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Activation of K^+ Channels by Lanthanum Contributes to the Block of Transmitter Release in Chick and Rat Sympathetic Neurons

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Summary. We studied the effects of lanthanum (La^{3+}) on the release of ^{3}H-norepinephrine (^{3}H-NE), intracellular Ca^{2+} concentration, and voltage clamped Ca^{2+} and K^+ currents in cultured sympathetic neurons. La^{3+} (0.1 to 10 μM) produced concentration-dependent inhibition of depolarization induced Ca^{2+} influx and ^{3}H-NE release. La^{3+} was more potent and more efficacious in blocking ^{3}H-NE release than the Ca^{2+}-channel blockers cadmium and verapamil, which never blocked more than 70% of the release. At 3 μM, La^{3+} produced a complete block of the electrically stimulated rise in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in the cell body and the growth cone. The stimulation-evoked release of ^{3}H-NE was also completely blocked by 3 μM La^{3+}. However, 3 μM La^{3+} produced only a partial block of voltage clamped Ca^{2+} current (I_{Ca}). Following La^{3+} (10 μM) treatment ^{3}H-NE release could be evoked by high K^+ stimulation of neurons which were refractory to electrical stimulation. La^{3+} (1 μM) increased the hyperpolarization activated, 4-aminopyridine (4-AP) sensitive, transient K^+ current (I_A) with little effect on the late outward current elicited from depolarized holding potentials. We conclude that the effective block of electrically stimulated ^{3}H-NE release is a result of the unique ability of La^{3+} to activate a stabilizing, outward K^+ current at the same concentration that it blocks inward Ca^{2+} current.

Key Words ion channels · Ca^{2+} transients · lanthanum · norepinephrine release · neuronal cultures

Introduction

Lanthanum (La^{3+}) was one of the first inorganic agents found to block the Ca^{2+}-dependent, presynaptic response at the neuromuscular junction (Miledi, 1966; 1971) and has since been shown to be an effective inhibitor of neurotransmitter release (Kirpekar et al., 1972; Wakade, 1981). This action is ascribed to La^{3+} block of Ca^{2+} channels. Indeed, La^{3+} was found to be the most potent inorganic cation to block Ca^{2+} channels in mouse neuroblastoma cells, exhibiting an apparent K_d of 1.5 and 0.9 μM for high and low voltage activated Ca^{2+} channels, respectively (Narahashi, Tsunoo & Yoshii, 1987).

In the present work we examine the mechanisms of La^{3+} blockade of stimulation-evoked ^{3}H-norepinephrine (^{3}H-NE) release in cultured sympathetic neurons of embryonic chick and neonatal rat. The effect of La^{3+} on neuronal Ca^{2+} was determined by monitoring intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) using fluorescent dye techniques and by measuring transmembrane ion fluxes under voltage clamp. We demonstrate for the first time that La^{3+} has the unique ability to enhance the hyperpolarization activated, transient outward K^+ current (I_A) at the same concentration that it inhibits inward Ca^{2+} current. The consequence of these two ionic effects is additive and accounts for the potent and long lasting block of stimulation-evoked ^{3}H-NE release by La^{3+} in cultured sympathetic neurons.

Materials and Methods

Cell Culture and ^{3}H-NE Release

Sympathetic neurons (75,000 cells/dish) derived from sympathetic ganglia of 10-day-old chick embryo (Wakade, Edgar & Theonen, 1982) or from the superior cervical ganglia (SCG) of newborn rats were used after 2 days in culture. Neurons were loaded with ^{3}H-NE (3 μCi/ml Krebs solution, 43.9 Ci/mmol) for 60 min in a CO_2 incubator. After 60 min washout, samples were collected, over 2 min periods, in the control medium to establish spontaneous release of ^{3}H-NE. Neurons were stimulated at 1 Hz for 30 sec (120 mA, 1.0 msec duration applied via platinum wire electrodes connected to a Grass stimulator) to determine evoked ^{3}H-NE release. Samples were then collected before and after stimulation in the presence of the indicated concentration of La^{3+}. Each concentration of La^{3+} was in contact with neurons for 5 to 10 min before collection of samples. All concentrations were tested in the same culture dish. The control Krebs solution contained (in mM): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2

KH_2PO_4, 25 $NaHCO_3$, 11 glucose, adjusted to pH 7.3 with NaOH. For details of studies on ^{3}H-NE release from cultured neurons *see* Wakade and Wakade (1988).

Ca^{2+} Current Measurement

Peak voltage-elicited Ca^{2+} currents were recorded from the same neuron during control and the indicated La^{3+} treatment as described before (Przywara et al., 1991). Briefly, cover slips with attached sympathetic neurons were placed in a 1.5-ml superfusion chamber mounted on the stage of an inverted microscope. The bathing solution contained (in mM): 120 NaCl, 5 KCl, 1 $MgCl_2$, 5 $CaCl_2$, 10 HEPES, 10 glucose, 10 tetraethylammonium-Cl, and 200 nM tetrodotoxin (TTX), pH 7.3 with NaOH. Electrodes of 0.8 to 2 MΩ resistance contained (in mM): 100 CsCl, 5 $MgCl_2$, 10 EGTA, 2 Mg-ATP, 40 HEPES, pH 7.3 with CsOH. Cells were voltage clamped at room temperature using the patch-clamp technique (Hamill et al., 1981). Cells without extensive neurites were chosen to insure adequate voltage control. Cells exhibiting signs of inadequate space clamp (notches or latency at the leading edge of the current trace or long lasting tails after return to holding potential) were not used. Cells were tested for current rundown before exposure to test agents and only those showing stable currents were used. Drugs were added by superfusing 5 ml of desired solution through the bath. Inward currents were elicited when cells held at −70 mV were depolarized to 0 mV for 200 msec. The signal from the patch-clamp amplifier (EPC7, List-electronic, Darmstadt, West Germany) was filtered at 3 kHz through an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) and digitally stored and analyzed using a personal computer (TL-1-125 kHz DMA interface and pCLAMP software, Axon instruments, Foster City, CA).

K^+-Current Measurement

For recording whole-cell K^+ currents the external solution contained (in mM): 120 NaCl, 5 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 HEPES, and 10 glucose. 60 μM $CdCl_2$ and 200 nM TTX were included to inhibit outward currents. The pH was adjusted to 7.3 with NaOH. The pipette solution contained (in mM): 125 KCl, 1 $MgCl_2$, 10 EGTA, 2 Mg-ATP, 10 HEPES, pH 7.3 with KOH. Current-voltage (*I-V*) relations were determined from −90 or −80 mV and −40 mV holding potentials by sequential 10-mV step depolarizations of 200 msec duration. 20 sec was allowed between each test depolarization.

Intracellular Free Ca^{2+}-Concentration

$[Ca^{2+}]_i$ was measured at rest and during electrical stimulation (*see above*) in control and La^{3+}-containing solution as described earlier (Wakade et al., 1990; Przywara et al., 1991). Cell bodies and growth cones were selected to show that each area is capable of exhibiting a Ca^{2+} response to electrical stimulation. Sympathetic neurons cultured on the glass cover slips were loaded with 0.25 μM indo-1-acetoxymethyl ester for 60 min and washed two times with Krebs solution at 37°C. From this point on the cells were maintained in HEPES solution at room temperature. The cover slip was placed in a Leiden chamber and secured on the stage of an ACAS laser photometer (Meridian Instruments, East Lansing, MI). Neurons were illuminated by laser light of 340 to 360 nm and indo-1 fluorescence recorded at 405 nm (Ca^{2+}-bound) and 485 nm (Ca^{2+}-free) wavelengths. Ratioing (405/485 nm) was used to eliminate possible artifacts due to variations in thickness or indo-1 distribution in the cells (Grynkiewicz, Poenie & Tsien, 1985). $[Ca^{2+}]_i$ was monitored in the entire cell body or growth cone using the image analysis mode of the ACAS photometer. Simultaneous images at the two wavelengths were obtained over 10-sec scanning periods at rest and coincident with electrical stimulation (1 Hz for 10 sec). $[Ca^{2+}]_i$ was also monitored continuously across a section of the cell using the photometer line scan mode. There was no difference in the cytosolic $[Ca^{2+}]_i$ determined by the two methods. Quantitative data shown in Results were obtained from line scan analysis. A 10-μm line crossing the cytosolic region of the cell body or growth cone was scanned at 1 μm/msec with a 500 msec delay between the simultaneous dual-wavelength scans. This yielded an average (across the cell) Ca^{2+} value every 500 msec. Continuous line scanning was performed before, during and after electrical stimulation (5 Hz for 2 sec) to monitor the stimulated rise in $[Ca^{2+}]_i$ and recovery. The experiment was then repeated in the presence of La^{3+} with each cell used as its own control. Neutral density filtering reduced photobleaching and allowed reproducible responses from the same sites. $[Ca^{2+}]_i$ was calculated by computer from a standard curve of fluorescence ratio *vs.* free Ca^{2+} in Ca^{2+}-EGTA buffer. Recording and optical parameters were identical for cell bodies and growth cones except for scan strength of 10 to 20% and 40 to 60% for bodies and terminals, respectively.

Drugs Used

4-aminopyridine (4-AP, Sigma, St. Louis, MO); indo-1-acetoxymethyl ester (Molecular Probes, Eugene OR); lanthanum-Cl (Fischer, Fair Lawn, NJ); ^{3}H-norepinephrine (New England Nuclear, Boston, MA); tetraethylammonium-Cl (TEA, Eastman Kodak, Rochester, NY); tetrodotoxin (TTX, Calbiochem-Behring, La Jolla, CA); verapamil (Knoll Pharmaceutical, Whippany, NJ).

Results

Release of ^{3}H-NE was evoked by field stimulation of cultured chick sympathetic neurons preloaded with ^{3}H-NE (Wakade & Wakade, 1988). The effect of La^{3+} on ^{3}H-NE release was compared with the effect of other Ca^{2+} channel blockers. Figure 1 shows that La^{3+} caused a concentration-dependent inhibition of ^{3}H-NE release evoked by stimulation (1 Hz for 30 sec). Unlike the Ca^{2+}-channel blockers cadmium and verapamil, which produced a maximum blockade of 69 ± 5% (n = 8) and 68 ± 3% (n = 3), respectively, La^{3+} was able to completely inhibit the stimulation-evoked release of ^{3}H-NE. The full effect was manifested between 3 and 10 μM.

We found that the inhibitory effect of La^{3+} on ^{3}H-NE release was accompanied by an inhibition of the stimulation-induced rise in $[Ca^{2+}]_i$ determined by indo-1 fluorescence (Fig. 2). Our on-going studies have revealed differential effects of test agents on Ca^{2+} channels of the cell body versus those of

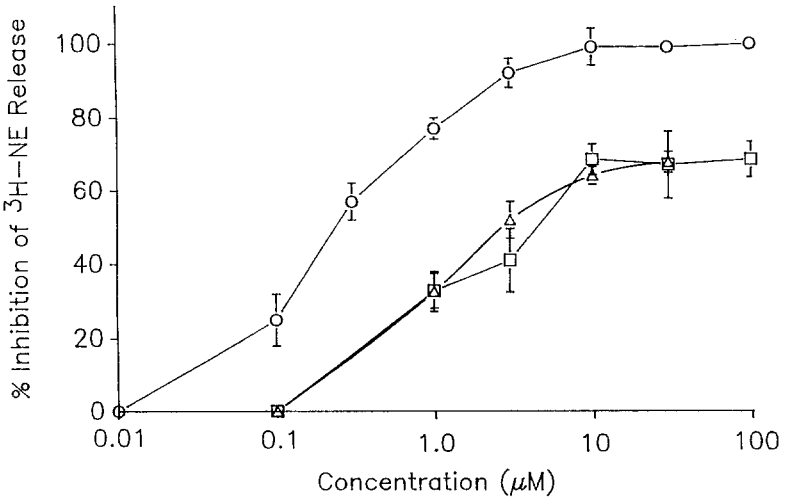

Fig. 1. La^{3+} is a potent and efficacious blocker of stimulated ^{3}H-NE release. Concentration-response curves for the block of electrically evoked (1 Hz for 30 sec) ^{3}H-NE release are shown for La^{3+} (circles), cadmium (squares) and verapamil (triangles) in chick sympathetic neurons. None of the drugs affected spontaneous background release of ^{3}H-NE. Each point represents the mean of three to eight experiments. Vertical bars show SEM. Error bars <10% of the symbol size are not shown.

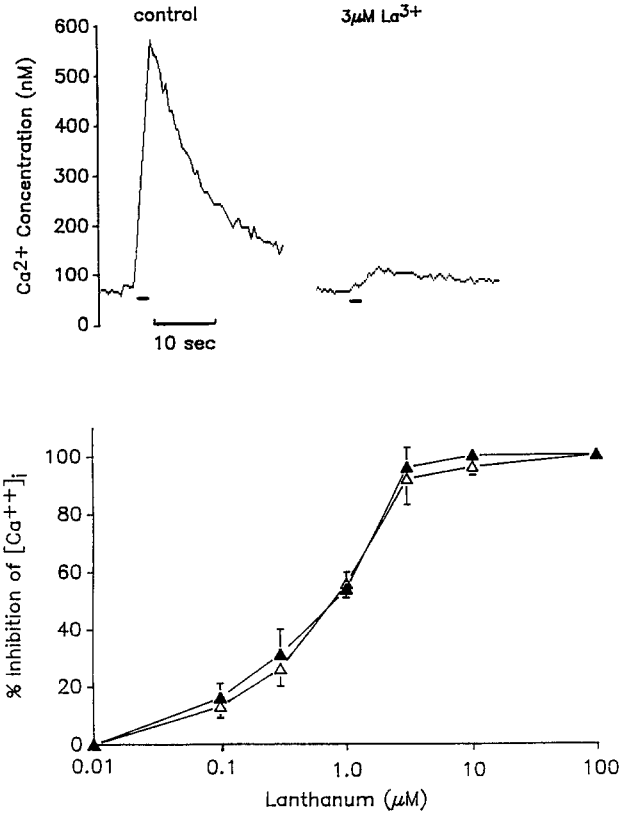

Fig. 2. La^{3+}-dependent inhibition of electrically stimulated rise in intracellular Ca^{2+} concentration. The upper panel shows the effect of La^{3+} on a representative Ca^{2+} transient response recorded in the cell body. The horizontal bar indicates the period of stimulation (5 Hz, 2 sec). The lower panel shows $[Ca^{2+}]_i$ monitored in cell bodies (open triangles) and growth cones (filled triangles) in chick sympathetic neurons. Each point represents the mean ± SEM of three to seven experiments, with each cell used as its own control. La^{3+} had no effect on $[Ca^{2+}]_i$ at rest.

growth cones of sympathetic neurons. Because growth cones have also been shown to be sites of transmitter release (Hume, Role & Fischback, 1983; Young & Poo, 1983) the effect of La^{3+} on $[Ca^{2+}]_i$ was compared in these two regions of sympathetic neurons. As shown in the lower panel of Fig. 2, La^{3+} was equipotent at the cell body and the growth cone. The increase in $[Ca^{2+}]_i$ initiated by electrical stimulation was inhibited in a concentration-dependent manner by La^{3+}. 3 μM La^{3+} completely blocked the stimulation-induced Ca^{2+} influx. The block was reversible. A representative example of La^{3+} effects on $[Ca^{2+}]_i$ is shown in the upper panel of Fig. 2.

Cell body Ca^{2+} currents (I_{Ca}) were studied using the whole-cell variation of the patch-clamp technique (Hamill et al., 1981). Cadmium (1 to 30 μM) caused a concentration-dependent depression of peak I_{Ca} with 90 ± 9% (n = 7) decrease at 30 μM. Cadmium caused a similar depression of the stimulated rise in $[Ca^{2+}]_i$ (93 ± 12% decrease at 30 μM, n = 5) in neuronal cell bodies. La^{3+} also produced a concentration-dependent decrease in voltage-clamped I_{Ca}. Figure 3 shows the superimposed dose-response curves for the La^{3+}-induced inhibition of ^{3}H-NE release, stimulated rise in $[Ca^{2+}]_i$ and voltage-clamped I_{Ca}. 3 μM La^{3+}, which produced 92 ± 7% and 92 ± 4% block of the rise in $[Ca^{2+}]_i$ and ^{3}H-NE release, respectively, caused only a 69 ± 8% inhibition of I_{Ca}. I_{Ca} was fully blocked (96 ± 8%) by 10 μM La^{3+}. A typical whole-cell I_{Ca} recorded before and during exposure to 1 μM La^{3+} is shown in the inset of Fig. 3.

The release of ^{3}H-NE induced by electrical stimulation and by high external K^+ was compared in the presence and after washout of La^{3+} to study the reversibility of La^{3+} action. Figure 4 shows the ef-

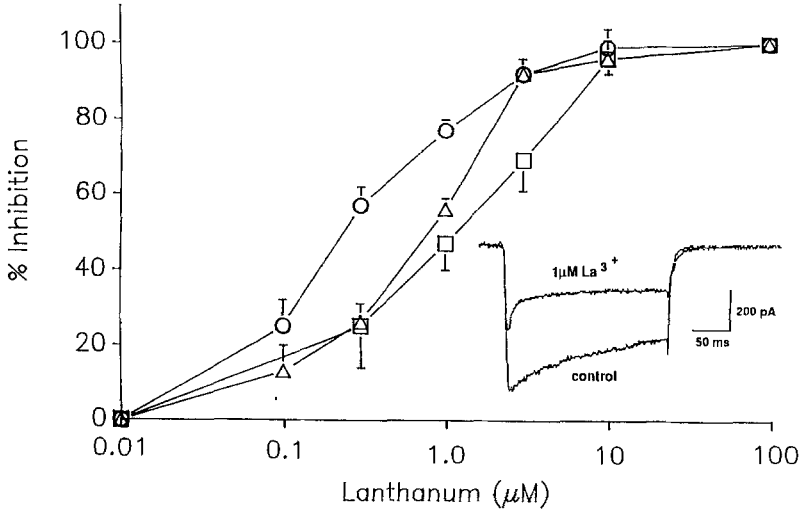

Fig. 3. Comparison of La^{3+} effects on Ca^{2+} influx and ^{3}H-NE release. Concentration-response curves for La^{3+} effects on voltage-clamped I_{Ca} (squares), ^{3}H-NE release (circles) and the rise in cell body $[Ca^{2+}]_i$ (triangles). ^{3}H-NE release and $[Ca^{2+}]_i$ data are taken from Figs. 1 and 2. Inset shows representative, leak-subtracted I_{Ca} and effect of 1 μM La^{3+} recorded in a chick sympathetic neuron.

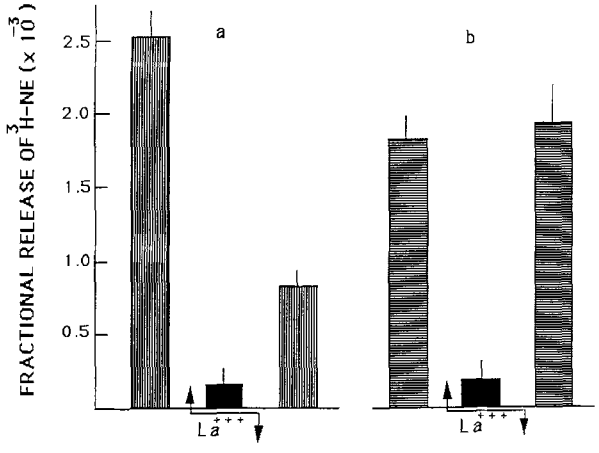

Fig. 4. Differential reversibility of La^{3+} effects on ^{3}H-NE release evoked by K^+ depolarization and electrical stimulation. ^{3}H-NE release before (hatched bar), during exposure (solid bar) and after 15 min washout (second hatched bar) of 10 μM La^{3+} in cells stimulated electrically (*a*) and with 25 mM external K^+ (*b*). Each bar represents a 2-min sample period and shows the mean ± SEM of four experiments.

fects of 10 μM La^{3+} on the onset and offset of ^{3}H-NE release induced by electrical stimulation (Fig. 4*a*) and by 25 mM K^+ (Fig. 4*b*). Although La^{3+} completely blocked the evoked release by both procedures, the pattern of recovery was clearly different. Electrically evoked release only partially recovered, whereas full recovery was obtained in the case of K^+-evoked release after 15 min washout of La^{3+}.

The observation that 3 μM La^{3+} produced a complete block of the electrically stimulated rise of $[Ca^{2+}]_i$ and ^{3}H-NE release but only a partial block of voltage-clamped I_{Ca}, and the finding that release could be evoked by K^+ stimulation of cells which remained refractory to electrical stimulation, suggested that La^{3+} may be affecting other steps of exocytosis or currents other than I_{Ca}. We considered the possibility that activation of outward current (K^+) could increase the effectiveness of La^{3+} block of electrical field-stimulated responses through a hyperpolarization or stabilization of membrane potential. Whole-cell voltage clamp was used to examine the actions of La^{3+} on neuronal K^+ currents. Tetrodotoxin (200 nM) and cadmium (60 μM) were used to block inward currents through Na^+ and Ca^{2+} channels. The effect of La^{3+} on whole cell K^+ currents recorded from the cell body of sympathetic neurons is shown in Fig. 5*a*. The voltage protocol, depolarization from −70 to 0 mV for 200 msec, was the same as that used to monitor I_{Ca}. La^{3+} (1 μM) caused an increase in the early, transient outward current with little effect on the late outward current. The results are representative of 12 experiments. To determine if the effect of La^{3+} on outward current was unique to embryonic chick sympathetic neurons, experiments were also done using SCG cells cultured from newborn rats. La^{3+} caused an increase of the early transient current peak in these cells as well, although the effect was not as prominent as seen in the chick neurons (Fig. 5*b*). The results are representative of nine experiments. Although not shown, La^{3+} completely blocked electrically evoked release of ^{3}H-NE and the rise $[Ca^{2+}]_i$ in SCG neurons.

A hyperpolarization-activated, transient outward K^+ current, first identified as I_A in molluscan neurons (Connor & Stevens, 1971) has been reported to occur in a variety of neuronal cell types (Rudy, 1988). Pharmacologically, I_A has been shown to be

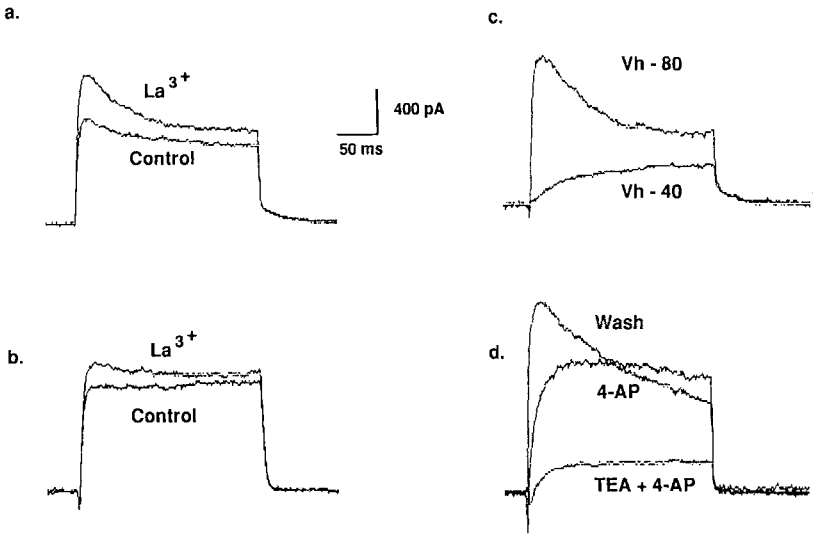

Fig. 5. Characterization of outward currents and activation by La^{3+}. Effect of 1 μM La^{3+} on transient peak K^+ current in chick sympathetic neuron (*a*) and rat SCG (*b*) held at −70 mV and depolarized to 0 mV. (*c*) K^+ currents evoked by depolarizations to −10 mV from holding potential of −80 or −40 mV in a chick sympathetic neuron are shown superimposed. (*d*) K^+ currents evoked in a single cell by depolarization to −10 mV from −80 mV are shown in the presence of TEA (10 mM) plus 4-AP (1 mM), in the presence of 1 mM 4-AP after washout of TEA, and following washout of both drugs (*Wash*). All depolarizing pulses were 200 msec duration. Currents shown are leak subtracted.

uniquely sensitive to block by mM concentrations of 4-aminopyridine (4-AP; Thompson, 1977). Because La^{3+} appeared to specifically activate an early, transient K^+ current we used electrophysiological and pharmacological means to identify the early outward current in chick sympathetic neurons. We found that the transient current peak was only observed from hyperpolarized holding potentials (−70 to −90 mV) and was most prominent with test pulses to between −20 and −5 mV (Fig. 5*c*). The transient current peak was not apparent from a holding potential of −40 mV (Fig. 5*c*). Both the early transient peak and the late sustained outward current were inhibited by a combination of 10 mM tetraethylammonium (TEA) and 1 mM 4-AP (Fig. 5*d*). However, only the transient peak current was eliminated by 1 mM 4-AP (Fig. 5*d*). These results support the idea that the early, transient current peak is due to activation of a current similar to that described as I_A in other neurons.

We determined the effects of La^{3+} under conditions which favor I_A and suppress other transmembrane currents. In addition to cadmium and TTX, to reduce Ca^{2+} and Na^+ currents, 25 mM TEA was included in the bath solution (NaCl adjusted accordingly) to suppress the delayed K^+ current. Cells were depolarized to −15 mV from holding potentials of −40 and −90 mV before and during exposure to 1 μM La^{3+}. The example in Fig. 6 is representative of six experiments using SCG neurons from newborn rats. La^{3+} caused an almost twofold increase in the early peak current evoked from a holding potential of −90 mV. No current flow was observed with depolarization from −40 mV during either control or La^{3+} exposure when TEA was included in the bath. Similar results were obtained with chick sympathetic neurons (*not shown*).

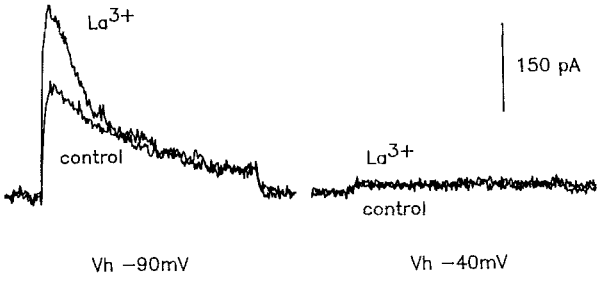

Fig. 6. La^{3+} activation of outward current under conditions which favor I_A. Superimposed, leak-subtracted outward current evoked by a 200-msec depolarization to −15 mV from the indicated holding potentials (*Vh*) are shown before and during exposure to 1 μM La^{3+}. All traces are from the same SCG cell. 60 μM Cadmium, 200 nM TTX and 25 mM TEA were included to suppress currents other than I_A. Scale bar represents 150 pA.

To better characterize the action of La^{3+} on K^+ currents, *I-V* curves were constructed by plotting the peak transient current elicited from hyperpolarized potentials (−80 or −90 mV) as well as the late (190 msec) sustained current elicited from a depolarized holding potential (−40 mV). Cadmium and TTX were used to suppress inward currents. TEA was not included in these experiments. Both sets of *I-V* data were obtained from the same cells before and during exposure to 1 μM La^{3+}. Typical results are shown in Fig. 7. Chick sympathetic neurons which exhibited little or no I_A during the control period showed a pronounced increase in I_A during exposure to La^{3+} (Fig. 7*a*). Rat SCG cells which did express I_A during control exhibited a negative shift in the

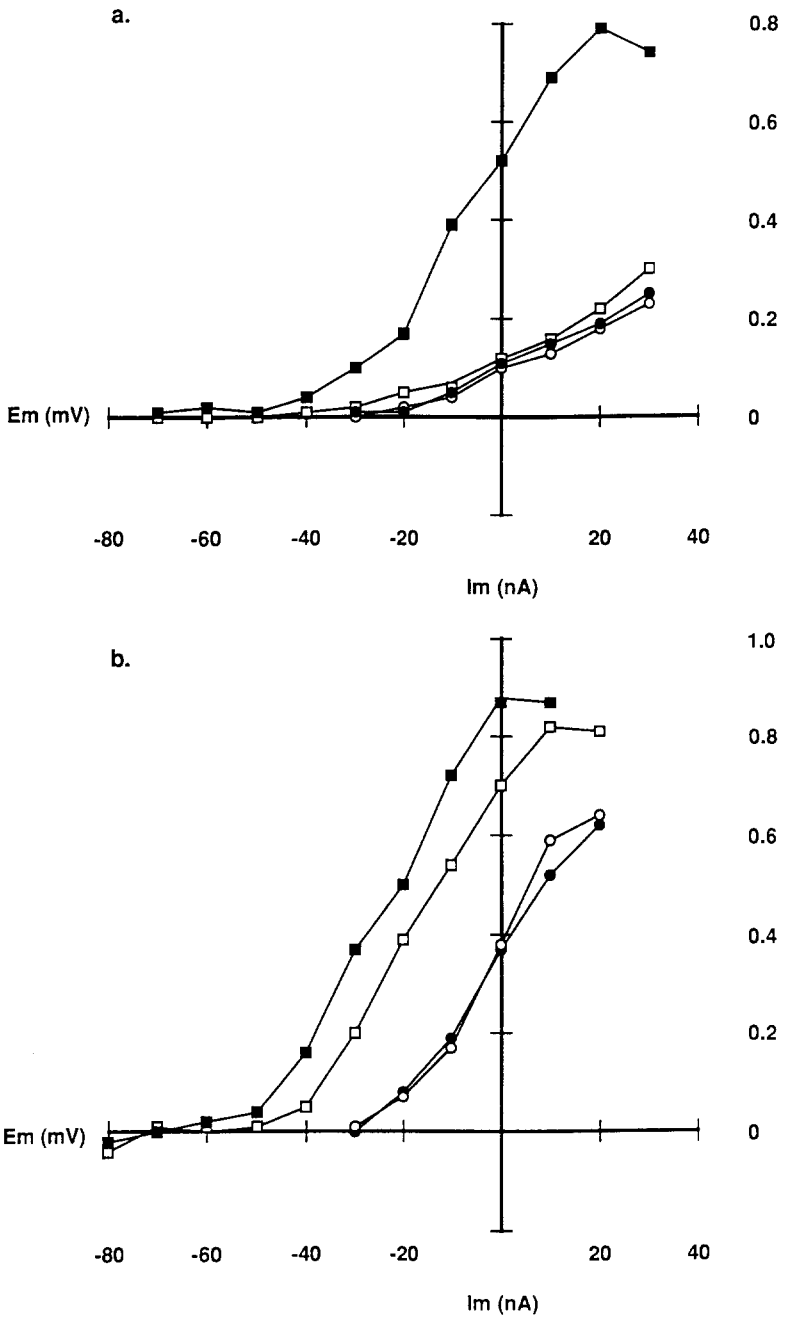

Fig. 7. La^{3+} specifically enhances the hyperpolarization-activated, transient K^+ current, I_A. Current-voltage relations of K^+ currents recorded from (*a*) a chick sympathetic neuron with no apparent I_A during control and (*b*) an SCG neuron exhibiting I_A during control. Transient peak current (squares) elicited by test pulses from hyperpolarized holding potentials (−80 mV in *a* and −90 mV in *b*) and sustained late current (circles) measured at 190 msec when voltage was stepped from depolarized (−40 mV) holding potential are shown before (open symbols) and during (filled symbols) exposure to 1 μM La^{3+}.

activation curve of I_A during exposure to La^{3+} (Fig. 7*b*). La^{3+} had no effect on the activation curve of late current evoked by test pulses from −40 mV (Fig. 7*a*,*b*).

Discussion

We have demonstrated that La^{3+} produces a specific activation of A-type K^+ current in cultured sympathetic neurons. The findings that La^{3+} caused a negative shift of I_A activation curves and enhanced the early peak current when I_A was isolated from other currents suggest that La^{3+} promotes A-type K^+-channel opening upon depolarization without affecting inactivation. The La^{3+}-induced activation of I_A occurs at the same concentration (1 μM) that blocks 50% of inward Ca^{2+} current. It is unlikely that these two opposite effects of La^{3+} are due to nonspecific neutralization of external surface charges. Surface-

charge effects would be expected to shift activation curves of all voltage-dependent currents to more positive potentials and cause nonspecific current inhibition. This is clearly not the case in the present experiments. Indeed, concentrations as high as 10 μM La^{3+}, which completely block I_{Ca} in voltage-clamped atrial cells, fail to exhibit surface-charge effects on Na^+ or K^+ currents in these cells (Nathan et al., 1988). Thus, we do not believe that nonspecific surface charge effects are involved in the high potency (IC_{50} = 0.3 μM) of La^{3+} on ^{3}H-NE release.

The differential sensitivity of transmitter release and Ca^{2+} influx to La^{3+} was not due to selective action at the nerve terminal (the principal site of transmitter release). When tested on growth cones La^{3+} blocked the stimulated increase in $[Ca^{2+}]_i$ in a concentration-dependent manner identical to that found in the cell bodies. We conclude that the effectiveness of low concentrations of La^{3+} in blocking transmitter release is due, at least in part, to the additive effects of reducing I_{Ca} and activating I_A. Both of these ionic actions would decrease membrane responsiveness to depolarizing stimulus and ultimately reduce the availability of Ca^{2+} to the secretory apparatus. Consistent with this interpretation is the observation that the electrically stimulated rise in $[Ca^{2+}]_i$ was inhibited more than 90% by 3 μM La^{3+} while voltage clamped I_{Ca} was inhibited less than 70% at the same concentration (Fig. 3). This is unlike Ca^{2+}-channel block by cadmium which decreased I_{Ca} and $[Ca^{2+}]_i$ to the same extent, as expected. The activation of K^+ current could well account for the greater sensitivity to La^{3+} of field stimulated Ca^{2+} entry compared to voltage clamped Ca^{2+} entry and would thus contribute to the high potency of La^{3+} in blocking stimulated [^{3}H]NE release. We do not mean to imply that [^{3}H]NE is in direct proportion to $[Ca^{2+}]_i$. The higher potency of La^{3+} in blocking [^{3}H]NE release than stimulated $[Ca^{2+}]_i$ may reflect a nonlinear, high-order $[Ca^{2+}]_i$ dependency of release as proposed by others (Augustine, Charlton & Smith, 1987) or it may be due to La^{3+} action on components of release down-stream from Ca^{2+} entry.

We speculate that the long lasting block of ^{3}H-NE release by high concentrations of La^{3+} may be due to incomplete washout of membrane-bound La^{3+}. Although a complete dose-response study of La^{3+} effects on I_A remains to be done, low levels of La^{3+} may activate A-channels sufficiently to block electrically evoked depolarization (and ^{3}H-NE release). It seems reasonable that such an effect might be more easily counteracted by elevated external K^+. In support, we did find a full restoration of ^{3}H-NE release by excess K^+ but not by field stimulation after washout of La^{3+}. In our earlier work we also observed incomplete recovery from La^{3+} blockade of electrically evoked release of NE from sympathetic neurons and catecholamines from adrenal medulla (Kirpekar et al., 1972; Wakade, 1981). In these same test preparations the effects of other Ca^{2+} channel blockers were fully reversible. The results of the present study offer an explanation for these earlier unresolved observations. It appears that La^{3+}, unlike other Ca^{2+} channel blockers, has the unique ability to activate K^+ channels in sympathetic neurons. It remains to be seen if this effect occurs in other types of cells.

In summary, we have shown that a Ca^{2+} channel blocker can have multiple effects which act in concert to block the release of neurotransmitter substances. We have demonstrated that La^{3+}, known for many years to block Ca^{2+} entry in neurons, also facilitates K^+ efflux to account for the long-lasting and complete block of NE release from sympathetic neurons. The mechanism(s) by which this apparently unique polyvalent cation activates one ion channel while blocking another is intriguing but remains unknown.

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