Inhibitory Effects of Cyanide on Mechanical and Electrical Activities in the Circular Muscle of Gastric Antrum of Guinea-Pig Stomach

Shu-Ming HUANG, Jalal U. CHOWDHURY, Koichi KOBAYASHI,
and Tadao TOMITA

*Department of Physiology, School of Medicine, Nagoya University,
Nagoya, 466 Japan*

Abstract Effects of cyanide (CN) on spontaneous mechanical and electrical activities were studied in the circular muscle of the guinea-pig gastric antrum. CN (0.05–0.5 mM) inhibited both mechanical and electrical activities, but the inhibition was stronger in the former than the latter (slow wave). When glucose was removed for more than 30 min, slow wave was also blocked by CN. It is likely that ATP necessary for the generation of spontaneous activity can be supplied through either glycolysis or oxidative phosphorylation. CN slightly depolarized the membrane and the inhibition by CN was not affected by glibenclamide, a blocking agent of ATP-regulated K^+ channel, suggesting that this channel is not activated by CN treatment. Basal level of intracellular Ca^{2+} measured with fura-2 tended to increase during the mechanical inhibition by CN. This was also clearly demonstrated in excess (60 mM) K^+ solution. These results suggest that intracellular Ca^{2+} regulation is impaired and that there is uncoupling between intracellular Ca^{2+} and tension development in the presence of CN.

Key words: smooth muscle, slow wave, cyanide, metabolic inhibition, stomach.

The circular muscle of the guinea-pig gastric antrum has a regular spontaneous contraction accompanied by membrane depolarization, generally called “slow wave” [1]. The mechanism underlying slow wave, including that found in gastrointestinal smooth muscles of other species, is still not clear [2, 3]. The frequency of the spontaneous activity in guinea-pig stomach, $3\text{--}5\text{ min}^{-1}$, is relatively resistant to changes in membrane potential [1] and to organic Ca^{2+} channel blockers, such as verapamil [4], but it is highly dependent on the temperature, suggesting an involvement of some metabolic process [1]. We have previously obtained preliminary evidence that the spontaneous activity can be supported by

Received on October 3, 1992; Accepted on February 25, 1993

glycolysis under the condition in which oxidative phosphorylation is blocked by cyanide or anoxia [5]. In the present experiments, we have further examined effects of cyanide on the spontaneous mechanical and electrical activities of guinea-pig gastric muscle.

METHODS

Guinea-pigs weighing 250–350 g of either sex were sacrificed by stunning and bleeding, and stomach was excised. After removing the mucosa, small muscle strips (1×7 mm) were dissected out in the direction of circular muscle fibers from the antral region. For isometric tension recordings with a strain gauge, the strips were mounted in a small organ bath (1 ml) and superfused with physiological solution prewarmed to 35°C at a constant rate of 3 ml min^{-1} .

A conventional microelectrode technique was used for measurements of intracellular membrane potential. A small muscle strip (1×4 mm) was mounted in an organ bath (0.5 ml), and mechanical activity was simultaneously recorded with a strain gauge. The organ bath was perfused with solution at a rate of 2 ml min^{-1} after prewarming to 35°C .

For measurements of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) one end of the circular muscle (1×4 mm) was fixed on a small plastic plate (1.0 mm in width) with a fine thread and mounted in a small chamber (0.3 ml) placed on the stage of the inverted microscope equipped with a fluorometer (Olympus, OSP-3). The other end of preparation was connected to a strain gauge for tension recording. Fura-2 was loaded by incubating preparations in physiological solution containing $10 \mu\text{M}$ fura-2 AM and 0.02% cremophor EL for 1–2 h at room temperature (20 – 25°C). The preparation was equilibrated for more than 30 min by superfusing with physiological solution (35°C) before starting the measurement. The size of measured spots was $150 \mu\text{m}$. Fluorescence was measured at intervals of 0.5 s. Relative changes of $[\text{Ca}^{2+}]_i$ were expressed by the ratio of fluorescence intensity excited at 340 nm to that at 380 nm (F_{340}/F_{380}). The experiment was carried out at 35°C .

Physiological solution contained (mM): 127 NaCl, 6 KHCO_3 , 2.4 CaCl_2 , 1.2 MgCl_2 , glucose 12, Tris buffer 10 (pH was adjusted to 7.4 at 35°C with HCl). When glucose was removed or when K^+ was increased, the osmolarity was compensated by altering the NaCl concentration. In many experiments, $0.3 \mu\text{M}$ meclofenamate, an inhibitor of prostaglandin synthesis (Parke-Davis), was applied throughout. NaCN, N^G -nitro-L-arginine, and glibenclamide were obtained from Sigma, and fura-2 AM was from Molecular Probes.

RESULTS

Circular muscles obtained from the antral region of guinea-pig stomach have regular spontaneous contractions (Fig. 1). CN (0.05–0.5 mM) reduced the ampli-

Fig. 1. Effects of cyanide (CN) on spontaneous mechanical activity in the presence (left) and in the absence of glucose (right column) in the circular muscle of guinea-pig gastric antrum. An increasing concentration (0.05–0.5 mM) of CN was applied at an interval of 30 min, as indicated by horizontal bars. Glucose was removed 10 min before each CN application and readmitted 10 min later. Meclofenamate ($0.3\mu\text{M}$) was present to inhibit endogenous prostaglandin synthesis.

tude of contractions concentration-dependently, with the IC_{50} of $85 \pm 15 \text{ nM}$ ($n = 12$). In the presence of glucose (12 mM), CN had a relatively weak effect on the frequency at a concentration less than 0.2 mM, but the activity was completely blocked at 0.5 mM in 7 out of 12 preparations.

Effects of glucose removal on mechanical activity varied to some degrees in different preparations. In some preparations, the frequency decreased by 30–70%, but the block of activity was never observed, even after more than 2 h in glucose-free solution ($n = 11$). In 5 out of 11 preparations, some irregularity in amplitude and frequency developed after glucose removal. The inhibitory effect of CN became stronger in the absence of glucose. The frequency was reduced during CN application at a lower concentration and the activity stopped at a concentration higher than 0.1 mM in all preparations examined ($n = 9$). The frequency had a

Fig. 2. Experiments similar to Fig. 1, but glucose (12 mM) was removed (b) or readmitted (c) during CN application, or it was simultaneously removed during CN application (d). Meclofenamate ($0.3 \mu\text{M}$) was present.

tendency to decrease following wash-out of CN, particularly after application of high CN concentrations in the absence of glucose.

Muscle tone was generally very low in the preparations taken from the pyloric region, but it was relatively high in those from the corporal region. When there was the muscle tone, CN strongly reduced it, in addition to the inhibition of phasic contractions. The inhibitory effect of CN was not affected by a pretreatment with $40 \mu\text{M}$ *N*-nitro-L-arginine, which inhibits nitric oxide synthesis [5].

As shown in Fig. 2, when glucose was removed during application of 0.2 mM CN, the remaining activity disappeared in 3–4 min (b). In the absence of glucose, 0.2 mM CN blocked the activity, but the spontaneous activity reappeared in 1–2 min after readmission of glucose (12 mM) in the presence of CN, even after 20 min in glucose-free solution (c).

Figures 3 and 4 show simultaneous recordings of electrical and mechanical activities (slow waves) from two different preparations. CN (0.5 mM) depolarized the membrane up to 5 mV, reduced the amplitude, and shortened the duration of

Fig. 3. Simultaneous recording of mechanical activity (upper) and slow wave (lower record) showing effects of CN in the presence of glucose (a, d) and in the absence of glucose (b, c). CN (0.5 mM) was applied at 20 min intervals. Glucose (12 mM) was removed (b) and readmitted 10 min before record (d). Membrane potential was recorded with an intracellular microelectrode. Continuous record from the same cell in the presence of $0.3 \mu\text{M}$ meclofenamate.

Fig. 4. Records similar to Fig. 3, but glucose removal was prolonged to 30 min in a different preparation. a and b were recorded from different cells in the same preparation. Meclofenamate was not added.

Fig. 5. Effects of glibenclamide ($0.5\mu\text{M}$) on the inhibition of mechanical activity (upper) and slow wave (lower trace) produced by CN (0.2mM). No clear effect of glibenclamide was observed. Meclofenamate was not added.

slow waves during the mechanical inhibition. There was no clear relationship between the degree of depolarization and the frequency of slow wave. In some preparations, the frequency was slightly increased, and this was mainly due to shortening of the slow wave duration, not to shortening of the interval between slow waves. The effects of CN on slow waves were potentiated by glucose removal and the mechanical activity was suppressed by CN in the absence of glucose (Fig. 3b, c), as described above. Slow waves were more resistant to CN than mechanical activities in the absence of glucose, as shown in Fig. 3. When an exposure to glucose-free solution was prolonged, however, electrical activities were also blocked by CN (Fig. 4), although the duration of glucose removal necessary for the blockade with 0.2mM CN varied in different preparations to some degree (10–30 min).

CN might activate ATP-regulated K^+ channels which are activated when intracellular ATP concentration is lowered [7]. Therefore, effects of glibenclamide, which is known to block this channel [8, 9], were examined. As shown in Fig. 5, glibenclamide ($0.5\mu\text{M}$) had little effect on mechanical and electrical activities and it did not significantly alter the inhibitory effect of CN on both mechanical and electrical activities. This concentration of glibenclamide completely reversed the hyperpolarization and relaxation produced by $3\mu\text{M}$ cromakalim, a K^+ channel opener in rat portal vein [10], and also in guinea-pig gastric muscle (unpublished observations).

$[\text{Ca}^{2+}]_i$ of muscle strips measured with fura-2 fluctuated synchronously with mechanical activity, but tension developments and changes in Ca^{2+} signal were not

Fig. 6. Simultaneous recordings of spontaneous mechanical activities (upper) and changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (lower record) and effects of CN, in the presence (a) and in the absence of glucose (b). $[Ca^{2+}]_i$ was measured using fura-2 and expressed as the ratio of fluorescence excited at 340 and 380 nm. The preparation was first treated with 60 mM K^+ for 2 min to increase spontaneous activity and 10 min later 0.5 mM CN was applied (a). Between records a and b, glucose was removed from superfusing solution for 15 min and it was absent throughout.

precisely correlated (Fig. 6). CN (0.5 mM) suppressed the mechanical activity and also inhibited the rhythmic fluctuation of Ca^{2+} signals, but the basal level of $[Ca^{2+}]_i$ was elevated during application of CN, although the muscle remained relaxed. In the absence of glucose the increase in $[Ca^{2+}]_i$ caused by CN was potentiated (Fig. 6b). This was confirmed in three other preparations.

Figure 7 shows effects of Ca^{2+} removal (a) and CN (b) on K^+ contracture and $[Ca^{2+}]_i$. When the external K^+ concentration was increased to 60 mM, a transient contraction was produced and tension decreased to a very low level (not shown). The decrease in tension seems at least partly due to nitric oxide released from intrinsic nerve fibers [11, 12], because in the presence of *N*-nitro-L-arginine (40 μ M), some tension could be maintained (Fig. 7). The tension was inhibited by removing the external Ca^{2+} , accompanied by a decrease in $[Ca^{2+}]_i$ (Fig. 7a). After loading fura-2 at a room temperature, spontaneous rhythmic activity was usually small, but this was potentiated by treating the preparation with 60 mM K^+ . During the second application of 60 mM K^+ , CN (0.5 μ M) was applied (Fig. 7b). This clearly inhibited the sustained phase of K^+ contracture, but this was accompanied

Fig. 7. Effects of Ca^{2+} removal (a) and 0.5 mM CN (b) on contracture (upper record) and changes in $[\text{Ca}^{2+}]_i$ (lower record) produced by 60 mM K^+ . Meclofenamate ($0.3 \mu\text{M}$) and *N*-nitro-L-arginine ($40 \mu\text{M}$) were present throughout. Sixty mM K^+ was applied at 20 min interval.

by an increase in $[\text{Ca}^{2+}]_i$ (Fig. 7b). Similar results were obtained from three other preparations.

DISCUSSION

In the absence of glucose, CN blocks spontaneous activities in the guinea-pig gastric muscle. Enough energy supply seems necessary for the generation of the spontaneous activity. Since CN at concentrations higher than 0.5 mM completely blocks O_2 consumption (unpublished observations), the activity can be maintained by ATP supplied from glycolysis, as previously concluded [5]. No clear evidence is, however, obtained for a decisive role of glycolysis. Repeated treatments with CN in the absence of glucose may deplete cellular glycogen, but rhythmic activities can still be maintained in the absence of glucose for more than 2 h provided that oxidative phosphorylation is not impaired. Therefore, it is likely that enough ATP supply is important for the generation of the rhythmicity, but that this ATP can be

supplied either by glycolysis or by oxidative phosphorylation. For the glycolytic process, however, glucose supplied from the external medium seems to be utilized more efficiently than intracellular glycogen, because the effects of removal and readmission of the external glucose appear in a few minutes in the presence of CN.

The plasma membrane is slightly depolarized by CN, particularly in the absence of glucose, but there is no close correlation between the degree of depolarization and changes in the frequency of slow wave. This supports the idea that the generation of the activity is only weakly controlled by the membrane potential [1]. It is clear that metabolic inhibition reduces the amplitude of slow wave and modifies its frequency, but further experiments are necessary before speculating the mechanism of the metabolic control of slow wave or the mechanism underlying the electrogenesis of slow wave.

In some smooth muscles, the presence of ATP-regulated K^+ channel, which is activated by lowering intracellular ATP, has been demonstrated [13]. If this channel were activated by metabolic inhibition with CN, one would expect membrane hyperpolarization. Hypoxic dilatation in guinea-pig coronary artery has been explained by hyperpolarization resulting from activation of ATP-regulated K^+ channel [14]. In the guinea-pig gastric muscle, however, no hyperpolarization was observed by CN treatments even in the absence of glucose. Furthermore, the inhibitory effect of cyanide was not affected by glibenclamide. These results suggest either that ATP-sensitive K^+ channels are lacking in this muscle or that CN treatment does not reduce $[ATP]_i$ low enough to activate ATP-regulated K^+ channel.

Although a reasonable correlation between contractions and changes in Ca^{2+} signals was found, there is no precise relationship between them. Some discrepancy may be partly due to that fact that the tension is recorded from the whole tissue and the Ca^{2+} signal is obtained from a small spot (150 μm in diameter) of the tissue. $[Ca^{2+}]_i$ is increased during the inhibition of mechanical and electrical activities with CN. A similar result has been obtained in the rat portal vein (Hellstrand, P., personal communication). Since the Ca^{2+} level is decreased during the relaxation on Ca^{2+} removal, the increase in Ca^{2+} signals with CN is not considered to be an artifact resulting from relaxation. The CN-induced increase in $[Ca^{2+}]_i$ is probably due to the release of Ca^{2+} from, and/or the reduced uptake of Ca^{2+} into, the intracellular stores. In addition, a decrease in ATP supply, intracellular acidosis, or some metabolites produced by CN treatments seems to have a strong inhibitory effect on a contractile process. A non-linear relationship between the amplitude of slow wave and the phasic contraction in the presence of CN might also be partly due to uncoupling between $[Ca^{2+}]_i$ and tension development during the metabolic inhibition.

REFERENCES

1. Tomita T: Electrical activity (spikes and slow waves) in gastrointestinal smooth

- muscle. In: Smooth Muscle: An Assessment of Current Knowledge, ed. Bülbring E, Barding AF, Jones AW, and Tomita T, Edward Arnold, London, pp 127–156, 1981
2. Huizinga JD: Action potentials in gastrointestinal smooth muscle. *Can J Physiol Pharmacol* **69**: 1133–1142, 1991
 3. Sanders KM: Ionic mechanisms of electrical rhythmicity in gastrointestinal smooth muscles. *Annu Rev Physiol* **54**: 439–453, 1992
 4. Golenhofen K and Lammel E: Selective suppression of some components of spontaneous activity in various types of smooth muscle by iproveratril (verapamil). *Pflügers Arch* **331**: 233–243, 1972
 5. Tomita T, Ashoori F, Kobayashi T, Takai A, and Tokuno H: Glycolysis and spontaneous activity in the guinea-pig stomach muscle. *Proc Int Union Physiol Sci* **15**: 455, 1983
 6. Moncada S, Palmer RMJ, and Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* **43**: 109–142, 1991
 7. Noma A: ATP-regulated K channels in cardiac muscle. *Nature* **385**: 147–148, 1983
 8. Zunker BJ, Lenzen S, Manner K, Panten U, and Trube G: Concentration-dependent effects of tolbutamide, meglitinide, glibenclamide and diazoxide on ATP-regulated K⁺ currents in pancreatic β -cells. *Naunyn-Schmiedberg's Arch Pharmacol* **337**: 225–230, 1988
 9. Winkquist RJ, Heaney LA, Wallace AA, Baskin EP, Stein RB, Garcia ML, and Kaczorowski GJ: Glybide blocks the relaxation response to BRL 34915 (cromakalin), minoxidil sulfate and diazoxide in vascular smooth muscle. *J Pharmacol Exp Ther* **248**: 149–156, 1989
 10. Hamilton TC and Weston AH: Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br J Pharmacol* **88**: 103–111, 1986
 11. Baccari MC, Calamai F, and Staderini G: L-N^G-nitro-arginine potentiates K⁺-induced contracture in the circular muscle of rabbit gastric corpus. *Acta Physiol Scand* **144**: 397–398, 1992
 12. Lefebvre RA, Baert E, and Barbier AJ: Influence of N^G-nitro-L-arginine on non-adrenergic non-cholinergic relaxation in the guinea-pig gastric fundus. *Br J Pharmacol* **106**: 173–179, 1992
 13. Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, and Nelson MT: Hyperpolarizing vasodilators activate ATP-sensitive K⁺ channels in arterial smooth muscle. *Science* **245**: 177–180, 1989
 14. Daut J, Maier-Rudolph W, von Beckerath N, Mehrke G, Günther K, and Goedel-Meinen L: Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science* **247**: 1341–1344, 1990