Hypoxia differentially modulates the activity of pacemaker and smooth muscle cells in the guinea pig stomach antrum

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Abstract

Effects of hypoxic solution (O2 tension, 161 ± 11 mmHg) on electrical responses of the membrane (slow waves), intracellular Ca2+-responses measured by Fura-2 fluorescence (Ca-transients) and isometric mechanical responses (phasic contraction) were observed in circular smooth muscles isolated from the guinea-pig stomach antrum. In normoxic solution (O2 tension, 362 ± 28 mmHg), muscle cells generated slow waves spontaneously, and switching to hypoxic solution caused an increase in frequency and decrease in duration of slow waves, with no significant change in the resting membrane potential. Hypoxia also reduced the amplitude and duration and increased the frequency of Ca-transients. The increase in frequency of slow waves by hypoxia was prevented by cyclopiazonic acid (CPA) but not by carbonyl cyanide m-chlorophenyl-hydrazone (CCCP), potassium cyanide (KCN) or low-Ca solution. The reduction by hypoxia of the duration of slow waves was prevented by CCCP or KCN but not by CPA or low-Ca solution. Hypoxia resulted in an increase in frequency and decrease in amplitude of phasic contractions, and the changes were prevented by CPA but not by CCCP. These results suggested that in antrum smooth muscle tissues, the increase in frequency of spontaneous activity by hypoxia is related to the enhanced function of the CPA-sensitive internal Ca-stores in pacemaker cells, while the inhibition in amplitude of phasic contractions by hypoxia may be mainly related to the decrease in Ca2+ release from the CPA-sensitive internal stores in smooth muscle cells. It is concluded that in hypoxic solution, the function of internal Ca2+ stores is enhanced in ICC-MY and is inhibited in smooth muscle cells in the guinea-pig stomach antrum.

Key words: hypoxia, internal Ca2+ store, slow wave, Ca-transient, phasic contraction

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Introduction

It is generally accepted that there is a causal relationship between smoking and gastric disorders, and that recovery from peptic ulcers is facilitated clinically by cessation of smoking (Gray, 1929). Sonnenberg and Husmert (1982) showed by using neutral red staining methods that smoking results in a reduction of blood flow and an associated lowering of acid secretion in the stomach. Changes in mucosal blood flow in the stomach during smoking have been examined by many investigators using different methods, and the causal relationship between smoking and the reduction of gastric circulation has been confirmed (Kawano et al., 1982; Kawano et al., 1989; Hayashi et al., 1992; Tsuji et al., 1994). Direct evidence indicating that smoking induces contraction of venules and produces ischemia in the entire stomach has also been reported (Kamada et al., 1982). Although the elevated nicotine level in serum during smoking was considered to be the main cause of the induction of gastric disorders (Sonnenberg and Husmert, 1982), a comparison of the effects of tobacco with or without nicotine content indicated that there was no causal relationship between nicotine in the serum and the reduction of gastric circulation (Hayashi et al., 1992). The importance of the role of the central nervous system is also suggested by the reduction of gastric circulation produced in patients exposed to a critical body stress, such as either a thermal or head injury (Kamada et al., 1982).

Thus, it seems likely that reduced blood circulation has a causal relationship with gastric disorders, probably due to local reduction of oxygen tension in the stomach. In isolated smooth muscle tissue, hypoxia induces many effects on smooth muscle activity, such as a reduction in both metabolism and force development and inhibition of the activity of ion channels (Paul et al., 1987; Ishida and Paul, 1990; Obara et al., 1997; Taggart and Wray, 1998). In the peripheral circulation, dilatation of vascular smooth muscle during hypoxia has also been noted (Spurway and Wray, 1987). Gastric smooth muscle is spontaneously active with periodic generation of “myogenic” contraction, based on spontaneous electrical activity demonstrated as slow waves and spike potentials (Tomita, 1981). This activity is considered to originate from two types of interstitial cells of Cajal (ICC) distributed in the gastric wall; ICC distributed in the myenteric layers (ICC-MY) and ICC distributed within the smooth muscle bundles (ICC-IM) (Sanders, 1996; Huizinga et al., 1997; Sanders et al., 1999; Suzuki, 2000; Hirst and Ward, 2003; Takaki, 2003). Both types of ICC are rich in mitochondria (Thuneberg, 1982; Komuro et al., 1999), suggesting that they are particularly sensitive to reduced oxygen tension in the stomach, since mitochondria may require a large consumption of oxygen for energy production.

In this study we have investigated the effects of hypoxia on the spontaneous activity of smooth muscle isolated from the guinea-pig stomach. Gastric smooth muscle tissue is composed of circular and longitudinal smooth muscle layers, with the myenteric nervous system and associated networks of ICC-MY in between these two smooth muscle layers (Thuneberg, 1982). Experiments were carried out to observe the effects of reduced oxygen tension on the electrical responses of the circular smooth muscle (slow waves) using conventional microelectrode techniques. Changes in the mechanical responses of the smooth muscle and of their intracellular calcium ion concentration ([Ca^{2+}]) in circular smooth muscle cells were also measured in preparations isolated from the antrum region of stomach, since the general
properties of electrical activity (Tomita, 1981; Suzuki, 2000; Hirst and Ward, 2003) and calcium responses (Fukuta et al., 2002) have been well documented in these tissues. The results indicated that stimulation of the smooth muscle tissue with hypoxic solution increased the frequency of electrical slow waves, with no change in the amplitude. These electrical changes were associated with a reduction in the amplitude and an increase in frequency of spontaneous phasic contractions. The different responses of electrical and mechanical activities of smooth muscle to hypoxia were considered to be due to the differential sensitivity of internal Ca\textsuperscript{2+} stores to hypoxia between smooth muscle cells and ICC, causing inhibition in the former and excitation in the latter. A brief report of part of this study was made to the 48\textsuperscript{th} annual meeting of the Japan Society of Smooth Muscle Research held in Okayama, Japan (Nakamura et al., 2006b).

**Materials and Methods**

**Animals and tissue preparation**

Male albino guinea-pigs, weighing 200–550 g, were anaesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (Sevoflurane; Maruishi Pharmaceutical Co., Osaka, Japan) and decapitated. Animals were treated ethically according to the Guiding Principles for the Care and Use of Animals in the Scientific Experiments, approved by The Experimental Animal Committee of the Nagoya City University Medical School. The stomach was excised and opened by cutting along the small curvature in oxygenated Krebs solution. The mucosal layer was removed by cutting with fine scissors, and a square block (300–500 \(\mu\)m square) of smooth muscle tissue containing 3–5 circular smooth muscle bundles was isolated from the antrum region.

**Recording of electrical responses of the membrane**

Electrical responses of smooth muscle cells were recorded by the methods reported previously (Nakamura and Suzuki, 2004). Briefly, segments of circular smooth muscle tissue containing 2–3 bundles (160–240 \(\mu\)m wide and 200–300 \(\mu\)m long), with attached longitudinal and myenteric layers, were immobilized on a Sylgard plate fixed to the bottom of the recording chamber (10 mm \(\times\) 20 mm long, 2 mm depth; capacity about 1 ml) using tiny pins, with the mucosal layer uppermost, and were superfused with warmed (35°C) oxygenated Krebs solution at a constant flow rate of 2 ml min\(^{-1}\). The recording chamber was covered with a plastic box which was filled with either O\(_2\)-CO\(_2\) or N\(_2\)-CO\(_2\) gas mixtures. After 1 h equilibration, membrane potentials were recorded from single circular smooth muscle cells using conventional microelectrode methods. The glass capillary microelectrodes (outer diameter 1.5 mm, inner diameter 0.8 mm, Hilgenberg, Germany) filled with 3 M KCl had a tip resistance which ranged between 30 and 50 M\(\Omega\). Electrical responses of the membrane were recorded via a high-input-impedance amplifier (Microelectrode Amplifier MEZ-8300, Nihon Kohden, Tokyo, Japan), displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Osaka, Japan) and stored on a personal computer for later analysis.
Isometric tension recordings

Segments of circular muscle tissue with 3–5 bundles (240–400 \( \mu \text{m} \) wide) and about 1.5 cm long, with attached longitudinal smooth muscle and myenteric layers, were prepared and tied at both ends with fine silk threads. The preparation was transferred to a cylindrical organ bath (1.0 cm diam. and 2.5 cm high, capacity about 2 ml) mounted at the center of the bath with thread, and superfused with warmed (35°C) oxygenated solution at a constant flow rate of 2 ml min\(^{-1}\). The upper thread was connected to the lever of a force transducer (FD pick-up, TB-612T, Nihon Kohdén, Tokyo, Japan) to measure the isometric tension produced by the circular muscle. Force changes were recorded on paper using an ink-writing recorder (VP-6524A, National, Osaka, Japan). The tissue was incubated in the bath with oxygenated Krebs solution for 60–90 min, after which the basal tension of approximately 2 mN was applied. Experiments were started after stabilization of tissue in the bath for a further 60–90 min.

Intracellular calcium measurements

Segments of circular smooth muscle containing 2–3 bundles (each bundle 80–100 \( \mu \text{m} \) wide and 200–300 \( \mu \text{m} \) long) were prepared by the mechanical removal of the longitudinal muscle and myenteric layers using fine forceps. These were pinned out on a block of Sylgard (silicone elastomer, Dow Corning Corporation, Midland, MI, U.S.A.) with a window of some 3 mm \( \times \) 3 mm opened at the centre. The Sylgard block was turned over and the tissue placed at the bottom of the recording chamber (a 5 cm round bath, 2 mm deep), so as to face the preparation against a glass cover-slip at the bottom of the chamber. After 30 min incubation with warmed (35°C) oxygenated Krebs solution, visual confirmation was made of the spontaneous movements of the tissues, and then the preparations were loaded with fluorescent dye, Fura-2 AM (10 \( \mu \text{M} \)), in nominally Ca\(^{2+}\)-free solution for 1 h at room temperature, as reported previously (Fukuta et al., 2002). After loading, the preparations were superfused with dye-free, oxygenated and warmed (35°C) Krebs solution for 30 min, at a constant flow (about 2 ml min\(^{-1}\)). Preparations loaded with Fura-2 were viewed under either an oil-immersion objective (UplanApo 40, Olympus, Tokyo, Japan) or a normal objective (UplanApo 10 and 20, Olympus, Tokyo, Japan), and were illuminated with ultraviolet light for two periods at wave lengths of 340 and 380 nm, alternating at a frequency of higher than 40 Hz. The ratio of the emission fluorescence (\( R_{340/380} \)) in a desired size of rectangular window (approximately 150 \( \times \) 150 \( \mu \text{m} \)) was measured through a barrier filter of 510 nm (sampling interval 25–100 ms), using a micro-photoluminescence measurement system (ARGUS/HISCA, Hamamatsu Photonics, Hamamatsu, Japan).

Solutions and oxygen tension

The ionic composition of the Krebs solution was as follows (in mM): Na\(^{+}\) 137.4, K\(^{+}\) 5.9, Mg\(^{2+}\) 1.2, Ca\(^{2+}\) 2.5, HCO\(_3\)\(^{-}\) 15.5, H\(_2\)PO\(_4\)\(^{-}\) 1.2, Cl\(^{-}\) 134, glucose 11.5. The solution was aerated with O\(_2\) containing 5% CO\(_2\). Hypoxic solution was prepared by bubbling the Krebs solution with N\(_2\) containing 5% CO\(_2\). The solutions were collected at the inflow of the recording chamber for microelectrode experiments and their gas tensions (O\(_2\) and CO\(_2\)) and pH were measured by blood gas analysis methods (GASTAT-601, Techno Medica, Kanagawa, Japan). Solutions with two different oxygen tensions were prepared; solutions with O\(_2\) aeration (normoxic solution) had...
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an oxygen tension of about 362 mmHg, while those with N₂ aeration (hypoxic solution) had a low oxygen tension of about 44% of the normoxic solution, both with similar pH of the solution (Table 1). Low-Ca solution ([Ca²⁺]₀ = 0.75 mM) was prepared by replacing a volume of CaCl₂ with MgCl₂. No significant change in pH was detected in the low-Ca solution.

Table 1. Oxygen tension and pH of the hypoxic Krebs solution

| Solution         | O₂ tension  | CO₂ tension | pH      | n  |
|------------------|-------------|-------------|---------|----|
| Normoxic solution| 362 ± 28 mmHg | 29 ± 5 mmHg | 7.2 ± 0.06 | 6  |
| Hypoxic solution | 161 ± 11 mmHg* | 29 ± 5 mmHg | 7.1 ± 0.03 | 6  |

*The Krebs solutions were aerated with either O₂ containing 5% CO₂ (normoxic solution) or N₂ containing 5% CO₂ (hypoxic solution), and the O₂ tension, CO₂ tension and pH of the solution flowing into the recording chamber for microelectrode experiments (see Methods) were measured. n=number of observations. *, significantly different to normoxic solution (P<0.05).

Chemicals

Nifedipine, carbonyl cyanide m-chlorophenyl-hydrazone (CCCP), potassium cyanide (KCN) and cyclopiazonic acid (CPA) were used in the present experiments (all chemicals purchased from Sigma, St. Louise, MO, USA). Nifedipine, CCCP and CPA were dissolved in dimethyl sulphoxide (DMSO) to make stock solutions, and were added to Krebs solution to make the desired concentrations just prior to their use. KCN was dissolved in distilled water. The final concentration of the solvent in Krebs solution did not exceed 1:1000. Addition of these chemicals to Krebs solution did not alter the pH of the solution.

Statistics

Experimental values were expressed as the mean value ± standard deviation (S.D.), with the n value representing the number of observations or the tissues obtained from different animals. Statistical significance was tested using Student’s t-test, and probabilities of less than 5% (P<0.05) were considered to be significant.

Results

Effects of hypoxia on slow waves

In normoxic solution, antral circular smooth muscle cells generated slow waves at a frequency ranging between 1 and 5 times min⁻¹ (mean, 4.7 ± 1.4 times min⁻¹; n=43) with an amplitude ranging between 25 and 40 mV (mean, 30.0 ± 5.2 mV; n=43). Exposure of tissues to the hypoxic solution increased the frequency of slow waves, with no alteration of either the resting membrane potential or the amplitude of the slow waves. Typical responses of the slow waves recorded before and during exposure to hypoxic solution are shown in Figs. 1A and 1B, respectively. Similar results were observed in all tissues examined (n=43), and the pooled data on the effects of hypoxia on the membrane potential, and the amplitude, frequency and duration of the slow waves are summarized in Fig. 1.

The slow waves consisted of two components; the 1st component which forms the base part
of the slow wave is the electrotonic propagation of the driving potential generated in the ICC-MY, while the 2nd component is formed by slow potentials produced in the circular muscle as a result of the summation of unitary potentials generated in the ICC-IM (Hirst and Ward, 2003). Hypoxia reduced the duration of the 1st and 2nd components of the slow waves by about 15–20% (Fig. 1F), with no alteration to the amplitude of either of the 1st and 2nd components of these slow waves (Fig. 1D). The frequency of the slow waves was also increased significantly in hypoxic solution (Fig. 1E). These changes in the slow waves produced by hypoxic solutions were reversible, but it required 1–2 min for complete recovery (data not shown).

Experiments were carried out to examine the effects of potassium cyanide (KCN), CCCP and a low-Ca solution on the responses produced by hypoxia in the antral smooth muscle tissue. KCN is a known inhibitor of cytochrome oxidase (complex IV) at the electron transport chain in mitochondria, and has been reported to reduce the frequency of slow waves in the gastric antrum (Huang et al., 1993; Nakayama et al., 1997; Nakamura et al., 2006a). CCCP is a mitochondrial protonophore, and its inhibition of proton transport secondarily prevents the transport of Ca\(^{2+}\) across the mitochondrial inner membrane (Duchen, 1999), and thus reduces the frequency of spontaneous activity in gastric smooth muscle (Ward et al., 2000; Fukuta et al., 2002; Suzuki et al., 2006). Low-Ca solutions could reduce the rhythmic activity of gastric
smooth muscle (Tomita and Hata, 2000), thereby suggesting that the reduced influx of Ca\(^{2+}\) may prevent the hypoxia-induced increase in the frequency of slow waves.

As reported previously (Nakamura et al., 2006a), application of KCN (1 mM) reduced the frequency of slow waves in the antral smooth muscle, with no significant alteration to either the resting membrane potential or the amplitude of slow waves (data not shown). Experiments were carried out to test the effects of hypoxia on slow waves in the presence of KCN, and the results are summarized in Fig. 2. Hypoxia increased the frequency of slow waves (Fig. 2C), with no alteration to the resting membrane potential (Fig. 2A) and either the amplitude (Fig. 2B) or duration (Fig. 2D) of both the 1st and 2nd components of slow waves. These results suggested that hypoxia-induced elevation of the frequency of slow waves was not causally related to alteration of mitochondrial metabolic activity.

The possible effects of reduction of [Ca\(^{2+}\)]\(_{o}\) (low-Ca solution, [Ca\(^{2+}\)]\(_{o}\) = 0.75 mM) on the responses produced by hypoxia were examined in gastric smooth muscle preparations. Low-Ca solution did not alter any of the parameters of membrane electrical activity, such as the membrane potential, or the amplitude, frequency and duration of the 1st and 2nd components of the slow waves (data not shown). In low-Ca solution, exposure of the tissue to hypoxic solution increased the frequency of slow waves and decreased the duration of the 2nd component of the slow waves (Fig. 2, G and H), with no change in either the resting membrane potential (Fig. 2E)
or the amplitude of slow waves (Fig. 2F). These results indicate that \([\text{Ca}^{2+}]_o\) was not an essential factor required for the increase in frequency of slow waves under hypoxic conditions.

Preliminary experiments revealed that slow waves were abolished during exposure to high concentrations of CCCP (>3 \(\mu\)M), with depolarization of the membrane by about 10–20 mV, while the generation of slow waves was maintained in the presence of lower concentrations (0.1–1 \(\mu\)M) of CCCP. Experiments were carried out to observe the effects of 1 \(\mu\)M CCCP on the modulation of slow waves by hypoxia, and one of the typical results is shown in Fig. 3. In this tissue, application of 1 \(\mu\)M CCCP depolarized the membrane by about 10 mV (control, –73.0 ± 2.3 mV; in CCCP, –61.2 ± 6.3 mV; \(n=3\); \(P<0.05\)), and reduced the amplitude and duration of slow waves while increasing their frequency (Fig. 3, D–F). In the presence of 1 \(\mu\)M CCCP, hypoxia further reduced the amplitude (Fig. 3D) and duration (Fig. 3F), but increased the frequency (Fig. 3E) of slow waves, with no significant change in the membrane potential (–58.6 ± 5.3 mV, \(n=3\), \(P>0.05\)). Effects of 1 \(\mu\)M CCCP on the hypoxia-induced changes in slow waves were observed in 3 different preparations, however, similar results were observed in all preparations. Thus, the results would indicate that CCCP did not prevent the hypoxia-induced increase in the

![Fig. 3.](image-url)
Experiments were carried out to test the effects of CPA on the slow waves induced by hypoxia. CPA (10 μM) depolarized the membrane by 5–12 mV and either abolished the slow waves (2 out of 9 preparations) or reduced both the frequency and amplitude of the slow waves (7 out of 9 preparations) in the antral smooth muscle. In the former, exposure of preparations to hypoxic solution in the presence of CPA did not elicit any electrical activity (data not shown).

In the presence of CPA, the hypoxic solution reduced the amplitude of the slow waves, mainly by the reduction in amplitude of the 2nd component (Fig. 4D). The frequency of slow waves was decreased by CPA, and it remained unaltered by exposure to the hypoxic solution in

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**Fig. 4.** Slow waves were recorded from antral circular smooth muscle cells, under normoxic conditions in the presence of 10 μM CPA (A), and under hypoxic conditions in the presence of 10 μM CPA (B). A and B were continuous recording from single cells, with an interval of about 15 min between each trace. Membrane potentials; A, −58 mV; B, −54 mV. The resting membrane potential (C), and the amplitude (D), frequency (E) and duration of slow waves (F) were measured from circular smooth muscle cells, in the absence and presence of 10 μM CPA and exposure to hypoxic solution in the presence of CPA, in 7 preparations. Slash, filled and open columns indicate the values in the absence of CPA in normoxic solution (Norm), in the presence of CPA in normoxic solution (CPA) and in the presence of CPA in hypoxic solution (CPA Hypo), respectively. Mean ± S.D. are shown. * and †, significantly different to control in the absence of CPA and from CPA under normoxic conditions, respectively (each for P<0.05).
the presence of CPA (Fig. 4E). The duration of slow waves (both 1st and 2nd components) was reduced by CPA, and they were reduced further by hypoxia (Fig. 4F). Thus, the important finding was that CPA prevented the hypoxia-induced increase in the frequency of slow waves.

**Effects of hypoxia on Ca-transients**

Changes in the intracellular concentration of Ca$^{2+}$ ([Ca$^{2+}$]) during exposure to a hypoxic solution were investigated in preparations loaded with Fura-2. In Fura-2 loaded preparations, [Ca$^{2+}$]$_i$ levels changed periodically (Ca-transients), as reported by Fukuta et al. (2002). Application of the hypoxic solution resulted in a decrease in both the amplitude and duration of the Ca-transients with an increase in the frequency of the Ca-transients, in a reversible manner. Similar results were observed in the 3 different preparations examined, as summarized in Fig. 5. The resting Ca$^{2+}$ level was 0.7 ± 0.06 R$_{F340/F380}$ in normoxic solution, and it tended to decrease in the hypoxic solution (0.6 ± 0.04 R$_{F340/F380}$, n=3, P>0.05).

**Effects of hypoxia on phasic contractions of smooth muscle**

Circular smooth muscle isolated from the guinea-pig stomach antrum was spontaneously active with a rhythmic generation of phasic contractions at a frequency of about 4 times min$^{-1}$ and an amplitude of 3 mN (n=10). Exposure of tissues to the hypoxic solution for 20 min resulted in a reduction in the amplitude and an increase in the frequency of these phasic
Hypoxia and gastric smooth muscle contractions, which was often associated with a slight reduction in the resting tension, as shown in Fig. 6A. It should be noted that a transient elevation of resting tension was often observed during the initial 5–10 min of exposure to different oxygen tensions (see Fig. 6A). In any case, sustained exposure of smooth muscle preparations to low oxygen tension resulted in a stable amplitude and frequency of the phasic contractions within 15–17 min. Once stabilized, there was a significant decrease in tension and an increase in the number of phasic contractions in all preparations examined (n=10, Fig. 6, B and C).

Attempts were made to test the effects of both CCCP and CPA on the hypoxia-induced changes in the phasic contraction of the antral smooth muscle. Figure 7 shows that application of CCCP (0.1 µM) for 30–60 min did not alter either the frequency or the amplitude of the phasic contractions. Hypoxic solution applied before and during exposure to CCCP for more than a period of 30 min, reduced the amplitude and increased the frequency of phasic contractions, either in the absence (Fig. 7A) or presence of CCCP (Fig. 7B). The summed data obtained from 7 preparations confirmed that CCCP did not prevent either the hypoxia-induced decrease in amplitude (Fig. 7C) or the increase in frequency (Fig. 7D) of the phasic contractions.

The effects of CPA on phasic contractions were observed at concentrations of both 0.1 and 1 µM. When 0.1 µM CPA was applied, the resting tension was slightly elevated in 2 out of 4 preparations, with a transient increase in the amplitude of the phasic contractions (Fig. 8B). In the other two preparations, there was no marked change in the resting tension, with no significant change in either the amplitude or frequency of the phasic contractions. In both these
preparations, in the presence of 0.1 µM CPA for 60 min, exposure of the preparation to the hypoxic solution for an additional 20 min did not produce any marked change in phasic contractions, but rather, increased the amplitude of the phasic contractions (Fig. 8B), in comparison with those seen in the absence of CPA (Fig. 8A). The resting tension was often elevated slightly in the hypoxic solution. Similar results were obtained when the concentration of CPA was increased to 1 µM (n=4 preparations). The summed data indicated that in the presence CPA, the averaged amplitude (Fig. 8C) and frequency (Fig. 8D) of the phasic contractions were not changed by hypoxia. Thus, CPA could, but CCCP could not, prevent the hypoxia-induced inhibition of the amplitude and increase of the frequency of phasic contractions in the antrum smooth muscle. These results strongly suggest that the reduction of oxygen tension produces a functional alteration of internal Ca\(^{2+}\) stores in the antral smooth muscle of the guinea-pig.

**Discussion**

In many types of smooth muscle, except that in pulmonary arteries (Wilson *et al.*, 2002), hypoxia inhibits contractile responses (Taggart and Wray, 1998). In the guinea-pig taenia caeci,
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The inhibition by hypoxia of the contractures produced by high-potassium solution is mainly due to a reduced activity of energy metabolism (Ishida and Paul, 1990). The present experiments indicated that in smooth muscle isolated from the guinea-pig stomach antrum, hypoxia reduced the amplitude of spontaneously generated phasic contractions, with an associated increase in their frequency. Measurement of electrical responses of smooth muscle cells using intracellular microelectrode techniques also showed that hypoxia increased the frequency and decreased the duration of slow waves, with no alteration in the amplitude of either slow waves or of the resting membrane potential. In the circular smooth muscle of the guinea-pig antrum, rhythmic generation of Ca-transients is associated with slow potentials, either in the absence or presence of nifedipine (Fukuta et al., 2002), suggesting that the hypoxia-induced changes in activity are intracellular events occurring in smooth muscle cells, with no direct relation to the influx of Ca\(^{2+}\) through voltage-sensitive Ca-channels. Therefore, the changes in phasic contractions are probably related to the alteration of intracellular mechanisms in smooth muscle cells. This is supported by the evidence that the membrane electrical responses produced by hypoxia are not altered in low-Ca solution, but can be modulated by chemicals known to modulate the handling of intracellular Ca\(^{2+}\) events, such as CPA or CCCP.

The reduction in amplitude of phasic contractions by hypoxic solutions may be causally

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**Fig. 8.** Effects of CPA on hypoxia-induced changes in phasic contractions of antral smooth muscle of the guinea-pig. Mechanical activity elicited by the hypoxic solution were recorded from circular smooth muscle preparations isolated from the guinea-pig stomach antrum isometrically, in the absence (A) and presence of 0.1 µM CPA (B). Both the amplitude (C) and frequency (D) of phasic contractions were measured from 4 preparations (mean values ± S.D.), under normoxic (filled column) and hypoxic conditions (open column), in the absence (left pair) and presence of CPA (0.1 µM, central pair; 1 µM, right pair). *, significantly different to values under normoxic conditions (P<0.05).
related to the reduced amplitude of Ca-transients. In antral circular smooth muscle, the Ca-
transient is a voltage-sensitive process, with membrane depolarization a prerequisite for this
event (Hirst et al., 2002). The Ca-transient may be produced mainly by the release of Ca\textsuperscript{2+} from
internal stores, and it is suggested that the inhibition of the amplitude of Ca-transients by
hypoxic solutions is causally related to the reduced amount of Ca\textsuperscript{2+} released from the internal
stores. The inhibition of the hypoxia-induced reduction of phasic contraction by CPA supports
this suggestion, since this chemical inhibits the release of Ca\textsuperscript{2+} from internal stores indirectly, by
reducing the content of stored Ca\textsuperscript{2+} as a result of inhibition of Ca-ATPase at the membrane
(Uyama et al., 1992). The decrease in duration of slow waves during low oxygen tension may
reflect a reduced release of Ca\textsuperscript{2+} from internal stores. Such a reduction may also make some
contribution to the reduced amplitude of the phasic contractions. However, the results indicate
that CPA did not inhibit the hypoxia-induced reduction in the duration of slow waves, suggesting
that the effects of duration of slow waves on the amplitude of Ca-transient are very little, if any.

The increase in frequency of phasic contractions during exposure to low oxygen tension is
consistent with the increased frequency of both Ca-transients and slow waves. The frequency of
slow waves is a voltage-sensitive event, and could be increased by depolarization (Nose et al.,
2000; Fukuta et al., 2002; Suzuki et al., 2006). In smooth muscle preparations isolated from the
guinea-pig stomach antrum, the pacemaker cells for the slow waves are the ICC-MY. These
generate the driving potentials which propagate to the circular smooth muscle cells in an
electrotonic manner to form the 1st component of the slow waves (Dickens et al., 1999; Dickens
et al., 2001; Hirst and Ward, 2003). We found that the hypoxic solution did not alter the resting
membrane potential in the antral smooth muscle cells. Although the present experiments did
not measure the change in membrane potential of the ICC-MY directly, both circular smooth
muscle cells and ICC-MY are electrically coupled (Cousins et al., 2003; Hirst and Ward, 2003),
suggesting that the increase in frequency of slow waves resulting from hypoxia was not causally
related to the change in membrane potential of the ICC-MY. The present experiments revealed
that the hypoxia-induced increase in frequency of slow waves is inhibited by CPA, but not by
CCCP or KCN. The latter two chemicals are known to inhibit mitochondrial activities in
different ways. CCCP prevents proton transport at the mitochondrial inner membrane (Duchen,
1999), while KCN inhibits electron transport processes (Huang et al., 1993). Thus, it is likely
that the increase in frequency of phasic contractions as a result of hypoxia is produced by an
elevated activity of internal Ca\textsuperscript{2+} stores in the ICC-MY.

The results obtained in the present experiments could be interpreted reasonably if the
internal Ca\textsuperscript{2+} stores are sensitive to low oxygen tension in both smooth muscle cells and ICC-
MY, but in a differential way. In considering the change in phasic contractions of the antral
smooth muscle in response to hypoxia, the increase in frequency may be related to the
responses of ICC-MY, while the reduction in amplitude may be related to the responses of
smooth muscle cells. In this case, the hypoxia-induced increase in frequency may be due to the
activation of CPA-sensitive processes in ICC-MY, while the reduction in amplitude by hypoxia
may be due to the inhibition of Ca-release from internal stores in smooth muscle cells. These
differences may produce the increased frequency and decreased amplitude of phasic
contractions in solutions with low oxygen tension. Thus, these results are the opposite of those
expected initially as the most sensitive site to hypoxia is likely to be the mitochondria in these cells. The central role of internal Ca\textsuperscript{2+} stores in the hypoxia-induced change in phasic contractions appears to be different to that involved in the slow potentials generated in circular smooth muscle bundles isolated from the guinea-pig stomach antrum. In the latter case, the amplitude and frequency of the slow potentials are regulated by [Ca\textsuperscript{2+}], in different ways; the amplitude is determined by the amount of Ca\textsuperscript{2+} released from internal stores while the frequency is related to the mitochondrial Ca\textsuperscript{2+} handling. This is supported by the evidence that the amplitude of the slow potentials is sensitive to CPA while the frequency of slow potentials is sensitive to CCCP (Nakamura and Suzuki, 2004; Suzuki et al., 2006). The properties of ICC-MY often differ from those of ICC-IM, and in general the sensitivity to chemicals is higher in ICC-IM than in ICC-MY. For example, the threshold concentration of CPA for inhibiting the generation of slow potentials (i.e., the activity of ICC-IM) is about 1 \textmu M (Suzuki et al., 2002), while CPA at a concentration of 10 \textmu M does not inhibit the generation of driving potentials in ICC-MY (Kito and Suzuki, 2002; Kito and Suzuki, 2003).

The responses of smooth muscle to hypoxia are considered to be similar to their responses to metabolic inhibitors (Taggart and Wray, 1998). However, this does not seem to be the case in gastric smooth muscle, for example the frequency of spontaneous activity is increased by hypoxia and decreased by metabolic inhibitors (Huang et al., 1993; Nakayama et al., 1997; Nakamura et al., 2006). It remains unclear why there are such differences in the handling of Ca\textsuperscript{2+}, and we have considered that a possible reason may be the different origins of these cells (Sanders, 1996). It is also reported that the difference in responses of smooth muscle to hypoxia between renal and pulmonary artery is causally related to the role of the Ca\textsuperscript{2+} entry mechanism in internal Ca\textsuperscript{2+} stores in the dog (Wilson et al., 2002), suggesting a possible involvement of differences in the functional development of the internal organelles in specific cells.

Hypoxia modulates many activities in smooth muscle cells, such as the inhibition of membrane excitability including ion channels at the plasma membrane, activities of intracellular organelles and metabolism (Taggart and Wray, 1998). The present experiments showed that in antral smooth muscle preparations isolated from the guinea-pig stomach, a reduction of oxygen tension increased the frequency of electrical slow waves and decreased the amplitude of phasic contractions. As both phenomena were CPA-sensitive, these differences were considered to be produced by the differential sensitivity of internal Ca\textsuperscript{2+} stores to low oxygen tension between ICC-MY and smooth muscle cells, and that the internal Ca\textsuperscript{2+} stores are activated in the former and inhibited in the latter.

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