DIFFERENTIAL ANTINOCICEPTIVE EFFECTS OF YOHIMBINE IN THE RAT FORMALIN TEST

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ABSTRACT

Although many pharmacological studies indicate that yohimbine antagonize the antinociceptive effects of α_2-adrenoceptor agonists, there are evidences that yohimbine by itself produces antinociceptive effects in the formalin test. However, its site of action on nociceptive processing is not fully understood. In this investigation, a series of experiments were designed to study the antinociceptive effects of intraperitoneal (i.p.), intraplantar (i.pl.) and intrathecal (i.t.) administration of yohimbine in the nociceptive processing. Yohimbine (2 and 4 mg/kg, i.p.) induced antinociception in the early phase (0-5 min) as well as in the late phase (10-60 min) of formalin test. While i.pl. yohimbine (5-100 μg) decreased the response in the early phase, i.t. yohimbine (30 μg) decreased pain behavior in the late phase of formalin test in rats.

In conclusion, our findings show that yohimbine induces antinociception in both phases of formalin test and its effects are produced at least in part through actions at the peripheral terminal of primary afferents or at the spinal level.

Keywords: Yohimbine; Formalin test; Antinociception, Rat

INTRODUCTION

Yohimbine, an indole alkaloid, is a relatively selective α_2-adrenoceptor antagonist, which is frequently used to assess the involvement of α_2-adrenoceptors in the mechanism of action of drugs (1). It has been reported that yohimbine eliminates or attenuates the analgesic effects of α_2-adrenoceptor agonists in different pain models (2-5). Interestingly, several studies have indicated that yohimbine dose not completely antagonize the effects of clonidine in the formalin test (6-8), and acts as an analgesic (9). It has been reported that yohimbine reverses clonidine antinociception in the tail-flick (3) and the hot-plate tests (2). These reports raise the possibility that the effects of yohimbine in the formalin test may be mediated by mechanisms other than blockade of adrenoceptors and are different from those of other nociceptive tests. However, the site of yohimbine action on nociceptive processing and the importance of local peripheral and spinal involvement in antinociception are unclear.

Numerous studies have examined the effects of pharmacological agents on the pain-related behaviors in the formalin test (10). Intraplantar injection of formalin evokes signs of nociception (flinching and licking of the injected paw) in the early stage (phase 1), followed by a quiescent period characterized by fewer pain behaviors, and late-hyperalgesic (phase 2) components that last for approximately 1 hr (11,12). It is generally agreed that the early phase results, at least in part, from direct activation of both low threshold mechanoreceptive and nociceptive types of peripheral nociceptors (13,14), whereas the late phase reflects induction of a spinal state of facilitation, central sensitization, development of inflammation, and enlargement of receptive fields (15-18).

The purpose of this study was therefore to evaluate the antinociceptive effects of i.p., i.pl. and i.t. administration of yohimbine in the nociceptive processing in order to further elucidate the site of yohimbine action.

MATERIALS AND METHODS

Drug

The solution of yohimbine hydrochloride (Tocris Cookson Ltd, UK) was prepared in a saline.

Animal maintenance and preparation

Male Wistar rats (Pasture institute laboratories, Tehran, Iran), weighing 225-290 g at the time of experiments, were kept in group cages and maintained on a 12 hr light/dark cycle with free access to food and water, except during the time of experiments. Rats were randomly divided into groups of 6-8 and each animal was used only once. For i.t. cannulation, rats were anesthetized with a mixture of ketamine hydrochloride (80 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) for

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induction of anesthesia and intraoperative analgesia. Rats were placed in a stereotaxic head holder and a polyethylene (PE-10) catheter filled with sterile saline was inserted through an incision in the atlanto-occipitial membrane (19, 20). They were then housed individually after surgery and allowed to recover for at least 6 days. Rats with neurological signs such as paralysis of fore paws or hind paws upon recovery from anesthesia were excluded from the experiment. Proper placement of the i.t. catheter was verified at the end of formalin test by the occurrence of fast hind limb paralysis after an i.t. injection of 15 µl of 2% lidocaine hydrochloride or by direct visualization of the catheter tip after laminectomy.

Drug injections

Yohimbine was freshly prepared and administered 20 min before formalin in the following volumes: i.p. in a volume of 1 ml/kg; i.t. in a volume of 5 µl and i.pl. in a volume of 100 µl. Each i.t. injection was followed by an injection of 10 µl of normal saline to flush the drug which was left in the catheter lumen over a period of 30 s.

Formalin test

Formalin test was used as reported by Dubuisson and Dennis (11). Before the test, the animals were placed individually in transparent Plexiglas testing chambers (30 x 20 x 20) and allowed to acclimate for at least 35-40 min. A mirror was placed at 45º angle below the observation box in order to allow the experimenter an unobstructed view of the injected paw. One hundred µl of 2.5% formalin was injected into the left hind paw using a microsyringe with a 29-gauge needle. Animals were immediately returned to the observation box and their behavior was continuously scored in 15 second intervals for a total of 60 min using a weighted score (11, 21, 22). Many different summarizing functions have been used to simplify numerical recipes of formalin test. In this experiment, the area under the curve (AUC) of pain score-time curves during 0-5 min was determined as the early phase and the 10-60 min interval was defined as the late phase of the formalin test.

Data Analysis

Pain behavior was calculated from the area under the curve (AUC) of pain score-time curve by the use of trapezoidal rule. Results are expressed as mean of AUC ± S.E.M. For statistical analysis, ANOVA test was used. Moreover, after ANOVA, the Tukey's HSD test for multiple comparisons was used. Probabilities of $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Fig. 1 shows the antinociceptive effects of i.p. yohimbine in the formalin test. Intraperitoneal administration of yohimbine (2 and 4 mg/kg) induced antinociception in the early [$F_{ANOVA}(2,18)=7.25$; $P<0.01$] and late phases [$F_{ANOVA}(2,18)=20.64$; $P<0.001$] of formalin test. The present study confirms previous findings (6) that doses of 1 and 4 mg/kg yohimbine produces antinociception in the rat formalin test but not in the tail-flick and hot-plate tests. It has been demonstrated (9) that yohimbine through activation of $5\text{-}HT_{1A}$ receptors produces antinociception. Our findings indicate that yohimbine produces antinociception parallel to α_2-adrenoceptor agonists. This suggests that, the effects of yohimbine in the formalin test may be mediated by mechanisms beyond adrenoceptor blockade. It has been reported that, neurotoxic destruction of noradrenergic pathways, either decrease the behavioral response to formalin (23-25) or have no effect in the formalin test (26).

One-way ANOVA showed that administration of α_2-adrenoceptor antagonist, yohimbine (10, 20, 30, 60, and 120 µg, i.t.) altered pain response in the late phase [$F(5,31)=5.1$, $P<0.01$], but not in the early phase of the test [$F(5,31)=0.29$, $P>0.05$]. However, while spinal noradrenergic fibers may play a predominant role in the antinociception (27), post hoc analysis indicated that yohimbine (30 µg/rat) has decreased pain behavior in late phase (Fig. 2). In agreement with our data, it has been shown that i.t. administration of yohimbine significantly reduces licking activity in both early and late phases of formalin test in mice (28). Furthermore, evidences have been provided (29) that nonsynaptic release of norepinephrine from the rat spinal cord slices is modulated via presynaptic α_{2A}-adrenergic autoreceptors. They observed that clonidine inhibited whereas yohimbine enhanced the release of norepinephrine in response to neuronal stimulation. As it has been depicted in Fig 2, it seems that yohimbine at the dose of 30 µg/rat, may act pre-synaptically to increase the release of norepinephrine and subsequently suppresses pain and at the dose of 120 µg/rat may acts postsynaptically to inhibit hyperpolarization of neurons and mask its pre-synaptic action. In contrast with these finding, it has been demonstrated (30) that pretreatment of rats with i.t. yohimbine following injection of 50 µl of 2% formalin enhances the flinching behavior and increases noradrenaline concentration in the dorsal horn of the spinal cord.

Fig. 3 represents the effect of i.pl. yohimbine on pain behavior evoked by formalin. Intraplantar administration of yohimbine reduced pain responses in animals in the early phase

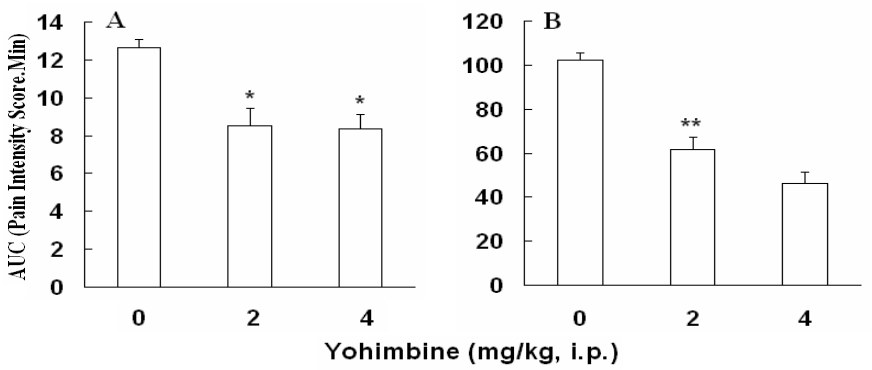

Figure 1. The effect of i.p. administration of yohimbine on pain behavior in the formalin test. Pain response was recorded between 0-5 (A; early phase) and 10-60 (B; late phase). Yohimbine was administered 20 min before formalin. Each bar represents the mean ± S.E.M. from six to eight rats. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to saline group.

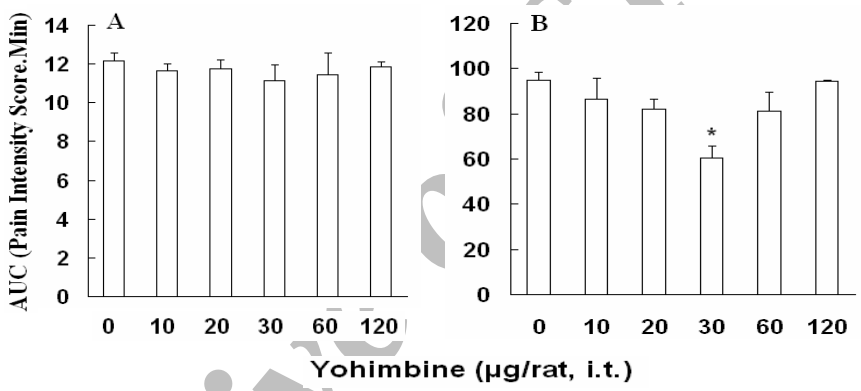

Figure 2. The effect of i.t. administration of yohimbine on pain behavior. Pain response was recorded between 0-5 (A; early phase) and 10-60 (B; late phase). Yohimbine was administered 20 min before formalin. Each bar represents the mean ± S.E.M. from six to eight rats. $^{*}P<0.001$ compared to saline control and also $P<0.01$ compared to 120 µg yohimbine group.

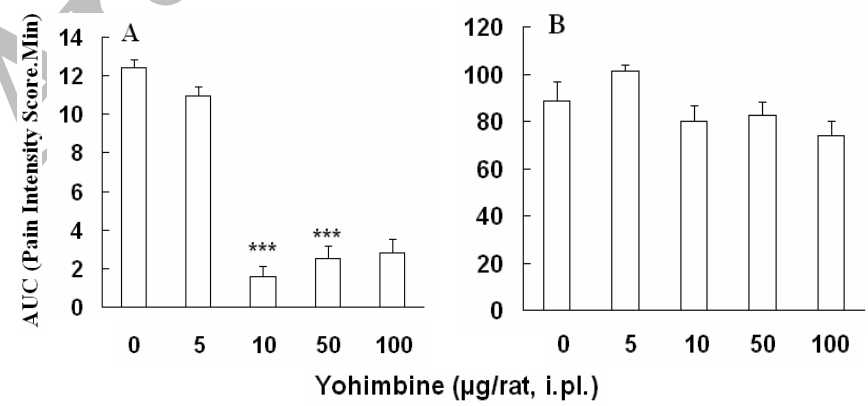

Figure 3. The effect of i.pl. yohimbine on pain behavior in the early (A) and late phase (B) of formalin test. Yohimbine was co-administered with formalin. Each bar represents the mean ± S.E.M. from six to eight rats. $^{***}P<0.001$ compared to control group.

[$F_{ANOVA}(4,28)=33.57$; $P<0.001$]. However, the pain response induced by formalin was not affected during the late phase [$F_{ANOVA}(4,28)=2.23$; $P>0.05$] of formalin. Co-administration of yohimbine with formalin locally produced antinociception in early phase, suggesting a predominant peripheral rather than central site of action for yohimbine. Whereas the early response to formalin mainly reflects the activation of nociceptors, it may be concluded that the antinociceptive effect of i.p. yohimbine may be related to the peripheral terminal of primary afferent. In agreement, the present study showed that, i.pl. yohimbine reduces pain intensity only, in the early phase of the test. It has been shown that yohimbine blocks firing of rat dorsal root ganglion neurons by inhibition of both tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ currents and vanilloid VR1 receptors at μM concentrations (31). The results of another study shows that high concentration of yohimbine markedly potentate the duration of tetrodotoxin block by an effect that dose not appear to be mediated by adrenergic receptors (32). These blocking effects may underlie the antinociceptive mechanism of yohimbine on primary afferent. Furthemore, yohimbine probably affects sympathetic nervous system after i.pl. injection. The sympathetic nervous system contributes to hyperalgesia following tissue injury and inflammation, but the nature of the involvement in this case differs from that in nerve injury (33). Inflammation does not lead to up-regulation of α_{2A}-adrenoceptors in dorsal root ganglia (34), and in this case, the enhancing effects of noradrenaline on the sensitivity of primary afferents may be mediated indirectly by actions on sympathetic postganglionic nerves (33, 35). It has been proposed that noradrenaline sensitizes nociceptors indirectly and involves an action on α_2-adrenoceptors on the terminals of post-ganglionic sympathetic neurons rather than on the nociceptive fibres themselves (35-37). Activation of these α_2-adrenoceptors elicits production and release of prostaglandins, in particular prostaglandin E_2 and prostaglandin I_2 by post-ganglionic sympathetic sympathetic neurons (38,39). These prosta-glandins have been shown to sensitize nociceptors (40).

In conclusion, the results of this study show that yohimbine exhibits antinociception in both phases of formalin test and its effects are produced at least in part, through its actions at peripheral terminal of primary afferents or at the spinal level.

REFERENCES

1. Goldberg MR, Robertson D. Yohimbine: a pharmacological probe for study of the alpha 2-adrenoreceptor. Pharmacol Rev 1983; 35:143-80.
2. Takano Y, Yaksh TL. Characterization of the pharmacology of intrathecally administered alpha-2 agonists and antagonists in rats. J Pharmacol Exp Ther 1992; 261:764-772.
3. Monroe PJ, Smith DL, Kirk HR, Smith DJ. Spinal nonadrenergic imidazoline receptors do not mediate the antinociceptive action of intrathecal clonidine in the rat. J Pharmacol Exp Ther 1995; 273:1057-1062.
4. Yaksh TL, Pogrel JW, Lee YW, Chaplan SR. Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. J Pharmacol Exp Ther 1995; 272:207-14.
5. Buerkle H, Yaksh TL. Pharmacological evidence for different α_2-adrenergic receptor mediating analgesia and sedation in the rat. Br J Anaesth 1998; 81:208-215.
6. Dennis SG, Melzack R, Gutman S, Boucher F. Pain modulation by adrenergic agents and morphine as measured by three pain tests. Life Sci 1980; 26:1247-1259.
7. Tasker RA, Melzack R. Different alpha-receptor subtypes are involved in clonidine-produced analgesia in different pain tests. Life Sci.1989; 44:9-17.
8. Shannon HE, Lutz EA. Effects of the I_1 imidazoline/ α_2-drenergic receptor agonist moxonidine in comparison with clonidine in the formalin test in rats. Pain 2000; 85:161-167.
9. Shannon HE, Lutz EA. Yohimbine produces antinociception in the formalin test in rats: involvement of serotonin(1A) receptors. Psychopharmacology (Berl) 2000; 149:93-7.
10. Porro CA, Cavazzuti M. Spatial and temporal aspects of spinal cord and brainstem activation in the formalin pain model. Prog Neurobiol 1993; 41:565-607.
11. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1977; 4:161-174.
12. Abbott FV, Franklin KBJ, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 1995; 60:91-102.
13. Puig S, Sorkin LS. Formalin-evoked activity in identified primary afferent fibers: systemic lidocaine suppresses phase-2 activity. Pain 1996; 64:345-355.
14. McCall WD, Tanner KD, Levine JD. Formalin induces biphasic activity in C-fibers in the rat. Neurosci Lett 1996; 208:45-48.

15. Woolf CJ, King AE. Dynamic alterations in the cutaneous mechanoreceptive fields of dorsal horn neurons in the rat spinal cord. J Neurosci 1990; 10:2717-2726.
16. Coderre TJ, Vaccarino AL, Melzack R. Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. Brain Res 1990; 535:155-158.
17. Coderre TJ, Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci 1992; 12:3665–3670.
18. Coderre TJ, Yashpal K, Henry JL. Specific contribution of lumbar spinal mechanisms to persistent nociceptive responses in the formalin test. Neuro Report 1994; 5:1337-1340.
19. Yaksh TL, Rudy TA. Chronic cathetrization of the spinal subarachnoid space. Physiol Behav 1976; 17:1031-36.
20. Hammond DL. Intrathecal administration: methodological considerations. Prog Brain Res 1988; 77:313-320.
21. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: An evaluation of the method. Pain 1992; 51:5-17.
22. Coderre TJ, Fundytus ME, McKenna JE, Dalal S, Melzack R. The formalin test: A validation of the weighted scores method of behavioral pain rating. Pain 1993; 54:43-50.
23. Fasmer OB, Berge OG, Tveiten L, Hole K. Changes in nociception after 6-hydroxydopamine lesions of descending catecholaminergic pathways in mice. Pharmacol Biochem Behav 1986; 24:1441-1444.
24. Tjolsen A, Berge OG, Hole K. Lesions of bulbo-spinal serotonergic or noradrenergic pathways reduce nociception as measured by the formalin test. Acta Physiol Scand 1991; 142:229-36.
25. Martin WJ, Gupta NK, Loo CM, Rohde DS, Basbaum AI. Differential effects of neurotoxic destruction of descending noradrenergic pathways on acute and persistent nociceptive processing. Pain 1999; 80:57-65.
26. Sawynok J, Reid AR, Doak GJ. Caffeine antinociception in the rat hot-plate and formalin tests and locomotor stimulation: involvement of noradrenergic mechanisms. Pain 1995; 61:203-13.
27. Willis WD, Westlund KN. Neuroanatomy of the pain system and of the pathways that modulate pain. J Clin Neurophysiol 1997; 14:2-31.
28. Kanui TI, Tjolsen A, Lund A, Mjellem-Joly N, Hole K. Antinociceptive effects of intrathecal administration of alpha-adrenoceptor antagonists and clonidine in the formalin test in the mouse. Neuropharmacology 1993; 32:367-71.
29. Umeda E, Satoh T, Nagashima H, Potter PE, Tarkovacs G, Vizi ES. α_{2A} subtype of presynaptic α_2-adrenoceptors modulates the release of [^{3}H]-noradrenaline from rat spinal cord. Brain Res Bull 1997; 42:129-132.
30. Omote K, Kawamata T, Kawamata M, Namiki A. Formalin-induced nociception activates a monoaminergic descending inhibitory system. Brain Res 1998; 814:194-198.
31. Dessaint, J, Yu W, Krause JE, Yue L. Yohimbine inhibits firing activities of rat dorsal root ganglion neurons by blocking Na^+ channels and vanilloid VR1 receptors. Eur J Pharmacol 2004; 485:11-20.
32. Kohane DS, Lu NT, Crosa GA, Kuang Y, Berde CB. High concentrations of adrenergic antagonists prolong sciatic nerve blockade by tetrodotoxin. Acta Anaesthesiol Scand 2001; 45:899-905
33. Janig W, Levine JD, Michaelis M. Interactions of sympathetic and primary afferent neurons following nerve injury and tissue trauma. Prog Brain Res 1996; 113:161-184.
34. Birder LA, Perl ER. Expression of α_2-adrenergic receptors in rat primary afferent neurons after peripheral nerve injury or inflammation. J Physiol (Lond) 1999; 515:533-542.
35. Levine JD, Taiwo YO, Collins SD, Tam JK. Noradrenaline hyperalgesia is mediated through interaction with sympathetic postganglionic neuron terminals rather than activation of primary afferent nociceptors. Nature 1986; 323:158-160.
36. Tracey DJ, Cunningham JE, Romm MA. Peripheral hyperalgesia in experimental neuropathy: mediation by alpha 2-adrenoreceptors on post-ganglionic sympathetic terminals. Pain 1995; 60:317-327.
37. Gonzales R, Goldyne ME, Taiwo YO, Levine JD. Production of hyperalgesic prostaglandins by sympathetic postganglionic neurons. J Neurochem 1989; 53:1595-1598.
38. Gonzales R, Sherbourne CD, Goldyne ME, Levine JD. Noradrenaline-induced prostaglandin production by sympathetic postganglionic neurons is mediated by alpha 2-adrenergic receptors. J Neurochem 1991; 57:1145-1150.
39. Sherbourne CD, Gonzales R, Goldyne ME, Levine JD. Norepinephrine-induced increase in sympathetic neuron-derived prostaglandins is independent of neuronal release mechanisms. Neurosci Lett 1992; 139:188-190.
40. Cohen RH, Perl ER. Contributions of arachidonic acid derivatives and substance P to the sensitization of cutaneous nociceptors. J Neurophysiol 1990; 64:457-464.