Stimulation of pulmonary C fibres by lactic acid in rats: contributions of H^+ and lactate ions

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1. The contributions of H^+ and lactate ions to the stimulation of single pulmonary C fibres by lactic acid were examined in anaesthetized and artificially ventilated rats.

2. Lactic acid injected into the right atrium caused a transient decrease in arterial blood pH (pH_a) and a short but intense burst of afferent activities in pulmonary C fibres, whereas sodium lactate had no effect. The fibre activity usually reached a peak within 1–1·5 s, with an onset latency of < 1 s, and returned to the baseline in 5 s.

3. The injection of hydrochloric acid at the same pH as that of lactic acid did not significantly decrease pH_a, nor did it stimulate any C fibres studied.

4. Formic acid has a pK_a value (the negative logarithm of the dissociation constant) almost identical to that of lactic acid; thus, its injection decreased pH_a to the same degree as did the injection of lactic acid. However, the response of C fibres to lactic acid was 134% stronger than that to formic acid.

5. We conclude that H^+ is primarily responsible for the activation of pulmonary C fibres by lactic acid, probably through a direct effect of H^+ on these afferent endings. The lactate ion, by itself, does not activate C fibres, but it seems to potentiate the stimulatory effect of H^+ on these afferents.

Low-pH solutions administered by aerosol inhalation or by systemic injection or perfusion have been shown to cause airway irritation and reflex bronchoconstriction (Allott, Evans & Marshall, 1980; Forsberg, Karlsson, Theodorsson, Lundberg & Persson, 1988; Satoh, Lou & Lundberg, 1993), to induce pulmonary chemoreflexes (Trenchard, 1986; Lee, Morton & Lundberg, 1997), and to release sensory neuropeptides (Franco-Cereceda & Lundberg, 1992) due to an activation of bronchopulmonary C fibres (Fox, Urban, Barnes & Dray, 1995; Lee, Morton & Lundberg, 1996). However, some of the previous studies (Allott *et al.* 1980; Forsberg *et al.* 1988) used certain acids that do not exist in significant amounts in the body, while others (Fox *et al.* 1995) used low-pH buffer solutions. Consequently, although all these acids or low-pH buffer solutions contain high concentrations of H^+, they do not provide appropriate conjugate bases (e.g. the lactate ion in lactic acid) that coexist with the H^+ in the body fluid during local or systemic acidosis. Lactic acid, which is dissociated into H^+ and lactate ions, is commonly produced in the body during physiological (e.g. severe exercise) or pathological (e.g. tissue hypoxia) anaerobic metabolism. Lactic acid has been shown to activate pulmonary C fibres (Lee *et al.* 1996) and to release neuropeptides from sensory nerves (Franco-Cereceda & Lundberg, 1992). However, the relative contributions of H^+ and lactate ions to the activation of C fibres by lactic acid is still not completely clear. Although a low-pH solution that does not contain any lactate ions can activate tracheal C fibres (Fox *et al.* 1995), several investigators have reported that the combination of H^+ and lactate ions is more potent than H^+ alone in the activation of C fibres in skeletal muscles (Thimm, Carvalho, Babka & Meier Zu Verl, 1984; Thimm & Baum, 1987) and abdominal visceral organs (Stahl & Longhurst, 1992), indicating an important role for the lactate ion.

The purposes of the present study were: (1) to determine whether H^+ is primarily responsible for the activation of pulmonary C fibres by lactic acid and whether the lactate ion, by itself, activates these afferents; and (2) to examine whether the stimulatory effect of H^+ on C fibres is altered by the presence of the lactate ion.

METHODS

Animal preparation

The procedures described below were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA and also were approved by the University of Kentucky Institutional Animal Care and Use Committee.

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Male Sprague–Dawley rats (325–409 g) were anaesthetized initially with an intraperitoneal injection of α-chloralose (~100 mg kg^{-1}, Sigma) and urethane (~500 mg kg^{-1}, Sigma) dissolved in a 2% borax (Sigma) solution; supplemental doses of the same anaesthetics were given, whenever necessary, to maintain abolition of pain reflex on paw-pinch. The left femoral artery and the left jugular vein were cannulated for recording arterial blood pressure and for injections, respectively. The jugular venous catheter was advanced until its tip was slightly above the right atrium; in some animals, the right carotid artery was also cannulated and the catheter was advanced until its tip was placed in the left ventricle. The positions of the catheter tips were monitored by pressure tracings and confirmed by post-mortem examination after the experiments. The trachea was cannulated, and the rats were artificially ventilated with a respirator (UGO Basile 7025, Comerio-Varese, Italy). The tidal volume (V_T) and the respiratory frequency were set at 8–10 ml kg^{-1} and 44 breaths min^{-1}, respectively, and were kept constant during the experiment; the end-tidal CO_2 concentration in such a setting was within physiological limits (4·5–5·1%). A midline thoracotomy was performed, and the expiratory outlet of the respirator was placed under 3 cmH_2O pressure to maintain a near-normal functional residual capacity. Body temperature was maintained at 36–37 °C throughout the experiment by means of a heating pad placed under the animal lying in a supine position. Animals were killed at the end of the experiments by an intravenous injection of an overdose of pentobarbitone.

Recordings of single pulmonary C fibres

The right cervical vagus nerve was separated from the carotid artery and sectioned rostrally. The caudal end of the cut vagus nerve was placed on a small dissecting platform and covered with mineral oil; its sheath was then removed. With the aid of a dissecting microscope and fine-tip forceps, a fine filament was teased away from the nerve trunk and placed on a platinum–iridium hook electrode. Action potentials were amplified (Grass P5-11K), monitored by an audio monitor (Grass AM8RS), and displayed on a video monitor (Gould ES2000). The thin filament was further split until the afferent activity from a single unit was electrically isolated. Both vagus nerves (and the oesophagus) were ligated just above the diaphragm to eliminate the electrical signals arising from visceral organs below the diaphragm. Pancuronium bromide (80 μg kg^{-1} I.V., Elkins-Sinn Pharmaceuticals) was given as a bolus to avoid any possible electrical artifact resulting from animal movement that might interfere with the recording of electrophysiological signals (Lee & Morton, 1995); the effect of pancuronium bromide at this dose lasted 40–60 min. During the neuromuscular blockade, supplemental doses of anaesthetics were administered intravenously whenever significant changes (> 10%) in heart rate or blood pressure were observed in response to paw-pinch.

Since vagal pulmonary C fibres usually have a sparse (< 1 impulse s^{-1}) and irregular discharge during artificial ventilation (Coleridge & Coleridge, 1984), hyperinflation of the lungs (3–4 V_T) was used as the first step in searching for these fibres to minimize the total dose of capsaicin used in each animal; in general, there was no clear adaptation in C fibre responses to hyperinflation (e.g. Fig. 1*A*). Once the afferent activity of a single unit was electrically isolated during hyperinflation, capsaicin was injected as a bolus (0·2 ml) via the venous catheter into the right atrium. Due to the different sensitivities, the dose of capsaicin (0·5–2 μg kg^{-1}) needed to evoke an afferent stimulation varied among C fibres. Only fibres that were activated within 2 s after the injection were studied. In nine C fibres identified by the immediate response to right-atrial injection of capsaicin, none were activated by a bolus injection of the same dose of capsaicin into the left ventricle (e.g. Fig. 1), confirming that they were pulmonary C fibres (Coleridge & Coleridge, 1984). To measure the conduction velocities in these afferent fibres, we transected the right clavicle of the rats and isolated the intrathoracic vagus nerve as caudally as possible. A pair of stimulating electrodes were placed under the vagus nerve close to the origin of the right subclavian artery to deliver rectangular constant-current pulses (duration, 1 ms; intensity, 0·3–3 mA) generated by a pulse generator (A310 Accupulser, World Precision Instruments) and a stimulus isolation unit (A360R-C, World Precision Instruments). The conduction velocity was then calculated by dividing the distance between the stimulating and the recording electrodes (15–20 mm) by the time elapsed between the onset of the electrical shock artifact and the action potential. Finally, the general locations of most C fibres (48/63) were identified by their responses to gentle pressing of the lungs with a cotton Q-tip or a blunt-ended glass rod after the experiment. The signals of the afferent activities, tracheal pressure (Validyne MP 45–28), and arterial blood pressure (Statham P23AA) were recorded on a Gould Thermal Writer (TW11) and on a videocassette recorder (Vetter 500H, PA, USA). Fibre activity (FA, in impulses s^{-1}) was analysed later by a computer for each 0·5 s interval. The baseline FA_{mean} (the mean value over the 5 s period just before the injection) and peak FA_{mean} (the mean value over the 2 s period immediately after the injection) were further calculated. A C fibre was considered to be activated if the peak FA_{mean} was 2 impulses s^{-1} higher than the baseline FA_{mean}.

Measurements of arterial blood pH (pH_a) and lactate levels

To avoid the electrical noises possibly generated by the procedure of collecting blood samples during the nerve recordings, changes in pH_a and blood lactate levels caused by acid injections were measured in different groups of rats prepared and ventilated identically to those in the nerve recording experiments. Through a carotid arterial catheter with its tip placed close to the left ventricle, we collected consecutive arterial blood samples immediately after the acid injection to detect the lowest pH_a that could best represent the immediate effect of acids on pH_a (however, it took ~5 s to obtain the minimum volume (~0·1 ml) of blood required by the pH–blood gas analyser; Instrumentation Laboratory 1306, Italy). Blood lactate levels before and after lactic acid injections were also measured (Ektachem 700 XR, Johnson & Johnson Clinical Diagnostics, Inc., NY, USA).

Experimental design

Four series of experiments were carried out to examine the effects of changes in the dose of lactic acid (study series 1), the dose of H^+ (study series 2 and 3), or the dose of lactate ions (study series 4) on the pulmonary C fibre afferent activities. L(+)-lactic acid (crystal or 30% solution, w/v), formic acid, and sodium lactate (all from Sigma) were dissolved in or diluted with distilled water. The final concentrations (0·325–0·409 mol l^{-1}) and pH (2·1–2·2) of the lactic acid or formic acid solutions at the dose of 0·2 mmol kg^{-1} varied slightly among animals because of the different body weights of the animals and the fixed volume (0·2 ml) of each injection. HCl (1 mol l^{-1}; Sigma) was diluted with saline to 0·01 mol l^{-1}, and the pH at this concentration was 2·1. All solutions were injected at room temperature (22–24 °C).

To achieve a balanced design, the sequence of the acid injections in each study series was alternated between C fibres (in nerve recording studies) or between animals (in pH_a measurements). A recovery time of at least 10 min was allowed between injections. Between the tests on two consecutive C fibres in the same animal, sodium bicarbonate (Sigma), equivalent to half to two-thirds of the

molar dose of the acid injected earlier, was given to restore the buffer base in the body.

Statistical analysis

Unless otherwise mentioned, a two-way ANOVA was used for the statistical analysis. One factor of the two-way ANOVA was the effect of acid injection; the other factor was the dose or the type of acid. When the two-way ANOVA showed a significant interaction, pairwise comparisons were made with a *post hoc* analysis (Fisher's least significant difference). Data are expressed as means ± S.E.M. P values $< 0{\cdot}05$ were considered significant.

RESULTS

A total of sixty-three pulmonary C fibres were studied; among them, only eight fibres were tested in more than one series of experiments. The locations of these C fibre endings were as follows: fourteen in the cranial lobe of the right lung; nine in the right middle lobe; fourteen in the right caudal lobe; nine in the right accessory lobe; and two in the vicinity of the right hilum. The locations of the remaining fifteen fibres were either not sought (11/15) or not identified (4/15). Although right-atrial injection of lactic acid at a dose of 0·2 mmol kg^{-1} activated 92% (58/63) of C fibres that responded to capsaicin, the relative sensitivities to capsaicin and lactic acid varied among individual C fibres; for example, the response to capsaicin (1 μg kg^{-1}) was stronger than that to lactic acid (0·2 mmol kg^{-1}) in 44% of C fibres while the opposite was true in the other 56%. Almost all C fibres (56/58) started discharge within 1 s after injection of lactic acid, and the average time for these fibres to reach the peak response after the injection was between 1 and

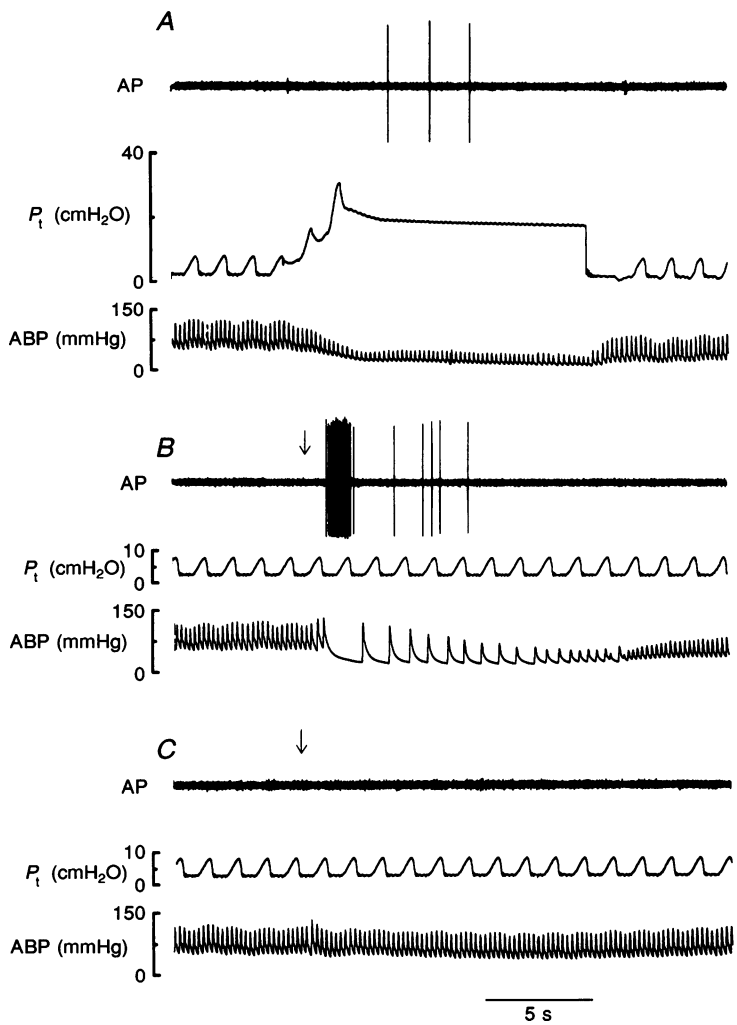

Figure 1. Experimental record illustrating the responses of a pulmonary C fibre arising from an ending in the accessory lobe of the right lung in an anaesthetized, paralysed and open-chest rat

AP, action potentials; P_t, tracheal pressure; ABP, arterial blood pressure. *A*, response to lung hyperinflation that was produced by occluding expiratory line of the respirator for three consecutive breaths and then prolonged by turning off the respirator. *B*, right-atrial injection of capsaicin (1 μg kg^{-1}); *C*, left-ventricular injection of capsaicin (1 μg kg^{-1}). Injectate (0·2 ml) was first slowly injected into the catheter (dead space, 0·3 ml) and then flushed, at the arrow, as a bolus with saline. At least 10 min elapsed between injections. Rat body weight, 386 g.

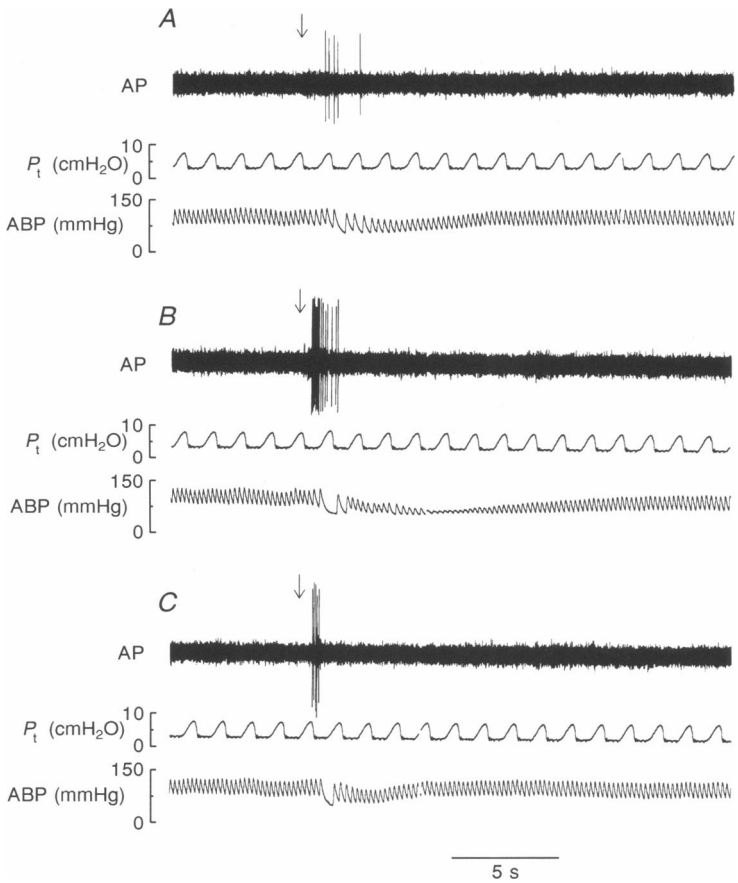

Figure 2. Experimental record illustrating the responses of a pulmonary C fibre arising from an ending in the right cranial lobe to the right-atrial injection of lactic acid in an anaesthetized, paralysed and open-chest rat

Traces show responses to injection of 1 μg kg^{-1} capsaicin (*A*), 0·2 mmol kg^{-1} lactic acid (*B*) and 0·1 mmol kg^{-1} lactic acid (*C*). See Fig. 1 legend for further explanation. Rat body weight, 409 g.

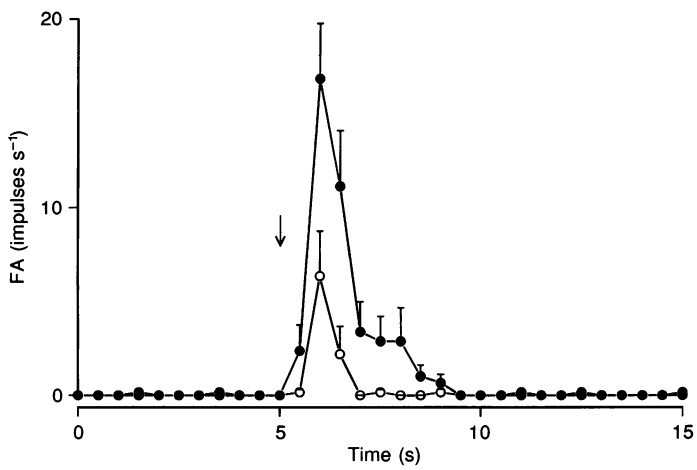

Figure 3. Average responses in 12 pulmonary C fibres from 5 anaesthetized, paralysed, and open-chest rats to lactic acid injected into the right atrium (arrow)

Fibre activity (FA) was measured in 0·5 s intervals. ○, 0·1 mmol kg^{-1} lactic acid; ●, 0·2 mmol kg^{-1}. Data are means ± S.E.M.

1·5 s. Afferent activities in most C fibres returned to their baselines within 5 s after the injection of lactic acid. The conduction velocity measured in twelve C fibres that were activated by both capsaicin and lactic acid was $1·0 \pm 0·1$ m s^{-1} (range, 0·65–1·5 m s^{-1}) and was within the range described for non-myelinated fibres (Coleridge & Coleridge, 1984).

Study series 1: dose dependence of lactic acid-induced C fibre activities, cardiovascular responses and pH_a

Right-atrial injections of lactic acid at a dose of 0·1 mmol kg^{-1} activated five of twelve C fibres from five rats (Figs 2 and 3), and the peak FA_{mean} after the injection of lactic acid ($2·2 \pm 0·8$ impulses s^{-1}) was significantly higher than the baseline FA_{mean} ($0·03 \pm 0·03$ impulses s^{-1}, $P < 0·05$; Fig. 4*A*). Immediately after injection, the heart rate decreased from a baseline of 313 ± 22 beats min^{-1} to 267 ± 30 beats min^{-1} and the mean arterial blood pressure dropped from a baseline of 87 ± 5 mmHg to 73 ± 5 mmHg ($P < 0·05$). Lactic acid at a dose of 0·2 mmol kg^{-1} activated eleven of these twelve pulmonary C fibres, and the baseline and peak FA_{mean} were $0·0 \pm 0·0$ and $9·2 \pm 1·6$ impulses s^{-1} ($P < 0·01$), respectively; the response was significantly higher than that after the dose of 0·1 mmol kg^{-1} ($P < 0·01$, Fig. 4*A*). Lactic acid at a dose of 0·2 mmol kg^{-1} also induced more intense and longer-lasting decreases in the heart rate (baseline, 324 ± 19 beats min^{-1}; after lactic acid, 194 ± 35 beats min^{-1}; $P < 0·05$) and in the mean arterial blood pressure (baseline, 92 ± 1 mmHg; after lactic acid, 50 ± 5 mmHg; $P < 0·05$; e.g. Fig. 2). Corresponding to the fibre activity and cardiovascular responses, pH_a after the injection of 0·2 mmol kg^{-1} lactic acid ($7·09 \pm 0·03$) was significantly lower than that after the injection of 0·1 mmol kg^{-1} lactic acid ($7·29 \pm 0·01$, $P < 0·01$, $n = 7$; Fig. 4*B*). The time course of pH_a changes after lactic acid injection (0·2 mmol kg^{-1}) is shown in Fig. 4*C*; pH_a measured at 10–15 s after injection ($7·44 \pm 0·02$) had already returned to the baseline ($7·47 \pm 0·04$, $P > 0·05$, one-way ANOVA). Blood lactate concentration was raised from a baseline of $2·1 \pm 0·1$ mmol l^{-1} to $4·2 \pm 0·6$ mmol l^{-1} ($P < 0·05$, paired t test; $n = 5$) at ~40 s after the injection of lactic acid (0·2 mmol kg^{-1}) and returned to the baseline within 20 min ($1·8 \pm 0·1$ mmol l^{-1}, $n = 3$).

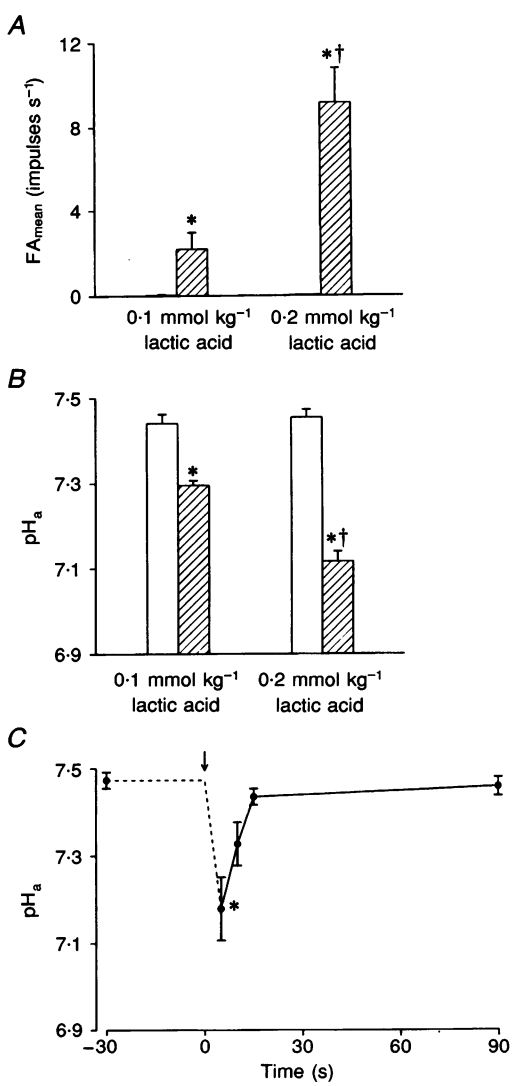

Figure 4. Dose dependence of lactic acid-induced pulmonary C fibre afferent activities and decreases in pH_a

A, the open bars representing the near zero values of baseline fibre activity (baseline FA_{mean}), averaged over the 5 s period before the injection, can barely be seen. The peak FA_{mean} (▨) is the average response over the 2 s period immediately after right-atrial injection of lactic acid in the same 12 C fibres from 5 anaesthetized, paralysed and open-chest rats. *B*, baseline pH_a (□) and the pH_a after injection of lactic acid (▨) in 7 rats. *C*, time course of pH_a changes after the injection of lactic acid (0·2 mmol kg^{-1}, arrow) in 4 of the 7 rats shown in *B*. * $P < 0·01$, compared with baseline (before injection); † $P < 0·01$, compared with the response after injection of 0·1 mmol kg^{-1} lactic acid. Data are means ± S.E.M.

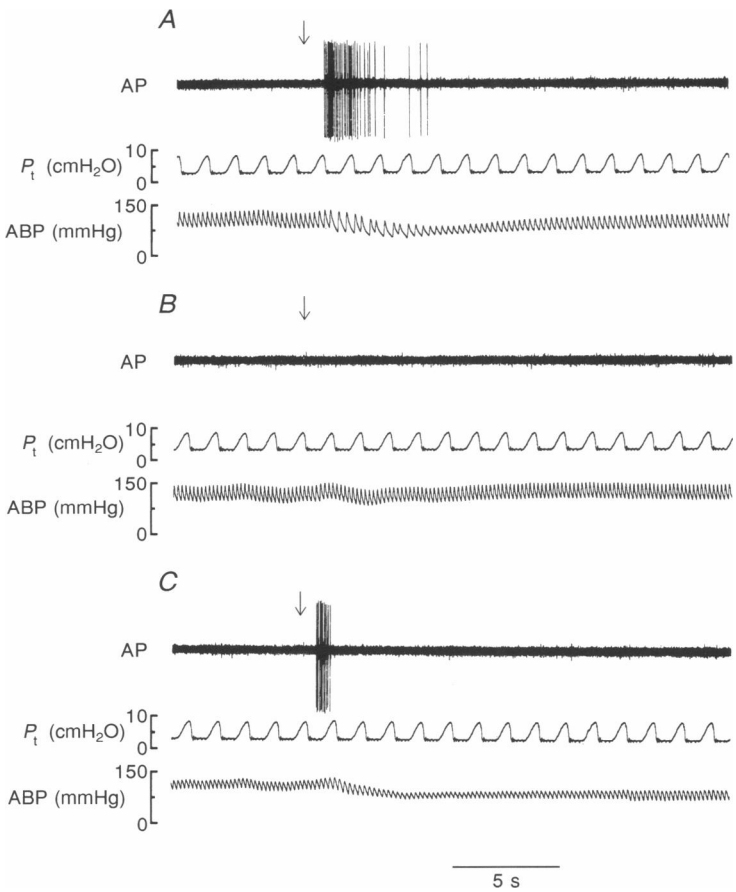

Figure 5. Experimental record illustrating the responses of a pulmonary C fibre arising from an ending in the middle lobe of the right lung to right-atrial injections of sodium lactate and lactic acid solutions in an anaesthetized, paralysed and open-chest rat

Traces show responses to injection of 2 μg kg^{-1} capsaicin (*A*), 0·2 mmol kg^{-1} sodium lactate (*B*) and 0·2 mmol kg^{-1} lactic acid (*C*). See Fig. 1 legend for further explanation. Rat body weight, 344 g.

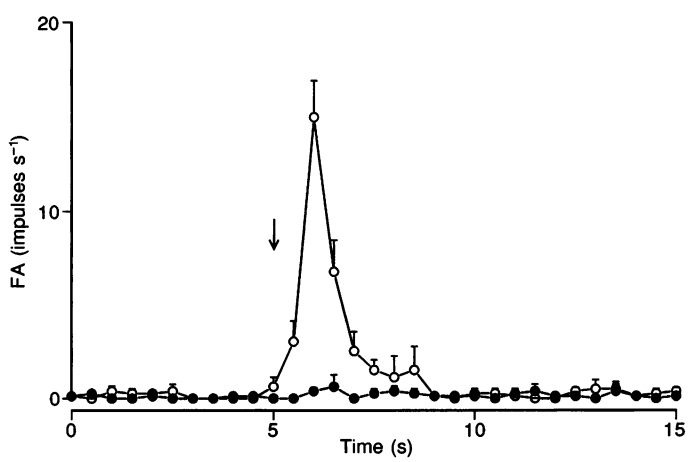

Figure 6. Average responses in 15 pulmonary C fibres from 6 anaesthetized, paralysed, and open-chest rats to lactic acid and sodium lactate injected into the right atrium (arrow)

Fibre activity (FA) was measured in 0·5 s intervals. ○, lactic acid (0·2 mmol kg^{-1}); ●, sodium lactate (0·2 mmol kg^{-1}).

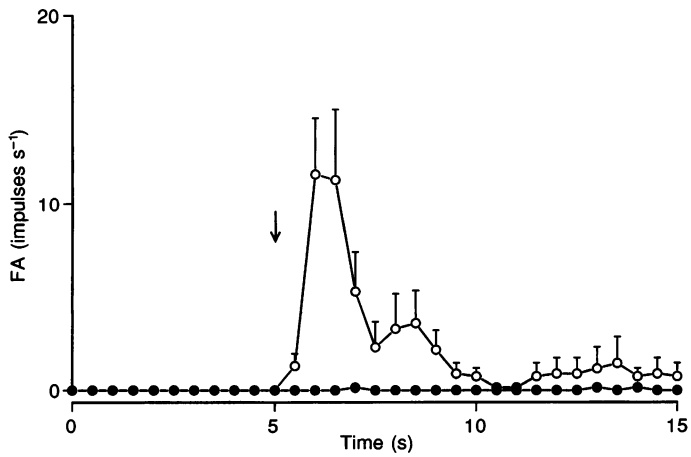

Figure 7. Average responses in 14 pulmonary C fibres from 5 anaesthetized, paralysed, and open-chest rats to lactic acid and hydrochloric acid injected into the right atrium

Rats were injected (at arrow) with 0·2 mmol kg^{-1} lactic acid (○) and 0·01 mmol l^{-1} hydrochloric acid (●, 0·2 ml). The pH (2·1–2·2) of these two acid solutions was approximately the same. Fibre activity (FA) was measured in 0·5 s intervals.

Study series 2: effects of lactic acid *vs.* sodium lactate on C fibre afferent activities

Right-atrial injection of 0·2 mmol kg^{-1} lactic acid activated fourteen of the fifteen fibres studied in six rats, whereas sodium lactate injection at the same dose did not activate any of them. Typical responses of a single C fibre and the average responses of these fifteen C fibres are shown in Figs 5 and 6, respectively. The values of baseline FA_{mean} before and peak FA_{mean} after the injection of lactic acid were 0·1 ± 0·1 and 16·7 ± 3·0 impulses s^{-1} ($P < 0·01$), respectively, whereas the baseline FA_{mean} before and peak FA_{mean} after sodium lactate injections were not significantly different ($P > 0·05$).

Study series 3: effects of lactic acid *vs.* HCl on pH_a and C fibre activities

Although the injection of 0·2 mmol kg^{-1} lactic acid (pH 2·1–2·2) activated twelve of fourteen C fibres from five rats, the same volume of HCl at the same pH (2·1) as lactic acid did not activate any of these C fibres (Fig. 7). Correspondingly, pH_a decreased significantly from a baseline of 7·45 ± 0·02 to 7·06 ± 0·05 ($n = 7$, $P < 0·01$) after lactic acid injection, whereas in the same animals the pH_a level after HCl injection (7·40 ± 0·02) was not significantly lower than the baseline (7·44 ± 0·02, $P > 0·05$, Fig. 8).

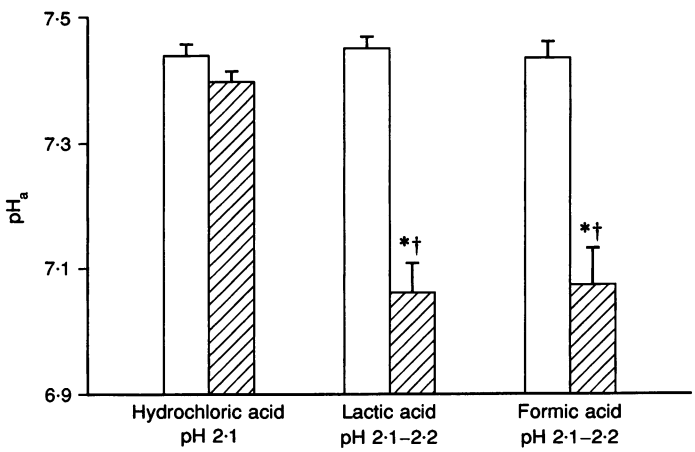

Figure 8. Effects of right-atrial injections of hydrochloric acid, lactic acid and formic acid on pH_a

Rats were injected with hydrochloric acid (0·01 mol l^{-1}, 0·2 ml), lactic acid (0·2 mmol kg^{-1}) and formic acid (0·2 mmol kg^{-1}). The pH of these three solutions was approximately the same. □, baseline pH_a; ▨, pH_a after injection of acids. * $P < 0·01$, compared with baseline; † $P < 0·01$, compared with pH_a after injection of HCl. $n = 7$ rats.

Study series 4: effects of lactic acid *vs.* formic acid on pH_a and C fibre activities

Right-atrial injection of formic acid (0·2 mmol kg^{-1}) decreased pH_a from a baseline of 7·44 ± 0·03 to 7·07 ± 0·03 ($n = 7$, $P < 0·01$), which was almost identical to the pH_a decrease after the injection of the same dose of lactic acid (from 7·45 ± 0·02 to 7·06 ± 0·05, $P < 0·01$, Fig. 8) in the same animals. Interestingly, seventeen of eighteen C fibres from nine rats were activated by lactic acid, whereas only nine of them were activated by formic acid (Fig. 9). Furthermore, the intensity of response to lactic acid of each of the C fibres was stronger than that to formic acid (Fig. 9*B*), and the peak FA_{mean} after lactic acid injection (7·0 ± 1·1 impulses s^{-1}) was 134% higher than that after formic acid injection (3·0 ± 0·8 impulses s^{-1}, $P < 0·01$, Fig. 9*B*).

DISCUSSION

According to the Henderson–Hasselbalch equation,

$$pH = pK_a + \log[A^-/HA],$$

where HA is an undissociated acid and A^- is its conjugate base, the degree of dissociation of a weak acid such as lactic acid and, therefore, the amount of H^+ provided by the acid is determined by the pH of the medium and pK_a (the negative logarithm of the dissociation constant, K_a) of the acid. Because extracellular pH under normal physiological conditions is 7·4, and because the pK_a of lactic acid is 3·79, more than 99·9% of lactic acid molecules are dissociated into H^+ and lactate ions in the body. The near-full dissociation should hold true even when pH_a drops to 7·06 (Fig. 4*B*) after lactic acid injections in the present study.

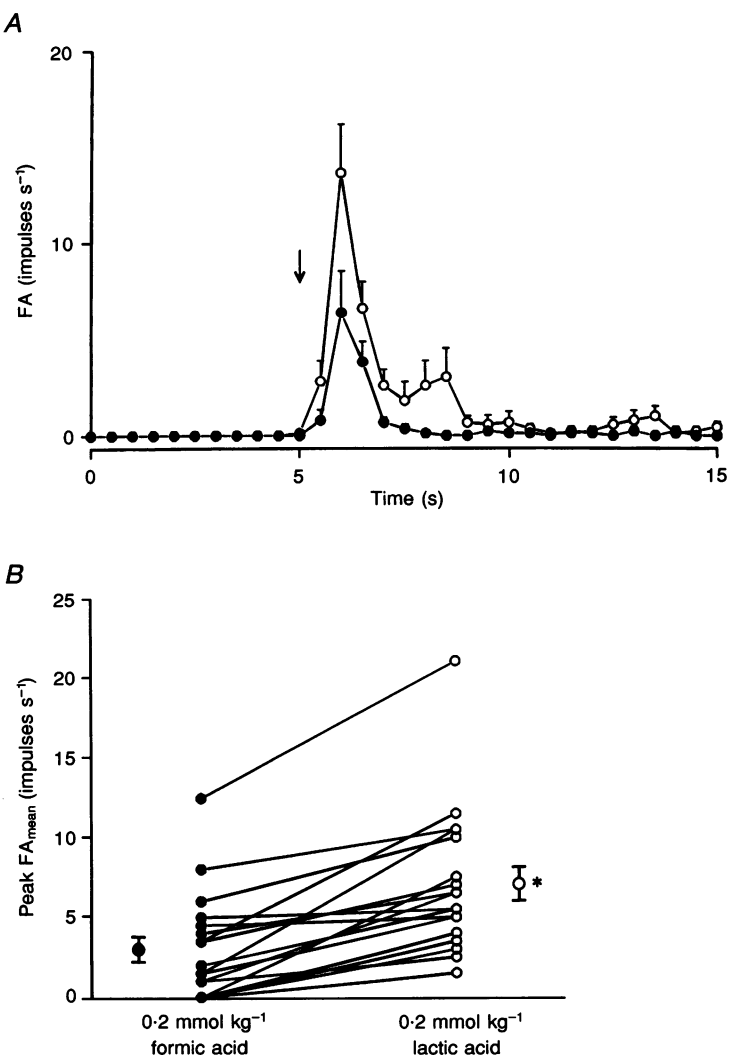

Figure 9. Comparison between responses to lactic acid and formic acid

A, average responses in 18 pulmonary C fibres from 9 anaesthetized, paralysed, and open-chest rats to lactic acid (○, 0·2 mmol kg^{-1}) and formic acid (●, 0·2 mmol kg^{-1}) injected into right atrium (arrow). Fibre activity (FA) was measured in 0·5 s intervals. *B*, comparison between peak FA_{mean}, averaged over the 2 s period immediately after injection of lactic acid or formic acid in each single C fibre (pair of circles). The two circles at both sides are means ± S.E.M. of 18 fibres. * $P < 0·01$, compared with the response after injection of formic acid.

Thus, any effect of exogenous or endogenous lactic acid could result from the effect of H^+, that of the lactate ion, or the interaction between the two. Although previous studies have shown that lactic acid induces pulmonary chemoreflexes (Trenchard, 1986; Lee *et al.* 1996) and sensory neuropeptide release (Franco-Cereceda & Lundberg, 1992) by activating bronchopulmonary C fibres (Fox *et al.* 1995; Lee *et al.* 1996), the relative contributions of H^+ and lactate ions to the stimulatory effect of lactic acid on these afferents were not studied. Our results showed that lactic acid activated 92% (58/63) of the pulmonary C fibres, whereas sodium lactate, which provides equimolar lactate ions but not H^+, did not activate any of these C fibres (Figs 5 and 6), indicating that H^+ is primarily responsible for the activation of C fibres by lactic acid, and that the lactate ion, by itself, does not activate C fibres. This conclusion is supported by the clear dose-dependent relationship of the lactic acid-induced changes in pH_a, cardiovascular reflex responses, and C fibre afferent activities (Fig. 4). The stimulatory effect of lactic acid on C fibre endings is not due to a change in blood osmolarity because equimolar sodium lactate, with a higher osmolarity than lactic acid, did not activate any C fibres (Figs 5 and 6). Our findings further demonstrate that the amount of H^+ available from the acid solution after it is injected into the blood, instead of the pH (H^+ concentration) of the acid solution, determines its potency in activating C fibres. Thus, injection of HCl solution with the same pH as that of the lactic acid, but at lower molar dose, did not significantly decrease pH_a (Fig. 8) and, as expected, did not activate the C fibres (Fig. 7), lending additional support to our conclusion.

The mechanism by which H^+ activates bronchopulmonary C fibres is not totally clear. Several studies have shown that the release of neuropeptides from sensory nerve endings by lactic acid and low-pH buffers is blocked by capsazepine, a 'capsaicin receptor' antagonist, suggesting that 'capsaicin receptors' are activated during acid stimulation (Franco-Cereceda & Lundberg, 1992; Lou & Lundberg, 1992). In addition, low-pH solutions activate a sustained cation conductance in a subpopulation of isolated rat dorsal root ganglion neurons that are capsaicin sensitive; the cation conductance and the capsaicin-induced current are coregulated by nerve growth factor, indicating a direct effect of H^+ on 'capsaicin receptors' on the soma membranes of C-type neurons (Bevan & Yeats, 1991; Bevan & Winter, 1995). It is also reported that the activation of vagal C fibres by a low-pH solution applied to their receptive fields in the trachea and bronchi is blocked by capsazepine (Fox *et al.* 1995). This blocking effect is not observed, however, in the depolarization of vagal C fibres caused by the same low-pH solution applied to the isolated whole vagus nerve (Fox *et al.* 1995), suggesting that the activation of C fibre terminals in the airways is mediated through an endogenous ligand acting as a 'capsaicin receptor' agonist, presumably released by the cells surrounding the nerve terminals upon acid administration (Fox *et al.* 1995). Similarly, other studies have suggested that cyclo-oxygenase products may be involved in mediating the effects of acid on non-myelinated afferent fibres in the lungs (Shams, Peskar & Scheid, 1988; Karla, Shams, Orr & Scheid, 1992), in the gastrointestinal system (Longhurst & Dittman, 1987; Longhurst, Rotto, Kaufman & Stahl, 1991), and in the heart (Franco-Cereceda, Källner & Lundberg, 1994). The involvement of acid-induced cyclo-oxygenase metabolites in the present study can be ruled out because, as shown in a recent study, indomethacin does not block pulmonary chemoreflex in rats induced by the same dose of lactic acid (Lee *et al.* 1996) as that used in the present study. Furthermore, the activation of most C fibres by lactic acid had a latency of less than 1 s (e.g. Figs 2 and 5), and the peak response was reached approximately 1–1·5 s after the injections (Figs 3, 6, 7 and 9); neither the latency nor the time required to reach the peak response was longer than that after the injection of capsaicin in the present study. In addition, in accordance with the immediate recovery of pH_a (Fig. 4*C*), the afferent activities in most C fibres induced by lactic acid returned to their baselines within 5 s after lactic acid injections (Figs 3, 6, 7 and 9). The rapid onset and the transient duration of C fibre responses seem to suggest that H^+ directly activates pulmonary C fibre endings.

Because formic acid has a pK_a (3·74 at 25 °C) almost identical to that of lactic acid (3·79 at 25 °C), it is expected to dissociate to the same degree in the blood, and thus to provide equimolar H^+ as lactic acid at the same dose. This expectation is confirmed by the fact that the decreases in pH_a after injections of formic acid and lactic acid were almost identical (Fig. 8). However, the C fibre response to lactic acid was 134% stronger than that to formic acid (Fig. 9), suggesting that the stimulatory effect of H^+ on pulmonary C fibres is stronger in the presence of lactate ions. The important role of lactate ions in the activation of C fibres by lactic acid in other organs has been previously reported. For example, lactic acid injected into the descending aorta in the cat decreases pH levels in the gastrointestinal tissues less than does hypercapnia, whereas lactic acid activates a larger proportion of ischaemically sensitive C fibres from the abdominal visceral organs than does hypercapnia (Stahl & Longhurst, 1992). In the vascularly isolated hindleg of rats, perfusion of low-pH Tyrode solution containing a higher lactate concentration induces a greater reflexogenic increase in heart rate, which is believed to be mediated through group III and IV (C fibres) afferent nerve fibres, than does perfusion with the same solution containing a lower lactate concentration (Thimm *et al.* 1984; Thimm & Baum, 1987). The mechanisms underlying the enhanced C fibre responses to H^+ in the presence of lactate ions are not known. Recently, it has been reported that a lactate–H^+ cotransporter in rat spinal roots is responsible for the interstitial acidification of these peripheral nerves during hypoxia by cotransporting H^+ and lactate ions out of the nerve axons and/or Schwann cells (Schneider, Poole, Halestrap & Grafe, 1993). Because this is a facilitated transport system, the direction and the rate of the transport process are dependent on H^+ and

lactate ion gradients across the cell membrane (Poole & Halestrap, 1993). If this cotransport system does exist on the plasma membranes of pulmonary C fibre terminals, as reported in other cultured neurons (Walz & Mukerji, 1988), the high concentrations of H^+ and lactate ions in the interstitial fluid after the injection of lactic acid could conceivably induce a greater intracellular acidification, which is known to activate sensory nerve terminals (Iturriaga, Rumsey, Lahiri, Spergel & Wilson, 1992), than that induced by injection of formic acid. Whether this mechanism is responsible for the stronger stimulation of C fibre endings by H^+ in the presence of lactate ions requires further investigation.

Lactic acid is commonly produced during anaerobic metabolism under a variety of conditions such as severe exercise, ischaemia, and hypoxia. The changes in pH_a and blood lactate levels after injections of lactic acid observed in our study are similar to the reductions in pH_a and the rises in blood lactate levels that have been reported during severe exercise (Sejersted, Medbø, Orheim & Hermansen, 1984; Stringer, Casaburi & Wasserman, 1992) and in various pathological conditions (Toffaletti, 1991; Mizock & Falk, 1992). Activation of pulmonary C fibre afferents elicits both central nervous system-mediated cardiopulmonary chemoreflexes (Dawes & Comroe, 1954; Coleridge & Coleridge, 1984; Coleridge & Coleridge, 1994) and local axon reflex (Lundberg & Saria, 1987), both of which are known to play an important role in the regulation of airway functions. Therefore, the findings in this paper, that the pulmonary C fibres are activated by H^+ and that the stimulating effect of H^+ is stronger in the presence of lactate ions, suggest an important role of the endogenously produced lactic acid in activating bronchopulmonary C fibres under certain physiological or pathophysiological conditions. Several important questions should then be raised. For example, does the dyspnea during severe exercise result, in part, from the activation of bronchopulmonary C fibres by lactic acid? Does lactic acid produced during severe exercise play a role in exercise-induced asthma? Does locally produced lactic acid play a part in eliciting cough and bronchoconstriction during airway inflammation?

In summary, our findings demonstrate that H^+ is primarily responsible for the activation of pulmonary C fibres by lactic acid, probably through a direct effect of H^+ on these afferent endings. Our results also demonstrate that the lactate ion, by itself, does not activate pulmonary C fibres, but it seems to potentiate the response of C fibres to H^+.

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