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Low dose aspirin attenuates escape/avoidance behavior, but does not reduce mechanical hyperalgesia in a rodent model of inflammatory pain

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Abstract

The present experiment examined the effect of aspirin on the escape/avoidance behavioral response to a mechanical stimulus (476 mN von Frey monofilament) in the place escape avoidance paradigm (PEAP) following subcutaneous administration of carrageenan (CARR). Forty-one male Sprague–Dawley rats received subcutaneous injection of CARR or saline in the left hindpaw and 3 1/2 h later were administered aspirin (0, 50 or 150 mg/kg). Thirty minutes later, animals were tested in the PEAP and then the mechanical paw withdrawal threshold was measured. Compared with Saline vehicle-treated controls, all CARR-treated animals displayed hyperalgesia, as reflected by enhanced responding to mechanical stimulation applied to the CARR-injected paw. Mechanical hyperalgesia was significantly reduced by the pre-treatment of 150 mg/kg, but not 50 mg/kg aspirin. In the PEAP, CARR vehicle-treated animals avoided a preferred location of the test chamber that was associated with mechanical stimulation of the hyperalgesic paw. The shift from a preferred dark side of the chamber to the light side was attenuated by pre-treatment with both doses of aspirin (50 and 150 mg/kg). The lack of anti-hyperalgesia and avoidance behavior with 50 mg/kg aspirin suggests a decrease in the aversive nature of mechanical stimulation of the afflicted paw. It is suggested that the mechanisms underlying the affective/motivational dimension of nociception (escape/avoidance) can be dissociated from the processing of nociceptive information related to withdrawal responding.

Keywords: Inflammation; Mechanical Hyperalgesia; Place Avoidance; Aspirin; Carrageenan; Affective nociceptive processing

Inflammatory pain conditions are characterized by a reddening of the skin, edema, spontaneous pain, decreased motor activity, hyperalgesia, and allodynia [1,14,15,17]. Common nociceptive tests such as the tail-flick, hot plate, and mechanical paw withdrawal tests assess the change in sensory threshold to thermal and mechanical stimuli [2,9,10,18]. These tests have been useful to examine the analgesic properties of treatments for the relief of inflammatory pain. For example, aspirin's effectiveness is largely based on its ability to block prostaglandin (PG) synthesis, thereby reducing inflammation and stimulus-evoked sensations of pain [17].

Although the ability of aspirin to decrease inflammation, attenuate hyperalgesia, and increase activity has been documented [12,16,17], aspirin's ability to attenuate the aversive quality of a noxious stimulus has been ignored. The recently developed place escape/avoidance paradigm (PEAP) is a behavioral test paradigm that measures the aversiveness of mechanical nociceptive stimuli [4,9,10]. This paradigm presents an animal with the 'choice' of remaining within the preferred dark side of the chamber where it receives mechanical stimulation to the hyperalgesic paw, or crossing over to the light side of the chamber where it is stimulated in the contralateral paw. The aversiveness of stimulus-evoked pain is characterized by the amount of time that the animal remains in the light side of the chamber. In the PEAP, compounds that decrease mechanical hyperalgesia also attenuate escape/avoidance behavior of a noxious stimulus. For example, morphine and gabapentin decrease mechanical allodynia, and in the PEAP, significantly attenuate the shift from the dark side of the chamber to the light side [10]. Therefore, decreased escape/avoidance of the noxious stimulus is directly related to the decrease in enhanced sensitivity to mechanical stimuli following nerve injury by morphine and gabapentin treatment. When accompanied with a sensory measurement of mechanical sensitivity, the

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PEAP provides a unique method to measure the relationship between the affective/motivational and sensory dimensions of pain processing [4]. Therefore, the purpose of the present study is to further characterize the PEAP by evaluating the anti-nociceptive and anti-aversive properties of aspirin in a rodent model of inflammatory pain induced by subcutaneous administration of carrageenan into the hindpaw.

Forty-one male Sprague–Dawley rats weighing 350–500 g (University of Texas at Arlington Vivarium) were housed in pairs and allowed free access to food and water throughout the study. Room temperature and humidity were maintained at 21°C and 70%, respectively. All procedures were approved by the University of Texas at Arlington Animal Use and Care Committee.

Aspirin and carrageenan (CARR) were prepared on the day of testing. An inflammatory condition was produced by the subcutaneous (s.c.) administration of 3% lambda CARR (0.12 ml) (Sigma Chemical Company, St. Louis, MO, USA) into the plantar surface of left hindpaw under light halothane anesthesia ($n = 30$). Eleven additional animals served as control animals and were administered an equal volume of saline (0.9%). Aspirin was dissolved in a 1% sodium bicarbonate solution to form either a 10 or 30 mg/ml solution and was delivered s.c. (5 ml/kg).

Animals randomly received a coded aspirin solution (0, 50 or 150 mg/kg) 3 1/2 h after CARR treatment. Thirty minutes later, animals were tested within the PEAP test for 30 min, as described previously [9,10]. Animals were allowed unrestricted movement within a chamber ($30 \times 30 \times 30$-cm Plexiglas chamber), half painted white (light area), half black (dark area). Testing started immediately with mechanical stimulation (476 mN von Frey monofilament) applied to the plantar surface of the hindpaws at 15-sec intervals and whenever the animal crossed from one side of the chamber to the other. Therefore, when the animal was within the dark side of the chamber, the mechanical stimulus was applied to the hyperalgesic paw; when the animal was within the light side of the chamber, the mechanical stimulus was applied to the contralateral paw. Following the PEAP testing, the threshold to respond to mechanical stimuli was assessed using the previously described up/down method [3,9,10] with eight von Frey monofilaments (5, 7, 13, 26, 43, 64, 106, and 202 mN). The experimenter was blind to the content of each solution and experimental conditions.

The amount of time spent within the light side of the test chamber during the PEAP test and mechanical paw withdrawal thresholds (MPWT) for right and left paws were analyzed using one-way ANOVAs followed by LSD post-hoc comparisons. General motor activity was analyzed using a repeated measure ANOVA, with individual one-way ANOVAs at each time point. The significance level for all analyses was set at 0.05.

The analysis of MPWT for all experimental conditions revealed a significant main effect for group ($P < 0.05$). There was not a significant difference in MPWT for the right (contralateral) paw ($P > 0.05$). However, there were significant differences in MPWT for the treated paw as all CARR-treated animals displayed significantly lower MPWTs for the left paw than saline-treated animals ($P < 0.0001$). Mechanical hyperalgesia of the left paw was significantly reduced by a pre-treatment with 150 mg/kg, but not 0 or 50 mg/kg of aspirin (Fig. 1).

The overall analysis of percent time spent within the light area of the PEAP chamber during the first 5 min of the test revealed no significant differences among groups ($P > 0.05$). However, there were significant differences between groups by the end of testing ($P < 0.05$). As illustrated in Fig. 2, the overall analysis of percent time spent within the light area of the test chamber for the entire 30-min test period revealed a significant main effect for group ($P < 0.007$). Further analysis of group differences in the PEAP indicated that CARR vehicle-treated animals avoided a preferred dark location of the test chamber significantly more than Saline vehicle-treated animals ($P < 0.05$). However, there was not a significant difference in the amount of time spent within the light side of the chamber for CARR-treated animals receiving 50 mg/kg or 150 mg/kg of aspirin ($P > 0.05$). In other words, aspirin pre-treatment attenuated the shift from a preferred dark side to the light side of the PEAP chamber.

The analysis of total crosses from the light to dark side of the chamber revealed a significant main effect for group ($P < 0.001$), time ($P < 0.001$) and a significant group × time interaction ($P < 0.001$). As illustrated in Fig. 3, Saline vehicle-treated animals made significantly more line crosses

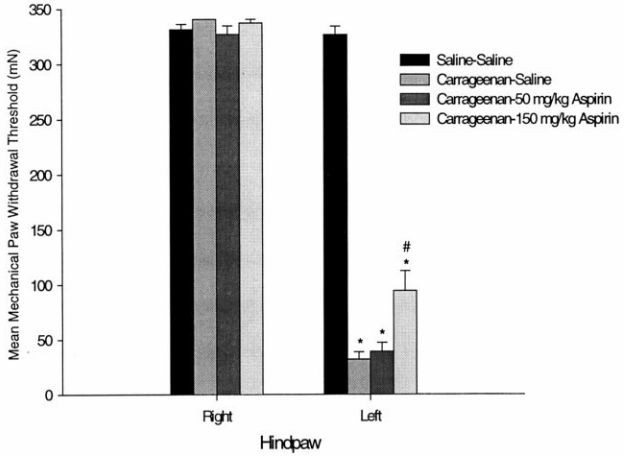

Fig. 1. Mean mechanical paw withdrawal thresholds for all experimental conditions. Data are expressed as the mean ± SEM mechanical paw withdrawal threshold (in mN) for the right and left paw. All intra-articular CARR-treated animals displayed significantly lower mechanical thresholds compared with i.a. Saline vehicle-treated animals. However, pre-treatment of 150 mg/kg, but not 50 mg/kg of aspirin significantly attenuated the mechanical hyperalgesia, but did not return thresholds to control levels. (*$P < 0.05$ compared with i.a. Saline vehicle-treated animals, #$P < 0.05$ compared with i.a. CARR Saline-treated animals).

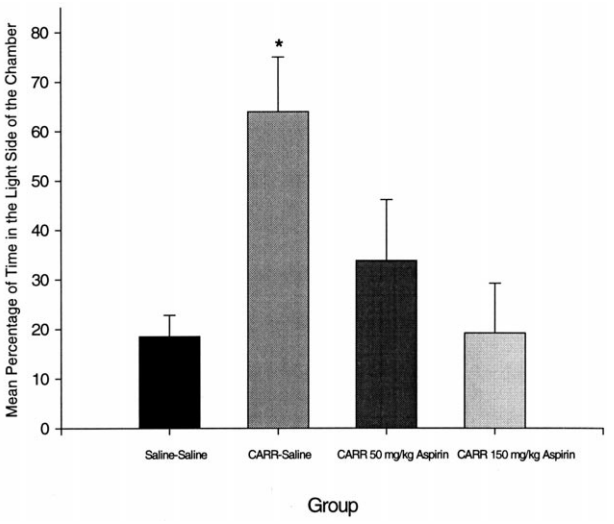

Fig. 2. Mean ± SEM percentage of time spent within the light side of the chamber during the PEAP. Data are expressed as average percentage of time spent within the light side of the chamber during the 30-min test period. Intra-articular CARR vehicle-treated animals avoided the presentation of a mechanical stimulus as animals spent significantly more time in the light side of the chamber compared with i.a. Saline vehicle-treated animals. However, aspirin (50 and 150 mg/kg) significantly reduced the amount of time spent within the light side of the chamber compared with CARR vehicle-treated animals. (*$P < 0.05$ compared with i.a. Saline vehicle-treated control animals).

than all CARR-treated groups ($P < 0.05$). However, there was not a significant difference between any CARR-treated animals ($P > 0.05$).

The present results support our previous findings that animals quickly escape/avoid a location in an environment associated with a noxious stimulus [9,10]. Subcutaneous injection of saline did not produce allodynic behavior to mechanical stimuli. In addition, these animals spent significantly less time in the light side of the PEAP chamber compared with CARR vehicle-treated animals. Aspirin (150 mg/kg) significantly attenuated mechanical hyperalgesia, and both 50 and 150 mg/kg aspirin-treated animals spent significantly less time in the light side of the PEAP chamber compared with CARR vehicle-treated animals. Since the CARR-50 mg/kg aspirin-treated animals are still hyperalgesic, but do not avoid the noxious stimulation of the hyperalgesic paw associated with the dark side of the chamber, it is suggested that a low dose of aspirin does not alter the sensory response to noxious stimulation, but does decrease the aversive quality associated with a noxious stimulus.

The mechanism by which aspirin produces its anti-inflammatory and anti-hyperalgesic effect primarily involves peripheral inhibition of prostaglandin (PG) synthesis [12,17]. It is of interest to note the dissociation of the anti-hyperalgesic and anti-aversive properties of aspirin. The lack of mechanical anti-hyperalgesia at the low dose of aspirin (50 mg/kg) likely reflects an insufficient block of PG synthesis. However, the attenuation of escape/avoidance behavior with both doses of aspirin (50 and 150 mg/kg) suggests that central mechanisms are involved in escape/avoidance behavior in the PEAP. The ventromedial hypothalamus (VMH) is a brain region that is involved in escape/flight behaviors [5,6,8], and has been shown to produce PGs [7,13]. It is possible that systemic administration of aspirin inhibits normal VMH PG synthesis. Therefore, blocking VMH PG synthesis may be the mechanism by which systemic aspirin attenuates escape/avoidance behavior with no attenuation in mechanical hyperalgesia.

Although it is possible that aspirin pretreatment decreased the amount of time spent in the light side of the chamber due to an effect on anxiety or an effect on motor activity, this is unlikely for two reasons. First, previous work demonstrates that aspirin does not possess anxiogenic properties, thereby reducing the likelihood that a lack of a shift from the dark side of the test chamber to the light side was due to a decrease in anxiety [11]. In fact, an anxiolytic effect of aspirin would likely be reflected as an increase in the amount of time that animals spent in the light side of the PEAP chamber. Second, analyses of line crossings indicate that both doses of aspirin did not produce an effect on motor activity compared with CARR vehicle-treated animals. Therefore, the failure to shift from the dark to the light side of the PEAP chamber cannot be explained by impaired motor activity. However, CARR aspirin-treated animals (50 and 150 mg/kg) demonstrated significantly elevated MPWTs compared with saline vehicle-treated animals. Therefore, the mechanical stimulus was less aversive following aspirin treatment, despite significant mechanical hyperalgesia in CARR-treated animals.

In conclusion, animals treated with aspirin (150 mg/kg) demonstrated a decrease in responsiveness to mechanical

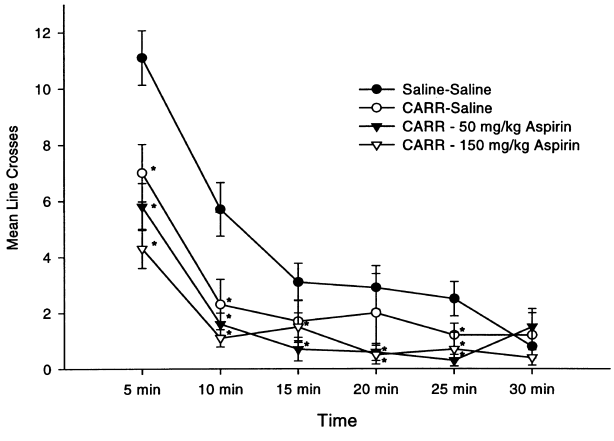

Fig. 3. General motor activity during the 30-min PEAP test. Data are expressed as the mean ± SEM transitions from one side of the test chamber to the other and provide a general measure of motor activity. Intra-articular saline vehicle-treated animals made significantly more transitions throughout the 30-min test session. (*$P < 0.05$ compared with i.a. saline vehicle-treated animals).

stimuli and a decreased avoidance of a suprathreshold stimulus compared with CARR vehicle-treated animals. Animals treated with a lower dose of aspirin (50 mg/kg) did not display decreased mechanical hyperalgesia, and did not avoid the suprathreshold mechanical stimulus in the PEAP. It is suggested that escape/avoidance behavior in the PEAP likely involves supraspinal processing of noxious information. Therefore, our data suggest that the mechanisms underlying the affective/motivational dimension of nociception (escape/avoidance) can be dissociated and studied separately from the processing of nociceptive information related to withdrawal responding.

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