Effects of Extracellular Sodium on the Slow Wave of the Antral Smooth Muscle in Guinea Pig Stomach

Sang Jin Lee, Ki Whan Kim and Woo Gyeum Kim

Department of Physiology, College of Medicine, Seoul National University, Seoul 110-880, Korea

Abstract: The characteristics of contractile and electrical responses to various concentrations of extracellular sodium in the antrum of guinea-pig stomach were investigated in order to elucidate the mechanisms generating and controlling the slow wave which is known to be responsible for the gastric motility. The results obtained were as follows:

1) Tonic contraction increased as the concentration of extracellular sodium decreased. However, phasic contraction gave a different response around 10 mM Na⁺.

2) In Na⁺-deficient solutions, prolongation in the second component and potentiation in the spike component of the slow wave and membrane depolarization were observed. Those changes became more marked with each decrement of Na⁺ concentration.

3) In solutions below 10 mM Na⁺, small slow waves whose components could not be clearly distinguished appeared at a faster frequency than it did in normal Tyrode solution.

4) In the presence of guanethidine and atropine the change described in 3) disappeared in solutions of Na⁺ concentration below 10 mM. Instead the same responses as described in 2) were observable.

From the above results following conclusions can be made: As the concentration of extracellular sodium decreases the configuration of the slow wave of the antral smooth muscle in guinea-pig stomach changed markedly, showing prolongation in the second component, potentiation in the spike component and depolarization in the membrane potential. These results are direct effects of extracellular sodium on the smooth muscle cell membrane. However, in low sodium concentration below about 10 mM, the influence of autonomic nervous system on the slow wave becomes remarkable and above changes of the slow wave cannot be found.

Key words: Guinea pig stomach, Slow wave, Depolarization, Second component, Spike component

INTRODUCTION

Rhythmic activity is characteristic of many smooth muscles, and frequently, electrical recording of the membrane potential reveals underlying slow rhythmic depolarizations. The spontaneous slow rhythmic depolarizations observed in gastrointestinal smooth muscles which have been given various names are called slow waves in general (Papasova et al. 1968; Prosser and Bortoff 1968). The typical slow wave in guinea-pig antrum is known to consist of an early part of the slow wave, the first component, and a following potential, the second component. The spike activity may appear on top of the second component. The first component occupies the bottom part of the slow wave and the second component the upper part. In this type of slow wave
the first component is believed to be generated by a potential-independent process and this component triggers the second component which is potential-dependent (Ohba et al. 1975, 1977).

Several hypotheses have been proposed for the mechanism of the generation of slow waves. The first component of the slow wave in the circular muscle of guinea-pig stomach is assumed to be generated by electrogenic Na\(^+\)-Ca\(^{2+}\) exchange mechanism, and the second component is thought to be due to an increase in Ca\(^{2+}\) conductance initiated by the depolarization of the first component (Ohba et al. 1975, 1976, 1977; Tomita and Sakamoto 1977). The slow wave of small intestine in cat and dog is known to be generated by periodic fluctuation of electrogenic Na\(^+\)-pump (Daniel 1965; Liu et al. 1969; Connor et al. 1974). And the two components of the slow wave in rabbit longitudinal muscle is explained by alternative increase of conductance to Na\(^+\) and Cl\(^-\) (El-Sharkawy and Daniel 1975).

In the great majority of cases, both the generation and the propagation of slow waves occur in the presence of tetrodotoxin (TTX) or guanethidine or atropine, and thus these processes are likely to be myogenic (Papasova et al. 1968; Liu et al. 1969; Szurszewski 1975; El-sharkawy et al. 1978). But, humoral substances like neurotransmitters or hormones which have an influence on slow waves have yet to be elucidated with respect to the contractile modulators.

After the existence of Na\(^+\)-Ca\(^{2+}\) exchange mechanisms were proved in heart muscle cell, many investigations on effect of Na\(^+\) ions on the contractions in smooth muscle cell were made. Nevertheless, the mechanisms generating slow waves are still unclear and remain to be established in near future.

Therefore, in this study the contractile and electrical responses to various concentrations of extracellular Na\(^+\) in the circular muscles of gastric antrum in guinea-pig were analyzed. Thus, the characteristics of the smooth muscle cell were investigated according to the components of slow waves, and an attempt was made to determine the mechanisms generating and controlling slow waves in relation to the autonomic nervous system.

**MATERIALS AND METHODS**

Guinea-pigs of either sex, weighing about 300g, were stunned and bled. The whole stomach was excised and placed in a bath containing oxygenated Tyrode solution. Again only the antrum region was obtained and cut in the longitudinal direction along the lesser curvature. After the contents of the stomach and mucosal layer were removed, patches of the muscle coat were obtained by cutting mucosa layer off in Tyrode solution at room temperature.

Circular muscle strips, 2 mm wide and 10 mm long, were dissected from the antrum region and used as a preparation for the experiment. The mechanical responses were recorded first in vertical chamber which had a capacity of 100 ml and then the mechanical and electrical responses were simultaneously recorded using conventional glass capillary microelectrode technique in a horizontal chamber. This chamber was made of lucite plate and had a capacity of about 2 ml. In the horizontal chamber the preparation was pinned out at one end with fine needles on a rubber plate which was fixed at the bottom of the chamber and was connected to the force transducer (Grass FT-03) at the other free end.

The tip resistance of the electrode filled with 3 M KCl ranged between 40-80 M\(\Omega\). The electrode was inserted from the mucosal side of the tissue, and the electrical activity was recorded on a pen recorder (Devices), and was perfused constantly with Tris-buffered normal Tyrode solution that was bubbled with pure oxygen gas and kept 35°C.

The ionic composition of Tyrode solutions was as follows (mM): NaCl 147, KCl 4, MgCl\(_2\) 6, H\(_2\)O 1.05, CaCl\(_2\) 2, H\(_2\)O 2, NaH\(_2\)PO\(_4\) 0.42, Na\(_2\)HPO\(_4\) 12H\(_2\)O 1.81 glucose 5.5 in phosphate-buffered Tyrode solution; NaCl 147, KCl 4, CaCl\(_2\) 2, H\(_2\)O 2, MgCl\(_2\) 6, H\(_2\)O 1.05, Tris HCl 5, glucose 5.5 in Tris-buffered Tyrode solution. The pH of the solution was kept at 7.35.

Na\(^+\)-deficient solutions were made by substituting Tris HCl in an equimolar basis for NaCl.

Drugs used were: Tetrodotoxin(Sankyo), Guanethidine sulfate(Tokyo Kasei) and Atropine sulfate(Sigma).
Fig. 1. Relationship between extracellular sodium concentration and mechanical contraction in the antral circular muscle strip of guinea-pig stomach.

Note that the responses are somewhat different between recordings of upper part (100, 50, 10 mM Na\(^+\)) and lower part (5.0 mM Na\(^+\)). In the upper part recordings showed relatively constant phasic contraction, but in the lower part phasic contractions increased in amplitude at first and then dropped to a new lower amplitude after a few minutes, with consequent increase of frequency of phasic contraction.

The relationship between extracellular Na\(^+\) concentration and the amplitude of tonic contraction in the antral circular muscle strip of guinea-pig stomach.

Each closed circle and bar represents the mean and standard error, respectively (mean ± S.E.D., n = 6).

Fig. 2. Relationship between extracellular sodium concentration and the amplitude of tonic contraction in the antral circular muscle strip of guinea-pig stomach.

RESULTS

Effect of extracellular Na\(^+\) on the contraction

The experiment was done to investigate the change of mechanical response as the concentration of extracellular Na\(^+\) ions was reduced to 0 mM. The concentrations used in this experiment were 147 (normal), 50, 10, 5 and 0 mM.

Fig. 1 shows various contractile responses to the changes in the extracellular Na\(^+\) concentration from normal concentration to 0 mM. The contractions were constituted of both tonic and phasic components. The amplitude of tonic component increased as extracellular Na\(^+\) concentration decreased. However, the phasic responses were different from the tonic ones in that they showed two distinct responses. In the concentration range of 100, 50, 10 mM Na\(^+\), the amplitude of phasic contraction increased as Na\(^+\) concentration decreased, while in the range of 10, 5, 0 mM Na\(^+\), the phasic contraction increased in amplitude at first and then dropped to a new lower amplitude after a few minutes, with consequent increase of frequency of phasic contraction.

The relationship between extracellular Na\(^+\) concentration and the amplitude of tonic contraction is illustrated in Fig. 2. The amplitude of tonic contraction in Na\(^+\)-free Tyrode solution was given the value 100% as a reference and then evaluated. The relative percentages were as follows: 6.37 ± 1.03 at 100 mM (mean ± S.E.D., n = 6), 26.37 ± 1.38 at 50 mM, 48.37 ± 2.21 at 10 mM, 69.16 ± 3.16 at 5 mM.

A graph denoting together the relative amplitude and the relative frequency of phasic contraction is represented in Fig. 3. Again the amplitude and the frequency of phasic contraction in Na\(^+\)-free Tyrode solution were given the value 100% as a reference respectively. The relative percentages for a given solution were as follows (%): 17.03 ± 1.61 (Mean ± S.E.D., n = 6), 74.48 ± 6.71 at 147 mM; 48.14 ± 4.52, 69.43 ± 2.25 at 100 mM; 91.69 ± 5.29, 64.23 ± 1.66 at 50 mM; 116.7 ± 5.91, 66.75 ± 2.13 at 10 mM; 107.52 ± 1.83, 80.52 ± 2.16 at 5 mM.

In the concentration range above 10 mM Na\(^+\), the amplitude of phasic contraction increased and the frequency decreased as Na\(^+\) concentration decreased, and in the concentration range
below 10 mM Na⁺, the amplitude decreased and the frequency increased.

In brief summary the responses were different below and above 10 mM Na⁺ and the relationship between the amplitude and the frequency was inversely related to each other.

**Effect of extracellular Na⁺ on the slow wave**

When the preparation was perfused with 100 mM Na⁺ solution made replacing NaCl with Tris · Cl the membrane potential showed about 10 mV hyperpolarization and the amplitude of slow wave was increased and frequency decreased (Fig. 4). The changes in configuration of slow waves were potentiation in the spike component and prolongation in the second component, when perfused with normal Tyrode solution the preparation recovered to normal state, showing depolarizing membrane potential, decreasing amplitude and increasing frequency.

When 100 mM Na⁺ solution was made by replacing NaCl with sucrose, the resulting outcome was similar to that of Tris-replacement in that membrane potential became hyperpolarized by 10 mV, but was different in that the amplitude was unchanged (Fig. 5). It is unclear whether the amplitude change was real. Therefore in the following experiments where Na⁺-deficient solutions were made by replacing NaCl with Tris · Cl, the data interpretation has focused primarily on the change of slow wave configuration and membrane potential.

In 50 mM Na⁺ solution the membrane potential was hyperpolarized by 5 mV and the amplitude of slow waves increased and the frequency decreased (Fig. 6). Note that potentiation in the spike component and prolongation in the second component became more marked than in 100 mM Na⁺ solution.
Fig. 5. Relationship between isometric tension and membrane potential in the concentration of 100 mM Na⁺ in the antral circular muscle strip of guinea-pig stomach (Sucrose replacement).

Fig. 6. The effects of 50 mM Na⁺ on the contractile and electrical activities in the antral circular muscle strip of guinea-pig stomach. (A): control (B); 50 mM Na⁺ (C); 50 mM Na⁺ after 10 min (D); wash-out with normal Tyrode.

In 10 mM Na⁺ solution the membrane potential either maintained nearly normal level or became slightly depolarized, and the amplitude increased while the frequency decreased (Fig. 7). Potentiation in the spike component and prolongation in the second component were most marked at this concentration. The two components of the slow wave which became clearly prominent evoked two component contraction in contractile activity.

As Na⁺-free Tyrode solution was introduced the membrane potential was hyperpolarized and the first component was markedly prolonged (Fig. 8). This brought about decrease in frequency. But after a few minutes the membrane potential became depolarized with appearance of some oscillatory electrical activities, which seemed to be the second component. When perfusion with normal Tyrode solution was done again, the depolarization disappeared and the first component was restored.

Effect of the change of extracellular Na⁺ concentration on the slow wave of the autonomic nerves

Introducing three neurotransmitter blockers, the effect on the slow wave of autonomic nerves ubiquitous in muscle layer of stomach
was analyzed as extracellular Na\(^+\) concentration was changed. The three blockers are \(3 \times 10^{-7}\) M tetrodotoxin, \(5 \times 10^{-6}\) M guanethidine and \(1 \times 10^{-6}\) M atropine. When the preparation was perfused with normal Tyrode solution containing 3 blockers for 10 minutes particular changes could not be found in slow waves. Thereafter it was perfused with 100 mM Na\(^+\) solution containing 3 blockers (Fig. 9). The observed changes were hyperpolarization of membrane potential, potentiation in the spike component and prolongation in the second component. These were similar to former findings as in Fig. 4, which were obtained in the absence of 3 blockers.

The response in Na\(^+\)-free Tyrode solution under the influence of 3 blockers was similar to that in 100 mM or 50 mM Na\(^+\) solution (Fig. 10): potentiation in the spike component, prolongation in the second component and decrease in frequency. These observations presented a striking contrast to the former findings of Fig. 8, which showed more complicated results.

To find out which blockers made such a difference in response of Na\(^+\)-free contracture, these 3 blockers were administered separately one after another (Fig. 11). When only tetrodotoxin was treated as in Fig. 11(A) particular changes could not be found; when guanethidine was treated as in Fig. 11(B) much reduction of the amplitude of both tonic and phasic contraction was observed; when atropine was treated as in Fig. 11(C) the most marked reduction of the amplitude of both tonic and phasic contraction was observed.

**DISCUSSION**

The slow wave usually triggers repetitive spike activity on top of the depolarization, and the strength of the mechanical response is closely related to the spike activity. So, the slow wave is reflected well in the contraction. The overall experiment results are represented in 100, 50, 10 mM Na\(^+\) solutions. But in low Na\(^+\) solutions below about 10 mM, influences of autonomic nervous system cannot be neglected and another experiment has to be done.

In the concentration range above 10 mM Na\(^+\),
neurotransmitter blockers had negligible influence on the slow wave and on the contraction. This fact means that the findings obtained in this concentration range is not from interaction of nerve and muscle but from muscle tissue itself. The findings in this range were potentiation of the spike component, prolongation of the second component, increase of the amplitude of overall slow wave, decrease of frequency and hyperpolarization of the membrane potential. Smooth muscle cells have a relatively low resting membrane potential in comparison with heart muscle cells. In other words, sodium channels are open in relatively much fraction so that sodium permeability is increased to some extent at resting membrane potential. If extracellular sodium concentration is decreased in step by step fashion, the hyperpolarization of the membrane ought to be increased theoretically to as much extent as Goldman–Hodgkin–Katz equation calculate. But, practical result indicates that the amplitude of hyperpolarization is clearly in decreasing direction. Therefore it is probably more correct to describe the membrane potential becoming depolarized rather than hyperpolarized as extracellular sodium concentration decreased. We cannot evaluate the degree of depolarization quantitatively, and can presume that the amplitude of the tonic component of contraction is related to that of depolarization of slow waves. For the present several hypotheses have been proposed for the depolarization. First, Na⁺ removal increases calcium permeability by unknown mechanism and this calcium influx into the cell contributes to the membrane depolarization (Osa 1971). Second, Na⁺ removal influences electrogenic ion pump to make membrane depolarized, with consequent calcium influx into the cell (van Breemen et al. 1977; Sakanoto and Tomita 1982). Besides, Na⁺–Ca⁺ exchange mechanism described in heart muscle cells may have something to do with the depolarization (Reuter and Seitz 1968; Baker et al. 1969; Brading et al. 1980; Brading 1981). The increase of the amplitude of slow waves is difficult to believe, because this observation is not
found in sodium deficient solutions made by sucrose replacement as well as in previous experiment of T. Tomita (Tomita 1981) that was performed using sucrose gap method. But the configuration change of slow waves (potentiation in the spike component and prolongation in the second component) and decrease of frequency are clear. These results are also found in experiment of excess calcium solution, where configuration changes of the slow wave and membrane potential changes were measured as extracellular calcium concentration increased (Rhie and Kim 1987; Ohba et al. 1977). From the above lines of evidence, it can be suggested that electrogenic Na\(^{+}\)-Ca\(^{2+}\) exchange mechanism is related to the generation of slow waves.

In solutions containing less than 10 mM Na\(^{+}\), especially in Na\(^{+}\)-free solution, the slow wave became initially hyperpolarized and gradually showed prolongation in the repolarizing phase (falling phase) of the first component and subsequent decrease of frequency. After a few minutes the first component was lost and replaced by persistent depolarization. On top of it certain waves which seemed to be the second component appeared in faster frequency. Exposure to normal Tyrode solution brought about complete recovery of slow waves showing repolarization to resting membrane potential, reappearance of the first component and decrease of frequency.

Here the depolarization by Na\(^{+}\) removal seems to be a prolongation of the first component and the oscillating activity on top of it the second component. The depolarization due to Na\(^{+}\) removal is difficult to explain. It may be that an increase in Ca\(^{2+}\) conductance, an increase in Cl\(^{-}\) conductance, a decrease in K\(^{+}\) conductance, suppression of the electrogenic Na\(^{+}\) pump, or some combination of these changes is responsible for the depolarization. However, contradictory evidence exists for all (Tomita 1981).

To study the effect of autonomic nerves which exist extensively in smooth muscle, neurotransmitter blockers (tetrodotoxin, guanethidine, atropine) were introduced. In normal Tyrode solution and in Na\(^{+}\)-deficient solutions containing more than 10 mM, the slow wave did not display remarkable discrepancy between the responses in the presence and in the absence of blockers. In Na\(^{+}\)-deficient solutions containing less than 10 mM the response pattern to Na\(^{+}\)-deficient solutions containing three blockers was basically identical with that to Na\(^{+}\)-deficient solutions containing more than 10 mM. Speculating from above results on Na\(^{+}\)-free contracture response it is assumed that the three blockers facilitated repolarizing phase of the first component. More specific effect can be examined on the basis of individual drug action. Tetrodotoxin (TTX) is a well known specific Na\(^{+}\) channel blocker. It seemed that the drug had no effect at all and the response had nothing to do with Na\(^{+}\) conductance. Guanethidine(GED) or adrenergic neuron blocker, and atropine or muscarinic receptor blocker, played a major role in changing the response of Na\(^{+}\)-free contracture. Guanethidine is a neuron blocker acting on presynaptic neuron and atropine is a receptor blocker acting on postsynaptic membrane. Therefore, effects of these two neurotransmitter blockers on receptor operated ion channel can be different. The above results suggest that Na\(^{+}\) removal induced the release of neurotransmitters from autonomic ganglia or autonomic nerve terminals by unknown mechanism and these neurotransmitters inhibited the repolarizing phase of the first component and this effect was abolished by the action of guanethidine and atropine. But we do acknowledge that further experiments are needed to assure these results.

REFERENCES


El-Sharkawy TY, Daniel EE. Electrical activity of small
Ohba M, Sakamoto Y, Tomita T. The slow wave in the circular muscle of the guinea-pig stomach. J. Physiol. 1975, 253:505-516
Ohba M, Sakamoto Y, Tomita T. Effects of sodium, potassium and calcium ions on the slow wave in the circular muscle of the guinea-pig stomach. J. Physiol. 1977, 267:167-180
Reuter H, Seitz N. The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. J. Physiol. 1968, 195:451-470
Szurszewski JH. Mechanism of action of pentagastrin and acetylcholine on the longitudinal muscle of canine antrum. J. Physiol. 1975, 252:335-361
세포외 Na⁺농도 변화가 기니피그 위 평활근 세포에 미치는 영향

서울대학교 의과대학 생리학교실

이상진·김기환·김우경

위의 농도를 유발시키는 세포의 발생과 조절근의 일관을 밝히기 위하여 기니피그(guinea-pig) 위에서 유문동 부분을 적출하여 세포외 Na⁺농도 변화에 따른 수축과 일기 활동의 특성을 비교 분석하여 다음의 결과를 얻었다.
1) 기계적 수축반응에서, 세포외 Na⁺농도가 감소함에 따라 강정성 수축 성분은 증가하였으나
위상성 수축 성분은 10 mM 농도를 전후하여 다른 반응을 나타내었다.
2) Na⁺농도가 정상보다 낮은 용액에서는 세포의 제2성분과 가시전압성분이 커졌으며 약전압은
탈분극되었다. 이와같은 변화는 Na⁺농도가 감소함에 따라 더욱 두려하게 나타났다.
3) Na⁺농도가 10 mM 이하인 용액에서는 성분구별이 분명한 세포가 정상시에 비하여 빠른 수
도로 나타났다.
4) guanethidine와 atropine에 의하여 Na⁺농도가 10 mM 이하에서도 3)과 같은 반응이 나타나
고 2)와 같은 반응이 나타났다.

이상의 실험결과를 토대로 다음과 같은 결론을 얻었다.

기니피그 유문동 음성근에서 세포외 Na⁺농도가 감소함에 따라 세포의 제2성분과 가시전압성
분이 커지고 약전압이 탈분극된 것은 세포외 Na⁺이 평활근 세포막에 직접 작용한 결과로 해석되
다. 그러나 Na⁺농도가 10 mM 이하일 때는 자율신경계의 영향이 상대적으로 크지면서 다른 반
응양상을 나타내었다고 판단된다.