Short Term and Long Term Effects of β-Adrenergic Effectors and Cyclic AMP on Nitrendipine-sensitive Voltage-dependent Ca²⁺ Channels of Skeletal Muscle*

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The effects of short term stimulation of β-adrenergic receptors and elevations in intracellular cyclic AMP on nitrendipine-sensitive voltage-dependent Ca²⁺ channels of skeletal muscle cells *in vitro* has been studied using both the ⁴⁶Ca²⁺ flux technique and [³H]nitrendipine-binding experiments. Isoproterenol increased the nitrendipine-sensitive ⁴⁶Ca²⁺ influx under depolarizing conditions. The effects of isoproterenol were additive to those of depolarization and were antagonized by alprenolol. Half-maximal inhibition of ⁴⁶Ca²⁺ influx induced both by depolarization and by isoproterenol occurred at a nitrendipine concentration of 1 nM. Treatments that resulted in an increased level of intracellular cyclic AMP, such as treatment with 1-methyl-3-isobutylxanthine, theophylline, dibutyryl cyclic AMP, or 8-bromocyclic AMP also resulted in an increased rate of ⁴⁶Ca²⁺ entry via nitrendipine-sensitive Ca²⁺ channel.

In contrast, long term treatment of myotubes in culture with isoproterenol and other compounds that increased intracellular cyclic AMP led to a large increase in the number of nitrendipine receptors. This increase was accompanied by a 4–10-fold decrease in the affinity of the receptors for nitrendipine. Alprenolol inhibited the long term effects of isoproterenol.

In *vivo* treatment of 7-day-old chicks with reserpine and alprenolol produced a decrease in the number of skeletal muscle nitrendipine receptors. This decrease in receptor number was accompanied by an increase in the affinity of nitrendipine for its receptor by a factor of 4 to 5. These effects on the nitrendipine receptor were prevented by simultaneous injection of isoproterenol.

The results are discussed in relation to the role of β-adrenergic receptors and intracellular cyclic AMP in the regulation of skeletal muscle Ca²⁺ channels.

The skeletal muscle cell contains voltage-dependent Ca²⁺ channels that are inhibitable by dihydropyridines such as nifedipine or nitrendipine (1–3). These Ca²⁺ channels appear to be primarily located in the T-tubular system (1, 4–6). Binding studies using [³H]dihydropyridines (3, 7–9) as well as with other tritiated Ca²⁺ channel blockers such as [³H]verapamil (10–13), [³H]D888 (14), or [³H]diltiazem (15) have shown that receptor sites for these different molecules are present in high density in T-tubule membranes where they are presumably associated with voltage-dependent Ca²⁺ channels. Because of the high density of dihydropyridine receptor sites in skeletal muscle T-tubule membranes as compared to other membranes (heart and smooth muscle) (16, 17), these membranes have been used in the first studies to purify and label the putative dihydropyridine-sensitive voltage-dependent Ca²⁺ channel (18–20).

Developmental properties of voltage-sensitive Ca²⁺ channels during *in vivo* myogenesis of chick muscle cells have been assessed using ⁴⁶Ca²⁺ flux experiments and [³H]nitrendipine-binding assays (3). Both approaches have shown that [³H]nitrendipine receptors, as well as functional dihydropyridine-sensitive and voltage-sensitive Ca²⁺ channels, are absent at the myoblast stage. However, both [³H]nitrendipine receptors and nitrendipine inhibitable Ca²⁺ flux appear in parallel with the fusion of myoblasts into myotubes (3).

There are two distinct phases in the appearance of [³H]nitrendipine receptors during myogenesis in *vivo* (3). The first one is observed during embryonic life and corresponds to the appearance of [³H]dihydropyridine sites during fusion of myoblasts into myotubes. The second phase occurs near hatching and corresponds to an increased maximum binding capacity for [³H]nitrendipine by a factor of 7. This increased number of [³H]nitrendipine-binding sites is accompanied by a decrease of affinity of nitrendipine for its receptor by a factor of 4–10. Ca²⁺ channels in other tissues, notably in cardiac muscle cells, are known to be regulated by cyclic AMP (21–24). In order to gain a further understanding of the skeletal muscle Ca²⁺ channel, we now have analyzed the effects of short term and long term elevations in cyclic AMP on both the activity and expression of dihydropyridine-sensitive Ca²⁺ channels and dihydropyridine receptors in skeletal muscle cells.

**MATERIALS AND METHODS**

**Cell Culture**—Primary cultures of skeletal muscle cells from chick embryos were prepared according to the method previously described (3, 25) using 11-day-old chick embryonic breast and leg muscles. Cells were grown in Dulbecco's modified Eagle's minimal essential medium and M199 medium (3:1) supplemented with 5% fetal calf serum, 200 units/ml penicillin, and 50 μg/ml streptomycin. Cells were plated either in gelatin-coated 60-mm diameter dishes (Corning tissue culture dish) or 24-well tissue culture plates (Nunc) at a density of 1.5 × 10⁶ cells/cm². Cultures were maintained at 37 °C in a water-saturated atmosphere of air/CO₂ (95/5). Differentiated myotubes were used for ⁴⁶Ca²⁺ flux experiments and binding assays.

**⁴⁶Ca²⁺ Flux Experiments**—The determination of nitrendipine-sensitive and -insensitive rates of ⁴⁶Ca²⁺ uptake by myotubes in culture was carried out in gelatin-coated 24-well tissue culture plates as...
previously described (3). Cells were cultured between 48 and 100 h before flux measurements were performed. The determination of rate of \( ^{45} \text{Ca}^2+ \) uptake first involved a preincubation of the cells for 15 min at 37 °C in a medium containing 40 mM K\(^+ \), 105 mM Na\(^+ \), 5 mM glucose, 25 mM Hepes-Tris at pH 7.4 in the presence or absence of reserpine, and the concentration of concentrations of nitrendipine and of the other molecules such as (-)-isoproterenol, \( \& \text{Ca}^2+ \) uptake was followed by removing the medium at different times of incubation between 0 and 1 min. In experiments done to obtain dose-response curves for nitrendipine action on \( ^{45} \text{Ca}^2+ \) uptake, the initial rate of \( ^{45} \text{Ca}^2+ \) uptake was measured after 20 s of incubation. At the end of the incubation, cells were washed three times in less than 5 s with 3 ml of washing medium containing 140 mM choline, 1 mM La\(^3+ \), and 25 mM Hepes-Tris at pH 7.4. After the third wash, 2 ml of 0.1 N NaOH were added to each well and the radioactivity that was incorporated by the cells was counted. Protein concentrations were measured according to Hartree (26).

Tissue Preparation—Skeletal muscles from legs of 7-day-old chicks were obtained from treated and nontreated animals at different stages of treatment. In all cases, muscles were removed and washed in an ice-cold buffer containing 20 mM Tris-Cl, 0.25 M sucrose, and 1 mM EDTA at pH 7.4 (TSE buffer). Subsequently the muscles were dissected, minced, rinsed once with the TSE buffer, and homogenized in ice-cold buffer containing 20 mM Tris-Cl, 0.25 M sucrose, and 1 mM EDTA at pH 7.4. Each homogenate was then filtered and centrifuged, and the supernatant was used as the initial cell homogenate. The crude homogenates were handled in a manner to minimize the exposure of the homogenate to light. Cells were cultured between 48 and 100 h and were used for all experiments.

**RESULTS**

Evidence for a Role of \( \beta \)-Adrenergic Receptors and \( c \text{AMP} \) in Regulating Dihydropyridine-sensitive \( ^{45} \text{Ca}^2+ \) Uptake in Chick Myotubes in Culture—The effect of isoproterenol on the nitrendipine-sensitive component of \( ^{45} \text{Ca}^2+ \) uptake in chick myotubes in culture has been investigated using a high external K\(^+ \) medium containing 40 mM KCl. Under these conditions, \( ^{45} \text{Ca}^2+ \) uptake in the presence of 10 \( \mu \text{M} \) isoproterenol, a concentration increasing the \( c \text{AMP} \) level in avian myotubes by approximately 3-fold (27), the \( ^{45} \text{Ca}^2+ \) uptake was higher than in the absence of isoproterenol. The same results were obtained with 1 \( \mu \text{M} \) isoproterenol (not shown). \( ^{45} \text{Ca}^2+ \) uptake in the presence or in the absence of isoproterenol was inhibited by nitrendipine. Addition of 10 \( \mu \text{M} \) alprenolol to the incubation medium prevented the effect of isoproterenol on the rate of \( ^{45} \text{Ca}^2+ \) uptake. Under the later conditions, the kinetics of \( ^{45} \text{Ca}^2+ \) uptake were identical to those obtained in the presence of both alprenolol and isoproterenol (Fig. 1). Dose-response curves for the action of nitrendipine on the initial rate of \( ^{45} \text{Ca}^2+ \) uptake in the presence and absence of isoproterenol are shown in Fig. 1, inset. The half-maximum effect of nitrendipine on the inhibition of \( ^{45} \text{Ca}^2+ \) flux was observed at 1 \( \mu \text{M} \) in both cases, indicating that the inhibition of \( ^{45} \text{Ca}^2+ \) uptake by nitrendipine was not affected by isoproterenol. We also tested the effects of other molecules that are known to elevate \( c \text{AMP} \) on the activity of the skeletal muscle \( \text{Ca}^2+ \) channel. Fig. 2 compares the effect of isoproterenol with the effects of B\(_{2}c\text{AMP} \), MIX, and theophylline on the initial rate of nitrendipine-sensitive \( ^{45} \text{Ca}^2+ \) uptake in myotubes in culture. Dibutyryl \( c \text{AMP} \) increased the initial rate of \( ^{45} \text{Ca}^2+ \) uptake (measured at 20 s) by 60%, MIX increased the initial rate of \( ^{45} \text{Ca}^2+ \) by 47%, whereas theophylline increased the rate of \( ^{45} \text{Ca}^2+ \) uptake by 56% as compared to the control. Under the same conditions, isoproterenol increased the initial rate of \( ^{45} \text{Ca}^2+ \) uptake by 65% as compared to control. In all cases \( ^{45} \text{Ca}^2+ \) uptake was inhibitable by nitrendipine (Fig. 2).

**Long Term Effects of Isoproterenol Treatment on Properties of \([\beta]H\text{Nitrendipine Binding to Membranes from Embryonic Chicks}**

![Image of graph showing the effect of isoproterenol on \( ^{45} \text{Ca}^2+ \) uptake](image-url)

**Fig. 1.** Short term effect of isoproterenol on nitrendipine-sensitive rate of \( ^{45} \text{Ca}^2+ \) uptake by myotubes differentiated in culture for 50 h. Main panel, time course of \( ^{45} \text{Ca}^2+ \) uptake was measured under depolarizing conditions (40 mM K\(^+ \)) in the presence (A) and the absence (C) of 10 \( \mu \text{M} \) isoproterenol and 1 \( \mu \text{M} \) nitrendipine. Inset, dose-response curves for nitrendipine inhibition of the initial rate of \( ^{45} \text{Ca}^2+ \) uptake under depolarizing conditions in the presence (A) and in the absence (C) of 10 \( \mu \text{M} \) isoproterenol. In this representation, nitrendipine-sensitive rates of \( ^{45} \text{Ca}^2+ \) uptake were obtained from 3 different experiments. Rates were calculated by subtracting the value obtained in the presence of 1 \( \mu \text{M} \) nitrendipine from the initial rate of \( ^{45} \text{Ca}^2+ \) uptake.
Chick Skeletal Muscle Cells in Culture: the Role of Cyclic AMP—Isoproterenol (10 μM) was added to cultures of chick skeletal myotubes two days after plating, at the time when the maximum extent of fusion reaches 80% (3). This isoproterenol treatment was repeated every 12 h. Then the myotubes were used at different lengths of the chronic isoproterenol treatment ranging between 1 and 50 h. Myotubes cultured in the presence of isoproterenol did not show any detectable morphological change as compared to control untreated cells. Fig. 3 shows typical Scatchard plots for specific [3H]nitrendipine binding to crude membranes from differentiated myotubes cultured for a total length of 85 h in the presence or absence of isoproterenol for 37 h. Membranes from control myotubes had a Kd value of 0.4 nM and a binding capacity of 137 fmol/mg protein. Scatchard plots for the specific binding of [3H]nitrendipine to myotubes treated chronically with isoproterenol for 37 h indicated that isoproterenol caused a large increase in the maximum binding capacity and a change in Kd value as compared to the control. After the chronic isoproterenol treatment, the Bmax was 427 fmol/mg protein and the Kd value was 1.5 nM. The Scatchard plots were linear for both the control and isoproterenol-treated myotubes indicating the presence of a single class of binding sites. The inset of Fig. 3 shows the time course for the isoproterenol-induced changes in [3H]nitrendipine-binding properties. The level of [3H]nitrendipine receptors remained relatively stable at a value of 130 ± 10 fmol/mg protein during the first 20 h of treatment with isoproterenol. Then the receptor level increased to reach a new plateau at 500 fmol/mg protein after 30 h of treatment. These variations in the number of [3H]nitrendipine-binding sites were accompanied by variations in the Kd values. The Kd was 0.4 ± 0.1 nM during the first 20 h of treatment, changed to 1.6 ± 0.2 nM at 30 h, and then remained essentially unchanged for the remaining period. Untreated myotubes have a level of [3H]nitrendipine receptors and a Kd value that remain stable at a value of 140 ± 10 fmol/mg protein and 0.4 ± 0.1 nM, respectively, during the time course of the culture (not shown).

The chronic effects of isoproterenol were dependent on the concentrations of isoproterenol used. Fig. 4, A and B, shows the dose-response curve for an isoproterenol treatment of 42 h on 92-h-old-myotubes. Isoproterenol was added every 12 h at concentrations ranging between 10 nM and 30 μM and once more 2 h before taking the cells for binding studies. At concentrations between 10 and 100 nM, isoproterenol did not induce significant changes in Kd and Bmax values for [3H]nitrendipine which remained at 0.4 ± 0.1 nM and 140 ± 15 fmol/mg protein, respectively. At concentrations of 3 μM and higher, isoproterenol induced changes both in Kd and Bmax values that reached 1.6 ± 0.2 nM and 425 ± 22 fmol/mg protein, respectively. Alprenolol (10 μM) produced a complete blockade of changes in [3H]nitrendipine-binding properties.

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induced by the highest dose of isoproterenol (Fig. 4, A and B).

Fig. 4C shows the results of competition experiments between unlabeled and labeled nitrendipine performed on membranes from myotubes cultured with and without isoproterenol. Half-maximal inhibition (Kd) for unlabeled nitrendipine is observed at 0.3 nM for untreated cells and at 3 nM for treated cells. The Kd values of the nitrendipine-receptor complex calculated from these experiments were 0.1 and 4.6 nM for myotubes cultured without and with isoproterenol, respectively.

In other experiments we determined the effects of long term treatment of myotubes with a series of molecules that lead to elevations in intracellular cAMP, either by inhibiting phosphodiesterase activity (like MIX and theophylline) or by activating adenylate cyclase activity (like isoproterenol). Molecules like 3',5'-cAMP and 8-bromo-cAMP also were tested. Results presented in Table I summarize the effects of these agents on specific [3H]nitrendipine binding to membranes from cultured myotubes. All these compounds caused a decrease in the Kd values, as compared to the control, and a parallel increase in the maximum binding capacity. The effect of isoproterenol on myotubes is given for comparison.

Effects of Treatments with Reserpine and Isoproterenol on the Properties of [3H]Nitrendipine-binding Properties in Chick Skeletal Muscle—Chicks (7-day postnatal) were treated with reserpine, which depletes norepinephrine and epinephrine and inhibits their synthesis (28). Chicks were divided into two different groups. The first group received injections of reserpine (5 mg/kg) on day 0 and day 2 and the second group received only the vehicle. Under these conditions, survival of the reserpine-treated group was 80%. Reserpine treatment caused a decrease in the maximum number of [3H]nitrendipine-binding sites from a control value of 885 to 565 fmol/mg protein (Table II). Under these conditions Kd values were 0.4 and 1.8 nM for the reserpine treated and for control, respectively. The decrease in maximum binding capacity and Kd value remained stable for 7 days under the treatment schedule used (not shown). Reserpine (10 μM) was found to have no direct effect by itself on [3H]nitrendipine binding.

Another series of experiments was carried out using membranes from chicks that received injections of isoproterenol alone (10 mg/kg) or isoproterenol plus reserpine (5 mg/kg) according to the protocol used for reserpine. Treatment with isoproterenol alone did not produce significant changes on Bmax or Kd values for [3H]nitrendipine binding as compared to control value (Table II). However, as shown in Table II simultaneous treatment with isoproterenol and reserpine prevented the effects caused by reserpine alone. Under these conditions the Kd value for [3H]nitrendipine binding was 1.7 nM and the Bmax was 900 fmol/mg protein, similar to the values obtained for control membranes.

**Table II**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bmax (fmol/mg protein)</th>
<th>Kd (nM)</th>
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<tbody>
<tr>
<td>Control (10 μM)</td>
<td>880 ± 40 (n = 12)</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Reserpine (10 μM)</td>
<td>565 ± 46 (n = 5)</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Isoproterenol (10 μM)</td>
<td>890 ± 46 (n = 2)</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Reserpine (10 μM) +</td>
<td>900 ± 30 (n = 3)</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Isoproterenol (10 μM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alprenolol (10 μM)</td>
<td>330 ± 30 (n = 3)</td>
<td>0.5 ± 0.1</td>
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DISCUSSION

The effects of β-adrenergic agonists and of other agents modulating intracellular cAMP levels on the activity of the voltage-dependent Ca2+ channel in heart cells have been extensively studied using electrophysiological techniques (23, 29–34). The mechanism of action of β-adrenergic agonists on cardiac Ca2+ channels can be summarized as follows. After binding of adrenergic agents to β-receptor, the adenylyl cyclase is activated via the guanine nucleotide-regulated N unit leading to an increase in intracellular cAMP levels (35). Cyclic AMP binds to the regulatory subunit of the cAMP-dependent protein kinase, thereby liberating the catalytic subunit of the enzyme. Then it is assumed that phosphorylation of the ion channels or of a protein closely associated with the channel occurs (23, 33, 36–38). This presumably causes a conformational change in the channel, the consequence of which is that there is a higher probability that the channel will open on membrane depolarization than in the dephosphorylated state (21, 23). As a result, β-adrenergic agents increase I Ca in cardiac cells (29–32). This causes an elevation of the plateau height of the cardiac action potential (39, 40). Kinetic analyses of I Ca in the presence of adrenergic agonists have shown that neither the voltage-dependent kinetics of the Ca2+ current nor the reversal potential are affected by the adrenergic agonists (31, 32, 41). The effects of β-adrenergic agents can be mimicked by the injection of cAMP into my-
ocardi al cells (42–44), by the application of BtZcAMP and 8-bromo-cAMP, by the use of phosphodiesterase inhibitors (22, 44, 45), and by injection of catalytic subunit of cAMP-dependent protein kinase into cells (21). The catecholamine effect on both Ca2+ current and plateau height of the action potential can be inhibited by β-receptor antagonists (36, 44). β-Adrenergic receptors have been identified in avian embryonic skeletal muscle cells both in vivo and in vitro (27, 46). Occupancy of these receptors by β-adrenergic agonists increases adenylate cyclase activity as well as cytoplasmic levels of cAMP (27, 46). The first part of the work presented in this paper has shown that isoproterenol, a well-known β-adrenergic agonist, increased the rate of nitrendipine-sensitive 45Ca2+ entry into depolarized chick skeletal muscle cells in culture. Depolarization by itself opens these Ca2+ channels (3). The effects of isoproterenol were additive to those of depolarization. Alprenolol, a potent β-adrenergic antagonist, prevented the effects of isoproterenol were additive to those of depolarization. Alprenolol, a potent β-adrenergic antagonist, prevented the effects of isoproterenol on 45Ca2+ uptake (Fig. 1). The half-maximum inhibition of 45Ca2+ influx induced by depolarization or by depolarization plus isoproterenol was observed at the same concentration of nitrendipine, (Kd0.5 = 1 nM). This Kd0.5 value is very similar to the dissociation constant of 0.4 nM previously found for specific [3H]nitrendipine binding to membranes from chick skeletal muscle cells differentiated in vitro and in vivo (3) (of course, isolated membranes are depolarized). The increased Ca2+-channel activity due to the short term effect of β-adrenergic agonists on skeletal muscle cells seems to occur by the same mechanism as that found for cardiac cells, i.e. the effect appears to be mediated via cAMP. Furthermore, treatments that resulted in an increased level of cAMP in cultured skeletal muscle cells, such as those caused by an inhibition of phosphodiesterase activity with MIX and theophylline, also resulted in an increased rate of 45Ca2+ entry via nitrendipine-sensitive Ca2+ channels (Fig. 2). Similar results were obtained with analogues of cAMP such as BtZcAMP. All these compounds caused results similar to those observed with isoproterenol.

Isoproterenol at a concentration of 1–3 μM caused an increase in the contractile force of isolated tibia skeletal muscle fibers from 5-day-old chicks. The development of the tension phase was faster than in the absence of isoproterenol and the peak tension was increased by 30–45% (not shown).

In addition to their short term effects, β-adrenergic agents also exerted long term effects. The long term effects may be directly related to the changes in [3H]nitrendipine-binding properties that occur in vivo near hatching (3). Binding characteristics of [3H]nitrendipine at two stages of development are summarized Table III. The postnatal form of nitrendipine receptors is characterized by a high binding capacity (880 fmol/mg protein) and a Kd value of 1.8 nM (3), whereas the fetal form is characterized by lower binding capacity (130 fmol/mg protein) and a Kd receptor antagonist of 0.4 nM (3). The hypothesis, suggesting a role of neurotransmitters in putative neurotransmitter-sensitive Ca2+ channels via the β-adrenergic system during chick skeletal muscle development, has been tested in vivo. Two different chronic treatments, that affect the β-adrenergic system carried out on 7-day-old chicks, support this conclusion. The first one was with reserpine, a molecule that depletes norepinephrine storage and inhibits norepinephrine and epinephrine synthesis (28). The second treatment was with alprenolol, a well-known β-adrenergic receptor blocking agent. Both treatments will of course suppress, or at least strongly decrease, β-adrenergic stimulation of the chick skeletal muscle cells. Reserpine decreased the number of receptors for [3H]nitrendipine by 64% and alprenolol by 40%. Both drugs also changed the affinity of the receptor for nitrendipine (Table III). The Kd values decreased by a factor of 4–5 in reserpine- and alprenolol-treated animals (Kd = 0.4 ± 0.1 nM) compared to control animals (Kd = 1.7 ± 0.1 nM). Therefore, skeletal muscle nitrendipine receptors in reserpine- and alprenolol-treated animals are more similar to “fetal” nitrendipine receptors than to the postnatal nitrendipine receptors. These results are clearly in favor of the conclusion that the physiological stimulation of β-adrenergic receptors regulate both the number of specific nitrendipine receptors and the affinity of the receptor for dihydropyridines. This conclusion is supported by the fact that if reserpine treatment was accompanied by repetitive isoproterenol injections then the reserpine effects on [3H]nitrendipine-binding properties were not seen (Table II). Although functional β-adrenergic receptors are present in chick skeletal myotubes in culture (27, 46), treatment of these cells with isoproterenol for periods of time shorter than 20 h modified neither Kd nor Bmax values for nitrendipine receptors. More prolonged treatments (30 h) with isoproterenol at concentrations higher than 1 μM induced an increase in Bmax values by a factor of 4 and a change in Kd values from 0.4 ± 0.1 nM in the control to 1.6 ± 0.2 nM in the isoproterenol-treated cells. The β-adrenergic antagonist alprenolol blocked the effects of the β-adrenergic agonist isoproterenol (Fig. 4). The long term β-adrenergic effects observed in vitro are clearly linked to changes in cytoplasmic levels of cAMP since they can be mimicked by inhibitors of phosphodiesterases such as MIX and theophylline, and by analogues of cAMP like BtZcAMP and 8-bromo-cAMP (Table I).

The results that are found here with skeletal muscle cells in culture have similarities with recent results obtained with different neuronal clonal cell lines (48). These cell lines have little or no voltage-sensitive Ca2+ channels under standard cell culture conditions. However, when their cellular level of cAMP is increased by using agents, like prostaglandin, BtZcAMP, forskolin, cholera toxin, etc., they acquire functional voltage-sensitive Ca2+ channels that can be blocked with dihydropyridines (47, 48). This effect of cAMP on the expression of voltage-sensitive Ca2+ channels in neurons has been associated with a possible role of cAMP in the regulation in synaptogenesis (47). Finally, it has been proposed that cAMP affects post-translational modifications of some species of glycoproteins (47), and it is now known that the dihydropyridine receptor from skeletal muscle is a glycoprotein since the solubilized receptor is retained on wheat germ agglutinin columns (18).

**Table III**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Kd</th>
<th>Bmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal (11 to 15 days in vivo)</td>
<td>0.4</td>
<td>130</td>
</tr>
<tr>
<td>Postnatal (7 days)</td>
<td>1.7–1.8</td>
<td>880–900</td>
</tr>
<tr>
<td>+ Reserpine</td>
<td>0.4</td>
<td>566</td>
</tr>
<tr>
<td>+ Alprenolol</td>
<td>0.4</td>
<td>530</td>
</tr>
</tbody>
</table>

**Acknowledgments**—We thank M. T. Ravier and N. Boyer for expert technical assistance, M. Valetti for skilful technical help, and Dr. M. Hovey for fruitful discussions and for a careful reading of the manuscript.

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β-Adrenergic Effects on Ca2+ Channels in Skeletal Muscle