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Central alpha-adrenoceptors contribute to mustard oil-induced central sensitization in the rat medullary dorsal horn

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Abstract

Our previous studies have demonstrated that application of the inflammatory irritant mustard oil (MO) to the tooth pulp produces trigeminal central sensitization that includes increases in mechanoreceptive field size and responses to noxious stimuli and decrease in activation threshold in brainstem nociceptive neurons of trigeminal subnucleus caudalis (the medullary dorsal horn, MDH). The aim of the present study was to test if central noradrenergic processes are involved in the central sensitization of MDH neurons and if α1-adrenoceptors or α2-adrenoceptors or both are involved. In urethane/α-chloralose anesthetized rats, the activity of extracellularly recorded and functionally identified single nociceptive neurons in the MDH was studied. Continuous intrathecal (i.t.) superfusion of the adrenergic modulator guanethidine and α-adrenoceptor blocker phentolamine or selective α1-adrenoceptor antagonist prazosin over the medulla strongly attenuated all three MO-induced parameters of central sensitization in the MDH nociceptive neurons, compared to phosphate-buffered saline (as vehicle control). In contrast, i.t. superfusion of the selective α2-adrenoceptor antagonist yohimbine had little effect on the mechanoreceptive field expansion and the decreased mechanical activation threshold, and indeed facilitated responses to noxious stimuli of sensitized nociceptive neurons. Superfusion of each of the four chemicals alone did not affect baseline nociceptive neuronal properties. These findings provide the first documentation of the involvement of central noradrenergic processes in MDH in the development of the central sensitization, and that α1- and α2-adrenoceptors may be differentially involved.

Introduction

Central sensitization is a crucial mechanism underlying the increased excitability of central nociceptive pathways following peripheral tissue injury and inflammation, and has been implicated in the development and maintenance of persistent pain (Dubner, 2005; Sessle, 2005, 2011; Woolf and Salter, 2006; Cao and Zhang, 2008; Nakagawa and Kaneko, 2010; Chiang et al., 2011). In the trigeminal system, we have demonstrated that application of the small-fiber excitant and inflammatory irritant mustard oil (MO) to the rat tooth pulp

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produces central sensitization in brainstem nociceptive neurons of trigeminal subnucleus caudalis (the medullary dorsal horn, MDH) that is glutamatergic and purinergic receptor-dependent and modulated by glial cell mechanisms (Chiang et al., 1998, 2005b, 2007, 2011; Hu et al., 2002; Xie et al., 2007; Itoh et al., 2011).

There are also data indicating an important role for central noradrenaline (NA) and α2-adrenoceptors in modulating dorsal horn neuronal activity. NA and α2-adrenoceptor agonists inhibit substantia gelatinosa neurons in the spinal dorsal horn through α2-adrenoceptors (Wolff et al., 2007; Roh et al., 2008; Ishii et al., 2008), and earlier experiments with intrathecally (i.t.) administered NA agonists or antagonists have demonstrated that NA plays a role in the modulation of nociceptive transmission, morphine analgesia and neuropathic pain (Stone et al., 1997; Pan et al., 2002; Kawasaki et al., 2003; Obata et al., 2005; Takeda et al., 2006; Hayashida et al., 2008; for review, see Pertovaara 2006; Taylor 2009). Moreover, a permanent reduction in the NA innervation of the rat spinal cord leads to a prolonged decrease in nociceptive threshold (Jasmin et al., 2003). However, some recent studies have reported that depletion of NA inhibits electrically-evoked pain in the skin of the human forearm (Drummond, 2008), that activation of α1- adrenoceptors augments thermal hyperalgesia in mildly burnt skin in humans (Drummond 2009) and that guanethidine applied subcutaneously for 3 days reduces the behavioral nociceptive responses induced by injection of formalin into the upper lip of the rat (Chichorro et al., 2004). So far, no studies have tested if NA modulates central sensitization in functionally identified spinal/medullary dorsal horn nociceptive neurons. Thus, the aim of the present study was to test if central noradrenergic processes are involved in central sensitization of MDH neurons and if α1-adrenoceptors or α2-adrenoceptors or both are involved. Preliminary data have been reported in abstract form (Chiang et al., 2005a, 2010).

Experimental Procedures

Animals

The experiments were performed in 36 male Sprague-Dawley adult rats. The methods used for animal preparation, stimulation, and neuronal recording and classification were similar to those described previously in detail (Chiang et al., 1998, 2007; Hu et al., 2002) and so will only be briefly outlined here. The animals were anesthetized by a single intraperitoneal injection of a mixture of α-chloralose (50 mg/kg) and urethane (1 g/kg). Then a tracheal cannula was inserted and the left external jugular vein was cannulated for intravenous (i.v.) injection of chemicals. To expose the pulp of the right maxillary first molar, an occlusal cavity was prepared with a dental drill (Rotex™ 780) and immediately filled with a small piece of cotton pellet soaked with normal saline. After the rat was placed in a stereotaxic apparatus, the caudal medulla was surgically exposed and the overlying dura and subarachnoid membrane were removed. Just before the recording session, a supplemental dose of urethane (200–300 mg/kg, i.v.) was administered and the rat was then immobilized with i.v. pancuronium bromide [initial dose, 0.2 ml of 2 mg/ml solution, followed by a continuous i.v. infusion of a mixture of 70% urethane solution (0.2 g/ml) and 30 % pancuronium solution (1 mg/ml) at a rate of 0.3–0.4 ml/h] and artificially ventilated throughout the whole experimental period. A deep level of anesthesia was confirmed periodically by the lack of spontaneous movements and responses to pinching the paw when pancuronium-induced muscle paralysis was allowed to wear off. Heart rate, percentage expired CO_2, and rectal temperature were constantly monitored and maintained at physiological levels of 330–430 beats/min, 3.5–4.5 %, and 37–37.5 °C, respectively. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada).

Chemicals

The chemicals used included MO (allyl isothiocynate, 95%; Sigma-Aldrich, St. Louis, MO), the adrenergic modulator guanethidine sulphate (2:1) (Sigma-Aldrich Canada, Oakville, ON), the α-adrenoceptor blocker phentolamine HCl (Sigma-Aldrich Canada, Oakville, ON), the α1-adrenoceptor antagonist prazosin HCl (Sigma-Aldrich Canada, Oakville, ON), the α2-adrenoceptor antagonist yohimbine HCl (Sigma-Aldrich Canada, Oakville, ON), and phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO) as vehicle. All chemicals except MO were freshly dissolved in PBS.

Superfusion of chemicals

A syringe filled with either PBS or one of the other chemicals was mounted on a Harvard digitally controlled infusion pump (Harvard apparatus Inc., Mass. U.S.A.) and connected with polyethylene tubing (PE50) for the continuous i.t. superfusion. The tip of the tubing was positioned at least 0.5 mm rostral to the microelectrode penetration site. During superfusion at a rate of 0.6 ml/h maintained continuously, the superfusing fluid flowed downstream along the caudal medulla and was continuously drained off, because the animal's head (held in a stereotaxic apparatus) was always higher than its body.

Electrophysiological experiments

Recording and stimulation procedures—Single unit neuronal activity was recorded extracellularly by means of an epoxy resin-coated tungsten microelectrode (5–15 MΩ, FHC, ME, U.S.A.). As the microelectrode was advanced with a rostral inclination of 23° into the right caudal medulla, 1.4–2.0 mm lateral to the midline and 1.5–2.0 mm behind the obex, natural stimuli (see below) were applied to the orofacial tissues to search for MDH nociceptive neurons receiving an orofacial sensory input. Neuronal activity was amplified, displayed on oscilloscopes and also led to a window discriminator connected to an A/D converter (CED 1401 plus, Cambridge Electronic Design, UK) and a personal computer. Data were analyzed off-line with Spike 2 software (Cambridge Electronic Design, UK). A wide range of mechanical (brush, pressure and pinch), and noxious thermal (radiant heat, 51–53 °C) stimuli was applied to the orofacial region to identify nociceptive-specific (NS) neurons (Chiang et al., 1998, 2005b, 2007). Neuronal spontaneous activity was determined (in Hz) over an initial 1 min recording period. It was noted that under a moderate dose of urethane (continuous infusion), few NS neurons had baseline spontaneous activity. As outlined in our previous studies (Chiang et al., 1998, 2005b, 2007), the cutaneous orofacial mechanoreceptive field(RF) of each NS neuron was determined. A burst response consisting of at least 2 spikes during each stimulus trial was accepted as the criterion for the RF boundary of the neuron tested (Yu et al., 1993). Noxious stimulation was used sparingly so as to avoid damage to the skin and peripheral sensitization. The activation threshold to a mechanical stimulus applied to the orofacial RF was assessed by a pair of force-monitoring forceps (with an attached strain gauge that monitored force levels up to 600 g/mm^2). The gradual increase in the mechanical force and the responses of the tested neuron were monitored and recorded simultaneously by the use of the Spike 2 program (Cambridge Electronic Design, UK). The NS neuronal responses to graded mechanical pinch stimuli delivered with a force-monitoring forceps (25 g, 50 g, 75 g, 100 g and 200 g, were applied in ascending order, each for 5 s at an interval of >45 s) to the neuronal orofacial RF, as previously described (Chiang et al., 1998, 2005b, 2007, 2008; Xie et al., 2007). The pinch-evoked responses of a given neuron were assessed by summing the number of spikes evoked by each of these graded stimuli during application of the stimulus

Experimental paradigm

1. Guanethidine and phentolamine experiments—These experiments involved PBS (which served as vehicle control), phentolamine (300 μM) and guanethidine (100 μM) groups (n=6 in each group). In all three groups, after baseline values of NS neuronal properties including RF size, threshold and responses to pinch stimuli were obtained, PBS or phentolamine or guanethidine was superfused over the MDH at a rate of 0.6 ml/h; their doses were chosen based on those used in the literature (Levine et al., 1986; Sawynok and Reid, 1992; Lavand'homme et al., 1998) and on our preliminary experiments where we found that higher doses could affect baseline NS neuronal properties. After two assessments of neuronal properties were performed during 30 min (PBS and guanethidine groups) or 15 min (phentolamine group) i.t. superfusion, the saline-soaked cotton pellet in the exposed pulp cavity was carefully removed from the molar pulp cavity and replaced with a segment of dental paper point soaked with MO (0.2 μl). The cavity was promptly sealed with CAVIT (3M ESPE, Germany) in order to prevent MO leaking out of the tooth and to ensure the chemical's sustained action on pulp afferents. Three min after MO application, the neuronal properties were re-assessed at 10-min intervals throughout a 50-min observation period. The superfusion of chemical continued until the end of each experiment. Only one neuron was tested with a single dose of chemicals in an experiment in all three groups. Then, recording sites were marked by electrolytic lesions (anodal current of 8 μA for 13 s), and verified histologically as previously described (Chiang et al., 1998, 2005b).

2. Prazosin and yohimbine experiments—These experiments used PBS (as vehicle control), prazosin (10 μM) and yohimbine (10 nM) groups (n=6 in each group), and a similar protocol to the above experiments was followed. While the 10 μM dose of prazosin was chosen based on the literature (Burnett and Gebhart, 1991; Ouseph and Levine, 1995), the 10 nM dose of yohimbine was chosen based on our preliminary experiments in which higher doses (10, 1 or 0.1 μM) affected baseline neuronal activity (e.g., generated sustained spontaneous activity and persistent discharges of nociceptive neurons to mechanical stimuli). Continuous i.t. superfusion of prazosin or yohimbine started after baseline values of neuronal properties were obtained and 15 min later another assessment of neuronal properties was performed prior to MO application to the tooth pulp.

Statistical analyses

All values were presented as mean ± S.E. The values of the assessment after chemical (PBS, guanethidine, phentolamine, prazosin, and yohimbine) superfusion i.e., prior to MO application were defined as baseline (100%) and the normalized data were treated statistically. Differences between the baseline values and values at different time-points after MO application were treated by 1-way repeated-measures (RM) ANOVA followed by Dunnett's test. Differences between the PBS group and each chemical group were treated by 2-way ANOVA followed by Dunnett's test. The level of significance was set at $P<0.05$.

Results

A total of 36 functionally identified NS neurons was tested. The recording sites of all NS neurons were histologically verified and were located in or close to the deep laminae of MDH (see Figs. 1A and 3D).

MO-induced effects

In the guanethedine/phentolamine experiments, only 2 of the 18 NS neurons had baseline spontaneous activity. After MO application to the pulp, no spontaneous or evoked activity appeared in any neurons in the rats receiving the adrenergic modulator guanethidine (n=6) or the α-adrenoceptor blocker phentolamine (n=6); however, 2 of the NS neurons in the PBS

group (n=6) were excited for 3–5 min following MO application (Fig. 1B). In the α1-adrenoceptor antagonist prazosin and α2-adrenoceptor antagonist yohimbine experiments, none of the 18 NS neurons initially had spontaneous activity; however, after MO application 2 neurons in the PBS group and 3 neurons in the yohimbine group (both n=6) developed spontaneous activity (0.1–16 Hz) lasting for 15 to 50 min.

Central sensitization was readily induced by MO application to the pulp during PBS (vehicle control) superfusion over MDH in 2 series of experiments (Tables 1, 2): MO application significantly increased the RF size, decreased the mechanical activation threshold and increased the pinch-evoked responses during the 50-min observation period (P<0.05–0.001 in Table 1 and P<0.02–0.001 in Table 2, 1-way RM ANOVA; Figs. 2, 3). In the PBS group of the guanethidine/phentolamine experiments, the RF size was significantly different from the baseline value at all post-MO time-points as was the activation threshold at 8, 18 and 28 min and the responses to the graded mechanical stimuli at 8, and 18 min after MO application (P<0.05, Dunnett's test, see Fig. 2). Similarly, in the PBS group of the prazosin/yohimbine experiments, the RF size was also significantly different from the baseline value at 18, 28 and 38 post-MO time-points as was the activation threshold at 8, 18 and 28 min post-MO time-points and the responses to the graded mechanical stimuli at 18 and 28 min after MO application (P<0.05, Dunnett's test, see Fig. 3).

Effect of guanethidine on the MO-induced central sensitization

Continuous i.t. superfusion of guanethidine over MDH did not affect baseline nociceptive neuronal properties during the 30 min pretreatment period (P>0.05 for pinch RF size, activation threshold, and pinch-evoked responses, 1-way RM ANOVA, n=6). On the other hand, guanethidine superfusion blocked the MO-evoked central sensitization since there were no significant changes in RF size, mechanical activation threshold or pinch-evoked responses after MO application to the pulp (P>0.4, 0.2, and 0.1 respectively,1-way RM ANOVA; Fig. 2; Table 1). The MO-induced effects in this group were significantly less than those in the PBS group (P<0.05–0.001, 2-way ANOVA; Table 1). Post-hoc analyses indicated that there were significant differences in values at some post-MO time-points between these two groups (see Fig. 2).

Effect of phentolamine on the MO-induced central sensitization

Continuous i.t. superfusion of phentolamine over MDH did not affect baseline nociceptive neuronal properties during the 15 min pretreatment period (P>0.05 for pinch RF size, activation threshold, and pinch-evoked responses, 1-way RM ANOVA, n=6). Phentolamine superfusion blocked the MO-evoked central sensitization since MO application to the pulp did not induce any significant changes in RF size, mechanical activation threshold or pinch-evoked responses under phentolamine superfusion (P>0.3, 0.4, 0.2; 1-way RM ANOVA; Table 1, Fig. 2). The MO-induced changes in RF size and pinch-evoked responses in this group were significantly less than those in the PBS group (P<0.05–0.001, 2-way ANOVA; Table 1), and there were significant differences in values at some post-MO time-points between these two groups (see Fig. 2).

Effect of prazosin on the MO-induced central sensitization

Continuous i.t. superfusion of prazosin, over MDH did not affect baseline nociceptive neuronal properties after 15 min of pretreatment (P>0.05 for pinch RF size, activation threshold, and pinch-evoked responses; 1-way RM ANOVA, n=6; Table2). As shown in Fig. 3, prazosin superfusion markedly attenuated the MO-induced increase in RF size (P>0.1), and also strongly attenuated the MO-induced decrease in activation threshold and increases in pinch-evoked responses (although MO application still produced a mild but significant decrease in activation threshold [P<0.01] and increases in pinch-evoked

responses compared to baseline values [P<0.02; 1-way RM ANOVA]). Compared to the PBS group, prazosin superfusion significantly attenuated the MO-induced RF size expansion, decrease in activation threshold, and increases in pinch-evoked responses (P<0.02–0.001, 2-way ANOVA; Table 2). There were significant differences in values at some post-MO time-points between these two groups (see Fig. 3).

Effect of yohimbine on the MO-induced central sensitization

Continuous i.t. superfusion of yohimbine over MDH did not affect baseline nociceptive neuronal properties after 15 min of pretreatment (P>0.05 for pinch RF size, activation threshold, and pinch-evoked responses; 1-way RM ANOVA, Table 2, n=6). During yohimbine superfusion, the MO-induced expansion of RF size and decrease in activation threshold were still significantly greater than the baseline values (P<0.05–0.01, 1-way RM ANOVA), although mildly attenuated, and the MO-induced increases in pinch-evoked responses were facilitated (P<0.05; Fig. 3). There were significant differences only in the MO-induced increase in RF size between the yohimbine group and the PBS group (P<0.001, Table 2), but not in changes of the activation threshold and pinch-evoked responses (P>0.1 and P>0.6, respectively, 2-way ANOVA; Fig. 3; Table 2).

Discussion

The present study has provided the first documentation that central endogenous NA processes may contribute to the central sensitization of functionally identified nociceptive neurons in the MDH. Application of the inflammatory irritant MO to the rat tooth pulp produced MDH central sensitization reflected in increases in RF size and responses to noxious stimuli and decrease in mechanical activation threshold, consistent with our earlier findings (Chiang et al., 1998, 2005b, 2007; Hu et al., 2002; Xie et al., 2007). Superfusion over MDH of guanethidine or phentolamine could markedly attenuate these three parameters of MO-induced central sensitization, suggesting its dependency on NA release and α-adrenoceptor activation. Superfusion of prazosin also strongly attenuated these three parameters of the MO-induced central sensitization, whereas yohimbine had little effect and indeed facilitated responses to noxious stimuli. None of the four chemicals superfused alone affected the baseline nociceptive neuronal properties. Collectively, the findings suggest that α1-adrenoceptors play a more important role than α2-adrenoceptors in the development of MDH central sensitization.

Anatomical studies indicate that the adrenergic innervation of the blood vessels, neurons, and glia within the parenchyma of the spinal cord originates mainly from the locus coeruleus in the brainstem (Commissiong, 1981), and those within dura and pia matter derive from peripheral sympathetic fibers (McNicholas et al., 1980; Amenta et al., 1990; Cohen et al., 1997). Thus, the adrenergic modulators used in the present study may have targeted adrenoceptors in MDH associated with innervation from either or both these origins.

Guanethidine, used for chemical sympathectomy, is commonly administered systemically where it causes an initial release of NA from neuronal terminals, leading eventually to depletion of NA after several days, but the transitional time (minutes to days) from NA release to NA depletion varies in different tissues and depends on the mode of drug administration. Our findings indicate that the marked attenuation of central sensitization occurred soon (within 1 h) after continuous i.t. application of guanethidine. These findings are consistent with other studies demonstrating that guanethidine or sympathetic ganglion blockade may attenuate formalin-induced nociceptive responses in rats (Chichorro et al., 2004) and capsaicin-induced cutaneous pain in humans (Drummond 2001), and that a stellate ganglion block with bupivacaine can inhibit the formalin-induced reduction of substance P in the superficial laminae of the spinal dorsal horn (Wang et al., 2005). These

findings collectively suggest that there may be a local release of pro-inflammatory substances from the NA system in exaggerated pain states, in accordance with earlier documentations that the sympathetic nervous system exerts a facilitatory role in the generation and processing of nociceptive signals, particularly in inflammatory pain states (Coderre and Melzack, 1987; Nakamura and Ferreira, 1987; for review, see Pertovaara, 2006).

It has been demonstrated that local injection of phentolamine into nucleus raphe magnus and lateral reticular nucleus produces a potent analgesia in the tail-flick test (Sagen and Proudfit, 1987). However, an action involving these nuclei is unlikely to explain our findings that phentolamine markedly attenuated MDH central sensitization since the tip of the superfusion tubing used was positioned at least 1 mm below the obex, and the superfusion flow was always in a caudal direction. Consequently, the superfused chemicals were unlikely to have diffused rostrally to influence these supraspinal nuclei. Notably, an earlier study has reported that the effects of phentolamine are mediated by its direct blocking of presynaptic serotonin autoreceptors (Limberger et al., 1989). A recent visceral pain study has demonstrated that the activation of presynaptic serotonergic 5-HT(1A) receptors in spinal GABAergic neurons can restrict GABA release and thereby disinhibit the excitatory glutamatergic neurons, producing a pronociceptive effect (Mickle et al., 2012). Therefore, the phentolamine-induced attenuation of MDH central sensitization can be explained by its blocking effect on presynaptic 5-HT receptors of GABAergic neurons; these mechanisms may also have been involved in guanethidine's effect in the present study.

Superfusion of the selective α1-adrenoceptor antagonist prazosin over the medulla in the present study strongly attenuated all three MO-induced parameters of central sensitization in the MDH nociceptive neurons. While there have been many studies on central α2-adrenoceptor mechanisms involved in spinal nociceptive processing (Pertovaara, 2006; Tully and Bolshakov, 2010), there have been fewer but nonetheless convincing studies showing that central α1-adrenoceptors exert their excitatory effects on GABAergic cells in the superficial laminae of the dorsal horn, producing analgesia (Baba et al., 2000a, b; Yuan et al., 2009) and that α1-adrenoceptors in the raphe magnus have a role in relaying descending antinociceptive influences from opioidergic neurons of the midbrain (Bie et al., 2003). In contrast, peripheral sympathetic nerve activation can facilitate sensory inputs activated by noxious stimuli. This is suggested by several findings: 1) mutant mice lacking the α1d-adrenergic receptor show longer tail-flick and hindpaw-licking latencies to noxious stimuli (Harasawa et al., 2003); 2) in spinal nerve-transected animals, cold allodynia is attenuated by phentolamine and prazosin (i.p.) but not by yohimbine (i.p.)(Kim et al., 2005); 3) local administration of α,β-methyleneATP (ligand for P2X3/P2X2/3 receptors) alone into the rat plantar hind paw produces few nociceptive behaviors, but when given in combination with phenylephrine (an α1-adrenoceptor agonist), but not clonidine (an α2-adrenoceptor agonist), increased flinching behavior results (Meisner et al., 2007); 4) The rolipram (phosphodiesterase inhibitor)-induced prolongation (> 3 days) of the mechanical hyperalgesia produced by prostaglandin E2 applied to the hairy skin of the rat's hindpaw is blocked by phentolamine and prazosin, but not by yohimbine, when given systemically or intradermally at the site of injection of prostaglandin E2 and rolipram, but not when administered i.t. (Ouseph and Levine, 1995). The i.t. superfusion of prazosin in the present study produced a strong attenuation of the MO-induced MDH central sensitization, which is inconsistent with the antinociceptive role of central α1-adrenoceptors (Baba et al., 2000a, b). It is possible that this marked attenuation of central sensitization by prazosin may at least partly be mediated by its blocking α1-adrenoceptors in astroglia, because it has been reported that α1-adrenoceptor agonists can evoke astroglial Ca^{2+} waves in brain slices by releasing inositoltriphosphate into the cytosol that causes Ca^{2+} release from intracellular stores on the endoplasmic reticulum (Shao and McCarthy, 1993; Duffy and MacVicar,

1995); these astroglial Ca^{2+} waves play an important role in the development of central sensitization (Cao and Zhang, 2008; Nakagawa and Kaneko, 2010; Hertz et al., 2010; Chiang et al., 2011; O'Donnell et al., 2012).

The selective α2-adrenoceptor antagonist yohimbine had weak effects on the MO-induced increase in RF size and decrease in mechanical activation threshold in the present study, and indeed facilitated the MO-induced increase in pinch-evoked neuronal responses. This facilitatory effect of yohimbine is not surprising, since the α2-adrenoceptor agonist clonidine is a potent inhibitor of presynaptic and postsynaptic neurotransmission and has been widely demonstrated in spinal nociceptive processing in association with descending inhibition and morphine analgesia (Stone et al., 1997; Pan et al., 2002; Kawasaki et al., 2003; Obata et al., 2005; Takeda et al., 2006; Hayashida et al., 2008; for review, see Pertovaara 2006; Taylor 2009). Furthermore, capsaicin-induced hypersensitivity was suppressed in wild-type but not in α2A-adrenoceptor knockout mice by a centrally acting α2-adrenoceptor agonist, whereas a peripherally acting α2-adrenoceptor agonist was without effect on hypersensitivity, indicating that central α2A-adrenoceptors may contribute to feedback inhibition of capsaicin-induced hyperalgesia (Mansikka et al., 2004). Thus these inhibitory central actions of α2-adrenoceptor agonists may provide an explanation for the α2-adrenoceptor antagonist yohimbine-produced facilitatory effect on the MO-induced pinch-evoked responses observed in the present study.

Also noteworthy are our recent observations that MO application to the tooth pulp induces immediate MDH neuronal discharges for 3–5 min accompanied by an increase in local medullary blood flow and that both the neuronal and vascular responses can be attenuated by i.t., but not i.v., administration of phentolamine and guanethidine (Chiang et al., 2008). This NA-related vasodilation effect seems to contradict the general view that local NA application produces vasoconstriction. However, recent studies provide strong evidence that *in vitro* cortical application of α1-adrenoceptor agonist phenylephrine can readily elicit Ca^{2+} waves in astroglia, which propagate into their end-feet where the synthetized epoxyeicosatrienoic acids (potent outward K^+ channel modulators) and the gliotransmitters ATP (and glutamate) are released; these agents mediate local vasodilation coupling with neuronal excitation (Pelligrino et al., 2011; Higashimori et al., 2010; Gordon et al., 2008; Shi et al., 2008; Mulligan and MacVicar, 2004; for review, see Attwell 2010). Thus, our previous and present results raise the possibility that the MO-induced MDH central sensitization is coupled with a local vasodilation required for energy supply, and that underlying mechanisms may include a central α-adrenoceptor-mediated astroglial-neuronal activation.

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1. Mustard oil (MO) induced central sensitization of NS neurons in MDH.
2. Guanethidine and phentolamine blocked the MO-induced central sensitization.
3. Prazosin significantly attenuated the MO-induced central sensitization.
4. Yohimbine facilitated the responses of sensitized NS neurons.
5. α1 and α2-adrenoceptor are differentially involved in MDH central sensitization.

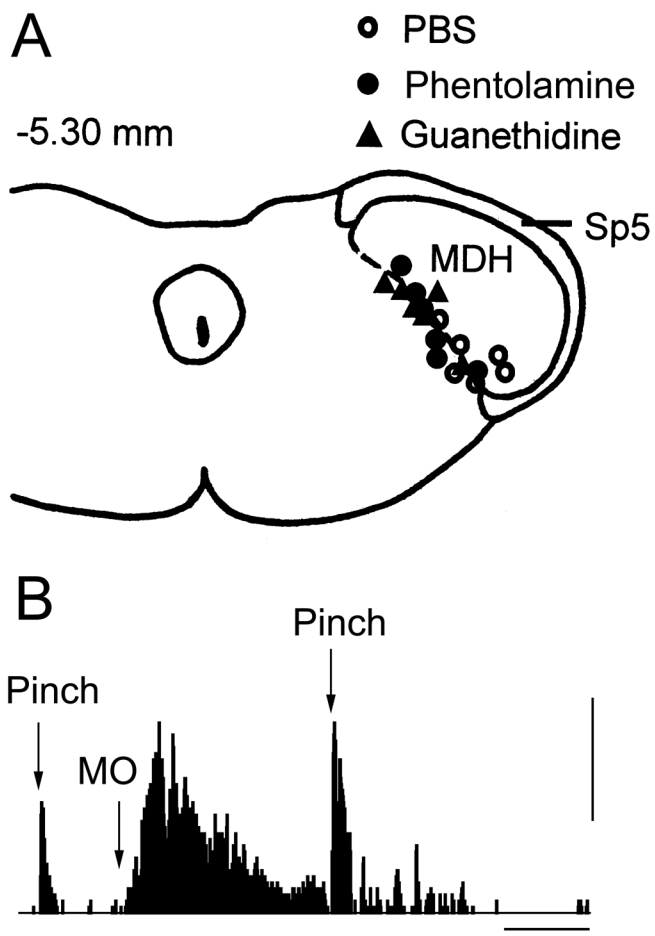

Fig. 1.

A. Histologically confirmed neuronal recording sites. Each of the 3 groups consisted of 6 NS neurons. The sites were plotted onto a section of the caudal medulla (−5.3 mm behind interaural line). Abbreviations: Sp 5: trigeminal spinal tract; MDH: medullary dorsal horn. Same abbreviations also apply to Fig. 3D. B. Activity of a NS neuron in the PBS group evoked by MO application to the tooth pulp. Note that an identical pinch stimulus delivered to the same RF site 5 min after MO application produced a pronounced increase in responses, compared to that evoked prior to MO application. Calibration: 20 Hz; 2 min.

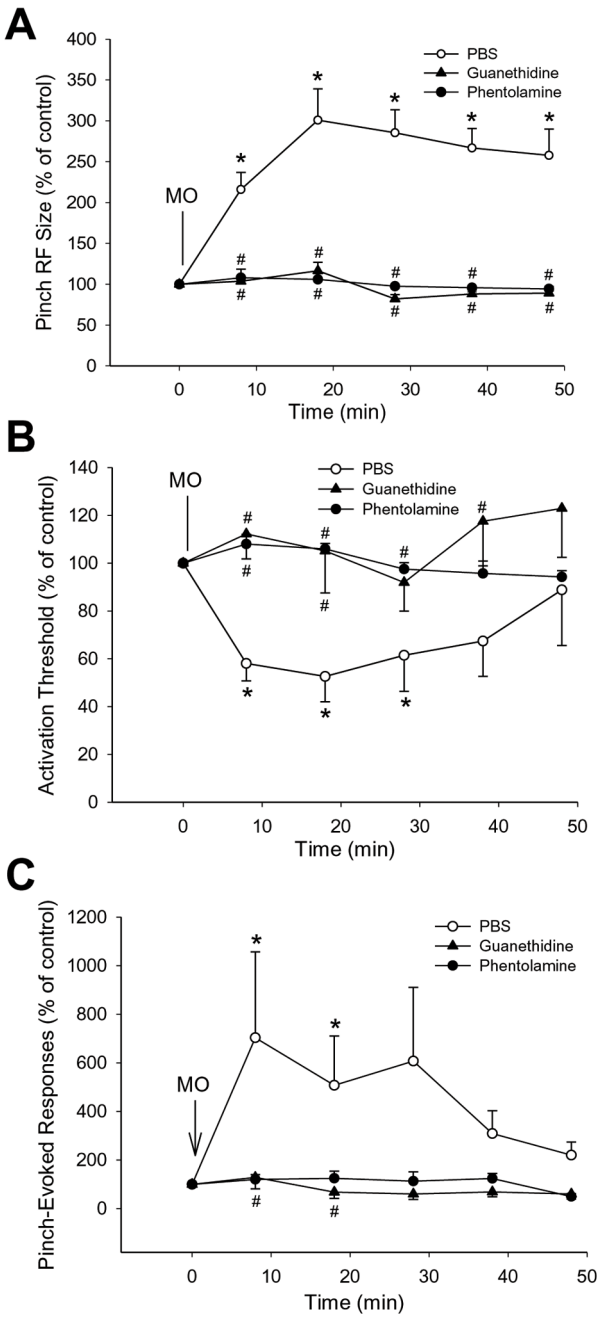

Fig. 2.

Changes in neuronal pinch RF size (A), mechanical activation threshold (B), and pinch-evoked responses (C) induced by MO application to the tooth pulp after continuous i.t. superfusion of PBS, guanethidine or phentolamine. Mean ± S.E. values are shown for each group at the different time-points. *P<0.05 compared to the baseline within the group (1-way RM ANOVA, post-hoc Dunnett's test); #P<0.05 compared to the PBS group at the different time-points tested (2-way ANOVA, post-hoc Dunnett's test).

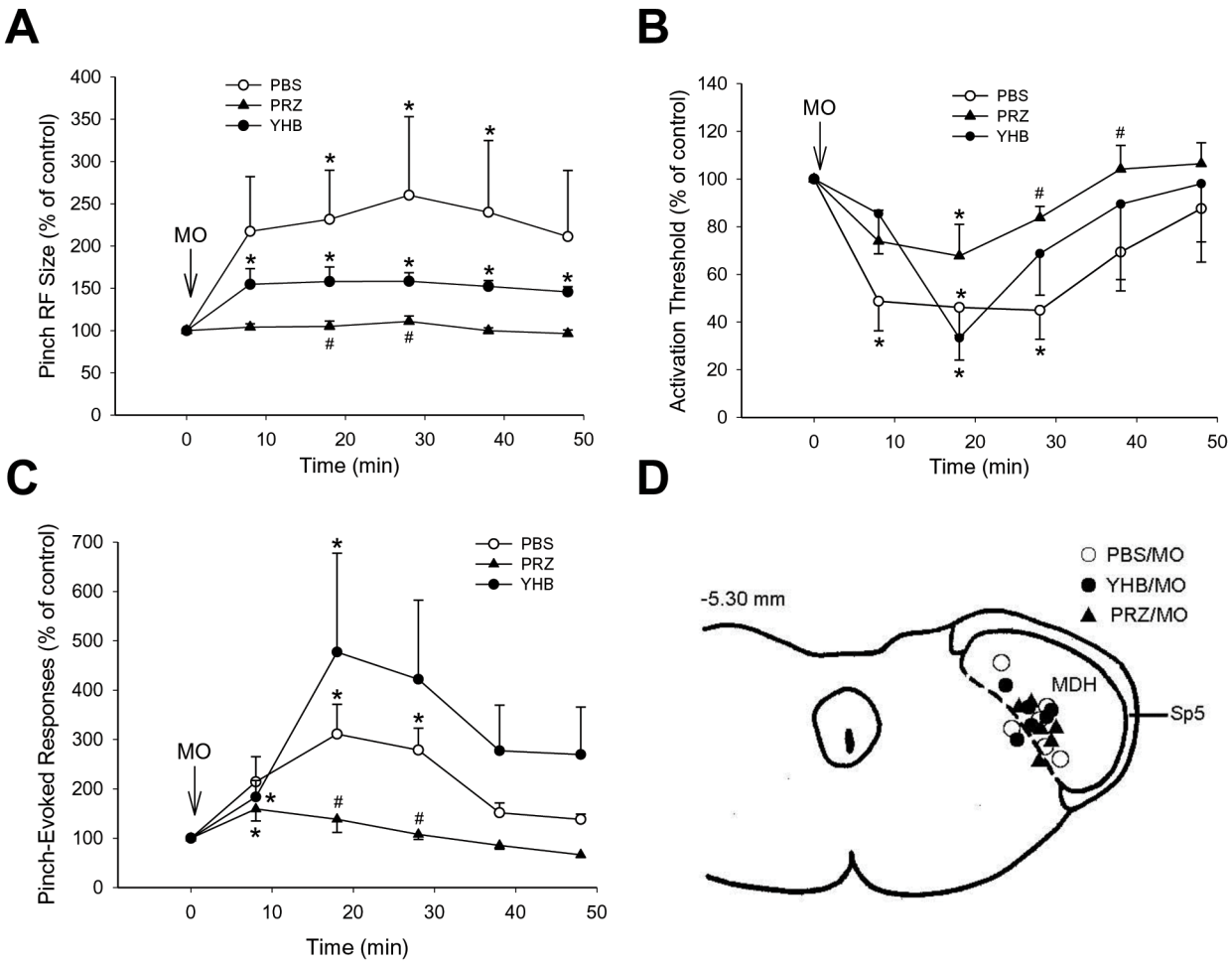

Fig. 3.

Changes in neuronal pinch RF size (A), mechanical activation threshold (B), and pinch-evoked responses (C) induced by MO application to the tooth pulp after continuous i.t. superfusion of PBS, prazosin or yohimbine. Mean ± S.E. values are shown for each group at the different time-points. *P<0.05 compared to the baseline within the group (1-way RM ANOVA, post-hoc Dunnett's test); #P<0.05 compared to PBS group at the different time-points tested (2-way ANOVA, post-hoc Dunnett's test). D. Histologically confirmed neuronal recording sites. Each of the 3 groups consisted of 6 NS neurons. The sites were plotted onto a section of the caudal medulla (−5.3 mm behind interaural line).

Table 1

Effects of guanethidine, phentolamine or PBS on mustard oil (MO)-induced changes in nociceptive neuronal properties in the rat MDH.

	Pinch RF (cm^2)	Activation threshold (g)	Response to noxious stimuli (# of spikes)
PBS group (n=6)			
Baseline values	1.9 ± 0.6	99.3 ± 38.6	36.1 ± 5.8
Values 30′ after PBS	1.9 ± 0.5	97.3 ± 35.4	35.7 ± 9.7
Values 18′ after MO	4.9 ± 0.9 *	49.5 ± 16.5 *	129.5 ± 40.8 *
Values 48′ after MO	4.2 ± 0.8 *	83.8 ± 30.4	87.3 ± 45.8
Guanethidine group (n=6)			
Baseline values	1.6 ± 0.7	108.8 ± 12.4	167.3 ± 34.5
Values 30′ after guanethidine	1.6 ± 0.7	106.3 ± 22.7	151.2 ± 39.2
Values 18′ after MO	1.6 ± 0.6	101.8 ± 17.2	79.2 ± 25.1
Values 48′ after MO	1.3 ± 0.6	140.1 ± 31.5	93.5 ± 30.1
Phentolamine group (n=6)			
Baseline values	2.1 ± 0.4	84.5 ± 24.9	151.5 ± 51.8
Values 15′ after phentolamine	2.0 ± 0.4	80.5 ± 23.5	164.3 ± 82.9
Values 18′ after MO	2.1 ± 0.4	68.0 ± 20.2	170.5 ± 84.3
Values 48′ after MO	1.9 ± 0.4	82.0 ± 23.6	152.2 ± 103.9
Comparison between groups			
Guanethidine versus PBS	F1,70 = 28.64; P<0.001	F1,70 = 16.22; P<0.001	F1,70 = 2.83; P<0.05
Phentolamine versus PBS	F1,70 = 23.11; P<0.001	F1,70 = 2.23; P>0.05	F1,70 = 4.57; P<0.05

All values are shown as mean ± S.E. n = number of tested neurons.

* = P<0.05, P values are based on 1-way repeated measures ANOVA followed by Dunnett's test. Differences in groups are treated by 2-way ANOVA.

Table 2

Effects of prazosin, yohimbine or PBS on mustard oil (MO)-induced changes in nociceptive neuronal properties in the rat MDH.

	Pinch RF (cm^2)	Activation threshold (g)	Response to noxious stimuli (# of spikes)
PBS group (n=6)			
Baseline values	2.3 ± 0.5	84.8 ± 16.9	79.7 ± 15.2
Values 8′ after PBS	2.4 ± 0.5	85.3 ± 18.8	82.8 ± 18.5
Values 18′ after MO	4.3 ± 1.0 *	40.8 ± 15.0 *	263.7 ± 94.1 *
Values 48′ after MO	3.3 ± 0.7	79.3 ± 21.5	116.5 ± 32.0
Prazosin group (n=6)			
Baseline values	2.5 ± 0.6	83.0 ± 10.1	55.8 ± 16.2
Values 8′ after PRZ	2.4 ± 0.6	98.7 ± 13.0	57.0 ± 16.3
Values 8′–18′ after MO	2.6 ± 0.8	71.7 ± 19.0 *	81.5 ± 24.7 *
Values 48′ after MO	2.3 ± 0.5	107.7 ± 18.8	41.0 ± 14.9
Yohimbine group (n=6)			
Baseline values	3.4 ± 0.6	72.8 ± 12.1	87.8 ± 21.4
Values 8′ after YHB	3.2 ± 0.4	65.0 ± 15.7	125.2 ± 50.0
Values 18′ after MO	5.2 ± 1.0 *	17.2 ± 3.8 *	358.3 ± 117.0 *
Values 48′ after MO	4.8 ± 0.7 *	46.5 ± 8.8	206.7 ± 40.5
Comparison between groups			
Prazosin versus PBS	$F_{1,70}$=5.89; P<0.02	$F_{1,70}$=9.93; P<0.002	$F_{1,70}$=15.59; P<0.001
Yohimbine versus PBS	$F_{1,70}$=30.5; P<0.001	$F_{1,70}$=2.71; P>0.1	$F_{1,70}$=0.18; P>0.6

All values are shown as mean ± S.E. n = number of tested neurons.

* = P<0.05, P values are based on 1-way repeated measures ANOVA followed by Dunnett's test. Differences in groups are treated by 2-way ANOVA.