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EFFECT OF INTRACELLULAR SODIUM ON CALCIUM UPTAKE IN ISOLATED GUINEA-PIG DIAPHRAGM AND ATRIA

SATOSHI YAMAMOTO * and KURT GREEFF

Institut für Pharmakologie der Universität Düsseldorf, D-4000 Düsseldorf (F.R.G.)

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(1) Effects of cellular sodium on the ^{45}Ca uptake of isolated guinea-pig diaphragm and atria were studied. (2) Cellular sodium and calcium contents were higher in diaphragm compared to atria after incubating the tissues in normal Krebs-Henseleit solution. (3) Cellular sodium content in atria and diaphragm were reduced signficantly by incubating the tissues in high potassium Krebs-Henseleit solution (K^+ = 34.7 mM), while it was increased by incubating the tissues in the ice-cold low potassium and low calcium Krebs-Henseleit solution (K^+ = 0.65 mM, Ca^{2+} = 0.2 mM). Cellular potassium content was changed inversely to the sodium content. (4) In atria, cellular content of calcium was not altered significantly by the above conditions. But in diaphragm, the cellular content of calcium was decreased slightly but significantly after incubation in the ice-cold low potassium and low calcium Krebs-Henseleit solution. (5) At normal cellular sodium levels, the ^{45}Ca uptake of both tissues was similar. (6) The reduction of the cellular sodium content caused a significant decrease in the ^{45}Ca uptake into both tissues. (7) When the cellular sodium content was increased in atrial preparations, a marked increase in the ^{45}Ca uptake was observed. On the other hand, in diaphragm preparations, only a slight increase was observed, even when cellular sodium content was much higher than the normal level. (8) These results indicate that even when the intracellular sodium is increased by some physiological of pharmacological events, calcium influx through Na^+/Ca^{2+} exchange mechanism is very slight and slow in diaphragm.

Introduction

Na^+/Ca^{2+} exchange is a mechanism by which transmembrane movement of calcium is directly coupled to a reciprocal movement of sodium [1–3]. In a variety of excitable tissues, the Na^+/Ca^{2+} exchange mechanism has been demonstrated and recognized as an important calcium entry or exit mechanism in response to various physiological and pharmacological stimuli [1–4]. In cardiac and smooth muscles, conditions which increase intracellular sodium concentration enhance the Na^+/Ca^{2+} exchange mechanism [3–7]. It has been suggested that in cardiac tissues, the Na^+/Ca^{2+} exchange mechanism contributes, at least partly, to the entry mechanism of contractile calcium on a beat-to-beat basis [8], and also plays an important role as a mediator of positive inotropic action of cardiac glycosides [9,10] and veratrum alkaloids [11,12]. In vascular smooth muscles, low potassium concentration medium or cardiac glycosides induce the muscle contraction and these contractions may be produced through a Na^+/Ca^{2+} exchange mechanism following the accumulation of cellular sodium by the above treatments [7,13]. It is widely known that cardiac [14] and some smooth muscles [15,16] depend upon extracellular calcium as a major source of contraction. On the other hand, skeletal

* To whom all correspondence should be addressed. Present address: Department of Pharmacology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan.

muscles are primarily dependent upon intracellular calcium stores [17], suggesting that transmembrane calcium movement is different in these two groups of tissues. However, information regarding Na^+/Ca^{2+} exchange of skeletal muscle is rather limited [3]. Therefore, we investigated the Na^+/Ca^{2+} exchange mechanism of skeletal muscle in comparison with cardiac tissue by employing isolated guinea-pig diaphragm and atria.

Materials and Methods

Intracellular sodium-activated ^{45}Ca uptake was determined according to the method of Glitsch et al. [5] with minor modifications.

Left atria and diaphragm strips (approx. 3 mm wide, 5 mm long and less than 1 mm thick) were obtained from male guinea-pigs weighing 250–300 g. Tissue preparations were preincubated for 30 min at 35°C in Krebs-Henseleit solution of the following composition (mM); 118.0 NaCl/24.9 $NaHCO_3$/4.7 KCl/1.2 NaH_2PO_4/1.2 $MgSO_4$/1.8 $CaCl_2$/11.1 glucose. Tissue preparations were either left in this solution for another 45 min or transferred into different solutions in order to alter the intracellular sodium content. Intracellular sodium was reduced by soaking the preparations for 45 min in modified Krebs-Henseleit solution containing 34.7 mM KCl, 30 mM NaCl of the solution being replaced by an equimolar amount of KCl. In order to increase the cellular sodium content but without increasing cellular calcium content, the tissue preparations were soaked in potassium- and calcium-poor Krebs-Henseleit solution (K^+ = 0.65 mM, Ca^{2+} = 0.2 mM) at 2°C for 45 or 90 min. At the end of the incubation, the cellular sodium, potassium and calcium contents of the tissues were determined by the method of Noack and Heinen [18], using [^{3}H]inulin to measure the extracellular space.

The uptake of ^{45}Ca in resting left atrial and diaphragm preparations with different intracellular sodium content was measured by incubating the preparations at 35°C for 10 min in radioactive Krebs-Henseleit solution with 0.65 mM KCl. The radioactivity of ^{45}Ca (New England Nuclear Corp.) in the medium was 2 μCi/ml and the total calcium concentration was 1.8 mM. In the experiments with atrial preparations, acetylcholine (final concentration, 0.5 μg/ml) was added to the above ^{45}Ca uptake medium in order to avoid spontaneous electrical and contractile activity, which are usually observed in a low potassium solution. The above concentration of acetylcholine has been shown to have no effect on ^{45}Ca uptake in resting atria [19]. After the 10 min incubation in radioactive solution, tissue preparations were rinsed with a large volume (total 5 l) of sodium- and calcium-free Krebs-Henseleit solution, the sodium of which was replaced by an equimolar amount of choline chloride, for 25 min to wash the calcium from the extracellular space, as described by Glitsch et al. [5]. The above rinsing period is also enough to wash out extracellular calcium from diaphragm preparations (data not shown). Then, the tissue preparations were gently blotted on filter paper and weighed. Tissue ^{45}Ca was extracted by soaking the preparations overnight in 1 ml of 0.5 M perchloric acid [18]. Tissue extracts (0.2 ml) were neutralized with 0.5 ml of 0.2 M NaOH, 10 ml of a scintillation cocktail (Quickszint 294®, Koch-Light Lab.) was added and radioactivity was assayed in a liquid scintillation spectrometer (Beckman LS 9000).

Statistical analysis was performed using the Student's *t*-test.

Results

The intracellular sodium content was altered by incubating the tissue preparations under various conditions. The total cellular contents of sodium, potassium and calcium were measured at the end of the incubation period. Sodium and calcium contents were higher in diaphragm than in atria after incubating the tissues in normal Krebs-Henseleit solution (Table I). Cellular sodium content in atria and diaphragm were reduced significantly by incubating the tissue preparations in the high potassium Krebs-Henseleit solution (K^+ = 34.7 mM), while it was increased by incubating the tissues in the ice-cold low potassium and low calcium Krebs-Henseleit solution (K^+ = 0.65 mM, Ca^{2+} = 0.2 mM) (Table I). Cellular potassium content was changed inversely to the sodium content (Table I). In atria, the cellular content of calcium was not altered significantly by the above conditions. But in diaphragm, the cellular content of calcium was decreased slightly but significantly after incubation in the ice-cold low potassium and low calcium Krebs-Henseleit solution (Table I).

TABLE I

INTRACELLULAR SODIUM, POTASSIUM AND CALCIUM CONTENTS OF LEFT ATRIAL AND DIAPHRAGM PREPARATIONS UNDER DIFFERENT EXTERNAL CONDITIONS

The total cellular ion content (mmol ion/kg cellular tissue) was calculated by subtracting the ion content in the extracellular fluid from the total tissue content. Values are mean ± S.E.

External condition (mM)	mmol ion/kg cellular tissue					
	Left atria			Diaphragm		
	Na^+	K^+	Ca^{2+}	Na^+	K^+	Ca^{2+}
Normal Na^+ = 144.1 K^+ = 4.7 Ca^{2+} = 1.8 at 35°C for 45 min	36.4 ± 1.1 (*n* = 10)	121.4 ± 2.5 (*n* = 10)	1.26 ± 0.07 (*n* = 6)	49.0 ± 1.8 (*n* = 19)	101.0 ± 2.2 (*n* = 19)	3.98 ± 0.36 (*n* = 7)
High K^+ Na^+ = 114.1 K^+ = 34.7 Ca^{2+} = 1.8 at 35°C for 45 min	26.9 ± 1.5 [a] (*n* = 8)	128.8 ± 2.0 [a] (*n* = 8)	1.26 ± 0.12 (*n* = 6)	32.5 ± 1.6 [a] (*n* = 15)	109.9 ± 1.3 [a] (*n* = 15)	4.47 ± 0.19 (*n* = 7)
Low K^+ and Ca^{2+} Na^+ = 148.1 K^+ = 0.65 Ca^{2+} = 0.2 at 2°C for 45 min	77.8 ± 4.6 [a] (*n* = 6)	71.9 ± 3.2 [a] (*n* = 6)	1.21 ± 0.10 (*n* = 6)	99.2 ± 2.3 [a] (*n* = 12)	39.0 ± 1.8 [a] (*n* = 12)	2.52 ± 0.14 [a] (*n* = 9)
Low K^+ and Ca^{2+} Na^+ = 148.1 K^+ = 0.65 Ca^{2+} = 0.2 at 2°C for 90 min	93.9 ± 5.5 [a] (*n* = 6)	65.0 ± 5.6 [a] (*n* = 6)	1.16 ± 0.13 (*n* = 6)	120.3 ± 4.0 [a] (*n* = 12)	26.6 ± 2.7 [a] (*n* = 12)	2.76 ± 0.17 [a] (*n* = 8)

[a] Significantly different from the value observed after incubation in normal Krebs-Henseleit solution for 45 min ($P < 0.05$).

Tissue preparations used for ^{45}Ca uptake measurements were first treated in exactly the same manner as those described in Table I. Therefore, the above cellular ionic contents listed in Table I indicate the ionic contents at the beginning of the uptake period. Cellular sodium contents may, however, subsequently decrease through the activity of the sodium pump during the ^{45}Ca uptake period. In order to avoid this possibility, ^{45}Ca uptake was carried out for 10 min in a low potassium Krebs-Henseleit solution (K^+ = 0.65 mM). The low potassium concentration of the medium itself has no significant effect on ^{45}Ca uptake [5]. Fig. 1 shows the effect of various cellular sodium contents on ^{45}Ca uptake in left atrial and diaphragm preparations. The values of cellular sodium contents are the same as those listed in Table I. At normal cellular sodium levels, the ^{45}Ca uptake of both tissue preparations were similar (Fig. 1). The reduction of the cellular sodium content caused a significant decrease in the ^{45}Ca uptake into both tissues. When the cellular sodium content was increased in atrial preparations, a marked increase in the ^{45}Ca uptake was observed (Fig. 1). On the other hand, in diaphragm preparations, only a slight increase was observed, even when the cellular sodium content was much higher than the normal level (Fig. 1).

Discussion

There are only a few publications concerning the Na^+/Ca^{2+} exchange mechanism in skeletal muscle. Cosmos and Harris [20] suggested the existence of intracellular sodium-activated calcium influx in frog

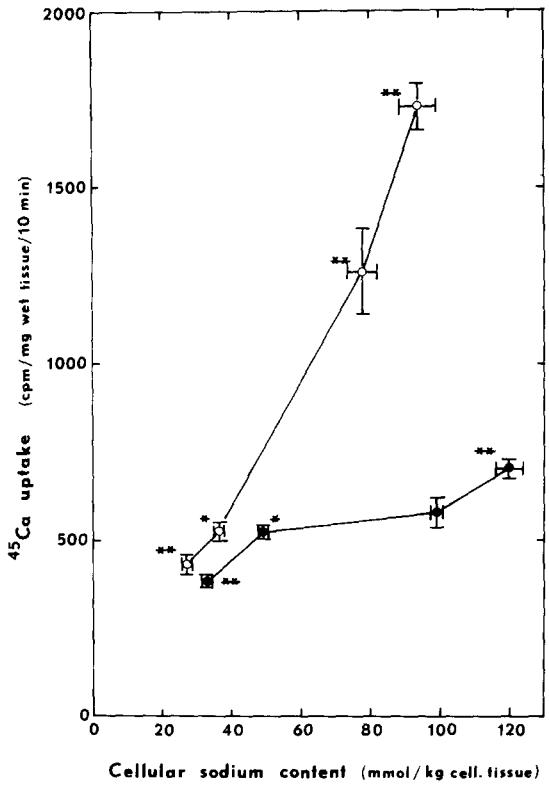

Fig. 1. Effect of intracellular sodium on ^{45}Ca uptake in resting isolated guinea-pig atrial (○) and diaphragm (●) preparations. First, tissue preparations were incubated in various external conditions in order to alter the intracellular sodium content, as described in Table I. Tissue preparations were then incubated at 35°C for 10 min in ^{45}Ca-containing (2 μCi/ml) modified Krebs-Henseleit solution (K^+ = 0.65 mM). Each point represents the mean of 6 to 23 experiments. Vertical and horizontal lines indicate the standard error. * ^{45}Ca uptake observed under normal intracellular sodium content. ** Values for ^{45}Ca uptake were significantly different from those observed with normal intracellular sodium content.

sartorius muscle by estimating cellular ionic concentrations after long incubation (6–16 h) of the tissues in K^+-free solution at 4°C. This type of experiment, however, does not provide an appropriate idea about the activity of Na^+/Ca^{2+} exchange mechanism of this tissue. After such long incubation periods in K^+-free solution, cellular calcium concentration may increase even if the activity of the exchange mechanism is very low. DiPolo [21] reported little increase in the calcium influx by changing the cellular sodium concentration from 15 to 90 mM in barnacle muscle fibers. Both of the above muscles are of lower vertebrate or invertebrate origin and are different types of muscle from mammalian cardiac and skeletal muscles. Recently, Brandt et al. [23] suggested that it is not the Na^+/Ca^{2+} exchange system, but the ATP-energized Ca^{2+} pump which is the major mechanism of Ca^{2+} efflux across the transverse tubuler membrane isolated from rabbit skeletal muscle. However, as far as we examined, we could not find publications regarding the Na^+/Ca^{2+} exchange mechanism of mammalian skeletal muscle in intact cell system. This is the first report which compares the activity of Na^+/Ca^{2+} exchange system in mammalian skeletal and cardiac muscle.

Our ^{45}Ca uptake studies using isolated guinea-pig diaphragm and atria indicate that with normal cellular sodium content, calcium uptake of diaphragm is similar to that of atria. When the cellular sodium content was increased above the normal level, a marked increase in the ^{45}Ca uptake was observed in atrial preparations. The result is consistent with that of Glitsch et al. [5]. On the other hand, the increase in intracellular sodium caused only a slight increase in the ^{45}Ca uptake in diaphragm preparations. Under our experimental conditions, when cellular sodium content was increased above the normal level, cellular calcium content was decreased slightly in diaphragm preparations (see Table I). However, low cellular calcium would not contribute to the low ^{45}Ca uptake in this tissue.

These results indicate that even when the intracellular sodium is increased by some physiological or pharmacological events, calcium influx through Na^+/Ca^{2+} exchange mechanism is very slight and slow in diaphragm. Since transmembrane calcium movements are usually a rapid process in excitable cells, it is unlikely that Na^+/Ca^{2+} exchange mechanism plays an important role in transmembrane calcium movements in skeletal muscle. A smaller surface to volume ratio of skeletal muscle fiber compared to that of cardiac cell may partly contribute to the low Na^+/Ca^{2+} exchange activity of the former tissue.

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