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PROPERTIES AND MODULATION OF CARDIAC CALCIUM CHANNELS

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SUMMARY

Voltage-dependent calcium channels are widely distributed in excitable membranes and are involved in the regulation of many cellular functions. These channels can be modulated by neurotransmitters and drugs. There is one particular type of calcium channel in cardiac cells (L-type) whose gating is affected in different ways by β-adrenoceptor and 1,4-dihydropyridine agonists. We have analysed single calcium channel currents (i) in myocytes from rat hearts in the absence and presence of isoproterenol or 8-bromo-cAMP. We have found that both compounds have similar effects on calcium channel properties. They increase the overall open state probability (p_o) of individual calcium channels while i remains unaffected. Analysis of the gating kinetics of calcium channels showed: (a) a slight increase in the mean open times of calcium channels, (b) a reduction in time intervals between bursts of channel openings, (c) an increase in burst length and (d) a prominent reduction in failures of calcium channels to open upon depolarization. These kinetic changes caused by isoproterenol and 8-bromo-cAMP can account for the increase in p_o. Since the macroscopic calcium current, I_{Ca}, can be described by $I_{Ca} = N \times p_o \times i$, the increase in p_o accounts for the well-known increase in I_{Ca} by β-adrenergic catecholamines. Cyclic AMP-dependent phosphorylation of calcium channels is a likely metabolic step involved in this modulation. Another class of drug that modulates calcium channel gating is the 1,4-dihydropyridines which can either enhance or reduce I_{Ca}, either by prolonging the open state of the channels or by facilitating the inactivated state. Both effects depend strongly on membrane potential and are independent of cyclic AMP-dependent phosphorylation reactions.

INTRODUCTION

Single-channel recordings by means of the patch-clamp technique provide a method for observing the kinetic properties of individual ion channels in cell membranes (Sakmann & Neher, 1983). Three distinct types of voltage-dependent Ca^{2+} channels have so far been characterized in muscle cells and neuronal tissues: T-type channels are responsible for a rapidly inactivating, transient Ca^{2+} current, while L-type channel openings produce a slowly inactivating, long-lasting Ca^{2+}

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current (Carbone & Lux, 1984; Nilius, Hess, Lansman & Tsien, 1985; Armstrong & Matteson, 1985; Bean, 1985; Fedulova, Kostyuk & Veselovsky, 1985). N-type channels have the kinetic features of neither T- nor L-type channels (Nowycky, Fox & Tsien, 1985). In addition to differences in their gating and conductance properties, the three types of channels can also be distinguished by their different sensitivities to pharmacological interventions (Bean, 1985; Nilius *et al.* 1985; Nowycky *et al.* 1985). The respective distributions of the various types of Ca^{2+} channels seem to vary from tissue to tissue. For example, more L-type Ca^{2+} channels may open during excitation in cardiac and smooth muscle cells than in nerve cells, where in turn the other channel types may prevail. In view of their functional significance, it will be of considerable interest to explore in greater detail the respective densities and sensitivities to neurotransmitters and drugs of various Ca^{2+} channels in different tissues. In this review we summarize briefly the basic properties of cardiac Ca^{2+} channels and the modulatory effects of catecholamines and 1,4-dihydropyridines on these channels.

PROPERTIES OF T- AND L-TYPE CALCIUM CHANNELS

The existence of transient and maintained Ca^{2+} currents has been shown in several cell types. However, direct comparisons of single-channel measurements with whole-cell recordings have only recently been done in a few cases (Carbone & Lux, 1984; Nilius *et al.* 1985; Nowycky *et al.* 1985). Fig. 1A–D shows examples of such recordings, while in Fig. 1E the current/voltage characteristics of T- and L-type Ca^{2+} channels have been plotted. The data in Fig. 1C–E have been obtained from the same cardiac cell. With Ba ions as charge carriers the single-channel conductance of T-type Ca^{2+} channels is only approximately one-third that of L-type channels. T-type Ca^{2+} channels pass Ba^{2+} and Ca^{2+} ions about equally well, while L-type channels have a higher permeability for Ba^{2+} ions (Bean, 1985). Correspondingly, with Ca^{2+} ions as charge carriers, T-channels may have a higher conductance than L-channels (Carbone & Lux, 1984), while the opposite holds true with Ba^{2+} ions as charge carriers (Fig. 1E) (Nilius *et al.* 1985). Moreover, T-type channels can be activated by smaller depolarizations (to about -60 mV) than L-type channels (to about -40 mV) (Fig. 1E). This agrees with Ca^{2+} current measurements in whole-cell recordings (e.g. Bean, 1985). T-type Ca^{2+} channels require a rather negative holding potential, V_H, for their activation by depolarizing voltage steps. They are inactivated at V_H positive to -40 mV. L-type channels inactivate much more slowly and over a more positive voltage range (Reuter, Stevens, Tsien & Yellen, 1982; Cavalié, Ochi, Pelzer & Trautwein, 1983). Openings of T-type Ca^{2+} channels occur primarily at the beginning of a voltage step (Fig. 1D) and thus produce the transient appearance of this current component. In contrast to L-type Ca^{2+} channels, T-type Ca^{2+} channel activity persists in excised membrane patches (Carbone & Lux, 1984; Nilius *et al.* 1985). Both channel types have rather different sensitivities to pharmacological agents. β-Adrenoceptor agonists and 1,4-dihydropyridines seem to have an effect primarily on L-type channels (Bean, 1985; Mitra & Morad, 1985; Nilius *et al.* 1985).

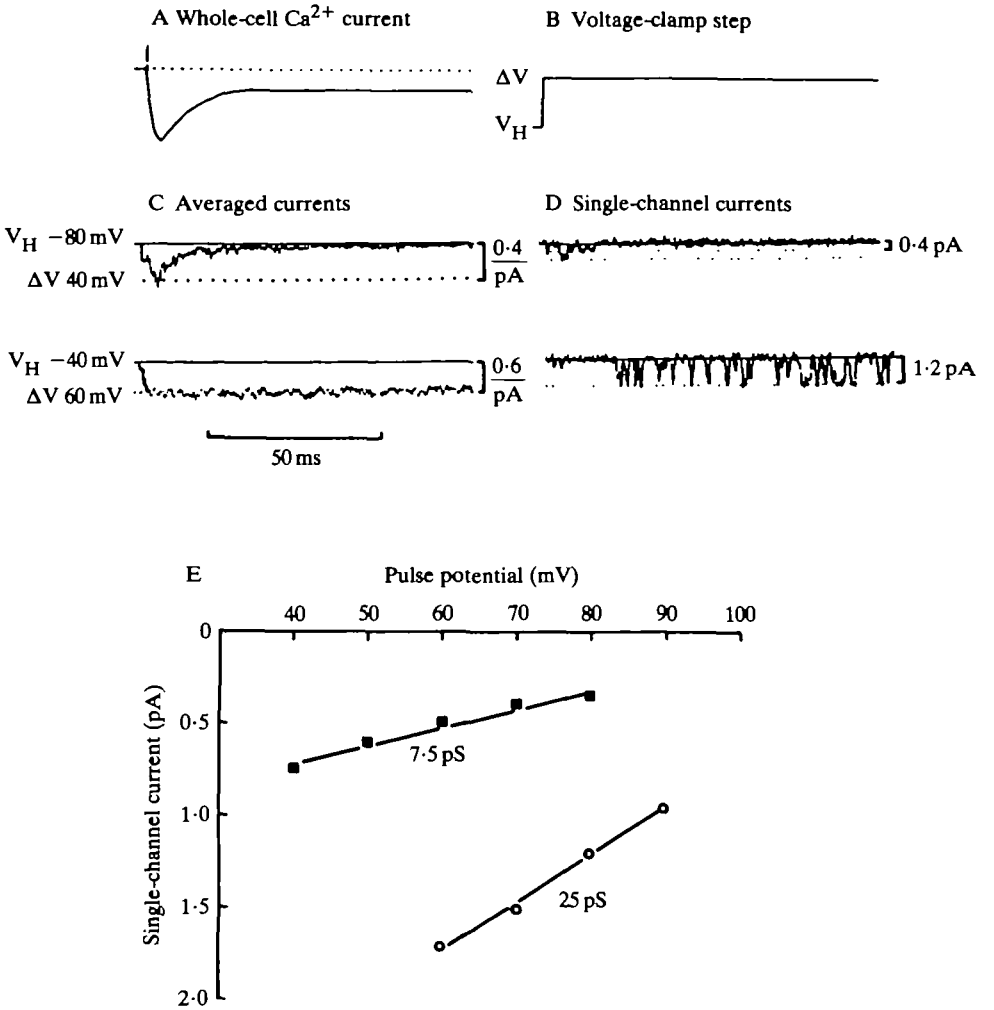

Fig. 1. (A) Whole-cell recording of Ca^{2+} current in a single cardiac cell. (B) Voltage-clamp step (ΔV) from a holding potential (V_H) is required for activation of Ca^{2+} channel activity. (C) Average currents with Ba^{2+} as charge carrier obtained from summation of about 50 single-channel records like that shown in D. Upper records in C and D show transient average current and single T-type Ca^{2+} channel activity, respectively; lower records show long-lasting average current corresponding to L-type Ca^{2+} channel activity. (E) Current/voltage relationships of T-type (■) and L-type (○) Ca^{2+} channels. Ba^{2+} currents flowing through both types of channels were measured in the same single cardiac cell. Abscissa, voltage-clamp pulse potential from a holding potential of about −80 mV; ordinate, amplitudes of single-channel currents.

The L-type Ca^{2+} channel is the most common and the one that has been best explored so far. It seems to be present in many cell types including cardiac cells. With isotonic $BaCl_2$ solution it has a conductance between 15 and 25 pS (see Fig. 1E). This type of voltage-dependent Ca^{2+} channel has distinct modes of gating (Reuter *et al.* 1982; Cavalié *et al.* 1983; Hess, Lansman & Tsien, 1984). Elementary currents flowing through a single Ca^{2+} channel which have been recorded from a

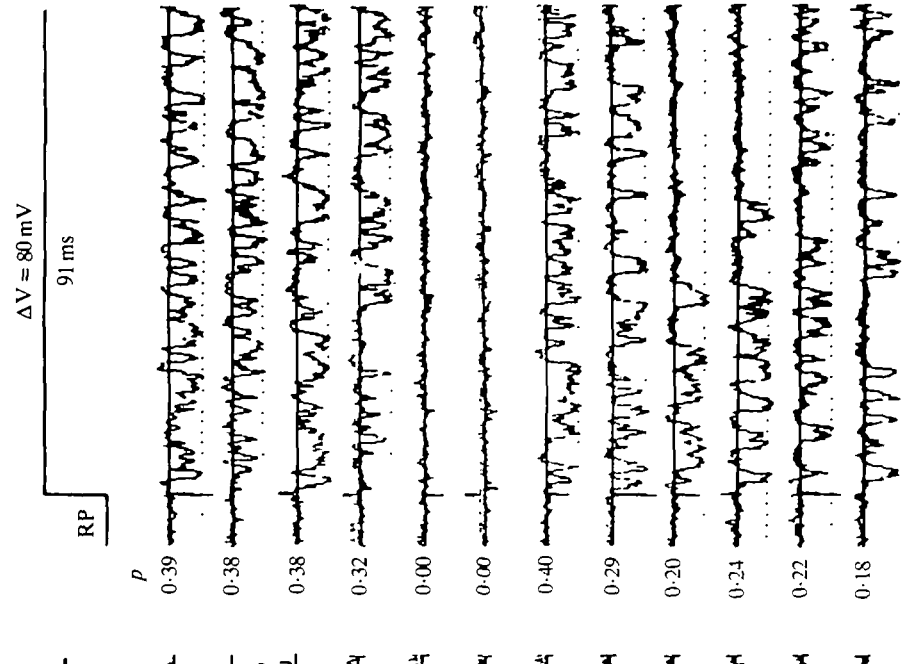

Fig. 2. Elementary Ca^{2+} channel currents recorded from a cell-attached membrane patch in a cultured cardiac cell. The pipette was filled with 96 mmol l^{-1} $BaCl_2$; thus the current carrier was Ba^{2+}. Depolarizing clamp steps (ΔV) of 70 mV (left) or 80 mV (right) were applied from a holding potential identical to the resting potential (RP; approx. −70 mV). Pulse duration was 91 ms. Twelve consecutive single-channel current records are shown for each pulse potential. The number on the left-hand side of each current record indicates the open state probability, p, of the channel during that pulse. The heavy lines indicate the closed state of the channel, the dotted lines the open state current.

cultured neonatal rat heart cell are shown in Fig. 2. The charge carriers were Ba^{2+} ions. Depolarizing step pulses of 70 or 80 mV amplitude were applied from a holding potential identical to the resting potential (approx. −70 mV). The probability, p, of the channel being in the open state during each trace is indicated on the left-hand side of each record. With $p = 1{\cdot}0$ the channel would be open throughout the voltage step, while with $p = 0$ it would remain shut. Ca^{2+} channel openings often occur in bursts separated by longer intervals of closures (mode 1; Hess *et al.* 1984). Quite rarely very long openings can also be observed (mode 2; Hess *et al.* 1984; see second and third current traces with $\Delta V = 70$ mV in Fig. 2). Sometimes the channel does not open at all during repetitive depolarizations (mode 0; Hess *et al.* 1984; see current traces with $p = 0{\cdot}00$ in Fig. 2). The probability of the channel entering an open state increases with increasing depolarization. Open times of Ca^{2+} channels during bursts (mode 1) are exponentially distributed with time constants (τ_o) that are only slightly voltage-dependent (Fig. 3). However, it should be noted that the rare very long openings (mode 2) last longer than would be expected from the exponential fits. Closed time histograms of Ca^{2+} channels can be fitted best by double exponentials (Fig. 3). The short time constant, τ_{C1}, of this double exponential curve corresponds to brief closures during the bursts of openings, while the longer time constant, τ_{C2}, describes the distribution of closed time intervals between the bursts. While τ_{C1} does not seem to be very voltage-dependent, τ_{C2} decreases greatly with increasing depolarizing step pulses. Failures of the channel to open (nulls; mode 0) are often grouped (Hess *et al.* 1984). Similar results have been obtained in other cell types (Fenwick, Marty & Neher, 1982; Hagiwara & Ohmori, 1983; Lux & Brown, 1984).

MODULATION OF L-TYPE CALCIUM CHANNELS

Catecholamines

L-type Ca^{2+} channels in cardiac cell membranes were the first potential-dependent ion channels that were shown to be modulated in their activity by neurotransmitters (see Reuter, 1983). Modulation of Ca^{2+} channels by β-adrenoceptor agonists causes an increase in Ca^{2+} current in intact cardiac preparations (Reuter, 1974). It occurs through a cascade of events (Reuter, 1979, 1983) finally leading to a cyclic AMP-dependent phosphorylation of one of the subunits of the channel protein (Curtis & Catterall, 1985). Fig. 4 shows membrane currents obtained from averaging many single-channel current traces, such as those illustrated in Fig. 2, in the absence and presence of isoproterenol ($0{\cdot}5\ \mu mol\ l^{-1}$), respectively. Isoproterenol approximately doubles the average currents. Assuming that the individual Ca^{2+} channels involved in this effect have identical properties and open and close independently, the average Ca^{2+} channel current, I_{Ca}, can be described as $I_{Ca} = N \times p_o \times i$, where N is the number of Ca^{2+} channels in the patch, p_o is the overall opening probability of these channels, and i is the current that flows through individual channels when they are open.

Several groups have analysed which of these factors leading to an increase in I_{Ca} are affected by catecholamines (Reuter, Cachelin, de Peyer & Kokubun, 1983; Bean,

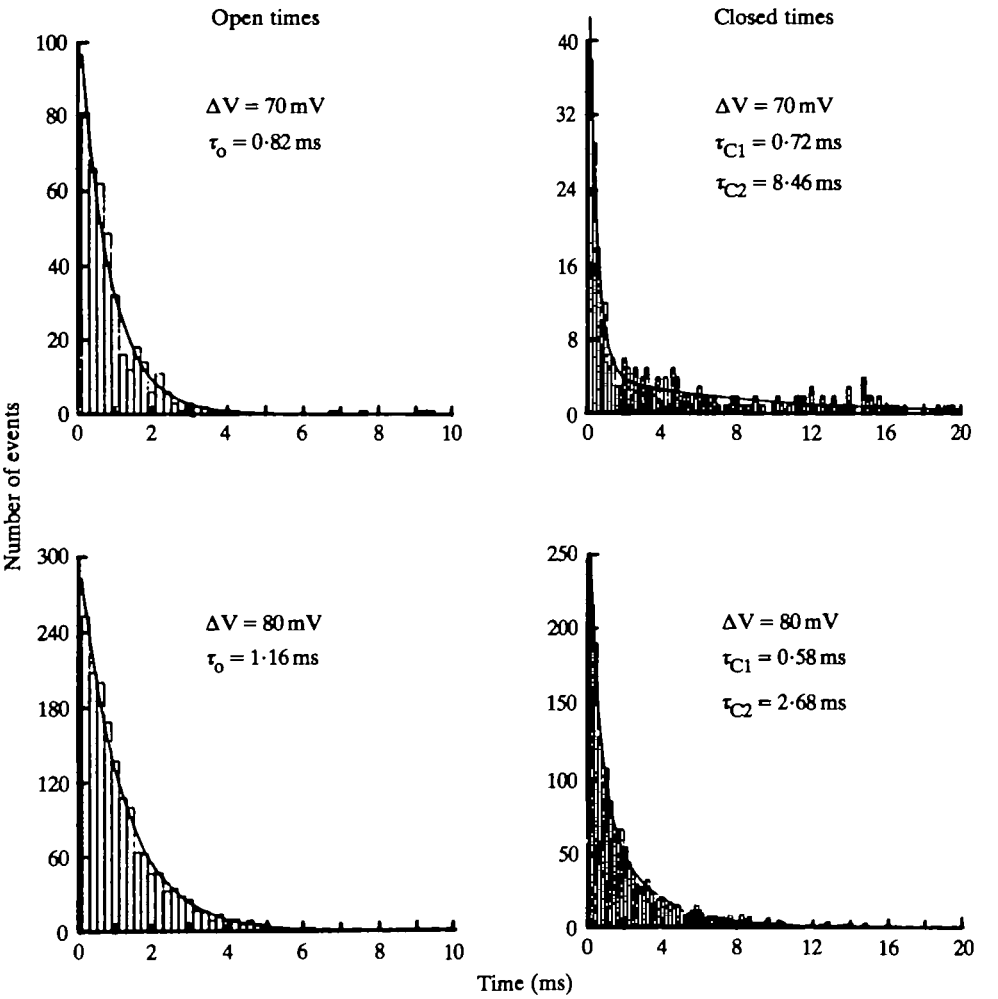

Fig. 3. Open-time histograms (left) and closed-time histograms (right) of Ca^{2+} channel openings and closings at two different membrane potentials (same experiment as in Fig. 2). Histograms were fitted by non-linear regression analysis with single exponentials (open-times) and the sum of two exponentials (closed-times). The respective time constants (τ) of the exponentials are indicated.

Nowycky & Tsien, 1984; Brum, Osterrieder & Trautwein, 1984) or 8-bromo-cAMP (Cachelin, de Peyer, Kokubun & Reuter, 1983; Reuter *et al.* 1983). Fig. 5 shows the principal results. From the current/voltage relationships, in the absence and presence of isoproterenol (Fig. 5A), it is clear that the unitary channel current, i, is not affected by the catecholamine. However, averaging the open state probability of many single-channel traces at each pulse potential shows a clear increase in p_o by the drug. This accounts for the increase in the average current in Fig. 4. The effect of β-adrenergic catecholamines on p_o is bigger if the opening probability of a channel is low in the absence of the drug. Very similar effects on single Ca^{2+} channels have been obtained with 8-bromo-cAMP (Cachelin *et al.* 1983). Opening and closing kinetics

(gating) of Ca^{2+} channels are affected by catecholamines in a characteristic way: (a) there is a slight increase in the mean open times (increase in τ_o), (b) time intervals between bursts of opening are reduced (decrease in τ_{C2}), (c) burst lengths are prolonged, and (d) the number of failures of the channels to open upon depolarization is considerably reduced. All these changes together account for the increase in p_o (Cachelin *et al.* 1983; Reuter *et al.* 1983; Brum *et al.* 1984). The main argument, that the opening probability rather than the number, N, of available Ca^{2+} channels is affected, comes from two observations. (1) Our group and Brum *et al.* (1984) have never been able to observe Ca^{2+} channel activity in the presence of the drug in patches where no channel openings were seen in the controls. This argues against the recruitment of previously silent channels in the patch. (2) If several channels were present in a patch, the superposition of opening levels of these channels was not larger than expected from an increase in p_o. However, as Tsien *et al.* (1986) pointed out, a distinction between channel availability and overall opening probability is to some extent a matter of definition of N and p_o. Failures of channels to open (mode 0) can be interpreted either as channels not being in a functional state, or as channels having a very low overall opening probability.

What is the mechanism by which p_o is increased by the catecholamines? In some elegant experiments Osterrieder *et al.* (1982) and Kameyama, Hofmann & Trautwein (1985) 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 application or cyclic AMP injection. This strongly supports

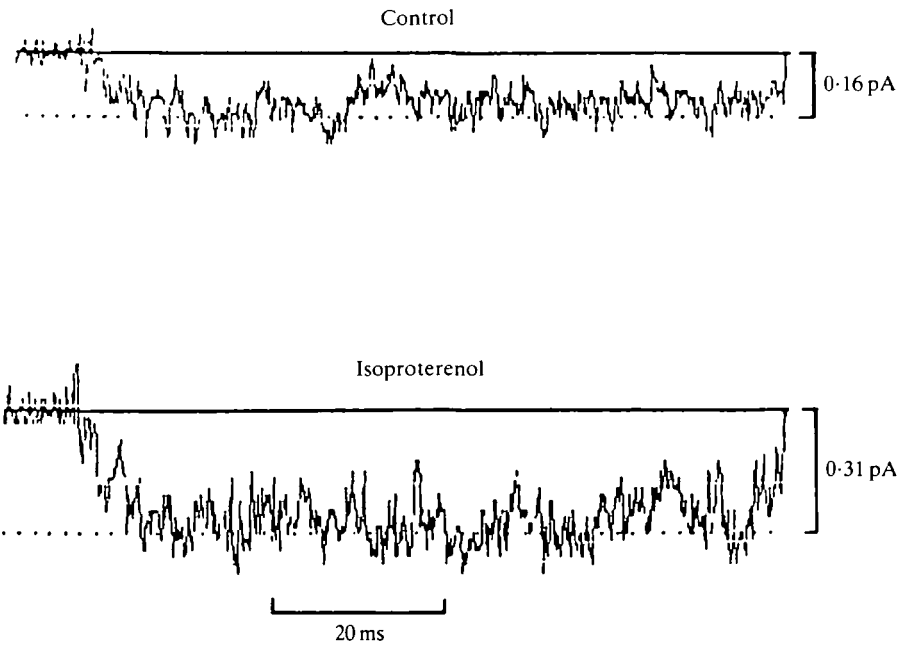

Fig. 4. Ensemble average currents obtained from 64 single-channel currents in the absence (above) and presence (below) of isoproterenol (0·5 μmol l^{-1}).

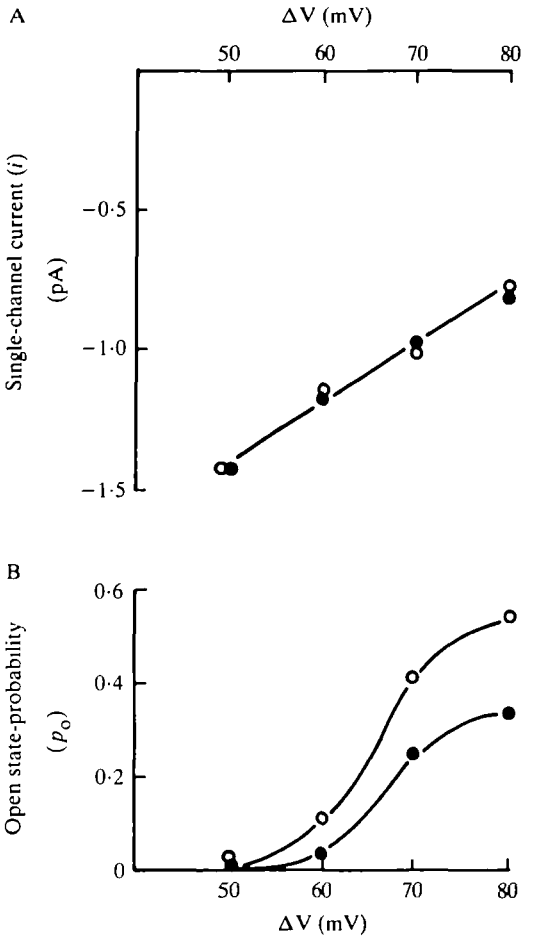

Fig. 5. Voltage dependencies of open Ca^{2+} channel current (A) and of overall open-state probability (B) in the absence (●) and presence (○) of isoproterenol. Each data point is the average of 64 single-channel current traces.

the hypothesis that openings of Ca^{2+} channels are facilitated if the channels are phosphorylated.

Dihydropyridines

Another group of drugs that modulates the voltage-dependent gating of L-type Ca^{2+} channels is the 1,4-dihydropyridines. Dihydropyridines are highly lipid-soluble compounds which seem to have access to individual Ca^{2+} channels through the lipid phase of the membrane (Kokubun & Reuter, 1984). Chemically closely related dihydropyridine derivatives can have opposite effects on Ca^{2+} channels. One group, which also has therapeutic applicability, reduces Ca^{2+} currents (antagonists), while the other group increases I_{Ca} (agonists). Even two enantiomers of the same compound can have opposite effects on Ca^{2+} currents (Williams *et al.* 1985; Reuter, Porzig, Kokubun & Prod'hom, 1985*a*). The blocking effects of dihydropyridine

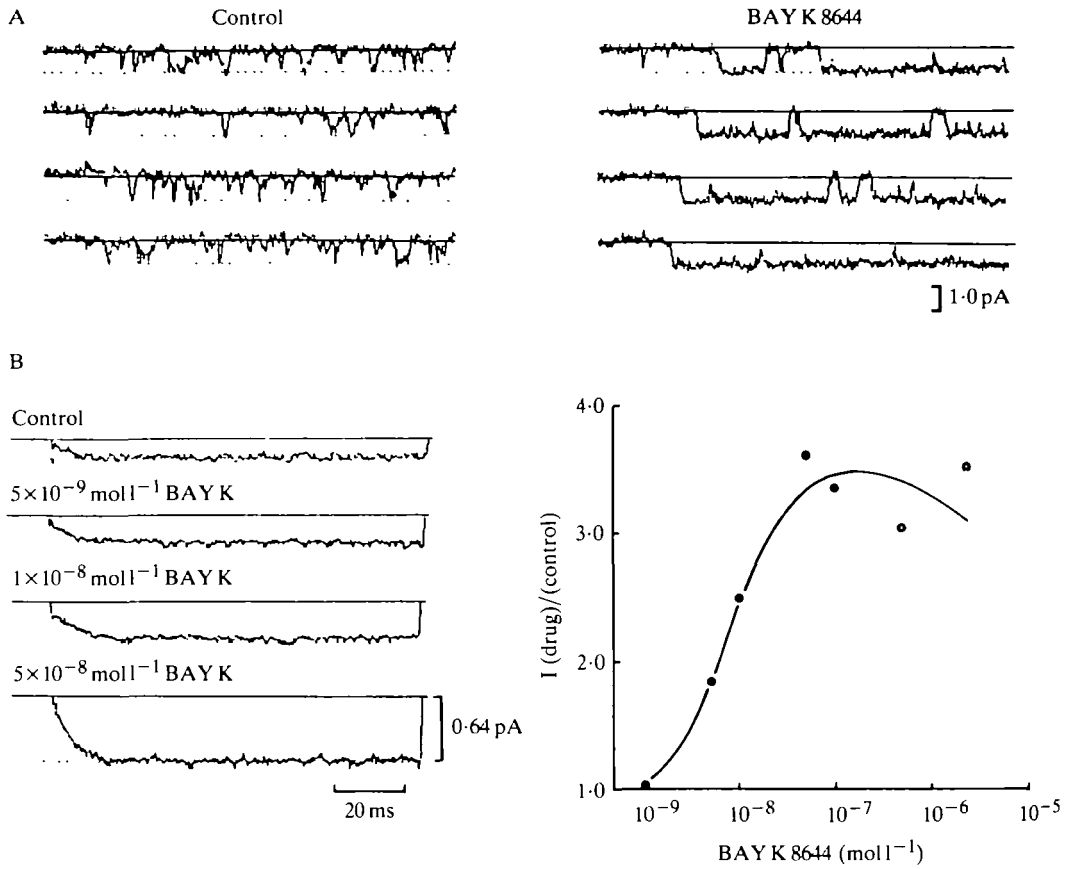

Fig. 6. (A) Four consecutive single Ca^{2+} channel currents in the absence (left) and presence (right) of the agonistic 1,4-dihydropyridine derivative BAY K 8644. Note long channel openings in the presence of the drug. (B) Ensemble average currents (left) obtained from 168–219 single-channel current traces as in A with increasing concentrations of BAY K 8644. Plot of dose/response relationship (right) of BAY K 8644 on ensemble average currents.

compounds such as nifedipine, nitrendipine or nimodipine result from a reduction of the opening probability of the channel by favouring mode 0 behaviour of gating (Hess *et al.* 1984). The agonist compounds (CGP 28392, BAY K 8644) increase Ca^{2+} currents by promoting long channel openings (mode 2; Fig. 6) (Kokubun & Reuter, 1984; Hess *et al.* 1984). Both effects depend strongly on membrane potential. The binding affinity for antagonists and agonists is greatly increased by membrane depolarization (Reuter *et al.* 1985*a*,*b*). Correspondingly, the blocking potency of antagonistic compounds is also increased (Bean, 1984; Reuter *et al.* 1985*a*), while the activator effect of the agonists turns into a blocking effect by increasing the failure rate of channels to open (mode 0) (Reuter *et al.* 1985*a*).

It is interesting to note that dihydropyridine agonists not only produce changes in Ca^{2+} channel kinetics which are distinctly different from those induced by catecholamines, but these changes are also independent of the cyclic AMP level in the

cell (Kokubun & Reuter, 1984; Böhm *et al.* 1985). This shows that 'openness' of Ca^{2+} channels can be modulated by very different mechanisms and in different ways.

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