Characterization of acute somatosensory pain transmission in P/Q-type Ca^{2+} channel mutant mice, *leaner*

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Abstract To study the role of the $Ca_v2.1/\alpha_{1A}$ (P/Q-type) Ca^{2+} channel in somatosensory pain processing, behavioral and electrophysiological studies were conducted using the *leaner* (tg^{la}/tg^{la}) mouse. Behavioral analyses in tg^{la}/tg^{la} revealed reduced responses to mechanical stimuli, and enhanced responses to heat stimuli. Electrophysiological analyses showed that tg^{la}/tg^{la} had a significantly reduced ability to evoke dorsal root potentials, suggesting a functional deficit in the spinal dorsal horn local circuitry responsible for presynaptic inhibition of primary sensory fibers. These results suggest the critical importance of the P/Q-type channel in modulation of acute somatosensory pain transmission in spinal cord.

Key words: Voltage-dependent calcium channel; P/Q-type calcium channel; Nociception; Pain; Dorsal root potential; Spinal cord

1. Introduction

Calcium entry through voltage-dependent Ca^{2+} channels (VDCCs) contributes directly to the elevation of intracellular Ca^{2+} concentration, and regulates many aspects of neural activities from short-term responses including neurotransmitter release, to sustained changes such as gene expression and cell differentiation [1]. VDCCs are generally classified into two groups, high-voltage-activated (HVA) and low-voltage-activated (LVA) channels [2]. HVA channel includes L, N, P/Q and R subtypes, which are distinguished by their voltage dependence, kinetics and pharmacology. Ca^{2+} currents through LVA channels are known as T-type currents.

The P/Q-type channel is suggested to participate critically in neural excitability, integration, and Ca^{2+}-dependent neurotransmitter release, based on their characteristic localization on somatodendritic membranes and presynaptic terminals of most central and peripheral neurons [2]. The discovery of P/Q-type channel mutations in mice and humans with inherited neurological diseases [3] further substantiates a pivotal role of P/Q-type channels in neural network functions.

Important roles of Ca^{2+} influx through VDCCs have also been proposed for pain signaling [4]. For example, P/Q-type as well as N-type channels are well known to be involved in the release of nociceptive neurotransmitters, such as glutamate, calcitonin gene-related peptide and substance P, from primary sensory neurons [5–7]. Furthermore, analgesia by narcotic drugs is believed to be mediated partly by the inhibition of P/Q- and N-type channels through the opioid receptor–G-protein systems, thereby decreasing nociceptive neurotransmitter release [8,9]. For these reasons, the modulation of P/Q- and N-type channel functions, directly by channel blockers or indirectly through receptor–G-protein pathways, has been explored extensively as a therapeutic means of controlling pain symptoms [4,10]. However, the importance of each Ca^{2+} influx through VDCC subtypes in physiological and pathological pain states remains to be characterized.

To understand the physiological role of the P/Q-type channel in somatosensory pain processing, we utilized the *leaner* (tg^{la}/tg^{la}) mutant mouse. This mouse has a mutation at the splice donor consensus sequence on the gene encoding the VDCC α_{1A} ($Ca_v2.1$) subunit [11,12], the pore-forming subunit of P/Q-type channels [2,13,14], which results in skipping the exon/intron or in failure to splice out the succeeding intron. In both cases, the tg^{la} mutation causes truncation of mRNA and expression of P/Q-type channels containing aberrant carboxy-terminal sequences. The mutation results in a dramatic reduction of Ca^{2+} current in cerebellar Purkinje cells [15–17], where the P-type contributes ~90% of the whole cell Ca^{2+} current. This seems to be partly caused by the reduction of open probability by the mutation [15]. Reduction of channel expression has also been reported [12], although there is another report showing no change of the channel expression [18]. Recently, the production of P/Q-type channel knockout mice has been reported by two groups [13,14]. The phenotypes of these mice were very similar to that of tg^{la}/tg^{la}, suggesting that the tg^{la}/tg^{la} phenotype is caused by the strong reduction of P/Q-type current. Therefore, we considered that the tg^{la}/tg^{la} mouse was useful in exploring the physiological role of P/Q-type channels in pain transmission.

2. Materials and methods

All experimental protocols involving pain behavioral studies were reviewed and approved by the Animal Care Committee of Tokyo Medical and Dental University.

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Abbreviations: ACSF, artificial cerebrospinal fluid; CAP, compound action potential; CV, conduction velocity; DRP, dorsal root potential; HVA, high-voltage-activated; LVA, low-voltage-activated; MSR, monosynaptic reflex; sVRP, slow ventral root potential; VDCC, voltage-dependent Ca^{2+} channel

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2.1. Animals

Breeding pairs of heterozygous *leaner* (tg^{la}/+) on the C57BL/6J background were originally obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Oligosyndactylism (*Os*) is a skeletal mutation tightly linked to the tg^{la} mutation. Heterozygous *Os*/+ mice, identified by the oligosyndactylism, were inferred to be heterozygous for the tg^{la} mutation. Mice with no oligosyndactylism were inferred to be homozygous for the tg^{la} mutation. Because homozygous *Os*/*Os* is embryonic lethal and the tg^{la}/+ strain we used has been backcrossed for 14 generations with the C57BL/6J strain (indicating more than 99.9% inbred), we used age-matched pups from C57BL/6J wild-type mating as wild-type (+/+) controls (Japan Clea, Shizuoka, Japan). tg^{la}/tg^{la} mice become ataxic beginning after postnatal day 10 (P10). Therefore, we conducted pain behavioral experiments between P7 and P9. Electrophysiological studies, except compound action potential (CAP) experiments, were conducted with younger animals (P1–P6) in order to obtain the higher integrity of electrical responses of the isolated preparations described below.

2.2. In vitro electrophysiological experiments

Isolated spinal cord preparations and isolated spinal cord–peripheral nerve preparations of neonatal mice were prepared as described previously [19]. The preparations were perfused (2.5 ml/min) with artificial cerebrospinal fluid (ACSF) saturated with a gas mixture of 95% O_2: 5% CO_2 at room temperature (25–27°C). The composition of ACSF was as follows (mM): NaCl 138.6; KCl 3.35; $NaHCO_3$ 20.9; glucose 10.0; $CaCl_2$ 1.25; $MgCl_2$ 1.15 (pH 7.4 after gas saturation). A tight-fitting suction electrode was used for extracellular recording from a ventral root (L3–L5) or a dorsal root (L4). Another suction electrode was used for electrical stimulation of the dorsal root of the same segment or peripheral (femoral) nerve. A single shock with a square pulse of 500 μs in duration and 15–20 V in amplitude, supramaximal for monosynaptic reflex (MSR) and slow ventral root potential (sVRP), was applied. Changes in potential in the ventral or dorsal root were monitored via a DC amplifier with inputs to a pen recorder and a personal computer running Axotape version 2 (Axon Instruments, Foster City, CA, USA). The spinal reflexes of fast time courses were stored in a transient memory device in AC mode and then recorded with the pen recorder using an expanded time scale.

For the CAP experiment, L5 dorsal root connecting to the sciatic nerve was isolated from P7–9 mice. Electrical stimulation (various intensities) was applied to the sciatic nerve and CAP was recorded from L5 dorsal root in the same manner as described above. Primary afferents could be divided into three distinct groups corresponding to Aα/β-, Aδ-, and C-fibers, on the basis of the activation thresholds and conduction velocities (CVs) of CAPs. At a pulse width of 10 μs and stimulus intensity of <10 V, only an Aα/β wave was detectable with maximum amplitude at 10–12 V (data not shown). When the pulse width was gradually increased (stimulus intensity was fixed at 12 V), several additional responses were elicited. A C-fiber volley, which was identified by its capsaicin sensitivity (1 μM), was evoked at around 150–250 μs. Thus, activation thresholds of Aα/β- and C-fibers were reported as voltage (V) and pulse width (μs) units. Although several intermediate components between Aα/β- and C-fiber volleys were observed, these components were relatively small and variable, and therefore we did not analyze these components.

2.3. Behavioral analyses

All behavioral experiments were performed in a sound-proof room during the light cycle. Mechanical sensitivity was measured as the frequency of foot withdrawals out of 20 trials elicited by a defined mechanical stimulus with calibrated Von Frey hairs (Stoelting, Wood Dale, IL, USA). Hindpaw thermal sensitivity was evaluated with a plantar test apparatus (Ugo Basile, Comerio, Italy). The heat stimulus shut off automatically when the hindpaw moved, providing hindpaw withdrawal latencies accurate to within 0.1 s. Four trials, at least 5 min apart, were conducted on each hindpaw, alternating between right and left hindpaws. Thermal sensitivity of the tail was evaluated by the method of Blass et al. [20]. The pup was placed on a piece of cardboard so that its tail extended beyond the cardboard's edge. The tail was then lowered onto the 48°C hotplate by setting the cardboard support down next to the heated surface. Latency (s) either to lift the tail or to curl it away from the heated surface was recorded.

2.4. Pharmacology

Bicuculline methiodide, capsaicin and cytochrome *c* were purchased from Sigma. ω-Agatoxin-IVA (ω-Aga-IVA), ω-conotoxin-GVIA (ω-CTx-GVIA), dynorphin A(1–17), endomorphin-1 and endomorphin-2 were from Peptide Institute, Osaka, Japan. Tetrodotoxin (TTX) was from Sankyo, Tokyo, Japan. GR82334 ([D-Pro^9,[spiro-γ-lactam]-Leu^{10},Trp^{11}]physalaemin-(1–11)) was from Peninsula Laboratories, Belmont, CA, USA. All drugs were made up as concentrated stock solutions in MilliQ (18.3 MΩ) water (or dimethyl sulfoxide for capsaicin), aliquoted and stored at −20°C. Aliquots were thawed and diluted to desired concentrations in ACSF immediately prior to use and applied to spinal cords by perfusion. The inhibitory effects of VDCC blockers, GR82334 and opioid peptides on the nerve-evoked MSR and sVRP were evaluated by comparing the average of three control responses with the average of three responses under the steady effects of these agents. In order to obtain steady depressant effects, the compounds were applied to the spinal cord for at least 10–15 min. The inhibitory effect of bicuculline on the dorsal root potential (DRP) was also evaluated in the same manner. For MSR, the peak amplitude was compared, whereas the magnitude of the integrated area (mV·min) of the depolarization was compared for sVRP. For DRP, the peak amplitude and the magnitude of the integrated area (mV·s) were measured. The VDCC blockers were applied in the presence of 0.1 mg/ml cytochrome *c* to saturate non-specific binding sites. Cytochrome *c* did not affect the spinal reflexes (data not shown). In the experiment examining the depolarizing effect of capsaicin on ventral root, capsaicin was applied to the spinal cord for 60 s at an interval of 40–50 min to minimize tachyphylaxis. The magnitude of the depolarizing response (estimated as the integrated area) was normalized by the magnitude of the depolarization evoked by a 10 mM KCl solution applied for 20 s in the presence of TTX (0.3 μM).

2.5. Statistical analysis

Experimental data are expressed as mean ± S.E.M. We used the Mann–Whitney test to compare data from +/+ and tg^{la}/tg^{la} mice and evaluate the effects of drugs on spinal reflexes. For a multiple comparison, we used Tukey's test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of VDCC blockers on the spinal reflexes of leaner mice in vitro

We first examined the effect of VDCC blockers on the dorsal root-evoked responses of ventral roots in the isolated spinal cord preparation in +/+ and tg^{la}/tg^{la} mice (Fig. 1). Electrical stimulation of a dorsal root at C-fiber strength evoked MSR followed by a prolonged depolarization lasting for about 30 s (sVRP) on the ipsilateral ventral root of the same segment. The average value of amplitude of MSR and integrated area of sVRP recorded from tg^{la}/tg^{la} (5.07 ± 0.27 mV and 0.216 ± 0.014 mV·min, respectively, $n=32$) were slightly smaller than those of +/+ (5.59 ± 0.18 mV and 0.269 ± 0.012 mV·min, respectively, $n=54$). The difference of sVRP magnitude reached a statistically significant level ($p<0.05$). The smaller sizes of MSR and sVRP may be due to the absence of a P/Q channel component of MSR and sVRP in tg^{la}/tg^{la} mice (see below). The reduced tachykininergic component in the tg^{la}/tg^{la} sVRP may also have contributed to the size reduction (see Section 3.4).

Bath application of the P/Q-type channel blocker ω-Aga-IVA (50 nM) to +/+ spinal cord produced a marked inhibition of sVRP (to $51.9 \pm 6.83\%$ of the control response, $P<0.001$, $n=6$) with a slight inhibition of MSR (to $89.5 \pm 3.68\%$ of the control response, $P<0.05$, $n=6$) (Fig. 1A,C). In contrast, the same dosage of ω-Aga-IVA had no effect on either the sVRP ($98.0 \pm 6.46\%$ of control response,

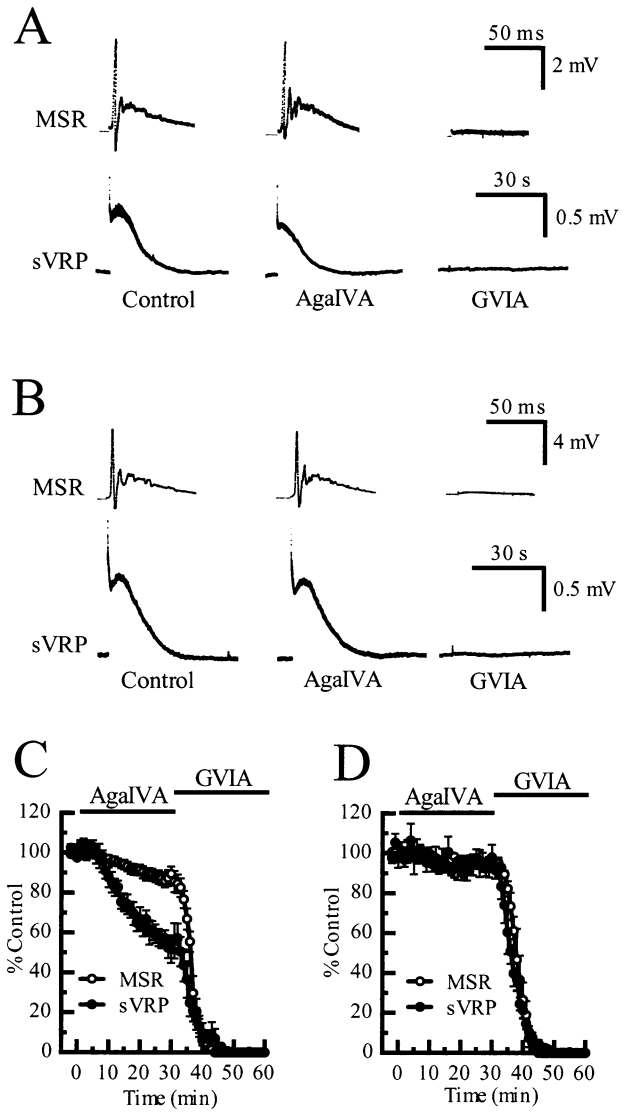

Fig. 1. Effects of VDCC blockers on the dorsal root-evoked spinal reflexes of neonatal +/+ and tg^{la}/tg^{la} mice in vitro. A,B: Effects of a P/Q-type VDCC blocker, ω-Aga-IVA, and an N-type blocker, ω-CTx-GVIA, on MSR (upper traces) and sVRP (lower traces). The upper traces show the record of the fast reflex responses during 78-ms post-stimulus periods. Initial sharp spikes represent the MSR. The lower traces show continuous chart records of the DC potential. Records in A and B were taken from +/+ and tg^{la}/tg^{la} spinal cord preparations, respectively. C,D: Time course of inhibition of MSR and sVRP by ω-Aga-IVA and ω-CTx-GVIA in +/+ (C) and tg^{la}/tg^{la} (D) mice (+/+: $n=6$–9; tg^{la}/tg^{la}: $n=4$–5). The ordinate scale represents the amplitude of MSR or magnitude of sVRP, which was expressed as percentage against averaged control responses.

$n=4$) or MSR (98.1 ± 0.74% of the control response, $n=4$) of tg^{la}/tg^{la} (Fig. 1B,D). Following the application of ω-Aga-IVA, further application of the N-type channel blocker ω-CTx-GVIA (100 nM) completely blocked the remaining components of both MSR and sVRP. These results suggest that the P/Q-type channel component in both MSR and sVRP is absent in tg^{la}/tg^{la} spinal cord. Furthermore, together with the finding that the sVRP magnitude in tg^{la}/tg^{la} is only slightly smaller than that in +/+ mice, the experiments with toxins strongly indicate that the N-type channel compensates most of the P/Q-type channel component in tg^{la}/tg^{la} spinal cord.

3.2. Altered acute nociception of tg^{la}/tg^{la} mice

Acute mechanical sensitivity was estimated by applying Von Frey filaments of three different bending forces (Fig. 2A). In +/+ mice, there was an increase in the number of withdrawal responses as the bending force increased. Compared with +/+, the numbers of withdrawal responses in tg^{la}/tg^{la} were greatly reduced. The tg^{la}/+ mice also showed significantly smaller numbers of responses with 27.5 and 166 mg Von Frey filaments.

In contrast to the mechanical response, tg^{la}/tg^{la} mice showed significantly enhanced thermal responses in paw flick (Fig. 2B) and tail flick (Fig. 2C) tests compared to +/+ and tg^{la}/+ mice. The +/+ and tg^{la}/+ mice showed similar thermal sensitivities in both tests.

3.3. Compound action potentials

The phenotype revealed by the above pain-related behavioral analyses suggests that tg^{la}/tg^{la} mice have some deficits in spinal nociceptive pathways, since these three pain-related behaviors are known to be tightly associated with the spinal reflex responses [21]. Therefore, we further attempted to evaluate the spinal reflexes of tg^{la}/tg^{la} and +/+ mice by using the isolated spinal cord preparations. Firstly, we tested whether the activation thresholds and CVs for A- and C-primary afferent fibers are normal in tg^{la}/tg^{la} mice. For this purpose, we recorded CAPs of L5 dorsal root evoked by electrical stimulation of the sciatic nerve, which innervates the plantar surface of the hindpaw, the region where mechanical and thermal stimuli were applied in the behavioral tests. The activation threshold and CV corresponding to the Aα/β-fiber (3.69 ± 0.20 V and 10.8 ± 1.34 m/s, respectively, $n=12$) and

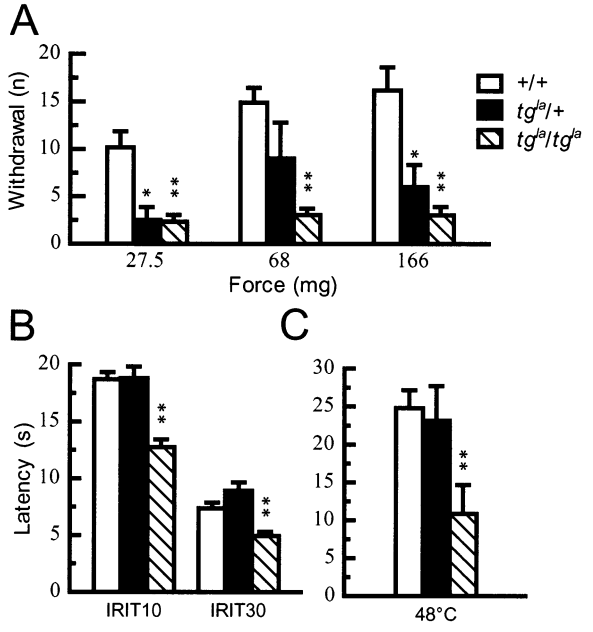

Fig. 2. Acute nociceptive responses. A: Number of hindpaw withdrawal responses out of 20 trials (ordinate scale) was plotted against each force of Von Frey filaments (+/+: $n=15$; tg^{la}/+: $n=5$; tg^{la}/tg^{la}: $n=16$). B: Paw flick test. Hindpaw withdrawal latencies to noxious thermal stimuli with two different heat intensities (infrared intensity (IRIT) 10 and 30) were measured (+/+: $n=19$, 36; tg^{la}/+: $n=8$, 8; tg^{la}/tg^{la}: $n=20$, 27). C: Tail flick test (+/+: $n=23$; tg^{la}/+: $n=6$; tg^{la}/tg^{la}: $n=11$). Significant differences: $^{*}P<0.05$ and $^{**}P<0.005$.

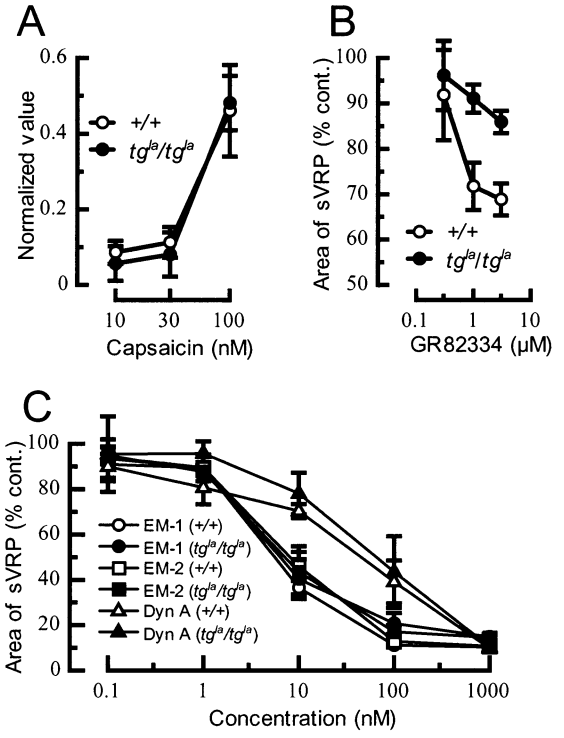

Fig. 3. Effects of capsaicin, a tachykinin receptor antagonist and opioid peptides. A: Concentration–depolarization curves for capsaicin. The ordinate scale represents the value of capsaicin-evoked response normalized by the response of KCl-evoked depolarization in the presence of TTX (0.3 μM) (+/+: $n=4$; tg^{la}/tg^{la}: $n=6$). B: Effect of GR82334, a tachykinin receptor antagonist, on the sVRP evoked by femoral nerve stimulation in the isolated spinal cord–peripheral nerve preparation (+/+: $n=5$; tg^{la}/tg^{la}: $n=5$). C: Effects of opioid peptides on the dorsal root-evoked sVRP. Concentration–inhibition curves for μ-opioid receptor agonists, endomorphin-1 (EM-1) and endomorphin-2 (EM-2), and a κ-opioid receptor agonist, dynorphin A(1–17) (Dyn A) (+/+: $n=4$; tg^{la}/tg^{la}: $n=4$). In B and C, the ordinate scale represents the magnitude of sVRP, which was expressed as percentage against the average of three control responses.

C-fiber (224 ± 32.8 μs and 0.39 ± 0.02 m/s, respectively, $n=15$) from +/+ were not significantly different from those of tg^{la}/tg^{la} (4.23 ± 0.49 V and 11.0 ± 2.18 m/s, respectively, for Aα/β-fiber, $n=12$; 165 ± 19.0 μs and 0.36 ± 0.02 m/s, respectively, for C-fiber, $n=15$). These results suggest that electrical activities of primary sensory axons in tg^{la}/tg^{la} mice appear to be normal.

3.4. Effects of capsaicin and a tachykinin receptor antagonist

To explore the possible functional deficit of C-fiber-mediated responses in tg^{la}/tg^{la} spinal cord, we examined the effects of capsaicin and a tachykinin receptor antagonist.

Capsaicin is known to excite a subset of primary sensory neurons (mainly small diameter nociceptive neurons), and cause a release and a depletion of sensory neuropeptides including tachykinins (substance P and neurokinin A) [22]. Bath application of capsaicin (10–100 nM) for 60 s to the spinal cord preparations from +/+ and tg^{la}/tg^{la} mice evoked nearly identical concentration-dependent ventral root depolarization, suggesting that the capsaicin-sensitive component in C-fiber response is not affected in tg^{la}/tg^{la} (Fig. 3A).

Several lines of evidence obtained from newborn rat spinal cord preparations suggest that the nerve-evoked sVRP represents a C-fiber-evoked nociceptive response in which tachykininergic primary afferents are involved (for review, see [23]). Similar to the newborn rat spinal cord [23,24], a tachykinin NK_1 receptor antagonist, GR82334, dose-dependently inhibited the femoral nerve-evoked sVRP in +/+ spinal cord. GR82334 also depressed the sVRP in tg^{la}/tg^{la}, but to a lesser extent (Fig. 3B).

3.5. Possible functional deficits in endogenous antinociceptive mechanisms in tg^{la}/tg^{la} spinal cord

The altered nociceptive behavior of tg^{la}/tg^{la} mice may result from functional deficits of endogenous antinociceptive mechanisms. To test this possibility, we first compared the inhibitory effects of several opioid peptides (μ-receptor agonists, endomorphin-1 and -2, and a κ-receptor agonist, dynorphin A(1–17)) on the dorsal root-evoked sVRP; however, the dose–response curves for these peptides were essentially identical in each case (Fig. 3C).

To further examine the mechanism of the altered nocicep-

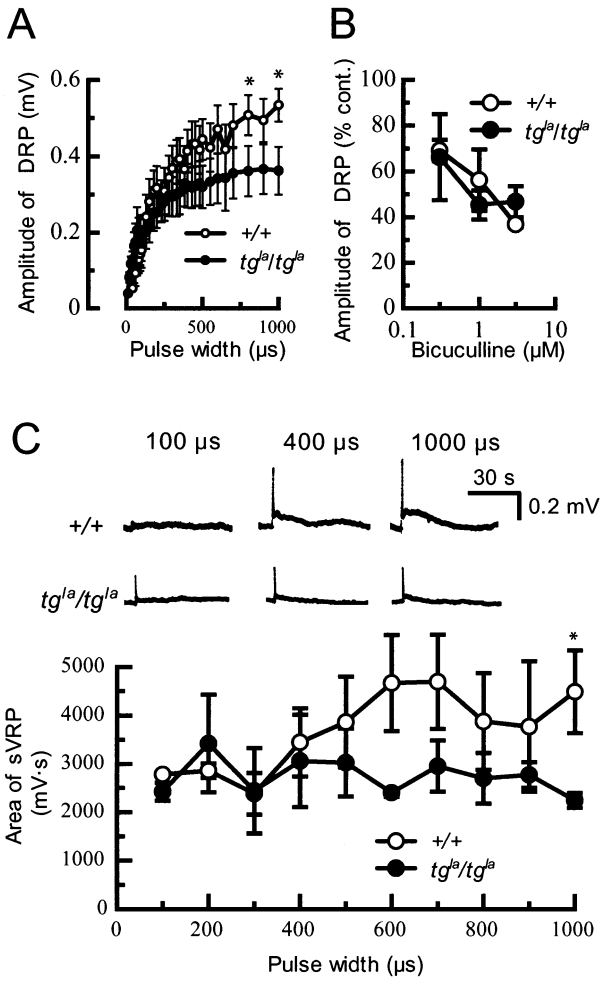

Fig. 4. Comparison of DRPs between +/+ and tg^{la}/tg^{la} mice. A: Stimulation–peak amplitude relationships. Stimulus intensities were fixed at 10–12 V (+/+: $n=8$; tg^{la}/tg^{la}: $n=5$). B: Effect of bicuculline, a $GABA_A$ receptor antagonist, on the peak amplitude of DRP (+/+: $n=7$; tg^{la}/tg^{la}: $n=4$). DRPs were evoked by intense stimulation of the femoral nerve with 800–1000 μs pulse width and 10–12 V intensity. C: Stimulation–response relationships of sDRP. Stimulus intensities were fixed at 10–12 V (+/+: $n=8$; tg^{la}/tg^{la}: $n=5$). Representative sample records from +/+ and tg^{la}/tg^{la} are shown in the upper panel. The magnitude of sDRP represented by the integrated area (mV·s) was plotted against pulse width (lower panel). *$P<0.05$, significant difference between +/+ and tg^{la}/tg^{la}.

tive behavior of tg^{la}/tg^{la} mice, we attempted to evaluate the function of another endogenous antinociceptive mechanism, γ-aminobutyric acid (GABA)-mediated inhibition. For this purpose, we recorded DRP and examined the stimulus–response relationships. DRP is derived from depolarization of the terminals of the primary afferent fibers (that is, primary afferent depolarization: PAD) and PAD is believed to be partly responsible for $GABA_A$ receptor-mediated presynaptic inhibition [25,26]. When the stimulation–response relationships were examined, the femoral nerve-evoked peak amplitude of tg^{la}/tg^{la} DRP was found to be smaller than that of +/+ DRP (significant differences at 800 and 1000 μs, $P < 0.05$) (Fig. 4A). However, the evoked DRP was inhibited to a similar extent by a $GABA_A$ receptor antagonist, bicuculline (Fig. 4B). In the DRP, there was a slow component (sDRP), which was evoked by relatively strong stimulation and prolonged by bicuculline treatment (data not shown). In +/+ mice, this sDRP was prominent and became larger when the pulse width of stimuli was increased. In contrast, sDRP tended to be smaller and no increase with the pulse width was observed in tg^{la}/tg^{la} mice (significant difference at 1000 μs, $P < 0.05$) (Fig. 4C).

4. Discussion

The principal finding of the present investigation is that tg^{la}/tg^{la} mice demonstrate altered acute mechanical and thermal nociceptive responses. These results suggest that the P/Q-type Ca^{2+} channel participates in the modulation of acute nociceptive transmission. However, these results are in contrast with our previous observations using mice lacking $Ca_v2.2/\alpha_{1B}$ (N-type channel) [27] or $Ca_v2.3/\alpha_{1E}$ (R-type channel) subunit [28]. These knockout mice represent almost no major deficits in acute somatosensory nociceptive tests, suggesting that each type of HVA Ca^{2+} channel participates differentially in nociceptive transmission.

We speculated that the altered phenotype of tg^{la}/tg^{la} might be related to some functional deficits of endogenous antinociceptive mechanism in the spinal cord. In particular, we observed the thermal hyperalgesic behavior irrespective of the slight ataxic nature of this mutant, because there are several reports suggesting that the P/Q-type channel is preferentially involved in inhibitory neurotransmission in spinal dorsal horn in the normal state [4,29,30]. Our electrophysiological studies showed a reduced ability for producing DRP in tg^{la}/tg^{la} mice compared with +/+ mice. The presynaptic inhibitory system including GABAergic interneurons is known to be responsible for DRP [25,26]. GABAergic and/or excitatory interneurons both depolarize primary afferent terminals leading to the inhibition of neurotransmitter release. Thus it is possible that the reduction of this antinociceptive system is responsible for the enhanced thermal sensitivity of tg^{la}/tg^{la} mice, although further studies are necessary to substantiate this possibility.

The results, showing that a relatively greater difference of peak DRP between +/+ and tg^{la}/tg^{la} mice was evident at a longer pulse width, might be due to the influence of the spillover of substances involved in the generation of sDRP, which also tended to be smaller in tg^{la}/tg^{la} mice. Although the neurotransmitters and the mechanism contributing to the generation of the sDRP remain to be clarified, possible participation of non-synaptic transmission by several substances such as glutamate and K^+ has been suggested [25,26].

The other antinociceptive system examined was the opioid system. In this study we could not observe any difference of the inhibitory effects of exogenously applied opioid peptides, suggesting that opioid (μ and κ) receptor-mediated signal transduction systems are normal in tg^{la}/tg^{la} spinal cord. However, further experiments are required to determine whether expression levels and/or release mechanisms of endogenous opioid peptides are affected.

It is worth noting that we found no functional contribution of P/Q-type channels on MSR and sVRP in tg^{la}/tg^{la} spinal cord in spite of the observation of a small amount of P-type Ca^{2+} current in cerebellar Purkinje cells from this mutant [15–17]. The tg^{la} mutation results in the expression of the P/Q-type channel containing aberrant carboxy-terminal sequences [11]. The carboxy-terminal region has been implicated in several important functions, including proper channel localization [31,32]. It is possible that a lower level of P/Q-type current, which was observed in the cerebellar Purkinje cells, may be present in spinal neurons expressing P/Q-type channels in tg^{la}/tg^{la} mice. This P/Q-type channel may not be localized in the proper site and/or the current may be under the threshold for eliciting MSR and sVRP.

Another prominent consequence of in vitro electrophysiological experiments was the apparent functional compensation of the P/Q-type channel defect with the N-type channel in the tg^{la}/tg^{la} spinal cord. The functional significance of this compensation should be clarified in future studies. Similar partial compensation with N-type channel and enhanced reliance on N-type channel in $Ca_v2.1/\alpha_{1A}$-null mutant mice are also observed [13,14].

An immunohistochemical study has shown that the distribution of P/Q-type channels complements rather than colocalizes with N-type channels in the superficial dorsal horn [33]. Furthermore, the study also demonstrated the presence of P/Q-type channels primarily in the nerve terminals in laminae II–VI, but unlike N-type channels, rarely in substance P-containing neurons. Electrophysiological experiments suggested that dorsal root ganglion (DRG) neurons express a variety of VDCCs [34,35]. Each group of DRG neurons classified by their sizes contains several types of VDCCs in different proportions and non-L/non-N (presumptive P/Q) current is shown to be relatively expressed in medium and large cells [34], which might be compatible with the reduced mechanical sensitivity in tg^{la}/tg^{la} mice, since large and medium DRG neurons constitute myelinated primary sensory neurons (Aβ- and Aδ-fibers) that contribute to tactile and touch sensations. The functional relationships between the P/Q-type channel mutation and the reduced mechanical response in tg^{la}/tg^{la} mice observed at P7–9 in this study requires further experimentation, because ataxic behavior starts to emerge after P10. Unrecognized motor and/or neurological deficits may affect the behavior even at P7–9. But the response to thermal stimuli is more prominent in tg^{la}/tg^{la} than in +/+ mice. It seems unlikely that the similar withdrawal response only to mechanical stimuli is deteriorated. Furthermore $tg^{la}/+$ mice showed a reduced response to mechanical stimuli compared to +/+. Even though further rigorous experiments might be needed to obtain conclusive evidence, we suggest here that the P/Q-type channel is also involved in acute mechanical pain transmission.

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