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Role of 5-HT_{1A} and 5-HT_2 receptors of dorsal and median raphe nucleus in tolerance to morphine analgesia in rats

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Abstract

Several studies indicate that central serotonergic neurons have important role in morphine analgesia and tolerance. The aim of this study was to investigate possible role of 5-HT_{1A} and 5-HT_2 receptors in dorsal and median raphe nucleus on development of tolerance to analgesic effect of morphine using hot plate test. Chronic injection of 5-HT_{1A} receptor agonist 8-OH-DPAT (8-hydroxy-2-[di-*n*-propylamino]tetralin) (2, 4 and 8 μg/rat/day) to dorsal raphe nucleus (DRN) delayed tolerance to morphine analgesia, whereas injection of the same doses of 8-OH-DPAT to the median raphe nucleus (MRN) did not alter tolerance to morphine. In addition, chronic administration of ketanserin (1.5, 3 and 6 μg/rat/day), as a 5-HT_2 receptors antagonist, in DRN and MRN did not produce any significant effect. We conclude that 5-HT_{1A} receptors of DRN are involved in tolerance to antinociceptive effect of morphine. However, the exact mechanism of interaction between serotonergic and opioidergic systems is not clear and remains to be elucidated.

Keywords: 5-HT_{1A} receptors; 5-HT_2 receptors; Morphine; Analgesia; Tolerance; Dorsal raphe; Median raphe; Hot plate

1. Introduction

The development of tolerance to analgesic effect is a major problem associated with chronic administration of opioid analgesics. The exact mechanism underlying the development of morphine tolerance remains controversial. Among them, serotonin (5-HT) is particularly well studied, and this transmitter has been shown to widely participate in pain pathways and play a major role in mediating the analgesic action of morphine (Nemmani and Mogil, 2003; Dyuizen et al., 2004; Fang et al., 2005). It is well accepted that opioids establish part of their analgesic effect through stimulation of the serotonergic system (Arends et al., 1998). It has been reported that acute morphine administration enhances serotonin turnover as evidenced by an increase in its synthesis, release and metabolism (Arends et al., 1998), in particular in projection areas of the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) (Jolas and Aghajanian, 1997), but upon chronic morphine administration, a decrease in the release of 5-HT from the nerve terminals is observed (Jolas et al., 2000). There have been controversial results about the role of 5-HT in the development of morphine tolerance. For example, evoked serotonin activity by systemic administration of L-tryptophan or 5-hydroxytryptophan was found to accelerate and attenuate morphine tolerance, respectively (Li et al., 2001).

Serotonin's role in pain control has long been recognized but the significance of particular receptor types remains unclear. The presence of 5-HT_{1A} and 5-HT_2 receptors in the spinal cord and in brain areas involved in pain processes suggests a possible involvement of these receptors in pain modulation. Remarkably, 5-HT_{1A} receptor activation has variously been reported to facilitate, to inhibit, or to fail to modify pain responses (Bardin et al., 2001). The 5-HT_2 receptors exhibit unusual regulatory responses to changes in 5-HT availability, and repeated administration of 5-HT_2 antagonists produced a down-regulation of 5-HT_2 receptors, rather than the expected up-regulation (Toth and Shenk, 1994).

DRN and MRN are major sources of serotonergic projections to the many areas of brain. It has been generally accepted that the bulbospinal serotonergic system which originates in several brainstem structures, including the midbrain periaqueductal gray

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(PAG) and rostral ventromedial medulla (RVM), is involved in the control of the transmission of noxious inputs at the spinal dorsal horn level (Guimarães and Prado, 1999; Jeong et al., 2001; Freitas et al., 2005). However, anatomical studies support a serotonergic innervation of ventrolateral PAG from the raphe magnus nuclei (Beitz et al., 1986). The midbrain area encompassing the DRN and MRN is also involved in adaptations to stress such as opioid-induced analgesia (Basbaum and Field, 1984). The opioids indirectly influence serotonergic neurons in raphe nucleuses by inhibiting both GABAergic (gamma-amino butyric acid) and glutamatergic afferents (Tao and Auerbach, 2003). The initial balance between the strength of GABAergic and glutamatergic inputs determined the increase or decrease in serotonin release from this area (Tao and Auerbach, 2003). It has been shown that chronic administration of morphine increases GABAergic in the DRN/PAG area (Jolas et al., 2000). In the present study, we examined the possible role of DRN and MRN located 5-HT_{1A} and 5-HT_2 receptors on the development of tolerance to antinociceptive effects of morphine.

2. Materials and methods

2.1. Drugs

All chemicals were obtained from Sigma Chemical Co. (USA) except for morphine, which was purchased from Temad Co. (Tehran, Iran).

Solutions were prepared fresh on the days of experimentation. Morphine, 8-OH-DPAT (8-hydroxy-2-[di-*n*-propylamino] tetralin) and ketanserin were dissolved in physiological saline (0.9% NaCl). Intraperitoneal morphine (5 mg/kg, i.p.) was administered once daily for 25 days. Hot plate test was performed 30 and 5 min after intraperitoneal and intranucleus injection of drugs, respectively. For intra-DRN and MRN administration, drugs were infused 0.5 μl/min in a total volume of 1 μl.

2.2. Animals

The experiments were carried out on male Wistar rats weighing 225–275 g. Animals were housed in standard polypropylene cages, eight per cage, under a 12:12 h L/D schedule at an ambient temperature of 25±2 °C and were allowed food and water ad libitum. Following intra-DRN and MRN implantation of guide cannula, the animals were housed individually in each cage to avoid possible displacement or disruption of the cannula. All rats were accustomed to the testing conditions for 2 days before the experiment was conducted. Experiments were carried out in accordance with the guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No 85-23, revised 1985).

2.3. Surgical procedures

To implant guide cannulae, the animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg), and additional anesthetics (4 mg/kg, i.p.) was given when necessary. After they were deeply anaesthetized (loss of corneal and toe pad reflexes), rats were mounted in a Stoelting stereotaxic frame in the flat skull position. Cannulas (8 and 10 mm length of 23 gauge stainless steel tubing for DRN and MRN, respectively) were implanted to serve as guides for subsequent insertion of injection tube into the DRN or MRN. The coordinates for these sites were based on rat brain atlas (Paxinos and Watson, 1982): DRN, AP-7.64, DV 7.3, ML 4.0 at a 32° angle lateral to midline; MRN, AP-7.64, DV 9.6, ML 4.0 at a 24° angle lateral to midline. Experiments started 5 days after surgery.

2.4. Histology

All rats with guide cannulas were sacrificed at the end of the experiments. The brain dissects were prepared for all animals to confirm the exact implantation of guide cannulae in DRN and MRN. The brains were fixed in 10% formalin for 1 week with the injecting tube in situ. The location of the tip of the injecting tube was verified in serial sections. Only the results from nociceptive tests where the tip of the injecting tube was within the DRN and MRN area were used for statistical analysis.

2.5. Induction of tolerance

To induce tolerance to analgesic effect of morphine, rats ($n=8$ per group) were injected daily with morphine (5 mg/kg, i.p.). This dose has been found to cause profound analgesia with no side effects in normal mice (Drouin et al., 2001). The responses to thermal stimuli 30 min after the morning dose were recorded by using hot plate test.

2.6. Hot plate test

In this test, animals were individually placed on a hot plate maintained at a constant temperature (52±0.5 °C). The latencies to first hindpaw withdrawal during thermal stimulation were measured in seconds as an index of nociceptive threshold with cut off time of 50 sec. This index was referred to as the latency time (LT) and was expressed as percentage of maximum possible effect (%MPE).

$$\%\mathrm{MPE} = [(\mathrm{TL}-\mathrm{BL})/(50-\mathrm{BL})] \times 100$$

MPE= Maximum possible effect
TL= Test latency time
BL= Base latency time

2.7. Expression of data and statistics

Descriptive statistics and comparisons of differences between each data set were calculated by use of InState software. The data were expressed as %MPE±S.E.M., and were analyzed by repeated measure one-way ANOVA in each experiment. Statistical significance was accepted at the level of $P<0.05$. In the case of significant variation ($P<0.05$), the values were compared by Tukey's test.

3. Results

In the present study, the effect of intra-DRN and intra-MRN injection of 8-OH-DPAT and ketanserin, as 5-HT_{1A} agonist and 5-HT_2 receptor antagonist, respectively, on development of tolerance to analgesic effect of morphine were examined using hot plate test. Histological studies confirmed that guide cannulas were located exactly in the DRN and MRN area.

3.1. Effect of intra-DRN injection of 8-OH-DPAT

A group of rats (n=8) received one daily dose of morphine (5 mg/kg, i.p.) for 25 days to develop tolerance to morphine analgesic effect. Effect of different test doses of 8-OH-DPAT (2, 4, and 8 μg/rat, intra-DRN) on analgesic effect of morphine was examined in other three groups. As it has been shown in Fig. 1, analgesic effect of morphine declined to base of MPE on day ten and disappeared completely from day fifteen. In the groups of animals, which were received daily dose of morphine and intra-DRN injection of 8-OH-DPAT (2, 4, and 8 μg/rat) analgesic effect of morphine remained (F(3,32)=230.05, P<0.001; Tukey's, P<0.05, 0.01 and 0.001) until day 20 and tolerance appeared on day 25.

3.2. Effect of intra-MRN injection of 8-OH-DPAT

Daily intra-MRN co-injection of 8-OH-DPAT (2, 4, and 8 μg/rat) with morphine (5 mg/kg, i.p.) did not alter (F(3,32) =1.61, P=0.212; Tukey's, P>0.05) development of tolerance to analgesic effect of morphine and, similar to the control group (morphine+vehicle), tolerance appeared on day 15 (Fig. 2).

3.3. Effect of intra-DRN and intra-MRN injection of ketanserin

Effect of intra-DRN (Fig. 3I) and intra-MRN (Fig. 3II) administration of ketanserin (1.5, 3 and 6 μg/rat), on development of tolerance was investigated. Results showed that ketanserin not only had not any significant effect (F(3,32) =2.46, P=0.07; Tukey's, P>0.05) on development of tolerance to analgesic effects of morphine but also, at the doses of 3 and 6 μg/rat, decreased (F(3,32)=4.37, P<0.05; Tukey's, P<0.05) percent of MPE on the first 5 days (Fig. 3I).

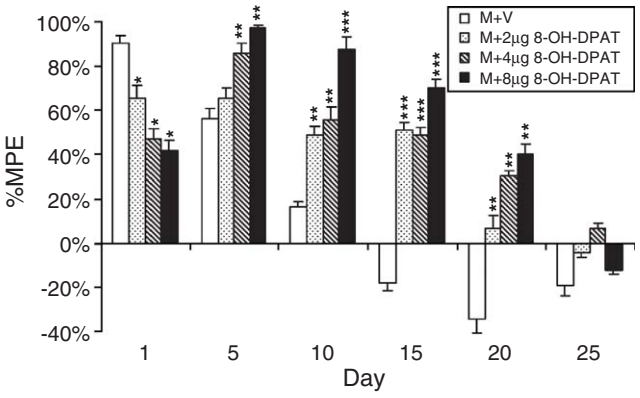

Fig. 1. The effect of daily intra-DRN injections of 8-OH-DPAT (2, 4 and 8 μg/rat) on tolerance to analgesic effect of morphine. Each bar represents % MPE±S.E.M. (n=8 per group), one-way ANOVA followed by Tukey's test. *P<0.05; **P<0.01; ***P<0.001 when compared with vehicle-treated rats. M=morphine, V=vehicle of 8-OH-DPAT.

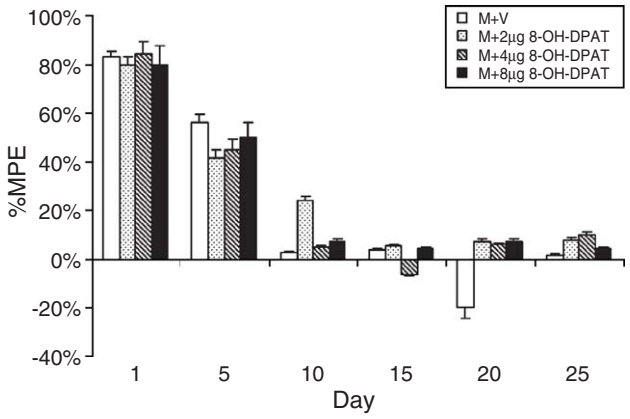

Fig. 2. The effect of daily intra-MRN injections of 8-OH-DPAT (2, 4 and 8 μg/rat) on tolerance to analgesic effect of morphine. Each bar represents % MPE±S.E.M. (n=8 per group), one-way ANOVA followed by Tukey's test. M=morphine, V=vehicle of 8-OH-DPAT.

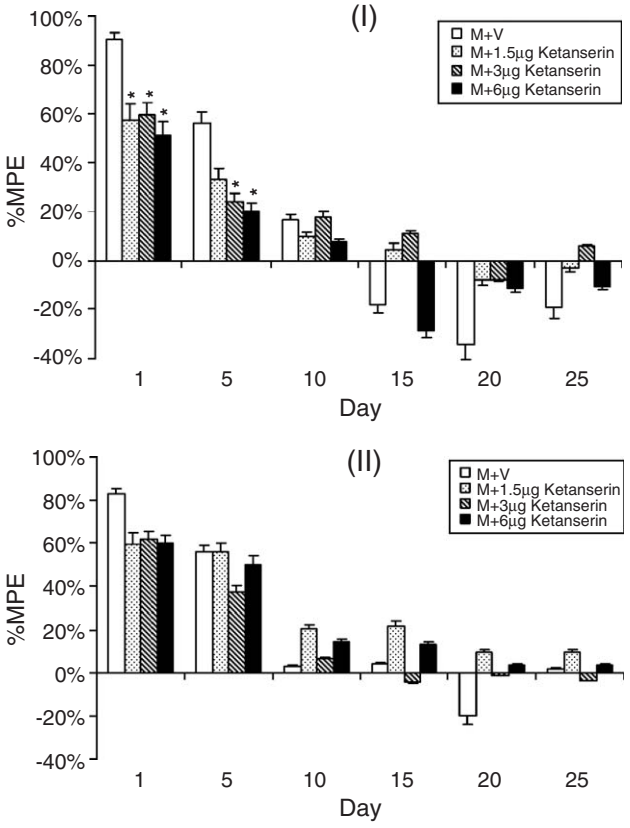

Fig. 3. The effect of daily intra-DRN (I) and intra-MRN (II) injections of ketanserin (1.5, 3 and 6 μg/rat) on tolerance to analgesic effect of morphine. Each bar represents %MPE±S.E.M. (n=8 per group), one-way ANOVA followed by Tukey's test. *P<0.05 when compared with vehicle-treated rats. M=morphine, V=vehicle of ketanserin.

4. Discussion

Midbrain serotoninergic neurons that project to the forebrain are mainly located in the DRN and MRN (Azmitta and Segal, 1978) and are involved in adaptations to stress such as opioid-induced analgesia (Basbaum and Field, 1984). It has been shown that chronic morphine administration leads to an increase in GABA tone and subsequently to the decrease in serotonergic activity in dorsal raphe nucleus (Jolas et al., 2000). Therefore, it may be hypothesized that dorsal and median raphe serotonergic system have important role in manifestations of morphine tolerance. Results obtained from our study show that, animals developed tolerance to the antinociceptive effect of morphine on day 15 and thereafter the degree of tolerance was enhanced. However, chronic intra-DRN microinjection of different doses of 8-OH-DPAT delayed the development of tolerance to the antinociceptive effect of morphine. These results are consistent to other studies suggesting a relation between tolerance to opioids analgesic effects and serotonergic system (Hynes and Fuller, 1982; Wang and Nakai, 1994; Arends et al., 1998; Singh et al., 2003; Bardin and Colpaert, 2004). Previous report shows that subcutaneous injection of 8-OH-DPAT, as 5-HT_{1A} receptor agonist produces an analgesic effect in the formalin model of tonic nociceptive pain (Bardin et al., 2001). It has also been reported that combined and continuous administration of morphine and 5-HT_{1A} receptor agonists inhibit the development of tolerance to morphine analgesia in trigeminal neuropathic pain (Deseure et al., 2004), but the effect of intra-nucleus (i.e., DRN and MRN) injection of 5-HT_{1A} receptor agonists on tolerance to morphine antinociceptive effects have not been well described. Although it is well appreciated that drugs can diffuse from the site of microinjection to adjacent neuronal structures, a few number of studies have been conducted to ensure that how far particular substances can travel in vivo. Accordingly, it can be suggested that 5-HT_{1A} receptors of DRN play an important role in the development of morphine tolerance. Serotonin–GABA interactions in the modulation of opioid analgesia at midbrain level, especially DRN, cannot be ruled out (Jolas et al., 2000; Kishimoto et al., 2001; Nemmani and Mogil, 2003).

We observed that 8-OH-DPAT on intra-MRN injection had not any significant effect on developing tolerance to analgesic effect of morphine. The contrast between the effect of morphine in the MRN and DRN is consistent with anatomical and behavioral evidence that different groups of serotonergic neurons have distinct functions. Morphine appears to act in the area of DRN to produce increased extracellular 5-HT in many forebrain sites, whereas in the area of MRN it is ineffective in enhancing 5-HT (Tao and Auerbach, 2003).

In our study, intra-DRN and MRN microinjection of ketanserin, as a 5-HT_2 receptor antagonist, has no effect on developing tolerance to morphine analgesia. This is in contrast to the results of an earlier study showing that systemic administration of ritanserine, as a 5-HT_2 receptor blocker, delays the development of tolerance to the opiate analgesic effect (Verbitskaia and Kudriashova, 1997). This controversy might be due to differences between drugs, route of administration, doses, analgesic tests and duration of treatment. It should be noted that ketanserin has also alpha-1-adrenoceptor antagonizing effect (Israilova et al., 2002). Thus, it is necessary to consider the possibility of alpha-1 receptors involvement in the observed effects.

Although serotonergic modulation of opioid analgesia is generally considered to occur in the spinal cord (Basbaum and Field, 1984; Sawynok, 1989; Kalyuzhny et al., 1996; Xu et al., 1998; Bardin and Colpaert, 2004), our present results offer a new mechanism for serotonergic modulation of opioid analgesia that also exists at the supra-spinal level. We propose that serotonin modulation may contribute to delay in the development of tolerance to morphine analgesia when administered in combination with antidepressants, especially selective serotonin reuptake inhibitors and 5-HT_{1A} receptor agonists. However, further research is required to ascertain the DRN location of these effects and elucidate the cellular features and neurophysiological pathways that opioids and 5-HT_{1A} ligands share in producing these effects.

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