Iranian Journal of Pharmaceutical Research (2006) 2: 117-121
Received: March 2005
Accepted: December 2005

Original Article

Effect of Spinal and Systemic Clonidine Administration on the Postoperative Analgesia in Morphine-dependent and Naïve Rats

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Abstract

Post operative pain and its control remains one of the most important issues in the field of surgery and health care system. Formalin test has been used as a method for assessing pain and analgesia in rats. Systemic and spinally administered clonidine, an α2-adrenergic agonist, is proved to enhance postoperative analgesia. It has also been shown that morphine-dependent rats experience more chronic pain than naïve rats.

In this study we have explored the effect of certain doses of i.t. and i.p. clonidine on postoperative pain in morphine-dependent and naïve rats. Rats were addicted by oral morphine. For one group, an intrathecal catheter was inserted 48 hours before surgery. A 1-cm longitudinal incision was made through skin, fascia and muscle of the plantar aspect of the right hind paw. Both morphine-dependent and naïve rats received i.t. (17μg.kg^{1}) or i.p. (0.7 mg.kg^{1}) clonidine immediately and 15 minutes befor formalin test, respectively. Then, formalin-induced behaviors were recorded. In both phases of formalin test in morphine-dependent rats, i.p clonidine produced more analgesic effect, compared to i.t. injection ($P<0.01$). On the other hand i.p. clonidine caused more analgesic effect in phase II of formalin test in morphine-dependent than naïve rats ($P<0.05$).

Clonidine may potentiate the analgesic effect of chronically administered morphine. Also it seems that clonidine acts better in supraspinal level, compared to the spinal level.

Keywords: Formalin test; Clonidine; Post-operative pain; Opioid-related disorders; Rat.

Introduction

Postoperative pain is a common form of acute pain. Much progress has been made in our understanding of acute pain through experimental animal models (1). The suggestion that peripheral sensitization, central sensitization, plasticity, and pre-emptive analgesia occur in postoperative pain, has been initiated by observations in pre-clinical models of chemical irritation, inflammation and thermal sensitization.

Post-operative pain control has two major goals. The first is to relieve the patient of as much suffering as possible. The second goal is to alleviate untoward effects of poorly managed pain, including excessive stress response, which in turn could have adverse physiologic effects on vital organ function, immune function, and ultimate outcome after a surgical procedure (2, 3).

Morphine is a potent analgesic with competitive agonist action at the μ receptor, which is thought to mediate many of its' other actions of respiratory depression, euphoria,

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inhibition of gut motility and physical dependence (4).

The standard pillar of post-operative treatment of severe pain is the use of opiates. One of the most commonly abused drugs in opioid group is morphine. Dependence is a biologic phenomenon often associated with "drug abuse" (5).

The substance abuse patient can manifest cross-tolerance to drugs, making it difficult to predict analgesic or anesthetic requirements (6, 7).

Clonidine, an α2-adrenergic agonist, has been used as an analgesic for post-operative pain. It has been demonstrated to produce anti-nociception, after epidural and intrathecal administration (8).

The results of a variety of studies have shown that the locus ceruleus possess not only opioid receptors, but also the largest cluster of noradrenergic neurons and noradrenergic neuronal activity within the LC plays a critical role in opioid dependence and withdrawal. Clonidine is a potent inhibitor of the firing rate of LC neurons, being highly active during morphine withdrawal (9).

Clonidine has a superior penetration via the Blood-Brain –Barrier, when administered i.p. However, it has been reported that spinal noradrenergic system modulates spinal nociceptive processing (10).

Initial management in opioid-dependent patients with cancer is difficult due to issues of distinguishing tolerance from disease progression, concurrent methadone, and drug-seeking behavioural patterns (11).

High doses of opiates could be administered (s.c., i.m., or p.o. infusion) for post-operative analgesia in opiate addicts with no fear of respiratory depression. Therefore, clonidine is a particularly good drug for the treatment of withdrawal (12).

In a previous study, we concluded that morphine-dependent rats experience significantly more post-operative pain than naïve rats in formalin test (13).

The present study compares postoperative pain scores in morphine-dependent and naïve rats and seeks the site of action of clonidine after intrathecal or intraperitoneal injection.

Experimental

Animals

All experiments were performed on adult male Sprague- Dawley rats, weighting 300 ±21 g. Before surgery, the animals were housed in pairs.

Animals were kept at constant ambient temperature (24±1ºC), under a 12-h light/dark cycle with free access to food and water. Rats were allocated to one of four groups (n=6). Groups I and II were morphine-dependent rats. Group III and IV were native rats. Groups I and III received a bollus of intrathecal clonidine, whereas groups II and IV received a bollus of intraperitoneal clonidine.

Drugs

Clonidine and morphine sulfate were purchased from sigma-Aldrich (St. Louis, MO). Clonidine was dissolved in a distinct volume of ethyl alcohol and saline (5%) (14).

Induction of morphine dependence

Oral administration of morphine was used to induce morphine dependence (15). Glucose 5% *w/v* in ordinary tap water was used to mask the bitter taste of morphine sulfate which was chronically administered in their drinking fluid. The opiate was given in an increasing concentration (48 h apart) of 0.1, 0.2, 0.3 and finally 0.4 $mg.ml^{1}$. the last concentration was used until the end of a 20-days experimental period (15).

Intrathecal catheter insertion

For implantation of intrathecal catheters, 48h before operation, rats were anesthetized with ketamin 50 $mg.kg^{1}$ and chlorpromazine 10$mg.kg^{1}$. The Atlanto-occipital membrane was incised at the midline, using the tip of an 18-gauge needle as the cutting edge. A catheter (PE-10) was inserted through the slit, into the subarachnoid space and advanced caudally to the L6-S1 level of the spinal cord. Correct placement of the catheter was checked by aspiration of cerebrospinal fluid from the catheter at the end of surgery and by dye injection at the end of the experiment. Only animals with a normal activity after the catheter insertion were studied.

Surgery

All rats were anesthetized with diethyl ether. A 1-cm longitudinal incision was made with a number 11 blade, through skin, fascia and muscle of the plantar aspect of the hind paw. After hemostasis with gentle pressure, the skin was apposed with 2 mattress sutures of 5-0 nylon on an HS-26 needle (1).

Formalin test

At the beginning of an experiment, the rats (morphine-dependent and naïve) were injected with a dose of clonidine, either intrathecally (13µg) or intraperitoneally ($0.7mg.kg^{1}$) . Immediately after intrathecal injection or 15 min after intraperitioneal injection, the rats were placed in restraint cylinders and administered formalin (50 µl of a 2.5% solution in saline) subcutaneously into the planter surface of the right hind paw, then placed individually in a glass cylinder (20 cm wide, 25cm long) on a flat glass floor and a mirror was arranged in a 45° angle under the cylinder to allow clear observation of the paws of the animals.

Pain response was scored immediately after formalin injection for a period of 50min. A nociceptive score was determined for each 5-min time block by measuring the amount of time spent in each of four behavioral categories: 0=the injected paw is not favoured, 1=the injected paw rests lightly on the floor and little or no weight is placed on it, 2=the injected paw is elevated and not in contact with any surface, and 3=the injected paw is licked, bitten or shaken. A weighed average nociceptive score, ranging from 0 to 3, was calculated by multiplying the time spent in each category by the category weight, and then dividing by the total time for each 5-min time block. The mean ±SEM of scores between 0- 5 and 15- 45min after formalin injection has been presented (16-22).

Statistical analysis

Behavioral data were compared using the nonparametric; Tukey's test (23). All the results are expressed as Mean ± SE, when appropriate. $P<0.05$ was considered statistically significant.

Results and Discussion

Throughout the experimental period and after intrathecal catheterization, the animals remained well groomed and appeared to maintain normal food and water intake.

In animals administered clonidine, in comparison with the control group which received solvent before formalin test, there was an initial dip in the pain intensity rating during the first 5-min block after formalin injection, followed by a decrease in the number of events in blocks 4 through to 9.

Anti-nociceptive effect of clonidine (i.t and i.p) on naïve rats has been shown in figure1.

Clonidine produced a significant antinociceptive effect, compared to the ($p^{*}<0.05$, $p^{**}<0.01$, $p^{***}<0.001$). Also i.p clonidine produced more anti-nociceptive effect that the i.t group, in phase II of the formalin test ($p^{+}<0.05$, $p^{++}<0.01$, $p^{+++}<0.001$).

I.p and i.t clonidine decreases pain intensity, compared to the vehicle, with $p^{***}<0.001$ and $p^{**}<0.01$, respectively, in morphine dependents rat.

Intraperitoneal administration of clonidine to morphine dependent rats,

results in a more significantly analgesic effect in both phases of formalin test, than the intrathecal administration ($p^{+++}<0.001$, $p^{+}<0.05$) (figure 2) .

Figure 3 shows that intrathecal clonidine significantly decreases the intensity of pain in both groups (naïve and morphine dependent), compared to the vehicle, in both phases ($p^{*}<0.05$, $p^{**}<0.01$, $p^{***}<0.001$) . I.t administration of clonidine has produced singificantly greater anti-nociception in naïve rats, than the mrorphine-dependent rats, during phase I of formalin test ($p^{+}<0.05$).

I.p clonidine caused a significant analgesic effect in both groups of rats (naïve and morphine –dependent), in comparison to the vehicle ($P^{***}<0.001$), and i.p clonidine produced a greater analgesic effect in both phases of formalin test in morphine-dependent rats, than the naïve group ($p^{+}<0.05$)(Figure 4)

The results of this study, in one strain of rats, showed that different routes of clonidine administration can produce different anti-nociceptive effects.

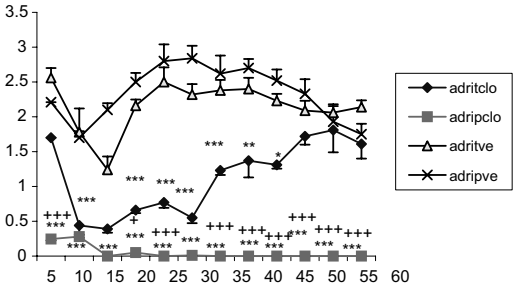

Figure 1. Comparison between the pain intensity rating by formalin test, after intrathecal and intraperitoneal injection of clonidine or vehicle in naïve rats.
Pain response was measured after i.t and i.p administration of clonidine or vehicle. I.t and i.p clonidine, both decrease pain intensity, significantly, compared to the vehicle (P^{+++}<0.001) in naïve rats. I.p clonidine showed a significant decrease in pain intensity, during the formalin test, compared to the i.t group .

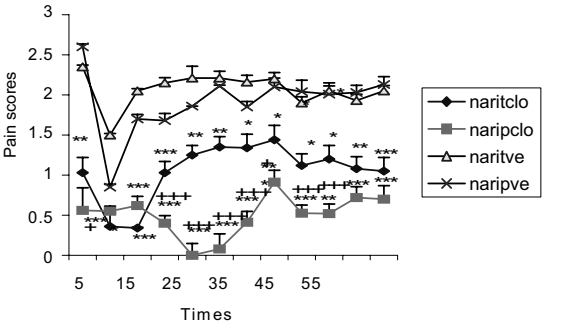

Figure 2. Comparison between the pain intensity rating by formalin test, after intrathecal and intraperitoneal injection of clonidine or vehicle in morphine dependent rats.
Pain response was measured immediately after making an incision, followed by an injection of intrathecal (i.t. group) and intraperitoneal (i.p. group) clonidine or vehicle. I.t and i.p clonidine both decrease pain intensity significantly, compared to the vehicle (P^{++}<0/01 and P^{+++}<0.001 respectively), in morphine-dependent rats. The i.p clonidine group showed a significant decrease in pain intensity during both phases of formalin test, in comparison with the i.t group

Following either intraperitoneal or intrathecal drug administration, the antihyperalgesic effect in the acute and chronic pain models differ significantly between the clonidine and solvent groups. The present finding indicates that clonidine produces anti-nociception in formalin test, after making an incision. Shannon et al. showed that the effects of clonidine in formalin test, appear to be mediated by mechanisms different from those which mediate its' effects in other nociceptive tests (8) .

Anesthesia and pain management in patients with previous opiate consumption or abuse, present a complex problem, regarding drug therapy (24, 25).

Casosola et al. used epidural bupivacaine, three times the normal dosage, to provide adequate post-operative analgesia in opiate-dependent patients (24).

The chronic effect of opioids appears to involve at least two steps. In the first step, called desensitization, the receptor becomes uncoupled from the GTP-binding subunit. In the second step, known as down regulation, the receptors are removed from the cell surface where they may be degraded or later recycled to the surface. This step then results in a loss of receptor binding sites. Reduction in B_{max} supports the author's previous study that revealed a higher perception of pain in morphine-dependent rats (26, 13).

Morphine-dependent and naïve rats achieved similar pain relief, after the intra-peritoneal clonidine injection in phase I of the formalin test. But in phase II, clonidine (i.p.) produced a greater analgesic response in morphine-dependent rats, than the naïve group. Nociceptive stimuli such as formalin-induced pain, attenuate tolerance to morphine (27). Activation of I_2 imidazoline receptors, enhances supraspinal morphine analgesia in mice (28).

It has been reported that the action of analgesics (e.g. opioids) differs in the first and late phases of the formalin test (21).

In the present study, the i.t. injection of clonidine produces anti-nociceptive effects in acute pain phase of naïve rats. these results suggest that α2-adrenoceptors and/or imidozoline receptors play an important role in spinal cord pain processing in naïve rats.

Spinally administered α2 adrenergic agonists alter pain transmission, by acting pres-ynaptically on C-fibers, to reduce transmitter release and post-synaptically to hyperpolarize dorsal horn nociceptors (29).

Although clonidine acts both spinally and supraspinlly in both phases of formalin test in morphine-dependent rats, i.p clonidine produced a greater analgesic effect comparing with the i.t. injection.

The differential effect on intrathecal and intraperitoneal clonidine, suggests that clonidine has a more efficient supraspinal than spinal effect. It has been shown that multiple α_2 adrenergic receptor subtypes have a role in the control of

nociception, motor behaviour and hippocampal synthesis of noradrenaline (30).

These findings indicate that potent α2-adrenocepor agonists, such as clonidine, may be useful alternatives to opioid-based agents for the control of acute post-operative pain and highlights the importance of assessing the risk-benefit ratio, when selecting the dosing regimens of analgesis.

Acknowledgement

The authors would like to acknowledge Dr. Motamedi, the head of Neuroscience Research Center and Dr. Khansefid for their kind assistance.

The authors also thank Ms. Masoudnia, for her worthy technical guidance.

This study was financially supported by the Neuroscience Research Center of Shaheed Beheshti University of Medical Sciences.

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