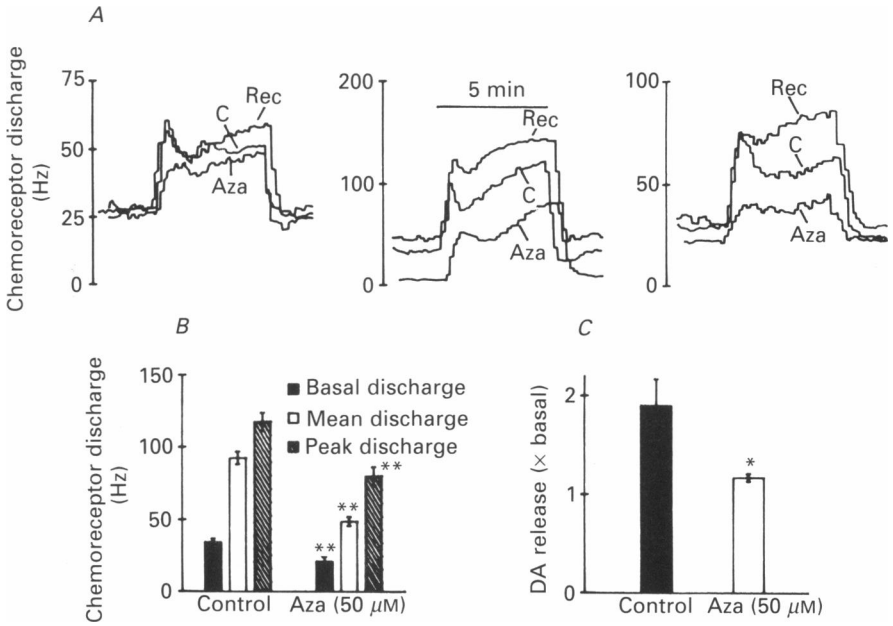

Fig. 7. Effects of carbonic anhydrase inhibition on carotid body responses. Carbonic anhydrase inhibition was achieved by superfusion of the carotid bodies with 50 μM-acetazolamide 1 h prior to and during the stimulus application. The recovery was assessed 1 h after superfusion with acetazolamide-free solution. Basal conditions: superfusion with 5% CO_2–pH 7·4 solution. Stimulus: 20% CO_2–pH 7·4 solution (see Table 1). *A*, results from three representative experiments showing the time courses of high P_{CO_2}-induced chemoreceptor discharge in control condition (C), during superfusion with acetazolamide (Aza), and after recovery (Rec). *B*, means ± S.E.M. for eight experiments similar to those in *A*. Since, on average, the chemoreceptor discharges during the recovery period were not different from the control, they were computed together. *C*, release of [^{3}H]dopamine in response to 20% CO_2–pH 7·4 in control conditions and during superfusion with acetazolamide. Data are means ± S.E.M. for eight experiments, expressed as evoked response times the basal release. $*P < 0·05$; $**P < 0·01$.

but the increase in the action potential frequency was not significantly different in both conditions. Superfusion with 5% CO_2-equilibrated media at pH 6·8 elicited responses smaller than those obtained with 20% CO_2 at either pH and comparable to those seen with HEPES-buffered media at pH 6·8 (compare with Fig. 2). As previously mentioned, the fact that the response with 5% CO_2 at pH 6·8 is considerably smaller than that with 20% CO_2 at pH 7·4 (the buffering capacity of the external medium being very much greater in the latter condition) does not support the involvement of external H^+ receptors in the activation of type I cells. In HCO_3^--buffered media, the release of DA exhibited the same Ca^{2+} dependence found in HEPES-buffered media (data not shown).

Effects of acetazolamide on high-P_{CO_2}-induced responses

Carbonic anhydrase has proved to be an important enzyme in setting the CB responses to high P_{CO_2} *in vivo*. Its inhibition abolishes the fast initial component and reduces the steady-state component of CSN activity when the animal breathes high P_{CO_2} gas mixtures (McCloskey, 1968; Torrance, 1977; Hanson *et al.* 1981). Since carbonic anhydrase inhibition in other structures abolishes the fast initial swing and reduces the steady-state increase in hydrogen ion concentration in response to high P_{CO_2} pulses (Thomas, 1976), it has been proposed that the changes observed in CSN activity upon carbonic anhydrase inhibition reflect the altered pattern in the genesis of H^+ (Torrance, 1974, 1977; Hanson *et al.* 1981). The intracellular location of carbonic anhydrase in type I cells (Rigual, Iñiguez, Carreres & Gonzalez, 1985) and the data of Figs 5 and 6 strengthened the concept that it is the H^+ concentration within these cells which is the detected parameter. Therefore, if DA is a type I cell neurotransmitter critical in setting CSN action potential frequency, its release should be also modified by carbonic anhydrase inhibition.

Figure 7*A* shows chart recordings of chemoreceptor discharge obtained in three representative experiments (total of eight). The three records shown in each box correspond to the time course of change in action potential frequency in the CSN during superfusion with 20% CO_2 at pH 7·4 in control conditions (C), in the presence of 50 μM-acetazolamide (Aza) and 1 h after acetazolamide removal (recovery; Rec). It is evident that the response of the CB to high P_{CO_2} in the *in vitro* preparation exhibits the basic traits of the *in vivo* response. Figure 7*B* shows means + S.E.M. of the chemoreceptor discharges in acatazolamide-free media (control) and while superfusing with the carbonic anhydrase inhibitor. It is evident that there is a significant reduction in basal, mean and peak discharge during enzyme inhibition. Figure 7*C* represents mean response for the evoked release of DA in control conditions and during superfusion with acetazolamide. It shows a reduction in DA release that parallels the reduction in the CSN electrical activity.

DISCUSSION

The data obtained in this study provide direct evidence that type I cells transduce changes of extracellular pH into the release of DA, a putative neurotransmitter at the synapse formed by type I cells and the sensory nerve endings. The ability of weak acids and high P_{CO_2}-containing media (at a pH of 7·4) to promote DA release and a parallel increase in CSN activity strongly suggests that an increase in H^+ concentration within type I cells is the effective stimulus at the CB chemoreceptors. This conclusion is reinforced by the observation that carbonic anhydrase inhibition reduces the response to elevated P_{CO_2}. The findings show also that a good correlation exists between the expected time courses of changes in H^+ concentration in type I cells, DA release and activity in the CSN.

The proposal that H^+ concentration within the type I cell is the sensed parameter in the CB, and the proposal that DA is involved in the genesis of activity in the CSN, both require further comment. Regarding the first point, it is necessary to consider the mechanisms by which an increase in H^+ concentration in the superfusing media

buffered with HEPES leads to an increase in the intracellular H^+ concentration. It is well known, in many cell types, that reducing external pH causes a significantly smaller fall of pH_i (Keynes, Rojas, Taylor & Vergara, 1973; Thomas, 1974; Aickin & Thomas, 1975, 1977; see Roos & Boron, 1981, Boron, 1986). In contrast, Buckler, Nye, Peers & Vaughan-Jones (1990) in a preliminary study carried out in isolated type I cells resuspended in HCO_3^--buffered medium have reported that these cells display a very steep dependence of pH_i on pH_o; they suggest that this high rate of transference of H^+ across type I cell membrane may be important in determining their chemoreceptor properties. However, it remains to be elucidated if a similar steep dependence of pH_i on pH_o occurs in HEPES-buffered medium.

Weak acids acidify the cell interior because their protonized forms freely cross plasma membranes in response to an imposed concentration gradient. The fall of intracellular pH is smaller at an extracellular pH of 7·4 than at a more acidic extracellular pH. This is because, at low pH_o, the concentration of the undissociated form of the weak acid increases in the extracellular media and also because, as pH_o falls, there is a significant inhibition of acid extrusion mechanisms (Roos & Boron, 1981). The intracellular acidifying power of high P_{CO_2}-containing media was suggested in the 1920s by Jacobs (1920) and measured by many authors in different cell types and with different methods (see Roos & Boron, 1981). It was also observed that the ability of CO_2 to acidify decreases as extracellular HCO_3^- increases (i.e. as pH_o increases), this effect being the result of HCO_3^- influx and the higher rate of operation of H^+ extrusion mechanisms (Roos & Boron, 1981; see our Fig. 6). The effects of carbonic anhydrase inhibitors on the acidifying effect of high P_{CO_2}-containing solutions has been commented on in the results section (see Results; see also Thomas, 1976; Roos & Boron, 1981).

Therefore under all experimental conditions tested in our experiments it appears that the release of DA from type I cells correlates with the expected fall in pH_i. We did not attempt to measure pH_i in our preparation, because the heterogeneity of CB tissue (McDonald, 1981) means that whole glomic pH_i can not readily be used to predict the pH_i of type I cells. In addition to the general good correlation between release of DA and the expected fall to pH_i, carbonic anhydrase inhibition in type I cells (Rigual *et al.* 1985) also modifies, as predicted, the release of this putative neurotransmitter. The mechanisms involved in the coupling of increased intracellular H^+ to the Ca^{2+}-dependent release of [^{3}H]DA are the subject of study in the accompanying paper (Rocher *et al.* 1991).

Throughout this study, a positive correlation is always found between the release of DA and the action potential frequency in the CSN. This finding for acidic stimuli, and the previously reported findings with low P_{O_2} (Fidone *et al.* 1982; Rigual *et al.* 1986) and different pharmacological stimuli (Obeso, Almaraz & Gonzalez, 1986, 1989), suggest that DA may be directly involved in the genesis of CSN activity. Especially relevant here are the observations made during inhibition of carbonic anhydrase: acetazolamide modifies in parallel the magnitude of DA release and CSN activity. There are, however, some deviations in the correlation between both parameters. For example in Fig. 2 it is evident that the ratio of action potential frequency to DA release is higher, the closer the pH is to 7·4, suggesting that DA is more effective at pH values near to the physiological one of 7·4. Among other possible

explanations (see Hille, 1984, but see also Krishtal & Pidoplichko, 1981), it is well known that the binding of transmitters and hormones to their receptors is maximal at physiological or near physiological pH (Enna, 1980; Hanley, 1985), so that a decrease in DA 'efficacy' is to be expected as the external pH decreases. The same interpretation can explain the differences observed with 20% CO_2-equilibrated media at pH_o of 6·8 and 7·4 (Fig. 6) and the high ratio of action potential frequency to DA release observed upon superfusion with weak acids at an external pH of 7·4 (see Fig. 5). Note also that in the experiments with Ca^{2+}-free media there was a greater reduction in the DA release than in action potential frequency in the CSN during low-pH_o stimulation. A similar observation has been also made previously using low P_{O_2} and different pharmacological stimuli (Fidone *et al.* 1982; Obeso *et al.* 1986, 1989). It has been interpreted as the result of a greater safety factor at this sensory synapse. This ill-defined safety factor could be related to the fact that in different structures it has been shown that in low-Ca^{2+} or Ca^{2+}-free media, DA antagonists and DA itself are more effective at blocking or eliciting physiological responses (Kato & Narahashi, 1982; Von Burskirk & Dowling, 1982). The interpretation of the findings reported here contrasts with the notion that DA may be an inhibitory transmitter (see Introduction). However, this interpretation is reinforced by the recent finding that exogenously applied DA to the *in vitro* preparation inhibits the basal and stimulus-induced release of DA by the CB (Gómez-Niño, Dinger, Gonzalez & Fidone, 1989).

In conclusion, the present paper provides strong evidence indicating that during acidic stimulation of the CB, it is the hydrogen ion concentration in type I cells which is the parameter sensed. The present data demonstrate that, during acidic stimulation, there is a good correlation between the amount of DA released and the action potential frequency recorded in the CSN, which favours the idea that DA plays a critical role in setting the level of activity in the chemoreceptor fibres of the CSN.

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