# TWO TYPES OF $Ca^{2+}$ CHANNELS IN CARDIAC MYOCYTES FROM SQUALUS ACANTHIAS

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Two types of $Ca^{2+}$ channels have been described in cardiac myocytes, which are characterized based on their threshold of activation and speed of inactivation (e.g., Bean, J. Gen. Physiol., 86:1, 1985; Mitra and Morad, PNAS 83: 5340, 1986). The low threshold cardiac t-channel has been described mostly in only mammalian atrial and ventricular myocytes, and its existence in other species has not been widely investigated. In this report, we have investigated whether t-type channels might exist in ventricular myocytes from the dogfish Squalus acanthias, and present evidence below for the existence of both t- and l-type $Ca^{2+}$ channels in this species.

Single myocytes were isolated enzymatically from shark heart (Squalus acanthius) as described elsewhere (Sorbera et al., this Bulletin) and were studied using the whole cell voltage clamp technique. In TTX-treated (10 uM) myocytes, depolarizing pulses frlom a holding potential of -80 mV, activated first a rapidly inactivating inward current which was maximally activated at -30 mV, which had a voltage dependence and kinetics of activation similar to the t-channel measured in mammalian cardiac myocytes. Depolarization to potentials positive to -20 mV also activated the slower inactivating high threshold $Ca^{2+}$ channel which attained its maximum value at 0 mV.

The two types of inward current showed a differential sensitivity to holding potential as is shown in Fig. 1. When the membrane was held at -80 mV, depolarizing pulses activated both types of inward current (Fig. 1A. However, if a conditioning pre-pulse was applied to -40 mV, only the slowly inactivating current could be activated (Fig. 1B). Subtraction of the currents recorded in Fig. 1A and Fig. 1B yielded a rapidly inactivating inward current alone (Fig. 1C). We further investigated the voltage-dependent inactivation of the rapidly inactivating inward current inward current by varying the duration of the conditioning pulse to -40 mV (Fig. 1D). Substantially all of this inward current was abolished following a 500 ms conditioning pre-pulse, the time constant for this process being 118 ms (Fig. 1E).

Further evidence for the existence of two types of $Ca^{2+}$ channel was obtained from the differential sensitivity of these channels to the inorganic $Ca^{2+}$ channel blockers $Cd^{2+}$ and $Ni^{2+}$. 100 uM of $Cd^{2+}$ blocked all the slowly inactivating inward current, leaving a rapidly inactivating $Ca^{2+}$ current whose voltage range was identical to the difference current shown in Fig. 1C (Fig. 2A). On the other hand, 500 uM of $Ni^{2+}$ preferentially blocked the rapidly inactivating component.

These data are consistent with the existence in shark heart of both l- and t-type $Ca^{2+}$ channels. The striking finding, however, is the relative size of the t-current compared to the l-current. As can be seen from Fig. 1, the peak currents are approximately equal. This is in stark contrast to the data obtained in mammalian preparations where the t-channel represents at most 30% of the total peak calcium current (Mitra and Morad). This suggests that the t-channel may play a more important role in the shark myocyte than in the mammal.

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