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Selective Postsynaptic Inhibition of Tonic-firing Neurons in Substantia Gelatinosa by μ-Opioid Agonist

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Background: **Spinal substantia gelatinosa (SG) is a site of action of administered and endogenous opioid agonists and is an important element in the system of antinociception. However, little is known about the types of neurons serving as specific postsynaptic targets for opioid action within the SG. To study the spinal mechanisms of opioidergic analgesia, the authors compared the action of μ-opioid agonist [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO) on SG neurons with different intrinsic firing properties.**

Methods: **Whole cell patch clamp recordings from spinal cord slices of Wistar rats were used to study the sensitivity of SG neurons to DAMGO.**

Results: **Three groups of neurons with distinct distributions in SG were classified: tonic-, adapting-, and delayed-firing neurons. DAMGO at 1 μM concentration selectively hyperpolarized all tonic-firing neurons tested, whereas none of the adapting- or delayed-firing neurons were affected. The effect of DAMGO on tonic-firing neurons was due to activation of G protein–coupled inward-rectifier K^+ conductance, which could be blocked by 500 μM Ba^{2+} and 500 μM Cs^+ but increased by 50 μM baclofen. As a functional consequence of DAMGO action, a majority of tonic-firing neurons changed their pattern of intrinsic firing from tonic to adapting.**

Conclusions: **It is suggested that tonic-firing neurons, presumably functioning as excitatory interneurons, are primary postsynaptic targets for administered and endogenous opioid agonists in spinal SG. Functional transition of cells in this group from tonic to adapting firing mode may represent an important mechanism facilitating opioidergic analgesia.**

SUBSTANTIA gelatinosa (SG) of the spinal cord is a site of termination of most fine-caliber primary afferent fibers and is therefore involved in pain conduction.[1–4] However, SG also represents one of the key elements in the system of pain control. High densities of enkephalin-containing neurons and axon terminals as well as opiate binding sites found in SG indicate its role in endogenous enkephalinergic antinociception.[5–8] Besides, SG is a site for the analgesic action of administered exogenous opioids.[9–11] It was shown that enkephalins inhibit SG neurons *via* a combination of presynaptic and postsynaptic mechanisms. The presynaptic effects are mediated *via* both μ- and δ-opioid receptors located in axons, whereas the postsynaptic inhibition is mostly attributed to activation of μ-opioid receptors in somatodendritic domains.[6,7,12,13] The postsynaptic μ-opioid receptors are specifically targeted to excitatory SG interneurons,[14] the inhibition of which may be important for the spinal mechanisms of antinociception.

The postsynaptic action of opioid agonists on SG neurons results in a robust membrane hyperpolarization associated with an increase in K^+ conductance.[13,15–17] This conductance mediated through G protein–coupled inward-rectifier K^+ (GIRK) channels could be blocked by low concentrations of external Ba^{2+} and Cs^+ but enhanced by the γ-aminobutyric acid type B ($GABA_B$) receptor agonist baclofen.[18,19]

There are several morphologic groups of SG neurons.[20–23] It was shown that the neurons of all these groups can be sensitive to the μ-opioid agonist [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO),[17,22] implying the lack of strict correlation between cell morphology and sensitivity to the drug. In addition to morphology, SG neurons are also distinguished on the basis of their membrane properties and intrinsic firing.[24–27] The actions of opioids on neurons with different patterns of intrinsic firing, however, have not been compared so far. Here, we report a striking correlation between the firing pattern of rat SG neurons and their sensitivity to DAMGO. The DAMGO-induced hyperpolarization was only observed in neurons with tonic-firing pattern, which possessed a functional combination of both μ-opioid receptors and GIRK channels. In addition, it has been found that the inhibition was facilitated by a transition of most neurons from tonic to adapting firing mode. It is suggested that the tonic-firing SG neurons can function as excitatory interneurons and that their selective inhibition by opioid agonists is involved in analgesic postsynaptic effects of endogenous enkephalins and administered opioids.

Materials and Methods

Tight-seal recordings were done using 200- or 300-μm coronal slices prepared from the lumbar enlargement of the spinal cord of 2- to 7-week-old Wistar rats.[28] The animals were killed in accordance with the national guidelines (Direcção Geral de Veterinária, Ministério da Agricultura, Lisboa, Portugal). After anesthesia by intraperitoneal injection of Na^+ pentobarbital (30 mg/kg), the vertebral column was quickly cut out and immersed

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Table 1. Parameters of Neurons and Their Responses to 1 μM DAMGO and 50 μM Baclofen

	V_R, mV	R_{IN}, GΩ	Responses to DAMGO	ΔV_{DAMGO}, mV	$R_{IN\text{-}DAMGO}/R_{IN}$	Responses to Baclofen
TFN	−71.3 ± 5.5 n = 53	1.67 ± 0.2 n = 53	53 of 53	−10.1 ± 3.0 n = 53	0.23 ± 0.07 n = 53	21 of 21
AFN	−71.5 ± 6.6 n = 46	1.48 ± 0.4 n = 46	0 of 30	−0.1 ± 0.6 n = 30	1.01 ± 0.12 n = 30	12 of 12
DFN	−76.8 ± 3.4 n = 47	0.77 ± 0.3 n = 47	0 of 31	0.0 ± 0.2 n = 31	1.02 ± 0.08 n = 31	12 of 12

Values are presented as mean ± SD. Responsiveness to baclofen was tested in voltage clamp mode, whereas all other measurements were performed in current clamp mode.

AFN = adapting-firing neuron; ΔV_{DAMGO} = membrane polarization in 1 μM [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin measured from −70 mV, at which each neuron was held in current clamp mode before the drug was applied; DFN = delayed-firing neuron; R_{IN} = input resistance; $R_{IN\text{-}DAMGO}$ = input resistance in 1 μM [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin; TFN = tonic-firing neuron; V_R = membrane resting potential.

in ice-cold oxygenated artificial cerebrospinal fluid. The segment of the lumbar enlargement was dissected and glued to the stage of the tissue slicer. Slices were prepared and incubated for 40–60 min in oxygenated artificial cerebrospinal fluid at 33°C. For recording, the slices were transferred into a 0.7-ml chamber and continuously perfused at a rate of 8 ml/min. All recordings were done at 22°–24°C. SG (lamina II) was identified as a translucent band in the dorsal horn. Each neuron was localized during recording according to the position of the pipette tip on the video image of SG.

Artificial cerebrospinal fluid contained 115 mM NaCl, 5.6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM glucose, 1 mM NaH_2PO_4, and 25 mM $NaHCO_3$ (pH 7.4 when bubbled with a 95%–5% mixture of oxygen–carbon dioxide). Standard pipette solution contained 6 mM NaCl, 128 mM KCl, 2 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES. The pH value was adjusted to 7.3 with KOH (final $[K^+]$ was 160.5 mM). In 36 experiments, guanosine 5'-triphosphate (100 μM) and adenosine 5'-triphosphate (2 mM) were added to the pipette solution. All chemicals were purchased from Sigma-Aldrich (Sintra, Portugal). DAMGO, baclofen, and naloxone (antagonist of μ-opioid receptors) were dissolved in distilled water and stored in aliquots of 4, 1.5, and 0.125 mM, respectively, at −20°C. Fresh dilutions were made with artificial cerebrospinal fluid just before the experiment. Adenosine 5'-triphosphate and guanosine 5'-triphosphate were dissolved in internal solution at final concentrations and kept in aliquots at −20°C until the experiment.

The patch pipettes had a resistance of 3–5 MΩ after fire polishing. An EPC-9 amplifier (HEKA, Lambrecht, Germany) was used in all experiments. The effective corner frequency of the low-pass filter was 3 kHz, and traces were digitized at 10 kHz. For measurements of transient K^+ (K_A) currents, a standard P/n protocol was used for transients and leakage subtraction. Offset and liquid junction potentials were corrected for in all experiments. In neurons subjected to detailed analysis, the series resistance was 6–20 MΩ and was compensated by 60%. Fast current clamp mode of the EPC-9 amplifier was used for voltage recording. Input resistance (R_{IN}) of SG neurons was measured in current clamp mode using negative 500-ms current pulses of 5–10 pA.

Special precautions were taken to correctly measure the resting potential in SG neurons. In current clamp mode, most commercially available patch clamp amplifiers inject into the cell a small uncompensated current of 5–20 pA, which can vary from day to day and cannot be compensated by users in a simple way (our personal observations with several EPC7, EPC9, and Axopatch 200B [Axon Instruments, Union City, CA] amplifiers). Such a current can depolarize by tens of millivolts the cells with R_{IN} in GΩ range. Therefore, before each experiment, we measured in current clamp mode a voltage decrease on a 0.5-GΩ resistor and determined the current needed to bring it to an expected 0 mV. In all following current clamp recordings, this sustained current was applied to the neuron and was considered zero current level. Under these conditions, the resting potential measured in current clamp mode was equal to the potential at which zero absolute current was recorded in voltage clamp mode. This correction may explain, at least in part, more negative resting potential values obtained here than in other patch clamp studies of dorsal horn neurons.[27,29]

All numbers in the text and figures are given as mean ± SEM; numbers in table 1 are given as mean ± SD. The parameters were compared using a paired or independent Student *t* test. The current study is based on recordings from 149 SG neurons.

Results

Classification of SG Neurons

Based on several criteria, SG neurons (n = 146) were separated into three groups (fig. 1): tonic-firing neurons (TFNs), adapting-firing neurons (AFNs), and delayed-firing neurons (DFNs). Three additional neurons could not be clearly classified and therefore were not subjected to study.

Tonic-firing neurons (n = 53) were able to support firing during 500-ms depolarization induced by a sustained current injection (fig. 1, *A1*). They had a low firing

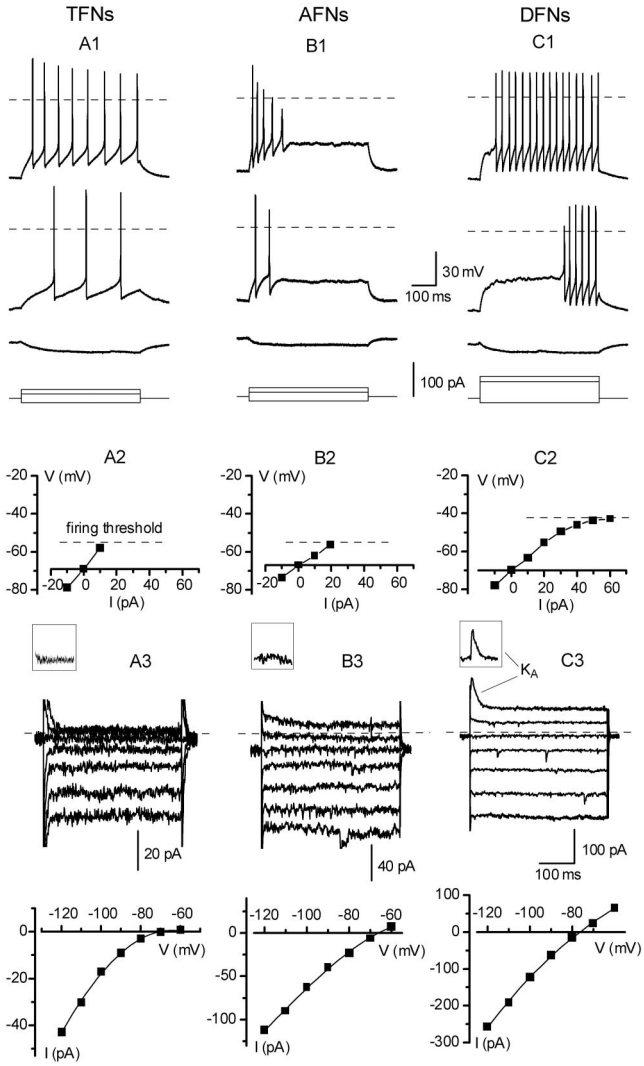

Fig. 1. Classification of three groups of substantia gelatinosa neurons: tonic-firing neurons (TFNs, *A*), adapting-firing neurons (AFN, *B*), and delayed-firing neurons (DFNs, *C*). (*A1*, *B1*, and *C1*) Each neuron was kept at −70 mV, and 500-ms hyperpolarizing or depolarizing current pulses were applied to test membrane responses and firing patterns. *Dashed lines* indicate 0 mV. (*A2*, *B2*, and *C2*) Subthreshold membrane polarization as a function of injected current. Neurons were kept at their resting potentials. (*A3*, *B3*, and *C3*) Voltage clamp recordings of membrane currents. All neurons were held at −80 mV, and 300-ms voltage pulses to potentials from −120 to −60 mV in 10-mV steps were applied. Transient K^+ (K_A) currents at −60 mV given in *insets* were obtained by subtracting the transients and leakage currents using a standard P/n protocol. Current and voltage clamp recordings are from different neurons.

threshold (−51.2 ± 1.7 mV, n = 11), and 10- to 20-pA current pulses were sufficient to evoke firing (fig. 1, *A2*). Under the voltage clamp condition, the current–voltage relations in all TFNs showed pronounced inward rectification (fig. 1, *A3*). A characteristic feature of AFNs (n = 46) was a burst-like firing of only two to six spikes at the beginning of depolarization (fig. 1, *B1*). At any stimulus intensity, AFN discharges could not continue during the whole pulse. The firing threshold of −50.7 ± 1.4 mV (n = 10) was not significantly different from that in TFNs ($P > 0.75$, independent Student *t* test). The membrane properties of AFNs (fig. 1, *B2* and *B3*) were fairly similar to those of TFNs. In both TFNs and AFNs, a K_A current was not seen at voltage steps from −80 to −60 mV (fig. 1, *A3* and *B3*, *insets*).

A principal difference of DFNs (n = 47) was a presence of large K_A current (137.9 ± 10.5 pA at voltage step from −80 to −60 mV, n = 10; $P < 0.001$, independent Student *t* test, for comparison with either TFN or AFN group; fig. 1, *C3*, *inset*), which substantially influenced the firing pattern (fig. 1, *C1* and *C2*). The spike threshold in DFNs (−36.4 ± 1.6 mV, n = 10) was considerably higher than in TFNs ($P < 0.001$, independent Student *t* test) or AFNs ($P < 0.001$, independent Student *t* test) and was reached at stimulation as strong as 50–70 pA because a large portion of injected current was compensated by activating K_A current. In DFNs, the first spikes typically occurred with a considerable time delay at the end of the pulse and moved to its beginning as the stimulation increased. R_{IN} in DFNs was approximately half of that in the other cell types ($P < 0.001$ for TFNs and $P < 0.001$ for AFNs, independent Student *t* tests), but a more negative resting potential (table 1; $P < 0.05$ for either TFN or AFN group, independent Student *t* tests) was closer to K^+ equilibrium potential of −84 mV, indicating a presence of larger resting K^+ conductance. An inward rectification was less pronounced in DFNs (fig. 1, *C3*). However, there were some minor variations in discharge patterns of DFNs at strong stimulation. Some cells discharged regularly during the whole pulse (fig. 1, *C1*), whereas others belonging to this group showed interrupted bursts of even single spikes (not shown).

In all three types of neurons, membrane response to hyperpolarizing voltages consisted of dominating fast inward-rectifier current. A slow hyperpolarization-activated I_H-like current carried by both K^+ and Na^+ ions[24,30,31] was negligible in majority of SG neurons studied here using both normal and adenosine 5'-triphosphate/guanosine 5'-triphosphate–containing (n = 36) pipette solutions.

Distribution of Neuron Types in SG

Each group of neurons had its specific distribution pattern within SG (figs. 2A and B). TFNs were relatively homogeneously distributed over the whole length of SG. Few AFNs were found in medial and intermediate region, but most of them were localized in a lateral SG, especially its ventral part. Although DFNs could be found in all SG regions, their density in the medial and lateral parts was higher. The sensitivity to DAMGO was tested for the neurons of each type located in different SG regions (indicated by crosses).

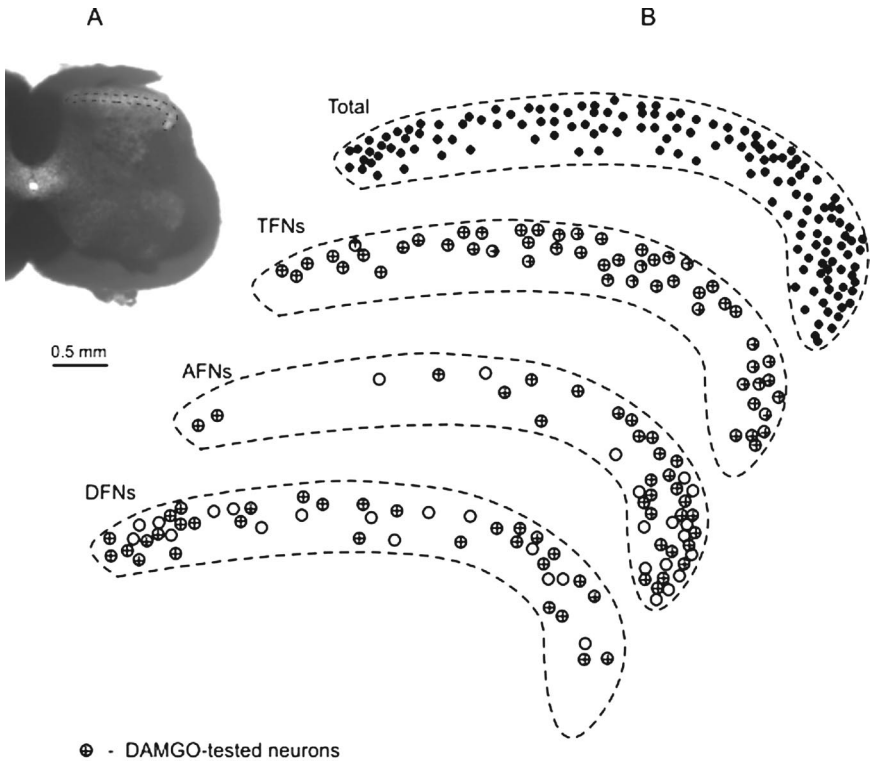

Fig. 2. Distribution of tonic-firing neurons (TFNs), adapting-firing neurons (AFNs), and delayed-firing neurons (DFNs). (*A*) Spinal cord slice from a 19-day-old rat with substantia gelatinosa appearing as a translucent band in the dorsal horn. (*B*) All neurons studied were plotted together as *circles* on a top schematic drawing of substantia gelatinosa. Each of three drawings below shows a distribution of one particular cell group. Neurons that were tested for their sensitivity to DAMGO are marked by *crosses*.

Sensitivity to DAMGO

Only one neuron per slice was tested for its sensitivity to DAMGO, and the slice was not exposed to the drug before beginning of the recording to avoid a desensitization of opioid responses.[16,32]

In each of 53 TFNs tested, a 30-s application of 1 μM DAMGO evoked a robust hyperpolarization ranging from 5 to 13 mV, which was accompanied by a considerable decrease in R_{IN} (fig. 3, *A1*, and table 1; $P < 0.001$, paired Student *t* test). Membrane currents were reversibly increased in such a way that the current–voltage curves in control and 1 μM DAMGO crossed each other at a po-

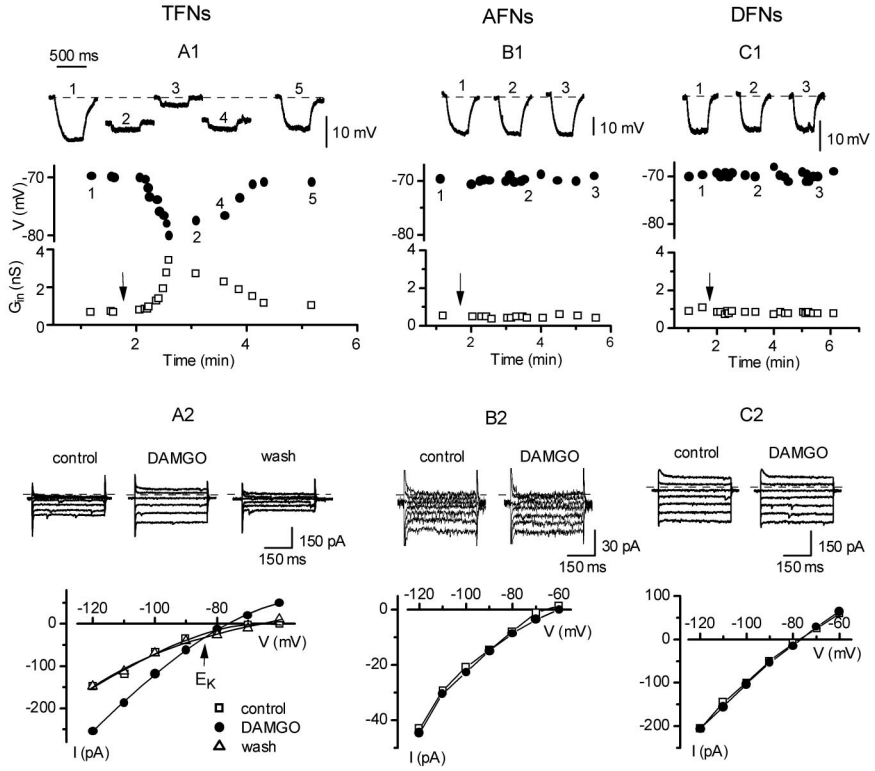

Fig. 3. Effect of DAMGO on tonic-firing neurons (TFNs, *A*), adapting-firing neurons (AFNs, *B*), and delayed-firing neurons (DFNs, *C*). (*A1*, *B1*, and *C1*) In current clamp mode, membrane potentials were uniformly adjusted to −70 mV (by a sustained current injection) before 1 μM DAMGO was applied for 30 s. The beginning of the application is indicated by an *arrow*. The effect of DAMGO was monitored by repetitive membrane hyperpolarization for 500 ms by 10-pA current. *Trace 3* in *A1* was recorded when additional current was injected into a DAMGO-hyperpolarized neuron to return membrane potential to −70 mV. Membrane conductance (G_{IN}) was calculated from changes in membrane potential during 10-pA hyperpolarizing current pulses. (*A2*, *B2*, and *C2*) Voltage clamp recordings of membrane currents (the same pulse protocol as in fig. 1, *A3*, *B3*, and *C3*) in the absence and presence of 1 μM DAMGO.

tential close to the K^+ equilibrium potential (fig. 3, *A2*), indicating an activation of K^+ selective current. Although the DAMGO-activated hyperpolarization lasted several minutes (up to 18 min) without remarkable inactivation, most TFNs responded to the drug only once, and only some cells responded to several applications. Presence of 2 mM adenosine 5'-triphosphate and 100 μM guanosine 5'-triphosphate in the pipette solution[16] in 9 of 53 tested TFNs did not improve their responsiveness to repetitive DAMGO applications. The hyperpolarization in TFNs was prevented if DAMGO was applied together with 100 nM naloxone (n = 7).

Neither AFNs (n = 30) nor DFNs (n = 31) responded to 1 μM DAMGO, and their R_{IN} did not change (figs. 3B and C and table 1; $P > 0.85$ for AFNs and $P > 0.8$ for DFNs, paired Student *t* tests). Increasing the duration of DAMGO application to 1–3 min in all AFNs and DFNs or inclusion of 2 mM adenosine 5'-triphosphate and 100 μM guanosine 5'-triphosphate to the pipette solution in 15 of 30 AFNs and 12 of 31 DFNs did not affect their sensitivity to DAMGO.

Therefore, only TFNs and not AFNs or DFNs were hyperpolarized by DAMGO, which activated K^+-selective conductance. The sensitivity of a neuron to DAMGO depended on its type rather than its location within the SG (fig. 2B).

Effects of Ba^{2+}, Cs^+, and Baclofen on Membrane Conductance

In experiments shown in figure 4, the effects of Ba^{2+}, Cs^+, and baclofen were studied. In seven TFNs, membrane currents were first activated by 1 μM DAMGO, and then 500 μM Ba^{2+} was added. In all these neurons, Ba^{2+} blocked the DAMGO-activated current (fig. 4, *A1*). Direct application of 500 μM Ba^{2+} (without DAMGO) blocked the resting membrane conductance in all cell types in a voltage-independent manner. At −120 mV, the inward current was blocked to 38.8 ± 4.3% in TFNs (n = 4, not shown; $P < 0.01$, paired Student *t* test), 32.4 ± 3.8% in AFNs (n = 8; fig. 4B1; $P < 0.001$, paired Student *t* test), and 30.9 ± 3.1% in DFNs (n = 11; fig. 4, *C1*; $P < 0.001$, paired Student *t* test). Application of 500 μM Cs^+ also blocked the resting K^+ conductance in all groups of neurons with stronger effects seen at more negative potentials (fig. 4, *A2*, *B2*, and *C2*). At −120 mV, an inward current was suppressed to 42.0 ± 3.9% in TFNs (n = 6; $P < 0.001$, paired Student *t* test), 43.9 ± 5.4% in AFNs (n = 8; $P < 0.001$, paired Student *t* test), and 30.4 ± 3.4% in DFNs (n = 5; $P < 0.001$, paired Student *t* test). All tested TFNs (n = 21), AFNs (n = 12), and DFNs (n = 12) responded to the $GABA_B$ receptor agonist baclofen (table 1), which, similar to DAMGO, was applied to one neuron per slice. In all three groups, 50 μM baclofen reversibly induced K^+ current (fig. 4, *A3*, *B3*, and *C3*). At −120 mV, the baclofen-induced current was −90.7 ± 7.1 pA in TFNs (n = 21), −73.4 ± 10.1 pA in AFNs (n = 12), and −72.9 ± 7.1 pA in DFNs (n = 12). In contrast to DAMGO, all neurons responded to several applications of baclofen. Therefore, all groups of SG neurons possessed GIRK conductance, which could be blocked by Ba^{2+} and Cs^+ but activated by baclofen *via* the $GABA_B$ receptor pathway.

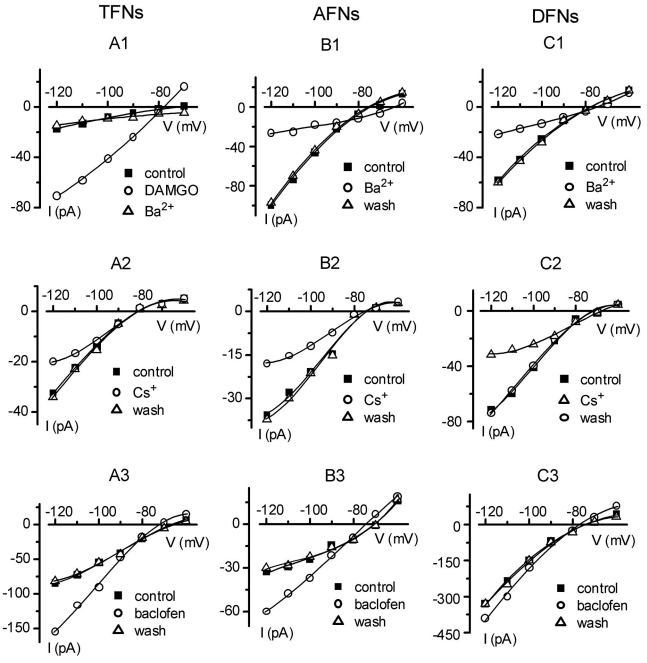

Fig. 4. Effects of external 500 μM Ba^{2+}, 500 μM Cs^+, and 50 μM baclofen on membrane currents in tonic-firing neurons (TFNs, *A*), adapting-firing neurons (AFNs, *B*), and delayed-firing neurons (DFNs, *C*). (*A1*) Current–voltage (I-V) curves obtained from one TFN in control solution and after addition of 1 μM DAMGO and Ba^{2+}. (*B1* and *C1*) I-V curves for AFNs and DFNs, respectively, in control, Ba^{2+}, and after wash. (*A2*, *B2*, and *C2*) Cs^+ exerted a voltage-dependent block in all three groups of substantia gelatinosa neurons. (*A3*, *B3*, and *C3*) Reversible increase in membrane currents by baclofen.

Modification of Discharge Pattern in TFNs by DAMGO

To study the functional consequences of postsynaptic effect of opioids, the firing patterns before and after 30-s application of 1 μM DAMGO were compared in all 53 TFNs. In 44 of them (83%), the DAMGO application reversibly induced a spike frequency adaptation in such a way that the neurons were not able to support tonic firing at any stimulation strength (fig. 5A). In addition, much stronger current injection was needed to reach the firing threshold of the first spike (fig. 5A). The discharge pattern modified by DAMGO was not improved when the membrane potential was returned to −70 mV by injecting persistent current through the recording pipette (fig. 5A, 5 min after DAMGO application). In the other 9 TFNs (17%), the tonic discharge pattern remained after DAMGO application. However, the firing characteristic constructed for those 9 TFNs was considerably shifted to the right (fig. 5B).

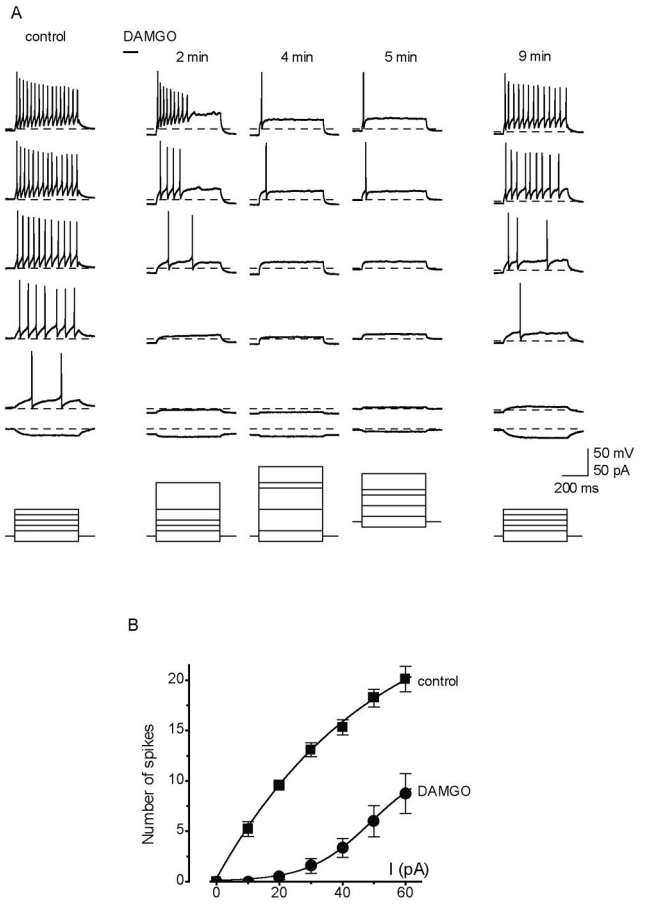

Fig. 5. DAMGO-induced modification of firing pattern in tonic-firing neurons. (*A*) Induction of spike-frequency adaptation observed in majority of tonic-firing neurons (44 of 53). The firing patterns were recorded 2, 4, 5, and 9 min (recovery) after DAMGO application. The recording at 5 min was done when the membrane potential was returned to −70 mV by current injection. *Dashed line* indicates −70 mV. (*B*) DAMGO-induced shift in firing characteristics for the rest (9 of 53) of the tonic-firing neurons. The characteristics were constructed as the number of spikes fired during 500-ms depolarization as a function of injected current. Each *point* is a mean of nine measurements. The data points are fitted by eye.

Discussion

The current results have shown that the μ-opioid agonist DAMGO selectively inhibits TFNs in SG, whereas it had no effect on AFNs and DFNs. Our finding implies that TFNs, which receive direct inputs from Aδ- and C-type afferents,[27] represent a primary postsynaptic target for both administered opioids and endogenous enkephalins in the spinal cord. Cell classification on the basis of firing patterns was important for this study. Our data support several patch clamp investigations showing that SG is formed by neurons with diverse intrinsic firing properties[23,27,33,34] but conflict with Ruscheweyh and Sandkuhler,[29] who have found only adapting discharge patterns in neurons from rat lamina II (SG). Our classification is also similar to those suggested on the basis of recordings with a sharp electrode.[25,26] However, it cannot be excluded that some TFNs from the current study would appear as AFNs if recorded with a sharp electrode introducing larger somatic shunt.

Each group of neurons had its specific distribution pattern within SG. The intermediate SG was dominated by TFNs, the medial region contained both TFNs and DFNs, and the lateral zone contained all three types of cells with a high percentage of AFNs. This is in good agreement with Melnick *et al.*,[23] who showed that in parasagittal spinal cord slices, including mostly the intermediate region of SG, TFNs represent 70% of the total neuronal population. The sensitivity of a given neuron to DAMGO depended on its firing properties rather than its location within the SG. Therefore, our results suggest that the percentage of neurons responding to opioids might vary along the medial–lateral axis of SG, being highest in the intermediate zone.

Agonists binding to μ-opioid receptors hyperpolarize membrane through the activation of GIRK (also known as Kir3) channels functioning as effectors.[19,35,36] It seems that all three types of SG neurons possess GIRK conductance, which could be blocked by Ba^{2+} and Cs^{+} as well as activated by baclofen *via* the $GABA_B$ receptor pathway.[18] Selective inhibition of TFNs by DAMGO could therefore be explained by a specific targeting of postsynaptic μ-opioid receptors to TFNs rather than AFNs and DFNs. Alternatively, it is possible that AFNs and DFNs also express μ-opioid receptors but that the G protein coupling them to GIRK channels is not present. Because the majority of SG neurons expressing μ-opioid receptors do not contain GABA or glycine and therefore are excitatory interneurons,[14] we suggest that TFNs can function as excitatory interneurons. The intrinsic firing properties would allow them to convert stronger synaptic inputs into higher discharge frequencies in a broad range of synaptic stimulation.

The current results show that the mechanism of postsynaptic action of opioids on SG neurons is more complex and effective than it was assumed so far. Membrane hyperpolarization due to activation of GIRK conductance resulted in increased stimulation intensity needed to reach the firing threshold. In addition, most TFNs showed a transition from tonic to adapting firing mode. Therefore, the input–output characteristics of the neurons were modified in such a way that stronger synaptic input could no longer be converted into increasing numbers of generated spikes. It should be also noted that the opioid-induced plasticity of firing behavior reported here can be a complex phenomenon, which, in addition to modulation of GIRK channels,[37] may also involve G protein–dependent regulation of some other ion channel systems.[38–40]

DAMGO-sensitive interneurons from SG send some of their axons to lamina I and V,[22] where most projection neurons that target supraspinal regions are located.[41,42] Therefore, μ-opioid agonists can inhibit SG neurons that

relay primary nociceptive afferent inputs to ascending projection neurons. Suppression of excitatory interneurons with a tonic-firing pattern by enkephalins may be an important mechanism of endogenous pain control because μ-opioid receptor–expressing dorsal horn neurons were shown to be located in close proximity to enkephalinergic terminals.[7,43] Under physiologic conditions, they may be involved in an endogenous, *i.e.*, stress-induced, analgesia.

Furthermore, TFNs possessing μ-opioid receptors are likely to participate in analgesic effects of administered opioids. High sensitivity of TFNs to μ-opioid agonist implies their involvement in analgesic effects of spinal, epidural, and systemic opioids, like morphine.[9–11,44] At low doses, opioids do not block voltage-gated Na^+ and K^+ channels,[45–47] and therefore, their specific action *via* opioid receptors in tonic-firing spinal sensory neurons may contribute to profound and prolonged relief of pain with virtually no motor blockade.

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