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Contamination of a cardiac sarcolemmal preparation with endothelial plasma membrane

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Preparation of sarcolemma from whole rabbit heart using the method of Jones et al. (Jones,L.R., Besch, H.R., Fleming, J.W., McConnaughey, M.M. and Watanabe, A.M. (1979) J. Biol. Chem. 254, 530–539) results in a 46-fold purification of the endothelial plasmalemma-specific marker angiotensin converting enzyme. This implies contamination of the sarcolemma with vascular endothelial plasmalemma. During preparation of sarcolemma from sheep heart, using the same method, angiotensin converting enzyme copurified with the general plasma membrane marker ($Na^+ + K^+$)-ATPase. The ratio of myocyte to endothelial plasma membrane in the final preparation is therefore similar to that in the whole heart homogenate. Ultrastructural analysis has shown that the myocyte / endothelial surface area is 70:30 in whole cardiac muscle. Comparison of angiotensin converting enzyme activity of an endothelial plasma membrane fraction with that of whole heart sarcolemma suggests an upper limit of 42% for endothelial contamination. Contamination by endothelial plasmalemma was dramatically reduced by preparing sarcolemma from myocytes produced by proteolytic disruption of whole hearts. Following disruption, myocytes were separated from non-muscle cells by sedimentation through 0.5 M sucrose. Sarcolemma prepared from sheep cardiac myocytes had approximately 15-fold less angiotensin converting enzyme activity than whole sheep heart sarcolemma but comparable ouabain-inhibitable ($Na^+ + K^+$)-ATPase activity.

Introduction

In recent years a number of procedures have been described for the purification of sarcolemma-enriched membrane fractions from mammalian heart [1]. Following early observations that cardiac membrane preparations contained marker enzyme activities thought to be associated with both sarcolemma and sarcoplasmic reticulum membranes, it was shown that these two populations could be separated using density gradient centrifugation [2–5]. These techniques produced low density membrane fractions rich in cell membrane marker enzymes and higher density fractions with activities characteristic of the sarcoplasmic reticulum.

Sarcolemmal preparations obtained in this way have been used in the study of a number of transport and regulatory systems ascribed to the cardiac muscle cell membrane. These include Na^+-Ca^{2+} exchange [6–9], Ca^{2+}-ATPase [10,11],

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Abbreviations: Tris, 2-amino-2-hydroxymethylpropane-1,3-diol; Hepes, 4-(2-hydroxyethyl)-1,1-piperazineethanesulphonic acid.

adenylate cyclase [12], protein kinase activity [12], substrates for phosphorylation [12,13], ionic channels [14] and angiotensin II receptors [15].

Whilst the membrane characterizations employed in these studies have clearly established the separation of cell surface membrane from intracellular membrane fractions, little or no attempt has been made to monitor possible contamination of sarcolemmal fractions with membrane systems derived from any of the various other types of cells present in the heart.

The fact that at least one preparation of cardiac sarcolemma is to some extent contaminated by another cell membrane fraction, the endothelial cell surface membrane, has now been demonstrated by Schrader and Londos [16]. These authors monitored the activity of a specific endothelial cell membrane enzyme, angiotensin converting enzyme (ACE), in a preparation of dog cardiac sarcolemma isolated using the method of Reeves and Sutko [9] and found a 10-fold enrichment of this activity in the sarcolemma fraction.

In this report we demonstrate that the cardiac sarcolemma membrane fraction prepared from whole rabbit and sheep heart by the widely used method of Jones et al. [4] also contains endothelial cell plasmalemma. Although the degree of contamination cannot be directly quantified we estimate that endothelial plasmalemma may account for as much as 30 to 40% of the final sarcolemma preparation.

In addition, we demonstrate that sarcolemma with greatly reduced endothelial plasmalemmal contamination may be prepared from isolated myocytes. To obtain sufficiently large quantities of myocytes for sarcolemma extraction we have developed a myocyte isolation procedure for use with large animals. The procedure is relatively inexpensive and does not involve complex surgical techniques. Following proteolytic disruption of the heart, myocytes are separated from other cardiac cells by density gradient centrifugation and sarcolemma is prepared by conventional methods. Some of these data have appeared in abstract form [17].

Materials and Methods

Rabbit heart

Homogenates. Ventricular homogenates were obtained by suspending washed ventricular tissue in 0.4 M sucrose at 4°C and homogenising for 2×3 s using an Ultraturrax homogeniser at speed setting 9.

Washed homogenates were obtained by sedimentation of tissue homogenates at $50\,000 \times g$(max) for 20 min. The pellet was resuspended in 10 mM Tris, 2 mM dithiothreitol (pH 7.5) with acetic acid and the washing procedure repeated. The final pellet was again suspended in Tris/dithiothreitol.

Preparation of sarcolemma from whole heart. Male New Zealand white rabbits were killed by a blow to the head. The hearts were rapidly excised and placed in 300 mM mannitol, 5 mM Hepes, 0.1 mM EDTA (pH 7.2) at 4°C. The coronary vasculature was flushed clear of blood by retrograde perfusion through the aorta with the same solution. The auricles and fat were trimmed away and the ventricles opened and flushed clear of any remaining blood. Sarcolemma was prepared from this tissue according to the method of Jones et al. [4] (procedure II). Sucrose density centrifugation was carried out for 16 h and the membrane fraction appearing at the interface of 0.25 M and 0.6 M sucrose was collected, washed with 150 mM KCl/5 mM Tris-HCl (pH 7.0) and resuspended in 0.4 M sucrose/5 mM Tris-HCl (pH 7.0).

Preparation of rabbit cardiac myocytes. Hearts were disrupted by perfusion with collagenase and hyaluronidase as described by Farmer et al. [18]. Myocytes obtained according to this procedure were washed twice by sedimentation through 4% bovine serum albumin (Sigma A 7906) in Krebs-Henseleit buffer ((mM): NaCl, 118; KCl, 4.74; KH_2PO_4, 0.93; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 10, made up in AnalaR water (British Drug Houses) and equilibrated with 95% O_2/5% CO_2 to pH 7.4) plus 50 μM Ca^{2+}. Sedimentation was at 400 rpm in an MSE 6L centrifuge for 1 min at room temperature. A final purification step was carried out by sedimenting washed myocytes through 0.5 M sucrose/5 mM Tris-HCl (pH 7.5) at $1000 \times g$ in an MSE 6L centrifuge for 10 min at 4°C.

Sheep heart

Preparation of sarcolemma from whole heart. Sarcolemma was prepared from sheep ventricular tissue following the method of Jones et al. [4]

(procedure II). 'Homogenate' (Table II) refers to material taken following the second homogenisation step of this procedure.

Preparation of isolated myocytes from sheep heart. Adult sheep were killed by exsanguination at a local abbatoir. Hearts were removed within 5 min of death and placed in St. Thomas' cardioplegic solution at 4°C (St Thomas' cardioplegic solution contains, mM: NaCl, 102; KCl, 21.4; $MgCl_2$, 16.0; sodium lactate, 28.7; $CaCl_2$, 2.4 and procaine 1.0). 1–2 h after death the hearts were perfused as described by Langendorff [19] at 37°C with high potassium Krebs-Henseleit buffer (mM: NaCl, 118; KCl, 20; KH_2PO_4, 0.93; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 10, in AnalaR water and equilibrated with 95% O_2/5% CO_2 to pH 7.4) containing 2.5 mM $CaCl_2$ for 5 min at a flow rate of 300 ml/min. Following this period of equilibration, the perfusate was switched to high potassium Krebs-Henseleit buffer containing no added calcium for a period of 5 min. 100 ml of high potassium Krebs-Henseleit buffer containing 0.1% collagenase (Worthington, 4176; 141–202 U/mg protein), 0.05% hyaluronidase (Sigma, H3506), 0.1% bovine serum albumin and 50 μM $CaCl_2$ was then perfused into the coronary circulation and flow stopped. The heart was then pulsed with 50-ml aliquots of this solution at one minute intervals for 10 min. A total volume of 300 ml of enzyme solution was used, the effluent from the heart being collected and passed through 3 μm filters before reuse. The heart was removed from the perfusion apparatus and the ventricular tissue chopped into approximately one centimeter cubes using scissors. This material was suspended in 80 ml of high potassium Krebs-Henseleit buffer containing 0.1% collagenase, 0.05% hyaluronidase, 2% bovine serum albumin and 50 μM $CaCl_2$ and shaken for 10 min at 37°C. At the end of this period the suspension was filtered through 300 μm nylon gauze. Undigested material was rinsed with 100 ml of high potassium Krebs-Henseleit buffer containing 2% bovine serum albumin and 50 μM $CaCl_2$ and returned to high potassium Krebs-Henseleit buffer containing 0.1% collagenase, 0.05% hyaluronidase, 2% bovine serum albumin and 50 μM $CaCl_2$. This was shaken for a further 10 min at 37°C. A total of five digestions were carried out. The filtrates from each digestion were diluted to 600 ml with high potassium Krebs-Henseleit buffer. Myocytes were sedimented at 400 rpm in an MSE 6L centrifuge at room temperature for 2 min and washed three times in high potassium Krebs-Henseleit buffer. Finally, myocytes from the five digestions were pooled and sedimented twice through 0.5 M sucrose, 5 mM Tris-HCl (pH 7.5) at 4°C for 10 min at $1000 \times g$ in an MSE 6L centrifuge.

Preparation of sarcolemma from isolated sheep cardiac myocytes. Myocytes were washed twice in 0.75 M KCl by sedimentation at $14000 \times g$ and resuspended in 10 mM $NaHCO_3$, 5 mM histidine (pH 7.2). The myocytes were then homogenised by subjecting them to sonication in an MSE probe sonicator (amplitude 13 μm for 90 s in 7-ml aliquots on ice). This procedure was taken as being equivalent to the second homogenisation step of the Jones et al. (procedure II), method [4]. The preparation of sarcolemma was then continued exactly as described by Jones et al. [4]. Sucrose gradient centrifugation was for 16 h and the material banding at the interface of 0.25 M and 0.6 M sucrose fraction was collected, washed and suspended in 0.4 M sucrose/5 mM Tris-HCl (pH 7.0).

Endothelial cell plasmalemma. Twenty to thirty segments of sheep aorta (5–20 cm in length) were brought from the abbatoir in 150 mM NaCl/5 mM Tris-HCl (pH 7.4) at 4°C. Segments were cut open longitudinally, rinsed in 150 mM NaCl, and the luminal surface scraped lightly with a razor blade to remove endothelial cells. The cells were suspended in 150 mM KCl/5 mM Tris-HCl (pH 7.5) and concentrated by sedimenting them at $175000 \times g$ for 30 min. The cells were resuspended in 2 ml of 150 mM KCl/5 mM Tris-HCl (pH 7.5) and homogenised by sonication for 3×30 s periods in an MSE probe sonicator (10 μm amplitude) on ice. Material was diluted to approx. 30 ml with 150 mM KCl/5 mM Tris-HCl (pH 7.5) and centrifuged at $10000 \times g$ at 4°C for 30 min. The supernatant was collected and sedimented at $175000 \times g$ at 4°C for 30 min. The pellet from the high-speed spin, representing the endothelial plasmalemma, was suspended in 1 M sucrose/5 mM Tris-HCl (pH 7.4). Approximately 1 μg of purified endothelial plasma membrane was obtained for each g of sheep aorta.

Enzyme activities

Angiotensin converting enzyme activity was measured using the ACE Microvial assay (Ventrex, Portland, Maine). 50 μl of membrane protein (5–250 μg) was added to 200 μl angiotensin converting enzyme assay buffer (0.05 M Hepes/0.1 M NaCl/0.1% sodium azide/0.6 M Na_2SO_4 (pH 8.0)). 50 μl of the diluted sample was added to 50 μl 10 nM [^{3}H]hippurylglycylglycine in a glass microvial. All reagents were prewarmed to 37°C. The reaction was stopped after 30 min at 37°C by addition of 50 μl 0.5 M HCl. Vials were cooled to room temperature and 1.5 ml Ventrex scintillation cocktail (scintillation fluid plus ethyl acetate) was added. The vials were vortex mixed for 30 s and then counted. Ethyl acetate extracts the product, [^{3}H]hippuric acid, from the aqueous phase. Blanks were run with either no membrane protein or with boiled protein. A control sample of serum with known angiotensin converting enzyme activity was included with each experiment. The assay was done in duplicate. Activity is expressed in U/mg protein: one unit is 1% substrate hydrolysed/min. The assay can detect as little as 0.2 units of activity, and is linear up to 50 μg total protein.

Ouabain inhibitable ($Na^+ + K^+$)-ATPase activity was monitored as described by Bers [2]. Protein concentrations were determined using either the method of Lowry et al. [20] or Bradford [21].

Results and Discussion

The endothelial plasmalemma content of an initial rabbit heart homogenate, a washed homogenate (see Materials and Methods) and the final sarcolemmal preparation from whole heart was assessed by monitoring the activity of a specific marker enzyme, angiotensin converting enzyme (ACE). It has been shown using immunocytochemical techniques that angiotensin converting enzyme is exclusively located in the vascular endothelium of rabbit heart [22,23]. The data obtained are shown in Table Ia. It is clear that the angiotensin converting enzyme activity is highly enriched in the sarcolemmal preparation (46-fold over initial homogenate). Attempts to separate endothelial plasmalemma and sarcolemma by density gradient centrifugation on either sucrose or Percoll were unsuccessful (data not shown).

An alternative approach was to reduce endothelial plasmalemma contamination by purifying cardiac myocytes from the heart and using these as the starting material for cardiac sarcolemma production. A number of methods for the production of viable cardiac myocytes are now described [18,24,25], all of which involve the perfusion of hearts with solutions containing low ionised calcium concentrations followed by proteolytic digestion. We adopted the method recently described by Farmer et al. [18] and were able to follow the separation of rabbit cardiac myocytes and endothelial cells by monitoring angiotensin converting enzyme activity (Table Ib). All of the data in Table Ib were obtained from washed homogenates produced by sedimentation of initial homogenates at $50\,000 \times g$. This was necessary to ensure the removal of extraneous soluble proteins (e.g. bovine serum albumin and hydrolytic enzymes) used during tissue disruption. Activities in Table Ib should therefore be compared with the washed homogenate value in Table Ia. Washing of homogenates yields some enrichment of angiotensin converting enzyme as other soluble cell proteins are removed (Table Ia). Comparison of the data of Tables Ia and Ib reveals that disruption of the heart lowered the angiotensin converting enzyme activity in the washed homogenate approx. 4-fold. This probably reflects flushing of endothelial cells from the heart during perfusion with collagenase. Angiotensin converting enzyme activity was further reduced in the cardiac myocytes obtained from the disrupted rabbit hearts by centrifugation (Table Ib) and still further by sedimenting the isolated myocytes through 0.5 M sucrose. These myocytes have approx. 19-fold less angiotensin converting enzyme activity than whole cardiac tissue. It should be noted that angiotensin converting enzyme activity was unaffected by prolonged exposure of material to the hydrolytic enzymes used for tissue disruption (results not shown).

Sarcolemma prepared from these myocytes should have greatly reduced endothelial plasmalemma contamination. However, the yields of myocytes obtained from rabbit hearts is low and it was not possible to obtain sufficient material to undertake routine sarcolemma production. It was clearly necessary to use hearts from larger animals.

However, our choice of species was restricted as the level of angiotensin converting enzyme activity of cardiac tissue varies considerably from species to species (e.g. rat < 4, rabbit 134, dog < 4, sheep 13, angiotensin converting enzyme activity of washed homogenate expressed as U/mg protein).

Subsequent experiments were conducted on sheep hearts. The angiotensin converting enzyme activity of this tissue, although not high enough to permit us to follow the separation of endothelial cells and myocytes as we had done for the rabbit, was sufficient for us to determine the degree of enrichment of endothelial plasmalemma in the final sarcolemma preparation. Plasma membrane was first prepared from whole sheep heart by the method described by Jones et al. [4]. Ouabain-inhibitable $(Na^+ + K^+)$-ATPase was used as a general plasmalemmal-membrane marker.

Activities of $(Na^+ + K^+)$-ATPase and angiotensin converting enzyme in whole heart sarcolemma are shown in Table IIa. Note that the homogenate described is a washed homogenate: the '-fold' purification for the sarcolemma will be less than that obtainable using an unwashed homogenate. $(Na^+ + K^+)$-ATPase activity was 15.1 μmol P_i/mg protein per h in the sarcolemmal fraction, similar to the 16.2 μmol/mg protein per h found by Jones et al. in dog heart sarcolemma [12]. $(Na^+ + K^+)$-ATPase activity was assayed without 'unmasking' agents [12]. As with the rabbit preparation, angiotensin converting enzyme activity of sheep heart was purified by a factor of approx. 10 from the washed homogenate to the sarcolemmal fraction. The purification factor for angiotensin converting enzyme (9.5) was slightly greater than that for $(Na^+ + K^+)$-ATPase (7.9).

Myocytes were prepared by scaling up the method used for the rabbit hearts as described in Materials and Methods. The procedure yielded 28.8 ± 5.7 g (S.E., $n = 5$) of myocytes (wet wt. of packed pellet)/heart, 33.4 ± 5.7% (S.E., $n = 5$) of which were calcium tolerant. Myocytes were washed and sedimented through 0.5 M sucrose, and then taken for sarcolemmal preparation as described in Materials and Methods. Angiotensin converting enzyme activity in the myocyte homogenate was not detectable. $(Na^+ + K^+)$-ATPase activities of myocyte homogenate and sarcolemma were not significantly different from those of whole heart (Table IIb). Angiotensin converting enzyme activity of myocyte sarcolemma was lower than that of whole heart by a factor of 15 (Table IIb). It is therefore possible to reduce angiotensin converting enzyme activity, and therefore endothelial contamination, without affecting $(Na^+ + K^+)$-ATPase. Adenylate cyclase was also monitored in these preparations [26] but the data obtained from myocyte sarcolemma was variable, probably reflecting the action of the hydrolytic enzymes, used during disruption, on this labile enzyme. The lower yield of sarcolemma from myocytes may reflect differences in the methods of homogenisation.

TABLE I

ANGIOTENSIN CONVERTING ENZYME ACTIVITIES IN VARIOUS FRACTIONS OF RABBIT HEART

(a) ENRICHMENT OF ACE IN RABBIT CARDIAC SARCOLEMMA PREPARED FROM WHOLE HEART

Tissue homogenate, washed homogenate and sarcolemma were prepared as described in Materials and Methods. Angiotensin converting enzyme (ACE) activity was determined using [^{3}H]hippurylglycylglycine as substrate; one unit is equivalent to 1% of the substrate hydrolysed/min. Further details are given in Materials and Methods

	ACE (units/mg protein) (mean ± S.E., $n = 5$)
Tissue homogenate	33.8 ± 13.6
Washed homogenate	133.6 ± 6.7
Sarcolemma	1565.0 ± 333.0

(b) ACE ACTIVITIES OF WASHED HOMOGENATES DURING PURIFICATION OF CARDIAC MYOCYTES FROM RABBIT HEART

Rabbit cardiac myocytes were prepared by disrupting hearts by perfusion with collagenase and hyaluronidase. Myocyte washing and purification procedures are described in Materials and Methods. The reduction on angiotensin converting enzyme (ACE) activity may best be assessed by comparing the activity in the washed homogenates presented here, with that of the whole heart washed homogenate in Table Ia. Enzyme disruption produces an approx. 4-fold reduction in ACE activity. ACE activity in washed myocytes is 7-fold lower and sedimentation through sucrose produces myocytes with approx. 19-fold lower ACE activity than whole cardiac tissue.

	ACE (units/mg protein) (mean ± S.E., $n = 6$)
Disrupted heart	32.5 ± 9.3
Washed myocytes	18.5 ± 4.2
Myocytes sedimented through 0.5 M sucrose	7.2 ± 2.7

Is it possible to quantify the degree of endothelial plasmalemma contamination in sarcolemma prepared from whole tissue? Without knowing the angiotensin converting enzyme activity of pure endothelial plasmalemma from the vascular bed of sheep heart it is impossible to do this. However, we can get an estimate of the degree of contamination using two independent, indirect methods. The first of these is based upon an ultrastructural analysis of rat papillary muscle, which gives a ratio of approx. 2.5 : 1 for the surface areas of myocytes to endothelial cells per given quantity of tissue [27]. The endothelial marker, angiotensin converting enzyme, copurifies with $(Na^+ + K^+)$-ATPase, an enzyme which is found on the plasma membrane of both myocytes and capillary endothelial cells [28] (Table II). This implies that the same ratio of endothelial to myocyte surface membrane will be found in the final preparations. We would, therefore, expect that our preparation of whole heart sarcolemma would contain approx. 70% myocyte sarcolemma to 30% endothelial plasmalemma. In support of this figure, we have obtained an upper limit of contamination by preparing plasmalemma from endothelial cells obtained by gently scraping sheep aorta with a razor blade (see Materials and Methods).

This preparation had an absolute angiotensin converting enzyme activity of 250 ± 28 (S.E., n = 4) U/mg protein. Taking this figure as the angiotensin converting enzyme activity of pure endothelial plasmalemma we arrive at a value of 42% for the endothelial plasmalemma content of sarcolemma prepared from whole sheep cardiac tissue. We have not assessed the purity of the endothelial plasma membrane preparation. However, it has been shown that the endothelial cells has an exceptionally large surface area in relation to its intracellular contents [27]. It is therefore likely that contamination of the membrane fraction by intracellular components is lower than that of the whole heart preparation. This would tend, if anything, to lead to an underestimate of endothelial contamination of whole heart sarcolemma. Because of the difficulty of determining the relative purity of the preparations, and the possibility that coronary angiotensin converting enzyme activity differs from aortic, this method of estimating endothelial contamination can only be regarded as approximate.

β-Adrenoceptor binding studies [29] on whole rat heart and rat myocytes provide further evidence to support our estimate of non-myocyte contamination. It was found that 30–35% of β-receptors in a whole heart homogenate were of the β_2-subtype, whereas only β_1-receptors were detected in a myocyte homogenate [29]. The β_2-receptors were not destroyed during the dissociation of the heart into single cells, but were recoverable on non-myocyte material. β_2-Adrenoceptors have been demonstrated on endothelial cells in culture [30]. If we assume that non-myocyte membrane contains no β_1-receptors, then 30–35% of plasma membrane in the whole heart homogenate consists of contaminating non-myocyte material. If non-myocyte membrane has a mixed β-receptor population then contamination may constitute an even greater proportion of the total. It is clear from this

TABLE II

CHARACTERISATION OF SHEEP SARCOLEMMA PREPARED FROM WHOLE HEART OR ISOLATED MYOCYTES

Sarcolemma was prepared from whole sheep heart following the method of Jones et al. [4] (procedure II). Homogenate refers to material taken following the second homogenisation step of this procedure and washed. Sheep cardiac myocytes were prepared and washed in 0.75 M KCl as described in Materials and Methods. Myocytes were then homogenised by sonication. This step was taken as equivalent to the second homogenisation step of the Jones et al. procedure II [4]. Myocyte sarcolemma was prepared and enzyme activities monitored as described in Materials and Methods. All values are mean values ± S.E. (n = 5). Angiotensin converting enzyme (ACE) activity was not detectable in the myocyte homogenate. Myocyte sarcolemma ACE activity is approx. 15-fold lower than that of sarcolemma produced from whole heart. n.d., not detectable.

	Ouabain-inhibitable $(Na^+ + K^+)$-ATPase (μmol P_i/mg protein/h)	ACE units/mg protein	Yield mg sarcolemma protein/g wet wt. muscle or myocytes
(a) Whole heart			
Homogenate	1.90 ± 0.71	11.0 ± 2.5	–
Sarcolemma	15.1 ± 0.8	104.7 ± 12.7	0.071 ± 0.011
(b) Isolated myocyte			
Homogenate	1.39 ± 0.67	n.d.	–
Sarcolemma	12.2 ± 2.4	6.8 ± 4.2	0.024 ± 0.003

study that whole heart homogenate is unsuitable for investigation of myocyte β-receptors.

In conclusion, we have demonstrated that cardiac sarcolemma, prepared by a widely used technique, contains at least one membrane fraction derived from another cardiac cell population. In fact, myocytes make up only 30–35% of the total number of cells in the heart [31] and it is likely that conventional preparations of cardiac sarcolemma will contain surface membrane from smooth muscle cells, fibroblasts etc. in addition to endothelial plasmalemma.

To our knowledge only one other report describes the purification of cardiac sarcolemma from myocytes [32]. Myocytes were prepared from canine heart by a technique requiring cannulation and perfusion of the heart in situ. However, no attempt was made to identify possible contaminating plasmalemma membranes or to separate myocytes from other cells following proteolytic disruption of the heart. As we have demonstrated, this step is required for optimal removal of endothelial plasmalemma from sarcolemma.

The results presented here represent a preliminary investigation into the production of mammalian cardiac sarcolemma free from contamination with plasmalemma membranes of other cardiac cell types. Further studies will involve a more complete characterization of cardiac sarcolemma prepared from isolated myocytes.

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