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EXTRACELLULAR pH SIGNALS AFFECT RAT VASCULAR TONE BY RAPID TRANSDUCTION INTO INTRACELLULAR pH CHANGES

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SUMMARY

1. Changes in extracellular pH (pH_o) are known to produce large effects on vascular tone, but the mechanisms involved are not understood. As changes in intracellular pH (pH_i) can also affect vascular tone, we have investigated the effects of changing pH_o upon both pH_i and tone.

2. Strips of rat mesenteric resistance vessels were loaded with the pH-sensitive fluorophore SNARF 1; thus tension and pH_i could be simultaneously measured as pH_o was altered.

3. Whenever pH_o was altered there was a corresponding alteration of pH_i. Furthermore, when pH_o was changed the pH_i change was more rapid than that reported to occur in other cells. The time to half-peak intracellular response was 38 ± 4 s ($n = 11$). The induced pH_i change was also less attenuated than in many other cells studied. Thus a ratio of 0·73 was obtained for the change in pH_i per unit pH_o change ($n = 7$).

4. An increase in pH_i produced by an increase in pH_o was accompanied by an increase in tension in the vascular strips. In other experiments pH_i was increased at constant pH_o by the addition of the weak base trimethylamine (40 mM). This also elevated tension in the strips. Conversely when pH_o was changed while pH_i was held at resting values, no change in vascular tone occurred.

5. It is concluded that the effects of pH_o on vascular tone are due to the induced change in intracellular pH, and that the vascular smooth muscle cell is functionally well adapted to respond to changes in tissue pH, thereby allowing blood flow to a tissue to be rapidly altered to meet changing needs.

INTRODUCTION

One of the major mechanisms for controlling the circulation is alteration of vascular tone. Changes in pH are known to alter vascular tone and thereby influence the circulation. It has been known for many years that both extracellular pH (pH_o) (Gaskell, 1880) and intracellular pH (pH_i) (Betz, Enzenroß & Vlahov, 1973; Kontos, Wei, Raper & Patterson, 1977; Wray, 1988) can alter tension. Their relative importance, however, is still debated and their mechanism of action is still unclear

(Lassen, 1968; Rooke & Sparks, 1981; Harder & Madden, 1985). The aim of this work was to investigate the mechanism whereby changes in pH_o alter vascular tone, in particular to determine what effect changes of pH_o may have on pH_i. By analogy with other tissues, it is generally assumed that when pH_o is altered: (i) pH_i will change only slowly, e.g. 10–40 min (Ellis & Thomas, 1976; Aickin, 1984); and (ii) the change in pH_i will only be a small percentage of the change in pH_o, e.g. 20–30% (Aickin, 1984; Tolkovsky & Richards, 1987). These assumptions, however, have not been tested on vascular smooth muscle. Clearly the effects of pH_o on pH_i must be known if the relative contributions of pH_o *vs.* pH_i are to be understood. No previous study of vascular smooth muscle has simultaneously measured pH_i and tension when pH_o is altered. We have made these measurements and report that the effects of pH_o on vascular tone are due to the induced change in pH_i. Furthermore, when pH_o is altered, then pH_i is affected much faster and with far less attenuation than in other cells. We suggest that this is a functional adaptation of the vascular cells to enable a rapid change in tissue pH_i when pH_o is changed, thereby allowing blood flow to tissues to be rapidly altered to meet changing needs. Some of these results have been communicated to the Physiological Society (Austin & Wray, 1993).

METHODS

Tissues and cells. Male Wistar rats were killed by cervical dislocation following chloroform anaesthesia. Strips of mesenteric artery branch vessels (radius 100–200 μm) were dissected and pH_i and isometric tension were simultaneously recorded. A ring of the vessel (0·5–0·8 mm wide) was opened and mounted circumferentially (i.e. the circumference of the vessel was between the two attachment points) under a resting tension of 0·5–1·0 mN; this approximates to the normal pressure in these vessels of around 40–50 mmHg (Bohlen & Gore, 1977) using Laplace's Law to relate pressure and tension. The endothelium was removed by gentle rubbing. These preparations contracted to KCl and sympathetic agonists. Single cells were produced by enzymatic digestion of mesenteric resistance vessels following previously published methods (Baro & Eisner, 1992). Tissues and cells were constantly superperfused at 37 °C with Krebs solution of the following composition (mM): NaCl, 154; KCl, 5·4; $MgSO_4$, 1·2; glucose, 11·7; $CaCl_2$, 3. In most experiments solutions were gassed with 100% O_2 and buffered with 11 mM Hepes, but all results were confirmed in HCO_3^-–CO_2 (22·4 mM HCO_3 and 95% O_2–5% CO_2) buffer. External pH was changed from a control value of 7·4 by addition of either NaOH or HCl. Intracellular pH was changed at constant pH_o by using weak acids (sodium butyrate, 40–60 mM) and bases (trimethylamine chloride, 40 mM) isosmotically substituted for NaCl. Control experiments were performed to ensure that the respective reductions in Cl^- and Na^+ had no significant effect upon the results. In some experiments, mentioned in the text, pH regulation was inhibited by using amiloride (1 mM) and 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS, 150 μM).

Intracellular pH measurement. The pH_i record was obtained from the ratio of fluorescent signal obtained at 580 and 650 nm following excitation at 540 nm, from strips and cells loaded for 1 h with 5 μM acetoxymethyl ester carboxy SNARF (Buckler & Vaughan-Jones, 1990) and placed on the stage of an inverting Nikon microscope. Shorter or longer loading periods did not make any difference to any of the results obtained. At the end of every experiment both the single cells and the strips were calibrated using the K^+–H^+ ionophore nigericin (Buckler, Vaughan-Jones, Peers, Lagadic-Gossmann & Nye, 1991). The calibrating solution was of the following composition (mM): NaCl, 24·6; KCl, 135; $MgSO_4$, 0·12; Hepes, 11; glucose, 11·7 and the pH set to known values.

Statistics. Figures given throughout are the mean values ± standard errors of the means. The n values indicate the number of animals.

RESULTS

The tension changes and pH_i changes produced as a result of alteration of pH_o were not significantly different in HCO_3^-–CO_2 and Hepes buffer, so the majority of experiments were performed in Hepes and all results confirmed in HCO_3^-–CO_2 buffer.

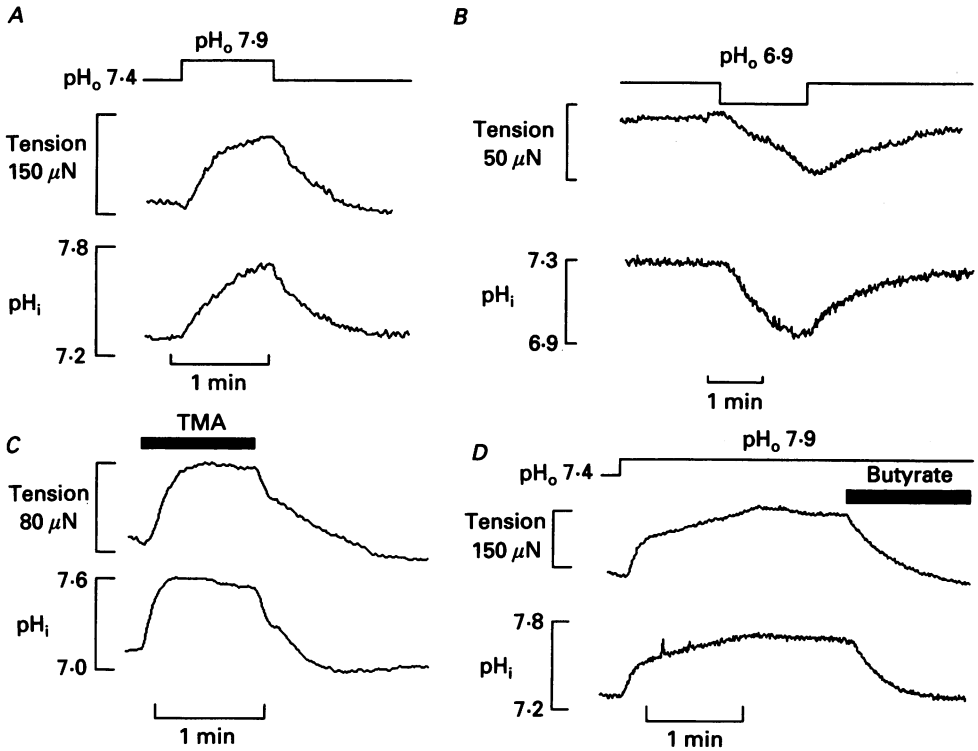

Fig. 1. The effects on tension of changing pH. *A*, in this and the other panels, the top trace shows tension and the bottom trace shows intracellular pH (pH_i). Elevation of external pH from 7·4 to 7·9 increased both pH_i and tension. Decreasing pH_o produced relaxation and a fall in pH_i. *B*, acidification of the perfusing solution to pH 6·9 produced a relaxation. *C*, elevation of pH_i at constant pH_o, produced by addition of 40 mM trimethylamine (TMA), caused an increase in vascular tension. *D*, tension increased when pH_o was elevated to 7·9 (HCO_3^-–CO_2 buffer), but not when pH_i was returned to resting levels by the addition of weak acid (sodium butyrate, 60 mM).

The mean resting pH_i value in the strips was $7{\cdot}10 \pm 0{\cdot}04$ in Hepes ($n = 25$) and $7{\cdot}07 \pm 0{\cdot}03$ ($n = 23$) in HCO_3^-–CO_2.

The effects of changing pH_o on pH_i and tension

In the vascular strips, when the external solution was changed from pH 7·4 (control) to 7·9, there was an increase in vascular tone as shown in Fig. 1*A*. The simultaneous pH_i record shows that this manoeuvre also affects pH_i. The cytoplasm

became alkaline over the course of about a minute (see below), even though the external pH (pH_o) change was made with a strong base. Lowering pH_o resulted in an intracellular acidification. This was accompanied in most tissues by no change in tension, but when there was a response, a small relaxation occurred, as shown in Fig. 1*B*. When the strips were contracted, e.g. with high-K^+ solution, all preparations relaxed upon lowering of pH_o.

The effects of changing pH_i on tension at constant pH_o

When pH_i was increased at constant pH_o by addition of a weak base such as trimethylamine (TMA), as shown in Fig. 1*C*, contraction occurred in all eleven vessels tested. It can be seen that the pH_i change produced by adding TMA was much quicker than that arising from alteration of pH_o. This is as expected due to rapid passage of undissociated weak base across the vascular cell surface membrane. Similar pH changes but in the opposite direction were obtained when the pH_i was reduced using the weak acid butyrate, although, as above, not all tissues produced a significant relaxation. All tissues showed pH regulation following addition of weak base or acid, the extent of which varied between preparations.

The effects of changing pH_o without changing pH_i

As Fig. 1*C* shows, changing pH_i *without* changing pH_o can affect contraction. As pH_i changes when pH_o is altered, the question arises whether the effects on tension are wholly or partly due to the induced pH_i change. To elucidate the effects of pH_o alone on tone, a weak acid (butyric) was added to the pH 7·9 bathing solution to return pH_i close to resting levels (Thomas, 1984). In all sixteen preparations changing pH_o to 7·9 elicited a contraction that could be decreased or abolished by returning pH_i to around resting levels at a continued pH_o of 7·9 (Fig. 1*D*). If pH_i was not allowed to change, i.e. by going from control (pH 7·4) to pH 7·9 plus butyrate, then no contraction occurred ($n = 4$). Thus in summary, raising pH_o to 7·9 only produced contraction when pH_i changed; extracellular pH *per se* has no effect on contraction.

The relation between pH_i and contraction is shown in Fig. 2*A*, for contraction being produced either by elevation of pH_o (○) or by addition of weak base to raise pH_i (*). It can be seen that the points are indistinguishable, i.e. force is related to pH_i regardless of the mechanism used to change pH_i. Figure 2*B* shows the mean relation between contraction and change in pH_i produced by elevation of pH_o. It can be seen that the relation is linear over a wide pH_i range, and is the same in both Hepes (■, $n = 8$) and HCO_3–CO_2 (□, $n = 9$) buffer.

The time course of pH_i change upon alteration of pH_o

The speed of the response to changing pH_o from 7·4 to 7·9 was investigated in both strips and isolated smooth muscle cells from mesenteric resistance vessels; pH_i was found to be altered rapidly in both. The mean times to half-peak intracellular response ($t_{0.5}$) were 38 ± 4 s ($n = 11$) and 33 ± 7 s ($n = 12$), respectively. For comparison, increasing pH_i by using weak base (TMA), gave a mean $t_{0.5}$ of 8 ± 1 s ($n = 10$) in cells and 10 ± 2 s ($n = 11$) in strips. The rapid changes in the vascular smooth muscle cells upon alteration of pH_o occurred in both the acid and alkaline

direction and over the pH range 0·2–1·0 pH unit change. The pH_i changes were equally rapid in Hepes- and HCO_3–CO_2- ($n = 9$) buffered external solutions and were not significantly affected by inhibitors of the two main pH regulating mechanisms in these cells; namely amiloride to inhibit Na^+–H^+ exchange and DIDS to inhibit Cl^-–HCO_3^- exchange (Aalkjaer & Cragoe, 1988).

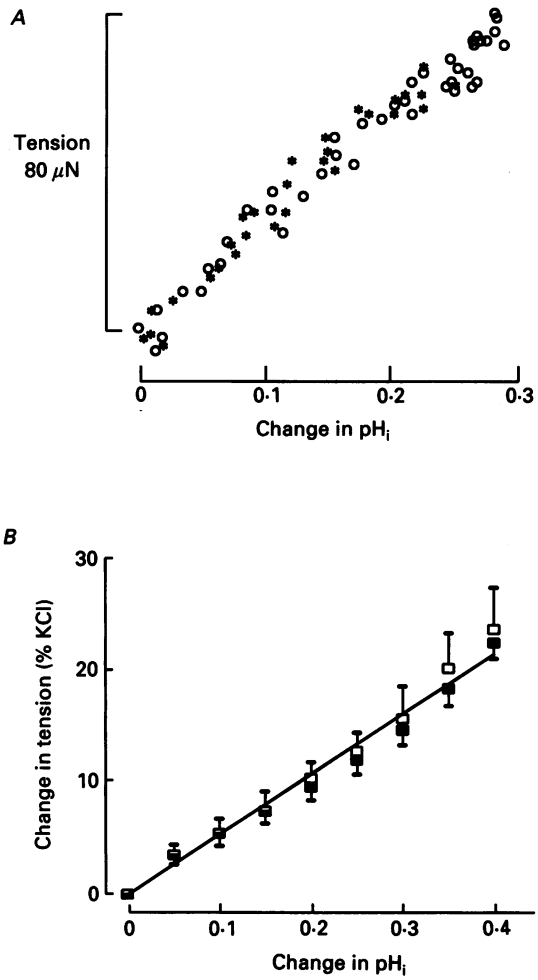

Fig. 2. The relation between vascular tone and pH_i. *A*, an example of the effects on tension produced by elevation of pH_i due to the addition of the weak base trimethylamine (TMA, 40 mM; *) and increase of pH_o to 7·9 (○). *B*, mean relation between the change in tension and increase in pH_i due to elevation of pH_o in the presence of Hepes (■, $n = 7$) or HCO_3^-–CO_2 (□, $n = 9$) buffer. The tension records were normalized to the KCl contraction obtained in each preparation. The vertical bars show the size of the standard errors of the means. The line through the data was fitted by linear regression; the r^2 value was 0·70 and the slope of the line was $53·43 \pm 1·52$; the slope of the best-fit lines to the Hepes data was $51·74 \pm 1·52$, and to the HCO_3^-–CO_2 data was $54·80 \pm 2·55$.

The relation between pH_i and pH_o

The extent of the change in pH_i following change in pH_o was investigated in the vascular cells and strips over a pH range up to a 1·0 pH unit change. Figure 3 shows a graph of change in pH_i *vs.* change in pH_o. A ratio of 0·73 for the change in pH_i per

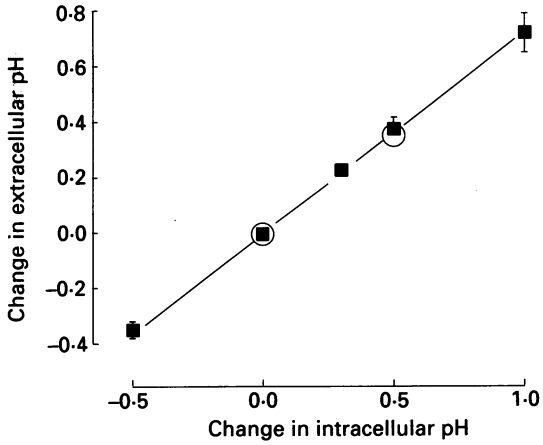

Fig. 3. The relation between the change in pH_o and the subsequent mean steady-state change in pH_i ($n = 7$). The initial pH_o was 7·4; shown as zero change on the figure. The regression line fitted has an r^2 of 0·99 and a slope of 0·73 ± 0·03. Vertical bars indicate the standard errors of the means. The circles represent data obtained in HCO_3^-–CO_2 buffer ($n = 10$). This was not significantly different from data in Hepes buffer.

unit change of pH_o was obtained. The relation appears linear over the pH_o range examined and is the same in Hepes and HCO_3^- buffers. Thus even small changes in pH_o will significantly alter pH_i, and therefore affect function.

DISCUSSION

By measuring simultaneously pH_i and tension when pH_o is changed it has been possible to show that the effects of pH_o on tension can be accounted for by the induced change in pH_i. Thus if the induced change in pH_i is prevented or abolished, no effects on vascular tension are seen. The effect on tension was related to the magnitude of the pH_i change, and no difference was found whether this pH_i change was brought about directly (by using weak acids and bases) or via changing pH_o. Thus any discussion concerning the mechanism of how pH_o affects pH_i should be focused upon how pH_i affects force in smooth muscle (see below).

It was found that pH_i changed rapidly ($t_{0.5}$ around 35 s) in vascular smooth muscle cells when the pH_o was altered. When pH_o is changed by using strong acid and base in other cells, much slower changes in pH_i occur, e.g. pH_i stabilizes in 15–40 min in cardiac cells (Ellis & Thomas, 1976) and 6–12 min in vas deferens smooth muscle (Aickin, 1984). Although the mechanism that underlies this rapid passage of H^+ across the vascular smooth muscle cell membrane is unclear, the results with amiloride and DIDS suggest that pH regulating mechanisms are not involved. The results are consistent with there being a large passive entry of H^+ across the

membrane. A high permeability in these vascular smooth muscle cells could be an adaptive feature, allowing rapid response of the vascular smooth muscle to changes in plasma pH. Meech & Thomas (1987) reported a large increase in H^+ permeability at positive membrane potentials in snail neurons. Further work is needed, however, to confirm a role of large passive H^+ entry across the smooth muscle vascular cell membrane. Previous studies of the effect of changing pH_o on contraction have noted how quickly tone changes in response to the alteration in pH_o. Indeed this speed was used as an argument against changes in pH_i being involved in the mechanism underlying the tone change, as it was assumed pH_i would change too slowly (e.g. Harder & Madden, 1985). However, the results reported in this paper show that pH_i changes rapidly enough to explain the tone changes observed.

It is not clear, in any cell, what determines the extent of the pH_i change when pH_o is altered, although different contributions from passive proton influx, active extrusion of protons and intracellular acid production will all be involved. The effect of changes of pH_i upon pH_o in the vascular smooth muscle cells was large ($0{\cdot}73 \pm 0{\cdot}03$), compared to most other cells. Previous studies have reported that, per unit change of pH_o, the change in pH_i has been 0·2–0·4 pH unit (Ellis & Thomas, 1976; Tolkovsky & Richards 1987; Wray 1988). The pH_i changes in the mesenteric smooth muscle cells are similar to those reported in a smooth muscle-like cell line (Putnam & Grubbs, 1990) and in carotid body cells, which are chemotransducers (Buckler *et al.* 1991). These last authors drew attention to the possible physiological implication of this high ratio, which allows changes of pH_o to be transmitted to the cytoplasm where they may then influence chemotransduction. The same argument also applies to the high ratio found in these vascular smooth muscle cells, i.e. small changes in plasma pH will affect vascular tone.

It is not known exactly how changes in pH_i affect contraction, and several possible mechanisms have been proposed (Wray, 1988). Harder & Madden (1985) found that elevation of pH_o in cerebral arteries resulted in membrane depolarization and reduction in outward K^+ conductance. Such depolarization would lead to Ca^{2+} entry, and indeed, if Ca^{2+} entry is blocked, the contraction to elevation of pH_o has been shown to be blocked in basilar artery (West, Leppla & Simard, 1992). pH_o has also been shown to modulate Ca^{2+} channel currents in this tissue (West *et al.* 1992). Thus it is suggested that alteration of pH_o causes corresponding alteration of pH_i, which in turn alters surface membrane excitation and Ca^{2+} entry, although other effects, e.g. on sarcoplasmic reticulum calcium release (Dettbarn & Palade, 1991) and myofilament sensitivity, cannot be excluded. Clearly, measurement of $[Ca^{2+}]_i$ in vascular strips (as opposed to single cells) with simultaneous tension measurement will add to our understanding of the mechanism.

These results show that the effects of a change in pH_o on vascular smooth muscle tone are mediated via a change of pH_i. The effects of pH_o on pH_i are faster and larger than in most other muscle and non-muscle cells. Thus small changes in pH_o, such as occur in exercise or hypoxia or with metabolic disturbance, will be functionally important. The exquisite sensitivity of pH_i to extracellular pH change in vascular smooth muscle, compared to cardiac muscle, may be functionally important for control of the circulation, as it allows a plasma acidosis to dilate arterioles without compromising cardiac output.

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