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SAXITOXIN AND OUABAIN BINDING ACTIVITY OF ISOLATED SKELETAL MUSCLE MEMBRANE AS INDICATORS OF SURFACE ORIGIN AND PURITY

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A simple biochemical method for identifying and distinguishing transverse tubule and sarcolemma membranes in preparations of skeletal muscle microsomes is proposed and evaluated. This method is based on the previous observation that the ratio of ouabain to saxitoxin binding sites is five-fold higher in the sarcolemma than the transverse tubule. We measured [^{3}H]saxitoxin and [^{3}H]ouabain binding to microsomes of frog, rat and rabbit muscle in the presence of detergents to expose latent sites. A high density fraction (30–40% sucrose) of membranes was identified as transverse tubule on the basis of a low ouabain/saxitoxin ratio and its association with sarcoplasmic reticulum. A low density fraction (20–30% sucrose) was identified as transverse tubule containing variable amounts of sarcolemma as judged by a higher ratio of ouabain/saxitoxin sites. Our results suggest that this ratio can be used to determine the surface origin of muscle membrane preparations. Several different methods for purifying transverse tubules were compared by this technique.

Introduction

The electrical and chemical control of muscle contraction is mediated by a complex system of functionally specialized membrane elements consisting of neuromuscular junctions, sarcolemma, (sarcolemma refers to the portion of muscle plasma membrane in apposition to the basement membrane), transverse tubule (T-tubule) and an internal network of sarcoplasmic reticulum. Previous studies of various biochemical markers have shown that microsomes isolated from homogenized muscle are a heterogeneous mixture of membrane vesicles originating from sarcoplasmic reticulum, T-tubules and sarcolemma [1–3]. Based on these observations, several purification schemes for sarcolemma and/or T-tubule membrane have been reported [4–8].

In this study, we address two major questions regarding the purification of muscle surface membrane (surface membrane refers to both sarcolemma and transverse tubule membrane). The first question concerns the buoyant density of T-tubules. One method for purifying T-tubules involves isolating triad junctions from a high-density (30–40% sucrose, all sucrose concentrations are expressed as % w/w unless otherwise indicated) fraction of rabbit muscle microsomes [5,9,10]. Another preparation involves isolating a low-density (28–32% sucrose) fraction of rabbit [7] or chicken [6] muscle microsomes. In view of these two different starting fractions, we compare the yield of surface membrane and reconcile the varia-

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Abbreviations: EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

bility in the relative amounts of T-tubule present in these fractions. The second question concerns the surface origin of muscle membrane preparations. In view of the reported purification of both T-tubule [5,6,7] and sarcolemma [4,8] from low-density membrane fractions, it is unclear which of these components predominates in light fractions.

Our approach to these questions is based on the work of Jaimovich et al. [11] and Venosa and Horowicz [12]. These workers found that the surface density of tetrodotoxin sites is about 4-fold higher in the sarcolemma than in the T-tubule, while the corresponding distribution of ouabain sites may be up to 25-fold higher in the sarcolemma. Therefore, we expect that a preparation enriched in T-tubule would have a low ouabain/saxitoxin binding site ratio compared to one enriched in sarcolemma. In the present study, we measure the binding of saxitoxin and ouabain to frog, rat and rabbit muscle microsomes under conditions selected to saturate all available binding sites. These markers are used to follow the purification of surface membrane by equilibrium density gradient centrifugation, French press treatment and calcium-phosphate loading. We find the highest ouabain/saxitoxin ratio in the low-density sucrose gradient fraction of frog and rat striated muscle, suggesting that these fractions contain sarcolemma as well as T-tubule. On the other hand, this ratio is low for the same fraction of rabbit skeletal muscle, indicating that, in this species, the light fraction is mainly of T-tubule origin.

Methods

Preparation and fractionation of crude muscle microsomes. Hind leg and back muscle (200–500 g) was obtained from either 1 male rabbit, 4–5 male rats, or 4 bullfrogs. The muscles were minced with scissors and combined with 3–4 volumes of buffer (0.25 M sucrose/20 mM histidine-Tris (pH 7.4)/0.2 mM phenylmethylsulfonylfluoride/0.2% NaN_3) and homogenized in a Waring blender for 30 s at low speed followed by 30 s at high speed. After centrifugation at $4000 \times g$ for 10 min, the supernatant was filtered through cheesecloth and the pellets were combined with 3–4 volumes of buffer, homogenized and centrifuged as before. Supernatants were combined and solid KCl was added to a final concentration of 0.6 M. This suspension was centrifuged at $8500 \times g$ for 10 min and the small pellets were discarded. The microsomes were pelleted from the 0.6 M KCl supernatant by centrifugation at $100\,000 \times g$ for 40 min. The pellets were resuspended in 100–200 ml buffer without KCl in a Dounce homogenizer. This suspension was centrifuged at $6000 \times g$ for 10 min to remove remaining mitochondria. The supernatant was pelleted at $100\,000 \times g$ and resuspended in 10–20 ml buffer. All preparative procedures were carried out at 0–4°C.

Continuous gradients were formed from equal volumes (16 ml) of 25% and 45% (w/v) sucrose in buffer using a linear gradient mixing apparatus. The gradient was underlayed with 2 ml 60% (w/v) sucrose, overlayed with 40–70 mg of microsome protein and centrifuged in an SW27 rotor at $85\,000 \times g$ for 20 h.

[^{3}H]Saxitoxin and [^{3}H]ouabain binding assays. [^{3}H]Saxitoxin and unlabeled standard saxitoxin were generously provided by Dr. Gary R. Strichartz of the Department of Anesthesiology, Harvard Medical School. In a standard assay of saxitoxin binding, 0.1–1.0 mg of membrane protein was incubated with 30–80 nM [^{3}H]saxitoxin, 1% Lubrol-PX and 40 mM Hepes-Tris, pH 7.5 at 0°C. The blank consisted of an identical sample containing 6 μM tetrodotoxin. After 10–30 min incubation, bound saxitoxin was separated from unbound by passage of 100 μl sample over Dowex 50X-200 cation exchange resin as described [13]. The sample was rapidly eluted at 4°C in 10–15 s from 1 ml Dowex columns by forcing 0.9 ml of 20 mM Tris-HCl, pH 7.2 wash medium through the columns with a syringe. The radiochemical purity of [^{3}H]saxitoxin was 80% as determined by the maximum fraction of total radioactivity that could be specifically bound by rat brain synaptosomes. The specific activity was 4000 dpm/pmol as determined by binding competition assay with standard saxitoxin.

Binding of [^{3}H]ouabain (18 Ci/mmol, New England Nuclear) was assayed as described [14]. Samples were incubated for 90 min at 37°C in the presence of 40 mM histidine-Tris (pH 7.4)/120 mM NaCl/10 mM $MgCl_2$/1 mM EGTA/0.05% sodium deoxycholate/1.2 μM [^{3}H]ouabain/10

mM ATP. The blank consisted of an identical sample containing 400 μM ouabain. Binding activity expressed per mg protein was based on a bovine serum albumin standard. Protein concentration was determined according to [15] after trichloroacetic acid precipitation of the samples as described [16].

Results

Assay of saxitoxin and ouabain binding in muscle microsomes

Fig. 1 shows the effect of a non-ionic degergent, Lubrol-PX, on the specific activity of [^{3}H]saxitoxin binding to crude microsomes isolated from rat and rabbit muscle. A 1.7 to 2-fold maximal enhancement of saxitoxin binding was observed in these preparations, suggesting that at least 50% of the total saxitoxin sites are present in intracellular-side-out, sealed vesicles. Under selected conditions (0°C, 10–30 min incubation), we find that saxitoxin binding in muscle microsomes is maximally stimulated by 1% Lubrol-PX and the activity is stable within this duration. The use of this degergent to expose latent binding sites does not appear to affect the kinetic parameters of saxitoxin binding. By displacing bound [^{3}H]saxitoxin with excess non-radioactive ligand, we measured first-order dissociation rate constants of 0.41, 0.34 and 0.13 min^{-1} for rat, rabbit, and frog, respectively. The k_{off} for rat microsomes is similar to the value of 0.35 min^{-1} reported by Barchi and Weigele [17] for a rat muscle membrane preparation assayed in the absence of detergent by a filtration assay. The concentration dependence of [^{3}H]saxitoxin binding to crude rabbit muscle microsomes in the presence of 1% Lubrol-PX is shown in Fig. 2. A Scatchard plot of these data corrected for the nonspecific component (Fig. 2, inset) conforms to a single site binding isotherm, as found in other species and tissues [18]. The observed dissociation binding constant of 1 nM is similar to that reported for saxitoxin binding to a rat surface membrane preparation in the absence of detergent [17]. With a dissociation constant of 1 nM, we calculate that our standard

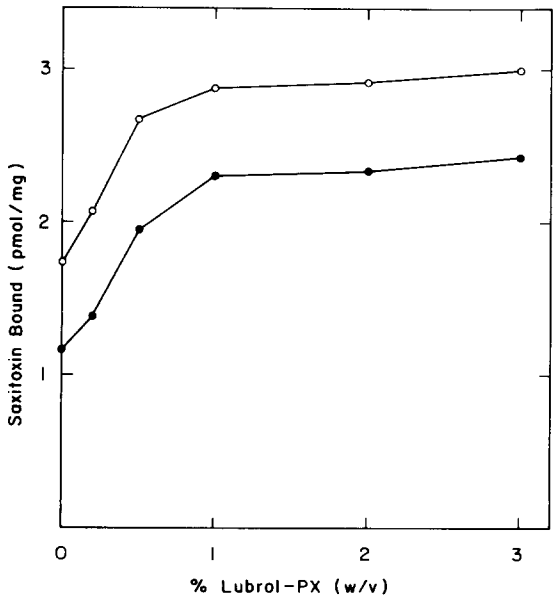

Fig. 1. Enhancement of [^{3}H]saxitoxin binding to muscle microsomes by Lubrol-PX detergent. Binding at 0°C in the presence of various concentrations of Lubrol-PX was assayed using 1 mg of crude rabbit (●) or rat (○) microsome protein and 65 nM [^{3}H]saxitoxin as described.

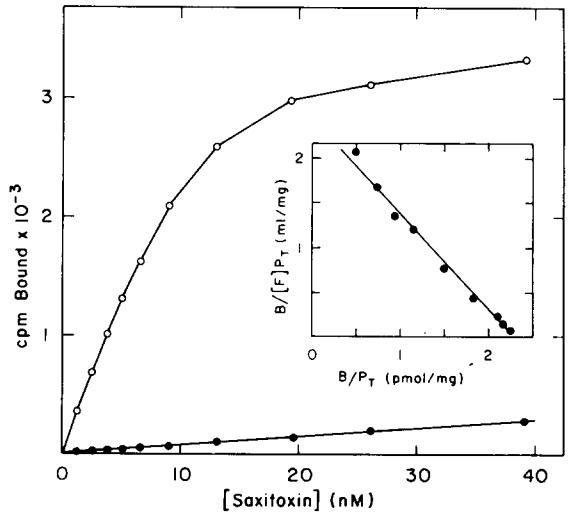

Fig. 2. Concentration dependence of [^{3}H]saxitoxin binding to rabbit muscle microsomes. Binding to crude rabbit microsomes (1 mg protein) was assayed at 0°C in the presence of 1% Lubrol-PX as described. The raw data are shown as cpm bound vs. the total concentration of [^{3}H]saxitoxin in the absence (○) or presence (●) of 6 μM tetrodotoxin. In the inset, the data are presented as a Scatchard plot, where the free (F) saxitoxin concentration was determined as the difference in the total and the bound (B). n = 2.3 pmol/mg, K_d = 0.96 nM.

assay conditions (30–80 nM saxitoxin) result in at least 95% saturation of the available sites. Therefore, assay of saxitoxin binding in the presence of 1% Lubrol-PX at 0°C provides a valid measure of the maximum specific activity of sodium channel binding sites in muscle microsomes, including those present on the inside surface of sealed vesicles.

In the present studies, we employed the [^{3}H]ouabain binding assay of Lau et al. [14] in the presence of deoxycholate. We have used a fixed [^{3}H]ouabain concentration of 1.2 μM which is at least 5-fold higher than the respective dissociation constants reported for rabbit (0.053 μM) [14], rat (0.21 μM) [19], and frog (0.22 μM) [12]. In the various microsome preparations, we observed up to an 8-fold stimulation of [^{3}H]ouabain binding by deoxycholate. We believe that these conditions are suitable for an estimate of the maximum number of ouabain sites in these preparations. We cannot interpret the orientation of these sites because detergent enhancement of ATP-dependent [^{3}H]ouabain binding is an ambiguous indicator of topology since this activity depends on accessibility of two impermeant ligands, ATP and ouabain, which bind on opposite sides of the membrane [20]. This ambiguity is avoided in the case of an assay where only one impermeant ligand is used, such as [^{3}H]saxitoxin.

Identification of low and high buoyant density populations of surface membrane in microsomes

When muscle microsomes from various species were applied to a continuous sucrose gradient and centrifuged for 20 h at 85 000 × g, at least half of the total protein banded in a narrow region with the peak fraction at 33–35% sucrose (Fig. 3A–C). The remainder of the protein was located in a small peak centered at 25% sucrose and a variable shoulder at 40% sucrose. Previous studies of Ca-ATPase and Ca transport in rabbit muscle microsomes have demonstrated comparable levels of these activities within a factor of 2–3 in all fractions from 25–43% sucrose [3,7,21]. Thus, the distribution of sarcoplasmic reticulum cannot be identified on the basis of these activities alone. The light fraction (25% sucrose), however, has been distinguished from sarcoplasmic reticulum on the basis of other criteria, including higher phospholipid and cholesterol content, a unique poly-

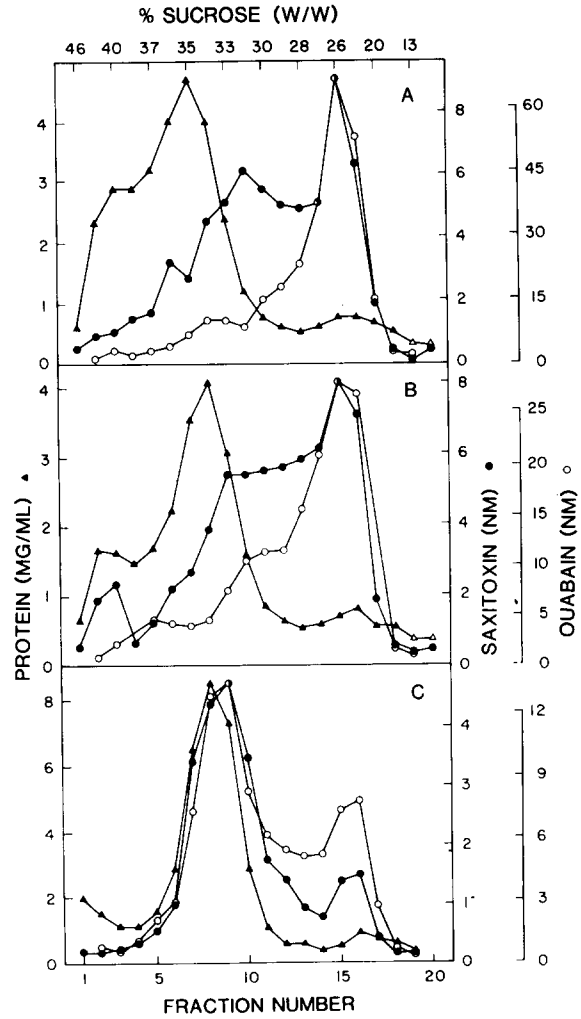

Fig. 3. Distribution of protein, saxitoxin and ouabain binding activity after centrifugation of muscle microsomes on continuous sucrose density gradients. Sucrose gradients were prepared and assayed as described. 52 mg rat (A), 60 mg rabbit (B), or 75 mg frog (C) crude microsome protein was loaded. The data are plotted as the concentration of protein (mg/ml) or binding sites (nM) measured in the fraction. The peak fraction of each activity is scaled to the same height for comparison between gradients. Recovery of protein and binding activity after centrifugation was greater than 70%.

peptide composition, a higher ratio of Mg-ATPase to Ca-ATPase, and an inability to accumulate Ca-phosphate or Ca-oxalate precipitates under optimal conditions for Ca-uptake by sarcoplasmic reticulum [3,7]. In agreement with these studies and the previous conclusion of a surface membrane origin for the light fraction, we consistently

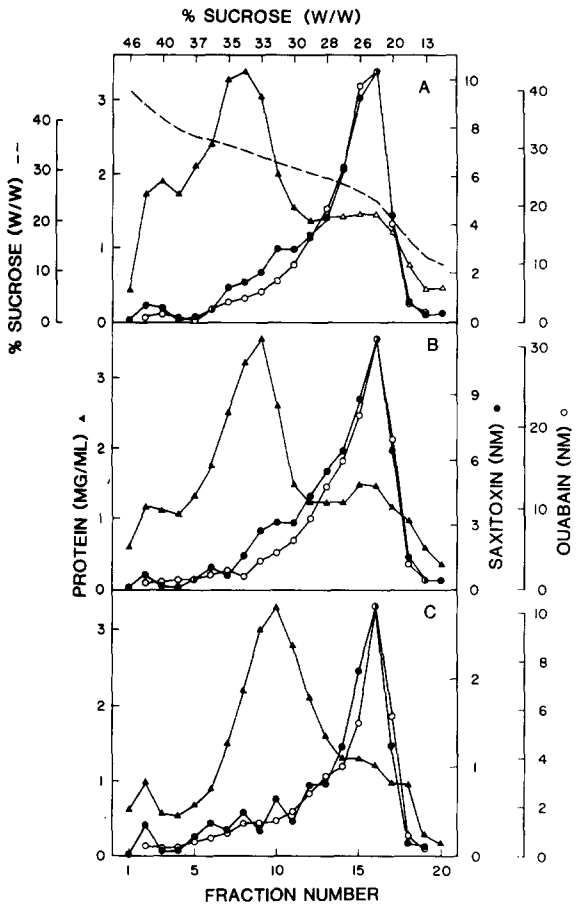

Fig. 4. Effect of French press homogenization on sucrose gradient profiles of muscle microsomes. The same microsome preparations shown in Fig. 3 A–C were passed through an Aminco French pressure cell at 11000 lb/inch^2 before loading on sucrose gradients. Conditions of centrifugation and assay were identical to those of Fig. 3, except that 44 mg of frog microsome protein was loaded on gradient C instead of 74 mg. A representative profile of sucrose concentration is shown in A.

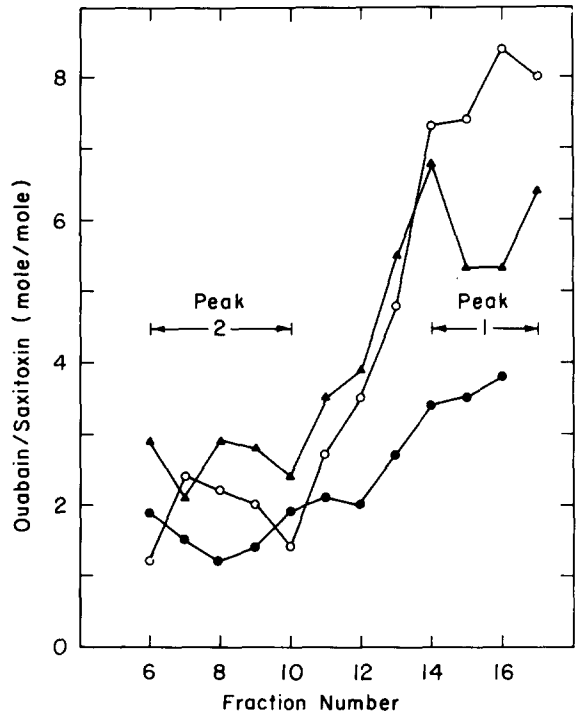

Fig. 5. Ratio of ouabain to saxitoxin binding activity measured in muscle microsomes fractionated in continuous sucrose density gradients. Binding data of Fig. 3A–C are presented as the molar ratio of ouabain/saxitoxin for rat (○), rabbit (●), and frog (▲), respectively. Peak 1 and peak 2 fractions are defined for the purpose of discussion as described in the text.

find that the highest specific activities of ouabain and saxitoxin binding peak at 24–26% sucrose (Fig. 3A–C). In addition, we observe that variable proportions of the total ouabain and saxitoxin binding sediment at a higher density coincident with intermediate or heavy sarcoplasmic reticulum. With frog microsomes (Fig. 3C), this high-density population banded in a discrete peak that lagged the main protein peak by only a single fraction. However, with rabbit or rat microsomes (Fig. 3A, B), we observed that the high density population tended to band in a shoulder between the light peak at 25% sucrose and the main protein peak at 33–35% sucrose.

Variability in the relative amounts of binding activity observed in the low and high density populations of surface membrane suggested that the final density distribution of binding sites may be dependent on the conditions of homogenization used during the microsome isolation procedure. This possibility was tested by analyzing parallel gradients using microsomes that had been subjected to homogenization in a French pressure cell at 11000 lb/inch^2 before loading. Such gradients exhibited a highly reproducible density distribution of binding activity regardless of the species (Fig. 4A–C). Both ouabain and saxitoxin binding activity banded in a sharp peak at 24% sucrose with about 20% of the total activity sedimenting in a tail region at 30% sucrose. However, the French press treatment also resulted in: (a) a loss of

approx. 20% of the binding activity of both ligands (see Ref. 7) and (b) a small shift of the main protein peak to lower density with a concomitant increase in the amount of protein banding in the low-density region at 25% sucrose. These two effects resulted in an average 40% decrease in peak specific activity for the French press gradient vs. the untreated microsomes.

If the surface density distribution of Na channels and (Na + K)-ATPase molecules is different in the sarcolemma vs. T-tubules and if muscle microsomes isolated by differential centrifugation contain a mixture of these surface membrane elements, we might expect variations in the ouabain/saxitoxin binding ratio after fractionation procedures capable of separating these membranes. Evidence for this type of heterogeneity was obtained in the density distribution of binding activities of microsomes that had not been subjected to the french press treatment. We consistently observe that the ratio of ouabain to saxitoxin binding activity is higher in the low density fractions than in the high density fractions. This is illustrated in the profile of this ratio plotted in Fig. 5 for the data of Figs. 3A–C. The regions labeled peak 1 and peak 2 in Fig. 5 correspond to the low-density binding activity peak and the high density protein peak, respectively. For a given gradient, the ouabain/saxitoxin ratios are relatively constant within the peak 1 and peak 2 regions, but this ratio is always higher in peak 1 and undergoes an increasing transition zone in the region between peak 2 and peak 1. Heterogeneity in the density distribution of the ouabain/saxitoxin binding ratio was more pronounced for the rat and frog microsomes than for the rabbit microsomes and was markedly reduced by the French press treatment.

Effect of Ca-phosphate loading on the purification of saxitoxin and ouabain binding activity

Attempts to further purify the light membrane fraction from contaminating sarcoplasmic reticulum are based on the ability of sarcoplasmic reticulum to precipitate Ca-oxalate or Ca-phosphate internally, under optimal Ca-transport conditions [6,7]. However, it is known that plasma membranes from many tissues contain a Ca-transporting ATPase [22,23] and it is not clear that this

TABLE I

SUMMARY OF RABBIT MUSCLE SURFACE MEMBRANE PURIFICATION

Results of binding determinations on various fractions obtained from the T-tubule purification procedure of Rosemblatt et al. [7] using discontinuous sucrose gradients. Fractions were diluted in buffer, pelleted, and resuspended at approx. 10 mg/ml protein before assay. Values are the mean of duplicate determinations.

Fraction	Protein (mg)	Saxitoxin			Ouabain			Ouabain/saxitoxin (mole/mole)
		pmol	pmol/mg	purification factor	pmol	pmol/mg	purification factor	
I. Microsomes	730	1400	1.9	1.0	2700	3.7	1.0	1.9
II. Step gradient [a]								
A. Light ($\leq$ 35%)	93	490	5.2	2.7	1200	12.4	3.4	2.4
B. Heavy (35–48%)	350	600	1.7		1100	3.0		1.8
III. Ca-P_i loading								
A. Light ($\leq$ 35%)	14	300	22	11.4	660	49	13.4	2.2
B. Intermediate (35–50%)	3.0	21	6.8		46	15		2.2
C. Heavy (pellet)	4.0	12	2.9		15	3.7		1.3

[a] Fractions refer to various sucrose layers (% w/v) as described in Ref. 7.

technique should fail to load muscle surface membrane vesicles with these precipitates. In order to evaluate the effectiveness of this procedure in the purification of surface membrane, we assayed ouabain and saxitoxin binding in various fractions that had been subjected to Ca-phosphate loading.

When rabbit surface membrane was purified by consecutive step sucrose gradient centrifugation and Ca-phosphate loading as previously described [7], the specific binding activities of ouabain and saxitoxin increased in parallel (Table I). Initial purification of crude microsomes by discontinuous sucrose gradient centrifugation resulted in a 3-fold increase of binding activities. Further purification of the light fraction by Ca-phosphate loading increased the specific activities of these markers to 11- to 13-fold. Only 10% of the total recovered binding activity shifted to higher density fractions after the loading procedure. Thus, the majority of surface membrane is unable to precipitate Ca-phosphate under these conditions. The specific binding activities of the two markers for the rabbit light fraction obtained after the Ca-phosphate loading procedure are: 49 pmol/mg for [^{3}H]ouabain and 22 pmol/mg for [^{3}H]saxitoxin. These values are higher than previously reported specific activities for mammalian muscle surface membrane purified by other methods [4,14].

Discussion

In studies of tetrodotoxin binding using normal and detubulated frog muscle, Jaimovich et al. [11] reported that the surface density of tetrodotoxin sites is 175 sites/μm^2 in sarcolemma and 41–52 sites/μm^2 in T-tubule. In similar binding experiments using [^{3}H]ouabain, Venosa and Horowicz [12] reported that the density of ouabain binding sites is 2500 sites/μm^2 in frog sarcolemma, while the value for the T-tubule was estimated at 4–5% of the sarcolemma density or 100–125 sites/μm^2 *.

The relative distribution of saxitoxin and ouabain sites in the sarcolemma and transverse tubule as measured by the physiological studies on intact muscle provides a criterion for identifying these two membrane elements in microsome fractions. From the values of the reported site surface densities [11,12], we calculate that the mole ratio of ouabain to tetrodotoxin sites is 14 for sarcolemma and 2–3 for transverse tubule. Strictly, these ratios are only applicable to frog sartorious since the measured surface density of ouabain or saxitoxin binding in muscles of different vertebrate species, or even in different muscles of the same species, varies by as much as a factor of two [25,26]. Despite this caution, from the standpoint of common physiological function, we will assume that the same relative distribution of (Na + K)-ATPase and sodium channels in frog sarcolemma and T-tubule also applies to skeletal muscle of other species.

In frog, rat, and rabbit, we consistently observed that the low sucrose density microsomal population exhibited a higher ouabain/saxitoxin site ratio than the high density population (e.g., Fig. 5). On the basis of the site ratios reported for whole frog sartorious, this result suggests that the low density fractions (peak 1, Fig. 5) are enriched in surface sarcolemma relative to the high density fractions (peak 2). Using the ligand binding ratios for frog, we calculate that the lowest density fractions of frog microsomes (peak 1) would be equivalent to a mixture of 30% surface sarcolemma and 70% T-tubule by surface area. If the same distribution of binding sites is applicable to other species, the lowest density fractions of rat microsomes in Fig. 5 would contain the greatest amount of sarcolemma (50%), while the same fractions of rabbit microsomes would contain the least amount of sarcolemma ($< 10\%$).

The average ouabain/saxitoxin ratios of the high sucrose density fractions (peak 2) for the three species studied range from 1.6 to 2.6. These values are consistent with the conclusion that the

* In both the cited binding studies with frog muscle, it was also concluded that the amount of tetrodotoxin or ouabain binding sites located in intracellular membranes is undetectable compared to sites accessible to the external medium. This conclusion was reached from experiments using cut fibers or muscle homogenates. A similar conclusion for ouabain binding was reached by Clausen and Hansen [19] using rat soleus. This finding is important in the present context, since it permits us to exclude the possibility of significant saxitoxin or ouabain binding sites in intracellular reticulum or organelles, such as has been found for glucose transport sites in adipocytes [24].

binding activity in this fraction is exclusively of T-tubule origin. The sensitivity of the high sucrose density population of binding activity to French press treatment also supports this conclusion as first proposed by Caswell et al. [9].

The 'light' fractions in Table I from step sucrose gradients in the purification procedure of Rosemblatt et al. [7] exhibits a ouabain/saxitoxin ratio of 2–3 which is close to the range expected for T-tubule. Our results support the previous conclusion [7] that rabbit surface membrane purified by this procedure is mainly of T-tubule origin. The detection of sarcolemma contamination in such preparations will require more sensitive techniques than those presently available.

The Ca-phosphate loading procedure appears to be quite effective in removing contaminating sarcoplasmic reticulum, as noted by the increase in specific binding activities found with this procedure in Table I. It is possible that the small amount of binding activity shifted to higher density after loading is due to a small population of T-tubule membrane attached to sarcoplasmic reticulum vesicles.

Previous measurements of the lipid composition of purified sarcoplasmic reticulum and T-tubule are reported at 0.6–0.9 and 1.6 μmol phospholipid/mg protein, respectively [7,10]. The higher lipid content of surface membrane vs. sarcoplasmic reticulum is probably the basis for the lower equilibrium density of surface membrane in sucrose gradients. An intermediate banding density for the heavy fraction of binding activity would be expected if the mass contribution of the lighter T-tubule vesicle is sufficient to cause a net reduction in the buoyant density of triad structures or vesicular aggregates containing higher density sarcoplasmic reticulum. This may explain the intermediate density of binding activity found between the major protein peak at 35% sucrose and the major binding activity peak at 25% sucrose observed for the rabbit and rat preparations of Fig. 3.

In conclusion, our results reconcile some of the differences noted in various studies of the purification of skeletal muscle surface membrane. The association of T-tubule with sarcoplasmic reticulum, ascribed to the morphological specialization known as the triad junction, is clearly dependent on the homogenization procedure, as shown by the effect of French press treatment in our work and that of Lau et al. [5]. Depending on the exact conditions of the initial muscle homogenization, variable amounts of T-tubule may be sheared from triad junctions and will band at low sucrose density. Relatively mild muscle homogenization will be expected to result in the greatest yield of intact triads that band at high sucrose density. Because of possible contamination of low-density fractions with sarcolemma, it appears that the purest preparation of T-tubules may be obtained by starting with high-density fractions of triads. However, for applications where yield is a consideration, the light microsome fraction may often contain the bulk of the surface membrane. Previous to this study, the only qualitative assays proposed for distinguishing T-tubules from sarcolemma in purified membrane preparations were based on microscopic examination of thin sections or freeze-fracture faces [2,5–8]. Our results suggest that the ouabain/saxitoxin binding ratio is a convenient biochemical criterion for determining the surface origin of muscle membrane preparations.

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References

1 Andrew, C.G. and Appel, S.H. (1973) J. Biol. Chem. 248, 5156–5163

2 Barchi, R.L., Bonilla, E. and Wong, M. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 34–38
3 Fernandez, J.L., Rosemblatt, M. and Hidalgo, C. (1980) Biochim. Biophys. Acta 599, 552–568
4 Barchi, R.L., Weigele, J.B., Chalikian, D.M. and Murphy, L.E. (1979) Biochim. Biophys. Acta 550, 59–76
5 Lau, Y.H., Caswell, A.H. and Brunschwig, J.P. (1977) J. Biol. Chem. 252, 5565–5574
6 Scales, D.J. and Sabbadini, R.A. (1979) J. Cell Biol. 83, 33–46
7 Rosemblatt, M., Hidalgo, C., Vergara, C. and Ikemoto, N. (1981) J. Biol. Chem. 256, 8140–8148
8 Seiler, S. and Fleischer, S. (1982) J. Biol. Chem. 257, 13862–13871
9 Caswell, A.H., Lau, Y.H. and Brunschwig, J.P. (1976) Arch. Biochem. Biophys. 176, 417–430
10 Lau, Y.H., Caswell, A.H., Bruschwig, J.P., Baerwald, R.J. and Garcia, M. (1979) J. Biol. Chem. 254, 540–546
11 Jaimovich, E., Venosa, R.A., Shrager, P. and Horowicz, P. (1976) J. Gen. Physiol. 67, 399–416
12 Venosa, R.A. and Horowicz, P. (1981) J. Membrane Biol. 59, 225–232
13 Rhoden, V.A. and Goldin, S.M. (1979) J. Biol. Chem. 254, 11199–11201
14 Lau, Y.H., Caswell, A.H., Garcia, M. and Letellier, L. (1979) J. Gen. Physiol. 74, 335–349
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
16 Peterson, G.L. (1977) Anal. Biochem. 83, 346–356
17 Barchi, R.L. and Weigele, J.B. (1979) J. Physiol. 295, 383–396
18 Ritchie, J.M. and Rogart, R.B. (1977) Rev. Physiol. Biochem. Pharmacol. 79, 2–50
19 Clausen, T. and Hansen, O. (1974) Biochim. Biophys. Acta 345, 387–404
20 Jones, L.R., Maddock, S.W. and Besch, H.R. (1980) J. Biol. Chem. 255, 9971–9980
21 Meissner, G. (1975) Biochim. Biophys. Acta 389, 51–68
22 Lew, P.D. and Stossel, T.P. (1980) J. Biol. Chem. 255, 5841–5846
23 Waisman, D.M., Gimble, J.M., Goodman, D.B.P. and Rausmussen, K. (1981) J. Biol. Chem. 256, 415–419
24 Karnieli, E., Zarnowski, M.J., Hissin, P.J., Simpson, I.A., Salans, L.B. and Cushman, S.W. (1981) J. Biol. Chem. 256, 4772–4777
25 Venosa, R.A. (1979) in Membrane Transport in Biology, Vol. II, (Tosteson, D.C., ed.), pp. 211–262, Springer Verlag, New York
26 Hansen Bay, C.M., and Strichartz, G.R. (1980) J. Physiol. 300, 89–103