TRPV1 Receptor Mediates Glutamatergic Synaptic Input to Dorsolateral Periaqueductal Gray (dl-PAG) Neurons

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Xing J, Li J. TRPV1 receptor mediates glutamatergic synaptic input to dorsolateral periaqueductal gray (dl-PAG) neurons. J Neurophysiol 97: 503–511, 2007. First published October 25, 2006; doi:10.1152/jn.01023.2006. The purpose of this study was to determine the role of transient receptor potential vanilloid type 1 (TRPV1) receptor in modulating neuronal activity of the dorsolateral periaqueductal gray (dl-PAG) through excitatory and inhibitory synaptic inputs. First, whole cell voltage-clamp recording was performed to obtain the spontaneous miniature excitatory postsynaptic currents (mEPSCs) and inhibitory postsynaptic currents (mIPSCs) of the dl-PAG neurons. As 1 μM of capsaicin was applied into the perfusion chamber, the frequency of mEPSCs was increased from 3.21 ± 0.49 to 5.64 ± 0.64 Hz (P < 0.05, n = 12) without altering the amplitude and the decay time constant of mEPSCs. In contrast, capsaicin had no distinct effect on mIPSCs. A specific TRPV1 receptor antagonist, iodo-resiniferatoxin (i-RTX, 300 nM), decreased the frequency of mEPSCs from 3.51 ± 0.29 to 2.01 ± 0.2 Hz (P < 0.05, n = 8) but did not alter the amplitude and decay time. In addition, i-RTX applied into the chamber abolished the effect of capsaicin on mEPSC of the dl-PAG. In another experiment, spontaneous action potential of the dl-PAG neurons was recorded using whole cell current-clamp methods. Capsaicin significantly elevated the discharge rate of the dl-PAG neurons from 3.03 ± 0.38 to 5.96 ± 0.87 Hz (n = 8). The increased firing activity was abolished in the presence of glutamate N-methyl-D-aspartate (NMDA) and non-NMDA antagonists, 2-amino-5-phosphono-pentanoic acid, and 6-cyano-7-nitroquinoxaline-2,3-dione. The results from this study provide the first evidence indicating that activation of TRPV1 receptors increases the neuronal activity of the dl-PAG through selective potentiation of glutamatergic synaptic inputs.

INTRODUCTION

The midbrain periaqueductal gray (PAG) is an important neural site for a number of physiological functions related to behavioral reactions, cardiovascular regulation, and pain modulation (Bandler et al. 1991; Behbehani 1995; Lovick 1996). Among regions of the PAG, the dorsolateral (dl) region receives abundant somatic afferent inputs from the dorsal horn of the spinal cord (Craig 1995; Keay et al. 1997) and also sends descending neuronal projections to the medulla in regulating cardiovascular activity and pain (McGaraughty et al. 2003; Tjen-A-Looi et al. 2006; Verberne and Guyenet 1992). Activation of the dl-PAG contributes to an increase in arterial blood pressure and antinociception (Bandler et al. 1991; Behbehani 1995).

Glutamate, the major excitatory neurotransmitter, appears in the dl-PAG region (Beitz and Williams 1991). The dl-PAG also has the high density of excitatory amino acid binding sites (glutamate receptor subtypes) including α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)/kainate, N-methyl-D-aspartate (NMDA), and metabotropic receptors (Albin et al. 1990; Cotman et al. 1987).

Transient receptor potential vanilloid type 1 (TRPV1) is widely found on small- and medium-size primary afferent neurons (Guo et al. 1999; Ma 2002) and mediates numerous sensory afferent activations (Caterina et al. 1997; Nault et al. 1999; Smith and McQueen 2001; Zahner et al. 2003). Recent studies have also shown that TRPV1 receptors are located in several regions of the CNS including hypothalamus, midbrain PAG, substantia nigra, and locus coeruleus (McGaraughty et al. 2003; Mezey et al. 2000; Toth et al. 2005). Moreover, activation of TRPV1 receptors induces hypoalgesia, similar to the effect of glutamate, and enhances glutamatergic synaptic transmission in the substantia nigra, locus coeruleus, and hypothalamus (Marinelli et al. 2002, 2003; Sasamura et al. 1998). Although capsaicin microinjected into the PAG produces antinociception by increasing glutamate release (Palazzo et al. 2002), the effect of TRPV1 receptors on glutamatergic synaptic inputs to the dl-PAG neurons has not specifically been studied using electrophysiological methods.

In this report therefore we used an in vitro whole cell recording technique in the midbrain slice to determine the role of TRPV1 in modulating the firing activity of the dl-PAG neurons through the excitatory glutamatergic inputs. We hypothesized that TRPV1 activation would increase discharge of the dl-PAG neurons through potentiation of glutamatergic synaptic inputs.

In addition, GABA-mediated neuronal elements constituting ~50% of the total population of neurons play a crucial role in the intrinsic neuronal circuitry of the PAG (Mugnaini and Oertel 1985; Reichling 1991). The GABA synaptic inputs make up ~50% of the synaptic innervation of the PAG neurons, and the majority of GABAergic neurons are tonically active interneurons (Strack et al. 1989). The release of GABA from those neurons may play a role in modulation of the synaptic inputs to the PAG neurons. Studies have further shown that GABA<sub>A</sub> receptors are dense within the PAG (Bowery et al. 1987; Chu et al. 1990). Thus the effect TRPV1 activation on the inhibitory GABAergic inputs to the dl-PAG neurons was also examined in this study.

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METHODS

Brain slice preparations

All procedures outlined in this study were approved by the Animal Care Committee of Penn State College of Medicine. Sprague-Dawley rats of either sex (4-6 wk old) were anesthetized by inhalation of isoflurane oxygen mixture (5% isoflurane in 100% oxygen) and were decapitated. Briefly, the brain was quickly removed and placed in ice-cold artificial cerebral spinal fluid (ACSF) perfusion solution. A tissue block containing the midbrain PAG was cut from the brain and glued onto the stage of the vibratome (Technical Product International, St. Louis, MO). Coronal slices (300 μm) containing the midbrain PAG were dissected from the tissue block in ice-cold ACSF solution. The slices were incubated in the ACSF at 37°C for an equilibrium period of 60 min. The slices were transferred to the recording chamber. During the procedures described above, ACSF was saturated with 95% O₂-5% CO₂. The ACSF perfusion solution contained (in mM) 124.0 NaCl, 3.0 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 1.4 NaH₂PO₄, 10.0 glucose, and 26.0 NaHCO₃ (Li et al. 2002, 2004).

Electrophysiological recordings

POSTSYNAPTIC CURRENTS OF DL-PAG NEURONS. A whole cell voltage-clamp technique was used to record postsynaptic currents in the dl-PAG neurons. Borosilicate glass capillaries (1.2 mm OD, 0.86 mm ID; World Precision Instruments, Sarasota, FL) were pulled to make the recording pipettes using a puller (Sutter Instrument, Novato, CA). The resistance of the pipette was 4-6 MΩ when it was filled with the internal solution (in mM: 130.0 potassium gluconate, 1.0 MgCl₂, 10.0 HEPES, 10.0 EGTA, 1.0 CaCl₂, and 4.0 ATP-Mg) (Li et al. 2002, 2004). The solution was adjusted to pH 7.25 with 1 M KOH and osmolarity of 280–300 mOsm. The slice was placed in a recording chamber (Warner Instruments, Hamden, CT) and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steel weight. The ACSF saturated with 95% O₂-5% CO₂. The ACSF perfusion solution contained (in mM) 124.0 NaCl, 3.0 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 1.4 NaH₂PO₄, 10.0 glucose, and 26.0 NaHCO₃ (Li et al. 2002, 2004).

RESULTS

At the end of each experiment, the recording site in the PAG tissue was examined. It was confirmed that all the cells included for data analysis in this experiment were located in the dl-PAG (anterior-posterior coordinates from AP: −7.1 to −7.9) (Swanson 1998). Whole cell patch-clamp experiments were performed and experimental data were collected from 55 dl-PAG neurons. The resting membrane potential was −66.7 ± 2.9 mV (from −78.5 to −60.8 mV), and amplitude of action potential was >60 mV. The input resistance was 511.7 ± 11.2 MΩ (from 320.1 to 678.6 MΩ).

Effect of capsaicin on glutamatergic mEPSCs

The spontaneous mEPSCs were recorded in the dl-PAG to determine the effect of TRPV1 activation on synaptic glutamate release onto the neurons. The mEPSCs were recorded in the presence of 1 μM TTX and 20 μM bicuculline. Capsaicin (1 μM) was perfused into the recording chamber (n = 12). This significantly increased the frequency of mEPSCs from 3.21 ± 0.49 to 5.64 ± 0.64 Hz (P < 0.05), but did not alter the amplitude and the decay time constant of mEPSCs in all neurons tested (Fig. 1). The mEPSCs recovered during wash-out of the perfusion solution and were completely abolished by blocking non-NMDA glutamate receptors after bath application of 20 μM CNQX (Fig. 1A).

The effect of capsaicin on mEPSCs was analyzed by measuring the time constant of the decay phase of mEPSCs. The decay time constant of mEPSCs after application of capsaicin (3.32 ± 0.50 ms) was not different from the control (3.56 ± 0.42 ms, P > 0.05 vs. capsaicin). As an average of 100 consecutive mEPSCs was superimposed during control and capsaicin application, the decay phase of mEPSCs was best fitted by a single exponential function (Fig. 1B). The cumulative probability analysis of mEPSCs shows that the distribution pattern of the interevent interval and amplitude of mEPSCs and mIPSCs was estimated using the Komogorov–Smirnov test (Li et al. 2002, 2004). Experimental data (frequency, amplitude, and decay time of mEPSCs and mIPSCs and the firing rate of dl-PAG neurons) were analyzed with one-way ANOVA. Tukey’s post hoc analyses were used to determine differences between groups, as appropriate. All values are expressed mean ± SE. For all analyses, differences were considered significant at P < 0.05. All statistical analyses were performed using SPSS for Windows, version 13.0.
data further show the effect of capsaicin on the frequency and amplitude of mEPSC of the dl-PAG neurons (Fig. 1, E and F).

**Effect of i-RTX on glutamatergic mEPSCs**

To determine tonic effect of endogenous TRPV1 activation on glutamatergic inputs to the dl-PAG neurons, 300 nM of i-RTX was applied into the recording chamber, and mEPSCs were examined (n = 8). i-RTX alone decreased the frequency of mEPSCs from 3.51 ± 0.29 to 2.01 ± 0.2 Hz (P < 0.05), without affecting the amplitude and decay time constant (Fig. 2, A–F). The inhibitory effect of i-RTX on the frequency of mEPSCs was no longer present after washout.

To confirm that the effect of capsaicin on mEPSCs was mediated through the TRPV1 receptor, i-RTX was perfused before capsaicin application (n = 10). Our results showed that capsaicin failed to increase the frequency of mEPSCs of the dl-PAG neurons in the presence of 300 nM of i-RTX (Fig. 3, A–E).

**Effect of capsaicin on GABAergic mIPSCs**

The spontaneous mIPSCs were recorded in the dl-PAG neurons in the presence of 1 μM TTX and 20 μM CNQX (n = 8). Capsaicin, in a concentration of 1 μM, did not produce a significant effect on the frequency and amplitude of mIPSCs in the dl-PAG neurons (Fig. 4). The mIPSCs were completely eliminated after bath application of 20 μM of bicuculline, blocking GABA_A receptors (Fig. 4A).

The effect of capsaicin on mIPSCs was analyzed by measuring the time constant of the decay phase of mIPSCs. As an average of 100 consecutive mIPSCs was superimposed during control and capsaicin application, the decay time constant of mIPSCs was best fitted by a double exponential function (Fig. 4B). The decay time constant was similar during control (16.38 ± 0.91 ms) and during capsaicin application (17.02 ± 1.09 ms, P > 0.05 vs. control). The cumulative probability analysis also shows that capsaicin did not alter the distribution pattern of either the interevent interval or the amplitude of the
mIPSCs (Fig. 4, C and D). Average data further show capsaicin had no effect on the frequency and amplitude of mIPSCs of the dl-PAG neurons (Fig. 4, E and F).

**Effect of capsaicin on discharge of dl-PAG neurons**

It was likely that capsaicin enhanced the activity of the dl-PAG neurons because capsaicin increased the excitatory glutamatergic inputs to the dl-PAG neurons without altering the inhibitory GABAergic synaptic activity. To test this hypothesis, the effect of capsaicin on the discharge of the dl-PAG neurons was examined using whole cell current-clamp recordings (n = 8). Capsaicin (1 μM) increased the discharge rate of the dl-PAG neurons from 3.03 ± 0.38 to 5.96 ± 0.87 Hz (P < 0.05). Application of capsaicin did not significantly alter the resting membrane potential of the dl-PAG neurons. The effect of capsaicin is shown in Fig. 5, A–C.

In addition, the role of the glutamatergic synaptic inputs and glutamate receptors in capsaicin activation of the dl-PAG neurons was determined (n = 9). The firing activities of the dl-PAG neurons were examined in the presence of glutamate NMDA and non-NMDA antagonists, AP-5 and CNQX, after application of capsaicin (Fig. 5, D–F). The spontaneous discharge activities of the PAG neurons were slightly decreased after perfusion of 20 μM of CNQX and 50 μM of AP-5 (4.24 ± 0.61 vs. 3.46 ± 0.67 Hz, P > 0.05). Subsequent application of 1 μM of capsaicin failed to increase the spontaneous neuronal activities of in the presence of CNQX and AP-5.

**DISCUSSION**

In this study, in vitro PAG slice preparation allowed us to determine regulatory actions of TRPV1 activation on excitatory glutamatergic and inhibitory GABAergic synaptic activity in the dl-PAG (Kabashima et al. 1997; Ozaki et al. 2000; Sulzer and Pothos 2000). Our results have shown that bath application of capsaicin significantly increased the frequency of mEPSCs of the dl-PAG neurons but had no distinct effect on the amplitude of mEPSCs (Fig. 1). These data suggest that TRPV1 activation increased the synaptic glutamate release in the PAG, and the site of action was likely at the presynaptic
glutamatergic terminals (Sulzer and Pothos 2000). Furthermore, the effect of capsaicin on mEPSCs of the dl-PAG was completely eliminated by the specific TRPV1 receptor antagonist, i-RTX (Fig. 3), suggesting that mEPSC enhancement of capsaicin was caused by TRPV1 receptors. In addition, i-RTX alone decreased the frequency of mEPSCs of the dl-PAG neurons (Fig. 2). This indicates that endogenous TRPV1 receptors tonically influenced glutamatergic inputs to the dl-PAG neurons.

In contrast to its actions on glutamatergic mEPSCs, capsaicin had no distinct effect on the frequency and amplitude of GABAergic mIPSCs recorded from the dl-PAG neurons (Fig. 4). This suggests the lack of TRPV1 effect on the synaptic GABAergic terminals. The similar action of TRPV1 on glutamatergic and GABAergic neurotransmission has been reported in the substantia nigra, locus coeruleus, and paraventricular nucleus in perfused brain slices (Li et al. 2004; Marinelli et al. 2002, 2003).

In addition, in this report, we also found that capsaicin significantly increased the discharge activity of the dl-PAG neurons (Fig. 5). This effect was abolished after blockade of NMDA and non-NMDA receptors with the prior application of AP-5 and CNQX (Fig. 5), indicating that TRPV1 activation elevated the activity of the dl-PAG neurons through augmentation of the excitatory glutamatergic synaptic inputs. It should be noted that AP-5 and CNQX did not significantly alter the firing activity in the dl-PAG neurons in this experiment. This suggests that the tonic glutamateric inputs might not be sufficient to alter the neuronal activity of the dl-PAG.

A prior study has shown that capsaicin injected into the PAG produces antinociception, and the effect is prevented by pretreatment with a TRPV1 antagonist, capsazepine (McGaraughty et al. 2003; Palazzo et al. 2002). TRPV1 receptor activation in the dl-PAG neurons affects neuronal activity of the rostral ventromedial medulla and contributes to descending modulation of nociception (McGaraughty et al. 2003).
The antinociceptive effect induced by capsaicin is also attenuated after a blockade of glutamate receptors within the PAG (Palazzo et al. 2002). Speculatively, the glutamate release is increased after TRPV1 activation and postsynaptic glutamate receptors are activated. Activation of glutamate receptors in the PAG has been reported to produce analgesia (Maione et al. 1998, 2000). The results from this study provide, for the first time, electrophysiological evidence that 1) TRPV1 activation within the dl-PAG neurons increases the spontaneous firing rate of the PAG cells, and 2) the excitatory action of TRPV1 on the neuronal activity is likely to be mediated through the synaptic glutamate release and activation of glutamate receptors.

A large body of evidence has suggested that the dl-PAG has descending neuronal projections to crucial cardiovascular areas in the rostral ventrolateral medulla and plays an important role in regulating cardiovascular activity (Tjen-A-Looi et al. 2006; van Bockstaele et al. 1991; van der Plas et al. 1995; Verberne and Guyenet 1992). Stimulation of the dl-PAG elicits potent increases in arterial blood pressure, heart rate, and sympathetic nerve discharge (Bandler et al. 1991). Studies have further shown that glutamate and glutamate-positive terminals appear throughout the PAG (Azkue et al. 1998). Glutamate and its receptors play a role in cardiovascular regulation in the PAG (Hall and Behbehani 1998; Maione et al. 1994). For example, increased glutamate in the dl-PAG elevates blood pressure, and the effect is significantly reduced by a pretreatment with AP-5 (Maione et al. 1994). The PAG is also involved in integrating cardiovascular responses to activation of somatic afferent and arterial baroreflex inputs (Li 2004; Li and Mitchell 2000, 2003). It has been reported that glutamate is accumulated in synaptic terminals ascending from the spinal cord to the PAG (Azkue et al. 1998). Furthermore, activation of somatic afferent inputs increases glutamate concentration in the dl-PAG (Li 2004).

**FIG. 4.** Effect of TRPV1 activation on GABAergic mIPSCs of the dl-PAG neurons (n = 8). A: representative tracings from a dl-PAG neuron show frequency of spontaneous mIPSCs was not altered by bath application of 1 μM capsaicin and that mIPSCs were completely abolished in presence of 20 μM of bicuculline, a GABA<sub>A</sub> receptors antagonist. B: decay time constant of mIPSCs was similar during control and during capsaicin application. C and D: cumulative probability analysis shows that capsaicin did not alter distribution patterns of interevent interval and amplitude of mIPSC. E and F: average data show capsaicin had no effect on frequency and amplitude of mIPSC of dl-PAG neurons.
This study suggests that TRPV1 activation enhances excitatory glutamatergic synaptic activity in the dl-PAG. Thus it is reasoned that TRPV1 receptors within the PAG may play a role in modulating cardiovascular responses during activation of somatic sensory afferents through glutamate release.

A significant finding from this study is that i-RTX decreased the mEPSCs of the dl-PAG neurons. The result suggests that TRPV1 receptors exert a tonic influence on glutamate release within the dl-PAG through endogenous factors. A number of endogenous capsaicin-like substances (i.e., proton, anandamide, and 12-hydroperoxyeicosatetraenoic acid) have been identified to activate and/or potentiate the activity of TRPV1 receptors (Hwang et al. 2000; Ryu et al. 2003; Zygmunt et al. 1999). For instance, evidence has shown that electrical stimulation of the dorsal regions of the PAG produces analgesia accompanied by a marked increase in the release of anandamide in the PAG (Walker et al. 1999). In the PAG, enhanced level of endogenous anandamide by inhibition of fatty acid amide hydrolase induces both CB1- and TRPV1-mediated analgesia (Maione et al. 2006). However, it is noted that a higher dose of anandamide is necessary to activate TRPV1 receptors compared with activation of CB1 receptors (Luo et al. 2002; Morisset and Urban 2001). A precise mechanism by which TRPV1 receptors are activated by endogenous factors needs to be determined.

It has generally been accepted that the glutamate increase by TRPV1 activation results from an increase in intraterminal Ca\(^{2+}\) concentration through Ca\(^{2+}\) influx into the nerve terminals, because the effect can be abolished after removal of extracellular Ca\(^{2+}\) (Li et al. 2004; Marinelli et al. 2002). Thus we speculate that the increased frequency of mEPSCs of the dl-PAG by capsaicin was caused by enhanced Ca\(^{2+}\) concentration in the nerve terminals in this study.

Finally, previous studies have shown that there exist TRPV1 receptors on presynaptic nerve terminals in the superficial
dorsal horn of the spinal cord and hypothalamus (Guo et al. 1999; Li et al. 2004). Whether TRPV1 receptors are localized on glutamatergic terminals of presynaptic sites has not, to our knowledge, been reported, although TRPV1 immunoreactivity and mRNA have been identified in the dl-PAG (McGaraughty et al. 2003; Roberts et al. 2004). Our data from this experiment showed that activation of TRPV1 receptors on presynaptic site increased glutamate release. This provides electrophysiological evidence that TRPV1 is likely to locate on presynaptic nerve terminals in the dl-PAG. It has been reported that there are neurons with TRPV1 immunostaining in the dl-PAG, and capsaicin injected into the dl-PAG induces analgesia (McGaraughty et al. 2003). Thus TRPV1 receptors speculatively exist on postsynaptic membrane. In this study, our purpose was to determine if activation of TRPV1 receptors would release glutamate from presynaptic nerve terminals. The data from this experiment support our hypothesis. Furthermore, our results show that the elevated glutamate stimulated glutamate NMDA and non-NMDA receptors on the dl-PAG neurons and increased neuronal activity. Glutamate appears within the dl-PAG as a major excitatory neurotransmitter (Beitz and Williams 1991). We believe that neurons tested in this experiment were likely glutamatergic. Nonetheless, those neurons had NMDA and non-NMDA receptors and were responsive to glutamate. The responsiveness of postsynaptic TRPV1 receptors in the dl-PAG to capsaicin requires additional experiments to be determined.

In summary, capsaicin significantly increases the frequency of glutamatergic mEPSCs but not GABAergic mIPSCs of the dl-PAG neurons through activation of TRPV1 receptors. The increased glutamatergic synaptic inputs augment the discharge of PAG neurons because this effect by capsaicin is blocked by glutamate NMDA and non-NMDA receptor antagonists. Our data suggest a mechanism by which TRPV1 modulates neuronal activity in the dl-PAG through synaptic glutamate. This study provides new information that the dl-PAG could be an important supraspinal site to be involved in TRPV1-related modulation of physiological functions.

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REFERENCES


Maione S, Bisogno T, de Novellis V, Palazzo E, Cristino L, Valenti M, Petrosino S, Guglielmotti V, Rossi F, Di Marzo V. Elevation of endocannabinoid levels in the ventrolateral periaqueductal gray through inhibition of fatty acid amide hydrolase affects descending nociceptive pathways via both cannabinoid receptor type 1 and transient receptor potential vanilloid type-1 receptors. J Pharmacol Exp Ther 316: 969—982, 2006.


