Acetate enhances the chemosensory response to hypoxia in the cat carotid body *in vitro* in the absence of CO_2-HCO_3^-

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To determine if intracellular acidosis enhances hypoxic chemoreception in the absence of CO_2-HCO_3^- at pH 7.4, the effects of sodium acetate (30 mM) were studied on the chemosensory responses of the cat carotid body to hypoxic, stagnant and cytotoxic hypoxia. Carotid bodies were perfused and superfused ***in vitro*** *with Tyrode's solution, free of CO_2-HCO_3^-, buffered with HEPES-NaOH, pH 7.40, at 36.5 ± 0.5° C and equilibrated at a PO_2 of 125 Torr (perfusate) and < 20 Torr (superfusate). In the absence of acetate, hypoxia (PO_2 25 Torr), flow interruption and NaCN (0.01-100 μg) augmented the chemosensory discharges. However, in the presence of acetate, the half-excitation time of these responses decreased and their amplitude increased. Thus, acetate enhances the chemosensory response to hypoxic, stagnant and cytotoxic hypoxia. It is suggested that the intracellular acidosis induced by acetate contributes to this potentiation by correcting the alkaline pHi caused by the absence of HCO_3^--HCO_2 in the perfusate.*

Key terms: *acetate, acidosis, carotid body, chemoreceptor, hypoxia, NaCN.*

INTRODUCTION

Hypoxia, hypercapnia and acidosis increase the frequency of the chemosensory discharges (f_x) from the carotid body (CB), which originate reflex ventilatory and cardiovascular adjustments. The discharges show a positive interaction between CO_2-H^+ and O_2 stimuli (Eyzaguirre and Koyano, 1965; Lahiri and DeLaney, 1975). A possible explanation for this interaction was first advanced long ago (Hayes *et al*, 1976) and has recently been ammended (Torrance *et al*, 1993). It proposes that a common mechanism of acidification, perhaps intracellular (Hanson *et al*, 1981), mediates the transduction of both O_2 and CO_2-H^+ stimuli. However, several studies have shown that hypoxia does not consistently reduce intracellular pH (pH_i), either in isolated rat, cat and rabbit glomus cells (He *et al*, 1991; Mokashi *et al*, 1995; Wilding *et al*, 1992) or in the whole CBs of cats and rabbits (Garcia-Sancho *et al*, 1978; Iturriaga *et al*, 1992). By contrast, all of these studies found that hypercapnia and acid stimuli do consistently lower pH_i in isolated glomus cells (Buckler *et al*, 1991; He *et al*, 1991; Mokashi *et al*, 1995; Wilding *et al*, 1992) and in the whole CB (Garcia-Sancho *et al*, 1978; Iturriaga *et al*, 1992). Thus, although transduction of hypoxic stimuli is not initiated by changes in intracellular acidity, the rapidity and amplitude of the hypoxic chemosensory response may nevertheless depend on the pH_i of the chemoreceptor cells. Not only do metabolic and respiratory acidosis potentiate the chemosensory response to hypoxia *in situ* (Lahiri and DeLaney, 1977; Pokorski and Lahiri, 1983) and *in vitro* (Eyzaguirre and

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Koyano, 1965; Iturriaga *et al*, 1993) but isohydric hypercapnia also does it. In fact, Iturriaga and Lahiri (1991) and Shirahata and Fitzgerald (1991) found that adding CO_2-HCO_3^- buffer to their normally HCO_3^--CO_2 free perfusate without changing its pH of 7.4, sped up and augmented the chemosensory response to hypoxia in the cat CB *in vitro*. These augmenting effects on hypoxic chemoreception of CO_2-HCO_3^- buffer at a constant perfusate pH of 7.4 seem to be mediated by a decreased pH_i (Iturriaga, 1993, for review). Further support for this interpretation is given by the observation that permeating inhibitors of carbonic anhydrase that slow the fall in pH_i in response to high CO_2 in isolated glomus cells (Buckler *et al*, 1991) also reduce the cat CB chemosensory response to hypercapnia and hypoxia both *in situ* (Hayes *et al*, 1976; Hanson *et al*, 1981) and *in vitro* (Iturriaga *et al*, 1993). These results taken together suggest that the chemosensory responses to hypoxia depend on the pHi setting of the chemoreceptor cells. If this is the case, a permeant weak acid such as acetic acid, that easily enters the cells in the unionized form and there ionizes (acetate + H^+) and so produces a predictable acidosis both in isolated glomus cells (Sato, 1994) and in the whole CB of cats (Iturriaga *et al*, 1992), should enhance the chemosensory response of the CB to hypoxia in the absence of CO_2-HCO_3^- at pH 7.4. I have therefore studied the chemosensory responses of the cat CB *in vitro* to hypoxia, flow interruption and NaCN, with and without 30 mM sodium acetate in the perfusate and superfusate Tyrode's solution, which was free of CO_2-HCO_3^- and always at a pH of 7.4.

METHODS

Experiments were performed on 5 male cats (3.4 ± 0.2 kg) anesthetized with sodium pentobarbitone (40 mg/kg, ip). One carotid bifurcation including the CB was perfused and superfused as previously described (Iturriaga *et al*, 1991). In brief, one carotid bifurcation including the CB and its carotid sinus nerve was excised. All the arteries originating from the carotid bifurcation except the ascending pharyngeal artery were ligated and the CB veins were left open. The common carotid was cannulated and the bifurcation was placed in a chamber. The carotid bifurcation with the CB was perfused by gravity (80-90 Torr) with a modified Tyrode's solution, equilibrated with room air (PO_2 of 120-125 Torr) and was simultaneously superfused (1 ml/min) with the same Tyrode's solution bubbled with 100% N_2 to equilibrate at PO_2 < 20 Torr. The temperature of the fluid in the chamber was maintained at 36.5 ± 0.5° C with a regulated heating device. The composition of the modified Tyrode's solution was in mM: 154 Na^+; 4.7 K^+; 2.2 Ca^{2+}; 1.1 Mg^{2+}; 42 glutamate; 123.3 Cl^- and 5.5 D-glucose. The Tyrode's solution was buffered with HEPES 5 mM, and its pH was adjusted to 7.40 with 2 mM NaOH. The PO_2 of the Tyrode's solution was measured by an oxygen electrode through a polarographic sensor.

The chemosensory discharge was recorded from the whole carotid nerve, placed on a pair of platinum electrodes and lifted into paraffin oil. Neural signals were preamplified, amplified, passed through band pass filters (10 Hz - 1 KHz) and a notch filter (50 Hz) and fed to an electronic amplitude discriminator and oscilloscope, which allowed the selection of action potentials of given amplitudes above the baseline noise. The resulting standardized pulses were counted with a frequency meter and the frequency of chemosensory discharges (f_x) was displayed as analog signals on a polygraph and printed as a digital signal (Hz).

Each CB was perfused first with Tyrode's solution without sodium acetate for about 45 min and then with Tyrode's solution containing 30 mM sodium acetate, replacing the same concentration of sodium glutamate. The chemosensory responses were tested as follows: 1) perfusion with hypoxic Tyrode's solution (PO_2 = 20-30 Torr) for 3-8 min; 2) interruption of the perfusate flow by clamping the perfusate line for 1-3 min, while the superfusate flow remained constant; and 3) injections of NaCN (0.01-100 μg) in boluses of 0.2 ml into the perfusate line.

Results are expressed as means ± SEM's. Statistical differences between paired samples were assessed by the Wilcoxon's signed rank test. To compare dose-response curves

with and without acetate, f_x was expressed as a percentage of the maximal response evoked by interruption of the perfusate flow in the presence of acetate. The data points from NaCN dose-response curves were fitted to the following logistic expression (De Lean *et al*, 1978):

$$R = \max f_x + \{[\text{bas } f_x - \max f_x] / [1 + (D/ED_{50})^s]\}$$

where: R = response; max f_x = maximal chemosensory response; bas f_x = basal f_x; D = arithmetic dose; ED_{50} = median effective dose; s = slope factor that determines the steepness of each curve. The curves were fitted through a computer program based on a simplex algorithm (Johnston, 1985).

RESULTS

Switching from Tyrode's solution without acetate to one with 30 mM sodium acetate increased basal f_x from 12.0 ± 2.6 Hz to 69.1 ± 18.0 Hz ($p < 0.05$; 5 CBs), even though the pH of the medium maintained constant at pH 7.4. Figure 1 shows the effects of hypoxic perfusion (PO_2 reduced from 125 to 25 Torr) on f_x in a CB perfused with normoxic Tyrode's solution without and with acetate. Without acetate, hypoxia slowly increased f_x to a stable level. Switching back to normoxic saline returned f_x to the basal values (Fig 1A). When the same CB was perfused and superfused with Tyrode's solution containing 30 mM sodium acetate, the basal f_x was greater and the same hypoxic stimulus increased f_x after a shorter latency to a higher level (Fig 1B). In the 5 CBs, the maximal f_x attained during hypoxia without acetate was 180.3 ± 57 Hz, and during hypoxia with acetate was 322.0 ± 28.3 Hz ($p < 0.05$). The half-excitation time decreased from 155.90 ± 36.7 s to 22.7 ± 8.0 s ($p < 0.05$).

Figure 2 shows the effects of interruption of perfusate flow on f_x in a CB perfused firstly with normoxic Tyrode's solution without acetate, and subsequently with saline containing 30 mM sodium acetate. Without acetate, perfusate flow interruption raised f_x to a stable level in about 2 min (Fig 2A). With acetate, the latency of the response decreased and f_x rose to a higher level in response to flow interruption (Fig 2B). In the 5 CBs, the half-excitation time decreased from 43.6 ± 6.8 s to 16.5 ± 5.2 s ($p < 0.05$) and the maximal f_x attained during flow interruption increased from 258.8 ± 56.8 Hz to 329.4 ± 26 Hz ($p < 0.05$).

Figure 3 shows the effects of three increasing doses of NaCN (0.01, 0.1 and 1 µg) on f_x in the absence and presence of acetate. Without acetate, only the larger dose of NaCN (1.0 µg) increased f_x (Fig 3A), but with 30 mM sodium acetate, the smallest dose of NaCN (0.01 µg) was able to increase f_x (Fig 3B). A systematic study of the f_x responses produced by increasing doses of NaCN (0.01 to 100 µg) was performed with the data obtained from the 5 CBs. The fitting of the maximal f_x attained during NaCN injections to sigmoidal functions is shown in Figure 4. Each data point was expressed as a percentage of the maximal response elicited

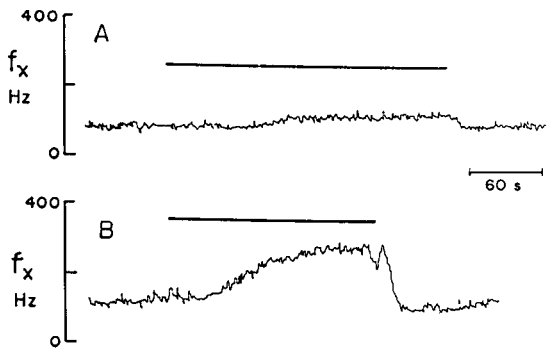

Fig 1. Carotid chemosensory responses to hypoxic perfusions (bars; PO_2 = 25 Torr) after perfusion with normoxic Tyrode's solution (PO_2 = 120 Torr), free of CO_2-HCO_3^-, at pH 7.40, without (**A**) and with (**B**) 30 mM sodium acetate.

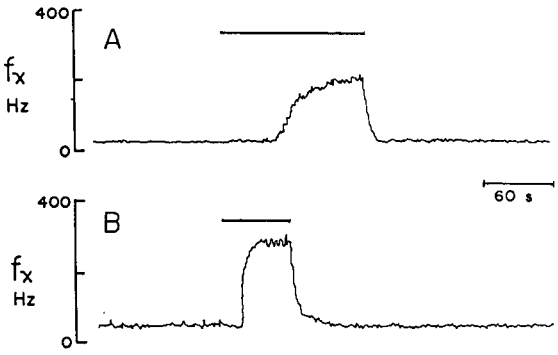

Fig 2. Carotid chemosensory responses to perfusate flow interruptions (bars) during perfusion with normoxic Tyrode's solution (PO_2 = 125 Torr), free of CO_2-HCO_3^-, at pH 7.40, without (**A**) and with (**B**) 30 mM sodium acetate.

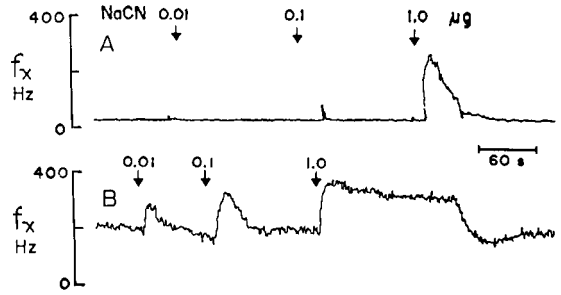

Fig 3. Carotid chemosensory responses to several NaCN doses (arrows) during perfusion with normoxic Tyrode's solution (PO_2 = 125 Torr), without CO_2-HCO_3^-, at pH 7.40, without (**A**) and with (**B**) 30 mM sodium acetate.

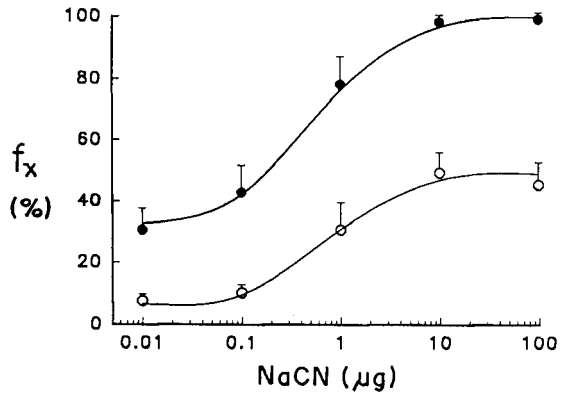

Fig 4. Dose-response curves for changes in chemosensory frequency (f_x) elicited by increasing doses of NaCN in the presence (filled circles) or absence (open circles) of 30 mM sodium acetate in the perfusate-superfusate media. Means + SEM's of 5 experiments. f_x expressed as percentage of maximal f_x produced by flow interruption during perfusion with acetate. Correlation coefficients for adjusted curves in absence and presence of acetate = 0.82 and 0.94, respectively ($p < 0.01$). Maximal reactivity significantly higher ($p < 0.02$) with acetate.

by flow interruption in the presence of acetate. The parameters defining the dose-response curves show that the maximal reactivity increased from 48.3 ± 5.7 % to 97.8 ± 2.1 % ($p < 0.02$) in the presence of acetate. The basal f_x augmented from 6.9 ± 1.6 % to 31.6 ± 4.4 % of the maximal response ($p < 0.02$). However, the ED_{50} and the slope factors were not significantly different with or without acetate; ED_{50}'s were 0.62 ± 0.2 and 0.56 ± 0.3 ($p > 0.05$), respectively.

DISCUSSION

Previously, Iturriaga and Lahiri (1991) have shown that if CO_2-HCO_3^- buffer is removed from the perfusate of the *in vitro* preparation of the cat CB, the response to hypoxia is slowed. My present results show that the presence of 30 mM sodium acetate in the external medium increases the basal chemosensory discharge and enhances the chemosensory responses to hypoxia, flow interruption and NaCN. These effects occurred in the absence of CO_2-HCO_3^- at pH 7.4 and are consistent with a reduction in the pH_i of the CB cells, as acetate produced the same effect in isolated rabbit glomus cells (Sato, 1994) and in the whole CB of cats (Iturriaga, 1992). Such an acetate-induced reduction of pH_i might reduce the threshold of the hypoxic response and increase the sensitivity of the chemoreceptor to a hypoxic stimulus. In contrast, the absence of CO_2-HCO_3^- from the perfused medium slowed the responses to hypoxia and flow interruption and decreased their amplitude (Iturriaga and Lahiri, 1991). Such an increase in the latency and reduction in amplitude of the chemosensory response to hypoxia in the absence of CO_2-HCO_3^- could be attributed to the alkaline pHi, which is found in the absence of CO_2-HCO_3^- at external pH of 7.4 in isolated rat glomus cells (Buckler *et al*, 1991; He *et al*, 1991; Wilding *et al*, 1992) and in the whole CB of cats (Iturriaga *et al*, 1992). Thus, the chemosensory responses to hypoxic stimuli may depend on the pH_i setting of the chemoreceptor cells.

The critical question is how might an acidification of the CB chemoreceptor cells enhance the change in f_x in response to hypoxia. While the prevailing hypothesis of chemoreception states that the glomus cells of the CB are the primary chemoreceptors for PO_2, PCO_2 and pH changes (see Gonzalez *et al*, 1994, for review), another hypothesis proposes that the nerve endings themselves are the receptors (Mitchell *et al*, 1972). The present results do not rule out a possible effect of acetate on the pH_i of the nerve endings. However, in other sensory systems such as the cat muscle spindle (Fukami, 1988) and the barnacle photoreceptor (Brown and Meech, 1979), the application of acid and high PCO_2 suppress the generation of action potentials by reducing pH_i in the nerve terminals. A possible vascular effect of acetate cannot be ruled out in this *in vitro*

preparation. A reduced PO_2 in the CB tissue caused by vasoconstriction may explain a fast chemosensory response. However, Lahiri *et al* (1993), using an optical method based on phosphorescence quenching by O_2, measured the tissue PO_2 of the cat CB *in situ* and found that it remains nearly constant when P_aCO_2 levels were changed from 20 to 100 Torr, during normoxic hypo- and hypercapnia. That study clearly shows that CO_2-H^+ does not have an important effect on the CB tissue PO_2.

In the last few years, several studies using patch-clamp techniques have shown that the glomus cells have an outward voltage-gated K^+ current that is reversibly reduced by hypoxia during a pre-imposed depolarization (Hescheler *et al*, 1989; López-López *et al*, 1989; Peers and Green, 1991; Stea and Nurse, 1991). Accordingly, O_2 transduction is believed to be initiated by a depolarization of the glomus cell due to the closing of PO_2-dependent K^+ channels and this depolarization would in turn open voltage-gated Ca^{2+} channels leading to a rise in intracellular calcium ($[Ca^{2+}]_i$) (Buckler and Vaughan-Jones, 1994; Sato *et al*, 1991) and transmitter release (see Gonzalez *et al*, 1994, for review). A plausible explanation for the enhancing effect of intracellular acidosis on the hypoxic response is that an acid stimulus converges on the O_2 transduction pathway. This explanation is supported by observations that intracellular acidosis of glomus cells, induced by acetate, propionate or CO_2-HCO_3^- at a constant extracellular pH of 7.4, reduces the PO_2-dependent K^+ current (Peers and Green, 1991; Stea and Nurse, 1991; Stea *et al*, 1991). But, more recently, Donnelly (1995) found in rat CB that brief hypoxia rapidly increased chemosensory discharge but only slightly decreased the outward current, suggesting that hypoxic control of glomus cells K^+ current is not the primary initiating factor of the chemosensory response to brief periods of hypoxia.

Acid stimuli depolarize glomus cells (Buckler and Vaughan-Jones, 1993; Eyzaguirre *et al*, 1989) and increase their $[Ca^{2+}]_i$ (Buckler and Vaughan-Jones, 1993; Sato, 1994). Thus, it is likely that intracellular acidosis enhances the increase in $[Ca^{2+}]_i$ produced by hypoxia. Indeed, Biscoe and Duchen (1990) found that an acid stimulus (HEPES buffered superfusate, pH 6.85 vs 7.3) enhanced the amplitude and sped up the onset of the $[Ca^{2+}]_i$ rise induced by hypoxia (PO_2 of 30 Torr) in the isolated rabbit glomus cells. This potentiation of the $[Ca^{2+}]_i$ rise would be expected to increase the release of the excitatory transmitter(s) from the glomus cells, and so finally to enhance the chemoreceptor nerve fibre response. According to this prediction, a weak but easy penetrating acid such as acetic acid or CO_2 should enhance the release of putative excitatory transmitter(s) from the glomus cells during hypoxia.

The present results also show that acetate reduces the half-excitation time of the chemosensory response to interruption of perfusate flow. In this preparation of the cat CB *in vitro*, f_x increases during flow interruption as PO_2 declines to close to 0 Torr due to O_2 consumption by the CB cells (Rumsey *et al*, 1991). Therefore, we cannot exclude the possibility that accumulation of acetate inside the cells increases the metabolism and the rate of O_2 disappearance from the CB. The enhanced response to NaCN found here in the presence of acetate suggests that a low pH_i could potentiate the hypoxic responses by changing the O_2 dependence of the cytochrome a_3 system (Wilson *et al*, 1988). Thus, a low pH_i may potentially enhance O_2 chemoreception not only by reducing the O_2-dependent K^+ current, but also by changing the O_2 dependence of the chemoreceptor respiratory chain. The effects of acetate on the chemosensory response to NaCN are similar to those of CO_2 on the response to NaCN, previously found in the same preparation of the cat CB *in vitro* (Iturriaga and Lahiri, 1991).

The present results confirm that hypoxia increases f_x in the absence of CO_2-HCO_3^- *in vitro* (Eyzaguirre and Koyano, 1965; Iturriaga and Lahiri, 1991; Iturriaga *et al*, 1991). In studies with isolated glomus cells performed in a medium without CO_2-HCO_3^-, hypoxia reduced the PO_2-dependent K^+ current (López-López *et al*, 1989) and increased $[Ca^{2+}]_i$ (Biscoe and Duchen, 1990) even when the pH_i of the glomus cells was alkaline (Buckler *et al*, 1991). These results

agree with the observation that the chemosensory response to hypoxia -although it is modest without CO_2-HCO_3^- does not absolutely require the participation of external CO_2-HCO_3^-. Furthermore, CO_2-HCO_3^- may not itself be essential for hypoxic chemoreception since its effect was mimicked by acetate. Weak acid stimuli, such as CO_2 or acetic acid, may modulate O_2 chemoreception by a common mechanism of reducing the pHi of the chemoreceptor cells.

In summary, the present results show that adding acetate to the CO_2-HCO_3^- free perfused and superfused medium of the CB *in vitro* enhances its chemosensory responses to hypoxia, flow interruption and NaCN at a pH of 7.4. These results strongly suggest that intracellular acidosis contributes to enhancing the hypoxic response.

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