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Enhancement of GABA_A Receptor-Mediated Conductances Induced by Nerve Injury in a Subclass of Sensory Neurons

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SUMMARY AND CONCLUSIONS

1. The effects of axotomy on the electrophysiologic properties of adult rat dorsal root ganglion (DRG) neurons were studied to understand the changes in excitability induced by traumatic nerve injury. Nerve injury was induced in vivo by sciatic nerve ligation with distal nerve transection. Two to four weeks after nerve ligation, a time when a neuroma forms, lumbar (L4 and L5) DRG neurons were removed and placed in short-term tissue culture. Whole cell patch-clamp recordings were made 5–24 h after plating.
2. DRG neurons were grouped into large (43–65 μm)-, medium (34–42 μm)-, and small (20–32 μm)- sized classes. Large neurons had short duration action potentials with ~60% having inflections on the falling phase of their action potentials. In contrast, action potentials of medium and small neurons were longer in duration and ~68% had inflections.
3. Pressure microejection of γ -aminobutyric acid (GABA, 100 μM) or muscimol (100 μM) onto voltage-clamped DRG neurons elicited a rapidly desensitizing inward current that was blocked by 200 μM bicuculline. To measure the peak conductance induced by GABA or muscimol, neurons were voltage-clamped at a holding potential of -60 mV, and pulses to -80 mV and -100 mV were applied at a rate of 2.5 or 5 Hz during drug application. Slope conductances were calculated from plots of whole cell current measured at each of these potentials.
4. GABA-induced currents and conductances of control DRG neurons increased progressively with cell diameter. The mean GABA conductance was 36 ± 10 nS (mean $\pm$ SE) in small neurons, 124 ± 21 nS in medium neurons, and 527 ± 65 nS in large neurons.
5. After axotomy, medium neurons had significantly larger GABA-induced conductances compared with medium control neurons (390 ± 50 vs. 124 ± 21 ; $P < 0.001$). The increase in GABA conductance of medium neurons was associated with a decrease in duration of action potentials. In contrast, small neurons had no change in GABA conductance or action potential duration after ligation. The GABA conductance of large control neurons was highly variable, and ligation resulted in an increase that was significant only for neurons $>50 \mu\text{m}$. The mean action potential duration in large neurons was not significantly changed, but neurons with inflections on the falling phase of the action potential were less common after ligation. There was no difference in resting potential or input resistance between control and ligated groups, except that the resting potential was less negative in small cells after axotomy.

6. Histograms of neuronal diameter distributions were constructed for control and ligated groups. The cell diameter distribution of control and ligated neurons were similar, but the ligated group had a decrease in the proportion of large neurons and a 27% increase in medium neurons, with no change in the relative proportion of other size classes. The 27% increase in the number of medium neurons was unlikely to be solely responsible for the 314% increase in GABA conductance seen in medium neurons after ligation, although some of the observed change could be attributed to a shift of large neurons (and their associated electrophysiologic properties) into the medium group.
7. These results indicate that axotomy resulted in a significant increase in GABA_A receptor-mediated conductance in specific size classes of sensory neurons. We hypothesize that this selective increase in GABA conductance results from an injury-induced increase in the density of GABA_A receptors on the soma or a change in the expression of specific GABA_A receptor subtypes with different single channel conductances.

INTRODUCTION

In response to nerve transection, electrophysiological changes occur proximal to the site of axotomy (for review see Titmus and Faber 1990) as well as at the site of injury. The somata of dorsal root ganglion (DRG) neurons can become a site of abnormal impulse generation after peripheral nerve injury (Burchiel 1984; DeSantis and Duckworth 1982; Devor and Wall 1990; Kajander et al. 1992; Wall and Devor 1983). Thus the clinical symptoms such as chronic pain and dysesthesias experienced after injury may result from changes in excitability at both the neuroma as well as more proximal somatic regions.

DRG neurons are heterogeneous in size, and soma diameter is roughly proportional to axon diameter (Harper and Lawson 1985a,b). Small DRG neurons give rise to nonmyelinated axons, whereas large neurons have myelinated axons (Harper and Lawson 1985a,b). DRG neurons of different size classes also have distinct electrophysiological properties. Activity of large diameter myelinated sensory fibers commonly contributes to unpleasant sensations after injury, even though nonmyelinated (C-fiber) and small diameter myelinated (A δ -fiber) axons constitute the classical pain pathways (Bowsher 1991). A common sensory symptom after nerve injury is a dysesthesia characterized by an uncomfortable “tingling,” which is thought to be mediated by ectopic impulse generation and hyperexcitability in sensory neurons with large diameter myelinated axons (Devor 1994; Merrington and Nathan 1949; Rasminsky 1978; Scadding 1981; Wall and Devor 1978).

DRG neurons have γ -aminobutyric acid-A (GABA_A) receptors on their cell bodies (White 1990), myelinated axons (Bhisitkul et al. 1990), and axon terminal regions (Barker and Nicoll 1973). Activation of GABA_A receptors results in membrane depolarization because of an increase in chloride conductance (White 1990). The functional role of GABA_A receptors on the soma and myelinated axons of DRG neurons is not clear; however, GABA_A receptors on presynaptic terminals in the spinal cord mediate presynaptic inhibition, which plays a role in the integration of sensory information (Curtis and Lodge 1982; Levy 1977). Ligation of the sciatic nerve has been shown to reduce GABA_A receptor-mediated depolarization of the central axonal extension (dorsal roots) of these neurons (Bhisitkul et al. 1990; Kingery et al. 1988), and to widen peripheral receptive fields (Devor and Wall 1978, 1981; Horch and Lisney 1981; Wall 1982; Wall and Devor 1981; Woolf and Wall 1982). It has been suggested that a reduction in GABA_A receptor-mediated inhibition at the presynaptic terminal may enhance transmission of sensory impulses into the CNS after chronic nerve injury, which may then contribute to sensory abnormalities (Bhisitkul et al. 1990).

In the present study, we have examined the effects of sciatic nerve ligation on GABA_A receptor-mediated conductances and action potential properties at the soma of DRG neurons as a model of neuronal receptor plasticity in response to nerve injury. In contrast to the reduction in GABA_A receptor-mediated responses observed in the axon terminals of DRG neurons after sciatic nerve ligation (Bhisitkul et al. 1990), we found that there was a prominent increase in GABA_A-receptor mediated conductance at the soma in a subpopulation (medium and some large, but not small neurons) of DRG neurons. These results demonstrate that injury-induced changes in DRG neuronal GABA receptors are both spatially heterogeneous and nonuniform across the DRG population, but occur primarily within a subpopulation of neuronal types.

Some of this work has previously been reported in abstract form (Eng et al. 1992; Oyelese et al. 1993).

METHODS

Nerve ligation and cell culture techniques

Wistar rats (140–160 g) were anesthetized with an intraperitoneal injection of ketamine (40 mg/kg) and xylazine (2.5 mg/kg). The sciatic nerve was exposed and ligated (4–0 silk suture) near the sciatic notch bilaterally (Kocsis et al. 1984). To prevent regeneration and promote formation of a neuroma, the nerve was sectioned immediately distal to the ligature site; a 10- to 15-mm section of the distal nerve was removed and the distal stump was retracted. A silicone cap was sutured to the end of the proximal stump. Not all neurons in the L4, L5 DRG were axotomized by this procedure and as many as 30% may have been unaffected (Himes and Tessler 1989) (Fig. 1). Unoperated animals were used as controls.

Two to four weeks after axotomy, cultures were prepared from DRG neurons supplying the injured nerve. Rats (180–240g) were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg), and decapitated. The L4 and L5 DRG were excised bilaterally and placed in cold oxygenated electrolyte solution (in mM): 124 NaCl, 3.0 KCl, 2.0 MgCl₂, 1.3 NaH₂PO₄, 26 NaHCO₃, 10 dextrose, 100 units/ml penicillin and streptomycin. DRG were desheathed and enzymatically digested for 25 min with collagenase (1 mg/ml) in the following solution (in mM): 137 NaCl, 5.3 KCl, 1 MgCl₂, 25 sorbitol, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 4.5 CaCl₂, 0.5 ethylenediamine tetraacetic acid (EDTA), and 0.2 mg/ml cysteine, pH = 7.2, at 40°C). Neurons were digested for an additional 25 min with papain (30 units/ml) and collagenase (1 mg/ml) in the same solution. The tissue was triturated in a solution of 45% Dulbecco's modified Eagle's medium (DMEM), 45% F12 medium, and 10% fetal calf serum (FCS) containing trypsin inhibitor (1.5 mg/ml) and bovine serum albumin (1.5 mg/ml). Cells were plated at a density of ~25 neurons/mm² on UV-irradiated glass coverslips coated with poly-L-ornithine (0.1 mg/ml) and laminin (9 µg/coverslip). One hour later the cells were fed with media containing 45% DMEM, 45% F12, 10% FCS, and 100 units/ml penicillin and streptomycin, followed by another feeding 12 h later.

Electrophysiological techniques

Electrophysiologic measurements were obtained 5–24 h after plating. Neurons were placed in a recording chamber on the stage of an inverted microscope (Leitz Fluovert), and superfused with Ringer solution containing (in mM): 124 NaCl, 3.0 KCl, 2.0 CaCl₂, 2.0 MgCl₂, 1.3 NaH₂PO₄, 26 NaHCO₃, 10 dextrose, pH = 7.4) at room temperature (20–24°C) at a rate of 3–5 ml/min. GABA (100 µM) or muscimol (100 µM) were mixed in the above solution and applied to individual cells by pressure microejection (Picospritzer 2, General Valve) using a micropipet positioned near the neuron with a hydraulic micromanipulator (Narishige). The perfusion micropipet was positioned between the cell and bath suction so that any residual

leakage did not reach the cell causing chronic desensitization of GABA receptors. Trypan blue (0.4%) was added to the solution to allow visualization of the stream so that the entire surface area of the neuron was bathed in drug (Fig. 2B). Bicuculline methiodide (200 μ M) was applied in the bath solution.

Whole cell recordings were obtained from DRG neurons using the patch-clamp technique (Hamill et al. 1981). Recording electrodes were fabricated from thin-walled, single filament borosilicate glass tubing (World Precision Instruments) with a micropipet puller (Model P-80/PC, Sutter Instruments). Electrodes were filled with a solution containing, (in mM): 140 KCl, 2 MgCl₂, 1 CaCl₂, 11 ethylene glycol-bis (β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 10 HEPES (pH 7.2, osmolarity 290–305 mosmol). Recordings from large and medium neurons were made with 1–2 M Ω electrodes; whereas those from small neurons were made with 3–4 M Ω electrodes. Seal resistances were ≥ 2 G Ω .

Voltage-clamp recordings were made with a patch-clamp amplifier (Axopatch ID, Axon Instruments) using either a standard headstage (CV-4 1/100) or a low gain headstage (CV-4 O.1/100). Data were digitized and stored on computer using a commercially available data acquisition system (TL-1 DMA interface, and PClamp software, Axon Instruments) and on a VCR using a digitizing unit (PCM-2, Medical Systems; or Neurocorder DR-484, Neurodata instruments). The culture procedure was optimized to yield spherical neurons that were either aneuritic or had only a single neuritic bud at the time of recording, to decrease space clamp artifact.

Action potential data were obtained in current clamp mode. A series of hyperpolarizing and depolarizing current pulses of 100-ms duration from -0.2 nA or -0.4 nA with 12 increments ranging from 0.1 to 0.4 nA were used to examine action potential characteristics of each neuron. Action potential width (at 50% of spike height) was analyzed for each neuron.

Estimation of access resistance

GABA-induced whole cell conductances were compared within small (20–32 μ m), medium (34–42 μ m) and large (43–65 μ m) neuron groups from ligated and control animals. Reliable quantitative comparison of whole cell GABA conductance between different cells required accurate estimation of access resistance, because GABA-induced currents could be large. Access resistance was estimated using three techniques: fitting a single exponential curve to the uncompensated current trace resulting from a voltage-clamp pulse from -60 to -70 mV to calculate access resistance, input resistance, and cell capacitance; measuring the time constant of voltage change in response to a small hyperpolarizing current step in current clamp to calculate input resistance and cell capacitance, then using this measure of capacitance to calculate access resistance from the time constant of current decay resulting from a voltage clamp pulse from -60 to -70 mV; or using the series resistance and capacitance compensation feature of the patch-clamp amplifier to estimate access resistance and cell capacitance. Values obtained using these three methods typically were within 10% of each other. When using the standard headstage, all three methods were used. When using the low gain headstage, only the two methods using the exponential fits could be used, because capacitance compensation is not available for that headstage. Only those neurons in which adequate series resistance compensation was achieved (>80%) were included in the analysis. Using this approach, the error in measurement of whole cell GABA-induced current was minimized in all but the cells with largest currents, which were in the ligated group. The size of the largest GABA-induced currents approached the limits of adequate series resistance compensation; however, the errors that resulted would underestimate the size of these large currents, and thus would only serve to blunt our finding of an increase in conductance in the ligated group compared with controls.

Conductance measurements

To measure membrane conductance at the peak of the response before desensitization, neurons were voltage clamped at a holding potential of -60 mV and test pulses (40 ms in duration to -80 and -100 mV) were applied (pulse protocol repeated at 2.5 or 5 Hz) throughout the application of GABA or muscimol (see Fig. 3A, 3, for example of protocol). The slope conductance was calculated from the steady-state current measured at -60, -80, and -100 mV. The conductance increase induced by drug application was calculated as the difference between the whole cell conductance at the peak of the GABA or muscimol response and the conductance during the baseline before drug application. This protocol was repeated during three applications of GABA to ensure that the response was stable and was completed within 20 min of recording, after which time the GABA-induced currents displayed a reduced response because of rundown. In some neurons, test potentials from -20 to -40 mV and 0 mV also were applied to determine the reversal potential and to evaluate whether there was rectification of the GABA *I-V* curve. However, this protocol activated voltage-dependent currents unless antagonists of K⁺ and Na⁺ currents were used. Since one purpose of these experiments was to measure resting potential and action potential waveform, solutions containing pharmacological agents were not routinely used.

Cell size distribution

Neurons obtained from normal and injured animals were maintained in culture for one day and analyzed for cell size distribution. Images of neurons were digitized using a CCD camera and image analysis system (Georgia Instruments). The diameters of cultured DRG neurons obtained from five control rats ($n = 2,457$ neurons) and six ligated rats ($n = 2,318$ neurons) were measured. The largest diameter was used in the case of cells that were not uniformly round and was usually within 2 μm of the smaller diameter. Histograms of cell diameter were constructed for a cell diameter range from 2 to 70 μm (bin size = 2 μm) for each animal. The number of cells in each size bin for each animal was normalized by dividing by the total number of cells from that animal to compensate for the variability in number of cells plated from one animal to another. Histograms were plotted as the mean $\pm$ SE fraction of total cell number per bin. Standard deviations and significance levels were determined with the Keuls-Student *t*-test.

RESULTS

DRG neurons exhibit GABA_A receptor-mediated currents

When GABA (100 μM) was pressure microejected onto DRG neurons voltage clamped at -60 mV, an inward current was elicited with a characteristic rapid desensitization (Fig. 2C). The inward current reached a peak within 200 ms and declined to a plateau despite continued application of GABA, reflecting desensitization of the GABA_A receptor. The plateau persisted until the end of GABA application and then returned to baseline approximately 3 s later. The GABA_A agonist muscimol elicited a response similar to that seen with GABA application. In the example shown (Fig. 2D), a series of voltage-clamp steps from a holding potential of -60 mV to test potentials of -80 and -100 mV (40-ms duration) were repeated at 2.5 Hz during muscimol application to measure the peak response before the onset of desensitization. Note that the inward current is accompanied by an increase in conductance. The competitive GABA_A antagonist bicuculline (200 μM) blocked the muscimol-induced current and conductance change (Fig. 2D, arrow). Responses in DRG neurons to GABA_A receptor agonists previously have been shown to be induced by activation of a GABA_A receptor-mediated increase in chloride permeability (Robertson 1990).

Axotomy increased GABA_A receptor-mediated conductance in medium and large neurons

In DRG neurons cultured from animals that previously had undergone sciatic nerve ligation (ligated neurons), GABA also induced an inward current; however, the amount of current that could be elicited tended to be larger than in control neurons. Figure 3 compares the whole cell current during application of GABA (100 μ M) in a 40- μ m control neuron (Fig. 3A) and a 40- μ m ligated neuron (Fig. 3B) under the same voltage clamp protocol as in Fig. 2D. At the holding potential of -60 mV, the peak current during GABA application for the control neuron was 11 nA (Fig. 3A, *I*) as compared with 75.7 nA for the ligated neuron (Fig. 3B, *I*). To calculate the GABA-induced conductance, the steady-state currents were measured at -60 mV and at the end of the test pulses to -80 and -100 mV, at the peak of the drug response (Fig. 3, A, 2, and B, 2, *bottom*) and during the baseline before drug application (Fig. 3, A, 2, and B, 2, *top*). The slope conductance was calculated from these three points at the peak of the response and at rest. The GABA-induced whole cell conductance was then calculated as the difference between the peak whole cell conductance and the resting whole cell conductance. For example, in these two size-matched medium-sized neurons, the GABA-induced whole cell conductance was 1,300 nS for the injured neuron and 205 nS for the control neuron (Fig. 4).

The increase in GABA-induced whole cell conductance found in chronically injured DRG neurons was seen in medium and large neurons, but not in small neurons (Table 1). For medium-sized neurons (34–42 μ m), the mean G_{GABA} was three times greater in the ligated group compared with controls and was also more heterogeneous [390 ± 50 nS ($n = 40$) vs. 124 ± 21 nS ($n = 32$); $P < 0.001$, Table 1]. In contrast, for small neurons (20–32 μ m), there was no difference in mean G_{GABA} [36 ± 10 nS in control neurons ($n = 17$) vs. 59 ± 27 nS in ligated neurons ($n = 14$)]. Of the ligated small neurons, only one cell was significantly different from controls and it appeared at 32 μ m, the upper limit for this size class (Fig. 5). In those neurons in which a full current-voltage (*I-V*) curve was obtained ($n = 18$), reversal potentials were close to 0 mV, which was expected for the solutions used with symmetric Cl⁻ concentration. Although rectification was seen in some small ($n = 6$) and medium ($n = 7$) control neurons, loss of rectification alone was not large enough to explain the increased slope conductance measured between -60 and -100 mV in medium ligated neurons. In addition, in the small ligated neurons in which a full *I-V* curve was obtained ($n = 5$), some rectification also was observed. With the exception of small neurons in which resting potential was less negative in injured neurons (-61 ± 5 mV) compared with controls (-82 ± 4 mV), resting potential and input resistance did not differ between control and ligated neurons (Table 1).

When whole cell GABA-induced conductance was plotted for the control and ligated groups versus neuron diameter (Fig. 5), it could be seen that there was no difference in conductance between small neurons (20–32 μ m) in the control and ligated groups, but the conductance of medium ligated neurons was increased compared with controls. There was much more scatter among the large control neurons, but there was a tendency for the large ligated neurons to have an increase in conductance. When neurons were separated into small-, medium-, and large-size groups (Fig. 6A), the difference in GABA-induced conductance between normal and ligated neurons was found to be statistically significant only for the medium-sized group. When the size of neurons was subdivided into smaller groups (Fig. 6B), it was found that the GABA-induced conductance of the ligated neurons was significantly increased in the 35–39 μ m and 40–44 μ m range (corresponding to the medium-sized class), as well as those neurons >50 μ m neurons (a subset of the large-sized class), but not in small neurons or in large neurons between 45 and 49 μ m.

Diameter distribution of DRG neurons after axotomy

One possible explanation for the increase in G_{GABA} observed in injured medium neurons is cell shrinkage, whereby large neurons retain the same number of GABA receptors but reduce

their surface area, shifting into the medium-sized class with increased receptor densities. To address this question, we examined neuronal size distributions for control and ligated populations. Neurons were used for morphometric analysis after 1 day in culture, which corresponds to the time of recording. Diameter distributions, plotted as a fraction of total population (see METHODS), were qualitatively similar for control ($n = 2,457$ neurons) and ligated ($n = 2,318$ neurons) neurons (Fig. 7); however, there was a 50% decrease in the percentage of neurons $>42 \mu\text{m}$ in the ligated cultures (11 vs. 22%), a 27% increase in the percentage of neurons $34\text{--}42 \mu\text{m}$ (21 vs. 16%) and no change in the percentage of neurons in the other size classes. A shift in cell size from large to medium neurons may have contributed to the increase in GABA conductance seen in medium neurons, but the observed increase in GABA_A conductances of medium neurons could not be accounted for by cell shrinkage alone (see DISCUSSION).

Axotomy-induced changes in GABA response correlated with a change in action potential shape

In addition to the increase in GABA-induced conductance of chronically injured medium and large DRG neurons, we also observed changes in the shape of action potentials in these same neurons. Action potentials were elicited by step depolarizations in current-clamp mode (Fig. 8), after the membrane potential was normalized to approximately -60 mV . Action potentials from medium and small control neurons (Fig. 8, A and C) were long in duration ($>2 \text{ ms}$), displayed an inflection on the downstroke, and had a slow rate of rise. In contrast, the action potentials of large control neurons were shorter in duration ($<2 \text{ ms}$) and had a rapid rate of rise (Fig. 8E). After ligation, action potentials from small neurons showed no difference in their duration, but a higher percentage were observed to have an inflection on the falling phase (Table 1, Fig. 8B). In contrast, medium neurons had a more rapid rise time and a significantly shorter duration after ligation (Table 1, Fig. 8D). Medium neurons were also less likely to have an inflection during the falling phase of the action potential after ligation (percentage of medium neurons with inflections: controls, 68%; ligated, 36%; Table 1). In large neurons, the action potential duration was not significantly altered by injury, but these neurons also showed a shift from inflected to noninflected action potentials with injury; (controls, 61%; ligated, 30%; Table 1).

Within the medium-sized class, if an individual neuron had a short-duration, noninflected action potential, it was more likely to have a large G_{GABA} . This was evident for both control and ligated neurons. In the control medium-sized class, the G_{GABA} for neurons with noninflected action potentials was $202 \pm 62 \text{ nS}$ compared with $69 \pm 9 \text{ nS}$ for neurons with inflected action potentials. After ligation, the G_{GABA} for medium neurons with noninflected action potentials was $553 \pm 74 \text{ nS}$ compared with $142 \pm 41 \text{ nS}$ for medium neurons with inflected action potentials. In the control large-sized class, the G_{GABA} for neurons with noninflected action potentials was $857 \pm 104 \text{ nS}$ compared with $268 \pm 55 \text{ nS}$ for neurons with inflected action potentials. After ligation, the G_{GABA} for large neurons with noninflected action potentials was $810 \pm 92 \text{ nS}$ compared with $462 \pm 116 \text{ nS}$ for large neurons with inflected action potentials. Thus the presence of a short duration, rapidly rising, noninflected action potential was an accurate predictor of whether a neuron would have a large GABA conductance, and injury increased the percentage of neurons having action potentials with these characteristics. In contrast, small DRG neurons did not show these changes in action potential characteristics after injury (Fig. 8, A and B, Table 1).

DISCUSSION

Changes in GABA_A receptor-mediated conductance and electrophysiological properties of DRG neurons resulting from axotomy were examined as a model of receptor plasticity in

response to chronic injury. The results indicate that axotomy induced a threefold increase in GABA_A receptor-mediated conductance in medium-sized neurons, with a smaller increase in large neurons, and no change in small neurons. Accompanying these changes was a change in action potential waveform and a change in cell size distribution. These results indicate that there is specificity in the injury-induced functional plasticity of sensory neurons within different classes of DRG neurons.

The functional type of individual DRG neurons could not be defined using the methods employed in these experiments. However, retrograde labeling studies indicate that a majority of large neurons correspond to muscle afferents, whereas a large proportion of medium and small neurons correspond to cutaneous and nociceptive afferents, respectively (Honmou et al. 1994; Lee et al. 1986; Peyronnard and Charron 1982). Moreover, small neurons give rise primarily to axons that are nonmyelinated, whereas axons arising from medium and large neurons tend to be myelinated. The increase in GABA-induced conductance seen in the present study primarily involved medium neurons, suggesting that injury-induced GABA_A receptor reorganization occurred primarily in neurons with myelinated axons.

The increase in somatic GABA_A conductances in DRG neurons after nerve injury could result from an increase in receptor density or a change in single channel conductance properties. Changes in receptor density could arise from an increase in receptor synthesis, a decrease in receptor degradation, a decrease in transport of newly synthesized receptor out of the soma with accumulation in the somatic membrane, or a reduction in cell size without a decrease in the number of receptors. Of these possibilities, a reduced rate of axonal transport of GABA_A receptors, resulting in increased incorporation of GABA_A receptors in the soma, appears to be the most consistent with existing evidence. It previously has been found that anterograde axoplasmic transport is reduced after axotomy (Hoffman and Lasek 1980; Hoffman et al. 1988) and dorsal root axons have been reported to have decreased GABA sensitivity after sciatic injury (Bhisitkul et al. 1990). Somal accumulation of the transganglionic marker BSI-B₄ with reduced axonal transport to the central terminals has been observed to occur in C fiber afferents but not in A β afferents (Kitchener et al. 1994). In the squid giant axon, the cells of origin in the giant fiber lobe do not normally express functional voltage-gated Na⁺ channels in their somata (Brismar and Gilly 1987). After axotomy, functional Na⁺ channels are expressed in the somatic membrane as a result of disruption of axonal transport, preventing the export of Na⁺ channels (which are synthesized in the somata) from incorporation in the giant axon and inappropriate proximal accumulation (Gilly and Brismar 1989; Gilly et al. 1990).

A reduction in cell size does occur in DRG neurons after long-term axotomy (Wells and Vaidya 1989); however, the significance of cell loss or cell shrinkage in the short period after ligation (2–4 wk) used in this study is unclear. Our morphometric analysis of plated neurons indicated that the cell diameter distribution of DRG neurons was altered at 2–4 wk postaxotomy. The largest neurons were reduced in number by 50%, and there was a 27% increase in medium neurons. However, from this analysis, it is not clear whether the decrease in percentage of large neurons was due to an actual shift of large neurons into the medium-sized class in vivo or whether there was a selective loss of large ligated neurons in vivo (Himes and Tessler 1989), or during the preparation of cultures.

Although a shift in cell size may have contributed to the increase in GABA_A receptor-mediated conductance in medium cells after ligation, this was unlikely to be sufficient to account for all of the differences observed for the following reasons. 1) In the 50–65 μ m neuron group where the greatest reduction in cell number was seen, the GABA conductance was significantly increased (Fig. 6B) and the action potential waveform was altered (Table 1), despite the fact that there was no pool of larger neurons available to shrink into this size class. This establishes

a precedent within a subpopulation of DRG neurons indicating that a form of GABA_A receptor remodeling other than cell size change may occur subsequent to injury. 2) The GABA conductance of medium ligated neurons was increased 314% over controls. If the increase in the GABA conductance of cells observed in the 34–42 μm range were due to a shift from the 43–65 μm group, given that there was no shift from the medium to small neurons (Fig. 7), the GABA conductance of medium neurons would be expected to increase by ~27% (the increase in the number of medium neurons) of the difference in GABA conductance between the large control (527 nS) and medium control (124 nS) groups, or an increase from 124 to 233 nS [i.e., $G_{\text{predicted}} \approx G_{\text{medium-control}} + 0.27 (G_{\text{large-control}} - G_{\text{medium-control}})$]. The GABA conductance of the ligated medium group increased from 124 to 390 nS or by 66% of the difference between large and medium control cells. Therefore, a shift in cell size cannot account entirely for the large increase in GABA_A receptor-mediated conductance observed in the axotomized medium neurons. Further studies on cells from identified functional classes in this size range (i.e., cutaneous and muscle afferents) will be necessary to confirm the interpretation of these data.

The increase in GABA_A conductance in medium neurons seen here could potentially have resulted from an increase in single-channel conductance of GABA_A receptors in this size class. The GABA_A receptor is composed of a combination of subunits (Burt and Kamatchi 1991), with different possible subunit combinations giving rise to the native receptor. Different subunit combinations can give rise to GABA_A receptors with different biophysical and pharmacological properties (Krishek et al. 1994; Sigel et al. 1990; Verdoorn et al. 1990). Our findings suggest that some form of reorganization of the GABA_A receptor or its regulatory elements must have occurred within medium neurons subsequent to sciatic nerve injury. Whether it is an upregulation of somatic GABA_A receptors (secondary to accumulation or increased synthesis) or an increase in single channel conductance resulting from an altered subunit composition or second messenger regulation is uncertain. Future experiments utilizing single-channel recordings in neurons of a specific size and functional class labeled with retrograde marker may help to determine whether our results reflect a change in individual GABA_A receptors or a change in number or distribution of receptors.

In addition to a change in GABA sensitivity, we found that the duration of action potentials was decreased in injured medium neurons. Small and large diameter neurons did not show changes in action potential duration, although ligated large neurons were more likely to have noninflected action potentials than control large neurons. The reasons for the change in action potential waveform are unclear. Axotomy-induced changes in action potential shape have been observed in other neuronal types. After axotomy of vagal parasympathetic efferent neurons there is a disappearance of the hump on the downstroke of action potentials with narrowing of spikes (Laiwand et al. 1988) similar to the changes we observed in axotomized medium DRG neurons. Several studies have described increases in both action potential amplitude and duration in sensory and motor neurons (see Titmus and Faber 1990 for review). The action potential characteristics of bullfrog C-type sympathetic neurons are not affected by axotomy (Shapiro et al. 1987), just as the action potential duration of small DRG neurons remained unchanged after axotomy in the present study.

Systemic injection of antinerve growth factor (NGF) antibodies into neonatal rats during the first week after birth leads to a conversion of functional subtypes of cutaneous neurons (Lewin et al. 1992; Ritter et al. 1991). The population of high threshold mechanoreceptor neurons decreases, but the populations of D-hair and “deep” A δ neurons increase without change in the total number of cutaneous neurons, indicating a conversion in phenotype. In parallel with changes in receptive field properties, the action potentials in cell bodies change to fast noninflected action potentials characteristic of D-hair and “deep” type neurons. Our findings show a similar conversion of broad, inflected action potentials in medium neurons to a narrow noninflected wave-form and increased expression of noninflected action potentials in large

neurons in the adult rat after injury. Although some investigators report the upregulation of trophic factors in the DRG after nerve injury (Sebert and Shooter 1993), it is unclear that these changes adequately compensate for the effects of axotomy. Axotomy results in a reduction of the retrograde transport of target derived trophic factors; therefore, it will be important to determine if deprivation of NGF, brain-derived neurotrophic factor (BDNF), NT3 or other neurotrophins after nerve ligation plays a role in the electrophysiologic changes reported here. The selective change in GABA_A receptor conductance in medium neurons is interesting in the context of recent work indicating an approximate differential distribution of tyrosine kinase receptors (Trk receptors) Trk A, Trk B, and Trk C on small, medium and large neurons, respectively (McMahon et al. 1994; Mu et al. 1993). The three types of Trk receptors are activated more selectively by different trophic factors (NGF, BDNF, and NT3, respectively) (for review see Meakin and Shooter 1992), suggesting that different classes of DRG neurons may respond differently to alterations in endogenous trophic factors. The TrkB receptor, which shows high affinity binding to BDNF, appears to be largely expressed in medium-sized sensory neurons (McMahon et al. 1994; Mu et al. 1993). It is possible that altered expression of the TrkB receptor plays a role in the observed phenotypic shift after injury. Our method of nerve injury, which isolates the neuron from target-derived trophic influences, provides a convenient model for future investigation of the possible role of specific trophic factors and their receptors in the regulation of injury-induced functional plasticity in sensory neurons.

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FIG. 1.

Schematic drawing of surgical technique for axotomy. Sciatic nerve of adult rats was ligated, a 15-mm section of nerve was removed, and a silicone cap was placed over the proximal stump to prevent regeneration. As can be seen in the figure, not all L4, L5 dorsal root ganglion (DRG) neurons are axotomized in this procedure. Two to 4 wk after ligation the dorsal root ganglia were dissociated and neurons plated on glass coverslips.

FIG. 2.

Application of γ -aminobutyric acid-A (GABA_A) receptor agonists induced an inward current in DRG neurons. *A*: video micrograph (Hoffman Modulation Contrast optics) of a DRG neuron within 1 day in culture. Note the lack of neurites. Patch-clamp recording electrode (*left*) and the microejection pipet (*right*) can be seen. *B*: trypan blue (0.4%) was added to the microejection pipet to ensure complete coverage of the cell during drug application. *C*: application of GABA ($100 \mu\text{M}$) for 3 s to a medium-sized DRG neuron elicited an inward current. Neuron was voltage-clamped at -60 mV . Note the rapid desensitization of the GABA response despite continued application. *D*: application of muscimol ($100 \mu\text{M}$) produced a similar response. Hyperpolarizing voltage-clamp steps to -80 and -100 mV were applied during drug application. Bicuculline ($200 \mu\text{M}$, arrow) antagonized the response to muscimol.

FIG. 3.

Comparison of GABA-induced currents in a control neuron and a neuron after axotomy. GABA-induced whole cell current of the control medium-sized DRG neuron (A) was less than that of the ligated medium-sized DRG neuron (B). Both neurons were voltage clamped at -60 mV and step pulses were applied to -80 and -100 mV. This protocol was repeated (2.5 Hz) throughout GABA application. A and B, 1: whole cell current during GABA application at a slow time scale. Note the increase in conductance during the GABA response. A and B, 2: individual pulse protocols at fast time base. *Top*: baseline current before GABA application; *bottom*: current at the peak of the GABA response. A and B, 3: voltage-clamp protocol.

FIG. 4.

Comparison of peak GABA-induced slope conductance for the same cells as in Fig. 3. GABA-induced currents were calculated as the difference between whole cell currents at the peak of the GABA response and whole cell currents during rest, for the control (●) and the ligated (■) neuron. Slope conductances were calculated to be 205 nS for the control and 1,300 nS for the ligated neuron.

GABA CONDUCTANCE vs CELL SIZE

FIG. 5.

Relationship between GABA-induced whole cell conductance (nanosiemens) and neuron diameter (micrometers) for control (▲) and ligated (○) neurons. Because of extensive overlap, some data points at 28, 36, and 40 μm were displaced by $\pm 0.5 \mu\text{m}$ to permit better visualization of individual data points.

FIG. 6.

Axotomy induced an increase in GABA-induced whole cell conductance for specific size classes of neurons. *A*: separation of neurons into 3 size classes. Note that the increase in G_{GABA} after axotomy is limited to the medium-sized neurons (34–42 μm). *B*: separation of neurons into smaller sized classes. Use of a smaller grouping revealed that the G_{GABA} was significantly increased in medium neurons (35–39 μm and 40–44 μm) as well as a subclass of large neurons (50–65 μm). Small neurons, and large neurons in the 45–49 μm range, did not significantly increase their GABA conductance after injury. Control, open bars; ligated, shaded bars; n given in parentheses; * $P < 0.05$; ** $P < 0.01$.

FIG. 7.

Axotomy resulted in a small difference in neuronal size distribution. Size distribution, represented as fraction of total cell number for ligated neurons, ($\blacksquare$; means $\pm$ SE from 6 rats; $n = 2,318$ neurons) was compared with control neurons ($\bullet$; means $\pm$ SE from 5 rats; $n = 2,457$ neurons). After axotomy, there was a decrease in the percentage of neurons $>50 \mu\text{m}$, a small increase in neurons from 38 to $40 \mu\text{m}$, and no change in the relative proportion of other size classes (error bars are shown in one direction for clarity).

FIG. 8.

Axotomy resulted in a change in morphology of action potentials in medium-sized neurons. Action potentials elicited by a depolarizing current pulse in control small (A) and ligated small neurons (B), control medium (C) and ligated medium neurons (D), control large (E) and ligated large neurons (F). Note the narrow action potential in the ligated medium neuron (D) as compared with control (C). Large neurons showed no difference in action potential duration, but fewer neurons had spikes with inflections after nerve ligation, whereas small neurons showed no difference in action potential duration after ligation (Table 1).

TABLE 1
Effect of sciatic nerve axotomy on GABA conductance (G_{GABA}), resting potential (RP), input resistance (RI), input resistance (RI) and action potential (AP) waveform in small, medium and large LA, L5 DRG neurons

	Small Neurons		Medium Neurons		Large Neurons	
	Normal	Ligated	Normal	Ligated	Normal	Ligated
G_{GABA} (nS) <i>n</i>	36 ± 40 (17)	59 ± 103 (14)	124 ± 118 (32)	390 ± 316 ^{**} (40)	527 ± 388 (36)	702 ± 406 (29)
RP (mV) <i>n</i>	-82 ± 18 (13)	-61 ± 18 [*] (14)	-54 ± 7 (25)	-52 ± 5 (40)	-58 ± 7 (35)	-55 ± 7 (29)
RI (MΩ) <i>n</i>	438 ± 277 (17)	384 ± 216 (14)	138 ± 102 (32)	105 ± 106 (40)	84 ± 51 (36)	91 ± 64 (29)
AP half-width (ms) <i>n</i>	3.19 ± 1.6 (14)	3.57 ± 2.88 (13)	2.58 ± 1.79 (25)	1.44 ± 0.99 ^{**} (32)	1.47 ± 0.76 (30)	1.24 ± 0.72 (28)
AP percent inflected	66.67%	84.6%	68%	36.36%	61.29%	32.14%

Small neurons were 20–32 μm; medium neurons, 34–42 μm; large neurons, 43–65 μm.
values are given as means ± SD.

* $P < 0.05$

** $P < 0.01$