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RAPID COMMUNICATION

The Differentiation of NSAIDs and Prostaglandin Action Using a Mechanical Visceral Pain Model in the Rat

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DELEO, J. A., R. W. COLBURN, D. W. COOMBS AND M. A. ELLIS. *The differentiation of NSAIDs and prostaglandin action using a mechanical visceral pain model in the rat.* PHARMACOL BIOCHEM BEHAV **33**(1) 253–255, 1989.—In order to determine the role of peripheral prostanoids in a newly developed mechanical visceral pain model, several NSAIDs were studied. Systemic acetylsalicylic acid and mefenamic acid, in doses known to produce cyclooxygenase inhibition, produced limited or no analgesia using a duodenal distension model and a behavioral scale for assessment. In contrast, indomethacin at 1 mg/kg, a dose 1/100th of the highest dose of the above compounds, had a marked analgesic effect in the visceral pain model (32% of control response). These data suggest that a duodenal distension stimulus does not have a peripheral prostaglandin E_2-mediated nociceptive mechanism. Furthermore, the results obtained with indomethacin support an alternate, possibly central nonprostanoid visceral antinociceptive action.

Visceral pain Duodenal distension Nociceptive tests NSAIDs, Prostaglandins Acetylsalicylic acid Mefenamic acid Indomethacin

THE role of prostaglandins in peripheral and central analgesia has been studied extensively in recent years. It has been observed that the intracerebral administration of PGE_2 causes hyperalgesia in the carrageenin-induced acute inflammatory pain model (5). The peripheral analgesic action of nonsteroidal antiinflammatory drugs (NSAIDs) or aspirin-like agents has been explained by the blockade of prostaglandin release that in turn prevents the sensitization of nociceptors to chemical or possibly mechanical stimulation (10). The tests that are most sensitive to antiinflammatory agents are those that are associated with tissue damage or inflammation and are analogous to that produced by a chemical stimulus. The chemical acetic acid writhing (AAW) model is a potent assay for determining the antinociceptive actions of antiinflammatory agents. Acetic acid elicits an indirect pain reaction by generating acute inflammation in the peritoneum with resultant locally increased levels of PGE_2 and $PGF_{2\alpha}$ (3, 4, 7, 12).

In the present study, we used a newly developed visceral pain model (VPM) to assess whether a prostanoid mechanism is involved in the nociceptive response to mechanical distension of the gut (2). We compared the actions of prostaglandin inhibitors in this test with those in the more established AAW pain assay. Surprisingly, we found that acetylsalicyclic acid (ASA) and mefenamic acid (MFA), reportedly both effective in the AAW test were ineffective in the VPM (8,14). These findings suggest that mechanical and chemical visceral pain models invoke different nociceptive mechanisms. Due to this, as well as the limitations of the AAW, the VPM may offer advantages over chemical induced writhing models.

METHOD

The surgical duodenal implantation has been previously described (2). Briefly, under inhalation anesthesia (N_2O, O_2 and halothane), male Sprague-Dawley rats, weighing 175–200 g, were

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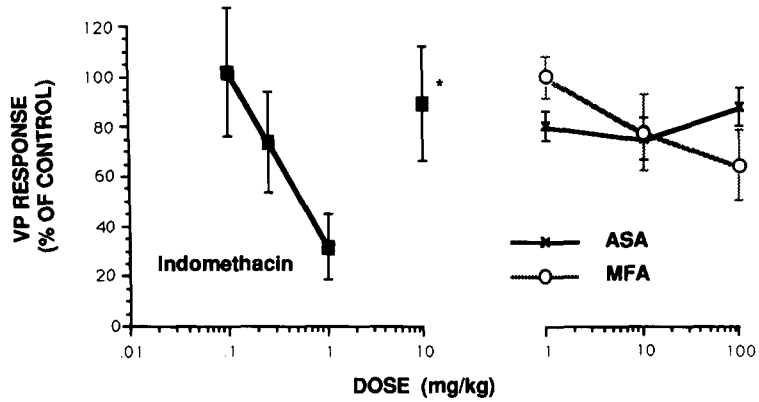

FIG. 1. Dose-response lines for indomethacin, ASA (acetylsalicylic acid) and MFA (mefenamic acid) in the mechanical visceral pain model (VP response). Data are expressed as percent of control response ($n \geq 6$ in each group). *Point eliminated from dose-response line due to 75% mortality with the highest dose and the marked deviation from the other 3 points. This value was not significantly different from control.

implanted with a latex rubber balloon catheter (7.5 mm long and distensible to greater than 2.0 ml fluid volume). Introduced by gastrostomy, the balloon was advanced through the pylorus into the first portion of the duodenum. The catheter was tunneled to the base of the skull, externalized, and anchored to the dermis with a silicone sleeve and suture. The animals recovered from anesthesia within 5 minutes postsurgery. Following a 4–5-day recovery period, the duodenal distension volume in test animals was determined by the mean threshold volume that produced writhing in a subset control group. This test volume (usually between 0.5 and 0.7 ml of saline) was then introduced to every animal in each group including the vehicle control group.

In this study, the animals were randomized and administered saline-vehicle, indomethacin (0.1, 0.25, 1, and 10 mg/kg, IP, Merck, Sharp and Dohme), mefenamic acid or acetylsalicylic acid (1, 10, 100 mg/kg, IP, Sigma) 90 minutes prior to the testing paradigm. The animals were then placed in a polypropylene box and challenged by inflating the balloon with saline using a 1 ml calibrated syringe, pulsed 5 times over 30 seconds and then distended for 1 minute. Behavioral responses, modified from a previous publication (2), were scored on a 0–4 scale as described below:

0 = Normal behavior defined as exploration, escape attempts and resting.
1 = Slightly modified behavior defined as cessation of exploration, focusing, wet-dog shake, excessive facial grooming, teeth chattering and deep breathing.
2 = Mildly to moderately modified behavior defined as retching-like activity, hunching, abdominal grooming or nipping and immobility of hindlimbs (disappears with removal of the stimulus).
3 = Severely modified behavior defined as stretching of the hindlimbs, arching and dorsoflexion of the hind paws.
4 = Intensive visceromotor activity defined as repetitive stretching of the body, extension of the hindlimbs, and pelvis, frequent rotating sideward, i.e., writhing as described by Collier *et al.* (3).

Statistics

The group VPM scores were plotted on semi log paper and the ED_{50} was determined by best line fit (Statworks, Cricket Software, Philadelphia, PA).

RESULTS

Dose-response lines in the VPM for indomethacin, acetylsalicylic acid and mefenamic acid, represented by percent of control response are shown in Fig. 1. The ED_{50} of indomethacin was 0.57 mg/kg. Indomethacin, at a dose 10 mg/kg, was devoid of antinociceptive activity and was associated with high mortality (75% within 48 hours of dosing). We tested the relative effectiveness of selected doses of indomethacin in an AAW assay, using the method previously described (13). In the AAW test, indomethacin at 1 mg/kg dose was ineffective in reducing the mean response score over control. However, in the VPM this same dose reduced the mean response to 32% of control. In contrast to indomethacin, ASA and MFA displayed little or no analgesia in the VPM at a dose 100-fold greater than the analgesic dose of indomethacin. The average mortality associated with the control groups (duodenal stimulation alone) was 2.7%.

DISCUSSION

The goal of this study was to determine if blockade of prostaglandin activity participates in antinociception in the mechanical visceral pain model. Our findings clearly indicate differences in NSAID analgesic responses between a chemically-induced writhing test reported in the literature and a mechanically-induced duodenal distension. Others have reported the ED_{50} of ASA to be 100 mg/kg in the acetic acid writhing test (4). It has been shown that at this dose prostaglandin generation is 95–98% inhibited between 1 and 6 hours after treatment with no evidence of mucosal lesions (9). ASA demonstrated no antinociceptive action in the VPM model at this same dose. This supports the hypothesis that peripheral prostaglandin inhibition has no effect on our mechanical visceral pain model. As a corollary to this finding, mechanical visceral distention of the upper intestine is not nociceptive via prostaglandin release.

Mefenamic acid was chosen in this study for its action as a direct antagonist at prostaglandin E_2 receptors (11). In addition, this agent is an inhibitor of both the 5-lipoxygenase and cyclooxygenase pathways of the arachidonic acid cascade (1). The observation that mefenamic acid, an agent structurally unique with respect to ASA and indomethacin, lacks analgesic effects in the VPM test is additional evidence that the antinociception associated with indomethacin is not mediated via either cyclooxygenase

inhibition or PGE_2 receptor antagonism. This study also gives preliminary indication that nociception resulting from duodenal distention is not mediated through a peripheral 5-lipoxygenase metabolite. Further work with selective lipoxygenase inhibitors is needed to verify this observation.

Of the NSAIDs used in this study, only indomethacin proved to possess any analgesic properties in the dose range 0.1 to 1 mg/kg, IP. The paradoxical absence of an effect of ASA and MFA, at doses equivalent to the ED_{50} of ASA in chemical visceral paradigms, suggest that indomethacin may have an analgesic action which is unrelated to its antiprostanoid activity. This antinociception may even result from a central rather than a peripheral locus. However, if mediated centrally, this paradox implies that a nonprostanoid mechanism may be operative. Indeed, intrathecal administration of NSAIDs has been previously shown to antagonize peripherally-induced nociceptive stimuli (5,6). Relatedly, the antinociceptive mechanism of indomethacin may originate from interactions with central opioid or nonopioid-dependent systems exclusive of prostaglandin mechanisms. In contrast, both ASA- and MFA-mediated analgesia have been strongly linked to prostaglandin inhibition. Ongoing research is being directed towards elucidation of the central mechanisms involved in indomethacin analgesia in the mechanical visceral pain model.

In summary, these results demonstrate differences between mechanically-induced and chemically-induced visceral pain models. The introduction of the mechanical visceral pain model may provide the impetus for further investigation into possible mechanisms of antinociceptive action of NSAIDs, in particular indomethacin.

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