Biochimica et Biophysica Acta, 551 (1979) 129—136

BBA 78275

TAURINE ENHANCEMENT OF CALCIUM BINDING TO RAT HEART SARCOLEMMA

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(Received July 3rd, 1978)

Key words: Taurine; Calcium binding; Verapamit; La^{3+}; (Sarcolemma)

Summary

The effect of taurine on calcium binding to isolated rat heart sarcolemmal membrane was examined. Taurine was observed to increase calcium binding to the low affinity sites in both high sodium-low potassium and low sodium-high potassium buffers. Taurine was also seen to antagonize the inhibition of calcium binding to the sarcolemma caused by both verapamil and lanthanum. Nevertheless, membrane structural changes due to taurine could not be detected using the spin label ESR probe 2N14. A possible regulatory role of taurine is discussed.

Introduction

Calcium is generally considered as the coupler of excitation and contraction in muscle [1]. In the heart, the amount of calcium required for maximal contraction is thought to be derived primarily from two separate pools, calcium stored in the sarcoplasmic reticulum and that supplied from extracellular fluids [2]. Some investigators believe that calcium bound to sarcolemmal sites provides the beat-to-beat supply of calcium from this extracellular pool [3]. This conclusion is deduced from the observation that agents such as verapamil [4,5] and lanthanum (La^{3+}) [3], which inhibit calcium binding to the sarcolemma, uncouple excitation and contraction. Conversely, enhanced calcium binding to the sarcolemma results in increased contractility [2].

Taurine (2-aminoethanesulfonic acid) has been shown to exert a positive inotropic effect on the heart [6,7], and several authors have suggested that this effect may be due to enhancement of calcium influx [8,9]. Several lines of

evidence support this conclusion. First, taurine antagonizes the negative inotropic effect of low calcium-perfused hearts [10]. Second, it acts to maintain calcium stores during washout studies [9,11]. Third, taurine increases the time to peak tension [8]. Fourth, the action potential in the presence of taurine is altered in a fashion consistent with an increase in intracellular calcium levels [12]. In this study, we provide further support to the hypothesis that taurine directly alters calcium distribution in the heart.

Materials and Methods

Plasma membrane preparation. Hearts were rapidly removed from male Wistar rats (280—360 g) following decapitation and immediately perfused at 37° C for 4—5 min with Krebs-Henseleit buffer (pH 7.4) to remove blood from the coronary system and ventricles. The ventricles were trimmed of atria, fat, and connective tissue, and the sarcolemma was isolated according to the procedure of Sulakhe et al. [13]. The final pellet was normally suspended in cold (4° C) 20 mM Tris-maleate buffer, pH 7.4, containing the following: 10 mM $MgCl_2$, 100 mM NaCl, and 5 mM KCl. Protein concentration was determined by the method of Lowry et al. [14], and the preparation was diluted to give 70—90 μg protein per reaction mixture. Cardiac sarcolemma isolated in this manner was assayed for purity using several enzyme markers. The preparations were shown to have a high ($Na^+ + K^+$)-ATPase activity, while exhibiting both low succinate dehydrogenase and glucose-6-phosphatase activities [15]. Each preparation was kept on ice and used within 24 h. No apparent change in binding activity was observed over this time period.

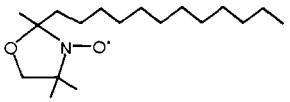
Calcium binding assay. Calcium binding was performed using a Millipore filtration system. Aliquots (70—90 μg protein/0.2 ml) of the sarcolemma suspension were preincubated for 10 min at 24° C in 20 mM Tris-maleate buffer (pH 7.4) containing 10 mM $MgCl_2$, 100 mM NaCl, and 5 mM KCl to allow thermal equilibrium to be established. This preincubation mixture contained one the following: (1) a control with no further additions; (2) 1 μM verapamil; (3) 10 mM taurine; (4) 1 μM verapamil plus 10 mM taurine; (5) 20 μM $LaCl_3$; or (6) 20 μM $LaCl_3$ and 10 mM taurine. Calcium binding was also determined using 100 mM KCl, 5 mM NaCl, and 10 mM $MgCl_2$ in the presence and absence of 10 mM taurine. The reaction was begun by adding 1—800 μM $^{45}CaCl_2$, allowed to incubate for an additional 10 min at 24° C, and terminated by the addition of 3.0 ml of ice-cold buffer and immediate vacuum filtration on cellulose acetate filters (0.2 μm pore size). The filters were washed three times with 3 ml ice-cold buffer and then counted using a Hewlett-Packard liquid scintillation counter. Each assay was performed in triplicate, and binding was corrected for non-specific interactions according to Cuatrecasas [16] by addition of 0.1 M unlabeled $CaCl_2$ to the preincubation medium. Non-specific binding was typically 5—15% of the total binding activity.

Electron spin resonance (ESR) studies. X-band ESR spectra were obtained using a Varian 4502 Spectrometer with a 12 inch Fieldial controlled magnet and a heated chamber to control the temperature within ±0.5° C. The rotational correlation time, Υ_c, was calculated from the following equation:

$$\Upsilon_c = Kw_1\left[\left(\frac{h_1}{h_{-1}}\right)^{1/2} - 1\right]$$

where w_1 is the width of the low field line, h_1, h_{-1} are the heights of the low and high field lines, respectively, and K is a constant having a value of $6 \cdot 10^{-10}$ s/G.

The spin label utilized was 2N14, whose structure is shown below:



Materials. Taurine was obtained from Aldrich Chemical Co., recrystallized twice from distilled water prior to use, and shown to be chromatographically pure. ^{45}Ca (as $CaCl_2$ in water, 4—30 Ci/g) was obtained from either New England Nuclear or Amersham Corp. Verapamil was a gift from Knoll Pharmaceutical Co. All other reagents were obtained from Fisher Scientific Co.

Statistical analysis. Statistical analyses were performed using paired Student's t-test, with a probability of $P < 0.01$ used to indicate a significant difference.

Results

In Fig. 1A, the calcium binding profile over a range of 1.0—800 μM Ca^{2+} is shown. Also shown are the binding profiles in the presence of 10 mM taurine, 1 μM verapamil, or both. Addition of taurine is seen to increase calcium binding to rat heart sarcolemma by as much as 30% in the range of 50—800 μM Ca^{2+}. On the other hand, verapamil decreases Ca^{2+} binding over the range of 200—800 μM Ca^{2+}, in agreement with results from other laboratories [5]. It is readily apparent that the inhibition of Ca^{2+} binding resulting from the presence of 1 μM verapamil is essentially reversed by the addition of 10 mM taurine.

The Scatchard plots of these data (Fig. 1B) yield hyperbolic curves. Several investigators [5,17,18] have suggested that this pattern indicates the existence of two classes of binding sites over this concentration range. These binding sites have association constants equal to about 10^5 and 10^2 M^{-1}, respectively, which is of the same order of magnitude as has been previously reported [5,18]. Both taurine and verapamil appear to affect the total amount of Ca^{2+} bound to the low affinity sites rather than the affinity of these sites for Ca^{2+}. However, the effect of taurine on Ca^{2+} binding may also be interpreted as an increase in the affinity of some of the low affinity sites to a third class of binding sites characterized by a K_a of about 10^4 M^{-1}.

To further define the taurine effect on Ca^{2+} binding to the sarcolemma, a more specific Ca^{2+}-binding inhibitor was utilized. Lanthanum (La^{3+}) has been shown to displace Ca^{2+} bound to both classes of binding sites [2,18], although this is somewhat controversial. Inhibition to only the low affinity sites has also been reported [19], but in that study the conditions, including the La^{3+} concentration used, were not well defined.

As shown in Table I, our results indicate that La^{3+}, at a concentration of 20 μM, affects the total amount of Ca^{2+} bound to both membrane sites. A Scatchard plot of the data suggests that La^{3+} primarily alters the number of Ca^{2+} binding sites available rather than the affinity of Ca^{2+} for these sites. Also in Table I is shown the effect of taurine on the inhibition of Ca^{2+} binding

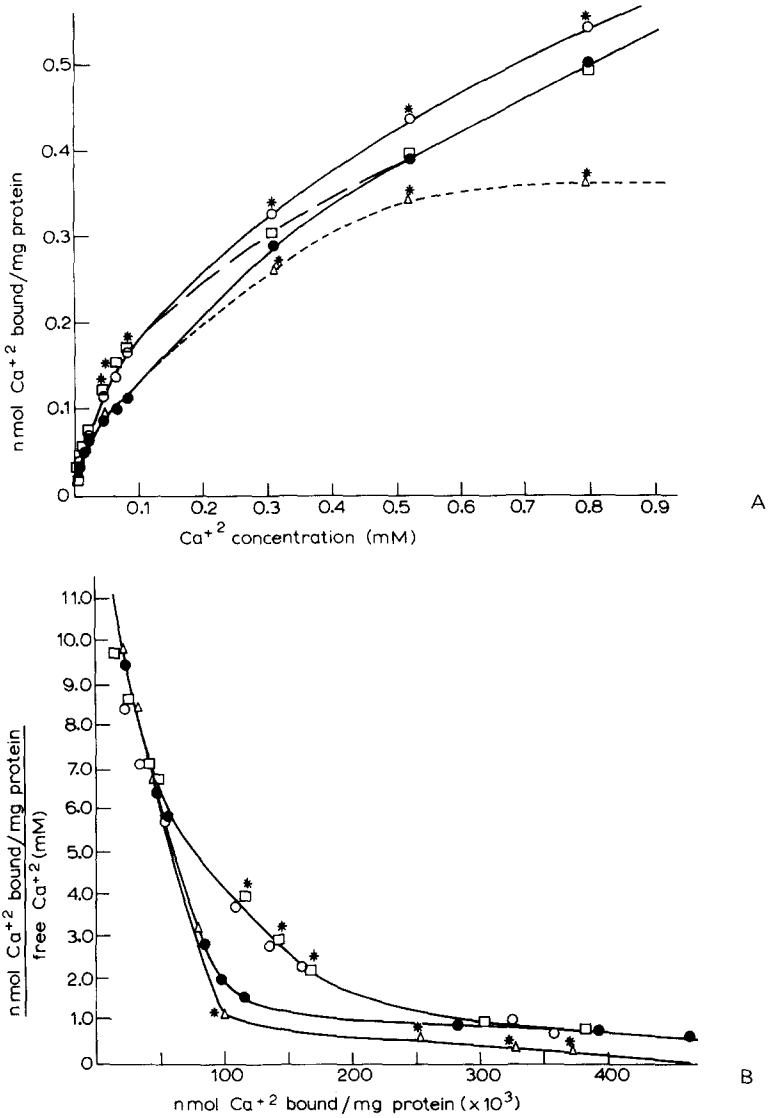


Fig. 1. (A) Calcium binding profiles of rat heart sarcolemma in the presence and absence of taurine, verapamil, or both. Sarcolemma protein (70—90 μg/0.2 ml) was preincubated for 10 min in 20 mM Tris-maleate, pH 7.4, with 10 mM $MgCl_2$, 100 mM NaCl, and 5 mM KCl, with or without 10 mM taurine, 1 μM verapamil, or both. $^{45}CaCl_2$ was added to a final concentration of 1.0—800 μM, incubated for a further 10 min, and filtered on a Millipore filtration system. Symbols contain mean ± S.E. An asterisk denotes significant difference from control ($P < 0.01$). ●, control; ○, 10 mM taurine; △, 1 μM verapamil; □, taurine plus verapamil. (B) Scatchard plot of the data in A. Symbols as in A.

caused by 20 μM La^{3+}. In the presence of 10 mM taurine, the inhibition by La^{3+} of Ca^{2+} binding to both the high and low affinity binding sites is effectively reversed.

Calcium binding to the sarcolemma has also been shown to be altered by varying Na^+ and K^+ concentrations [18]. Therefore, it was of interest to determine the effect of these ions on the taurine-mediated changes in Ca^{2+} binding.

TABLE I

TAURINE ENHANCEMENT OF Ca^{2+} BINDING IN THE PRESENCE OF La^{3+} OR IN A HIGH K^+-LOW Na^+ MEDIUM

Calcium binding to rat heart sarcolemna in the presence of 20 μM La^{3+}, 20 μM La^{3+} plus 10 mM taurine, and under high K^+-low Na^+ conditions in the presence and absence of 10 mM taurine was performed as described in Fig. 1. An asterisk denotes a significant difference from the appropriate control ($P < 0.01$). Results are expressed as nmol Ca^{2+} bound per mg protein × 100.

Ca^{2+} concentration (μM)	Control [a] (5 mM K^+, 100 mM Na^+)	20 μM La^{3+}	20 μM La^{3+} plus 10 mM taurine	Control [b] (100 mM K^+, 5 mM Na^+)	10 mM taurine (100 mM K^+, 5 mM Na^+)
2.1	4.07 ± 0.23 [c]	3.55 *	3.99 ± 0.15	5.63 ± 0.33	4.40 ± 0.27 *
4.2	7.20 ± 0.41	5.87 ± 0.28 *	7.23	—	7.98
5.6	—	7.26 ± 0.55	—	13.2 ± 1.1	10.6
7.1	11.0	8.86 ± 0.36 *	10.9 ± 0.54	—	13.0
14.1	18.1	—	17.3 ± 1.2	21.7 ± 1.2	22.4 ± 0.67
50.0	35.8 ± 1.4	22.1 ± 0.41 *	31.1 ± 1.0	35.8 ± 1.2	—
83.3	41.4 ± 1.3	27.9 ± 3.7 *	38.9	49.8 ± 8.5	62.6 ± 2.6 *
99.1	42.3	27.1 ± 1.4 *	—	51.4 ± 7.3	68.6 ± 14.0
198	—	43.1 ± 1.7	75.3 ± 1.3	81.1 ± 1.4	100.0 ± 7.1 *
297	101.0 ± 7.4	57.7 *	94.6 ± 7.3	91.3 ± 5.5	121.0 ± 11.0 *
395	119.0 ± 10.0	68.4 ± 3.2 *	124.0	93.9 ± 3.4	132.0
495	—	75.8 ± 8.4	136.0 ± 4.2	107.0 ± 8.3	142.0 ± 7.2 *

[a] Control for the La^{3+} studies.

[b] Control for the high K^+-low Na^+ studies.

[c] Values given are the means ± S.E.

When the high Na^+-low K^+ buffer was replaced with a buffer containing 5 mM Na^+ and 100 mM K^+, taurine enhancement of Ca^{2+} binding appeared even more pronounced (Table I). Taurine again is seen to increase the amount of Ca^{2+} bound to the low affinity sites, but, in this case, does not mediate a change in Ca^{2+} affinity to those sites. The data do, however, show a slight decrease in both the affinity and amount of Ca^{2+} bound to the high affinity sites ($P < 0.01$, only at 2.1 μM Ca^{2+}). Thus, taurine appears to affect the low affinity Ca^{2+} binding sites when the concentration of Na^+ and K^+ approximate either intracellular or extracellular conditions, but may inhibit Ca^{2+} binding to the high affinity sites under intracellular conditions.

These results suggest that taurine alters Ca^{2+} binding to the membrane. Changes of ligand binding due to activators or inhibitors have often been associated with conformational changes in the binding factor, whether seen by binding profiles [17] or more direct methods [21]. We therefore examined the effect of taurine on membrane structure using the ESR probe 2N14. This probe becomes embedded with the *N*-oxyl group near the interface of the polar head groups and the hydrocarbon chains of the membrane lipids. The rotational correlation time (Υ_c) of this spin label is a measure of the local viscosity within the membrane. We determined Υ_c in the presence and absence of 40 mM taurine at various temperatures. A membrane phase change due to the presence of taurine would be seen as a change in the slope of an Arrhenius plot of ln Υ_c versus reciprocal temperature. This was not observed and it is concluded that taurine does not affect the fluidity of the membrane under these conditions.

Discussion

Taurine is the most abundant free amino acid in the heart [22], but its function in this organ is as yet ill-defined. Read and Welty [23—25] demonstrated that taurine prevents the development of epinephrine or digoxin-induced premature ventricular contractions in dogs. It was shown that restoration of normal sinus rhythm was associated with retention of cellular K^+ levels [24,25].

Several studies suggest that the effects of taurine are primarily linked to alterations of cellular Ca^{2+} rather than K^+ levels [8—11]. It was shown that the rate at which intracellular Ca^{2+} levels and contractile force fell following perfusion of guinea pig [11] and rat hearts [9] with Ca^{2+}-free Tyrode was less when taurine was included in the buffer. Dolara et al. [26] further showed that taurine increased both the total accumulation of Ca^{2+} by sarcoplasmic reticulum vesicles and the rate of Ca^{2+} binding to those vesicles isolated from guinea pig hearts. These authors suggested that the taurine effects may be linked to an increased affinity of sarcoplasmic reticulum for Ca^{2+}. In contrast, both Chubb and Huxtable [9] and Entman et al. [27] found that taurine had no effect on either Ca^{2+} binding or (Ca^{2+} + Mg^{2+})-ATPase activity of sarcoplasmic reticulum isolated from rat heart and dog heart, respectively.

The present results are consistent with the observed positive inotropic effects of taurine [6,7]. It can be argued that enhanced binding of Ca^{2+} to the low affinity sites of the sarcolemma may be the primary mediator of the positive inotropic effects. Several authors suggest that these low affinity sites are the

contributors of 'activator' Ca^{2+} from the 'pool I' (extracellular) Ca^{2+} stores [5,19].

Recently, taurine has been shown to bind to cardiac sarcolemma at two distinct classes of binding sites [28]. The high affinity sites were proposed to be related to the β-amino acid transport system. The low affinity sites appear to show positive cooperativity, which is associated with conformational changes of the binding factor [29]. Although we were not able to observe a change in membrane fluidity with the ESR probe 2N14, the two observations are not contradictory. The possibility of extremely localized membrane structural changes or of protein-protein interactions not affecting membrane fluidity is not ruled out by the ESR data. Since taurine reverses the inhibitory effects of both La^{3+} and verapamil on Ca^{2+} binding to the sarcolemma, it is likely that a close association exists between the taurine and Ca^{2+} binding sites.

Taurine also appears to regulate other sarcolemmal functions. It has been shown that addition of 10 mM taurine to perfused rat heart leads to a transient decrease in tissue cyclic AMP levels [7]. Since Ca^{2+} is known to inhibit adenylate cyclase [30], it is possible that the taurine-mediated changes in cyclic AMP levels might be linked to the enhanced binding of Ca^{2+} to the sarcolemma.

Further evidence linking taurine, Ca^{2+}, and cyclic AMP in the heart has been reported. Catecholamines, which increase cellular cyclic AMP levels [31], enhance both taurine [9] and Ca^{2+} [32] influx and K^+ efflux from the heart [25]. In addition, dibutyryl cyclic AMP enhances taurine uptake by the heart [9]. All of these results suggest the existence of a complex regulatory system in the heart involving taurine, Ca^{2+}, cyclic AMP, and K^+.

Acknowledgements

This work was supported in part by U.S. Public Heatlth Service Grant HL-18554. The authors wish to thank Juli Russ for her technical assistance and Mary Ann Elgin for the typing of the manuscript.

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