Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 2858–2862, March 1994
Neurobiology

Functional dependence of Ca^{2+}-activated K^+ current on L- and N-type Ca^{2+} channels: Differences between chicken sympathetic and parasympathetic neurons suggest different regulatory mechanisms

(dihydropyridine/nifedipine/BAY K 8644/ω-conotoxin/perforated patch)

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Communicated by Lloyd M. Beidler, December 6, 1993 (received for review September 30, 1993)

ABSTRACT **The influx of Ca^{2+} ions controls many important processes in excitable cells, including the regulation of the gating of Ca^{2+}-activated K^+ channels (the current $I_{K[Ca]}$). Various $I_{K[Ca]}$ channels contribute to the regulation of the action-potential waveform, the repetitive discharge of spikes, and the secretion of neurotransmitters. It is thought that large-conductance $I_{K[Ca]}$ channels must be closely colocalized with Ca^{2+} channels (I_{Ca}) to be gated by Ca^{2+} influx. We now report that $I_{K[Ca]}$ channels can be preferentially colocalized with pharmacologically distinct subtypes of voltage-activated Ca^{2+} channel and that this occurs differently in embryonic chicken sympathetic and parasympathetic neurons. The effects of various dihydropyridines and ω-conotoxin on voltage-activated Ca^{2+} currents (I_{Ca}) and Ca^{2+}-activated K^+ currents ($I_{K[Ca]}$) were examined by using perforated-patch whole-cell recordings from embryonic chicken ciliary and sympathetic ganglion neurons. Application of nifedipine or ω-conotoxin each caused a 40–60% reduction in I_{Ca}, whereas application of *S*-(−)-BAY K 8644 potentiated I_{Ca} in ciliary ganglion neurons. But application of ω-conotoxin had little or no effect on $I_{K[Ca]}$, whereas nifedipine and *S*-(−)-BAY K 8644 inhibited and potentiated $I_{K[Ca]}$, respectively. These results indicate that $I_{K[Ca]}$ channels are preferentially coupled to L-type, but not to N-type, Ca^{2+} channels on chicken ciliary ganglion neurons. Chicken sympathetic neurons also express dihydropyridine-sensitive and ω-conotoxin-sensitive components of I_{Ca}. However, in those cells, application of ω-conotoxin caused a 40–60% reduction in $I_{K[Ca]}$, whereas nifedipine reduced $I_{K[Ca]}$ but only in a subpopulation of cells. Therefore, $I_{K[Ca]}$ in sympathetic neurons is either coupled to N-type Ca^{2+} channels or is not selectively coupled to a single Ca^{2+}-channel subtype. The preferential coupling of $I_{K[Ca]}$ channels with distinct I_{Ca} subtypes may be part of a mechanism to allow for selective modulation of neurotransmitter release. Preferential coupling may also be important for the differentiation and development of vertebrate neurons.**

Most neurons express K^+ currents whose gating is controlled primarily by changes in the intracellular concentration of free Ca^{2+} ions ($I_{K[Ca]}$) (1, 2). In most cells, these currents are eliminated by removal of extracellular Ca^{2+} or by application of agents that block the influx of Ca^{2+} ions. Parasympathetic neurons of the chicken ciliary ganglion also express $I_{K[Ca]}$ channels that depend primarily on Ca^{2+} influx through voltage-activated Ca^{2+} channels for their activation (3). In ciliary ganglion neurons, as in many other cells, the various components of $I_{K[Ca]}$ contribute to the repolarization of the action potential, the amplitude and duration of the hyperpolarizing afterpotential, and to the regulation of repetitive firing (3, 4).

It has been proposed that for large-conductance $I_{K[Ca]}$ channels to be activated by Ca^{2+} influx through voltage-activated Ca^{2+} channels, $I_{K[Ca]}$ channels must be located close to Ca^{2+} channels in the plasma membrane. This is because, in most cases, large-conductance $I_{K[Ca]}$ channels have a relatively low sensitivity to activation by Ca^{2+}, often requiring micromolar concentrations for significant activity (1, 2). In vertebrate hair cells, functional and ultrastructural evidence suggests that large-conductance $I_{K[Ca]}$ and Ca^{2+} channels cluster together at presynaptic active zones (5). In U cells of *Helix* neurons, where $I_{K[Ca]}$ is involved in spike repolarization (6), Gola and Crest (7) found that those large-conductance $I_{K[Ca]}$ channels that were *not* colocalized with Ca^{2+} channels remained silent during action potential firing. These quiescent $I_{K[Ca]}$ channels could only be detected after intracellular injection of Ca^{2+} or after excision of inside-out patches of the plasma membrane. Several investigators (8–11) have proposed various Ca^{2+} microdomain hypotheses in which regions of elevated free Ca^{2+} are highly localized to the immediate vicinity of individual Ca^{2+} channels and in which there is very little change in the bulk Ca^{2+} concentration of the cytosol. Each of these schemes would predict that large-conductance $I_{K[Ca]}$ channels must be located within Ca^{2+} microdomains to be activated by Ca^{2+} influx.

Many excitable cells express more than one class of voltage-activated Ca^{2+} channel (for review, see ref. 12). The different classes of Ca^{2+} channels can be distinguished by differences in their voltage-dependence, kinetics, and sensitivity to various pharmacological agents. For example, L-type Ca^{2+} currents are inhibited or, in some cases, potentiated by various dihydropyridine compounds (DHPs). By contrast, N-type Ca^{2+} currents are relatively insensitive to DHPs but are instead blocked by low concentrations of the venom neurotoxin ω-conotoxin (ω-CgTX). Many cell types express both of these classes of Ca^{2+} currents (I_{Ca}), and in some cells, specific Ca^{2+}-channel subtypes have been shown to have different physiological functions. For example, in rat sympathetic neurons, the N-type Ca^{2+} channels play a dominant role in the Ca^{2+}-dependent release of norepinephrine (13). However, in both rat (14) and chicken (15) sensory neurons, it is the L-type Ca^{2+} channels that are essential for neurotransmitter release. Moreover, in some cells containing both L- and N-type Ca^{2+} channels, it is possible to modulate the channels independently by different G-protein-dependent mechanisms (for review, see ref. 16).

Chicken ciliary ganglion neurons also express more than one component of voltage-activated Ca^{2+} current (17–19).

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Abbreviations: $I_{K[Ca]}$, K^+ current gated by Ca^{2+}; I_{Ca}, Ca^{2+} current(s); DHP, dihydropyridine compound; ω-CgTX, ω-conotoxin.
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The first component is sensitive to DHPs, whereas the second is blocked by ω-CgTX. These components of I_{Ca} therefore correspond to the L- and N-type Ca^{2+} channels described in other cell types. Results to be described below indicate that chicken lumbar sympathetic neurons also express DHP-sensitive and ω-CgTX-sensitive components of I_{Ca}. In ciliary ganglion neurons, the two components of I_{Ca} appear to have different physiological functions. For example, at the time of hatching, the K^+-evoked release of acetylcholine is preferentially sensitive to ω-CgTX, whereas the release of somatostatin is exclusively sensitive to DHPs (20). Moreover, the pharmacology of Ca^{2+}-dependent acetylcholine release changes during embryonic development (21). Early in development, at embryonic day 8, acetylcholine release is insensitive to blockade by either DHPs or by ω-CgTX. However, by embryonic day 14 acetylcholine release is sensitive to both DHPs and ω-CgTX, and after hatching acetylcholine release is sensitive only to ω-CgTX.

The purpose of the present study was to determine the roles of multiple Ca^{2+}-channel subtypes in the regulation of $I_{K[Ca]}$ in sympathetic and ciliary ganglion neurons of the chicken embryo. The results indicate that in chicken ciliary ganglion neurons, $I_{K[Ca]}$ preferentially depends on Ca^{2+} influx through L-type but not through N-type Ca^{2+} channels. But in chicken sympathetic neurons, N-type Ca^{2+} channels and in some cases, both types of Ca^{2+} channels are functionally linked to $I_{K[Ca]}$. This result suggests that the preferential colocalization of different types of ionic channels is an important factor in the regulation of the electrophysiological properties of vertebrate neurons and that this process is regulated differently in different populations of neurons.

MATERIALS AND METHODS

Cell Isolation. Neurons were isolated acutely as described (3). Briefly, chicken lumbar sympathetic or ciliary ganglia were obtained from embryos on embryonic days 12–19. Because these ganglia develop at different times, ciliary ganglia were used at embryonic days 12–14, and sympathetic ganglia were used at embryonic days 16–19. Ganglia were dissected and placed in a saline nominally free of divalent cations and containing collagenase at 1 mg/ml (Sigma type II). Ganglia were incubated at 37°C for 10–30 min. The collagenase was removed by aspiration, and the ganglia were rinsed once in a cell-culture medium consisting of Eagle's minimal essential medium/10% heat-inactivated horse serum/2 mM glutamine/penicillin at 50 units/ml/streptomycin at 50 μg/ml. The ganglia were then resuspended in cell-culture medium and triturated with 8–12 passes through a fire-polished Pasteur pipette. Dissociated neurons were subsequently plated onto poly(D-lysine)-coated coverslips and allowed to settle for at least 30 min. Neurons were used within 4 hr of plating, at which time the cells were essentially free of neurites.

Perforated-Patch Whole-Cell Recordings. Perforated-patch whole-cell recordings were made essentially as described by Horn and Marty (22) using a commercially available patch-clamp amplifier (Axopatch 1C; Axon Instruments, Foster City, CA). Pipettes were pulled in two steps from hard borosilicate glass (Boralex; Rochester Scientific), coated to within 100 μm of the tip with Sylgard resin, and fire polished. For recordings of K^+ currents, pipette tips were filled by immersion in a solution consisting of 55 mM KCl, 70 mM K_2SO_4, 7 mM $MgCl_2$, 10 mM Hepes·KOH, and 5 mM glucose (pH 7.35). For recordings of Ca^{2+} currents, the pipette tips were filled with a solution containing 125 mM CsCl, 7 mM $MgCl_2$, 10 mM Hepes·NaOH, and 5 mM glucose (pH 7.35). Pipettes were then backfilled with the same solutions containing nystatin at 150–250 μg/ml prepared fresh from stock solutions within 2 hr of use. After formation of a stable cell-attached patch, nystatin molecules are incorporated into the plasma membrane allowing for intracellular contact within 5–20 min. In useful recordings the access resistance was 10–20 MΩ. In many cases, it was possible to compensate up to 90% of this series resistance without introducing oscillations into the recordings, but when this was not possible, the cell was abandoned. Normal external salines consisted of 145 mM NaCl, 5.3 mM KCl, 5.4 mM $CaCl_2$, 0.8 mM $MgCl_2$, 13 mM Hepes, and 5.6 mM glucose (pH 7.4). In all experiments, 250 nM tetrodotoxin was present to block voltage-activated Na^+ currents. For Ca^{2+}-free salines, all external Ca^{2+} was replaced on an equimolar basis with $MgCl_2$. For recordings of I_{Ca}, 10 mM tetraethlyammonium and 5 mM 4-aminopyridine were added to the external saline to block K^+ currents. Cells were mounted in a recording chamber with a total volume of 500 μl and superfused at a rate of 3–5 ml/min. All drugs and chemicals were obtained from Sigma except ω-CgTX (lot CB 492B) and *S*-(−)-BAY K 8644 (lot TC 1092), which were obtained from Research Biochemicals (Natick, MA). In some experiments, ω-CgTX (2 μM) was applied by whole-bath perfusion. In other experiments, ω-CgTX (20 μM) was delivered to the cell by pressure injection [2 psi (1 psi = 6.9 kPa)] from a micropipette positioned to within 50 μm of the cell. No differences were observed with these two methods of ω-CgTX application. The effects of ω-CgTX were irreversible during the course of these experiments. DHPs were always applied by whole-bath perfusion.

Data Analysis. Whole-cell currents were filtered with a 4-pole Bessel filter at 2–5 kHz before analysis. Data were digitized on-line and stored on magnetic hard discs. Voltage commands and computer acquisition of data were performed with PCLAMP version 5.1 (Axon Instruments), a Tecmar Labmaster 12-bit, 125-kHz A/D board, and a personal computer as described elsewhere (3, 23). Currents dependent on external Ca^{2+} ions were obtained by digital subtraction using PCLAMP software, as described (3, 23, 24). With CsCl pipettes, Ca^{2+}-dependent currents are caused by I_{Ca}, but with KCl in the pipette, Ca^{2+}-dependent currents are due to both I_{Ca} and $I_{K[Ca]}$. However, in these cells, $I_{K[Ca]}$ was invariably much larger than I_{Ca}.

RESULTS

Ciliary Ganglion Neurons Express L-Type and N-Type Ca^{2+} Currents. We have confirmed previous observations indicating that ciliary ganglion neurons express multiple subtypes of high-threshold Ca^{2+} currents (17–19). Fig. 1 shows Ca^{2+} currents evoked by a depolarizing voltage step to 0 mV from a holding potential of −40 mV. This voltage protocol evoked a large I_{Ca} that showed very little tendency to inactivate during the course of a maintained 25-ms depolarization. Application of 10 μM nifedipine caused a significant blockade of I_{Ca} (Fig. 1*A*). This concentration appeared to be saturating, as higher concentrations did not produce any additional reduction in I_{Ca} (data not shown). Similar results were obtained in each of 11 other cells tested, with the percentage blockade ranging from 30 to 60% (44% ± 11%, mean ± SEM). The effects of nifedipine were fully reversible (data not shown). Application of 2 μM ω-CgTX also caused a substantial and consistent blockade of I_{Ca} as shown in a different cell (Fig. 1*B*). Similar results were obtained in nine other cells with typical blockade ranging from 40 to 80% (mean 55% ± 12%). Higher concentrations of nifedipine or ω-CgTX did not produce additional blockade of I_{Ca}, indicating that the concentrations used in the present experiments were saturating. Application of both blockers to the same neuron caused a very large blockade of I_{Ca} in all cells tested (Fig. 1*C*). In this example, nifedipine again blocked a large portion of I_{Ca}, and the remaining Ca^{2+} current was nearly eliminated by ω-CgTX. The order of application of the drugs did not affect the results. As described (17), as much as 10% of the

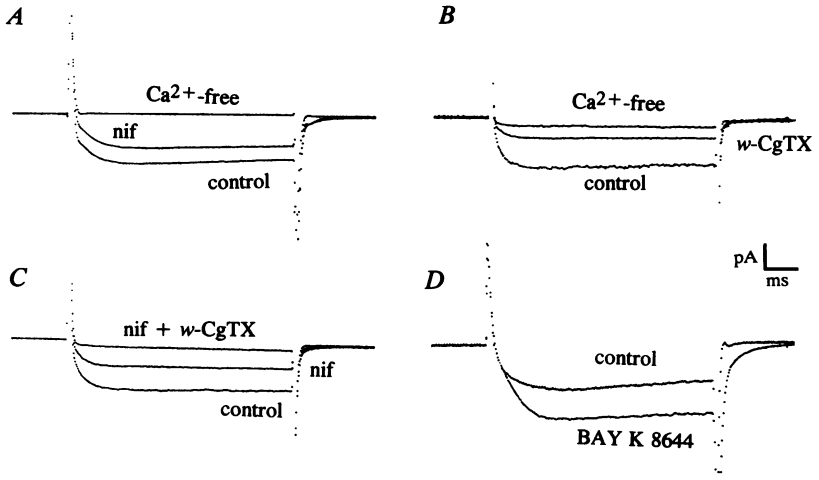

FIG. 1. Multiple pharmacologically distinct components of I_{Ca} in acutely isolated chicken ciliary ganglion neurons. Ca^{2+} currents were evoked by a depolarizing voltage step to 0 mV from a holding potential of −40 mV. Voltage- and Ca^{2+}-activated K^+ currents were eliminated by the use of CsCl recording pipettes and by addition of tetraethylammonium and 4-aminopyridine to the bath saline. All records shown are the average of six traces. (*A*) I_{Ca} evoked in normal saline (control), normal saline containing 10 μM nifedipine (nif), and in Ca^{2+}-free saline (Ca^{2+}-free). (*B*) I_{Ca} evoked in normal saline (control), normal saline containing 2 μM ω-CgTX, and in Ca^{2+}-free saline (Ca^{2+}-free). (*C*) Effects of application of nifedipine and ω-CgTX to the same cell. Currents were evoked in normal saline (control), normal saline containing 10 μM nifedipine (nif), and nifedipine-containing saline after application of 2 μM CgTX (nif + ω-CgTX). (*D*) I_{Ca} evoked in normal saline (control) and in control saline containing 1 μM *S*-(−)-BAY K 8644. [Bar = 200 pA, 4 ms (*A*, *C*, and *D*) and 140 pA, 3 ms (*B*).]

sustained inward current remained in some cells after exposure to both nifedipine and ω-CgTX. This residual current was abolished by application of Ca^{2+}-free salines (data not shown). In other experiments, we found that application of the L-type Ca^{2+} channel agonist *S*-(−)-BAY K 8644 (1 μM) caused a reversible potentiation of I_{Ca} (Fig. 1*D*). Thus ciliary ganglion neurons express a DHP-sensitive, ω-CgTX-insensitive L-type Ca^{2+} current and a ω-CgTX-sensitive, DHP-insensitive N-type Ca^{2+} current. In many cells, a small residual sustained inward Ca^{2+} current was present that did not fit into either of these groups.

$I_{K[Ca]}$ Channels Are Functionally Linked to L-Type Ca^{2+} Channels in Ciliary Ganglion Neurons. A different picture was observed when we examined the effects of the DHPs and ω-CgTX on $I_{K[Ca]}$. Fig. 2 shows Ca^{2+}-activated K^+ currents evoked by a depolarizing step to 0 mV from a holding potential of −40 mV. From this holding potential, there is minimal activation of voltage-activated K^+ currents (3) and substantial activation of I_{Ca} (as shown above) and $I_{K[Ca]}$. We had expected that blockade of a significant amount of I_{Ca} would reduce $I_{K[Ca]}$, and, in fact, application of 10 μM nifedipine caused a significant blockade of $I_{K[Ca]}$ in nine out of nine cells tested (Fig. 2*A*). The mean blockade of $I_{K[Ca]}$ caused by nifedipine was 57% ± 9%. In addition, application of 1 μM *S*-(−)-BAY K 8644 potentiated $I_{K[Ca]}$ (Fig. 2*B*), consistent with its actions on I_{Ca}. The opposite effects of these two structurally related DHPs suggest that their effects on $I_{K[Ca]}$ are caused by altered Ca^{2+} influx through L-type channels. This is an important point, as previous studies have shown that DHPs can cause a direct blockade of other voltage-activated ionic channels (25). By contrast, application of 2 μM ω-CgTX had little effect on $I_{K[Ca]}$ in 9 out of 11 ciliary ganglion neurons tested (Fig. 2*C*), even though it caused a significant reduction of I_{Ca}. The mean reduction of $I_{K[Ca]}$ caused by ω-CgTX was 7% ± 3%. In many cells, as in the example shown, ω-CgTX caused a slight increase in the net outward current due to inhibition of I_{Ca}. When nifedipine and ω-CgTX were applied to the same cell, ω-CgTX had little or no effect, whereas subsequent application of nifedipine produced a substantial blockade of $I_{K[Ca]}$ (Fig. 2*D*). These results indicate that the Ca^{2+} influx required for the gating of $I_{K[Ca]}$ channels in ciliary ganglion neurons is primarily through L-type and not through N-type Ca^{2+} channels. In addition, Ca^{2+} influx through some other type of high-threshold Ca^{2+} channel may play a role in the regulation

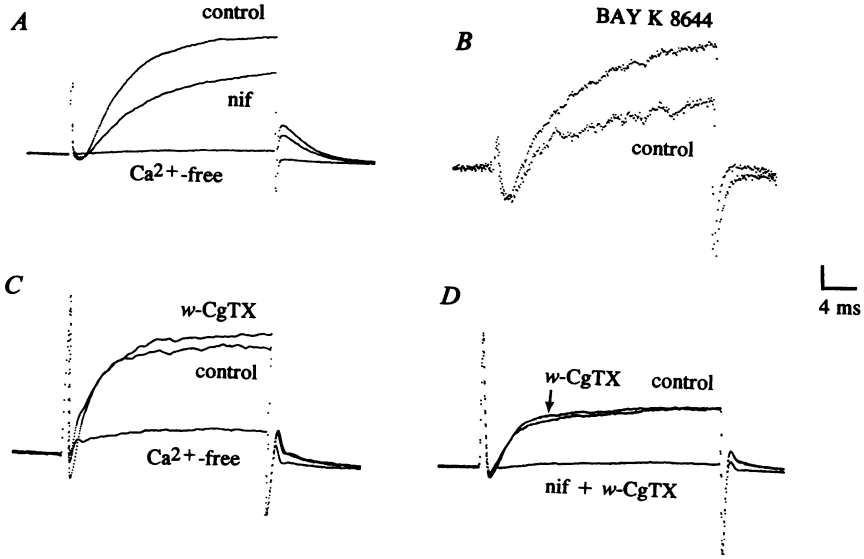

FIG. 2. Effects of agents affecting Ca^{2+} channels on whole-cell $I_{K[Ca]}$ in chicken ciliary ganglion neurons. Total whole-cell currents were evoked by a depolarizing voltage step to 0 mV from a holding potential of −40 mV. All records shown are the average of six traces. (*A*) Currents evoked in normal saline (control), normal saline containing 10 μM nifedipine (nif), and in Ca^{2+}-free saline (Ca^{2+}-free). (*B*) Currents evoked in normal saline (control) and in control saline containing 1 μM *S*-(−)-BAY K 8644. (*C*) Currents evoked in normal saline (control), normal saline containing 2 μM ω-CgTX, and in Ca^{2+}-free saline (Ca^{2+}-free). (*D*) Application of nifedipine and ω-CgTX to the same cell. Currents were evoked in normal saline (control), after application of ω-CgTX, and in saline containing nifedipine after application of ω-CgTX (nif + ω-CgTX). [Bar = 450 pA (*A*); 150 pA (*B*); and 400 pA (*C* and *D*).]

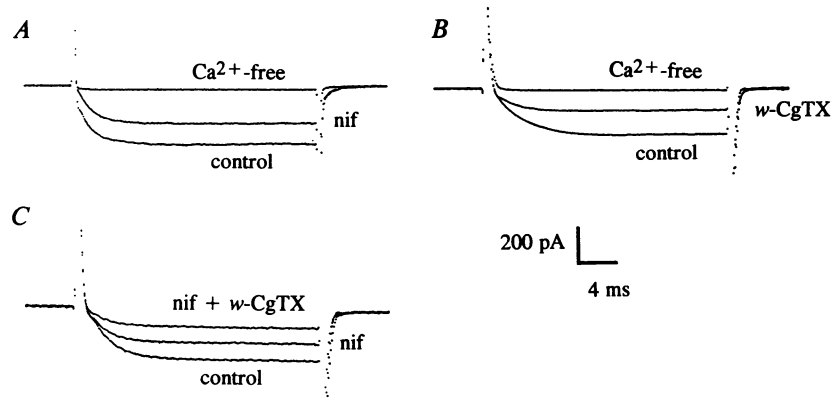

FIG. 3. Multiple pharmacologically distinct components of I_{Ca} in acutely isolated chicken sympathetic neurons. I_{Ca} was evoked by a depolarizing voltage step to 0 mV from a holding potential of −40 mV. All records shown are the average of six traces. (*A*) I_{Ca} evoked in normal saline (control), normal saline containing 10 μM nifedipine (nif), and in Ca^{2+}-free saline (Ca^{2+}-free). (*B*) I_{Ca} evoked in normal saline (control), normal saline containing 2 μM ω-CgTX, and in Ca^{2+}-free saline (Ca^{2+}-free). (*C*) Effects of nifedipine and ω-CgTX applied to the same cell. Currents were evoked in normal saline (control), normal saline containing 10 μM nifedipine (nif), and in nifedipine-containing saline after application of ω-CgTX (nif + ω-CgTX). Note that in this cell, some I_{Ca} remained after exposure to a combination of nifedipine and ω-CgTX.

of $I_{K[Ca]}$ gating, as nifedipine does not cause a complete blockade of $I_{K[Ca]}$ in most cells.

Sympathetic Ganglion Neurons Also Express N-Type and L-Type Ca^{2+} Currents. Voltage-activated Ca^{2+} currents in chicken lumbar sympathetic ganglion neurons are similar to those of ciliary ganglion neurons. Fig. 3 shows Ca^{2+} currents evoked as described above. Application of 10 μM nifedipine caused a partial blockade of I_{Ca} (Fig. 3*A*), as did application of 2 μM ω-CgTX (Fig. 3*B*) in all sympathetic neurons tested. Nifedipine caused a 30–60% blockade (mean, 39% ± 7%, n = 8 cells), whereas ω-CgTX caused a blockade of 30–80% (mean, 41% ± 12%, n = 7 cells). When both nifedipine and ω-CgTX were applied to the same cell, most of the Ca^{2+} current was blocked, although in many cells a small component of the sustained inward current remained (Fig. 3*C*). This residual current was abolished by Ca^{2+}-free salines (data not shown). Therefore, sympathetic neurons also express a DHP-sensitive L-type Ca^{2+} current, a ω-CgTX-sensitive N-type Ca^{2+} current, and, in some cells, a small residual inward current that is resistant to both blockers.

Both L-Type and N-Type Ca^{2+} Channels Are Linked to $I_{K[Ca]}$ Channels in Sympathetic Neurons. Fig. 4 shows $I_{K[Ca]}$ evoked in chicken sympathetic neurons as described above. Application of 10 μM nifedipine caused a partial blockade of $I_{K[Ca]}$ in 4 out of 11 cells tested but had no effect on outward currents in 7 out of 11 cells. An example of a neuron where nifedipine reduced $I_{K[Ca]}$ is shown in Fig. 4*A*, and an example where nifedipine was ineffective is shown in Fig. 4*B*. Mean blockade of $I_{K[Ca]}$ caused by nifedipine in all cells tested was only 12% ± 11%, but the mean blockade in the four cells that responded to nifedipine was 39% ± 7%. In sympathetic ganglion neurons, application of 2 μM ω-CgTX caused a significant blockade of $I_{K[Ca]}$ in seven out of eight cells tested, with a mean blockade of 41 ± 11% (Fig. 4*C*). In some cells, application of both drugs to the same cell caused large and additive reductions in $I_{K[Ca]}$ (Fig. 4*D*), but in others, nifedipine did not increase the blockade caused by ω-CgTX (data not shown). These results indicate that Ca^{2+} influx through N-type Ca^{2+} channels typically regulates the gating of $I_{K[Ca]}$ in chicken sympathetic neurons and that Ca^{2+} influx through L-type channels regulates $I_{K[Ca]}$ in a subpopulation of cells.

DISCUSSION

These experiments show that in chicken ciliary ganglion neurons, the L-type Ca^{2+} channels are preferentially coupled to $I_{K[Ca]}$, although both L-type and N-type Ca^{2+} channels are present. By contrast, in sympathetic neurons, N-type Ca^{2+} channels and in some cells both types of Ca^{2+} channels are functionally linked to $I_{K[Ca]}$. Previous studies have suggested that in order for large-conductance $I_{K[Ca]}$ channels to be activated by Ca^{2+} influx, $I_{K[Ca]}$ channels and Ca^{2+} channels must be closely colocalized within the plasma membrane (5, 7). Therefore, our results suggest that at least some vertebrate neurons have the capacity to preferentially colocalize $I_{K[Ca]}$ channels with specific Ca^{2+}-channel subtypes. It should be noted that the interpretation of these results depends on the specificity of the DHPs and ω-CgTX for Ca^{2+} channels. There are no reports or indications that ω-CgTX can cause a direct blockade of K^+ channels, and, indeed, this toxin had no obvious effect on whole-cell $I_{K[Ca]}$ in ciliary ganglion neurons. However, nifedipine has been reported to cause direct blockade of K^+ channels and Na^+ channels in bullfrog sympathetic neurons (25). But nifedipine had no effects on outward currents in the majority of sympathetic ganglion neurons examined in the present study. Moreover, we have also examined the effects of the structurally related DHP *S*-(−)-BAY K 8644. This DHP caused potentiation of both I_{Ca} and $I_{K[Ca]}$ in ciliary

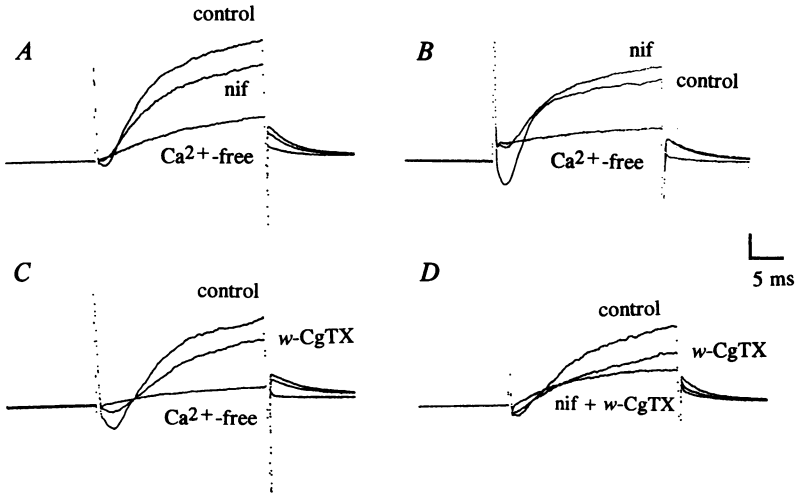

FIG. 4. Effects of Ca^{2+}-channel blockers on $I_{K[Ca]}$ in chicken sympathetic neurons. Whole-cell currents were evoked by a depolarizing voltage step to 0 mV from a holding potential of −40 mV. All records shown are the average of six traces. (*A*) Currents evoked in normal saline (control), normal saline containing 10 μM nifedipine (nif), and in Ca^{2+}-free saline (Ca^{2+}-free). (*B*) Currents evoked in a different sympathetic neuron in normal saline (control), normal saline containing 10 μM nifedipine (nif), and in Ca^{2+}-free saline (Ca^{2+}-free). (*C*) Currents evoked in normal saline (control), normal saline containing 2 μM ω-CgTX, and in Ca^{2+}-free saline (Ca^{2+}-free). (*D*) Effects of nifedipine and ω-CgTX applied to the same cell. Currents were evoked in normal saline (control), normal saline containing 2 μM ω-CgTX, and in nifedipine-containing saline after application of 2 μM CgTX (nif + ω-CgTX). [Bar = 200 pA (*A* and *C*); 300 pA (*B*); and 180 pA (*D*)].

ganglion neurons; this result strongly suggests that the effects of nifedipine are not caused by direct blockade of K^+ channels but are, instead, due to altered Ca^{2+} influx.

The finding that $I_{K[Ca]}$ channels can be preferentially colocalized with specific Ca^{2+}-channel subtypes has several consequences for the functional development of vertebrate neurons. For example, in chicken ciliary ganglion neurons, whole-cell $I_{K[Ca]}$ cannot be evoked by depolarizing voltage steps early in embryonic development or in neurons that develop *in vitro* (24). However, nifedipine-sensitive L-type Ca^{2+} currents are present in these cells. Moreover, preliminary results indicate that $I_{K[Ca]}$ channels are present in excised membrane patches of chicken autonomic neurons at early developmental stages or after development *in vitro* (S. Raucher and S. E. Dryer, unpublished data). Therefore, it is possible that under those conditions, both L-type Ca^{2+} channels and $I_{K[Ca]}$ channels are present in the membrane but are not properly colocalized. In other words, colocalization of these ionic channels may itself be a developmentally regulated phenomenon.

What is the functional significance of this preferential coupling of ionic channels? One possibility is that it could be part of a mechanism to provide for more selective modulation of neurotransmitter secretion. For example, in those chicken sympathetic cells, in which both N- and L-type Ca^{2+} channels are linked to $I_{K[Ca]}$ channels, a reduction of current through either Ca^{2+}-channel subtype would lead to a reduction in $I_{K[Ca]}$ and thus to broadening of the action potential. Although the rate of Ca^{2+} entry will be reduced (due to the Ca^{2+}-channel blockade), the unblocked channels will remain open longer due to the spike broadening. In this case, the total Ca^{2+} influx per spike may not change significantly. Indeed, at frog neuromuscular junctions, inhibition of $I_{K[Ca]}$ causes broadening of the spike and increases in neurotransmitter release (26). Thus, in many sympathetic neurons, modulation of Ca^{2+} current *by itself* may have a minimal effect on neurotransmitter release. In those cells, presynaptic inhibition may require simultaneous inhibition of Ca^{2+} channels and a concurrent potentiation of K^+ channels. Ciliary ganglion neurons represent a different situation; in those cells, inhibition of the N-type Ca^{2+} current would have little or no effect on $I_{K[Ca]}$ or on spike repolarization and would, therefore, result in a significant decrease in neurotransmitter release, as is observed (21). But inhibition of the L-type Ca^{2+} current would reduce $I_{K[Ca]}$, resulting in broadening of the spike. Under these conditions, one might expect neurotransmitter release to be relatively unaffected. This result is supported by the observation that the K^+-evoked release of acetylcholine from posthatched chicken ciliary ganglion neurons is unaffected by inhibitors of L-type Ca^{2+} channels (21). There are many examples of modulation of L- and N-type Ca^{2+} channels in vertebrate neurons by neurotransmitters acting through G-protein-coupled receptors. Moreover, in some cells, these Ca^{2+} channels can be modulated independently (16). It would be interesting to know whether there is preferential coupling of a given Ca^{2+}-channel subtype to $I_{K[Ca]}$ in those cases and whether the inhibitory neurotransmitters also modulate K^+ channels involved in spike repolarization.

The recordings in the present study were made from the cell bodies of embryonic neurons where one would not necessarily expect to find significant neurotransmitter release under physiological conditions. However, the functional coupling of I_{Ca} and $I_{K[Ca]}$ channels may be important for other processes that are not directly related to synaptic transmission. For example, voltage-sensitive Ca^{2+} channels are known to regulate several aspects of the normal embryonic development of neurons, including the regulation of gene expression (27), the initiation and guidance of neurite outgrowth (28, 29), the choice of neurotransmitter phenotype (30, 31), and the dependence of embryonic neurons on neurotrophic factors (32–34). Therefore, the functional coupling of I_{Ca} and $I_{K[Ca]}$ is a potentially important regulator of neuronal differentiation and development.

In summary, we have found that $I_{K[Ca]}$ is preferentially coupled to L-type channels but not to N-type channels in embryonic chicken parasympathetic neurons. $I_{K[Ca]}$ is functionally linked to N-type Ca^{2+} channels and, in some cases, to both types of Ca^{2+} channels, in chicken sympathetic neurons. The functional colocalization of I_{Ca} and $I_{K[Ca]}$ channels may be an important regulatory mechanism in developing and mature vertebrate neurons.

Dr. Sanja Raucher participated in some of these experiments. This work was supported by National Institutes of Health Grant NS-27013.

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