MODIFICATION OF THE TONUS AND DRUG-INDUCED CONTRACTION OF THE ISOLATED RAT ILEUM BY REMOVAL OF Na FROM BATHING MEDIA

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Abstract—Effect of removal of Na from bathing media on the tonus and contractile response to drugs of strips of the rat ileum was investigated. Removal of Na from the bathing media by replacing NaCl with sucrose or choline Cl produced a transient contraction, which was not altered by treatment with atropine. This contraction was attenuated when preparations were exposed to Ca-free media. After a 30 min exposure to Na⁺-free media, contractions caused by ACh and Ba and the phasic contraction by K were decreased, whereas the tonic contraction by K was increased. In preparations in which contractions by removal of Na⁺ were abolished by repeated treatments with Na⁺-removal in Ca-free media, the contractions were restored when the preparations were incubated with Ca⁺⁺ and then with Na⁺-containing solutions. This restoration was not obtained when preparations were soaked in Na-free media after incubation with Ca⁺⁺. It appears that removal of Na from bathing media inhibits uptake of Ca by intracellular storage sites and increases the influx of Ca⁺⁺, resulting in an increase in the amount of cellular active Ca⁺⁺, and that Na⁺ plays an important role in binding Ca⁺⁺ in intracellular storage sites.

During the past decade numerous studies on interactions of extracellular Na with Ca responsible for mechanical activities of smooth muscles have accumulated. It is generally agreed that in various organs and animal species reduction in extracellular Na elicits an increase in inward movements of Ca across cell membranes (1-5). However, little information (6, 7) is available concerning the effect of reduced Na on the contractility of intestinal smooth muscles. The present study includes findings showing that removal of Na from bathing media not only increases the influx of Ca into muscle cells but also accelerates the release of Ca from intracellular storage sites.

MATERIALS AND METHODS

Male rats, weighing approx. 300 g, were used. The animals were sacrificed by a blow on the neck and the ileum was dissected. Strips of the ileum, approx. 1 cm long, were fixed vertically in the preparation bath containing 30 ml of nutrient solutions. Isotonic contractions were recorded on a smoked paper with a magnification of approx. 10 times. The solution was maintained at $27.0 \pm 1.0^\circ\text{C}$ and continuously bubbled with air. The composition of the solution was as follows (mM); NaCl, 154; KCl, 5.6; CaCl₂, 2.2;

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glucose, 5.6; NaHCO_3 , 4.8. In the Na-free solution used, NaCl was replaced with isotonic sucrose or choline Cl. When Ca was totally removed, osmotic adjustment was not made. Preparations were allowed to equilibrate for 60 to 90 min in bathing solutions before experiments were begun.

Acetylcholine chloride (Tokyokasei), barium chloride (Wakojunyaku) and *l*-isoproterenol hydrochloride (Proteranol-L, Nikkenkagaku) were used. Drugs were added directly to the bathing medium. Concentrations of the drug are expressed in terms of g/ml of the salts. Concentrations used were 10^{-8} ACh, 10^{-4} Ba and 2×10^{-3} g/ml K, which were obtained as ED_{50} in an earlier report (8).

RESULTS

1) Effect of the removal of Na from bathing media

When NaCl in the bathing media was replaced with sucrose, a transient contraction appeared, and a return to the initial tonus was usually observed about 30 min later. This contraction was reproducible at an interval of 20–30 min and was strongly inhibited by a pre-application of 10^{-7} isoproterenol, in all of 7 cases (Fig. 1). A similar contraction was produced when NaCl was replaced with choline Cl. The transient contraction induced by Na removal was reduced by $24.6 \pm 2.5\%$ ($N=7$) by removal of Ca^{+2} from bathing solutions. In the Ca-free media the contraction gradually decreased by repeated treatments with Na removal and within 4 hr disappeared completely.

FIG. 1. Effect of isoproterenol (Iso) on the contractile response to removal of Na from bathing media.

↓ : Washout with the normal medium.

2) Modification by Na removal of the effect of exogenous Ca

The addition of Ca in concentrations of 2 mM or higher always revealed a relaxation in the normal fluid but a contraction in the Na-free media. The Ca-induced contraction was reduced by $39.8 \pm 3.3\%$ ($N=10$) after 30 min exposure to Na^+ -free media, as compared with that after 10 min exposure (Fig. 2).

In preparations in which K-induced contractions were abolished by repeated additions of K in Ca-free solutions, the addition of 2 mM Ca to Ca-free media produced a contraction. This contraction was potentiated by $35.7 \pm 4.9\%$ ($N=6$) 1 min after removal of Na from the Ca-free media. The effect of Na removal was reversed by washing preparations with normal solutions.

FIG. 2. Effect of exogenous Ca on the tonus. (a), normal bathing medium; (b), 10 min after removal of Na from the normal bathing medium; (c), 30 min after. $\downarrow$: Washout with the normal medium.

FIG. 3. Effect of Na removal from the normal bathing medium on the contractile response to stimulating agents. (a), normal bathing medium; (b), one min after Na removal from the normal bathing medium; (c), 30 min after.

3) Modification by Na removal of the contractile response to various agents

The addition of K (2×10^{-3} g/ml), ACh (10^{-8} g/ml) and Ba (10^{-4} g/ml) to the bathing media caused a rapidly-developing contraction (phasic contraction) followed by slow contraction (tonic contraction). In the contractile responses to ACh and Ba the phasic and tonic phases could not actually be distinguished. The contractions by these agents were not significantly influenced 1 min after removal of Na from the bathing media except that the tonic contraction by K increased by $21.5 \pm 2.1\%$ ($N=6$), as compared with that in the normal media. Thirty min after removal of Na, the contractions by ACh and Ba and the phasic contraction by K were inhibited by $61.1 \pm 4.9\%$ ($N=6$), $34.7 \pm 2.2\%$ ($N=6$) and $83.4 \pm 3.7\%$ ($N=11$), respectively, whereas the tonic contraction by K was increased by $21.0 \pm 2.8\%$ ($N=11$) (Fig. 3). These changes in the drug action were completely reversed within 20 min after returning preparations to the normal medium.

4) Restoration of the response to removal of Na^+

Preparations in which the contraction by Na removal had disappeared by repeated

FIG. 4. Reappearance of the response to Na removal by the addition of Ca. $\downarrow$ and $\uparrow$ indicate the washout with $\text{Ca}(-)$, $\text{Na}(+)$ and $\text{Ca}(-)$, $\text{Na}(-)$ bathing medium, respectively. Note the difference in the solution by which the preparation was washed.

treatments with Na removal in Ca-free media, were used. The addition of 2 mM Ca to the Ca-free, Na-free media produced sustained contraction. After 20 min incubation with Ca^{++} the preparations were incubated for 30 min with the Ca-free, Na-normal solution. In these preparations the contractile response to removal of Na was restored. A typical example of this series of experiments is shown in Fig. 4(a). When Na removal from the media was repeated at 25 min interval, this contraction was gradually reduced. However, after subsequent treatment for 20 min with 2 mM Ca^{++} , the contractile response to Na-removal was again restored. Regardless of whether the time of incubation with 2 mM Ca^{++} was 20 or 25 min, the contractile response to removal of Na was the same.

After incubation for 20 min with 2 mM Ca^{++} , the preparations were treated for 5 min with Ca-free, Na-free solutions, instead of Ca-free, Na-normal solutions in the above-mentioned series of experiments, and then for 25 min with Ca-free, Na-normal solutions. Following this treatment, contractile response to removal of Na^+ was slightly restored (Fig. 4(b)) ($20.0 \pm 1.9\%$ ($N=4$) relative to that obtained in the above-mentioned series of experiments, as demonstrated in Fig. 4(a)).

DISCUSSION

The present study demonstrated that the removal of Na from bathing media by replacing NaCl with isotonic sucrose produced contractions in rat excised ileum, which persisted for approx. 30 min. Since this contraction was not influenced by treatment with atropine, cholinergic mechanisms would not be involved. When NaCl was replaced with choline Cl in the presence of atropine, similar contractions were obtained, which persisted for approx. 15 min. Thus, it can be considered that the induced contractions are due to a reduction of extracellular Na but not to decreased Cl.

The contraction induced by removal of Na was significantly reduced in preparations exposed to Ca-free solutions. This finding supports the hypothesis that removal of Na from the bathing media increases the inward movement of Ca responsible for the smooth

muscle contraction. It has been demonstrated that Na and Ca share channels of the cell membrane through which these ions enter into cells (9, 10). Thus, lowering extracellular Na may increase the permeability of membranes for Ca. In addition, reduced Na would increase the Na efflux-Ca influx coupling system and decreases the Na influx-Ca efflux coupling system, as suggested in mesenteric artery and aorta (5), vascular smooth muscle (1, 12), cardiac muscle (11) and giant axon (13). In guinea pig taenia coli (14) and guinea pig taenia caecum (7), removal of Na from bathing media produces depolarization, probably resulting in increased influxes of Ca, as does a membrane-depolarizing ion, K. On the other hand, contractions by removal of Na were not completely abolished by treatment of preparations with Ca-free media. In preparations in which K-induced contractions were abolished by repeated additions of K in Ca-free media, the Na removal still caused contractions. Contractions induced by Na removal were greatly inhibited by treatment with isoproterenol, which had been suggested to selectively attenuate the release of Ca^{++} from storage sites (8). These results suggest that contractions by removal of Na are due partly to the release of Ca from intracellular storage sites and that the release occurs from not only the site sensitive to K but also that resistant to the ion. Possible importance of Na in binding Ca in storage sites will be discussed later.

In Ca-depleted preparations in which contractions by Na removal were abolished by repeated treatments with Na removal in Ca-free solutions, the contractile response to removal of Na was restored following incubation in 2 mM Ca and then for 5 min in Na-normal, Ca-free media. Thus, addition of Ca appears to replenish Ca in intracellular storage sites. However, when the preparations were incubated for 5 min in Na-free, Ca-free media after treatment with Ca, the response to Na removal was restored only slightly. These findings indicate the importance of the presence of Na^+ in the bathing media to replenish and bind Ca in the storage sites. According to Arora (15), an increase in intracellular Na accelerates the uptake of intracellular Ca by storage sites in toad atria. If this applies also to the case of intestinal smooth muscles used in the present study, it can be considered that removal of Na from the bathing media decreases intracellular Na, resulting in release of Ca from storage sites and inhibition of Ca from re-binding in the sites and that the ability of binding Ca in storage sites is restored by raising Na in bathing media, possibly as a result of an increase in intracellular Na.

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REFERENCES

- 1) BRIGGS, A. AND MELVIN, S.: *Am. J. Physiol.* **201**, 365 (1961)
- 2) BOHR, D.F.: *Pharmacol. Rev.* **16**, 85 (1964)
- 3) BAKER, P.F.: *J. Physiol.* **192**, 43P (1967)
- 4) PRASADO, K.: *Can. J. Physiol. Pharmacol.* **48**, 241 (1970)
- 5) SITRIN, M.D. AND BOHR, D.F.: *Am. J. Physiol.* **220**, 1124 (1971)
- 6) KARAKI, H., URAKAWA, N. AND IKEDA, M.: *Japan. J. Pharmacol.* **16**, 423 (1966)
- 7) TAKAGI, K. AND TAKAYANAGI, I.: *Japan. J. Pharmacol.* **21**, 129 (1971)
- 8) TANIYAMA, K.: *Kobe J. Med. Sci.* **19**, 67 (1973)

- 9) WILBRANDT, W. AND KOLLER, H.: *Helv. Physiol. Pharmac. Acta* **6**, 208 (1948)
- 10) LÜTTGAU, H.C. AND NIEDERGERKE, R.: *J. Physiol.* **143**, 486 (1958)
- 11) REUTER, H. AND SEITZ, N.: *J. Physiol.* **195**, 451 (1968)
- 12) BOHR, D.F., SEIDEL, C. AND SOBIESKI, J.: *Microvascular Res.* **1**, 335 (1969)
- 13) BAKER, P.F., BLAUSTEIN, M.P., HODGKIN, A.L. AND STEINHARDT, R.A.: *J. Physiol.* **200**, 431 (1969)
- 14) BÜLBRING, E. AND KURIYAMA, H.: *J. Physiol.* **166**, 29 (1963)
- 15) ARORA, H.R.K.: *Am. J. Physiol.* **222**, 333 (1972)