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Ectopic α_2-Adrenoceptors Couple to N-Type Ca^{2+} Channels in Axotomized Rat Sensory Neurons

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Dorsal root ganglion (DRG) neurons from control rats or from rats in which the sciatic nerve had been sectioned were studied by whole-cell recording techniques. Noradrenaline (10–100 μM) activated β-adrenoceptors and increased L-type Ca^{2+} channel current in control DRG cells, but this had little effect on excitability (the number of action potentials generated by a pulse of current at rheobasic strength). By contrast, in cells from nerve-damaged animals, noradrenaline activated α_2-adrenoceptors, suppressed N-type Ca^{2+} channel current, and increased excitability. In axotomized cells, it also reduced total outward current recorded at +70 mV. Because noradrenaline did not affect total outward current recorded in the presence of the Ca^{2+} channel blocker Cd^{2+} (0.5–1 mM), its effects on excitability may result from reduction of Ca^{2+}-sensitive K^+-conductance(s) following suppression of N-type Ca^{2+} channel current. The strongest effects of noradrenaline were seen in small cells and in cells from animals that exhibited autotomy, a self-mutilatory behavior that can accompany peripheral nerve damage. Because many of these small DRG cells may be involved in the transmission of nociceptive information, changes in coupling between Ca^{2+} channels and adrenoceptors may contribute to the generation of the ectopic sensory nerve activity that has been implicated in the etiology of neuropathic pain.

Key words: pain; nerve injury; spinal ganglion; sodium channel; norepinephrine; acutely dissociated neurons

Although mammalian sensory nerves normally seem insensitive to noradrenaline (NA; Xie et al., 1995), α_2-adrenoceptors appear on the cell bodies of neurons in dorsal root ganglia (DRG) after their axons are damaged (Nishiyama et al., 1993; Devor et al., 1994; Xie et al., 1995). Also, in rats, injury or section of the sciatic nerve promotes sprouting of sympathetic nerve fibers into the DRG, where they abut the cell bodies of sensory neurons (McLachlan et al., 1993; Zhou et al., 1996). NA from these fibers interacts with the ectopic α_2-adrenoceptors, excites the DRG neurons, and facilitates the generation of spontaneous activity in damaged sensory nerves (McLachlan et al., 1993; Devor et al., 1994; Xie et al., 1995). The following experiments were undertaken to elucidate the mechanism of this noradrenergic excitation. The results may be relevant to understanding the etiology of the sympathetically maintained causalgias and chronic pain syndromes that are seen in humans who have received peripheral nerve injuries (Wall et al., 1979; Bonica, 1990; Devor et al., 1994).

MATERIALS AND METHODS

The left sciatic nerve of adult male Sprague Dawley rats (120–170 gm) under sodium pentobarbital anesthesia (50–55 mg/kg) was sectioned proximal to its bifurcation into the tibial and the peroneal divisions. A 5–10 mm segment was removed to prevent regeneration. Animals were housed in separate cages and examined and weighed twice daily for the first 3 d after surgery and then once daily for the remainder of the experimental period. Section of peripheral nerves can invoke a behavior known as "autotomy" that involves self-mutilation of the denervated foot (Rodin and Kruger, 1984; Coderre et al., 1986). In addition to the physical examination to determine the presence or extent of autotomy-induced damage, we also checked whether the animals exhibited other signs of postoperative stress. These included extreme vocalization on handling, lack of grooming, lethargy, and/or arched back. Had any of these signs been observed or if animals had failed to gain weight (i.e., if they were 5% lighter than age-matched controls), they would have been killed by intraperitoneal pentobarbital, followed by decapitation. It was not necessary to implement this procedure for any of the animals used in this study. The extent of autotomy was scored according to a scale derived by Wall et al. (1979), and although the maximum score that can be attained on this scale is 11, none of the animals used in this study exceeded a score of 8. A score of 1 was given for the removal of one or more nails. The score was increased by 1 for injury to each distal digit and another 1 for injury to each proximal digit. Because there was considerable variation among different individual animals in the rate of onset of autotomy, "axotomized rats exhibiting autotomy" were defined as those that exhibited autotomy scores of 4 or more 2–7 weeks after axotomy. Thus, any individual that developed autotomy exceptionally rapidly such that it exhibited advanced lesions 2 weeks postoperatively would have been used immediately. Because the development of autotomy is progressive and we were waiting for animals to develop an autotomy score of 4 so that we could use them, there was no reason to maintain animals that exhibited scores of 7 or 8 for any length of time. Only animals that had failed to attain an autotomy score of 4 were allowed to survive after two weeks. Some of these animals had to be maintained for 7 weeks for them to attain a score of 4. The axotomy group comprised those animals that had not developed autotomy within 2–7 weeks after sciatic nerve section.

For electrophysiological analysis, rats were decapitated, and DRG from L4 and L5 were dissociated as described by White et al. (1989). These ganglia receive the majority of fibers from the sciatic nerve (Swett et al., 1991). Cells were plated into poly-L-lysine-coated Petri dishes and used for recording within 2–10 hr. The cells were superfused with various extracellular solutions at ~2 ml/min. For action potential (AP) recording, external solution contained (in mM): 150 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES-NaOH, pH 7.4, and 10 D-glucose (osmolarity 330–340 mOsm). Internal solution contained (in mM): 130 K-gluconate, 2 Mg-ATP, 0.3 Na-GTP, 11 EGTA, 10 HEPES-KOH, pH 7.2, and 1 $CaCl_2$, (osmolarity 310–320 mOsm). Single APs were generated by using a 2 msec pulse of depolarizing current, and spike width was measured at 50% of maximum amplitude. Excitability was measured by counting the number of APs that discharged in response to 200 msec pulses of current at rheobasic strength (the minimum current required to discharge an AP). Ba^{2+} (I_{Ba}) was used as the charge carrier to record Ca^{2+} channel currents (I_{Ca}). For these experiments, external solution contained (in mM): 160

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TEA-Cl, 10 HEPES, 2 $BaCl_2$, 10 glucose, and 200 nM TTX, adjusted to pH 7.4 with TEA-OH; internal solution contained (in mM): 120 $CsCl_2$, 5 Mg-ATP, 0.4 Na-GTP, 10 EGTA, and 20 HEPES-CsOH, pH 7.2. I_{Ba} was evoked at −10 mV from a holding potential of −90 mV. After the pulse, the voltage was stepped to −40 mV to slow I_{Ba} tail currents so that they could be measured conveniently. In early experiments, I_{Ba} was leak-subtracted by applying a 20 mV hyperpolarizing pulse, multiplication by four, and addition. Because it was noted that leak subtraction failed to alter the numerical value of I_{Ba}, this practice was discontinued in later experiments. For recording K^+ currents (I_K), external solution contained (in mM): 145 *N*-methyl-D-glucamine (NMG)-Cl, pH 7.4, 10 KCl, 2.5 $CaCl_2$, 10 HEPES, 1.0 $MgCl_2$, and 10 D-glucose; internal solution contained (in mM): 100 K-gluconate, 40 NMG-Cl, pH 7.2, 2 Mg-ATP, 0.3 Na-GTP, 11 EGTA, 10 HEPES, and 1.0 $CaCl_2$. For recording Na^+ currents (I_{Na}), external solution contained (in mM): 100 NaCl, 5 KCl, 4 $MgCl_2$, 10 HEPES, and 60 D-glucose, adjusted to pH 7.4 with NaOH; internal solution contained (in mM): 140 $CsCl_2$, 10 NaCl, 2 Mg-ATP, 0.3 Na-GTP, 2 EGTA, 10 HEPES, and 2 $MgCl_2$, adjusted to pH 7.2 with NaOH.

Whole-cell recordings were made at 22°C with an Axoclamp 2A amplifier in discontinuous voltage-clamp or bridge-balance current-clamp mode. Borosilicate glass patch electrodes had DC resistance of 4–6 MΩ for AP recording or 1–3 MΩ for current recording. With these low resistance electrodes it is possible to attain sampling rates of 30–60 kHz.

Because discontinuous voltage-clamp method allows the cell to be clamped to the measured membrane voltage, it circumvents some of the series resistance problems that could be encountered if a conventional patch-clamp amplifier had been used (Jones, 1987). The quality of the clamp was confirmed by the rapidity of I_{Ba} tails recorded at −40 mV and by the fact that they readily could be described by a single exponential function. Because Ba^{2+} was used as the charge carrier, we never observed the slow tail currents that result from activation of Ca^{2+}-dependent Cl^- conductances ($g_{Cl,Ca}$; Mayer, 1985). Because we did not use leak subtraction and appropriate compensation circuitry is not available in the Axoclamp 2A amplifier, the illustrated data records that were filtered to −3 dB at 1 or 3 kHz display large capacitance transients. Under current clamp, input capacitance (C_{in}) was calculated from the input resistance (R_{in}) and the membrane time constant (τ_m) via the equation $\tau_m = C_{in} \cdot R_{in}$. Under voltage clamp, C_{in} was measured by integrating the area of capacitative current transients that were generated by 10 mV commands (ΔV); this yielded the charge, Q, that is related to C_{in} by $Q = V \cdot C_{in}$. Data were acquired and analyzed by pClamp software (version 5.5.1). Drugs were applied by superfusion, and all data are presented as mean ± SEM. Statistical significance was assessed by Student's paired or unpaired *t* test, as appropriate. NA-induced percentage changes in AP duration, I_{Ba}, and outward current were calculated only from those cells that responded rather than from the whole population of cells tested. This is because some subgroups of cells may not exhibit adrenoceptors either before or after nerve injury (see Discussion).

RESULTS

Classification of DRG cells

Rat DRG cells were classified into three groups according to their size and AP shape: "large" cells were defined as those with AP duration <3 msec and C_{in} >90 pF; "medium" cells had an AP duration of 3–5 msec and C_{in} of 70–90 pF; "small" cells had an AP duration >5 msec and C_{in} <70 pF. Both medium and small cells exhibited a "hump" on the falling phase of their AP.

Effects of noradrenaline on action potentials and excitability of DRG cells

The excitability of large, medium, or small cells from control animals was unchanged or slightly decreased by noradrenaline (NA; 10–100 μM). Some sample data records are shown in Figure 1A_1. AP discharge was invoked by using a 200 msec pulse of current at rheobasic strength in the presence or absence of NA. The records for a control small cell in Figure 1A_1 illustrate an experiment in which NA decreased excitability. By contrast, in cells isolated from axotomized rats, NA increased excitability (Fig. 1A_2). This effect was seen most frequently in small cells (in 11 of 18 or 61.1%), less frequently in medium cells (in 6 of 13 or 46.2%), and only occasionally in large cells (in 2 of 15 or 13.3%). The prevalence of the excitatory effect of NA also was increased in cells from rats that exhibited autotomy (Fig. 1A_3); in this group, excitability was increased in 14 of 16 small cells (87.5%), in 9 of 12 medium cells (75.0%), and in 10 of 15 large cells (66.7%). In addition, NA invoked its strongest effects on the excitability of small cells and cells from animals that exhibited autotomy. The magnitude of the effects of NA and the frequency of occurrence of increased excitability in different cell types are reflected in the graphic summary of data from all cells (i.e., those sensitive to and those insensitive to NA) in Figure 1*B*.

The was no obvious difference between the resting membrane potential (RMP) of small, medium, or large cells from the control, axotomy, or autotomy groups (Table 1) and no consistent effect of NA on RMP on any of the cell types under any condition.

NA (up to 100 μM) did not affect the AP duration of large control cells, but it increased that of 66.7% of medium cells and 80.6% of small cells (Table 2 and Fig. 2*A*). A greater increase in spike width was seen in small cells than in medium cells (Table 2). The effect of 10 μM NA on spike width after axotomy was the opposite of its effect on control neurons; AP duration was decreased in 81.8% of small cells and in 63.0% of medium cells. Not only were small axotomized cells affected more frequently than medium cells, but the magnitude of the NA-induced decrease in AP duration was greater in this cell type (Table 2). Large axotomized cells were unaffected (Fig. 2*B*). As with the effect of NA on excitability, its effect on spike width was more pronounced in cells from rats that exhibited autotomy (Fig. 2*C*). Thus, under these conditions, 10 μM NA decreased the AP duration of almost all small cells by 51.8 ± 4.3%, that of the majority of medium cells by 43.5 ± 3.2%, and that of approximately one-half of the large cells by 31.26 ± 5.2% (Table 2).

The hump on the falling phase of the medium cell AP and the long duration of the AP in small cells reflect voltage-dependent Ca^{2+} influx (Holz et al., 1986). Cd^{2+} (0.5–1 mM) attenuated the hump of the AP in small and medium cells and therefore shortened AP duration (Fig. 2*D*). The AP duration of all three cell types was increased by the L-channel ($I_{Ca,L}$) activator BAY K 8644 (2 μM; Fig. 3*A*), and that of small and medium cells (but not large cells) was reduced by nifedipine (1 μM; Fig. 3*B*). In control cells, BAY K 8644 increased the AP duration of large cells by 181.2 ± 8.9% (n = 5), medium cells by 249.5 ± 17.1% (n = 4), and small cells by 297.8 ± 13.3% (n = 5). Nifedipine (1 μM) decreased the AP duration of small control cells by 64.5 ± 7.5% (n = 4) and medium control cells by 52.1 ± 7.0% (n = 4).

The similarity between the effects of Ca^{2+} channel modulators and NA suggested the involvement of Ca^{2+} channels in the actions of NA. This idea is supported by the observation that NA-induced prolongation of the AP in small and medium control cells was prevented when Ca^{2+} channels were blocked with Cd^{2+} (1 mM; Fig. 3*C*). Cd^{2+} also prevented NA-induced AP shortening in cells from axotomized animals (Fig. 3*D*). Last, blockade of I_{Ca} with Cd^{2+} (instead of NA) increased the excitability of all cell types (Fig. 4*A*). The effects of NA on I_{Ba} in neurons from control rats and from axotomized rats, therefore, were examined.

Effects of noradrenaline on DRG cells under voltage clamp

NA (10–100 μM) did not affect I_{Ba} in large control cells but potentiated that in 7 of 13 medium cells by 18.5 ± 2.8%. Potentiation was seen more frequently and the effect of NA was greatest in small control cells (Table 3). Typical experiments are illustrated

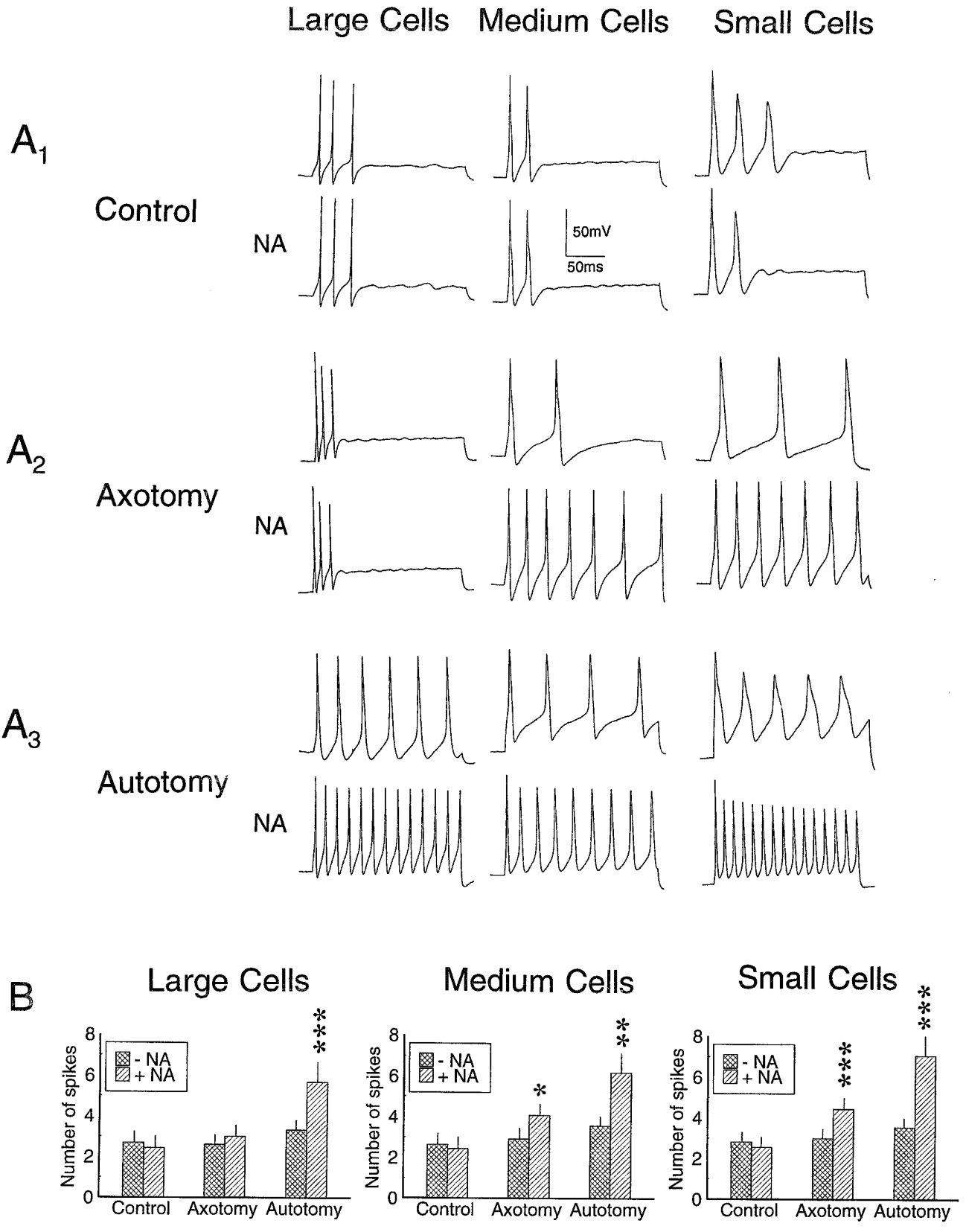

Figure 1. Effect of noradrenaline on the excitability of rat DRG neurons. Each cell was depolarized with a 200 msec pulse of current at rheobasic strength (current trace not shown) in the absence and presence of 10 μM noradrenaline (*NA*). Shown are typical recordings from large, medium, and small cells: A_1, control animals; A_2, axotomized animals; A_3, animals exhibiting autotomy. *B*, Graphic summary of data collected from all cells (i.e., those responding and those insensitive to *NA*). The excitatory effects of *NA* (number of spikes discharged in response to a 200 msec pulse of rheobasic current) are seen most clearly in small cells and in cells from animals that exhibit autotomy. Error bars represent SEM. $^{*}p < 0.025$, $^{**}p < 0.002$, $^{***}p < 0.001$ (paired *t* test for number of spikes before and after NA application to each cell).

Table 1. Resting membrane potentials (in mV) of different DRG cell types

Cell type	Control	Axotomy	Autotomy
Large	−59.1 ± 1.5 (n = 147)	−57.6 ± 1.8 (n = 100)	−58.4 ± 1.6 (n = 50)
Medium	−58.6 ± 1.4 (n = 40)	−58.2 ± 1.3 (n = 49)	−56.4 ± 1.6 (n = 35)
Small	−56.8 ± 1.3 (n = 28)	−54.8 ± 1.3 (n = 54)	−55.4 ± 1.5 (n = 37)

Table 2. Percentage change in spike width in response to NA

Cell type	Conrol	Axotomy	Autotomy
Large	0 (0/34)	0 (0/28)	−31.3 ± 5.2%*** (12/26)
Medium	+60.4 ± 6.8%*** (20/30)	−31.9 ± 2.8%*** (17/27)	−43.5 ± 3.2%*** (20/24)
Small	+75.1 ± 6.3%*** (29/36)	−40.7 ± 1.9%*** (27/33)	−51.8 ± 4.3%*** (29/31)

Negative changes indicate decreases in spike width, and positive changes indicate increases in spike width. Numbers in parentheses indicate number of cells responding relative to number of cells tested. Percentage changes are calculated only from those cells that responded. ***$p < 0.001$. Comparison of control spike widths with spike widths seen in the presence of NA (paired t test).

in Figure 5A_1. NA-induced potentiation of I_{Ba} was occluded by nifedipine. Figure 5B_1 illustrates the time course of changes in amplitude of I_{Ba} in a small control cell after treatment with NA and/or nifedipine. NA (10 μM) increased peak I_{Ba} from 5.6 to 7.5 nA. After NA was washed out, application of 2 μM nifedipine reduced the current to 3.4 nA. Reapplication of NA failed to potentiate the remaining current. NA thus selectively potentiates $I_{Ca,L}$. Superimposed original data records from the experiment are shown in the inset to Figure 5B_1.

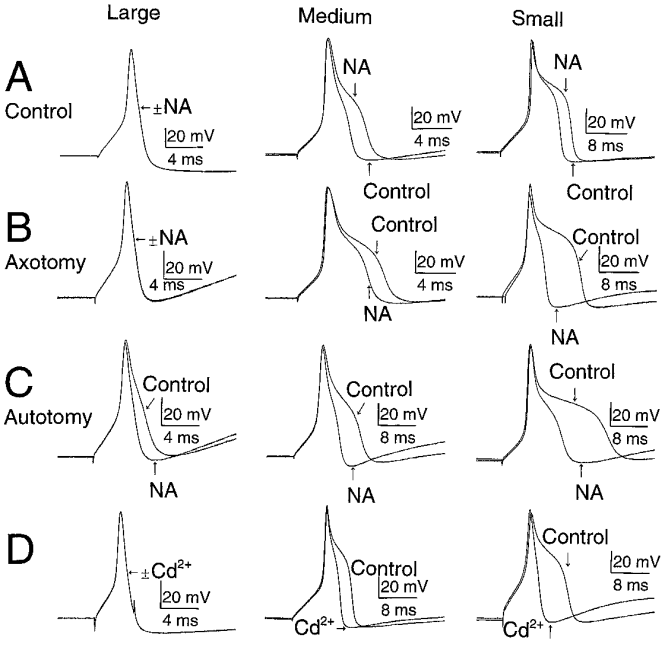

Figure 2. Effects of NA and Cd^{2+} on spike width of DRG cells from (*A*) control animals, (*B*) axotomized animals, and (*C*) axotomized animals that exhibited autotomy. The three columns of typical data records illustrate effects of 10 μM *NA* on spike width in large, medium, and small cells. *D*, Lack of effect of 1 mM Cd^{2+} on spike width of a control large cell and reduction of spike width observed in a medium and in a small cell.

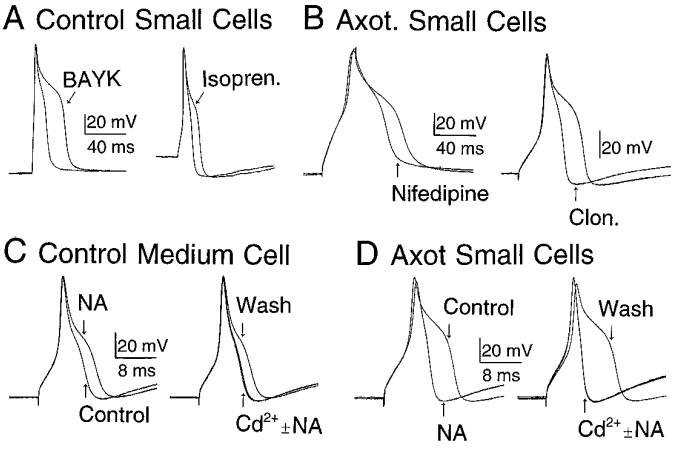

Figure 3. Effects of adrenergic drugs, Cd^{2+}, and dihydropyridines on spike width of DRG cells from control animals, axotomized animals, and axotomized animals that exhibited autotomy. *A*, Increase in AP duration of small control cells induced by 2 μM BAY K 8644 or 10 μM isoprenaline (*Isopren.*). *B*, Decrease in spike width of small axotomized cells induced by 1 μM nifedipine (*Nifedipine*) or 10 μM clonidine (*Clon.*). *C*, Occlusion by 1 mM Cd^{2+} of the increase of AP duration invoked in a medium control cell by 10 μM NA. *Left trace* shows initial NA-induced increase in spike width; *right trace* was obtained after washout of NA; Cd^{2+} decreased AP duration, and subsequent application of NA was unable to restore or increase AP duration. *D*, Occlusion by 1 mM Cd^{2+} of the decrease of AP duration invoked in a small axotomized cell by 10 μM NA. *Left trace* shows initial NA-induced decrease in spike width; *right trace* was obtained after washout of NA; Cd^{2+} decreased AP duration, but subsequent application of NA was unable to invoke a further decrease.

The effect of NA on I_{Ba} in axotomized cells was opposite to that seen in control cells. Thus, NA decreased the current in small, medium, and large axotomized cells (Fig. 5A_2). Again, these effects were least intense in large cells and most intense in small cells and in cells from rats that exhibited autotomy (Table 3 and Fig. 5A_3). NA selectively inhibited N-type I_{Ca} ($I_{Ca,N}$) because its effects on axotomized cells were occluded by ω-conotoxin GVIA (ω-cntxGVIA; Fox et al., 1987; Scroggs and Fox, 1992) (Fig. 5B_2). Because it did not potentiate the remaining ω-cntxGVIA-insensitive current, NA neither potentiated $I_{Ca,L}$ in axotomized cells nor did it affect P- or Q-type current (Rusin and Moises, 1995). T-type Ca^{2+} current, evoked at −40 mV from a holding potential of −90 mV (Fox et al., 1987), was not affected by NA in the control or the experimental rats.

To confirm that NA was exerting its stimulatory or inhibitory effects directly on I_{Ba} and that changes in leak conductance and/or other voltage-sensitive conductances were not involved, we examined its effects on I_{Ba} tails. Tail current amplitudes were estimated by measuring a single point on the current record 500 μsec after a step to −40 mV. Percentage changes in the tail current amplitudes induced by NA are summarized in Table 4. These results are in good agreement with those obtained from peak I_{Ba} measurements (Table 3). Thus, NA potentiates I_{Ba} in control cells yet attenuates it after axotomy. Small cells are affected more than medium cells, which are affected more than large cells. Attenuation of I_{Ba} is more pronounced in cells from animals that exhibit autotomy.

NA (up to 100 μM) did not affect Na^+ current (I_{Na}) in any of the cell types under any of the experimental conditions (data not shown); neither did it affect total outward current at +70 mV

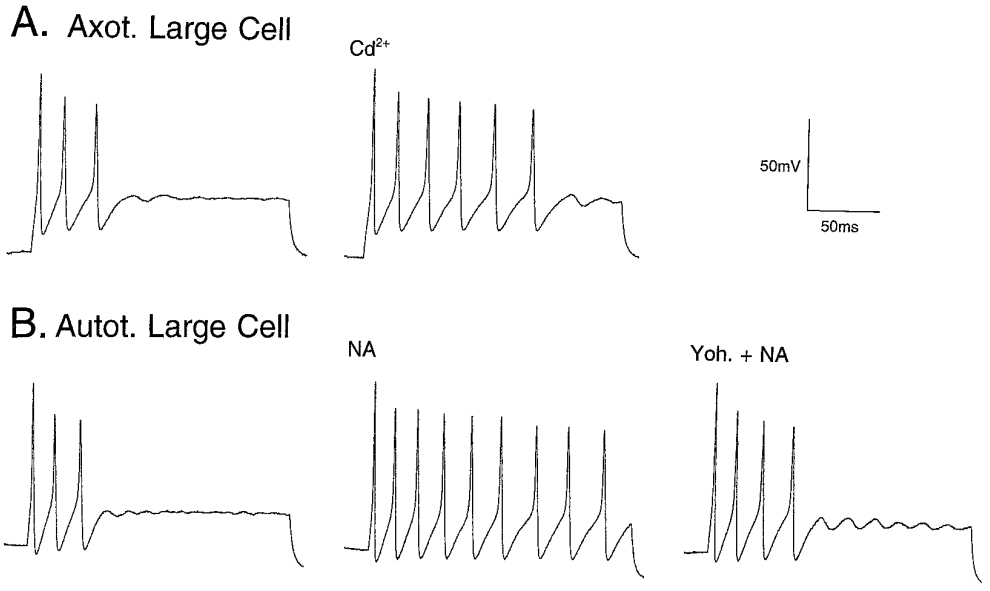

Figure 4. Increases in excitability invoked by blocking Ca^{2+} channels with Cd^{2+} or by activation of α_2-adrenoceptors. *A*, Increase in excitability of a large cell from an axotomized animal induced by 1 mM Cd^{2+}. *B*, Antagonism by 1 μM yohimbine (*Yoh.*) of NA-induced enhancement of excitability in a large cell from an animal that exhibited autotomy. Both cells were stimulated with a depolarizing current pulse at rheobasic strength.

recorded in the presence of Cd^{2+} from a holding potential of −90 mV (n = 8–16 for each condition in each cell type). This current is assumed to reflect outward movement of K^+ through delayed rectifier and A-type K^+ channels (Akins and McCleskey, 1993). In the absence of Cd^{2+}, however, NA (10 μM) decreased the total outward current in cells from axotomized rats yet did not affect that of control cells (Fig. 6*A*). NA was most effective in inhibiting current recorded from small and medium axotomized cells and from cells from animals that exhibited autotomy. Thus, it reduced outward current in 78% of small axotomized cells, 80% of medium cells, and in only 20% of large axotomized cells (Table 5). In cells from animals that exhibited autotomy, NA reduced current in 66.7% of large cells, 91% of medium cells, and in all small cells (Table 5). This table also shows that the greatest effects on outward currents were seen in small and medium cells and in cells from animals that exhibited autotomy. Data from a typical experiment on a small axotomized C-cell are plotted in Figure 6B_1. Outward currents were recorded when the cell was stepped from −90 mV to +70 mV at 20 sec intervals throughout the experiment. The current was reduced in the presence of NA, but once the Ca^{2+}-dependent component was removed by the addition of 1 mM Cd^{2+}, NA was ineffective. Superimposed original records from the experiment are shown in Figure 6B_2,B_3. These results suggest that after axotomy NA may affect Ca^{2+}-activated K^+ conductance(s) ($g_{K,Ca}$) and perhaps $g_{Cl,Ca}$ indirectly as a consequence of its action on $I_{Ca,N}$.

Table 3. Percentage change in peak I_{Ba} in response to NA

Cell type	Control	Axotomy	Autotomy
Large	0 (0/14)	−19.9 ± 2.6%* (3/12)	−29.2 ± 2.2%*** (8/12)
Medium	+18.5 ± 2.8%*** (7/13)	−26.4 ± 2.0%** (11/12)	−34.8 ± 2.5%*** (12/12)
Small	+34.5 ± 2.4%*** (12/14)	−30.2 ± 1.9%*** (13/13)	−36.4 ± 2.0%*** (13/13)

Negative changes indicate decreases in I_{Ba} amplitude, and positive changes indicate increases. Numbers in parentheses indicate number of cells responding relative to number of cells tested. Percentage changes are calculated only from those cells that responded. *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$. Comparisons of control currents with currents seen in the presence of NA (paired t test).

Pharmacology of the effects of noradrenaline

In small and medium control cells, the NA-induced increase in AP width and potentiation of $I_{Ca,L}$ was blocked by 1 μM propranolol (n = 4 for both effects on small cells; n = 2 or 3 for effects on medium cells) and was mimicked by 10 μM isoprenaline (n = 4 or 6 for small cells; n = 4 for both effects on medium cells). Typical experiments are illustrated in Figures 3*A* and 5A_1. α-Adrenoceptor agonists and antagonists were ineffective in mimicking or blocking, respectively, the effects of NA in small and medium control cells (1–10 μM prazosin, yohimbine, clonidine, or U.K.14304 was tested on 4–6 small cells and on 2–4 medium cells for effects related to NA modulation of AP parameters; for effects related to NA-induced potentiation of I_{Ba}, each of the four drugs was tested on 3–4 small cells or on 3–4 medium cells.) By contrast, in cells from axotomized rats, the effects of NA on excitability, spike width, and $I_{Ca,N}$ were blocked by 1 μM yohimbine, (n = 2–4 for each effect on each cell type) and were mimicked by the α_2-adrenoceptor agonists, clonidine (10 μM; in 2 of 6 small cells and 1 of 4 medium cells) and U.K.14304 (10 μM; in 3 of 6 small cells, 2 of 4 medium cells, and 1 of 3 large cells). Some typical experiments are illustrated in Figures 3*B*, 4*B*, and 5A_2. The effects of NA in cells from axotomized rats were insensitive to prazosin (α_1-adrenoceptor antagonist up to 10 μM; n = 4–5, depending on cell type and protocol) and propranolol (n = 5–6, up to 10 μM, depending on cell type and protocol) and were not mimicked by isoprenaline (n = 5). Yohimbine (1 μM; n = 5) blocked the NA-induced reduction in outward current (at +70 mV) seen in cells from axotomized animals (Fig. 6*C*), whereas prazosin (n = 5; up to 10 μM) and propranolol (n = 6; up to 10 μM) were ineffective.

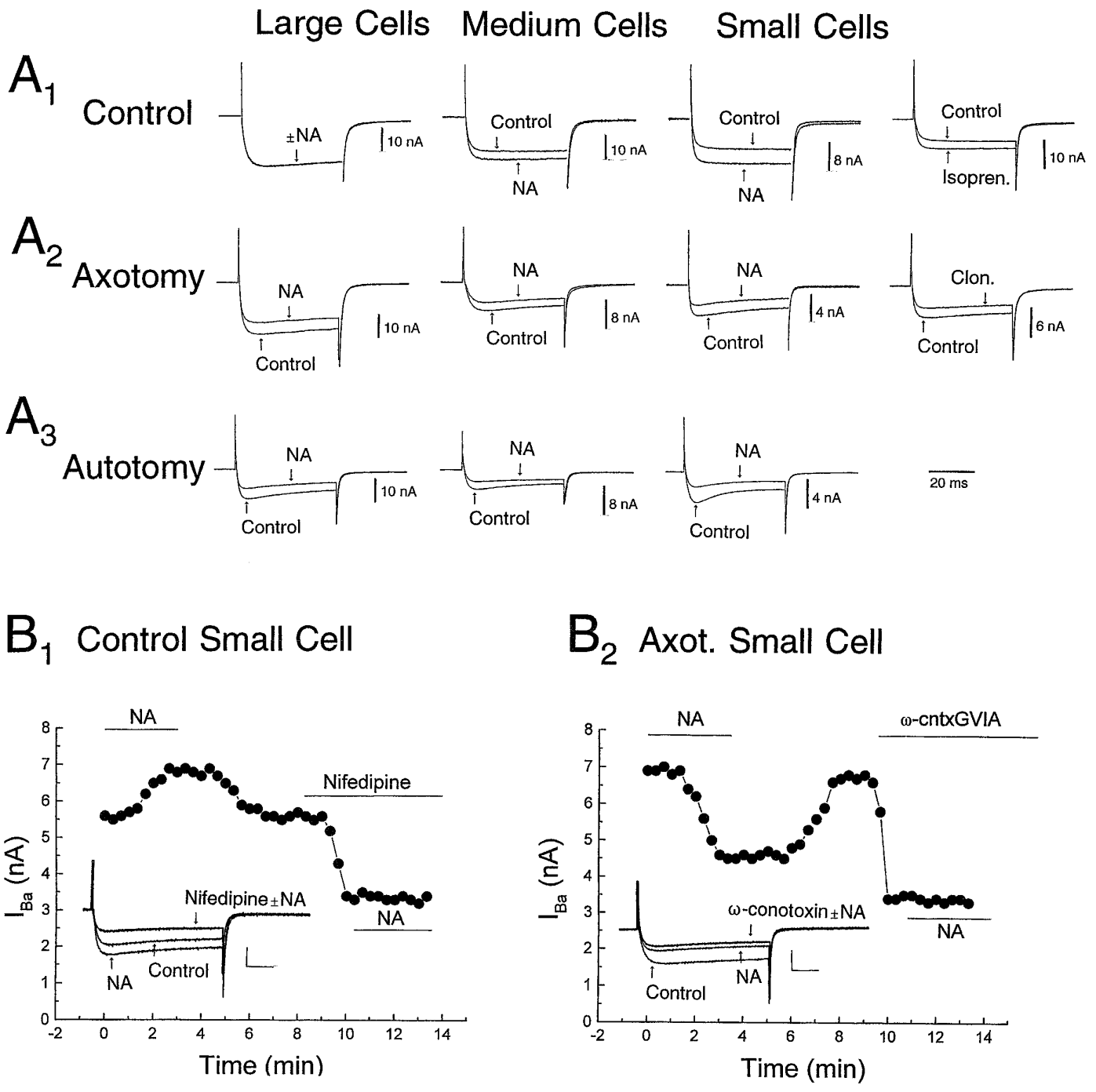

Figure 5. Effects of adrenergic drugs and Ca^{2+} channel blockers on Ba^{2+} currents of DRG cells from (A_1) control animals, (A_2) axotomized animals, and (A_3) axotomized animals that exhibited autotomy. Shown are effects of 10 μM NA on I_{Ba} evoked from a holding potential of −90 mV and recorded at −10 mV for each of the three experimental groups. Note NA-induced potentiation of I_{Ba} in control cells and attenuation of the current in cells from the axotomized and axotomized-autotomy groups. NA exerts the most profound potentiation or suppression of I_{Ba} in small cells, and suppression of I_{Ba} is most pronounced in cells from animals that exhibit autotomy. Potentiation of I_{Ba} in a small control cell by isoprenaline (*Isopren.*, 10 μM) and suppression in a small cell from an axotomized animal by clonidine (*Clon.*, 10 μM) also are illustrated in A_1 and A_2, respectively. Calibration (20 msec) refers to all records in A_1, A_2, and A_3. B_1, Time course of potentiation of I_{Ba} by 10 μM NA in a small control cell. Subsequent superfusion of 2 μM nifedipine suppresses $I_{Ba,L}$ and prevents NA-induced potentiation. Data points indicate peak I_{Ba} after a step to −10 mV from a holding potential of −60 mV. Sample data records are shown in the *inset*; calibration is 3 nA/10 msec. B_2, Time course of suppression of I_{Ba} in a small cell from an axotomized animal by 10 μM NA and further suppression of $I_{Ba,N}$ by 1 μM ω-cntxGVIA. NA fails to affect the current recorded in the presence of the toxin. Voltage commands are as in B_1. Sample data records are shown in *inset*; calibration is 4 nA/10 msec.

Table 4. Percentage change in I_{Ba} tails in response to NA

Cell type	Control	Axotomy	Autotomy
Large	0 (0/14)	−18.3 ± 1.9%* (3/12)	−25.9 ± 2.4%*** (8/12)
Medium	+22.2 ± 2.7%** (7/13)	−24.7 ± 1.7%*** (11/12)	−31.2 ± 2.3%*** (12/12)
Small	+37.2 ± 2.0%*** (12/14)	−26.8 ± 1.7%*** (13/13)	−32.3 ± 1.8%*** (13/13)

Negative changes indicate decreases in tail current amplitude, and positive changes indicate increases. Numbers in parentheses indicate number of cells responding relative to number of cells tested. Percentage changes are calculated only from those cells that responded. *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$. Comparisons of control current with currents seen in the presence of NA (paired t test).

DISCUSSION

These data show that NA acts via a β-adrenoceptor in control DRG cells to increase $I_{Ca,L}$ and spike width without altering excitability. After axotomy, however, effects mediated via β-adrenoceptors are lost. NA then acts on α_2-adrenoceptors to decrease $I_{Ca,N}$ and spike width. Excitability is increased, and this may reflect attenuation of $g_{K,Ca}$ after suppression of Ca^{2+} influx. This conclusion is supported by the observations that suppression of I_{Ca} with Cd^{2+} increased excitability in much the same way as NA (Fig. 4) and that α_2-adrenoceptor activation suppressed Ca^{2+}-sensitive outward current in axotomized cells (Fig. 6*C*). It is also possible that, under our experimental conditions, effects mediated via $g_{Cl,Ca}$ (Mayer, 1985) may contribute to NA-induced increased excitability of axotomized cells. This is because $g_{Cl,Ca}$ would produce outward current at the recorded RMP of DRG cells (−55 to −60 mV and for our recording conditions; estimated $E_{Cl} = -110$

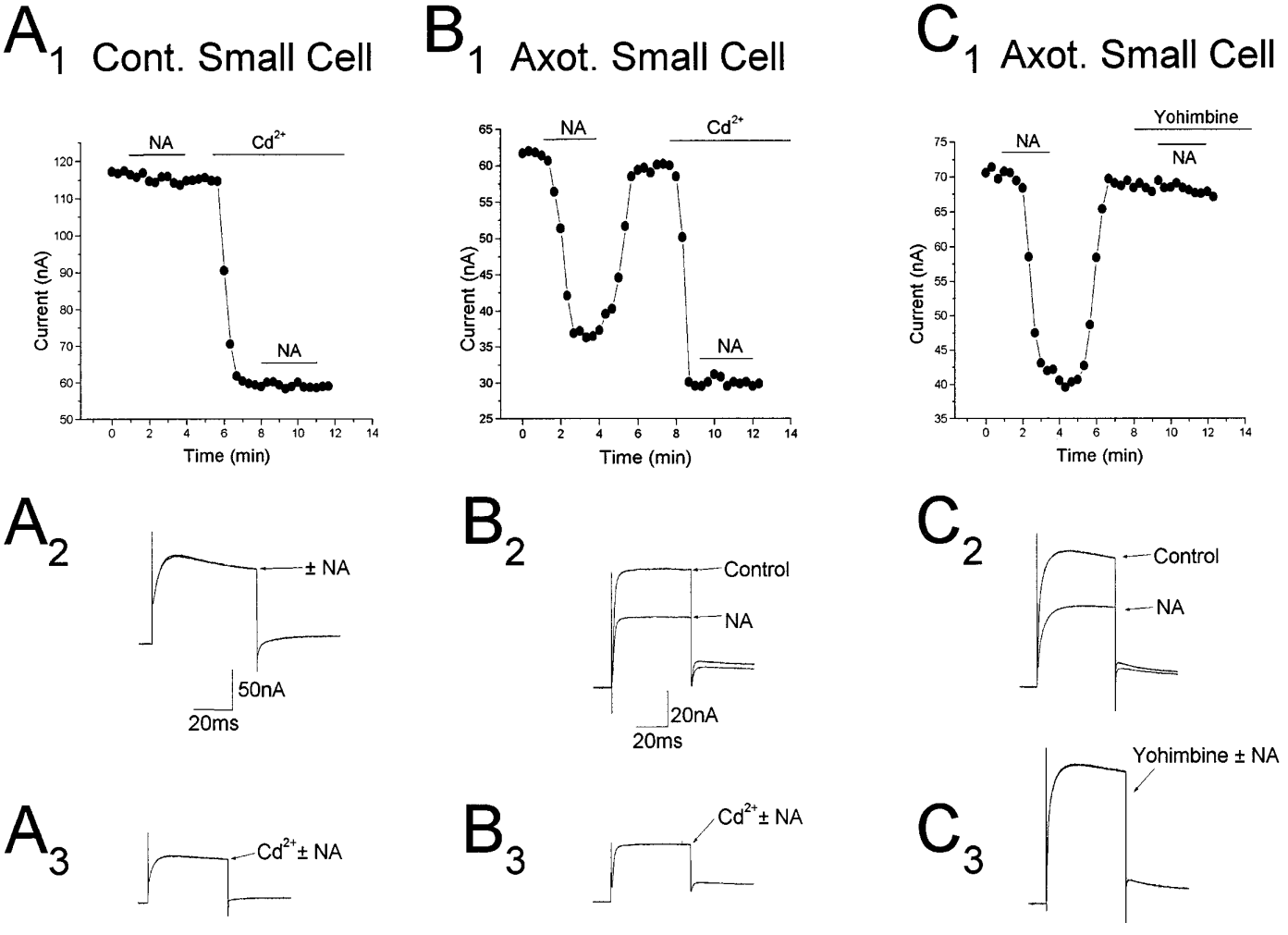

Figure 6. Lack of effects of NA on outward currents in DRG cells from control animals and suppression of Ca^{2+}-sensitive portion of current in cells from axotomized animals. A_1, A_2, A_3, Lack of effect of 10 μM NA on total outward current recorded in a small cell at +70 mV from a holding potential of −90 mV in the absence and in the presence of 1 mM Cd^{2+}. A_1, Graphic representation of the time course of the experiment; each point represents the amplitude of the outward current response to a test pulse to +70 mV evoked once every 20 sec. A_2, Superimposed current responses recorded in the presence and absence of NA. A_3, Superimposed responses recorded in Cd^{2+} in the presence and absence of NA. B_1, B_2, B_3, Reduction of Ca^{2+}-sensitive component of outward current in an axotomized small cell by 10 μM NA (voltage commands as in *A*). B_1, Graphic representation of the time course of the experiment; note that NA only suppresses the outward current before the addition of 1 mM Cd^{2+}. B_2, B_3, Superimposed data records to illustrate action of NA and its occlusion by Cd^{2+}. C_1, C_2, C_3, Pharmacology of reduction of outward current in an axotomized small cell by 10 μM NA (voltage commands as in *A* and *B*). C_1, Graphic representation of the time course of the experiment; note that NA does not suppress the outward current in the presence of yohimbine (1 μM). C_2, C_3, Superimposed data records to illustrate antagonism of the action of NA by yohimbine. Calibration (20 nA/20 msec) in *B* refers to both *B* and *C*. All voltage traces were omitted for clarity.

Table 5. Percentage change in outward current in response to NA

Cell type	Control	Axotomy	Autotomy
Large	0	−24.5 ± 5.0%*	−31.4 ± 2.1%***
	(0/11)	(2/10)	(8/11)
Medium	0	−33.6 ± 2.6%**	−41.6 ± 3.5%***
	(0/13)	(8/10)	(10/11)
Small	0	−37.2 ± 3.1%***	−45.9 ± 2.6%***
	(0/14)	(11/14)	(12/12)

Negative changes indicate decreases in amplitude recorded at +70 mV from a holding potential of −90 mV. Numbers in parentheses indicate number of cells responding relative to number of cells tested. Percentage changes are calculated only from those cells that responded.

*$p < 0.05$; **$p < 0.005$; ***$p < 0.001$. Comparisons of control currents with currents seen in the presence of NA (paired t test).

mV because gluconate was used as the internal anion). Suppression of this conductance secondary to NA-induced decreases in I_{Ca} would, therefore, be expected to initiate depolarization and to increase excitability. The contribution of a change in $g_{Cl,Ca}$ *in vivo* is likely to be minor, because under these conditions E_{Cl} would be expected to be closer to the RMP.

Under current clamp, we found that criteria based on spike width, spike shape, and C_{in} divided DRG cells into three distinct groups, which we have termed small, medium, and large. Although C_{in} is the only parameter available for classification of neurons under voltage clamp (see Scroggs and Fox, 1992), the cells that were sampled seemed to fit into the same three groups. This was because the magnitude of the effect of NA seen in each group of cells under voltage clamp corresponded with effects seen in the same, better-characterized group studied under current clamp. For example, the suppression of I_{Ba} by NA in axotomized neurons under voltage clamp is greatest for small and medium cells and weakest for large cells (Tables 3 and 4). Under current clamp, NA increases the excitability and attenuates the spike width of small axotomized cells more than that of medium and large cells (Table 2).

There is not, however, an exact correspondence between the number of cells of each type that respond to NA under each experimental condition. For example, I_{Ba} is reduced by NA in all small cells in the axotomy group (Tables 3 and 4), whereas increases in excitability occur in only 61.1% of the cells in this group. The probable reason for this difference is that current is a continuous variable, whereas excitability (number of spikes) is a discontinuous variable. Thus, a measurable change in I_{Ba} may not, necessarily, be reflected as a change in the number of APs generated by a depolarizing current command. A related possibility to consider is that some subgroups of cells may fail to develop sensitivity to α_2-adrenoceptor agonists after injury. This may be especially likely for large cells because, even in the autotomy group, NA only reduced the spike width of approximately one-half of the cells studied (12 of 26; Table 2). As part of another study (our unpublished observations), we subdivided large cells into six subgroups on the basis of differences in the amplitude, shape, duration, and rate of onset of AP afterhyperpolarization (see Villière and McLachlan, 1996). Because NA affected ~50% of the cells in four of these six subgroups, it is unlikely that its actions are confined to one particular subset of large neurons. The number of cells in the remaining two subgroups of large cells was too small to assess the sensitivity of these populations to NA accurately.

Because cells with broad APs generally have slowly conducting axons (Villière and McLachlan, 1996), the small cell population likely includes C-fiber nociceptive afferents (Bessou and Perl, 1969). It is also likely that some of the medium-sized cells that display a hump on the falling phase of their APs subserve a nociceptive role (Koerber et al., 1988). NA, therefore, seems to have its largest effects on populations of cells that include those that fulfill a nociceptive function. We therefore suggest that altered coupling between adrenoceptors and Ca^{2+} channels may provide a mechanistic basis for the sympathetically induced activity in damaged sensory nerves that has been implicated in the etiology of chronic pain (Devor et al., 1994).

The idea that an interaction between sympathetic fibers and nociceptive afferents occurs in the DRG is controversial because, after injury, arborations of sympathetic nerves form only around the large cells (McLachlan et al., 1993; Zhou et al., 1996). Although most large cells normally do not transmit nociceptive information, nerve injury may cause reorganization of their spinal projections (Woolf et al., 1992) so that they contact ascending pain pathways. The result that small cells become especially sensitive to NA is consistent with results from extracellular recordings of C-fiber polymodal nociceptors (Sato and Perl, 1991). Because stimulation of sympathetic nerves excites afferent C-fibers (Xie et al., 1995) in nerve-lesioned rats, it is possible that the transfer or generation of nociceptive information could be facilitated if NA were to diffuse to small cells after its release from the sympathetic arborations that abut the large cells. Indeed, a similar mechanism operates in another peripheral ganglion; the LHRH-like peptide that is released from C-fiber arborations around C-cells in bullfrog sympathetic ganglia evokes a postsynaptic response in B-cells that do not have physical contact with C-fiber arborations (Jan et al., 1979).

NA-induced reduction of AP duration and suppression of I_{Ca} in DRG neurons previously has been described in embryonic chick DRG neurons growing in tissue culture (Dunlap and Fischbach, 1978; Holz et al., 1986). Data from the present paper suggest, however, that the actions of NA on avian neurons in culture may correspond only to those seen in freshly dissociated, mature, mammalian DRG neurons after axotomy. The effect of NA on DRG cells from control rats is a β-adrenoceptor-mediated increase in $I_{Ca,L}$. Although β-adrenoceptor-mediated enhancement of I_{Ca} has been described in hippocampal neurons (Gray and Johnston, 1987), it has not been described previously in DRG (Cox and Dunlap, 1992). Because β-adrenoceptor stimulation does not have pronounced effects on the excitability of DRG cells, this would explain why NA seemed to be without effect in experiments performed with extracellular recording techniques (Xie et al., 1995). Because the α_2-adrenoceptor-mediated suppression of $I_{Ca,N}$ increases the excitability of axotomized sensory neurons and this may facilitate the generation of spontaneous activity, modulation of $I_{Ca,N}$ in DRG cell bodies may affect the processing of sensory information. This conclusion represents a departure from the view that modulation can be exerted only at primary afferent terminals in the spinal cord or at the peripheral receptors of sensory neurons (see Dunlap and Fischbach, 1978; Holz et al., 1986; Scroggs and Fox, 1992).

In the B-cells of bullfrog sympathetic ganglia, N-type Ca^{2+} channels seem to be in close proximity to high conductance, voltage-dependent, Ca^{2+}-sensitive K^+ channels (I_C channels; Jassar et al., 1994). If a similar situation obtains for rat DRG neurons, this may explain why suppression of $I_{Ca,N}$ evokes a pronounced increase in excitability, whereas enhancement of $I_{Ca,L}$ has minimal effects; the $I_{Ca,L}$ channels may not be close enough to

alter the Ca^{2+} concentration at the inner face of I_C or other Ca^{2+}-sensitive K^+ channels.

The observation that noradrenergic effects are more pronounced in cells from animals that exhibit autotomy is consistent with the hypothesis that this behavior is a response to spontaneous, aberrant activity in damaged sensory nerves (Coderre et al., 1986) (but also see Rodin and Kruger, 1984). Although it is impossible to determine whether this aberrant activity actually is perceived by the animal as pain, the fact that NA excites afferents associated with nociception seems consistent with this possibility. A further aspect of the data is that the α_2-adrenoceptors that appear in cell bodies after axotomy become associated with N-type Ca^{2+} channels, whereas the β-adrenoceptors that normally are present are associated with L-type Ca^{2+} channels. This presumably affects affinity of the receptors for appropriate G-proteins, because both N- and L-type Ca^{2+} channels are present before and after axotomy (our unpublished observations). It is also significant that the β-adrenoceptor mechanism is abolished after axotomy. This raises the possibility that the emergence of α_2-adrenoceptor mechanism in the cell body somehow suppresses effects mediated via β-adrenoceptors.

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