Calcium channel modulation by β-adrenergic neurotransmitters in the heart

by H. Reuter

Department of Pharmacology, University of Bern, Friedbühlstrasse 49, CH-3010 Bern (Switzerland)

Summary. Calcium ions play a crucial role in the regulation of the heart beat. During each action potential Ca^{2+} ions flow into the cell and are directly and indirectly involved in generation of pacemaker potentials and of contractile force. Adrenergic and cholinergic neurotransmitters modulate Ca^{2+} influx. The most detailed analysis has been made on the mechanism of the β-adrenergic effect on calcium channels in cardiac cell membranes. This is briefly summarized in a personal account, while for more detailed information the reader is referred to more extensive recent reviews[16,22].

Key words. Calcium channels; catecholamines; β-adrenoceptors; cyclic AMP.

In April of 1964 I came to the Department of Physiology (Hallerianum) in Bern to work in Silvio Weidmann's laboratory on electrophysiological effects of adrenaline on cardiac Purkinje fibers. My interest in this topic arose from two experimental observations:

1) Electrophysiological studies in various heart tissues had provided evidence for a) an elevation of the plateau height of cardiac action potentials[11,21] and b) an increase in the steepness of diastolic pacemaker potentials[11,21]. These effects had been explained by an increase in the sodium permeability (P_{Na}) by the neurotransmitter[24].

2) Before I came to Weidmann's laboratory I had worked on ^{45}Ca fluxes in isolated guinea pig atria and had found that ^{45}Ca uptake is greatly enhanced by adrenaline (fig. 1)[12]. This suggested that catecholamines increase the calcium permeability (P_{Ca}) of cardiac cells.

To distinguish between these possibilities (i.e. a change in P_{Na} or in P_{Ca}), I superfused calf Purkinje fibers with Na-free, but Ca-containing solution and measured electrotonic responses of the cells during application of constant current pulses. Adrenaline greatly enhanced calcium-dependent regenerative electrotonic potentials in Purkinje fibers (fig. 2), which was consistent with an increase in P_{Ca}[13].

Voltage clamp experiments confirmed that β-adrenergic catecholamines greatly increase an inward current carried by calcium ions (I_{Ca}) in various cardiac preparations (fig. 3)[14,15,23]. This accounts for the elevation of the plateau height of the action potentials and is also involved in acceleration of pacemaker activity[9,14,23]. Elaborate analysis of the effect of catecholamines on I_{Ca} showed that the kinetics of this current component were not much affected by these drugs and we concluded that the increase in the calcium conductance of the membrane was due to an increase in the availability of functional Ca channels[17]. This hypothesis could be tested after introduction of the patch clamp method[6] which allowed the resolution of currents flowing through individual Ca channels.

In cardiac cells two types of Ca channels have so far been identified: T-type and L-type channels[8]. With isotonic Ba^{2+} ions as charge carriers the T-type channel has a conductance of about 8 pS[8,19] and the L-type channel of about 25 pS[8,19,20].

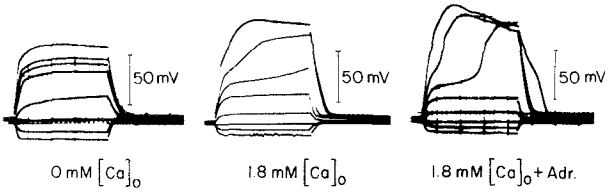

Figure 2. Electrotonic potential changes elicited by injection of constant current pulses (800 ms) into short (~ 1 mm) calf Purkinje fibers superfused with Na-free (choline Cl) bathing solution. Note regenerative responses in the presence of extracellular Ca ($[Ca]_o$ = 1.8 mM) plus adrenaline (1 μM/l). Such an effect cannot be observed in the absence of $[Ca]_o$ (from Reuter[13]).

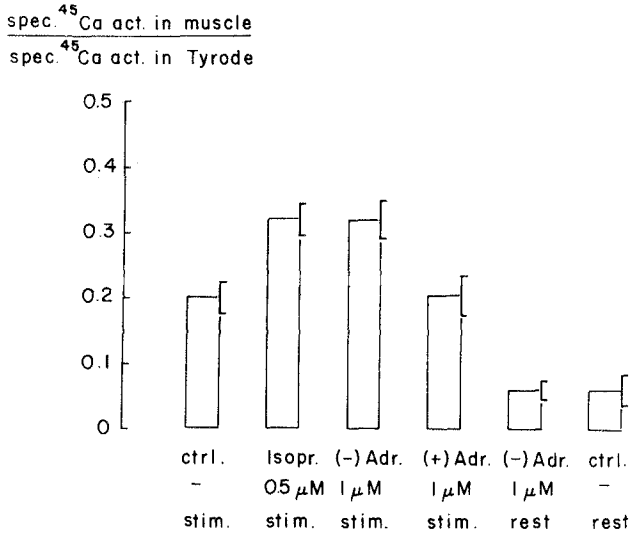

Figure 1. Effects of isoproterenol and of adrenaline stereoisomers on ^{45}Ca uptake (relative specific ^{45}Ca activity in muscle after 5 min incubation periods) in stimulated (stim.) and unstimulated (rest) guinea pig auricles (modified from Reuter & Wollert[18]).

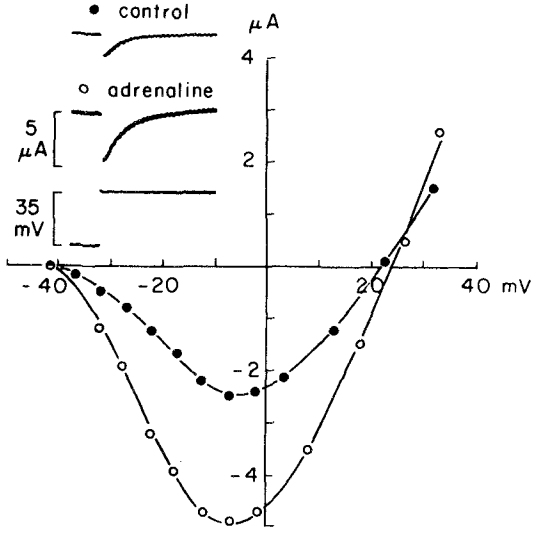

Figure 3. Current-voltage relationships of I_{Ca} measured under voltage-clamp conditions from a cow ventricular trabecula in the absence (closed circles) and presence (open circles) of adrenaline (0.5 μM/l; Tyrode solution with 1.8 mM/l $CaCl_2$). Original current traces are shown as inset and correspond to a membrane potential of −7 mV; clamp duration 250 ms. The holding potential was set at −42 mV, in order to inactivate I_{Na}.

The T-type channel activates and inactivates over more negative potential ranges than the L-type channel. T-type Ca channels are responsible for a rapidly inactivating, transient Ca current component[8, 19], while openings of L-type channels produce slowly inactivating, long-lasting Ca currents[8, 19, 20]. Gating of both channels is steeply voltage-dependent.

L-type Ca channels in cardiac cell membranes were the first potential-dependent ion channels that were shown to be modulated by neurotransmitters. As mentioned above, modulation of these channels by β-adrenoceptor agonists causes an increase in I_{Ca} in intact cardiac preparations[14, 15, 23]. This modulation occurs through a cascade of events, finally leading to a cyclic AMP-dependent phosphorylation of Ca channels[16]. This has been shown most convincingly by Trautwein's group[2, 7, 10]. They have injected the catalytic subunit of cyclic AMP-dependent protein kinase into single cardiac cells and found effects on I_{Ca} identical to those seen with isoproterenol. In purified and reconstituted Ca channels from skeletal muscle they could show effects of cyclic AMP-dependent protein kinase similar, if not identical, to those seen with isoproterenol or 8-Br-cyclic AMP on Ca channels in intact cells[5].

What is the effect of cyclic AMP-dependent phosphorylation on individual Ca channels that finally leads to an increase in I_{Ca} in the heart? The macroscopic Ca current can be expressed as $I_{Ca} = N \cdot p_o \cdot i$, where N is the number of functional Ca channels, p_o is the opening probability of these channels, and i is the current that flows through the channel when it is open. Several groups have analyzed which of these factors, leading to an increase in I_{Ca}, are affected by catecholamines or cyclic AMP derivatives[1, 3, 4]. From current-voltage relationships in the absence and presence of these drugs it is clear that the single channel current, i, is not affected by these drugs. However, during repetitive depolarization a single channel does not always open. Catecholamines and cyclic AMP reduce these failures of the channel to open and increase the opening probability during single depolarizing sweeps. It is this effect on the overall open state probability, p_o, that is responsible for the increase in I_{Ca} (fig. 4)[1, 3, 4]. There is no direct evidence for recruitment of new channels, but individual channels are more easily available to open during depolarization. With thousands of Ca channels in a cell this leads to an enhanced Ca current because more channels will have a chance to be open at the same time.

Future work will have to be directed towards the following questions: What is the structre of a Ca channel? How is voltage-dependent gating of a channel related to its structure? How does phosphorylation affect gating? There is a good chance that these questions can be answered in the not too distant future.

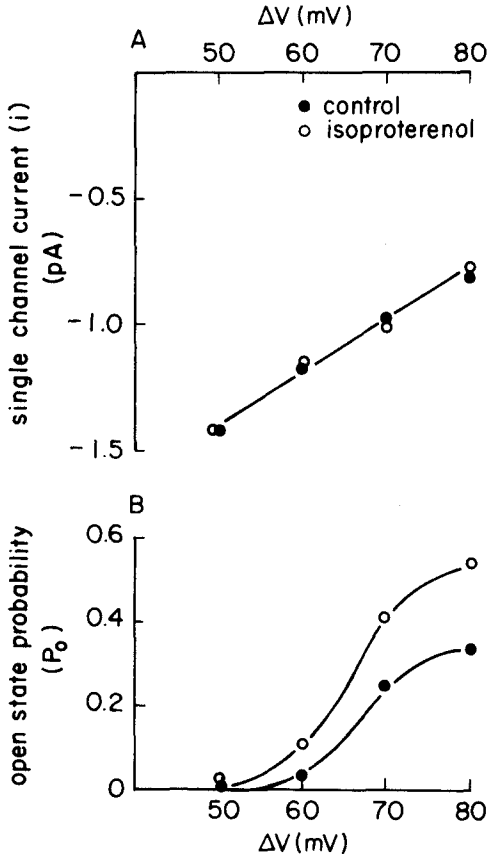

Figure 5. Voltage dependencies of open Ca channel current (above) and of overall open state probability (below) in the absence (●) and presence (○) of isoproterenol (0.1 μM/l). Each data point is the average of 64 single channel current traces. The overall open state probability includes the number of failures of the channel to open during a series of depolarizing clamp steps (nulls). The number of nulls decreases in the presence of isoproterenol.

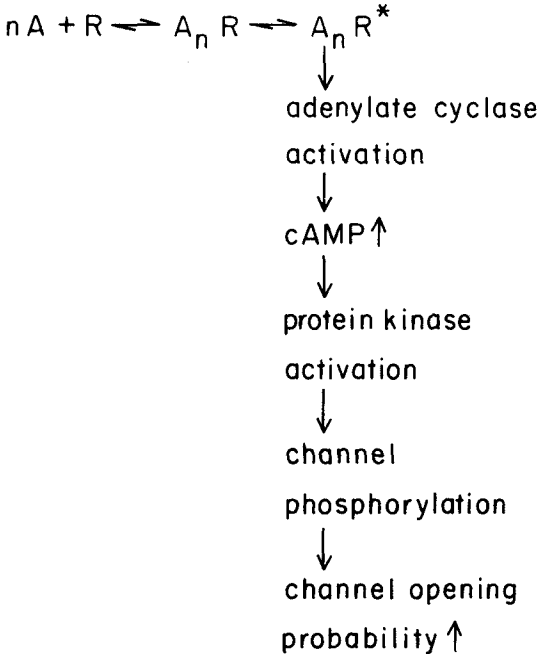

Figure 4. Ca channel modulation by β-adrenoceptor agonists. A = agonist; R = receptor; n = number of agonist molecules reacting with R; A_nR^* = 'activated' agonist-receptor complex. After binding of the agonist to the β-receptor, the adenylate cyclase is activated via the guanine nucleotide-regulated G_s-protein. This results in an increase in cyclic AMP which binds to the regulatory subunit of a cyclic AMP-dependent protein kinase, thereby releasing the catalytic subunit of the enzyme. As a result, phosphorylation of the ion channel, or of a protein closely associated with the channel, occurs. This causes a conformation of the channel where it is more easily available to open upon membrane depolarization (modified from Reuter[16]).

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Potassium currents in cardiac cells

by E. Carmeliet, G. Biermans, G. Callewaert and J. Vereecke

Laboratory of Physiology, University of Leuven, Gasthuisberg, Herestraat, B-3000 Leuven (Belgium)

Summary. The kinetic properties of the inwardly rectifying K current and the transient outward current in cardiac cells were investigated.
In sheep Purkinje fibers superfused with Na-free K-free solution, time-dependent changes in the conductance of the inward rectifier are described. In patch clamp experiments the inward rectifier inactivates during hyperpolarization, as can be seen by a decrease in the open state probability. Using whole cell clamp on ventricular myocytes it is demonstrated that the inactivation during hyperpolarization is due to blocking of the channel by external Na, Mg and Ca.
The channels responsible for the transient outward current in cow, sheep and rabbit Purkinje fibers are identified using single channel recording. It is demonstrated that in all three preparations the channels are K-selective. The channel in cow Purkinje cells has a large conductance and is regulated by voltage and internal Ca concentration. The channels identified in the sheep and rabbit cells have a much smaller conductance.
Key words. Heart; membrane; electrophysiology; K current; voltage clamp; single channel.

Introduction

A number of different K currents have been described in cardiac cells:
Cardiac cells at rest are mainly K-selective, so that the membrane potential is largely governed by the K equilibrium potential. The channel responsible for the K-selectivity of the membrane was first described as a purely voltage-dependent channel which passes inward current more easily than outward current[19]. It has been called the inwardly rectifying K current (i_{K1}). This current is prominent in ventricular cells. It is present to a lesser extent in atrial cells, and its amplitude is very small in the sino-atrial node.
A transient outward current (i_{to}) has been found in sheep[27] and cow Purkinje fibers[31], and in rat ventricular cells[12]. This current causes a fast repolarization to the plateau level in Purkinje cells, and contributes to the final repolarization in the rat ventricular cells.
Another current, the delayed outward current, affects the duration of the plateau. It was first described in Purkinje fibers and called i_{x1}[22], later it was renamed i_x. In ventricular cells a delayed outward current has been described, and identified as carried by K; therefore it was named i_K[20]. The same terminology is now also often applied for the delayed outward current in Purkinje cells, since deviation of its reversal potential from the K equilibrium potential appears to be related to K accumulation in the narrow clefts between the cells.