Evidence for Inward Calcium Current in the Absence of External Sodium in Rat Myocardium

Since the observations of GIEBISCH and WEIDMANN[1] on voltage clamped sheep ventricle fibres, there has been a growing weight of evidence that a delayed inward current contributes to the plateau phase of the cardiac action potential. This current is probably carried by Ca^{++} ions[2,3]. There is also evidence that calcium ions moving in during the plateau contribute directly, or indirectly, to the rise in free intracellular calcium through increasing an internal releasable store. This is thought to be responsible for tension development in the myocardium[4,5].

The evidence for inward calcium current has come largely from studies on trabecular tissue from sheep[1] or frog[6] myocardium. Experiments on other species such as cat[7] and rat[8] have also been reported.

Our interest has been centred on the rat myocardium, particularly rat papillary muscle, which has been used extensively as a model system in interpreting myocardial mechanics[9–11]. This is perhaps unfortunate since it is clear that the rat heart behaves differently to most other mammalian hearts in several respects. Increasing the frequency of stimulation gives a negative inotropic response under most conditions[12]. It is relatively insensitive to cardiac glycosides[13] and has a short action potential with almost no plateau phase[12]. A direct comparative study of the rat and cat myocardium led to the suggestion that the intrinsic control mechanics for contractility in the rat may be quite different[14]. Most of these differences point to the possibility that the rat myocardium behaves as a 'closed system' with respect to calcium. On the other hand the work of LEOTY et al.[15] clearly shows a manganese-sensitive slow inward current in strips of rat myocardium. We have attempted to find further direct evidence for a potential dependent Ca^{++} channel in the myocardial membrane of rat ventricle.

Material and methods. Papillary muscles taken from the left ventricle of rats were equilibrated in Tyrode solution of the following composition Na, 149.2; K, 5.4; Mg, 0.5; H_2PO_4, 0.4; HCO_3 11.9; Cl 146.9 meq/l and glucose 5 g/l, equilibrated with 5% CO_2, 95% O_2. When *tris* solutions were used, all Na was replaced by *tris* or *tris* + Ca and the pH adjusted with HCl, other buffers (HCO_3 and H_2PO_4) were eliminated and the solution gassed with oxygen. The sucrose solution was made by adding 100 g sucrose and 5 g glucose to 1 l distilled water. The chamber contained a single, central sucrose gap 1.5 mm wide which was separated from the 2 end compartments by thin rubber membranes. The muscle was sucked into a thin plastic cannula which was passed through the holes in the two rubber membranes and gently withdrawn to leave the tip of the papillary muscle projecting into the voltage measuring compartment. Current pulses passed through the fibres in the sucrose gap from the base of the muscle could be used to change the potential of fibres in the tip of the papillary muscle. Alternatively, current from a feedback amplifier could be used to control transmembrane potential in the tip of the papillary muscle measured by a microelectrode. The arrangement was similar to that described by GIEBISCH and WEIDMANN[16].

After perfusing the 3 chambers with Tyrode solution for 1 h, one end of the muscle was stimulated and potentials between the end compartments were measured. Action potentials of about 30 mV were reached at this time. The centre compartment was then perfused with sucrose. The *trans* gap potential increased during the next 20 min until the ratio of the *trans* gap action potential to the transmembrane potential recorded by microelectrode reached about 0.95. This corresponds with the average value of 0.94 reported by MCGUIGAN[17] using sheep and calf heart trabeculae. The ratio decreases slowly with time to reach a level of about 0.8 after 2 h. Undoubtedly this is due to the progressive rise in internal longitudinal resistance as reported by KLEBER[18]. This further complicates the interpretation of current voltage relationships in prolonged experiments. The transmembrane voltage change, in response to square current pulses was measured at different intervals along the projecting tip with a microelectrode.

λ was estimated by fitting curves for a sealed end cable[19] to the observed data. Estimates of 0.9 mm for depolarizing pulses and 0.8 mm for hyperpolarizing pulses were obtained. The projecting tip of the papillary muscle must therefore be no more than 0.5 mm in order to obtain spatial voltage control of $\pm 10\%$ when the microelectrode impales the central region of the projecting fibres and when transmembrane resistance (R_m) is uniform and close to that seen in the resting state. When potential dependent changes in R_m occur, current flow distribution will become non-uniform. The range of transmembrane voltage over which ionic channels open will thus be subject to considerable errors in measurement. The method is still however valuable in identifying current channels when different ions are substituted in the external solution.

Results and discussion. Using short muscle lengths (0.4–0.5 mm) current voltage relations were measured in different external solutions. In Tyrode, peak inward current showed an abrupt transient increase to a maximum value with step depolarization from the resting potential (-70 to -80 mV) to about -50 mV (Figure 1). The sharp increase in current is almost certainly a reflection of poor spatial control of potential at the point where sodium conductance starts to increase[20]. A net initial inward current persists up to step depolarizations of about $+20$ mV. At this point, outward current exceeds inward current and estimates of inward current become dependent upon the estimated time course of outward current. The curve shown in Figure 1B is based on the assumption that there is a steady net outward current, independent of

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time, during the 150 msec depolarizing pulse. This probably results in some underestimate of the inward current since there appears to be a small initial transient outward current component after sodium has been washed out (Figure 1A, column 2).

Within 1–2 min of the start of the wash out with sodium-free *tris* solution the initial inward transient disappears. It may be argued that a small delayed inward current remains even after 10 min soaking in Na-free solution since outward current passes through a minimum at about 50 msec. For the sake of completeness, this component was calculated assuming a steady outward current equal to the net current at the end of the pulse. In this experiment a small amount of calcium (0.1 m*M*) was added to the *tris* solution. This helped to maintain stability of membrane potential during the prolonged wash out periods.

Upon replacement of this *tris* solution with a solution containing large amounts of calcium (10–50 m*M*) large inward currents became apparent within 1–2 min and persisted for as long as the experiment continued (30 min). Here again there is evidence of poor spatial control at clamp potentials between −20 mV and zero. The sudden increase in inward current between −20 mV and −10 mV and the small hump in the voltage record are typical indicators of this. While these artefacts are an undesirable feature for precise clamp analysis they nevertheless are a consequence of the rather large calcium dependent inward current. Net inward current was again only maintained for potentials up to about +20 mV. At higher potentials there appeared to be an initial inward current component superimposed on a steady outward current. The inward component persisted even at voltage steps of +80 mV.

The arguments against the inward current component in Ca-*tris* solution being due to sodium, seem considerable. First the inward current disappears within 1–2 min of switching to Na-free *tris* solutions indicating a reasonably rapid sodium wash out. Secondly the time course of the inward current in high Ca is quite different to that in high sodium and thirdly the apparent reversal potential of the inward current in the presence of external Ca is so

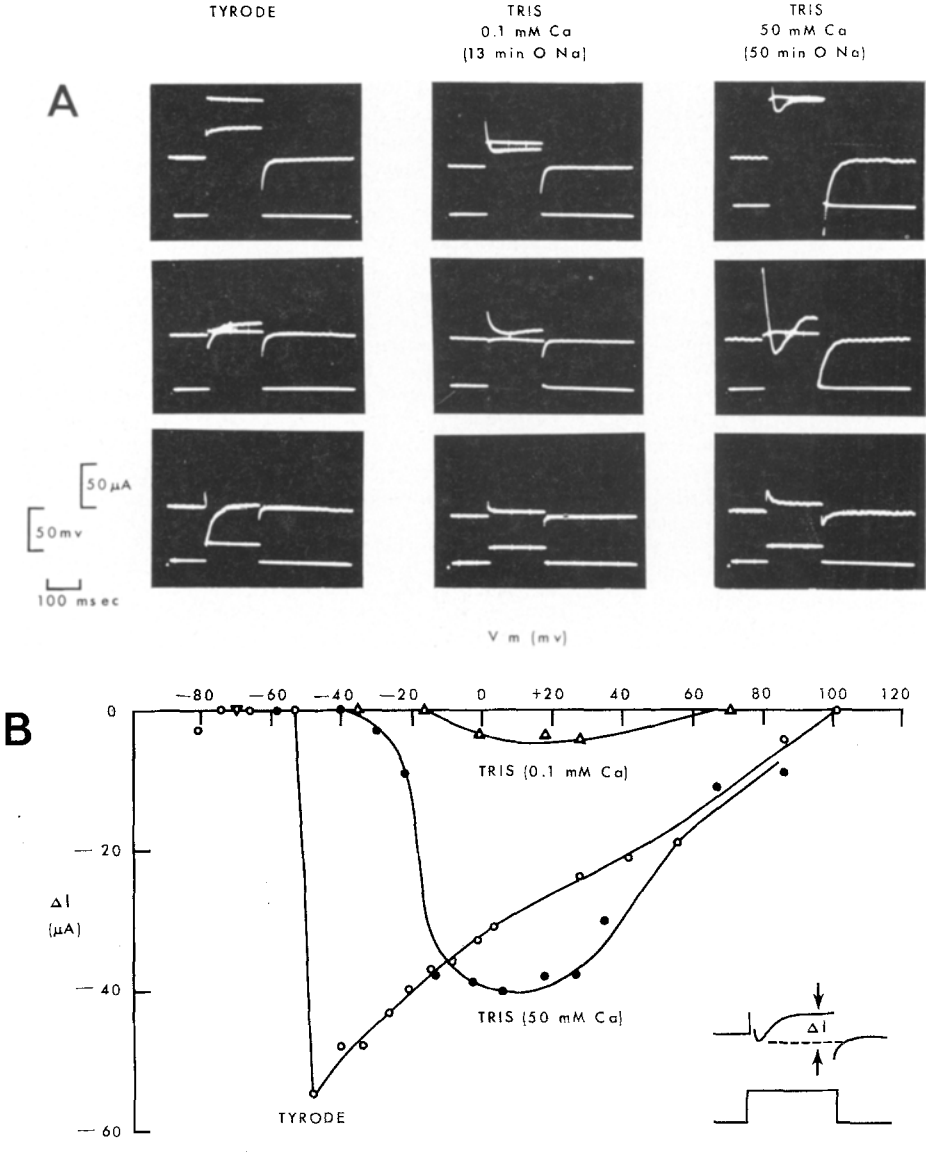

Fig. 1. A) Typical current and voltage records from rat papillary muscle (see text). Lower trace; microelectrode recording of transmembrane potential during step depolarizations. Upper trace; current records. After washing out tyrode solution with *tris*, the initial inward current component disappears (column 2). After 20 min, the 0.1 m*M* Ca-*tris* solution is replaced by 50 m*M* Ca-*tris*. Large inward currents appear within a few minutes and persist for at least 30 min after the change (column 3). B) A plot of inward current (ΔI; see inset) as a function of transmembrane potential in the 3 different solutions, sequence as in A.

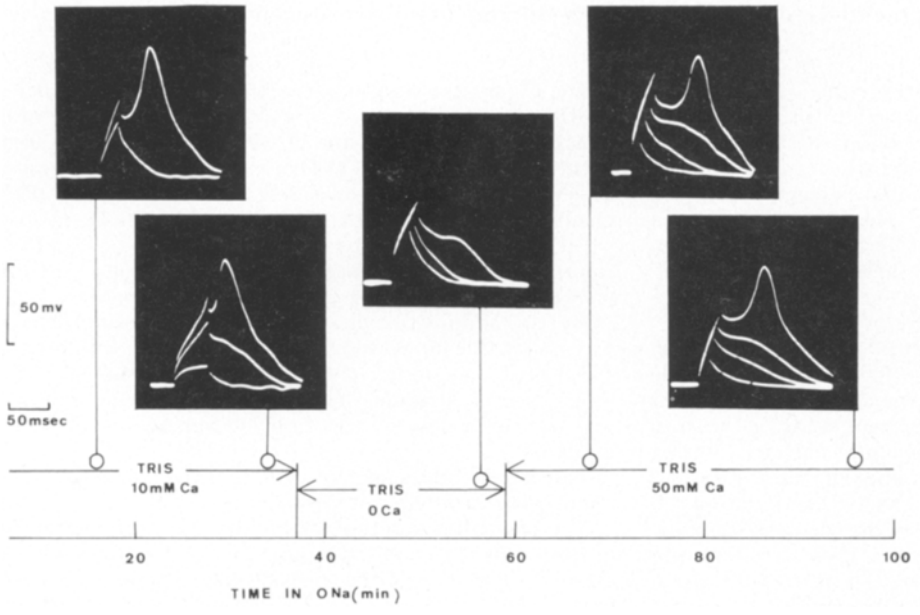

Fig. 2. Recordings of membrane potential responses to depolarizing pulses in rat papillary muscle. At zero time the tyrode solution was replaced by 10 m*M* Ca-*tris* solution. Subsequent changes as indicated.

high that it would exceed the sodium equilibrium potential even if no sodium were washed out of the extracellular space in 50 min.

It follows that the large inward currents seen in the high calcium-*tris* solution should lead to regenerative spikes if large enough depolarization pulses are passed through the preparation. The typical action potential in response to small depolarization pulses (15–20 mV, 2 msec) disappears with the same time course as that required for the disappearance of the transient inward current when Tyrode is replaced with *tris* solutions. If the duration of the depolarizing pulse is increased to about 30 msec and the amplitude of the pulse progressively increased, a potential is reached at which regenerative spikes appear. A typical experiment is shown in Figure 2. At time zero, Tyrode is exchanged for *tris* + 10 m*M* Ca. The spikes are generated by an initial depolarization of about 50 mV and can still be generated after about 30 min in this solution, with a slight decrease in peak amplitude. After washing with *tris* containing no calcium, the regenerative spikes disappear in about 5 min and cannot be obtained with larger depolarizing pulses. Membrane potential begins to decline slowly after about 10 min. Upon readmission of Ca-*tris*, regenerative spikes can again be elicited and have been observed for at least another 40 min.

The calcium dependency of both the inward current in the clamp condition and the regenerative spikes in non-clamped tissue seem to preclude the possibility that the inward current is carried by other ions. The possibility that the inward current is carried by *tris* ions seems unlikely as such a channel would have to be dependent upon external calcium levels.

Two questions remain unanswered. a) Is there still a considerable inward Ca^{++} current in the presence of the normal constituents of Tyrode solution? b) Why does the rat myocardial action potential have such a brief plateau in the presence of an inward calcium current? Our observations on steady state current suggest that the range of anomalous rectification is considerably less in rat myocardium than in other species. The larger outward potassium current may account for the brief action potential even in the presence of inward calcium current.

The experiments reported here provide further support for the existence of a channel in the rat myocardial membrane which carries an inward calcium current. In Ca-*tris* solutions an inward current is generated at depolarization steps above about −20 mV. It remains to be seen how important this current is, as a component of the normal rat myocardial action potential.

Zusammenfassung: An Papillarmuskeln von Rattenherzen ist bei Depolarisation mittels Spannungsklemme ein beträchtlicher Einwärtsstrom messbar, dessen Stärke in Na^+-freier Lösung von der extrazellulären Ca^{2+}-Konzentration abhängt. Die kurze Dauer des Aktionspotentials kleiner Nagetiere wird darauf zurückgeführt, dass die K^+-Konduktivität bei Depolarisation der Zellmembran nicht so stark absinkt wie bei den meisten andern Herzen.

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