Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1518–1522, March 1986
Neurobiology

Dihydropyridine-sensitive Ca^{2+} channels in mammalian skeletal muscle cells in culture: Electrophysiological properties and interactions with Ca^{2+} channel activator (Bay K8644) and inhibitor (PN 200-110)

(voltage clamp/Ca^{2+} agonists and antagonists)

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Communicated by J.-M. Lehn, October 3, 1985

ABSTRACT **The whole-cell patch-clamp technique has been used to analyze the properties of the dihydropyridine-sensitive Ca^{2+} channel in rat skeletal muscle cells (myoballs) in culture. The potential dependence of Ca^{2+}-channel activation is similar to that observed in cardiac cells. However, the skeletal muscle Ca^{2+} channel is activated more slowly (by a factor of about 10). The voltage dependence of Ca^{2+}-channel inactivation indicates a half-maximal inactivation ($V_{h0.5}$) at −72 mV as compared to $V_{h0.5}$ = −35 mV for cardiac cells. Blockade of the skeletal muscle Ca^{2+} channel by the dihydropyridine (+)-PN 200-110 is voltage dependent, with a half-maximal effect ($K_{0.5}$) of 13 nM for an application of the drug to the myoball membrane held at −90 mV and of 0.15 nM for an application at a potential of −65 mV. The 100-fold difference in apparent affinity is interpreted as a preferential association of PN 200-110 with the inactivated form of the Ca^{2+} channel. The $K_{0.5}$ value found from electrophysiological experiments for the binding to the inactivated state ($K_{0.5}$ = 0.15 nM) is nearly identical to the equilibrium dissociation constant found from binding experiments with (+)-[^{3}H]PN 200-110 using transverse-tubular membranes (K_d = 0.22 nM). The dihydropyridine activator Bay K8644 acts by increasing Ca^{2+} current amplitude and by slowing down deactivation.**

Voltage-sensitive Ca^{2+} channels are of critical importance in a large number of tissues. In smooth and cardiac muscle, these channels provide Ca^{2+} for muscle contraction. In a number of endocrine cells and neurones, they provide Ca^{2+} for hormone and neurotransmitter release. Slow voltage-dependent Ca^{2+} channels also have been identified in skeletal muscle and shown to reside in the transverse-tubular (T-tubule) system (1–5). However, the exact physiological function of these Ca^{2+} channels is still a matter of debate (6).

A variety of Ca^{2+} channel inhibitors is now known. Tritiated dihydropyridines ([^{3}H]DHPs) and [^{3}H]verapamil have permitted the biochemical identification of membrane receptors to which they bind with a relatively high affinity. It has been found recently that T-tubular membranes of skeletal muscle have a high density of a homogeneous population of receptors for specific blockers of Ca^{2+} channels, such as DHPs (7, 8). Because of this observation, mammalian skeletal muscle has become the favorite source for the biochemical isolation of putative Ca^{2+} channels (9–11). However, the biophysical and pharmacological properties of mammalian skeletal muscle Ca^{2+} channels have not been extensively characterized at the electrophysiological level.

This paper presents the analysis of the properties of Ca^{2+} channels of mammalian skeletal muscle cells (myoballs) in culture by using the whole-cell patch-clamp technique and describes the properties of the interaction of the channel with inhibitors and activators belonging to the DHP family.

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MATERIALS AND METHODS

Cell Cultures. Primary cultures of thigh muscle of newborn rats were prepared as described (12). Myoballs were obtained by adding colchicine (10 nM) to the culture medium when the cells reached the early myotubular stage of development. These myoballs contract under stimulation in a Ca^{2+} medium.

Solutions. Tetraethylammonium$^+$ was substituted for Na^+ and K^+ in the external (bath) solution and Cs^+ was the predominant cation in the internal (pipette) solution. Ba^{2+} was used as a substitute for Ca^{2+} for the following reasons: (*i*) Ba^{2+} is known for carrying more current than Ca^{2+} through the Ca^{2+} channel in frog muscle (3), (*ii*) Ba^{2+} is believed to minimize or suppress Ca^{2+}-dependent outward currents in skeletal muscle cells (13), (*iii*) Ca^{2+} is responsible for a Ca^{2+}-dependent inactivation process in cardiac cells that is not observed with Ba^{2+} (14).

External solutions with 2.5 mM Ba^{2+} or 2.5 mM Ca^{2+} contained 1 mM $MgCl_2$ and 150 mM tetraethylammonium chloride. External solutions containing 10 mM Ba^{2+} or 0.25 mM Ba^{2+} contained 1 or 3.25 mM $MgCl_2$ and 140 or 150 mM tetraethylammonium chloride, respectively. All external solutions also contained 10 mM glucose and 10 mM Hepes (at pH 7.5). The internal solution had the following composition: 150 mM CsCl, 5 mM EGTA, 10 mM Hepes (at pH 7.2), and 10 mM glucose. The osmolarity was adjusted to 300 mosM with sucrose.

Current and Voltage Recordings. Myoballs were voltage- and current-clamped by using the whole-cell patch-clamp method (15). Currents through the pipette were converted to a voltage, amplified and low-pass-filtered (cutoff frequency, 1 kHz) by a built-in two-pole Bessel filter of the recording apparatus (EPC-5, List Electronic). Signal was then sampled (sampling frequency, 2 kHz), stored, and analyzed with a minicomputer system. In all recordings presented here linear leak currents, but not capacitive currents, have been subtracted from linear extrapolation of leak current amplitude for small depolarizing pulses.

All experiments were carried out at 20 ± 2°C with myoballs having a diameter of about 20 μm. Time constants were determined by semilogarithmic computer plotting of the current time course. Statistical data are expressed as mean ± SD × $t_{0.05}$ ($t_{0.05}$ = Student–Fischer variable with a safety coefficient of 0.95).

Abbreviation: DHP, dihydropyridine.

Biochemical Assays. T-tubular membranes were isolated from rat hind leg skeletal muscle as described (16).

Binding experiments with (+)-[^{3}H]PN 200-110 were carried out as described for [^{3}H]nitrendipine (8).

RESULTS

Voltages and Time Dependence of the Inward Current. No rundown of Ca^{2+} currents was observed with rat myoballs under conditions described in this paper for periods as long as 1 hr.

In the standard solution containing 2.5 mM Ba^{2+}, a myoball responded to a depolarizing current step by a long-lasting (2.5 s) action potential, which reached a peak at +30 mV (V_h = −80 mV) in about 250 ms (Fig. 1*A*). Under voltage-clamp conditions (Fig. 1*B*), when a myoball was depolarized beyond a threshold level (−30 mV) by a long-lasting pulse (500 ms), a slowly activating inward current was elicited (step to −18 mV). Larger depolarizations caused the inward current to become larger and the time-to-peak to decrease (step to −10 and −2 mV). A further increase in depolarization amplitude (step to +10 and +30 mV) decreased the peak amplitude.

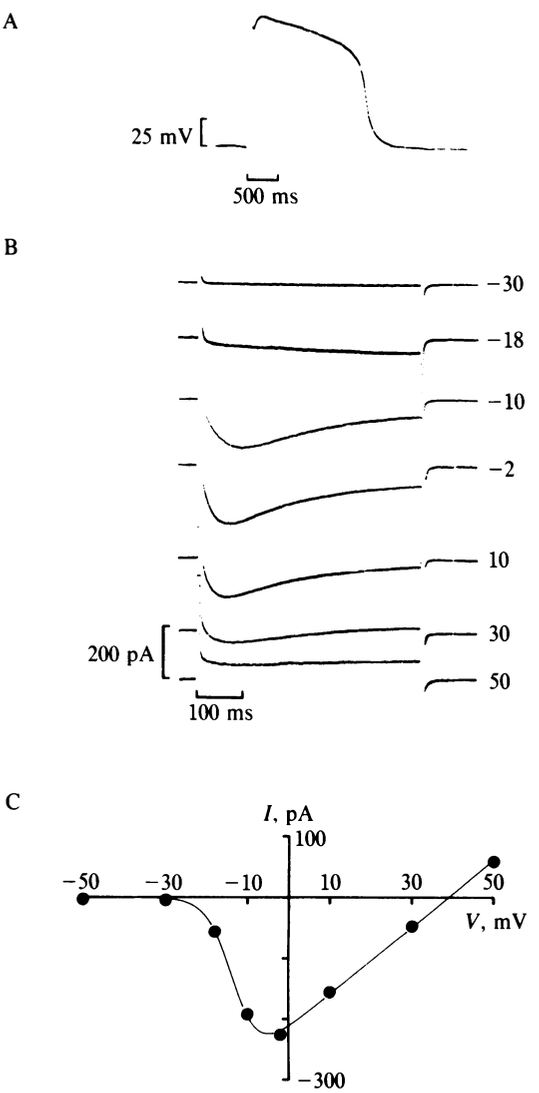

FIG. 1. (*A*) Long-lasting action potential recorded in current clamp condition in 2.5 mM Ba^{2+} external solution. V_h = −80 mV. (*B*) Whole-cell patch-clamp currents during step depolarizations to the potentials indicated (in mV). V_h = −90 mV. External solution: 2.5 mM Ba^{2+}. (*C*) Dependence of the maximal inward current amplitudes measured in *B* on membrane potential.

Measurement from 33 different myoballs gave a maximal amplitude of the inward current of 333 ± 54 pA at −2 mV.

The peak current–voltage (*I–V*) relationship of this inward current is illustrated in Fig. 1*C*. The inward current was activated for a threshold potential of −30 mV and reached a maximum at 0 mV.

Demonstration that the slow inward current was carried by Ba^{2+} comes from the following observations (not shown). (*i*) The current was not carried by Na^+ since Na^+ was replaced by tetraethylammonium$^+$ and inward currents were not sensitive to tetrodotoxin (20 μM). (*ii*) Replacement of the 2.5 mM Ba^{2+} solution by a 10 mM Ba^{2+} solution led to an increase of the inward current by a factor 2. Conversely, the current amplitude was drastically reduced (by a factor of 10) in 0.25 mM Ba^{2+}. (*iii*) The inward current was blocked by millimolar concentrations of Cd^{2+}, Mn^{2+}, or Co^{2+}, which are the usual inorganic blockers of Ca^{2+} channels.

No significant difference was observed in the peak current amplitude in 2.5 mM Ba^{2+} or in 2.5 mM Ca^{2+} (not shown). However, the current decayed more rapidly in 2.5 mM Ca^{2+}. This difference could be due to the presence of a Ca^{2+}-dependent inactivation process or to the presence of a Ca^{2+}-dependent outward conductance, which would not be functional in a 2.5 mM Ba^{2+} solution.

Steady-state inactivation of the inward current was studied by measuring the inward current peak amplitude at a fixed potential (−2 mV) after holding the membrane potential at various conditioning levels for a sufficiently long time (>30 s) to reach a steady state (Fig. 2*A*). Ca^{2+} channels were half-inactivated at a holding potential of −72 mV and were almost fully inactivated at −40 mV (Fig. 2*B*).

Pharmacological Properties of the Ca^{2+} Channel. The blocking effect of the DHP (+)-PN 200-110 on the Ca^{2+} channel was investigated after application of the drug to the prepa-

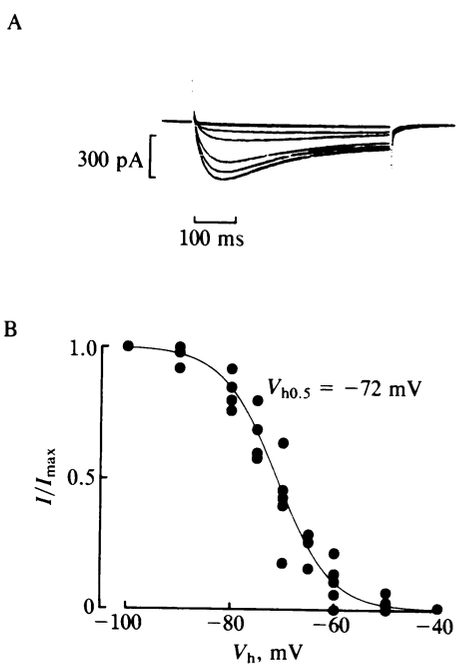

FIG. 2. Steady-state inactivation of the inward current in 2.5 mM Ba^{2+} solution. (*A*) Superimposed inward currents elicited by depolarizing pulses to −10 mV after shifting the holding potential for 30 s. V_h was successively (from lower to upper trace) −90, −80, −75, −65, −60, −50, and −40 mV (mingled with the current trace to −50 mV). (*B*) Peak inward current amplitude plotted against holding potential. Pooled data obtained on six myoballs. Experimental points are fitted by a Boltzmann distribution (solid line): $I = I_{max}[1 + \exp(V - V_{h0.5})/k]^{-1}$, with a potential where the inward current was half-inactivated, $V_{h0.5}$ = −72 mV and k = 5.4 mV.

ration at different membrane potentials. First, the membrane was held at −90 mV. Under these conditions the Ca^{2+} current was almost completely blocked at 1 μM (+)-PN 200-110 (Fig. 3*A*, group a). The corresponding dose–response curve (Fig. 3*B*) yielded a $K_{0.5}$ value of 13 nM.

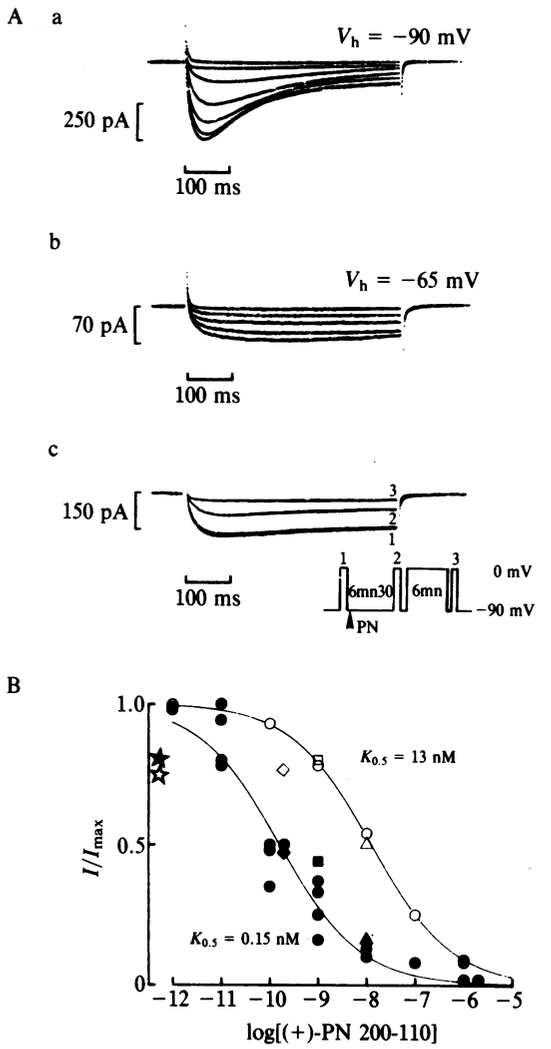

FIG. 3. Voltage-dependent blocking effect of (+)-PN 200-110 on Ca^{2+} channels (2.5 mM Ba^{2+}). (*A*) Group a: effect of increasing concentrations of (+)-PN 200-110 on inward current elicited by test pulses to −2 mV from a holding potential V_h = −90 mV. (+)-PN 200-110 concentrations were successively (from lower to upper trace) 0 (without drug), 0.1 nM, 1 nM, 10 nM, 100 nM, 1 μM, and 1 μM + 1 mM Co^{2+}. Time exposure to each concentration was 6 min. Group b: same experiments on another myoball with V_h = −65 mV. (+)-PN 200-110 concentrations were successively (from lower to upper trace) 0 (without drug), 0.01 nM, 0.2 nM, 1 nM, and 10 nM. Time exposure to each concentration was 8 min. Group c: blocking effect of 10 nM (+)-PN 200-110 before and after the membrane was held at 0 mV for 6 min. (*Inset*) Schematic diagram of pulse protocol with the test pulses to −2 mV (1, 2, 3), the time interval between test pulses 1 and 2 (6.5 min), and the transient shift for 6 min of the holding potential from −90 mV to 0 mV. Ten micromolar (+)-PN 200-110 (PN) was added at the time indicated. Lower trace (1): current elicited by a test pulse to −2 mV from V_h = −90 mV. Middle trace (2): current after addition and steady-state effect of 10 nM (+)-PN 200-110 (time exposure, 6.5 min). Test pulse to −2 mV from V_h = −90 mV. Upper trace (3): current elicited by a test pulse to −2 mV after a transient shift of V_h to 0 mV during 6 min and a return of V_h to −90 mV for 30 s. The control current trace elicited by a test pulse to −2 mV after the same sequential V_h shifting (−90 mV to 0 mV for 6 min and then −90 mV for 30 s) in the absence of (+)-PN 200-110 is mingled with the lower trace (1) described above. (*B*) Dose–response curves for blocking effect of (+)-PN 200-110 on Ca^{2+} channels under polarized (○) (V_h = −90 mV, $K_{0.5}$ = 13 nM) and depolarized (●) conditions (V_h = −65 mV, $K_{0.5}$ = 0.15 nM). Results corresponding to experiments similar to those of *A*, group c, are also plotted. The current amplitudes elicited by test pulses to −2 mV before (◇, □, △) and after (◆, ■, ▲) a transient shifting of the holding potential to 0 mV and a return to −90 mV for 30 s were obtained in experiments with 0.2 nM (◇, ◆), 1 nM (□, ■), and 10 nM (△, ▲) (+)-PN 200-110. Stars along the ordinate represent the recovery of the current amplitude after a 15-min wash with a 2.5 mM Ba^{2+} solution without drug following exposure to (+)-PN 200-110 under conditions of the experiments in *A*, group a (☆), and in *A*, group b (★).

Similar experiments were performed by clamping myoballs at −65 mV, where 75–80% of Ca^{2+} channels were in the inactivated state during the exposure to (+)-PN 200-110 (Fig. 3*A*, group b). Under these more depolarized conditions, the dose–response curve (Fig. 3*B*) yielded a $K_{0.5}$ value at 0.15 nM. This voltage-dependent effect of (+)-PN 200-110 was confirmed by experiments (Fig. 3*A*, group c) in which the cell was exposed to a fixed concentration of (+)-PN 200-110 at V_h = −90 mV until a steady-state effect on the current was reached. Then, the membrane was held for 6 min at 0 mV and then returned to −90 mV for 30 s before the test pulse. In passing to −90 mV, one should expect to see a further blocking effect of (+)-PN 200-110 due to the transient holding of the Ca^{2+} channel at 0 mV. This hypothesis was indeed verified, as shown in Fig. 3*A*, group c, and *B*.

Fig. 4*A* illustrates equilibrium binding of (+)-[^{3}H]PN 200-110 to rat skeletal muscle T-tubular membranes. Scatchard plots for the specific binding (Fig. 4*A Inset*) are consistent with the existence of a single class of sites with an equilibrium constant (K_d) of 0.22 ± 0.05 nM and a maximal binding capacity (B_{max}) of 41 ± 5 pmol/mg of protein. The specific binding of (+)-[^{3}H]PN 200-110 was inhibited by unlabeled (+)- and (−)-PN 200-110 (Fig. 4*B*). K_d values determined from these competition experiments are 0.1 nM and 300 nM for (+)- and (−)-PN 200-110, respectively.

Application of the DHP Ca^{2+} channel activator Bay K8644 (1 μM) to rat myoballs held at −90 mV induced no significant increase in peak current amplitude (not shown). In contrast, when the same experiment was performed at a less negative holding potential (−65 mV) (Fig. 5*A*), the peak amplitude was increased and tail currents were slowed down under repolarization. The current–voltage relationship is shown in Fig. 5*B*. The effect of Bay K8644 is particularly important for low depolarizing potentials (Fig. 5*C*).

DISCUSSION

The main contributions of this paper are (*i*) the demonstration that the voltage- and time-dependence properties of mammalian skeletal muscle cells can easily be studied in culture by using the whole-cell patch-clamp technique and (*ii*) the electrophysiological analysis of the pharmacological properties of inhibitors and activators of the Ca^{2+} channel belonging to the class of the DHPs and the comparison of these properties with those found from biochemical experiments.

The voltage dependence of the Ca^{2+} current in rat myoballs (Fig. 1*C*) was similar to that found for frog and rat muscle fibers (2–5). The threshold is observed between −40 and −30 mV, and the peak potential is observed between −10 and 0 mV. This voltage dependence is also similar to that found in cardiac cells (17, 18).

The mean of the Ca^{2+} current amplitude in myoballs was 30 ± 5 μA/cm^2 (n = 33) in 2.5 mM Ba^{2+}. In 10 mM Ba^{2+}, the current amplitude was increased by a factor of 2 to 60 μA/cm^2, a value that is of the same order of magnitude as the values of 80 μA/cm^2 and 140 μA/cm^2 found in 10 mM Ca^{2+} for frog and rat muscle fibers, respectively (1–4). Using a unitary conductance of 25 pS (19), one calculates that a

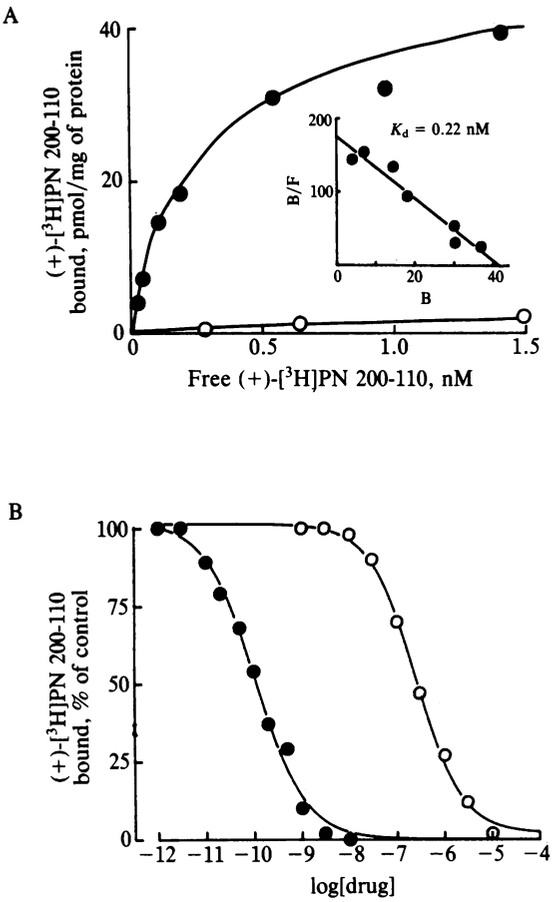

FIG. 4. Equilibrium binding of (+)-[^{3}H]PN 200-110 (78 Ci/mmol; 1 Ci = 37 GBq) to T-tubular membranes and inhibition by unlabeled (+)- and (−)-PN 200-110. (*A*) Binding of (+)-[^{3}H]PN 200-110 to membranes (5 μg/ml) was measured in the absence (total binding) (●) and in the presence (nonspecific binding) (○) of 1 μM unlabeled (+)-PN 200-110. (*Inset*) Scatchard plot for specific (total − nonspecific) (+)-[^{3}H]PN 200-110 binding component. B (bound), pmol/mg of protein; F (free), nM. (*B*) Inhibition of (+)-[^{3}H]PN 200-110 (0.055 nM) binding by increasing concentrations of (+)-PN 200-110 (●) or of (−)-PN 200-110 (○).

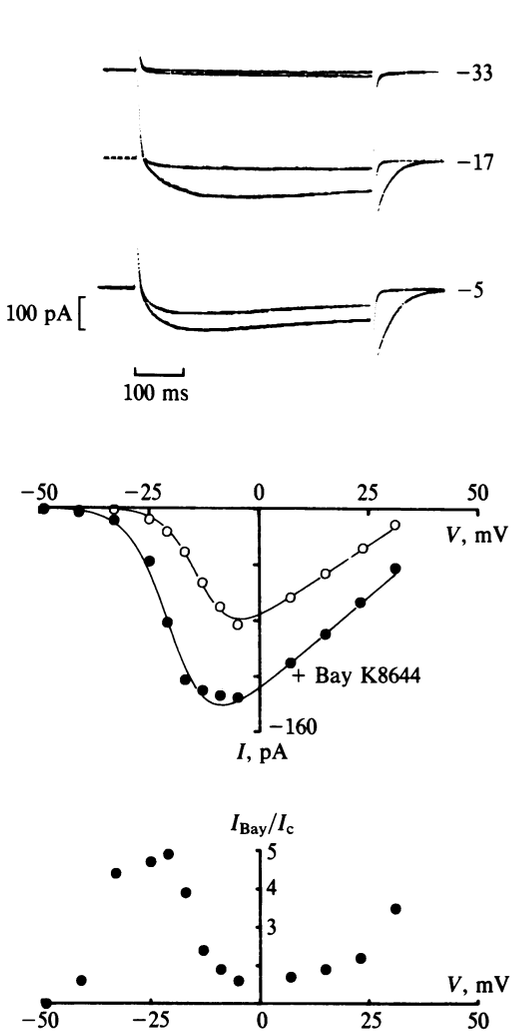

FIG. 5. (*A*) Membrane currents after voltage jumps from V_h = −65 mV to −33, −17, and −5 mV before (upper traces) and after (lower traces) application of Bay K8644 (1 μM) for 5 min. Bath solution: 2.5 mM Ba^{2+}. (*B*) *I–V* curve in the absence (○) and in the presence (●) of Bay K8644 (1 μM). (*C*) Voltage dependence of the ratio (I_{Bay}/I_c) of currents obtained in the presence and in the absence of Bay K8644.

myoball with a diameter of 20 μm has about 300 Ca^{2+} channels.

The time to reach half of the maximal peak current amplitude ($t_{1/2}$) with depolarizing pulse to 0 mV is 30 ms in rat myoballs in 10 mM Ba^{2+} (not shown). This $t_{1/2}$ value is relatively short as compared to values of 110 and 100–300 ms, which have been reported for rat and frog skeletal muscle fibers, respectively (1–4). However, the rate of activation of the Ca^{2+} channel in rat skeletal muscle cells in culture is much slower than that found for guinea pig heart myocytes, for which the $t_{1/2}$ value is near 2 ms (17).

The voltage dependence of the steady-state inactivation observed for rat muscle cells in culture was different from that observed in frog muscle. Half-inactivation was observed at −72 mV for rat myoballs and at −35 to −44 mV (20) for frog muscle (in 10 mM Ca^{2+}). The voltage dependence of the steady-state inactivation observed herein is also different from that found with rat cardiac cells, for which half-inactivation is around −35 mV (21).

Pharmacological tools are essential to analyze the molecular properties of Ca^{2+} channels at the biochemical level. Receptors for DHPs, for verapamil and analogs, and for diltiazem and bepridil have now been well identified (8, 16, 22, 23) in T-tubules. Much less is known concerning the electrophysiological properties of skeletal muscle Ca^{2+} channel inhibition, although it is known that these channels are blocked by DHPs (24, 25), verapamil, and bepridil (26). One of the problems in relating electrophysiological and biochemical data has been the observation that biochemically determined K_d values for the complex formed by nitrendipine and (+)-PN 200-110 with skeletal muscle Ca^{2+} channels are 1.8 nM and 0.2 nM, respectively (8–10), whereas half-maximal blockade of Ca^{2+} channel activity has been observed at 0.7 μM nitrendipine and at 0.4 μM PN 200-110 (25)—i.e., at much higher concentrations.

Results presented in this paper show that (+)-PN 200-110 is a very potent Ca^{2+} channel blocker when associated with myoballs depolarized at −65 mV—i.e., when Ca^{2+} channels are 80% inactivated. The apparent dissociation constant, $K_{0.5}$, then measured by electrophysiology is 0.15 nM. When (+)-PN 200-110 was associated with myoballs at a holding potential of −90 mV, a $K_{0.5}$ value of 13 nM was obtained. At −90 mV, the Ca^{2+} channel is in the resting state. These results strongly suggest that the affinity of (+)-PN 200-110 for the Ca^{2+} channel depends on the state of the channel such that binding to the resting state is less potent by a factor of about 100 than binding to the inactivated state. These electrophysiological observations concerning skeletal muscle

Ca^{2+} channels are very similar to those that have been reported recently for cardiac Ca^{2+} channels (19, 27).

An important observation is that the apparent affinity of (+)-PN 200-110 for the inactivated skeletal muscle Ca^{2+} channel measured by electrophysiology ($K_{0.5}$ = 0.15 nM) is nearly identical to the affinity of (+)-[^{3}H]PN 200-110 for isolated rat muscle cell membranes (which are, of course, depolarized). The K_d value for the interaction of (+)-[^{3}H]PN 200-110 with its receptor was 0.22 nM. This observation makes it most likely that the receptor of DHPs identified by biochemists on skeletal muscle membranes really corresponds to skeletal muscle Ca^{2+} channels.

Recently, Schwartz *et al.* (25) performed binding experiments with [^{3}H]PN 200-110 in intact frog sartorius muscle and found that there was a voltage-dependent effect on the maximal binding capacity for PN 200-110 but not on the dissociation constant. The most likely interpretation of these results in light of the work reported herein is that (*i*) in the frog muscle preparation, as in mammalian skeletal muscle, inactivated Ca^{2+} channels have high-affinity binding sites for PN 200-110, which are converted to lower-affinity binding sites when the channel is itself converted to its resting state, and (*ii*) the sites identified in ref. 25 are high-affinity sites and the decrease in their maximal number in passing from −16 mV to −88 mV reflects the conversion of part of them into low-affinity binding sites. These low-affinity sites could not be identified by Schwartz *et al.* (25) because of the existence of a large component of nonspecific binding of [^{3}H]PN 200-110 to intact frog muscle fibers.

Bay K8644 operates as a Ca^{2+} channel activator in cardiac and smooth muscle (28–33). It was of interest to see whether Bay K8644 would also be an activator of Ca^{2+} channel in skeletal muscle. Fig. 5 shows that Bay K8644 had three main effects on Ca^{2+} channels in rat myoballs: (*i*) it increased the inward Ca^{2+} current amplitude, (*ii*) this increase was voltage dependent and the maximal increase (a factor of 4–5) was seen near −25 mV, and (*iii*) it slowed down the tail currents, which corresponds to an increase of the time constant of deactivation under repolarization. All of these effects are similar to those seen with cardiac muscle (29–32). The use of Bay K8644 to open the Ca^{2+} channel in skeletal muscle might possibly help to solve the problem of the function of this channel in muscle contraction (6).

In conclusion, although there are differences between cardiac and skeletal muscle Ca^{2+} channels, analogies in functioning and pharmacology of these channels make it likely that the protein structures of Ca^{2+} channels in skeletal muscle and other tissues will be similar. Therefore, it is probable that the available information on the structural properties of the putative skeletal muscle Ca^{2+} channel (9, 10) may also apply to cardiac Ca^{2+} channels.

We thank Dr. R. P. Hof from Sandoz, Basel, for a generous gift of (+)- and (−)-PN 200-110 and Dr. G. Franckowiak from Bayer AG, F.R.G., for a gift of Bay K8644. [^{3}H]PN 200-110 was from Amersham. Thanks are due to M. T. Ravier for technical help, to Dr. M. M. Hosey for reading the manuscript, and to M. Valetti and C. Roulinat-Bettelhiem for secretarial assistance. This work was supported by the Association des Myopathes de France, the Centre National de la Recherche Scientifique, the Ministère de l'Industrie et de la Recherche (Grant 83.C.0696), and the Mutuelle Générale de l'Education Nationale ("Système Nerveux en Neurobiologie").

1. Stanfield, P. R. (1977) *Pflügers Arch.* **368,** 267–270.
2. Sanchez, J. A. & Stefani, E. (1978) *J. Physiol.* **283,** 197–209.
3. Almers, W. & Palade, P. T. (1981) *J. Physiol.* **312,** 159–176.
4. Donaldson, P. L. & Beam, K. G. (1983) *J. Gen. Physiol.* **82,** 449–468.
5. Potreau, D. & Raymond, G. (1980) *J. Physiol.* **303,** 91–109.
6. Ildefonse, M., Jacquemond, V., Rougier, O., Renaud, J. F., Fosset, M. & Lazdunski, M. (1985) *Biochem. Biophys. Res. Commun.* **129,** 904–909.
7. Lazdunski, M., Barhanin, J., Borsotto, M., Fosset, M., Galizzi, J.-P., Renaud, J. F., Romey, G. & Schmid, A. (1985) in *Membranes and Muscle*, ed. Whelan, W. J. (ISCU Press, Miami, FL), pp. 121–132.
8. Fosset, M., Jaimovich, E., Delpont, E. & Lazdunski, M. (1983) *J. Biol. Chem.* **258,** 6086–6092.
9. Borsotto, M., Barhanin, J., Norman, R. I. & Lazdunski, M. (1984) *Biochem. Biophys. Res. Commun.* **122,** 1357–1366.
10. Borsotto, M., Barhanin, J., Fosset, M. & Lazdunski, M. (1985) *J. Biol. Chem.* **260,** 14255–14263.
11. Curtis, B. M. & Catterall, W. A. (1984) *Biochemistry* **23,** 2113–2118.
12. Frelin, C., Vigne, P. & Lazdunski, M. (1983) *J. Biol. Chem.* **258,** 7256–7259.
13. Barrett, J. N., Barrett, E. F. & Dribin, L. B. (1981) *Dev. Biol.* **82,** 258–266.
14. Kass, R. S. & Sanguinetti, M. C. (1984) *J. Gen. Physiol.* **84,** 705–726.
15. Hamill, O., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391,** 85–100.
16. Galizzi, J.-P., Fosset, M. & Lazdunski, M. (1984) *Eur. J. Biochem.* **144,** 211–215.
17. Lee, K. S. & Tsien, R. W. (1982) *Nature (London)* **297,** 498–501.
18. Reuter, H. (1983) *Nature (London)* **301,** 569–574.
19. Sanguinetti, M. C. & Kass, R. S. (1984) *Circ. Res.* **55,** 336–348.
20. Cota, G., Nicola Siri, L. & Stefani, E. (1984) *J. Physiol.* **354,** 99–108.
21. Reuter, H., Stevens, C. F., Tsien, R. W. & Yellen, G. (1982) *Nature (London)* **292,** 501–504.
22. Glossman, H., Linn, T., Rombusch, M. & Ferry, D. R. (1983) *FEBS Lett.* **160,** 226–232.
23. Galizzi, J.-P., Fosset, M. & Lazdunski, M. (1984) *Biochem. Biophys. Res. Commun.* **118,** 239–245.
24. Almers, W. & McCleskey, E. W. (1984) *J. Physiol.* **353,** 585–608.
25. Schwartz, L. M., McCleskey, E. W. & Almers, W. (1985) *Nature (London)* **314,** 747–751.
26. Kerr, L. & Sperelakis, N. (1982) *J. Pharmacol. Exp. Ther.* **222,** 80–86.
27. Bean, B. P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6388–6392.
28. Schramm, M., Thomas, G., Towart, R. & Franckowiak, G. (1983) *Nature (London)* **303,** 535–537.
29. Hess, P., Lansman, J. B. & Tsien, R. W. (1984) *Nature (London)* **311,** 538–544.
30. Brown, A. M., Kunze, D. L. & Yatani, A. (1984) *Nature (London)* **311,** 570–572.
31. Thomas, G., Chung, M. & Cohen, C. J. (1985) *Circ. Res.* **56,** 87–96.
32. Kokubun, S. & Reuter, H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4824–4827.
33. Renaud, J. F., Méaux, J. P., Romey, G., Schmid, A. & Lazdunski, M. (1984) *Biochem. Biophys. Res. Commun.* **125,** 405–412.