Inhibition of [^{3}H]catecholamine release and Ca^{2+} currents by prostaglandin E_2 in rabbit carotid body chemoreceptor cells

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1. Basal release of [^{3}H]catecholamine ([^{3}H]CA) from rabbit carotid bodies (CBs), previously incubated in the presence of [^{3}H]tyrosine, was not significantly modified by prostaglandin E_2 (PGE_2). On the contrary, PGE_2 (3–300 nM) produced a dose-dependent inhibition of the low P_{O_2}-evoked release of [^{3}H]CA. The inhibition was greatest (55 %) at a low intensity of hypoxic stimulation (incubating solution $P_{O_2} \approx 66$ mmHg) and decreased with increasing intensities of hypoxia. Chronic denervation of the CB did not modify the response to PGE_2.
2. The release of [^{3}H]CA induced by incubating the CBs in a hypercapnic–acidic solution ($P_{CO_2} \approx 132$ mmHg; pH = 6·60) and by dinitrophenol (100 μM) was not significantly modified by 300 nM PGE_2.
3. PGE_2 (300 nM) inhibited the release of [^{3}H]CA elicited by incubating the CBs in a high K^+ (35 mM)-containing solution. The release response elicited by high K^+ (25 mM) was strongly augmented by a dihydropyridine agonist of Ca^{2+} channels, Bay K 8644, at a concentration of 1 μM. The Bay K 8644 effect was partly inhibited by PGE_2 (300 nM).
4. Using whole-cell recordings in freshly dispersed or short-term cultured chemoreceptor cells from adult rabbits it was found that Ca^{2+} currents (I_{Ca}) were reversibly inhibited by bath application of PGE_2. A good parallelism exits between the dose–response curves for PGE_2 inhibition of I_{Ca} in isolated chemoreceptor cells and high extracellular [K^+]- or hypoxia-evoked release of [^{3}H]CA from the whole CB.
5. When recordings were made with an internal solution lacking GTP and containing 100 μM GDP-β-S, a GDP analogue which inhibits G-protein cycling, PGE_2 did not inhibit I_{Ca} in chemoreceptor cells.
6. These results indicate that PGE_2 inhibits the release of [^{3}H]CA induced by hypoxic and high extracellular [K^+] stimulation in adult CB chemoreceptor cells by reducing the entry of Ca^{2+} through voltage-dependent Ca^{2+} channels. This effect of the prostanoid on the Ca^{2+} channels appears to be mediated by a G protein-dependent mechanism.

The carotid body (CB) is an arterial chemoreceptor that is activated when arterial blood P_{O_2} or pH decrease or when P_{CO_2} increases. The CB type I or chemoreceptor cells release catecholamines (CA), mostly dopamine (DA), and probably other neurotransmitters during natural stimulation. The released neurotransmitters in turn set the activity in the carotid sinus nerve (CSN). The stimulus-induced release of CA is Ca^{2+} dependent and proportional to the frequency of action potentials simultaneously recorded in the CB sensory nerve (CSN) (Fidone, González & Yoshizaki, 1982; Obeso, Almaraz & González, 1986; Rigual, López-López & González, 1991). Recently, stimulus-specific models for sensory transduction in chemoreceptor cells have been proposed (Biscoe & Duchen, 1990; González, Almaraz, Obeso & Rigual, 1992).

The transduction of low P_{O_2} into a neurosecretory response appears to involve depolarization of chemoreceptor cells and activation of Ca^{2+} channels, because dihydropyridine agonists and antagonists of Ca^{2+} channels potentiate and inhibit, respectively, the release of CA induced by hypoxia (Obeso, Fidone & González, 1987; Shaw, Montaigne & Pallot, 1989; Obeso, Rocher, Fidone & González, 1992); low P_{O_2} would depolarize the cells by reversibly inhibiting a specific K^+ current sensitive to P_{O_2} (López-Barneo, López-López, Ureña & González, 1988; see González *et al.* 1992). In spite of the dependence of the release of CA on extracellular Ca^{2+} and its sensitivity to dihydropyridines, it has also been proposed that the Ca^{2+} needed to trigger the release of neurotransmitters has its origin in intracellular deposits (Biscoe & Duchen, 1990). In adult rabbits the transduction

of hypercapnic–acidic stimuli does not seem to involve depolarization of chemoreceptor cells or Ca^{2+} channel activation, since the release of neurotransmitters elicited by these stimuli is not sensitive to hydropyridines (Rocher, Obeso, González & Herreros, 1991; Obeso *et al.* 1992); it appears that the Ca^{2+} supporting the release of neurotransmitters enters the cells via the Na^+–Ca^{2+} exchanger, as the release is dependent on extracellular Na^+ and Ca^{2+} ions (Rocher *et al.* 1991; see Gonzalez *et al.* 1992). However, in chemoreceptor cells from neonatal rats, acidic stimuli seem to cause depolarization (Buckler & Vaughn-Jones, 1993).

The information available on the regulation of the steps of the transductive cascades is scarce. It is known that cAMP turnover is increased during natural stimulation of the CB, and that drugs that elevate the cAMP content of the organ augment the release of [^{3}H]CA evoked by several stimuli (Pérez-García, Almaraz & González, 1990, 1991; Wang, Cheng, Yoshizaki, Dinger & Fidone, 1991; Delpiano & Acker, 1991). More recently it has been found that bath application of a permeant analogue of cAMP inhibits the O_2-sensitive K^+ current, leading to the proposal that cAMP may be involved in the regulation of the early steps of the O_2 transduction cascade (López-López, de Luis & González, 1993). In the preceding article (Gómez-Niño, Almaraz & González, 1994) we have provided evidence indicating that endogenously released prostaglandin E_2 (PGE_2) modulates in an inhibitory manner the transductive cascades for hypoxic and acidic stimuli which culminate in the release of catecholamines, but it is not known which are the modulated steps.

In other catecholaminergic structures it has been shown that PGE_2 exibits species- and tissue-specific actions. Thus, PGE_2 inhibited the release of CA from sympathetic nerve endings (Hedqvist & Persson, 1975; Malik & Sehic, 1990), bovine and rat adrenal medulla (Gutman & Boonyaviroj, 1979) and rabbit brain slices (Reimann, Steihauer, Hedler, Starke & Hertting, 1981). In all these systems it has been suggested that PGE_2 produces a decrease in Ca^{2+} entry, and thereby a decrease in the availability of Ca^{2+} at the exocytotic machinery (Hedqvist & Persson, 1975; Gutman & Boonyaviroj, 1979; Malik & Sehic, 1990). However, in cultured bovine adrenomedullary cells, PGE_2 has a facilitatory action on the release of CA by raising the intracellular calcium concentration ($[Ca^{2+}]_i$); in these cases, several mechanisms, including an activation of protein kinase C and voltage-independent Ca^{2+} channels, seem to be involved (Koyama, Kitayama, Dohi & Tsujimoto, 1988; Ito, Mochizuki-Oda, Hori, Ozaki, Miyakawa & Negishi, 1991; Mochizuki-Oda, Mori, Negishi & Ito, 1991).

Based on the results of our preceding study (Gómez-Niño *et al.* 1994) and on the suggestion that PGE_2 actions on the release of CA are mediated by modulation of the pathways for Ca^{2+} entry into the cell, in the present work we have investigated the modulation by exogenous PGE_2 of the [^{3}H]CA secretion from the intact rabbit CB. Additionally, using freshly dissociated or short-term cultured chemoreceptor cells, we have tested the hypothesis that PGE_2 effects on neurotransmitter release are mediated by changes in chemoreceptor cell Ca^{2+} currents.

METHODS

Surgical procedures and [^{3}H]CA release experiments, including procedures for [^{3}H]CA analyses, have been described in the accompanying paper (Gómez-Niño *et al.* 1994).

Chemoreceptor cell culture and electrophysiological recordings

Isolation and culture of chemoreceptor cells from adult rabbit CBs was done following protocols described previously (Pérez-García, Obeso, López-López, Herreros & González, 1992). In brief, CBs were incubated for 20 min at 37 °C in 2 ml of nominally Ca^{2+}- and Mg^{2+}-free Tyrode solution (mM: NaCl, 140; KCl, 5; *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes), 10; glucose, 5; pH 7·2) containing trypsin (2 mg ml^{-1}, type II; Sigma, Alcobendas, Madrid, Spain) collagenase (2 mg ml^{-1}, type IV; Sigma), and deoxyribonuclease (DNase; 0·5 mg ml^{-1}, type II; Sigma). At 10 and 20 min of incubation the organs were subjected for 1 min to gentle mechanical disruption by repeated aspiration through a fire-polished Pasteur pipette. The medium was then changed for a new one containing collagenase (4 mg ml^{-1}), DNase (0·5 mg ml^{-1}) and bovine serum albumin (BSA; 5 mg ml^{-1}; Sigma) and incubation proceeded for an additional 30 min. The tissues were subjected to mechanical disruption at 15 and 30 min. After centrifugation (800 *g*) the pellet was resuspended in 12 ml of enzyme-free Tyrode solution and centrifuged again. The pellet was then resuspended in 250 μl of growth media (Dulbecco's modified Eagle's medium nutrient mixture F-12 Ham supplemented with 5 % fetal calf serum, 2 mM L-glutamine, 100 μg ml^{-1} streptomycin and 40 μg ml^{-1} gentamycin). Aliquots of the cell suspension were plated on small poly-L-lysine-coated coverslips and incubated in a Petri dish at 37 °C with an atmosphere of 5 % CO_2 in air. Two hours later, when the cells were attached, 2 ml of medium were added to the dish.

Freshly dissociated or short-term cultured (3–72 h) chemoreceptor cells were used for the electrophysiological experiments. Whole-cell currents were recorded at room temperature using fire-polished pipette electrodes (2–4 MΩ) filled with (mM): CsCl, 130; $MgCl_2$, 2; Hepes, 10; EGTA, 10; MgATP, 2; MgGTP, 2; pH 7·2. In some experiments (see Results) a MgGTP-free solution containing 0·1 mM GDP-β-S was used. The composition of the control bathing solution was (mM): NaCl, 140; KCl, 5·4; $CaCl_2$, 10; $MgCl_2$ 2; Hepes, 10; glucose, 5; tetrodotoxin (TTX), 5×10^{-5}; pH, 7·42. Superfusion was made by gravity at a rate of 2–3 ml min^{-1}, and the recording chamber had a volume of 0·5 ml; the half-time for solution renewal in the recording chamber, measured as half-time of radioactivity washing out, was 9–13 s. Membrane currents were recorded using a patch-clamp amplifier (EPC-7, List Medical, Darmstadt, Germany). In all of the experiments the holding potential was −80 mV, and 10 or 20 ms depolarizing pulses (range −60 to +70 mV) were applied. After each pulse, two pulses to −120 mV were applied to subtract the leakage currents. Membrane currents were filtered with an 8-pole Bessel filter at 3 kHz, and stored for

later analysis in a PC-AT computer at a sampling rate of 16 kHz. Pulse generation, data acquisition and analysis were made through a computer interface (CED 1401, Cambridge, UK) commanded by software provided by J. Dempster (Voltage Clamp Analysis Program, VCAN, Strathclyde, UK).

RESULTS

Effect of PGE_2 on the [^{3}H]CA release from the CB

Figure 1*A* shows the release of [^{3}H]CA elicited by application of two successive mild hypoxic stimuli ($P_{O_2} \approx 66$ mmHg) from a control drug-free CB and its contralateral CB incubated in the presence of PGE_2 (300 nM) during the second stimulation. The inhibition of low P_{O_2}-induced release of [^{3}H]CA by PGE_2 is evident. A dose–response curve for PGE_2 on the release of [^{3}H]CA elicited by a mild hypoxic stimulus ($P_{O_2} \approx 66$ mmHg) was obtained in groups of five to eight similar experiments for each concentration of PGE_2, and the results are shown in Fig. 1*B*. Maximum inhibition of low P_{O_2}-induced release was observed with a concentration of PGE_2 of 300 nM. At higher concentrations (3 μM) the inhibition appears to be less.

Figure 2*A* shows the effect of PGE_2 (300 nM) on the release of [^{3}H]CA from the CBs elicited by hypoxic stimuli of different intensities. Maximal effect (55 ± 10 % inhibition, mean ± S.E.M.; $n = 6$) was observed with a mild hypoxic stimulus ($P_{O_2} \approx 66$ mmHg), and the inhibitory effect of PGE_2 decreased as the intensity of the hypoxic stimuli increased. No significant inhibition was found during incubation in a hypoxic solution equilibrated with 2 % O_2–5 % CO_2–93 % N_2 ($P_{O_2} \approx 13$ mmHg; pH 7·42). In one experiment, high-performance liquid chromatography (HPLC) analysis of the [^{3}H]CA released from four pooled control CBs and their contralateral, PGE_2-treated CBs, was performed (Fig. 2*B*). It can be seen that basal [^{3}H]DA + [^{3}H]DOPAC release was approximately 5 times higher than that of [^{3}H]NA. The stimulation of the CBs with a hypoxic solution ($P_{O_2} \approx 33$ mmHg) produced a potent secretory response, which was more than 90 % due to the release of [^{3}H]DA and its metabolite. Therefore, the inhibition of [^{3}H]DA release accounts for most of the observed decrease in total [^{3}H]CA release.

Since [^{3}H]DA release is a specific response of chemoreceptor cells (Fidone & González, 1986), HPLC analysis demonstrated that PGE_2 was modifying the activity of these cells. In another group of experiments carried out with control *vs.* chronically denervated CBs, it was explored whether the effects of PGE_2 on chemoreceptor cells were produced directly or via the innervation. Figure 3 shows that the inhibitory effect of 300 nM PGE_2 on the release of [^{3}H]CA elicited by hypoxia ($P_{O_2} \approx 33$ mmHg) was not statistically different in six control CBs compared with five CSN-denervated, five sympathectomized or ten sham-operated CBs, indicating that PGE_2 acted directly on chemoreceptor cells.

Table 1 summarizes the effect of PGE_2 (300 nM) on the release of [^{3}H]CA from the CB evoked by other stimuli. The release evoked by incubation of the CB in a hypercapnic–acidic solution ($P_{CO_2} \approx 132$ mmHg; pH = 6·60) was not significantly modified by PGE_2, although a tendency to decrease could be observed. Similar results were obtained when dinitrophenol (100 μM), an intracellularly acidifying

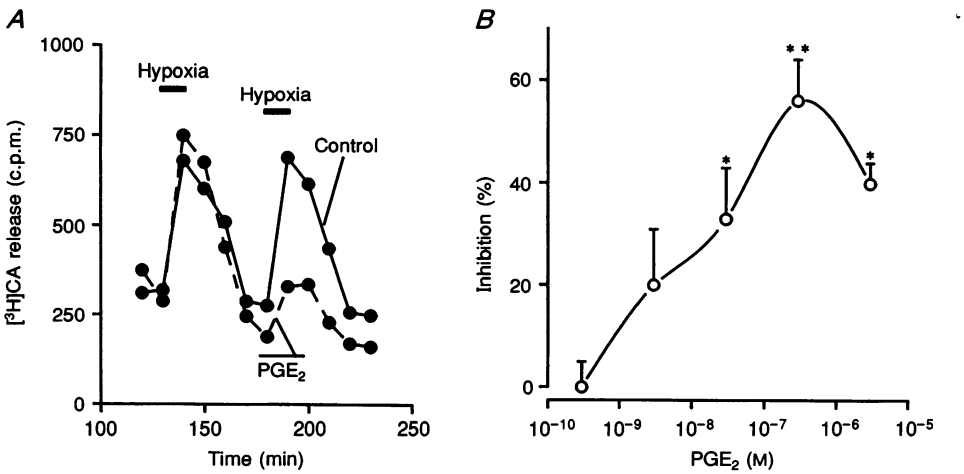

Figure 1. Effect of PGE_2 on basal and low P_{O_2}-evoked [^{3}H]CA release
A, single experiment with a pair of CBs subjected to two hypoxic stimulation cycles (P_{O_2} in the incubating solution was ~66 mmHg; horizontal bars). During the second stimulation cycle one of the CBs (experimental; dashed line) had 300 nM PGE_2 added to the incubation solution during the 10 min period prior to and the 10 min period of incubation with the hypoxic solution. Observe the marked inhibition of the low P_{O_2}-evoked response. *B*, dose–response curve for PGE_2 on low P_{O_2} (~66 mmHg)-evoked release of [^{3}H]CA. Each point is the mean ± S.E.M. of 5–8 individual values. $*P < 0.05$; $**P < 0.01$.

Table 1. Effect of 300 nM PGE_2 on the [^{3}H]CA-evoked release from rabbit carotid body

Stimulus	S1	S2	S2/S1	n
20 % CO_2, pH 6·6	0·61 ± 0·10	0·52 ± 0·08	1·00 ± 0·20	10
20 % CO_2, pH 6·6 + PGE_2	0·53 ± 0·08	0·44 ± 1·10	0·83 ± 0·09	10
DNP (0·1 mM)	5·36 ± 0·86	4·80 ± 1·06	0·90 ± 0·10	8
DNP (0·1 mM) + PGE_2	5·82 ± 1·56	4·20 ± 0·85	0·83 ± 0·11	6
35 mM K^+	4·55 ± 0·59	4·26 ± 0·68	0·94 ± 0·10	8
35 mM K^+ + PGE_2	4·17 ± 0·36	2·31 ± 0·28	0·55 ± 0·07*	8

When indicated, PGE_2 was present only in S2 (see Fig. 1). S1 and S2 represent the [^{3}H]CA-evoked release in each cycle of stimulation and are expressed as a percentage of the [^{3}H]CA present in the carotid body immediately before stimulus application (see Fig. 1). Data are means ± S.E.M. of individual CB values for S1, S2 or S2/S1 ratio. Twenty per cent CO_2 equals a P_{O_2} of ~132 mmHg. DNP, dinitrophenol. *$P \leq 0{\cdot}01$.

protonophore (Rocher *et al.* 1991), was used as stimulus. On the contrary, incubation of the CB in an osmotically balanced solution containing 35 mM K^+ produced a secretory response that was 42 % blocked by 300 nM PGE_2 (a full dose–response curve for PGE_2 effects on 35 mM K^+-induced release of [^{3}H]CA is shown below in Fig. 6). The fact that PGE_2 inhibited the release of [^{3}H]CA elicited by stimuli (hypoxia and high extracellular K^+), which activate Ca^{2+} channels, and did not affect the release evoked by acidic–hypercapnic stimuli or dinitrophenol, which do not appear to activate Ca^{2+} channels, suggested that these ionic channels could be involved in mediating the effects of PGE_2. This suggestion was explored in the experiments that follow.

Evidence for the involvement of voltage-operated Ca^{2+} channels in the action of PGE_2

Figure 4*A* shows the release of [^{3}H]CA elicited by low P_{O_2} (~33 mmHg) in a CB incubated with 2 μM indomethacin (a blocker of the PGE_2 synthesis), and in its contralateral CB incubated with 2 μM indomethacin plus 1 μM nisoldipine (a dihydropyridine blocker of Ca^{2+} channels). Results from five experiments showed that nisoldipine completely reversed the potentiating effect of indomethacin on low

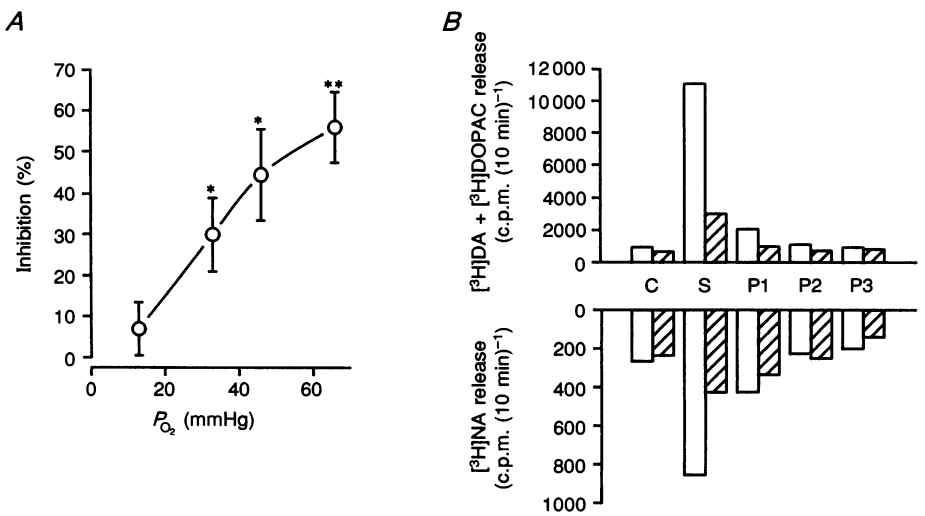

Figure 2. Effects of PGE_2 on the release of [^{3}H]CA elicited by hypoxic stimuli of increasing intensity and HPLC profile of the released material

A, the percentage inhibition of the hypoxia-induced release of [^{3}H]CA produced by PGE_2 (300 nM) at different values of P_{O_2} is shown. Experimental protocols are as in Fig. 1. Each value is the mean ± S.E.M. of 4–6 data points. *$P < 0{\cdot}05$; **$P < 0{\cdot}01$. *B* shows the chromatographic analysis of the [^{3}H]CA released from two groups of 4 CBs during one stimulation cycle ($P_{O_2} \approx 33$ mmHg) in the absence (□) or the presence of 300 nM PGE_2 (▨). Each bar corresponds to a 10 min incubation period: C (control or basal), S (stimulus), and P1–P3 (post-stimulus or recovery periods). See preceding article for chromatographic technique. Note the different scales on the ordinates.

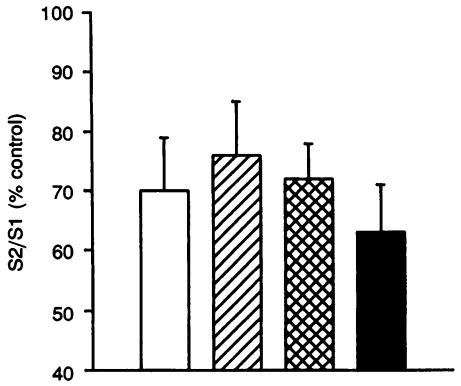

Figure 3. Effect of chronic removal of the superior cervical ganglion or the CSN on the effect of PGE_2 on low P_{O_2}-induced [^{3}H]CA release
Experimental protocols as in Fig. 1; P_{O_2} stimulus was ~33 mmHg. Each value is the mean ± S.E.M. of 5 or more data points. □, unoperated; ▨, sham operated; ▩, sympathectomized; ■, CSN denervated.

P_{O_2}-induced release. The ratios of the evoked release of [^{3}H]CA in the second to the first presentation of the stimulus, S2/S1, were 2·41 ± 0·42 and 0·66 ± 0·11 (means ± S.E.M.; $P < 0·025$) for indomethacin and indomethacin plus nisoldipine-treated CBs, respectively. Figure 4*B* shows that the [^{3}H]CA release evoked by incubation of the CB in a K^+-rich solution (25 mM) was strongly potentiated by the addition of 1 μM Bay K 8644, a dihydropyridine agonist of the L-type voltage-gated Ca^{2+} channels, and that Bay K 8644 action could be partly inhibited by PGE_2 at 300 nM. In four similar experiments it was found that PGE_2 (300 nM) reduced the action of Bay K 8644 by an average of 40 %. S2/S1 ratios were 0·72 ± 0·20 for control CBs, 7·02 ± 1·04 for Bay K 8644-treated organs (means ± S.E.M., $P < 0·001$) and 4·13 ± 0·86 for the CBs incubated with Bay K 8644 plus PGE_2 ($P < 0·05$, for the last two groups). These results indicated that voltage-dependent Ca^{2+} channels are indeed involved in mediating the effects of PGE_2.

Effect of PGE_2 on type I cell Ca^{2+} currents

Figure 5 shows the inward currents recorded in a chemoreceptor cell dialysed with a 130 mM Cs^{2+}, 2 mM GTP, 2 mM ATP solution, and superfused with a solution containing 10 mM Ca^{2+} and 5×10^{-8} M TTX. The currents were obtained by application of a 20 ms pulse to +10 mV from a holding potential of −80 mV. The presence of 300 nM PGE_2 in the superfusing solution reduced the calcium current (I_{Ca}) by nearly 50 %, this effect being completely reversible. Examination of the peak current–voltage (*I–V*) relationship

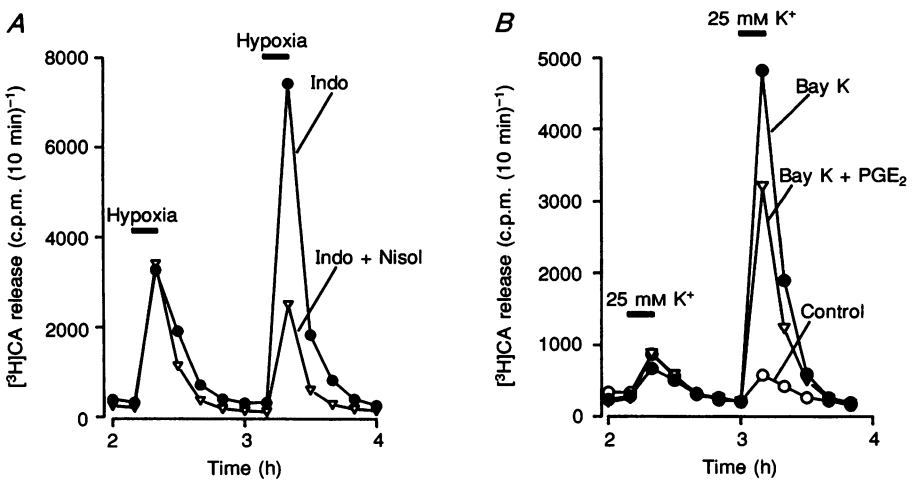

Figure 4.
A, nisoldipine (Nisol) blockade of the potentiating action of indomethacin (Indo) on low P_{O_2}-induced release of [^{3}H]CA (representative experiment with a pair of CBs). Indomethacin concentration was 2 μM, nisoldipine 1 μM, and P_{O_2} stimulus ~33 mmHg. *B*, inhibition by PGE_2 of the potentiating effect of Bay K 8644 on high K^+-induced release of [^{3}H]CA (typical experiment with 3 CBs). Bay K 8644 concentration was 1 μM, PGE_2 was 300 nM and incubating solution K^+ was 25 mM. In *A* and *B*, drugs were present in the incubation media for 20 min (nisoldipine and PGE_2) or 10 min (indomethacin and Bay K 86442) prior to and during the presentation of the second stimulus.

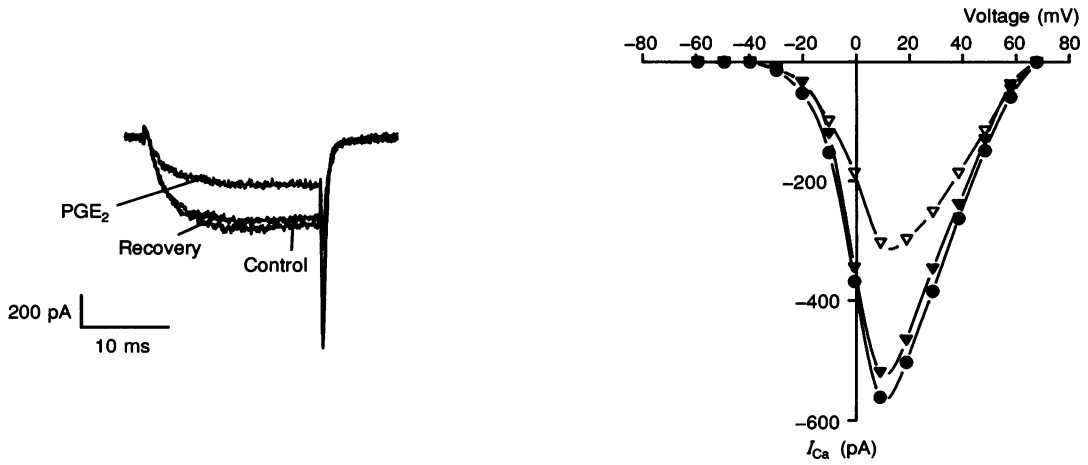

Figure 5. Effect of PGE_2 on Ca^{2+} currents of chemoreceptor cells
Ca^{2+} currents recorded during voltage steps to +10 mV from a holding potential of −80 mV while perfusing with control solution (Control), in the presence of 300 nM PGE_2 (PGE_2), and 2 min after returning to control conditions (Recovery). The figure shows also *I–V* relationships (currents were measured at their peak) obtained in control conditions (●), during perfusion whith 300 nM PGE_2 (▽), and after returning to control conditions (▼).

from the same experiment reveals that the PGE_2-induced decline in I_{Ca} was similar at all tested voltages, implying that no significant shifts in the voltage dependency of Ca^{2+} currents are produced by PGE_2. An effect of quite similar magnitude was obtained in four additional cells, two of which were perfused with a TTX-free solution to assess simultaneously any possible action of PGE_2 on Na^+ currents; PGE_2 did not modify Na^+ currents at any tested voltage. The dose dependence of the inhibition of I_{Ca} by PGE_2 in a chemoreceptor cell is presented in Fig. 6*A*. A marginal inhibition of the current was obtained with 3 nM PGE_2, and 30 and 300 nM produced a decline in the current of 40 and 54 %, respectively. No further inhibition was observed with higher doses (3 μM). Additional features of the response are noticeable in this figure. With the higher concentrations, PGE_2 effects were well developed 30 s after

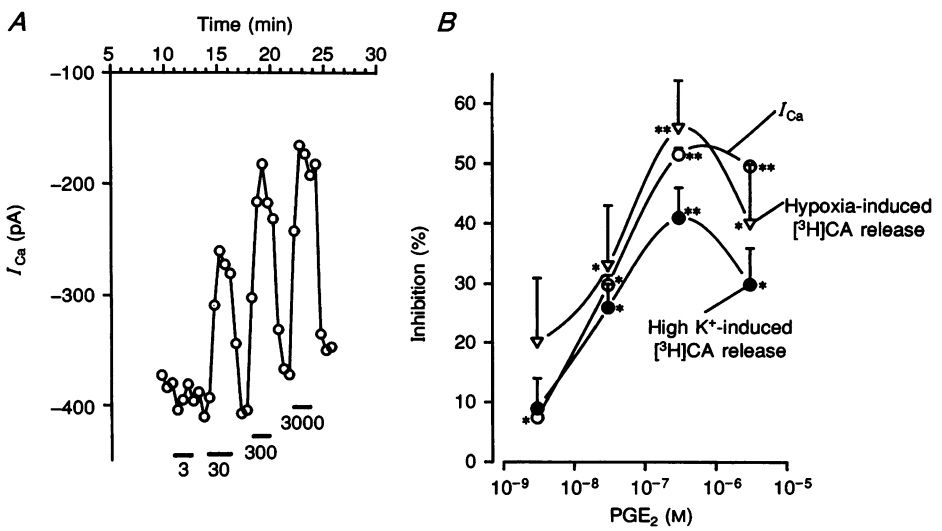

Figure 6. Dose–response curves for the action of PGE_2 on the Ca^{2+} current of chemoreceptor cells and on the release of [^{3}H]CA elicited by high K^+ and hypoxia in the whole CB
A shows peak amplitude of Ca^{2+} currents obtained on 20 ms pulses to +10 mV applied every 15 s. PGE_2 was present in the superfusing solution at the concentrations (nM) and times indicated by the horizontal bars. Time scale indicates time after establishing whole-cell recording. *B*, comparison of the dose–response curves for PGE_2 on I_{Ca} and [^{3}H]CA release. Hypoxic stimulus was ~66 mmHg and high K^+ was 35 mM. Each point represents the mean ± S.E.M. of 4 or more individual data points. $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

the onset of the perfusion with the prostanoid, were fully developed in 1 min, and completely disappeared 1 min after reperfusion with control solution. Figure 6*B* shows the mean inhibition of I_{Ca} obtained in five cells exposed to different concentrations of PGE_2, and compares it with the inhibition produced by the same concentrations of PGE_2 on the release of [^{3}H]CA elicited by mild hypoxia ($P_{O_2} \approx 66$ mmHg) and high extracellular K^+ (35 mM). It should be mentioned that, in addition to these cells in which the full dose–response curve could be obtained, the effects of different concentrations of PGE_2 were tested in no less than thirty cells; inhibition of I_{Ca} was obtained in all the cases at concentrations of PGE_2 above 3 nM.

The possibility that PGE_2 actions on Ca^{2+} channels involved G-protein-mediated events was assessed by the use of an internal solution that contained 100 μM GDP-β-S instead of GTP (Birnbaumer, Abramowitz & Brown, 1990). PGE_2 at 300 nM did not inhibit I_{Ca} in GDP-β-S dialysed cells at any tested voltage in four chemoreceptor cells in which a complete I–V relationship could be obtained, or in six additional cells with incomplete I–V curves.

DISCUSSION

The data presented in this paper demonstrate that bath application of PGE_2 produces an inhibition of low P_{O_2}- and high K^+-induced release of [^{3}H]CA (mostly [^{3}H]DA) from the CB chemoreceptor cells, while acidic stimulus-induced release is not significantly affected. PGE_2 inhibition of the release of [^{3}H]CA results from inhibition of Ca^{2+} currents in chemoreceptor cells, which in turn is mediated by a G-protein-dependent mechanism.

The proposed cause–effect relationship between Ca^{2+} current inhibition and the release of [^{3}H]CA is in line with suggestions made in other catecholaminergic structures (Malik & Sehic, 1990) and directly supported from known facts regarding the CB chemoreceptor cells. First, the inhibition by PGE_2 of the release of [^{3}H]CA is restricted to that elicited by high K^+ and low P_{O_2}, which is known to be mediated by Ca^{2+} entering through voltage-dependent, dihydropyridine-sensitive L-type Ca^{2+} channels (Almaraz, Obeso & González, 1986; Shaw, Montaigne & Pallot, 1989; Ureña, López-López, González & López-Barneo, 1989; Peers & Green 1991; Obeso, Rocher, Fidone & González, 1992). Acidic stimulation-evoked release of [^{3}H]CA is unaffected by either dihidropyridine agonists or antagonists (Rocher *et al.* 1991), and also is unaffected by PGE_2. Second, in the *in vitro* preparation, the sensitivity of the low P_{O_2}-induced release of [^{3}H]CA to dihydropyridine blockers decreases with increasing intensity of hypoxia (Obeso *et al.* 1992), and PGE_2 exhibits a similar inhibitory pattern. This particular relationship between intensity of hypoxia and sensitivity to dihidropyridines has been attributed to the progressive intracellular acidification of chemoreceptor cells *in vitro* as P_{O_2} decreases (Delpiano & Acker, 1985); then, as P_{O_2} decreases there is a progressive change in the quality of the stimulus, from true hypoxia to asphyctic-like stimulation (hypoxia plus acidosis). The same explanation would hold true for PGE_2 effects at increasing levels of hypoxia. However, it should be noted that in the natural *in vivo* situation hypoxia is accompanied by respiratory alkalosis due the low P_{O_2}-triggered CB-mediated hyperventilation, and thus the modulatory role of PGE_2 could cover the full range of physiological hypoxia. In this regard it should also be mentioned that Ca^{2+} channel blockers markedly reduce the increase in CSN discharge induced by intense hypoxia in the *in vivo* preparation of the cat CB (Shirahata & Fitzgerald, 1991). Third, the potentiation of the hypoxic [^{3}H]CA release during blockade of the prostaglandin synthesis by indomethacin is due to an increase in Ca^{2+} entry to the cell through voltage-dependent Ca^{2+} channels, since it is completely inhibited by nisoldipine. PGE_2 mimics this effect of nisoldipine (see preceding paper, Gómez-Niño *et al.* 1994). Fourth, the inhibition of high K^+-induced release of [^{3}H]CA and the reduction of the potentiating effect of Bay K 8844 produced by PGE_2, add further support for a cause–effect link between the inhibition of Ca^{2+} currents and the inhibition of the release of [^{3}H]CA, since Bay K 8644 is well known to be an agonist of Ca^{2+} channels (García *et al.* 1984; Hess, 1990). Fifth, basal release is insensitive to dihydropyridines (Obeso *et al.* 1992) and to PGE_2.

The modulation of ionic channels by G-proteins is a well established fact (Birnbaumer *et al.* 1990; Hess, 1990). Dihydropyridine-sensitive Ca^{2+} channels have been shown to be inhibited through GTP-binding proteins by GABA, dopamine, opioids, neuropeptide Y, somatostatin and α-adrenergic agonists (Birnbaumer *et al.* 1990). Our data show that, as in sympathetic neurons (Ikeda, 1992), a G-protein-mediated inhibitory control of these channels by PGE_2 is present in chemoreceptor cells, since the effect of this prostanoid on the I_{Ca} was abolished by adding GDP-β-S to the internal solution. However, our data do not allow us to distinguish whether the effect of the G-protein activated by PGE_2 on the Ca^{2+} channels is direct or indirect, with involvement of a second messenger system. In the CB, as well as in other tissues (Axelrod, Burch & Jelsema, 1988), PGE_2 produces activation of adenylate cyclase (Pérez-García, Gómez-Niño, Almaraz & González, 1993), but a participation of cAMP in mediating the effect of PGE_2 on Ca^{2+} channels in chemoreceptor cells does not seem plausible, because cAMP would be expected to have a facilitory effect instead of an inhibitory effect on Ca^{2+} channels (Hess, 1990; Birnbaumer *et al.* 1990). In addition, in recording conditions similar to those used in the present experiments, neither forskolin nor dibutyryl-cAMP modified voltage-sensitive Ca^{2+} current in chemoreceptor cells (López-López *et al.* 1993). Further experiments are required to work out the specific mechanism of action of PGE_2 on Ca^{2+} channels.

Compiling the data from this and the preceding article (Gómez-Niño *et al.* 1994), a new concept in CB chemoreception emerges, namely that PGE_2 is a potent modulator of the O_2 chemoreception process. To appreciate this statement fully, it should be recalled that the release of [^{3}H]DA from chemoreceptor cells is a valid index of the chemoreception process (Fidone, González, Obeso, Gómez-Niño & Dinger, 1990; González *et al.* 1992). Thus, although the role played by DA at the synapse between chemoreceptor cell and sensory nerve ending is in dispute (see Fidone *et al.* 1990), it has been shown that, in all experimental conditions tested, the release of [^{3}H]DA parallels the simultaneously recorded action potential frequency in the CSN (see Fidone & González, 1986; Fidone *et al.* 1990). In the only study in which PGE_2 has been tested on CSN discharge, it was found that PGE_2 injected into the common carotid artery reduces in a dose-dependent manner the resting CSN discharge, and that in the '*in vitro*' preparation comparable doses of PGE_2 are without effect on the same parameter (McQueen & Belmonte, 1974). This discrepancy might be explained by the fact that the resting level of CSN discharge was quite different in both preparations, being several times lower in the *in vitro* conditions. Thus, it appears that the lack of effect of PGE_2 on CSN discharge *in vitro* correlates with its lack of effect on the basal release of [^{3}H]CA presented in this article (see also the preceding article).

In comparing the findings from this and the preceding article (Gómez-Niño *et al.* 1994), an apparent contradiction emerges, namely that PGE_2 is able to prevent the large potentiating effect of indomethacin on the release of [^{3}H]CA induced by acidic stimuli and yet it reduces only marginally the acidic stimulus-induced release. It may be suggested that during acidic stimulation PGE_2 reaches saturating concentrations at the chemoreceptor cell PGE_2 receptors due to the high level of PGE_2 production during acid stimulation (see Fig. 2 in Gómez-Niño *et al.* 1994) and to the higher affinity of the receptors for PGE_2 at low pH (Pralong, Vesin & Droz, 1990). If this is the case, it should be expected that exogenously added PGE_2 would have no effect on the release of [^{3}H]CA, as is observed (Table 1). Alternatively, during indomethacin inhibition of cyclo-oxygenase, arachidonate might be metabolized by other pathways (e.g. lipoxygenase pathway) and overproduction of other arachidonate-derived messengers can occur; if this is the case, the presently undefined messenger(s) would be responsible for the potentiating action of indomethacin on the release of [^{3}H]CA and the added PGE_2 would counterbalance its action. In indomethacin-free conditions, endogenously released PGE_2 would counterbalance the stimulatory action of this postulated messenger. These alternatives, and probably others, need to be resolved experimentally.

In summary, we report that PGE_2 reduces specifically the dihydropyridine-sensitive CA release from chemoreceptor cells by inhibiting the Ca^{2+} entry through voltage-activated Ca^{2+} channels. PGE_2 effects on Ca^{2+} channels are mediated by a GTP-binding protein. It could be through this mechanism that endogenous PGE_2 would modulate hypoxia-driven CSN chemoreceptor activity, and consequently the hypoxic ventilatory response.

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