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Effects of Extracellular Na^+ on K-Free Contracture in Taenia Coli and Gastric Antrum of Guinea Pig[1]

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=Abstract=To study the role of Na-Ca exchange mechanism which controls the intracellular free Ca concentration, the changes in contractile force and membrane potential of G-I smooth muscle were measured. Guinea-pig's taenia coli and gastric antrum were isolated, and all experiments were performed in Tris-buffered Tyrode solution aerated with 100% O_2 and kept 35°C. After application of K-free solution which is known to increase the intracellular Na concentration due to inhibition of Na-K pump, Na-free or low Na solution were applied. Contractions were recorded with force transducer, and membrane potentials were measured by single sucrose-gap method. In the taenia coli, the extent of relaxation of K-free contracture was inversely proportional to external Na concentration. As the external Na concentration was decreased, the membrane potential was more hyperpolarized. In the gastric antrum, the extent of relaxation of K-free contracture increased proportionately to the decrease of external Na concentration. Membrane potential was maximally hyperpolarized at Na-free solution. As extracellular Na concentration was increased, the degree of hyperpolarization decreased, and at 125 mM Na the membrane potential was depolarized. When Ca antagonist (verapamil) was applied, K-free contracture did not develop in the taenia coli. It is concluded that Na-Ca exchange mechanism would play little role in the control of intracellular free Ca concentration in the taenia coli and gastric antrum.

Key words: ***Taenia coli, Gastric antrum, Na-Ca exchange, K-free contracture***

INTRODUCTION

There is a close relationship between the intracellular free Ca concentration and the contraction(Ruegg 1971), but the mechanisms that increase the intracellular free Ca concentration, so induce the contraction are different one another in the various muscle tissues. The depolarization in the skeletal muscle and the Ca current through Ca channel in the cardiac muscle release Ca from the sarcoplasmic reticulum, and thus, increase the intracellular free Ca concentration which leads to contraction. Smooth muscle is the only muscle where the diffusional distance between extracellular space and myofilaments is short enough, and the rate of tension development is slow enough for Ca influx to play a major role in activation of contraction (van Breemen *et al.* 1982).

The mechanism controlling the intracellular free Ca concentration that is closely related to contraction has been studied long before. Since the demonstration of a link between transmembrane Na and Ca movements in heart(Reuter & Seitz 1968), work has been done in many tissues to investigate the existence and the role of Na-Ca exchange. In cardiac muscle, physiologists have strong evidence for the existance of a

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Na-Ca exchange mechanism and have become increasingly enthusiastic about its role in the control of intracelular free Ca. The exchange is thought to be electrogenic and voltage sensitive, and thus to affect not only tension development (Chapman 1974; Chapman & Tunstall 1980; Eisner *et al.* 1983; Vassort 1973) but also the membrane potential itself(Coraboeuf *et al.* 1981; Mullins 1981). In contrast, smooth muscle physiologists have less direct evidence for the exchange, although the importance of Na in determining the contractile state of the tissues has been frequently investigated. Initially, such studies led research workers to look favourably on the idea that a Na-Ca exchange was involved (Bohr *et al.* 1973). This outlook was reinforced by the demonstration of Na-dependent Ca efflux in arterial smooth muscle and culminated in speculations on the involvement of Na-Ca exchange in the etiology of hypertension(Blaustein 1977). Several workers have become increasingly reluctant to assign much importance to a Na-Ca exchange in the regulation of intracellular free Ca(Casteels & van Breemen 1975; Droogmans & Casteels 1979; Raeymaekers *et al.* 1974; van Breemen *et al.* 1978).

There have been many arguments whether or not Na-Ca exchange would play a major role in the control of intracellular free Ca in taenia coli and gastric antrum of guinea-pig. In taenia coli the evidence that Na-Ca exchange is important was mainly obtained by the flux experiments(Brading 1978; Brading & Widdicombe 1977; Katase & Tomita 1972; Ma & Bose 1977). Results that deny the existence of Na-Ca exchange and favor the role of a Ca pump were obtained in other studies (Casteels & van Breemen 1975; van Breemen *et al.* 1975, 1982). In gastric antrum, Na-Ca exchange has been reported to play a major role in the control of the intracellular free Ca in the longitudinal muscle, and Ca pump in the circular muscle (Kuriyama *et al.* 1975). Na-Ca exchange is also considered as a mechanism of the slow wave(Ohba *et al.* 1975, 1977). We therefore tried to study the ability of low Na and Na-free to induce contractions by Na-Ca exchange in guinea-pig taenia coli and gastric antrum with normal and elevated intracellular Na, the susceptibility of these response to Ca-antagonist durgs and the changes in the membrane potential that accompany them.

MATERIAL AND METHODS

1. Preparation

White guinea-pig weighing about 300 g were stunned and bled. The stomach and taenia coli were cut and removed from the animal. The stomach was excised and cut in the longitudinal direction along the lesser curvature. The content within stomach and the mucosal layer were removed from the muscle layers in phosphate-buffered Tyrode solution at room temperature. The strips of circular muscle preparation, 2 mm in width and 10 mm long, were isolated together with the longitudinal layer. The circular muscle and serosal fat were carefully removed from the longitudinal taenia coli muscle. The strips of taenia coli were prepared 2 mm in width and 10 mm long. Both strips were fully relaxed for experiments.

2. Solutions

A Tris-buffered normal Tyrode solution contains NaCl 147 mM, KCl 4 mM, $CaCl_2$-$2H_2O$ 2 mM, $MgCl_2$-$6H_2O$ 1.05 mM, Tris-HCl 5 mM, glucose 5.5 mM; equilibrated with 100% O_2, pH 7.4 at 35°C. Na-free soutions were made by replacing NaCl isosmotically with Tris-Cl, sucrose, or choline chloride. Variations in the Na concentration were made by replacing NaCl isosmotically with Tris-Cl.

3. Experimental apparatus and protocol

Tissues were allowed to relax at the horizontal chamber for at least 1 hour in Tris-buffered Tyrode solution at 35°C before being exposed to the experimental solution. Then isometric contractions were recorded by using a transducer (Device force transducer). When spontaneous activity reached the steady-state, length-tension curves were obtained and all experiments were performed at optimal length.

The membrane potentials were recorded by single sucrose-gap method. The single sucrose-gap method was modified from the rubber membrane method by using material, which was made from very elastic rubber and X-ray film, to partition off the horizontal chamber(volume 2 ml) to three compartments. The sucrose-gap was 2 mm in width. Tissues were allowed to relax at the horizontal chamber for at least 1 hour in Tris-buffered Tyrode solution at 35°C before being exposed to sucrose and isosmotic KCl.

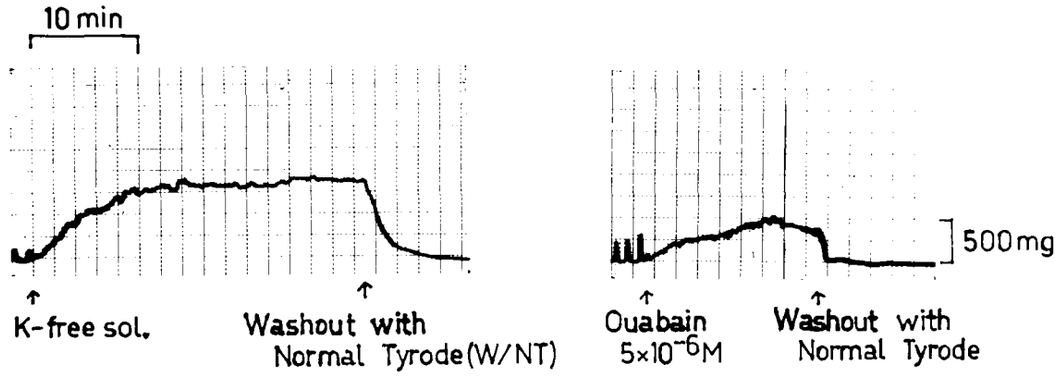

Fig. 1. Comparison of K-free contracture with ouabain-induced contracture in taenia coli. K-free contracture appeared immediately and developed a constant amplitude of tonic contracture 10 min after the application of K-free Tyrode solution.

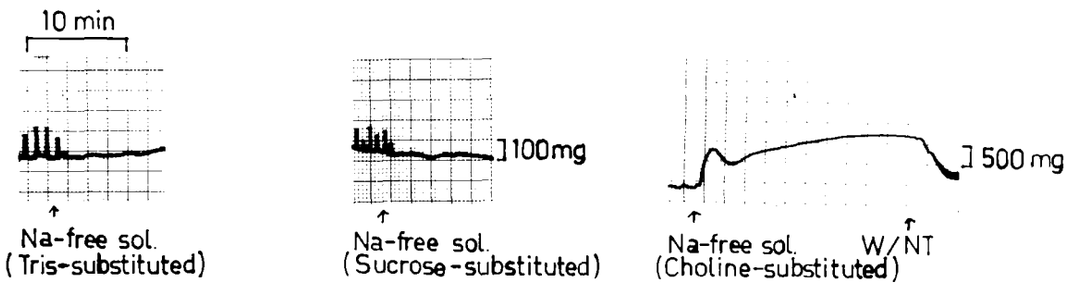

Fig. 2. Effects of some substitutes for extracellular Na in taenia coli. Na-free solution substituted by Tris or sucrose led to the disappearance of spontaneous contraction, while choline-substituted Na-free solution induced contracture.

The electrical signal that was preamplified ten times was connected to a DC amplifier, and recorded by the pen recorder(Device physiograph). Membrane potential recorded by intracellular glass microelectrode is about −53 mV in the taenia coli(Bülbring & Kuriyama 1963; Tomita 1966), and about −63 mV in the gastric antrum-(Komori & Suzuki 1986). Membrane potential recorded by single sucrose-gap was about −25 mV in the taenia coli, and about −30 mV in the gastric antrum. In principle, if the resistance of the sucrose-gap is infinite, membrane potential recorded by single sucroose-gap method would be equal to membrane potential recorded by glass microelectrode. Comparing the above results implied that there must be short-circuit across the sucrose-gap(Kuriyama & Tomita 1970). This method, therefore, represents the direction of membrane potential change qualitatively, not the quantitative change of membrane potential.

RESULTS

1. Effects of K-free solution

Changes in tension development by K removal from the Tyrode solution is shown in Fig. 1. The K-free contracture reached steady-state in about 10 minutes and sustained itself until K-free solution was replaced by normal Tyrode solution. It is known that the K-free contracture developed due to the inhibition of the Na-K pump(Casteels 1966; Tomita & Yamamoto 1971), similarly contracture also developed with digitalis. Membrane potential depolarized after application of K-free solution(Fig. 6).

2. Effects of substances substituted to Na to make Na-free solution

NaCl was replaced isosomtically with Tris-Cl, sucrose, or choline cholride. When Tris-substituted and sucrose-substituted Na-free solution were applied, spontaneous contraction disappeared, but when choline-substituted Na-free

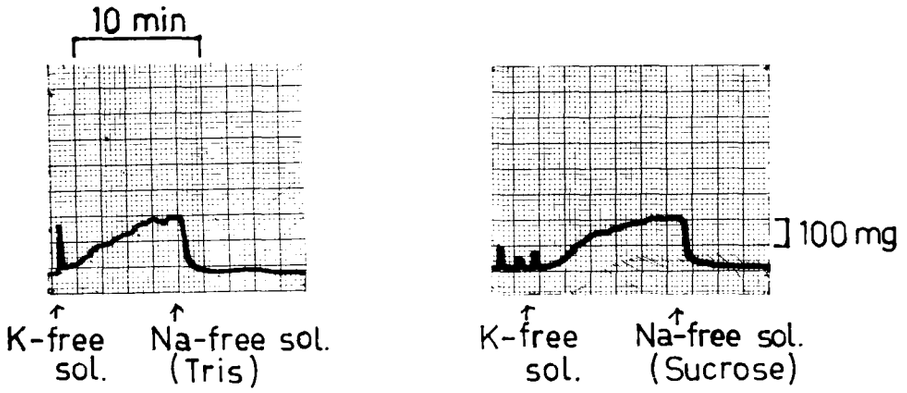

Fig. 3. Effects of extracellular Na on K-free contracture in taenia coli. K-free contracture was completely relaxed immediately after the application of Na-free solution. The response of Na-free solution on K-free contracture was similar in both Tris- or sucrose-substituted solution.

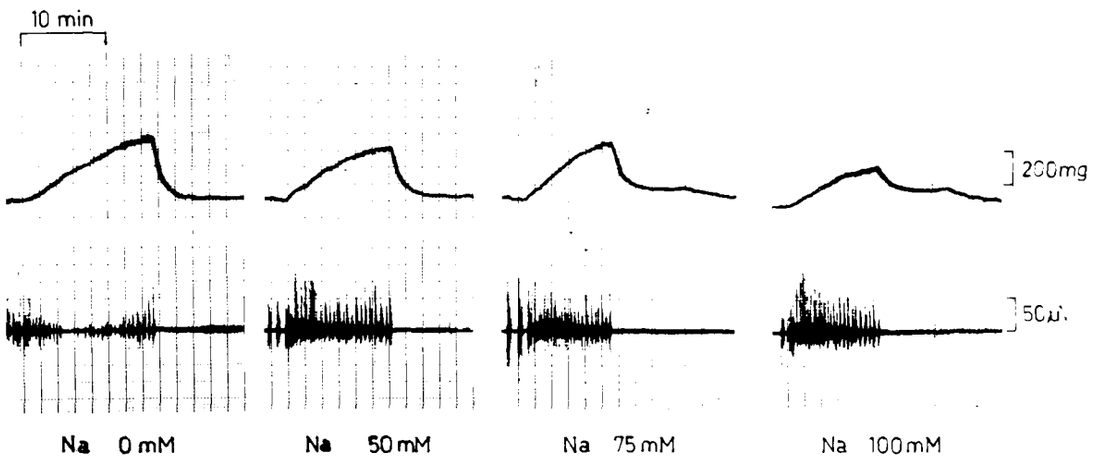

Fig. 4. Effects of extracellular Na on K-free contracture in taneia coli. K-free contracture was completely relaxed immediately after the application of 0 mM Na Tyrode solution. The relaxation extent of K-free contracture was extracellular Na-dependent; the basal tone remained was increased proportionately to extracellular Na.

solution was applied, changes of membrane activity occurred and contractures developed. Even though atropine 10^{-6}M, which is known to inhibit the effect of acetylcholine at the synapse-(Holman 1957), was applied before the application of Na-free solution, choline-substituted Na-free contracture didn't disappear.

K-free contracture were relaxed by either Tris-substituted Na-free solution or sucrose-substituted Na-free solution(Fig. 3).

3. Effects of extracellular Na on K-free contracture

In the taenia coli. 0 mM. 50 mM Na solutions completely relaxed K-free contracture immediately after aplication. But 75 mM, 100 mM Na solution incompletely relaxed the contracture, and the basal tone was remained to be increased proportional to extracellular Na concentration(Fig. 4). As the maximal rate of relaxation and the relaxation time constant are considered to be the reference of relaxation, the relation between Na concentration and extent of relaxation is linear (Fig. 5). The membrane potential recorded by single sucrose-gap method is shown in Fig. 6. The membrane potential became more hyperpolarized as the Na concentra-

tion was decreased; that is, 2 mV more negative than resting membrane potential at 100 mM Na, 4 mV at 75 mM, 5 mV at 50 mM, 7 mV at 25 mM, and 14 mV at 0 mM. The changing rate of membrane potential was 5.5 mV/decade(Fig. 7).

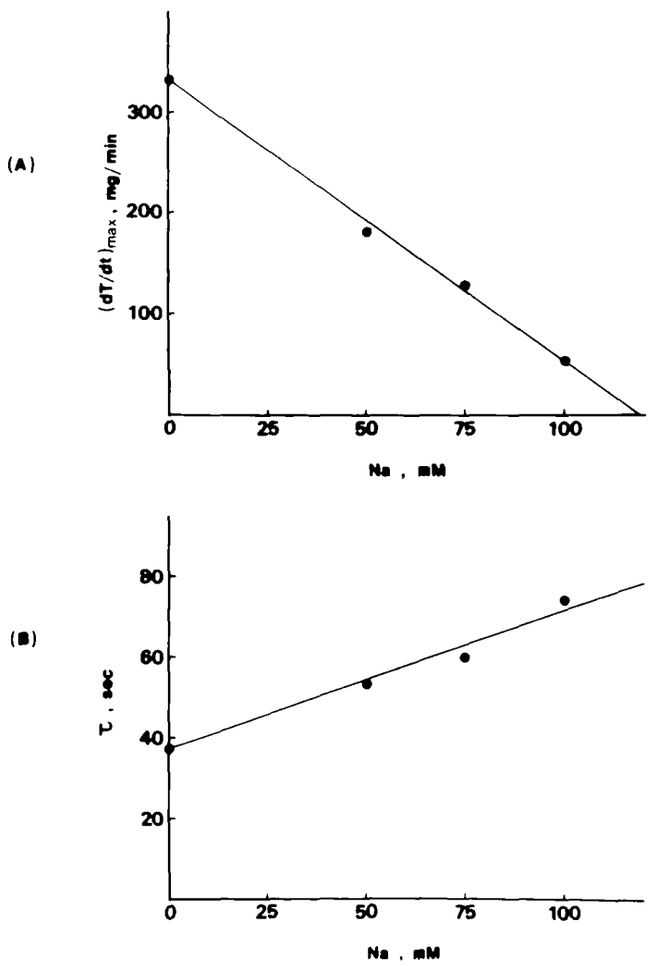

Fig. 5. Relationship between the extent of relaxation and extracellular Na in the taenia coli. $(dT/dt)_{max}$ or τ was linearly proportional to extracellular Na. (dT/dt)max: the maximal rate of relaxation; τ : relaxation time constant.

In the gastric antrum, the extent of relaxation in the K-free contracture was increased as Na concentration was decreased(Fig. 8). The membrane potential was maximally hyperpolarized at 0 mM Na. The degree of hyperpolarization were decreased along with increasing the Na concentration. At 100 mM Na, the membrane potential returned to the resting membrane potential, and a little depolarized at 125 mM(Fig. 9).

4. Effects of Ca antagonist

When the Ca antagonist(verapamil) was applied, there was no change of contraction due to K-free and Na-free state(Fig. 10).

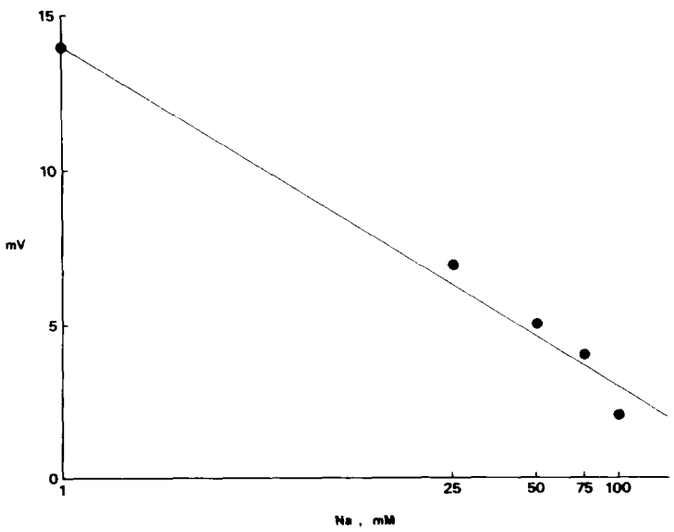

Fig. 7. Relationship between extracellular Na and membrane potential in taenia coli. The changing rate of the membrane potential was 5.5 mV/decade.

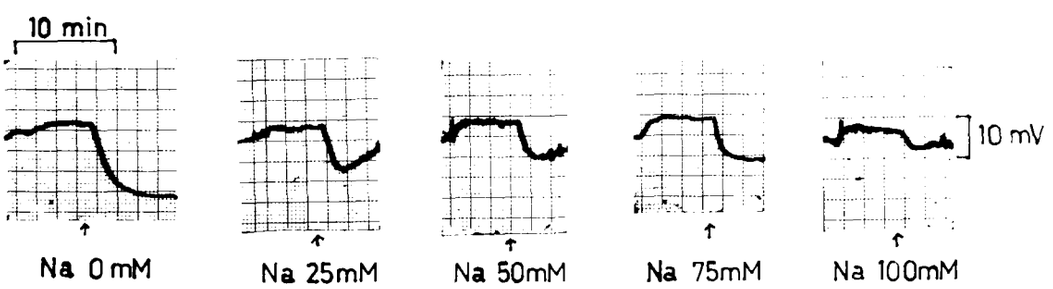

Fig. 6. Effects of extracellular Na on the membrane potential developed during K-free contracture in taenia coli. The membrane potential was more hyperpolarized as extracellular Na was decreased.

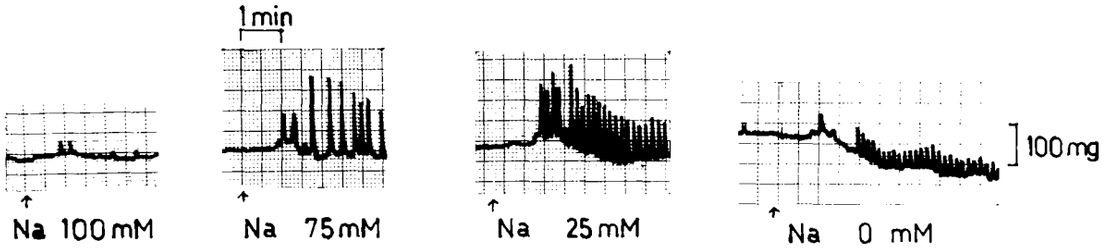

Fig. 8. Effects of extracellular Na on K-free contracture in the anturm. The extent of relaxation in the K-free contracture was increased as extracellular Na was decreased.

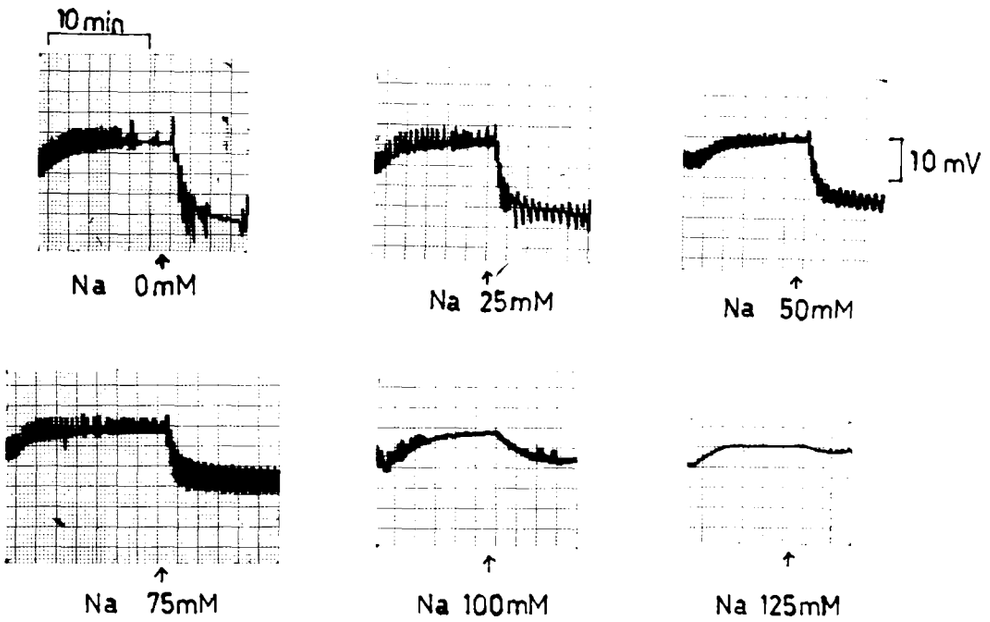

Fig. 9. Effects of extracellular Na on the membrane potential in antrum. The membrane potential was maximally hyperpolarized at 0 mM Na, after which the degree of hyperpolarization was decreased. The membrane potential returned to the resting membrane potential at 100 mM Na, and slightly depolarized at 125 mM Na.

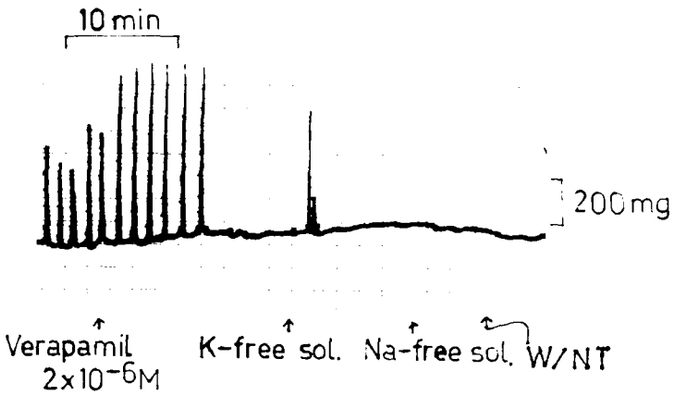

Fig. 10. Effects of Ca antagonist(verapamil) on K-free contracture in taenia coli. There were no changes of contraction due to K-free and Na-free state when verapamil was pretreated.

DISCUSSION

In taenia coli, what is known to play major roles in the control of the intracellular free Ca are: 1) Ca flux through Ca channel 2) Na-Ca exchange, and 3) ATP-dependent Ca pump(van Breemen *et al.* 1982). First, there is a body of evidence for role of Ca flux. Spike potentials are considered as Ca spikes in the taenia coli (Inomata & Kao 1976; Yashida & Yabu 1985). The diffusional distance between extracellular space and myofilaments is short enough, and the rate of tension development is slow enough for Ca influx to play a major role in activation of contractions(van Breemen *et al.* 1982). In addition,

amplitude of contraction is proportional to spike frequency(Kim & Kim 1985), and as extracellular Ca was decreased, spike frequency and amplitude of contractions were reduced. At 0 mM Ca spontaneous contraction disappears(Brading *et al.* 1969; Kim & Kim 1985). Second, there are evidence for existence of Na-Ca exchange. High K-contracture was relaxed by Na while membrane potential continuously depolarized, and increase of intracellular Na concentration increased Ca influx. The above facts suggest that Na-Ca exchange play a certain role in the control of the intracellular free Ca(Brading 1978; Brading & Widdicombe 1977; Katase & Tomita 1972; Ma & Bose 1977). But others insisted that K-free solution and 3×10^{-5} M ouabain did not cause significant increase in total cellular Ca content, and Na-Ca exchange is nonspecific, that is, Li and K instead of Na could decrease the intracellular Ca accumulated when Na-free solution applied. They, therefore, denied the important role of Na-Ca exchange (van Breemen *et al.* 1982). Third, there are indirect evidence for Ca pump. ATP depletion, which is induced by lowering temperature and using metabolic inhibitor, led to rapid Ca accumulation toward euqilibrium well before the Na gradient had changed enough to significantly affect Ca transport(van Breemen *et al.* 1982). With the results denying the existence of Na-Ca exhcange, above results suggest that ATP-dependent pump play a certain role.

In Fig. 10, K-free contracture did not develop when verapamil, which is known as blocker of Ca channel, was pretreated. In Fig. 6, K-free contracture was not potentiated but relaxed by Na-free solution. Our results thus suggest that Na-Ca exchange play little role in the control of the intracellular free Ca. However the possiblities either that when receptor-operated channel increases the Ca permeability Na-Ca exchange might a certain role (Brading 1971, 1977), or that Na-Ca exchange might operate in different pattern compared with vascular smooth muscle and cardiac muscle are remained.

It is thought that K-free solution depolarizes the membrane potential by 3-5 mV, and depolarization releases Ca from sarcoplasmic reticulum that may be closely related to the membrane potential, also open Ca channel through which Ca enters into the cells. As a result, the intracellular free Ca increase and K-free contracture develops. In the taenia coli, it appears that sarcoplasmic reticulum that exist near the membrane might be closely related to membrane potential, and important to control intracellular free Ca(Brading 1977). As the maximal rate of relaxation and relaxation time constant are considered the reference value of relaxation, the relation between Na concentration and extent of relaxation is linear. The reason might be Na permeability is larger than other excitable cells(Brading 1971; Casteels 1966), thus contributions of Na permeability to membrane potential is larger. In the present experiment membrane potential became more hyperpolazied exponentially as Na concentration was decreased(Fig. 7). In the taenia coli, therefore, Na-Ca exchange play little role in the control of intracellular free Ca, and sarcoplasmic reticulum closely related to the membrane potential controls the intracellular free Ca along the membrane potentials.

In the grastric antrum, Na-free solution caused the K-free contracture to relax. Na-free solution didn't maintain the K-free contracture like the vascular smooth muscle, and decreased the contracture, because Na permeability is larger, so Na-free solution causes the membrane potential to hyperpolarized and Ca pump decreases the intracellular free Ca and relaxation occurs, rather than Na-Ca exchange changes the membrane potential and this change causes the Na-Ca exchange to change and relaxation occurs(Kuriyama *et al.* 1975).

It is suggested that in taenia coli and gastric antrum Na-Ca exchange play little role in the control of intracellular free Ca and in the taenia coli Ca spike and sarcoplasmic reticulum closely related to the membrane potential are important, in the gastric antrum Ca pump is important in the control of intracellular free Ca.

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= 국문초록 =

결장뉴와 위 유문동에서 K-제거 경축에 대한 세포밖 Na의 영향

서울대학교 의과대학 생리학교실

서인석 · 김기환 · 김우겸

Na-Ca 교환 기전이 평활근의 세포내 칼슘 이온 농도 조절에 기여하는 정도를 연구하기 위하여 위장관 평활근의 수축과 세포막전압의 변화를 관찰하여 분석하였다.

guinea-pig의 결장뉴와 위 유문동을 적출하여 35°C에서 100% 산소로 평형을 이룬 Tris-완충 Tyrode 용액에서 이들 조직이 일정한 빈도와 크기로 자발적 수축을 하게 되면 세포내 Na 농도를 증가시키는 K-제거 용액(K-free Tyrode solution)으로 전처치하여 K-제거 경축을 유발시키고 여기에 Na-제거 용액과 여러 수준의 Na 농도 용액을 관류시켜 수축곡선의 변화를 기록하였다. 단일 자당액 간극법(single sucrose-gap method)으로는 세포막전압 변화를 기록 분석하여 다음과 같은 결과를 얻었다.

1. 결장뉴에서 K-제거 경축이 이완되는 정도는 세포밖 Na 농도에 반비례하였다.
2. 결장뉴에서 세포밖 Na 농도가 높을수록 과분극되는 정도가 적었다.
3. 유문동에서도 결장뉴처럼 K-제거 경축이 Na-제거 용액에서 이완되었고, 세포밖 Na 농도를 증가시키면 K-제거 경축을 이완시키는 정도가 작아졌고, 세포막전압이 과분극되는 정도도 세포밖 Na 농도가 높을수록 작았다.
4. Ca 억제제인 varapamil을 1 mg/l 전처치하면 K-제거 경축이 거의 생기지 않았다.

이상의 결과로 보아 결장뉴와 위 유문동에서는 세포내 칼슘 이온농도 조절에 Na-Ca 교환 기전의 역할이 중요하지 않는 것으로 생각된다.