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Short communication

Dipyrone into the nucleus raphe magnus inhibits the rat nociceptive tail-flick reflex

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Abstract

Recent studies suggest that non-steroidal anti-inflammatory drugs have central sites of action which contribute to their analgesic efficacy. In the present study microinjections of the non-steroidal anti-inflammatory drug, dipyrone, were made into the medullary nucleus raphe magnus of lightly pentobarbital-anesthetized rats; 25, 50, 100 and 200 μg of dipyrone dose-dependently inhibited the nociceptive tail-flick withdrawal reflex. These results suggest that dipyrone modulates bulbospinal neurons in the nucleus raphe magnus to inhibit spinal nociceptive reflexes; thus, new routes of administration and methods of application may be possible for these analgesic agents.

Keywords: Descending inhibition; Nociception; Non-steroidal anti-inflammatory drug; Raphe magnus

1. Introduction

Non-steroidal anti-inflammatory drugs traditionally are thought to produce their analgesic effects by inhibiting prostaglandin synthesis in the periphery; as a result, they commonly are referred to as 'peripheral analgesics'. However, evidence has accumulated which suggests that non-steroidal anti-inflammatory drugs also have central sites of action in the spinal cord and brainstem which may contribute to their analgesic efficacy (e.g., Bannwarth et al., 1995; Carlsson et al., 1986; Carlsson and Jurna, 1987; Malmberg and Yaksh, 1992; Neugebauer et al., 1994; Tortorici and Vanegas, 1994; Tortorici et al., 1996). It is well-known that supraspinally organized descending inhibitory systems exist, which, when activated, can powerfully modulate spinal nociceptive transmission (e.g., Gebhart, 1982 for review). The midbrain periaqueductal gray and a bulbospinal relay in the medullary nucleus raphe magnus have been established to play important roles in the centrifugal modulation of spinal nociceptive transmission (e.g., Basbaum and Fields, 1978, 1984; Gebhart, 1982; Hosobuchi et al., 1977; Mayer, 1979; Yaksh and Rudy, 1978).

Several studies suggest that non-steroidal anti-inflammatory drugs may activate descending inhibition from the midbrain periaqueductal gray to produce antinociception (Carlsson et al., 1986; Carlsson and Jurna, 1987; Tortorici and Vanegas, 1994; Tortorici et al., 1996). However, to date, no studies have examined directly the role that the medullary nucleus raphe magnus plays in the antinociceptive effects produced by non-steroidal anti-inflammatory drugs. In the present study microinjections of the non-steroidal anti-inflammatory drug, dipyrone, were made into the nucleus raphe magnus to determine if dipyrone dose-dependently inhibits the nociceptive tail-flick withdrawal reflex.

2. Materials and methods

Experiments were performed on adult, male Sprague-Dawley rats (Sasco Labs, Omaha, NE, USA), weighing between 300–400 g. Rats initially were deeply anesthetized with pentobarbital (45 mg/kg, i.p.) for craniotomy and cannulation of the femoral artery and vein. The rats subsequently were maintained in a lightly anesthetized state (i.e., corneal and flexion reflexes present) throughout

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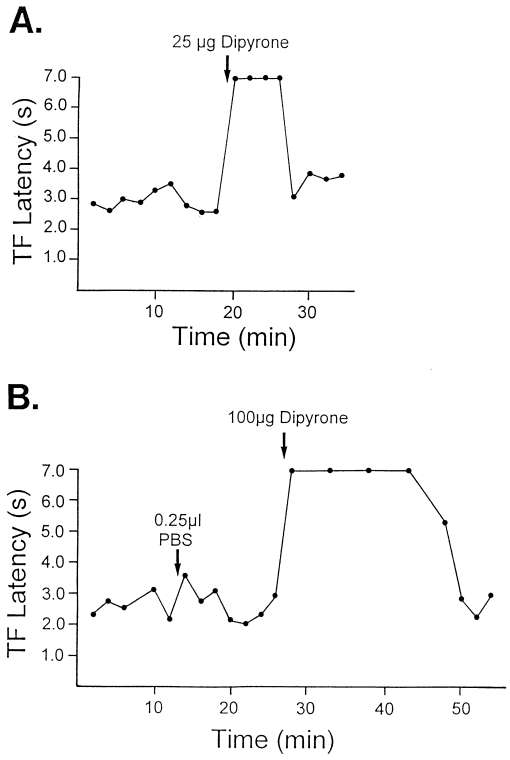


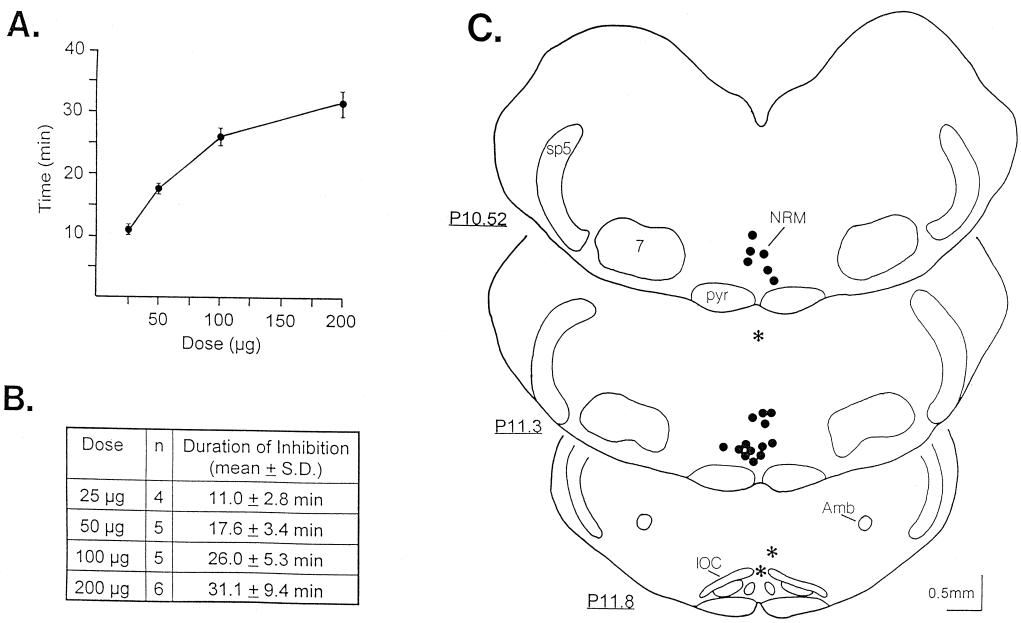
Fig. 1. Effects of dipyrone (25 and 100 μg, 0.25 μl) microinjected into the nucleus raphe magnus (NRM) on the tail-flick (TF) latency. In A and B, ● represent the TF latency before and after the dipyrone microinjections; they are connected by a solid line. In B, the vehicle (0.001 M PBS, 0.25 μl) was microinjected into the same site in the NRM as dipyrone; no changes in TF latency were observed.

the duration of the experiments with a continuous infusion of pentobarbital (3–6 mg/kg per h, i.v.). Mean arterial blood pressure was monitored throughout the experiments and body temperature was maintained at 36–38°C.

Rats were placed in a stereotaxic instrument with the incisor bar set 3.3 mm below the horizontal plane. A guide cannula (26-gauge) was placed in the medulla stereotaxically directed at the nucleus raphe magnus. Microinjections were made into the medulla through an injection cannula (33-gauge) inserted through the guide cannula; the injection cannula was measured to extend 2 mm beyond the end of the guide cannula.

The tail-flick reflex was evoked by focused radiant heat applied to the dorsal surface of the tail every 2 min at one of 6 tail positions approximately 1 cm apart; this protocol produced stable tail-flick latencies throughout the course of an experiment (see Fig. 1). To minimize damage to the skin of the tail, inhibition of the tail-flick reflex was defined as a tail-flick latency ≥ 7 s. Following the establishment of a stable baseline tail-flick latency, microinjections of dipyrone (25, 50, 100 and 200 μg; 0.25 μl each, Sigma) were made into the nucleus raphe magnus. The injections were made over a period of several minutes, and the injection cannula was left in place for an additional minute after the injection was complete. The dipyrone was dissolved in 0.001 M phosphate-buffered saline and the pH was adjusted to 7.4.

At the conclusion of the experiments the rats were killed with an overdose of sodium pentobarbital (100



Dose	n	Duration of Inhibition (mean ± S.D.)
25 µg	4	11.0 ± 2.8 min
50 µg	5	17.6 ± 3.4 min
100 µg	5	26.0 ± 5.3 min
200 µg	6	31.1 ± 9.4 min

Fig. 2. Effects of varying doses of dipyrone microinjected into the nucleus raphe magnus (NRM) on the tail-flick (TF) latency. Mean durations of inhibition for 25, 50, 100 and 200 μg doses of dipyrone are shown in A and B. Microinjection sites for the data portrayed in A and B are shown in C and are indicated by ●. * indicate sites of dipyrone microinjections that did not inhibit the TF reflex.

mg/kg, i.v.). Anodal electrolytic lesions (75 μA DC for 10 s) were made to mark the sites of microinjection in the medulla. The brains were removed, fixed in 10% formalin, cut in 50 μm coronal sections, mounted on glass slides and counterstained with cresyl violet for histologic verification of the site of microinjection. All data are presented as means ± S.E.M.

3. Results

Representative examples of inhibition of the tail-flick reflex following the microinjection of dipyrone into the nucleus raphe magnus are shown in Fig. 1. In the examples shown, control tail-flick latencies were 2–3 s; dipyrone was microinjected into the nucleus raphe magnus and, when tested 1 min later, the tail-flick reflex was inhibited. Inhibition of the tail-flick reflex lasted approximately 7 min and 16 min for the 25 μg (Fig. 1A) and 100 μg (Fig. 1B) doses of dipyrone, respectively.

Control microinjections of vehicle (0.001 M phosphate-buffered saline, 0.25 μl, pH 7.4) into the nucleus raphe magnus had no effect on the tail-flick latency (see Fig. 1B). Similarly, control microinjections of phosphate-buffered saline (0.1 M, 0.25 μl, pH 7.4, 507 mOsm), having an osmolality comparable to that of 100 μg of dipyrone (503 mOsm), had no effect on the tail-flick latency. Thus, the inhibition of the tail-flick reflex produced by dipyrone was not the result of mechanical stimulation produced by the microinjection, and was not due to the osmolality of the dipyrone solution.

The mean durations of inhibition of the tail-flick reflex for varying doses of dipyrone microinjected into the nucleus raphe magnus are shown in Fig. 2A and B. The sites of microinjection in the medulla for the data portrayed in panels A and B are shown in panel C; in one experiment two microinjections of dipyrone were made into the same site. The duration of inhibition of the tail-flick reflex for varying doses of dipyrone occurred in a dose-dependent manner; increasing doses of dipyrone produced increasing durations of inhibition. Microinjections of dipyrone in sites *outside* of the nucleus raphe magnus failed to produce inhibition of the tail-flick reflex (see asterisks in Fig. 2C); thus, inhibition of the tail-flick reflex produced by dipyrone exhibited site specificity for the nucleus raphe magnus.

Dipyrone microinjections into the nucleus raphe magnus frequently were associated with decreases in blood pressure; however, since in some animals inhibition of the tail-flick reflex occurred in the absence of any changes in blood pressure, a blood pressure decrease was not requisite for inhibition of the tail-flick reflex. Microinjections of 25, 50, 100 and 200 μg of dipyrone into the nucleus raphe magnus produced mean decreases in blood pressure of 12.5 ± 10.4, 10.0 ± 6.1, 25.0 ± 12.8 and 44.2 ± 19.6 mmHg, respectively.

4. Discussion

Dipyrone is a pyrazolone derivative; it commonly is classified as a peripherally acting drug since its major metabolites, 4-methylaminoantipyrin and 4-aminoantipyrin, inhibit prostaglandin synthesis in a manner similar to aspirin (Shimada et al., 1994). Dipyrone has significant analgesic and antipyretic effects; however, in contrast to aspirin, it is devoid of significant anti-inflammatory effects. The reasons for the differences between dipyrone and aspirin are unclear; however, it has been hypothesized that the analgesic and antipyretic effects produced by dipyrone may be centrally mediated effects. As a result, dipyrone has been the focus of several studies investigating possible central antinociceptive effects. To date, relatively few studies investigating possible sites of action in the brainstem have been done; those studies that have been done have focused on the possible involvement of the midbrain periaqueductal gray in the antinociceptive effects produced by dipyrone (Carlsson et al., 1986; Carlsson and Jurna, 1987; Tortorici and Vanegas, 1994; Tortorici et al., 1996).

Carlsson et al. (1986) examined the effects of dipyrone in intact and spinally transected rats to determine a possible central site of action for dipyrone. Dipyrone administered either by intraperitoneal (10–40 mg/kg) or intrathecal (50–400 μg) injection to intact rats dose-dependently prolonged the tail-flick latency. However, when administered to spinally transected rats, intrathecally administered dipyrone failed to inhibit the tail-flick reflex. The authors concluded that the antinociceptive effects produced by dipyrone were due to a supraspinal site of action. To investigate possible supraspinal sites of action, neuronal activity was recorded in the periaqueductal gray and in the substantia nigra. Intravenously administered dipyrone increased neuronal activity in the periaqueductal gray and decreased neuronal activity in the substantia nigra. Dipyrone administered by microinjection directly into the periaqueductal gray (15–100 μg) prolonged the tail-flick latency and depressed C-fiber-evoked activity in ascending axons. The authors concluded that the activation of descending inhibition originating in the periaqueductal gray may contribute to the antinociceptive effects produced by dipyrone.

In a follow-up study, Carlsson and Jurna (1987) further examined the role that the periaqueductal gray plays in the antinociceptive effects produced by the pyrazolone derivatives, aminophenazone and dipyrone; interactions with the opioid, morphine, also were examined. Microinjections of the local anesthetic, procaine, into the periaqueductal gray abolished the increase in tail-flick latency produced by intraperitoneally administered dipyrone and aminophenazone, but had no effect on the antinociceptive effects produced by morphine. Threshold doses of intrathecally administered morphine potentiated the effects of threshold doses of dipyrone administered either intraperi-

toneally or by microinjection directly into the periaqueductal gray. Similarly, intrathecally administered morphine potentiated the effects of intravenously administered dipyrone on noxious-evoked activity in ascending axons.

Tortorici and colleagues (Tortorici and Vanegas, 1994; Tortorici et al., 1996) recently examined the effects of dipyrone on off- and on-cell activity in the rostral ventromedial medulla; off- and on-cells in the medulla have been proposed to inhibit and facilitate nociceptive transmission, respectively (e.g., Fields et al., 1983). Intravenously administered dipyrone and microinjections of dipyrone into the periaqueductal gray produced a dose-dependent increase in off-cell activity; the activity of on-cells remained at a low level. The authors suggest that dipyrone inhibits the tail-flick reflex by altering the mechanisms that decrease off-cell activity and increase on-cell activity.

In the present study, dipyrone microinjected directly into the nucleus raphe magnus dose-dependently inhibited the nociceptive tail-flick reflex. These results support the findings of the above-mentioned studies. The present results are consistent with the suggestion that dipyrone activates a periaqueductal gray-nucleus raphe magnus bulbospinal pathway to centrifugally modulate spinal nociceptive transmission.

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