α₂-Adrenoceptors Modulate NMDA-Evoked Responses of Neurons in Superficial and Deeper Dorsal Horn of the Medulla

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Kai-Ming Zhang, Xiao-Min Wang, Angela M. Peterson, Wen-Yan Chen, and Sukhibir S. Mokha. α₂-Adrenoceptors modulate NMDA-evoked responses of neurons in the superficial and deeper dorsal horn of the medulla. J. Neurophysiol. 80: 2210–2214, 1998. Extracellular single unit recordings were made from neurons in the superficial and deeper dorsal horn of the medulla (trigeminal nucleus caudalis) in 21 male rats anesthetized with urethan. NMDA produced an antagonist-reversible excitation of 46 nociceptive as well as nonnociceptive neurons. Microiontophoretic application of a preferential α₂-adrenoceptor (α₂AR) agonist, (±)-2,6-dichloro-2-imidazoline hydrochloride (clonidine), reduced the NMDA-evoked responses of 86% (6/7) of nociceptive-specific (NS) neurons, 82% (9/11) of wide dynamic range (WDR) neurons, and 67% (4/6) of low-threshold (LT) neurons in the superficial dorsal horn. In the deeper dorsal horn, clonidine inhibited the NMDA-evoked responses of 94% (16/17) of NS and WDR neurons and 60% (3/5) of LT neurons. Clonidine facilitated the NMDA-evoked responses in 14% (1/7) of NS, 9% (1/11) of WDR, and 33% (2/6) of LT neurons in the superficial dorsal horn. Idazoxan, an α₂AR antagonist, reversed the inhibitory effect of clonidine in 90% (9/10) of neurons, whereas prazosin, an α₁-adrenoceptor antagonist with affinity for α₂AR, and α₂C-AR, were ineffective. We suggest that activation of α₂ARs produces a predominantly inhibitory modulation of the NMDA-evoked responses of nociceptive neurons in the medullary dorsal horn.

INTRODUCTION

The dorsal horn of the medulla (trigeminal nucleus caudalis) is an important relay for nociceptive and thermosensory information from the orofacial region (reviewed in Light 1992; Sessle 1987). Glutamate, a putative excitatory neurotransmitter (Watkins and Evans 1981), is present in trigeminal primary afferent fibers (Clements and Beitz 1991). Glutamate produces its actions by acting on modulate N-methyl-D-aspartic acid (NMDA), non-NMDA ionotropic [(±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)/Kainate], and metabotropic receptors in the spinal cord. The superficial dorsal horn of the medulla contains high densities of NMDA (Tallaksen-Greene et al. 1992) and non-NMDA ionotropic (Kondo et al. 1995; Tallaksen-Greene et al. 1992) and metabotropic (Tallaksen-Greene et al. 1992) receptors. NMDA receptors are involved in mediating nociceptive neurotransmission and neuroplasticity (hyperalgesia) in the dorsal horn (reviewed in Wilcox 1993). (±)-2,6-Dichloro-2-imidazoline hydrochloride (clonidine), an α₂-adrenoceptor (α₂AR) agonist, is reported to be effective in treating neuropathic pain in humans (Eisenach et al. 1995; Tamsen and Gordh 1984) and in attenuating hyperalgesia induced by nerve injury or inflammation in animal studies (Kayser et al. 1995; Post et al. 1987; Puke et al. 1994; Solomon et al. 1989; Xu et al. 1992). Autoradiographic studies demonstrated the presence of α₂ARs in the dorsal horn, particularly in the superficial dorsal horn of the spinal cord (Roudet et al. 1994; Unnerstall et al. 1984; Sullivan et al. 1987) and the medulla (Unnerstall et al. 1984). Microiontophoretically applied norepinephrine (NE) is reported to selectively inhibit nociceptive responses of deep dorsal horn neurons (Belcher et al. 1978; Fleetwood-Walker et al. 1985; Headley et al. 1978; reviewed in Jones 1991). Consistent with data from behavioral studies (reviewed in Jones 1991), the selective inhibitory effect of NE was suggested to involve α₂ARs (Davies and Quinlan 1985; Fleetwood-Walker et al. 1985). However, NE was also reported to produce nonselective inhibitory modulation of primate spinothalamic tract neurons (Willcockson et al. 1984) and interneurons in the superficial dorsal horn of the spinal cord in the rat (Howe and Ziegglänsberger 1987; Todd and Millar 1983). Although behavioral studies in mice indicated the importance of adrenoceptors in modulating biting and scratching behavior induced by intrathecal NMDA (Aannonsen and Wilcox 1987), no previous electrophysiological studies investigated the role of α₂-noradrenergic receptors in modulating the NMDA-evoked responses of physiologically characterized nociceptive neurons in the brain. This study was therefore designed to investigate the α₂AR-mediated modulation of NMDA-evoked responses of neurons in the superficial and deeper dorsal horn of the medulla.

METHODS

This study was based on data obtained from 21 male Sprague-Dawley rats (230–360 g) anesthetized with urethan (1.5 g/kg ip). Methods for animal preparation, monitoring the level of anesthesia, neuronal recording, and characterization of trigeminal neurons were similar to those described previously (Mokha 1992, 1993; Zhang et al. 1996). The rat’s face was carefully shaved for adequate stimulation and mapping of receptive fields. To improve the stability of extracellular recordings from the superficial dorsal horn of the medulla, a small metallic brass plate was fixed to the skull, and the head was ventroflexed. The dorsal surface of the medulla was exposed and covered with warm agar (3–4% agar in normal saline at 40°C). All protocols were approved by the Institutional Animal Care and Use Committee of Meharry Medical College.

Recordings were made with the central barrel (2 M NaCl or 2% pontamine sky blue in 0.5 M sodium acetate) of a seven-barrel
microelectrode (tip diameter, 4–10 μm; impedance, 2–15 MΩ, Medical Systems). One barrel of the seven-barreled glass microelectrode contained 2 M NaCl for automatic current balancing, and the remaining barrels were filled with drug solutions for microiontophoretic application with the Neurophore BH2 system (Medical Systems). The solutions were either made up fresh before an experiment or thawed from a frozen state. The drug barrels contained solutions of the following drugs: NMDA (50 mM in 150 mM NaCl, pH 8.2), dl-2-amino-5-phosphonovaleric acid (AP-5, 50 mM in 150 mM NaCl, pH 8.0), clonidine (100 mM in double-distilled water, pH 4.5), idazoxan hydrochloride (100 mM in double-distilled water, pH 4.5), and (1-[4-amino-6,7-dimethoxy-2-quinozalinyl]-4-[2-furanyl-carbonyl]piperazine) hydrochloride (prazosin, 1.5 mM in double-distilled water, pH 4.5). All chemicals were obtained from Sigma Chemical. All drugs were ejected with positive current except NMDA and AP-5, which were ejected with negative current. Retaining currents were adjusted between 2 and 10 nA to prevent drug diffusion. Application of microiontophoretic currents ≤ 140 nA through a vehicle (150 mM NaCl, pH 4.5 and/or pH 8.0) containing barrel did not alter significantly the responses of neurons.

Single unit extracellular recordings were made from neurons in the superficial (laminae I and II) and deeper dorsal horn of the medulla. The procedures for recording from neurons in the superficial versus the deeper dorsal horn and the responses and receptive fields of neurons were described and discussed previously (Mokha 1992, 1993; Zhang et al. 1996). In addition, recording sites were also marked by electrophoretic injection of pontamine sky blue (10 μA for 3 min) from the central recording barrel or one of the outer barrels as illustrated in Fig. 2B. Action potentials were recorded and amplified with conventional means and were continuously monitored on an oscilloscope to allow for discrimination of single units. Application of NMDA at low current was used to search for single units. Brush, pressure, pinch, squeeze, and radiant heat stimuli were used to characterize the responses of neurons to receptive field stimulation. Neurons were classified as selectively nociceptive [nociceptive specific (NS), responding only to noxious stimuli], multireceptive [wide dynamic range (WDR), responding to both noxious and innocuous stimuli], and nonnociceptive [low threshold (LT), responding only to innocuous stimuli] as described previously (Mokha 1992, 1993; Zhang et al. 1996). Neural activity was fed to a window discriminator and to a microcomputer to allow analysis of the data on-line and off-line (MI² software). The histogram data files were transferred into ASCII files for further analysis with Microsoft Excel, SigmaStat, and SigmaPlot for Windows. The number of spikes was counted before, during, and after drug ejection. Stable control responses evoked by cyclical administration of NMDA (5–10 s on, 5–50 s off) were compared with responses during drug application. The effect of a test drug was defined as inhibitory or excitatory only when the NMDA-evoked responses differed from the mean of the control response by ≥ 2 SD in the same direction. The time to one-half recovery (t_{1/2}) after the termination of the drug application was calculated. Grouped data are presented as the mean ± SE. Statistical analysis was performed by using paired t-test and one-way analysis of variance followed by Student-Newman-Keuls test. A probability level of P < 0.05 was considered significant.

RESULTS

Forty-six neurons were recorded extracellularly from the superficial (7 NS, 11 WDR, and 6 LT) and deeper (1 NS, 16 WDR, and 5 LT) dorsal horn. All neurons had an ipsilateral receptive field, and spontaneous activity was essentially absent. Responses evoked by NMDA were related to the intensity of the microiontophoretic current and displayed a short latency (< 5 s). NMDA-evoked firing outlasted the injection period by 3–10 s. AP-5 (10–30 nA), a selective NMDA receptor antagonist, blocked the NMDA-evoked responses in 100% of neurons (6/6) tested.

Clonidine (30 ± 3 nA, n = 41) reduced the NMDA-evoked responses of 86% (6/7) of NS neurons, 82% (9/11) of WDR neurons, and 67% (4/6) of LT neurons in the superficial dorsal horn (Figs. 1 and 2). It produced a peak inhibitory effect of 80 ± 4% (n = 19, P < 0.0001). Clonidine facilitated (153 ± 98%, n = 4, P < 0.05) the NMDA-evoked responses of 14% (1/7) of NS neurons, 9% (1/11) of WDR neurons, and 33% (2/6) of LT neurons in the superficial dorsal horn. In the deeper dorsal horn, clonidine reduced the NMDA-evoked responses of 94% (16/17) of NS and WDR neurons and 60% (3/5) of LT neurons. It produced a peak inhibitory effect of 71 ± 5% (n = 19, P < 0.0001). Biphasic effects were observed in 9% (1/11) of WDR neurons in the superficial dorsal horn and 40% (2/5) of LT neurons in the deeper dorsal horn. The inhibitory effect of clonidine exhibited longer lasting (t_{1/2} = 2.262 ± 285 s, n = 5, range 1.710–3.345 s) or shorter lasting (t_{1/2} = 255 ± 48 s, n = 19, range 35–800 s) time course. There was no significant correlation (P > 0.05, n = 24) between the time course and the dosage of clonidine (iontophoretic current × duration of application). Idazoxan, administered at currents that had no effect (P > 0.05, n = 10) when given alone, reduced the magnitude and/or the time course of the inhibitory effect of clonidine in 9 of 10 neurons (Fig. 1C). Idazoxan produced a significant reduction of 62 ± 10% in the time course of the inhibitory effect of clonidine (P < 0.05, n = 10, paired t-test). Significant reduction in the inhibitory effect of clonidine by idazoxan was also revealed in the combined data on the time course and the peak inhibitory effect of clonidine (P < 0.05, n = 10, paired t-test). Idazoxan reduced the peak inhibitory effect of clonidine by 69 ± 15% in 6 of 10 neurons. Prazosin, administered at currents that had no effect when given alone, did not alter the effect of clonidine in two of two neurons tested (Fig. 1B).

DISCUSSION

This is the first in vivo electrophysiological demonstration of the α₂-AR-mediated modulation of the NMDA-evoked responses of physiologically characterized neurons in the brain. The results presented here demonstrate that iontophoretically applied clonidine, an α₂-AR agonist, produces a predominantly inhibitory modulation of the NMDA-evoked responses of nociceptive and nonnociceptive trigeminal neurons. This is consistent with the previously reported observations demonstrating the predominantly inhibitory modulation of glutamate-evoked responses by microiontophoretically applied NE in the dorsal horn of the spinal cord (Howe and Ziegglansberger 1987; Willcockson et al. 1984). Inhibitory modulation of the NMDA-evoked responses of nociceptive neurons may contribute to the antinociceptive actions of clonidine observed in human (Eisenach et al. 1995; Tamsen and Gordh 1984) and animal studies (Kayser et al. 1995; Post et al. 1987; Solomon et al. 1989; Xu et al. 1992). NMDA receptor activation is intimately involved in mediating nociceptive neurotransmission and neural plasticity (hyperalgesia) after nerve injury or inflammation (reviewed in Wilcox 1993). Clonidine also facilitated the
FIG. 1. α₂-adrenoceptor activation mediates the inhibitory effect of clonidine on the N-methyl-D-aspartic acid (NMDA)-evoked responses of a wide dynamic range (WDR, excited by brush and pinch applied to the cutaneous receptive field) neuron in the superficial dorsal horn of the medulla. A: (2-[2,6-Dichloroaniline]-2-imidazoline) hydrochloride (clonidine) reduced the NMDA-evoked responses. B and C: inhibitory effect of clonidine was antagonized by idazoxan and not by prazosin. Neither prazosin nor idazoxan administered alone affected the NMDA-evoked responses.

NMDA-evoked responses of some neurons in the superficial dorsal horn. This is consistent with previously reported observations on the glutamate-evoked responses of neurons in the superficial dorsal horn of the spinal cord (Millar and Williams 1989; Millar et al. 1993). Neurons facilitated by clonidine may represent a population of small inhibitory interneurons that can gate the transmission of nociceptive signals. The population of neurons facilitated by clonidine in our investigation is much smaller than that reported previously (Millar and Williams 1989; Millar et al. 1993). Several factors, such as the characteristics of recording electrodes and the iontophoretic paradigms, may contribute to this discrepancy. Although LT neurons are encountered less frequently in the superficial dorsal horn, nevertheless these are reported to be present in the superficial dorsal horn. Some of the superficial LT neurons in our study may include recordings made from neurons in the deeper dorsal horn because histological localization was not obtained for all LT neurons.

The modulation of NMDA-evoked responses by clonidine was significantly reduced by idazoxan, α₂AR antagonist. The α₂ARs exist in three pharmacologically distinguishable receptor subtypes (α₂A, α₂B, and α₂C) (Bylund et al. 1994). Prazosin, which has high affinity for the α₁, α₂B, and α₂C adrenoceptors, did not alter the effects of clonidine on the NMDA-evoked responses. It is therefore likely that the α₂A subtype of α₂ARs is involved in modulating the NMDA-evoked responses of neurons in the medullary dorsal horn. This is consistent with the previous anatomic findings indicating that the α₂AAR appears to be the dominant receptor subtype present in neurons in the spinal dorsal horn (Nicholas et al. 1993; reviewed in Nicholas et al. 1996; Uhlen and Wikberg 1991). In the medullary dorsal horn, α₂AARs have been shown to be present predominantly in neurons in the superficial dorsal horn (Nicholas et al. 1993; Talley et al. 1996), whereas the α₂C ARs are primarily located on neurons in the descending tract of the trigeminal nerve (Rosin et al. 1996). Receptor subtype dominance appears to be species specific because in the human spinal cord the α₂B appears to be the dominant adrenoceptor (Smith et al. 1995). Evidence obtained recently in mouse strains with either a point mutation in the α₂A AR gene or null mutation in the α₂B and α₂C AR genes demonstrated clearly that agonists at the α₂ARs produce antinociceptive action by acting primarily at the α₂A ARs (Hunter et al. 1997; Lakhlani et al. 1997; Stone et al. 1997).

Agonists at the α₂ARs which are imidazoline (clonidine, idazoxan) or imidazole (dexmedetomidine) also display...
moderate to high affinity binding to imidazole or imidazoline binding sites (reviewed in Codd et al. 1995). However, the antinociceptive action of clonidine in the spinal cord (Monroe et al. 1995) or PAG stimulation (Peng et al. 1996) was reported to be mediated by its action at the α2ARs rather than on imidazoline receptors. Lack of analgesic effect of α2 agonists in mice with a point mutation in the α2AR gene strongly argues against the role of imidazoline receptors in contributing to the analgesic effects of α2 agonists (Hunter et al. 1997; Lakhiani et al. 1997; Stone et al. 1997).

α2ARs are G-protein (G_16/G_9) coupled receptors and produce their actions by reducing the calcium conductance and enhancing the potassium conductance. Catecholamine terminals have been shown to make postsynaptic contacts with spinothalamic tract neurons (Westlund et al. 1990). Activations of α2ARs increases potassium conductance in dorsal horn neurons, which produces hyperpolarization, decreases excitability, and contributes to analgesia (Grudt et al. 1995; North and Yoshimura 1984; O'can and Baeysen 1993). Similar postsynaptic hyperpolarization induced by increased potassium conductance might contribute to the inhibitory modulation of NMDA-evoked responses observed in this investigation. This suggestion is consistent with previous observations reporting a nonselective inhibitory action of NE and clonidine in the dorsal horn (Höwe and Zieglgänsberger 1987; Peng et al. 1996; Willcockson et al. 1984). NE acting at α1ARs was reported to produce γ-aminobutyric acid (GABA)-mediated inhibitory postsynaptic potentials in dorsal horn neurons (Grudt et al. 1995); nevertheless it is possible that clonidine-induced facilitations might be produced by postsynaptic inhibition of neurons containing GABA or glycine. Although direct axo-axonic contacts of noradrenergic terminals were not demonstrated on primary afferent fiber terminals (Doyle and Maxwell 1991a,b), electrophysiological studies suggest the involvement of presynaptic mechanisms in mediating the actions of NE and α2 agonists (Jeflinja et al. 1981; Travagl and Williams 1996). Further, mRNA for α2ARs (α2A, α2B, and α2C) is present in dorsal root ganglion cells (Gold et al. 1997; reviewed in Nicholas et al. 1996), and ganglionectomies reduce the binding of α2ARs in the spinal cord. Activation of α2ARs in dorsal root ganglion cells inhibits the N-type calcium channels (Cox and Dunlap 1992), which will be expected to reduce neurotransmitter release. Indeed, α2 agonists are known to inhibit the stimuli-evoked release of substance P (Kuraishi et al. 1985; Pang and Vasko 1986) and glutamate (Kamisaki et al. 1995; Millar et al. 1995) in the spinal cord. Therefore antinociceptive actions of α2 agonists presumably involve both presynaptic and postsynaptic mechanisms.

The long-lasting (t_{1/2} = 2.262 ± 285 s, n = 5, range 1,710–3,345) effects of clonidine observed in this investigation are consistent with previous observations in the spinal cord and the trigeminal system (Cahusac et al. 1995; Millar et al. 1993; Zhao and Duggan 1988). These longer-lasting effects presumably involve second messenger systems. Although the α2ARs are known to reduce the production of cAMP in the spinal cord, raising levels of cAMP with forskolin did not alter the antinociceptive action of α2 agonists (Uhlen et al. 1990).

Predominantly inhibitory modulation of NMDA-evoked responses in the medullary dorsal horn may contribute to the antinociceptive effects of clonidine observed in human and animal studies.

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REFERENCES


HUNTER, J. C., FONTANA, D. J., HEDLEY, L. R., JASPER, J. R., LEWIS, R., LINDE, R. E., SECCHI, R., SUTTON, J., AND EALEN, R. M. Assessment of the role of α2-adrenoceptor subtypes in the antinociceptive, sedative and...


