Expression of Adenosine A$_{2A}$ Receptors in the Rat Lumbar Spinal Cord and Implications in the Modulation of N-Methyl-D-Aspartate Receptor Currents

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BACKGROUND: The presence of A$_{2A}$ receptors in the dorsal horn of the spinal cord remains controversial. At this level, activation of N-methyl-D-aspartate (NMDA) receptors induces wind-up, which is clinically expressed as hyperalgesia. Inhibition of NMDA receptor currents after activation of A$_{2A}$ receptors has been shown in rat neostriatal neurons. In this study, we sought to establish the presence of adenosine A$_{2A}$ receptors in the lamina II of the rat lumbar dorsal horn neurons and investigated whether the activation of A$_{2A}$ receptors is able to modulate NMDA receptor currents.

METHODS: Experiments were conducted in the rat lumbar spinal cord. The presence of adenosine A$_{2A}$ receptor transcripts inside the lumbar spinal cord is assessed with the reverse transcriptase polymerase chain reaction (RT-PCR) technique. Western blot experiments are performed at the same level. The RT-PCR technique is also performed specifically in the lamina II, and the presence of adenosine A$_{2A}$ receptor transcripts is assessed in neurons from the lamina II with the single-cell RT-PCR technique. The effect of adenosine A$_{2A}$ receptor activation on NMDA receptor currents is studied by the whole-cell configuration of the patch clamp technique.

RESULTS: RT-PCR performed on the lumbar spinal cord revealed the presence of adenosine A$_{2A}$ receptor transcripts. Western blot experiments revealed the presence of A$_{2A}$ receptors in the lumbar spinal cord. RT-PCR performed on the substantia gelatinosa also revealed the presence of adenosine A$_{2A}$ receptor transcripts. Finally, single cell RT-PCR revealed the presence of adenosine A$_{2A}$ receptor transcripts in a sample of lamina II neurons. Patch clamp recordings showed an inhibition of NMDA currents during the application of a selective A$_{2A}$ agonist.

CONCLUSIONS: These results demonstrate the presence of A$_{2A}$ receptor on neurons from the substantia gelatinosa of the rat lumbar dorsal horn and the inhibition of NMDA-induced currents by the application of a selective A$_{2A}$ receptor agonist. Therefore, A$_{2A}$ receptor ligands could modulate pain processing at the spinal cord level.

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Adenosine is an endogenous neuromodulator that acts on four different subtypes of G protein-coupled receptors (A$_1$, A$_{2A}$, A$_{2B}$, and A$_3$).[1] The presence of A$_{2A}$ receptors is well established in the central nervous system. *In situ* hybridization studies demonstrated that the A$_{2A}$ receptor mRNA is predominant in the striatopallidal γ-amino butyric acid (GABA)ergic projection neurons of the striatum.[2,3] The weak expression of A$_{2A}$ receptors in all the other regions of the brain needed immunohistochemical localization and reverse transcriptase polymerase chain reaction (RT-PCR) techniques to investigate their distribution. These methods revealed expression of the A$_{2A}$ receptor in the cortex, hippocampus, hypothalamus, thalamus, cerebellum, and amygdala.[4,5] In contrast, the distribution of A$_{2A}$ receptors in the spinal cord remains controversial. Tissue autoradiography demonstrated the distribution of A$_1$ and A$_2$ receptors in the spinal cord, predominantly in the substantia gelatinosa.[6,7] Kaelin-Lang et al. located the A$_{2A}$ receptor in the rat cervical dorsal root ganglia,[8] but found no expression of the receptor in the entire rat spinal cord.[9] Immunochemical studies revealed the presence of A$_{2A}$ receptors in the dorsal horn neurons of the thoracic spinal cord.[10]

The effects of adenosine in pain pathways are complex, and are either pronociceptive or antinociceptive depending on the activated receptors.[11] Spinally administered adenosine exhibits antinociceptive properties; these are described in acute nociceptive pain

models, inflammatory pain models, and neuropathic pain models and seem to be related to the activation of the A_1 receptor.[12–16] A1 receptor activation is not related to the activation of a pronociceptive pathway. On the contrary, the role of A_{2A} receptors is more controversial. A_{2A} receptor activation has been described to be related to pronociceptive and antinociceptive pathways. Activation of A_{2A} receptor in the periphery seems to be pronociceptive.[17] However, A_{2A} receptor activation exhibits antiinflammatory properties[18] and was shown to be involved in the modulation of the early (peripheral) and the late phase (central) responses of the formalin test.[19] Nevertheless, in three studies, A_{2A} receptor agonists injected intrathecally in acute nociceptive pain models, inflammatory pain models, and neuropathic pain models, produce analgesia.[20–22] Finally, it is noteworthy that in rat neostriatal neurons, activation of A_{2A} receptors has been shown to inhibit the conductance of N-methyl-D-aspartate (NMDA) receptors through an intracellular pathway.[23,24] Therefore, a similar mechanism in the spinal cord could be relevant in regard to the involvement of the NMDA receptors in pain pathways. Indeed, the plastic phenomenon called wind-up is related to the activation of NMDA receptors and is responsible for the development of hyperalgesia.[25]

In the present study, our aim was to demonstrate the presence of A_{2A} receptors in the dorsal horn of the spinal cord and its role in the pain pathways. First, we performed RT-PCR in the entire lumbar spinal cord to assess the presence of adenosine A_{2A} receptor transcripts inside the lumbar enlargement. Western blot experiments were also conducted in the entire lumbar spinal cord. Second, we performed RT-PCR with samples obtained from microdissections of the substantia gelatinosa. Afterwards, we performed single-cell RT-PCR to detect the presence of A_{2A} receptor mRNA in individual substantia gelatinosa neurons. Finally, we recorded the membrane current from these neurons using the whole-cell patch clamp technique. We investigated whether the activation of A_{2A} receptors has any modulatory effect on the activity of NMDA receptors.

METHODS

Animal experimentation committee approval was obtained and all efforts to avoid animal suffering were made according to the standards of the Institutional Ethical Committee of the School of Medicine of the Université Libre de Bruxelles.

RT-PCR

RT-PCR Performed on the Entire Lumbar Spinal Cord

After being anesthetized with halothane, 10-day-old Wistar rats (Iffa Credo, Reims, France) were killed by cervical dislocation, a lumbar laminectomy was performed inside ice-cold Krebs solution saturated with 95% oxygen and 5% carbon dioxide, and the lumbar enlargement was removed. Total RNA was removed using the Rneasy Mini kit (Qiagen, Westburg bvBV, Leusden, The Netherlands) and treated with DNAse I (On Column Dnase, Qiagen) according to the manufacturer's instructions to avoid genomic DNA contamination. RT was performed on 100 ng of RNA with random Hexamer using Superscript II ™ (Invitrogen, Gent, Belgium) according to the manufacturer's instruction in a final volume of 20 μL. The resulting cDNAs of A_{2A}R and β actin contained in 20 μL RT reaction were amplified separately. To 1 μL of RT reaction Taq polymerase (2.5 U) (Qiagen, Hilden, Germany) and 10 pM of forward and reverse primers were added in the buffer supplied by the manufacturer (final volume 50 μL), and 40 cycles (94°C, 1 min; 60°C, 1 min; 72°C, 1 min) of PCR were run. The following sets of primers were used (from 5′ to 3′): A_{2A}R forward TTTGTCCTGGTCCTCACGCAG, A_{2A}R reverse CGCCAGAAAAATCCGTAGGTAGAT yielding a predicted PCR product of 356bp; β actin forward AGCTTCTTTGCAGCTCCTTCGT, β actin reverse CACATGCCGGAGCCGTT yielding a predicted PCR product of 461 bp.

RT-PCR Performed on the Substantia Gelatinosa

The lumbar enlargement was removed using the protocol described above, immersed in Sakura Tissue-Tek OCT Compound (Bayer, Brussels, Belgium) and frozen in isopenthane (Fluka, Bornem, Belgium) at −80°C. Fifteen micrometer thick transversal sections were obtained with a cryostat (Leica CM 3050, Wetzlar, Germany) on palm membranes slides NF (Palm microlaser technology, Bernried, Germany) and frozen again at −80°C. Slides were then colored with cresyl violet and the substantia gelatinosa was located (see paragraph Electrophysiology) and dissected with a microdissector (Palm microlaser technology). Eighty samples were harvested in palm adhesive caps (Palm microlaser technology). RT was performed on 78 ng with the protocol described above.

Single Cell RT-PCR Performed on Neurons from the Substantia Gelatinosa

The cytoplasm of neurons was retrieved by aspiration into a recording pipette. Harvesting was completed within 2 min after the execution of the seal to avoid the degradation of the RNA. The pipette solution contains: 144 mM KCl, 3 mM MgCl$_2$, 0.2 mM EGTA, and 10 mM HEPES, 4 mM NaATP, and 0.4 mM NaGTP, (pH adjusted at 7.2 with KOH). The contents were expelled into a test tube where the RT reaction was performed in a final volume of 10 μL,[26] left overnight at 37°C. PCR amplification was performed for detection of the adenosine A_{2A} receptor and β actin c-DNA was used as a control to assess the integrity of the RNA and prevent false-negative results.

The possibility of contamination was eliminated by inclusion of a template minus negative control (extracellular solution near harvested neurons).

The set of primers used was located in different exons to exclude genomic DNA amplification by size criterion. The two steps of multiplex PCR were performed as described previously.[26] The resulting cDNAs of $A_{2A}R$ and β actin contained in 10 μL RT reaction were first amplified simultaneously. Taq polymerase (2.5 U) (Qiagen) and 10 pmole of each of the 14 primers were added in the buffer supplied by the manufacturer (final volume 100 μL), and 20 cycles (94°C, 1 min; 60°C, 1 min; 72°C, 1 min) of PCR were run. Second rounds of PCR were then performed using 2 μL of the first PCR as a template (final volume 50 μL). In this second PCR, each cDNA was amplified individually using its specific primer pair by performing 40 PCR cycles as described above. Twenty microliter of each individual PCR were then run on a 2% agarose gel. The efficiency of this RT-multiplex PCR protocol was tested on 10 pg of whole brain total RNA.[27,28] The $A_{2A}R$ primers are the same as above. The β actin forward AGCTTCTTTGCAGCTCCTTC-GT, β actin reverse CTGGATGGCTACGTACATG-GCT yielding a predicted PCR product of 100 bp.

Western Blot Analysis Performed on the Entire Lumbar Spinal Cord

After being anesthetized with halothane, four 10-day-old Wistar rats (Iffa Credo) were killed by cervical dislocation, a lumbar laminectomy was performed inside ice-cold Krebs solution saturated with 95% oxygen and 5% carbon dioxide and the lumbar enlargement was removed. The samples were homogenized in lysis buffer (20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, pH 7.5) containing protease (Sigma, Bornem, Belgium) and phosphatase inhibitors (Pierce, Aalst, Belgium). The homogenates were centrifuged in a Sigma 3K15 centrifuge at 30,996g for 20 min at 4°C, and the supernatants were frozen at −80°C. After thawing on ice, protein concentrations were established by using the Bradford method. Protein samples (30 μg) were separated on SDS-Page gel (4%–15% gradient gel) and transferred to nitrocellulose membranes (Millipore, Bedford, MA). The membranes were blocked for 1 h in 5% dry milk in PBST (290 mM NaCl, 509 mM KCl, 20.3 mM Na_2HPO_4, 3.5 mM KH_2PO_4, Tween-20 0.1%) at room temperature and incubated overnight at 4°C with anti-adenosine A_{2A} receptor antibody (mouse monoclonal, 1:500, in 5% albumin; Upstate-Campro Scientific, Veenendaal, The Netherlands). In another membrane, loaded with the same samples, the primary antibody was omitted. After three washes of 10 min, the blots were incubated for 1 h at RT with horseradish peroxidase-conjugated secondary antibody (Amersham Bioscience, Eire, UK 1:3000). Detection was done by using Western Lightning Chemiluminescence reagents (PerkinElmer Life Sciences, Boston, MA) for 1 min and exposure to hyperfilm (Amersham Biosciences, Eire, UK) for 5 min.

Afterwards, both membranes were treated with Restore Western Blot Stripping Buffer (Pierce, Aalst, Belgium) to remove the primary antibody. Stripping was verified by exposing the membrane to hyperfilm (Amersham Biosciences, Eire, UK) for 10 min. The blots were then reincubated with βactin antibody (Sigma) at room temperature for 2 h, rinsed three times for 10 min, and exposed to HRP-conjugated secondary antibody (Amersham Bioscience; 1:3000) for 1 h at room temperature. Detection was again done by using Western Lightning Chemiluminescence reagents (PerkinElmer Life Sciences, Boston, MA) for 1 min and exposure to hyperfilm (Amersham Biosciences) for 1 min.

Electrophysiology Performed on Neurons from the Substantia Gelatinosa

Whole-cell patch-clamp recordings were conducted on lumbar spinal cord slices of 300-μm-thick obtained from 10-day-old Wistar rats (Iffa Credo). The lumbar enlargement was removed, cleared of any remaining dura membrane, embedded in a 10% gelatin block, and glued to the stage of a vibrating microslicer (Leica, Wetzlar, Germany) with cyanoacrylate glue (Roth, Karlsruhe, Germany). The spinal tissue was immersed in ice-cold Krebs solution and saturated with 95% oxygen and 5% carbon dioxide throughout all these procedures. Slices were maintained in an incubation chamber at room temperature for at least 1 h before recordings. Krebs solution used for this procedure had the following composition: 206 mM sucrose; 25 mM $NaHCO_3$; 25 mM D-(+) glucose; 2.5 mM KCl; 1.25 mM NaH_2PO_4; 1 mM $CaCl_2$; 2 mM $MgCl_2$.[29]

Membrane currents were recorded in the whole-cell configuration of the patch-clamp technique[30] at room temperature inside the lamina II of the dorsal horn.

Neurons from lamina II (substantia gelatinosa) were identified using a Zeiss (Oberkochen, Germany) Axioskop II FS MOT microscope. At low magnification (×100), location of substantia gelatinosa is facilitated by the macroscopic aspect of the spinal slices and the entrance of the dorsal roots. Because of its relatively high neuronal density, lamina II could be readily visualized at low magnification (×100). Lamina II could also be distinguished from lamina I characterized by a striated appearance.[31] At higher magnification (×400), morphologically distinct interneurons stalk and islet cells served as markers for the boundaries of lamina II, outer and inner, respectively.[32,33]

During the patch-clamp recordings, slices were perfused with standard Krebs-Henseleit solution containing: 125 mM NaCl; 25 mM $NaHCO_3$; 25 mM D-(+) glucose; 2.5 mM KCl; 1.25 mM NaH_2PO_4; 1 mM $CaCl_2$; 10 μM bicuculline; 0.5 μM strychnine; 0.1 μM tetrodotoxin; and had a pH of 7.4 (95% oxygen and 5% carbon dioxide). Bicuculline is used as a blocker of the GABA A receptor, strychnine as a blocker of glycine

receptors, and tetrodotoxin as a blocker of sodium channels.

The following components were diluted into the former solution: 10 μM NMDA; 10 μM Glycine, 0.1 μM CGS 21680. Patch pipettes were pulled from borosilicate glass capillaries (Harvard Apparatus, Edenbridge, UK) and had a resistance of 6–10 M[Omega]. The intracellular solution contained: 130 mM Cs^+ gluconate; 6.8 mM CsCl; 0.1 mM EGTA; 10 mM HEPES; 2 mM Mg_2ATP; pH adjusted to 7.3 with NaOH, osmolarity adjusted to 305–310 mOsm with sucrose. Type A porcine gelatin, glycine, HEPES, EGTA, glutamate and D-gluconic acid lactone were obtained from Sigma, NMDA, bicuculline, CGS 21680 from Tocris (Bristol, UK), strychnine from RBI (Bornem, Belgium), and tetrodotoxin from Alexis (Zandhoven, Belgium).

Whole-cell currents were recorded with an EPC-8 amplifier (Heka Elektronik, Lambrecht/Pfalz, Germany) in voltage clamp mode at a membrane potential of −60 mV.

Data were obtained using the acquisition software Pulse (Heka) and were analyzed with IGOR software (WaveMetrics, Lake Oswego, OR). Passive cellular parameters were extracted in voltage clamp by analyzing current relaxation induced by a 10 mV step change from a holding potential of −60 mV. Visually identified neurons were selected based on a low and stable leak current variation inferior to 20 pA.

Statistical Analysis

Data are reported as means ± SEM and statistical comparisons were performed using paired Student's t-test for electrophysiological results ($P < 0.05$ was considered significant). Each cell was analyzed to define if the NMDA-induced current was sensitive to the application of the CGS 21680. Therefore, 30 points recorded during the NMDA application were compared with 30 points recorded during the application of NMDA and CGS 21680 using a paired Student's t-test. Each group of 30 points was selected when the current reached its steady-state value.

RESULTS
Expression of A_{2A} Receptors in Neurons from the Substantia Gelatinosa

To assess for the presence of the adenosine A_{2A} receptor in the lumbar spinal cord we first performed RT-PCR on the lumbar enlargement. Results from these experiments reveal the presence of A_{2A} receptor transcripts. These results indicate that the A_{2A} receptor might be present in the lumbar spinal cord (Fig. 1).

Western blot analysis was also performed in this region. We used the anti-adenosine receptor A_{2A} antibody (mouse monoclonal) that is described as specifically recognizing the A_{2A} receptor and yielding a band at a molecular weight of 43 kDa. After separation of the protein samples, the application of the
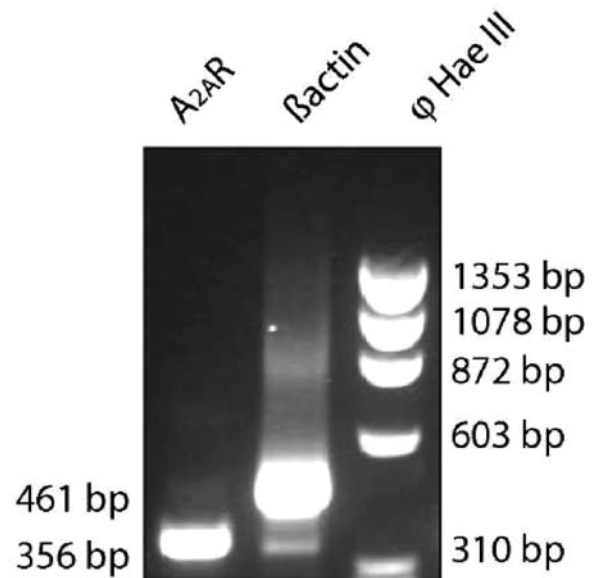

Figure 1. Reverse transcriptase polymerase chain reaction (RT-PCR): expression of A_{2A} receptor transcripts in the lumbar spinal cord. Agarose gel electrophoresis of the RT-PCR products from the total lumbar spinal cord. Lane A_{2A} shows the expression of A_{2A} receptor transcripts. Lane β actin shows expression of β actin transcripts as a positive control of RNA integrity.
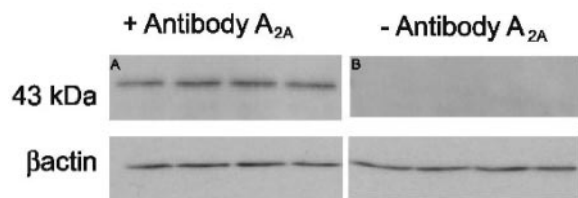

Figure 2. Western blot: presence of the adenosine A_{2A} receptor in the lumbar spinal cord. A, The application of the anti-adenosine receptor A_{2A} antibody reveals the presence of A_{2A} receptors at a molecular weight of 43 kDa. Application of β actin antibody results in uniform bands, which confirms equal loading. B, When the anti-adenosine receptor A_{2A} antibody is omitted no bands are visualized. In order to assess the validity of this negative control, β actin antibody is applied. The antibody reveals the presence of β actin in each lane in equal amount.

anti-adenosine receptor A_{2A} antibody revealed the presence of the A_{2A} receptor at this molecular weight (Fig. 2A). A negative control was performed without the presence of the anti-adenosine receptor A_{2A} antibody. The resulting films did not exhibit any bands, whereas all the loaded lanes showed the presence of β actin (Fig. 2B). The presence of equal amounts of β actin in each lane confirms that our examination of A_{2A} receptors was performed under the same conditions (Fig. 2A). Presence of β actin on the film that did not exhibit any bands in the absence of the anti-adenosine receptor A_{2A} antibody confirms that the result is not due to the absence of proteins (Fig. 2B). These results, added to the one obtained with the RT-PCR technique, indicate that the A_{2A} receptor is present in the lumbar spinal cord without evidence of the exact location of the receptor.
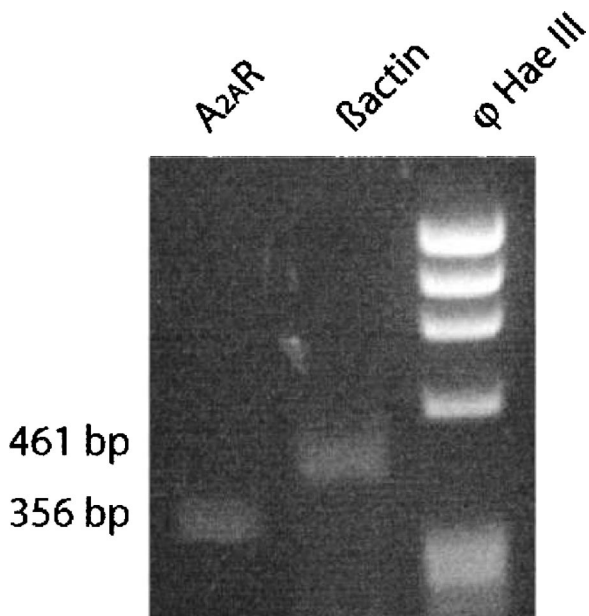
Figure 3. Reverse transcriptase polymerase chain reaction (RT-PCR): expression of A_{2A} receptor transcripts in the substantia gelatinosa. Agarose gel electrophoresis of the RT-PCR products from the microdissected substantia gelatinosa. Lane A_{2A} shows the expression of A_{2A} receptor transcripts. Lane β actin shows the expression of β actin transcripts as a positive control of RNA integrity. In the lane [varphi] Hae III digestion of [varphi] $\times$ 174 by Hae III indicates the DNA scale.

Considering the presence of adenosine A_{2A} receptor transcripts and proteins inside the lumbar spinal cord, we performed RT-PCR specifically inside the dorsal horn. This region, selected using a laser microdissector, also revealed the presence of adenosine A_{2A} receptor transcripts. Again, β actin c-DNA was used to confirm the integrity of RNA preparations (Fig 3).

Finally, we performed single cell RT-PCR to specifically establish the expression of the receptor in neurons from the lamina II. Neurons from the substantia gelatinosa were selected based on the technique described in the method section. RT-PCR performed on single cell units reveals the presence of the adenosine A_{2A} receptor transcripts in five neurons of a group of 32 cells (Fig. 4 A,B). Only positive β actin c-DNA neurons were collected in order to prevent false negatives. These results indicate that the A_{2A} receptor is present in neurons from the substantia gelatinosa and that the neuronal population of this region is divided in two groups regarding the presence or the absence of the A_{2A} receptor.

Modulation of NMDA Receptors by the Activation of the A_{2A} Receptors

Electrophysiological recordings were performed from neurons that were visually identified inside the lamina II as described in the Methods section. Membrane capacitances (25.2 $\pm$ 4.4 pF) and resistances (1.8 $\pm$ 0.3 G[Omega]) of the selected cells were extracted and exhibited little cell-to-cell variations which underline, as in our previous study, the ability of the
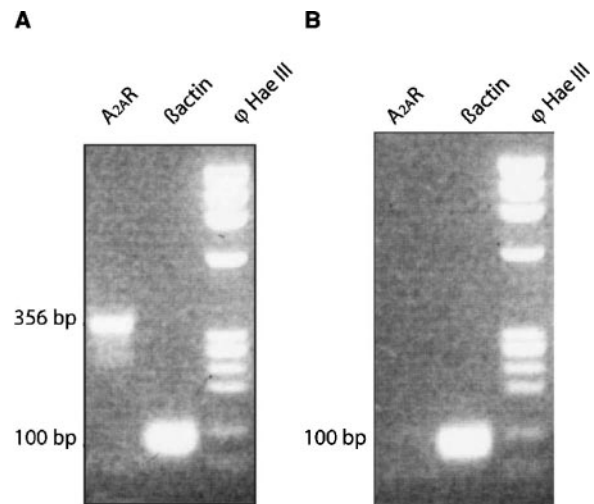

Figure 4. Single cell reverse transcriptase polymerase chain reaction (RT-PCR): expression of A_{2A} receptor transcript in neurons from the substantia gelatinosa. A, Agarose gel electrophoresis of the RT-PCR products from a representative neuron harvested in the substantia gelatinosa. Lane A_{2A} shows the expression of A_{2A} receptor transcripts. Lane β actin shows the expression of β actin transcripts as a positive control of RNA integrity. B, Agarose gel electrophoresis of the RT-PCR products from a representative neuron harvested in the substantia gelatinosa. A_{2A} receptor transcripts are absent whereas lane β actin shows the expression of β actin transcripts attesting RNA integrity in the sample.

method to select, record, and analyze a homogenous population.[34]

After the first step that consisted of demonstrating the presence of the A_{2A} receptor in neurons from the substantia gelatinosa, electrophysiological recordings of these neurons were performed to define if the activation of the A_{2A} receptor by a selective agonist, the CGS 21680, has any modulatory effect on a NMDA-induced current.

Whole-cell current is first recorded in control conditions. When the current is stable for 100 s, NMDA receptors are activated by 10 μM NMDA and 10 μM glycine. The induced current is significantly different from the one recorded during the control condition (-152.30 ± 33.20 pA vs -29.46 ± 6.15 pA; $P < 0.05$, $n = 7$). In 5 neurons out of 7, the current recorded during the application of the association NMDA-glycine and CGS 21680 was significantly different from the one induced in the absence of CGS 21680 (-115.00 ± 34.1 pA vs -166.60 ± 45.6 pA; $P < 0.05$, $n = 5$) showing a 31% $\pm$ 0.25% decrease of the NMDA-induced current. Washout of CGS 21680 leads the current to a level similar to the one observed in the presence of NMDA-glycine (Fig. 5A). Two cells of seven did not exhibit any variation of the NMDA-induced current after the application of the CGS 21680 ($P > 0.05$) (Fig. 5B).

These results and the ones obtained with RT-PCR and Western blot techniques indicate that the A_{2A} receptor is present in a sample of neurons from the substantia gelatinosa and that the activation of this receptor is able to inhibit an induced NMDA current.
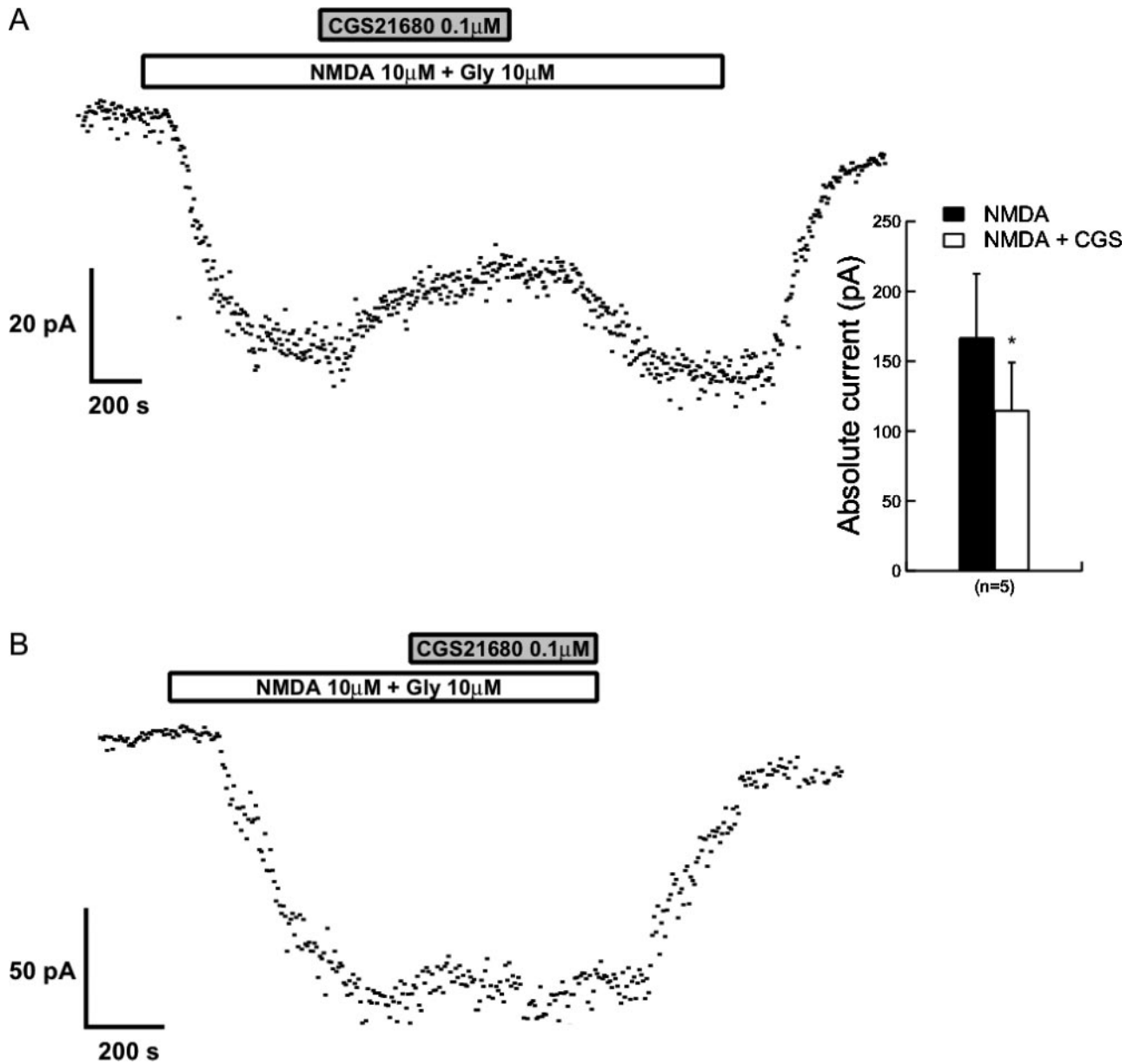
Figure 5. Electrophysiology: modulation of *N*-methyl-D-aspartate (NMDA)current by CGS 21680. A, Representative trace of the decrease of NMDA-induced currents by application of 100 nM of CGS 21680. Whole-cell current is first recorded in control conditions (Ctrl). Ten micromole NMDA and 10 μM glycine added to the perfusing bath induce a NMDA current. When this current remains stable 100 nM of CGS 21680 is added to the perfusing bath. Application of CGS 21680 decreases NMDA currents. Current returns to the NMDA-induced current level after washout of the CGS 21680 and to the initial level after washout of NMDA. Histogram in inset shows a significant difference between the NMDA-induced current and the NMDA-induced current in the presence of CGS 21680 ($n = 5$, *$P < 0.05$). B, Representative trace of an NMDA-induced current that is not modulated by the application of CGS 21680. Whole-cell current is first recorded in control conditions (Ctrl). 10 μM NMDA and 10 μM glycine added to the perfusing bath induce a NMDA current. When this current remains stable 100 nM of CGS 21680 is added to the perfusing bath. Application of CGS 21680 does not modify NMDA current. Current returns to the initial level after washout ($n = 2$, $P > 0.05$).

DISCUSSION

This study provides strong evidence revealing the expression of the A_{2A} receptor in the rat lumbar dorsal horn. Expression of this receptor was demonstrated in the lumbar spinal cord, the substantia gelatinosa and in a sample of neurons from the lamina II. Moreover, electrophysiological recordings performed on these neurons show a functional relevance of A_{2A} receptor activation. Indeed, patch clamp recordings show that the activation of the A_{2A} receptor by a selective agonist inhibits NMDA-induced currents.

Neurons were selected on a morphological basis for the single cell PCR and the electrophysiological recordings. Electrophysiological characteristics reveal a homogenous population of the sample of neurons. The recorded membrane capacitances of the neurons that are proportional to the surface of the cells are homogenous and similar to the ones described previously.[34,35]

In the present study, RT-PCR revealed the presence of A_{2A} receptor transcripts in the rat lumbar spinal cord. This first result is underlined by Western blot experiments that reveal the presence of A_{2A} receptors at the same level. RT-PCR also shows the presence of A_{2A} receptor transcripts in the substantia gelatinosa and in a sample of neurons from the dorsal horn.

Our results assessed the presence of A_{2A} receptor transcripts in neurons from the lumbar rat dorsal horn, but no conclusion can be drawn about the amount of A_{2A} receptors expressed in the studied population. Indeed, the efficiency of the reverse transcriptase is related to the transcripts' secondary structure, the protein-mRNA complex formation and the total RNA copy number. Therefore, the efficacy of the single cell PCR can be as weak as 1%–30%.[36,37] Electrophysiological recordings also confirm the presence of the A2A receptor in a sample of dorsal horn neurons. Again, the heterogeneity of the response after the application of CGS 21680 suggests that neurons from the substantia gelatinosa could be divided into two groups, one group with the receptor and the other without it. A similar situation is described with striatal neurons that are divided in two populations depending on the presence or the absence of the A_{2A} receptors.[24] In the striatum, 25% of induced NMDA currents are unaffected by subsequent application of CGS 21680. Moreover, confocal fluorescence immunochemistry demonstrated that these neurons are devoid of A_{2A} receptors.

Our study isolated a group of dorsal horn neurons in which NMDA-induced currents were significantly inhibited by the application of an A_{2A} receptor selective agonist. The application of CGS 21680 on dorsal horn neurons was described previously as unable to affect the resting membrane current.[38] Therefore, the decrease of the current recorded during the application of both the selective agonist of NMDA receptors and the selective agonist of A_{2A} receptors corresponded to a modulation of NMDA-receptor induced current through activation of A_{2A} receptors.

CGS 21680 has been described as able to bind to the A1 receptor in hippocampus and to behave as an agonist when applied at a concentration more than 11 μM.[39] The binding affinity of CGS 21680 is high for A_{2A} compared to A_1 receptor (K_i values $\pm$ sem: 22 $\pm$ 4.3 vs 3100 $\pm$ 470 nM).[40] In this study, CGS 21680 was infused at 100 nM. This concentration is very low compared with the concentrations able to bind and to activate the A_1 receptor. Moreover, even if a high dose of CGS 21680 seems to be able to activate the A_1 receptor,[41] it has been shown in hippocampus that modulation of the NMDA receptor through the activation of the A_1 receptor is very unlikely.[42]

On the contrary, A_{2A} receptors activation has been described as being able to inhibit the activation of the NMDA receptor in the neostriatum. The mechanism is mediated through the activation of phopholipase C, inositol 1,4,5 triphosphate receptor, and calcium/calmoduline and calmoduline kinase II pathways.[23,24]

Nevertheless, the role of the A_{2A} receptor in the spinal pain pathways remains controversial. Allodynic pain states induced by drugs such as strychnine or by nerve injury mimicking neuropathic pain are not sensitive to opioïds but are reversed by NMDA antagonists and adenosine agonists.[12,43] Interestingly, CGS 21680 has been described as being able to inhibit allodynic pain states induced by L5 and L6 nerve ligation.[22] CGS 21680 has also been described as able to reduce inflammation-induced thermal hyperalgesia.[21] However, incision-induced hyperalgesia is not reduced by an intrathecal infusion of CGS 21680.[44] Finally, it is noteworthy that A_{2A} receptor knockout mice have a important reduction in NMDA receptor binding in the whole spinal cord.[45]

Our results underline the ability of A_{2A} receptors to play a role in the regulation of NMDA receptor activity through a postsynaptic mechanism. This property could partly explain the efficiency of A_{2A} receptor agonists in alleviating allodynic pain.

In summary, A_{2A} receptors are present inside the dorsal horn of the spinal cord on a subpopulation of neurons from the substantia gelatinosa. Activation of these receptors inhibits NMDA receptor activity. Therefore, adenosine could exert, through the activation of A_{2A} receptors, a control of the wind-up phenomenon and, consequently, of hyperalgesia that remains a challenge in pain management.

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