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Potentiation by cyclooxygenase inhibitors of the release of catecholamines from the rabbit carotid body and its reversal by prostaglandin E_2

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Salicylates, at the high therapeutic doses used in the treatment of rheumatoid arthritis, produce an increase in ventilation and augment the carotid body reactivity to hypoxic stimulus, leading to an exaggerated hyperventilation during hypoxia. These effects had been related to the action of salicylates as uncouplers of oxidative phosphorylation. In the present study, carried out in an in vitro preparation of the rabbit carotid body, we show that acetylsalicylic acid and indomethacin, two anti-inflammatory drugs that are also powerful inhibitors of cyclooxygenase, the prostaglandin-synthetizing enzyme, produce an increase in the [^{3}H]catecholamine release evoked by low oxygen stimulation. The drugs did not affect basal normoxic release, a finding that suggests that at the concentration used these anti-inflammatory agents do not have uncoupling actions, and that their effects on hypoxic-induced release of [^{3}H]catecholamines is mediated by their specific action as cyclooxygenase inhibitors. In agreement with this suggestion we found that prostaglandin E_2 completely prevented the effects of both anti-inflammatory agents. In addition, our data indicate that endogenously synthetized prostaglandins are powerful modulators of chemoreceptor cell function.

Salicylates at high doses stimulate ventilation, the effect being mediated by peripheral and central chemoreceptors [6]. It has been reported also that salicylates potentiate the carotid body-mediated hyperventilation produced by hypoxia in man [10]. The mechanism of action of salicylates at the carotid body (CB) level has been related to their well known respiratory uncoupling effect [3, 6].

On the other hand, it is well known also that salicylates are potent and irreversible inhibitors of cyclooxygenase, an enzyme involved in the synthesis of prostaglandins [4]. Since it has been reported [5] that prostaglandin E_2 in vivo inhibits ongoing chemoreceptor discharges in the carotid sinus nerve, we have tested the possibility that at least part of the CB-mediated effects of salicylate on ventilation could be produced by a specific effect of the anti-inflammatory drugs by inhibiting prostaglandin synthesis. Taking into consideration that all chemostimulant agents tested increase in parallel the action potential frequency in the carotid sinus nerve and the release of catecholamines (CAs) from chemoreceptor cells [1, 7–9], we have measured the release of catecholamines as an index of the CB chemoreceptors activation.

The experiments were performed in CBs isolated from adult New Zealand rabbits anesthetized with sodium pentobarbital (30–40 mg/kg, Sigma) administered via the lateral vein of the ear. After tracheostomy, the bifurcation of the carotid artery was dissected, removed as a block and placed in a Lucite chamber filled with ice-cold saline. The CBs were identified and cleaned of surrounding connective tissues under a dissecting scope. Thereafter the catecholamine deposits of the CBs were labeled by incubating the organs with the labeled natural precursor [^{3}H]tyrosine as previously described [11]. After this loading incubation, the CBs were incubated in precursor-free solution for 2 h (solution renewal every 30 min), and finally the collection of the incubating media for analysis in their [^{3}H]CA content was started (see Fig. 1 for protocol of sample collection). The isolation and quantification of [^{3}H]CA in the collected media and in the CB at the end of the release experiments was performed as previously described [11]. The incubating solution in these experiments was a CO_2/HCO_3^--buffered saline with the following composition (in mM): NaCl 116, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1.1, $NaHCO_3$ 24, HEPES 10, glucose 5.5. The solutions were equilibrated with a 5% CO_2-containing gas mixture (see results) and adjusted, if needed, at a pH

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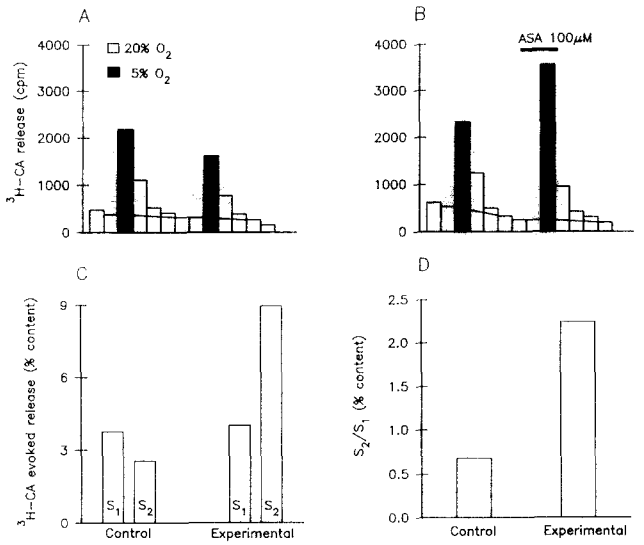

Fig. 1. Effects of acetylsalicylic acid on low oxygen-induced release of [^{3}H]CA in the rabbit CB. Single experiment. A shows the release of [^{3}H]CA from a CB (control) incubated with a 20% O_2/5% CO_2-equilibrated solution (empty bars), and subjected to two periods of 10 min of hypoxic stimulation (incubation in a solution equilibrated with 5% O_2/5% CO_2, filled bars). B: another CB (experimental) was similarly incubated but the 10 min period prior to and during the second hypoxic stimulation the incubating solutions contained 100 μM of acetylsalicylic acid (ASA). C shows the evoked release (cpm above the horizontal lines throughout the bars), expressed as percent of [^{3}H]CA tissue content, for both presentations of the stimulus (S_1 and S_2) and for control and experimental CBs. D shows the ratios of the evoked release in the second stimulus to that in the first stimulus (S_2/S_1 ratios) for control and experimental CBs.

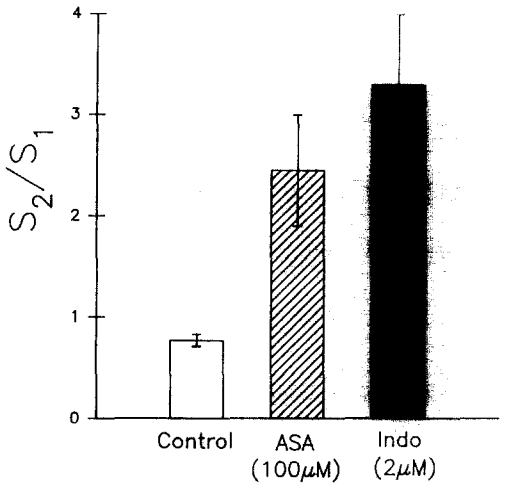

Fig. 2. Effects of acetylsalicylic acid and indomethacin on low oxygen-induced release of [^{3}H]CA. The figure shows means ± S.E.M. of the S_2/S_1 ratios found in control, acetylsalicylic acid (ASA; 100 μM) and indomethacin (Indo; 2 μM)-incubated CBs. Protocols and hypoxic stimulus intensity as in Fig. 1. $^*P < 0.005$.

of 7.40. Statistical significance of the differences observed was assessed by using a *t*-test for unpaired data.

Fig. 1 shows the release of [^{3}H]CA from a pair of CBs subjected to two consecutive hypoxic stimulations (incubation for 10 min in a 5% O_2/5% CO_2 rest N_2-equilibrated solution; filled bars). In the experimental CB (Fig. 1B), the 10 min prior to and during the second presentation of the stimulus the solution contained 100 μM of acetylsalicylic acid. It is evident that acetylsalicylic acid potentiates low oxygen-induced release of [^{3}H]CA. The effect of the drug at this concentration on the basal normoxic release (20% O_2/5% CO_2-equilibrated solutions) was somewhat variable. In some experiments we observed a moderate increase in the basal release while in others (like the one in the figure) there was not any significant effect. No effect on the basal release was observed with lower concentrations of acetylsalicylic acid (20 μM). Fig. 1C shows the stimulus-induced release (above horizontal lines in A and B) for control and experimental CBs and for both presentations of the stimuli, S_1 and S_2, respectively, expressed as percent of endogenous [^{3}H]CA content. In Fig. 1D are represented the S_2/S_1 ratios of the [^{3}H]CA-induced release for both CBs. This form of presenting the data eliminates individual variations in the absolute amounts of the evoked release and allows a better dissection of the effects of the drug. In this particular experiment acetylsalicylic acid raised the S_2/S_1 ratio to 350% of that seen in the control CB. The rest of the experiments to be presented were performed following this protocol.

Fig. 2 shows mean S_2/S_1 ratios obtained in 8 control CBs, 4 CBs treated with 100 μM acetylsalicylic acid and 8 CBs treated with 2 μM indomethacin, another anti-inflammatory agent and potent inhibitor of cyclooxygenase. It is evident that both agents increased markedly the release of [^{3}H]CA induced by hypoxic stimulation ($P < 0.005$ in both cases). At this concentration indomethacin did not modify the basal normoxic release of [^{3}H]CA.

At the concentrations used, neither acetylsalicylic acid nor indomethacin have effect as uncouplers of oxidative phosphorylation [3], but they inhibit markedly cyclooxygenase, the prostaglandin-synthesizing enzyme [4]. Therefore we tested for the reversibility of the effects produced by both agents by the addition of prostaglandin E_2. We chose this particular prostaglandin because it is known that it inhibits the stimulus-induced release of CA in different catecholaminergic structures and in different species including the rabbit [12]. Table I shows that 300 nM of prostaglandin E_2 applied simultaneously with indomethacin completely reversed the effect of the latter agent. The same reversibility was observed for the acetylsalicylic acid effect (not shown). Prostaglandin E_2, at these concentrations, did not modify the basal release of [^{3}H]CA.

TABLE I

REVERSIBILITY OF INDOMETHACIN EFFECTS BY PROSTAGLANDIN E_2

Protocols for the experiments as in Fig. 1. Prostaglandin E_2 (PGE_2) was added to the incubation solution of the experimental CBs at the same time as indomethacin. Data are means ± S.E.M. of 5, 6 and 4 values for control, indomethacin and indomethacin plus PE_2, respectively.

Experimental condition	S_1	S_2	S_2/S_1
Control	5.00 ± 0.45	4.17 ± 0.46	0.84 ± 0.06
Indomethacin (2 μM)	4.80 ± 0.52	10.42 ± 1.71*	2.25 ± 0.44*
Indomethacin + PGE_2 (300 nM)	5.28 ± 0.36	1.34 ± 0.39*	0.24 ± 0.06*

*$P < 0.01$ in all the cases.

The present results show that acetylsalicylic acid and indomethacin potentiated low pO_2-induced release of [^{3}H]CA without affecting the basal release. This effect is completely reversed by simultaneous application of prostaglandin E_2, suggesting that the actions of these anti-inflammatory drugs are not the result of their uncoupling properties, but produced via inhibition of cyclooxygenase with the subsequent decrease in endogenous prostaglandin production. Supporting this interpretation, we have found that average prostaglandin E_2 released to the incubating medium from 7 pairs of CBs was 134.5 ± 46.9 pmol/mg prot. in basal normoxic conditions (20% O_2/5% CO_2), and 198.7 ± 62.0 in hypoxic conditions (7% O_2/5% CO_2) ($P<0.02$). In two additional CBs incubated in the presence of indomethacin, prostaglandin E_2 release was halved in normoxia and the hypoxia-induced release was abolished.

These data show, for the first time, that prostaglandins are modulators of chemoreceptor cell function and suggest that, at least in part, the inhibitory actions of prostaglandin E_2 on carotid sinus nerve activity observed by Belmonte and McQueen [5] were mediated via chemoreceptor cells and not solely mediated by changes in the CB blood flow. It should be mentioned, however, that in the same article these authors reported that, contrary to the in vivo situation, in the vascularly isolated CB preparation when the spontaneous activity in the carotid sinus nerve was low, prostaglandin E_2 did not have any effect. This latest finding is in agreement with the observation on the lack of effect of prostaglandin E_2 on the basal release in different preparations, including ours, in which this prostaglandin inhibits the stimulus-induced release [2, 12].

Regarding the mechanism of action of salicylates on the production of hyperventilation, it will appear that the rapid hyperventilation observed immediately (within 1 min) after a high dose of the drug [6] could in fact be due to an uncoupling effect, but the long lasting hyperreactivity to hypoxia [10] can be adequately explained on the basis of our findings. Thus, an inhibition of prostaglandin synthesis will, on the arrival of a hypoxic stimulus, lead to an exaggerated release of catecholamines (and probably of other neurotransmitters) and a concurrent exaggerated activity in the carotid sinus nerve and ventilatory response.

The mechanism by which prostaglandin E_2 produces its inhibitory effect remains to be established. Due to the fact that it only affects the stimulus-induced release, and that the effect can be at least partially abolished by increasing the extracellular concentration of Ca^{2+} [12], it is tempting to suggest that prostaglandins interfere with the stimulus-activated influx of Ca^{2+} into chemoreceptor cells.

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