An Enkephalinase Inhibitor, SCH 32615, Augments Analgesia Induced by Surgery in Mice

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Background: Stress-induced analgesia is a well recognized phenomenon in animals and humans in which endogenous opioids have been implicated. However, analgesia induced by surgical stress has not been reported. The purpose of this study was to determine whether surgery evokes analgesia and to examine the effect of SCH 32615, an inhibitor of one of the enzymes (enkephalinase) responsible for the degradation of enkephalins, on this analgesia, in mice.

Methods: Analgesia was tested using the hot-plate test. Animals were tested before any procedure was done and then at hourly intervals thereafter. Under halothane anesthesia, the anterior abdominal wall was incised, and the abdominal aorta was compressed against the vertebral column for 1 s. This was repeated for a total of three times at 5-s intervals. At the end of the procedure, the following drug(s) were administered subcutaneously to different groups of animals: (1) no drugs, only surgery (n = 15); (2) 5 mg/kg naloxone (n = 15); (3) 150 mg/kg SCH 32615 (n = 14); (4) 150 mg/kg SCH 32615 plus 5 mg/kg naloxone (n = 15); and (5) SCH 32615 vehicle (0.9% methylcellulose; n = 13). Two more groups of animals were included as controls and were anesthetized, but no surgical procedure was performed. One control group (n = 15) received 0.9% methylcellulose and the other 150 mg/kg SCH 32615 (n = 12).

Results: Hot-plate latency was significantly longer after surgery (hot-plate latency at 4 h after surgery 29.3 ± 3.2 (SE) s and at 5 h 30.7 ± 5 s versus baseline 15.8 ± 7 s, P < 0.05). Naloxone (5 mg/kg) inhibited this analgesic effect of surgery. SCH 32615 significantly enhanced this analgesia (percentage of maximal possible effect (%ME) at 4 h 33.7 ± 8.7%, at 5 h 27.5 ± 4.7%, and at 6 h 23.2 ± 4.7%; P < 0.05 compared to all other groups), and naloxone antagonized its effect. Anesthesia without surgery did not evoke subsequent analgesia, and SCH 32615 was not analgesic in the absence of antecedent surgery.

Conclusions: Surgery activated endogenous analgesia, the development of which was prevented by naloxone. SCH 32615, an enkephalinase inhibitor, significantly enhanced this analgesia. (Key words: Analgesia, endogenous opioid. Antagonists: naloxone. Enkephalinase inhibitor: SCH 32615.)

ANALGESIA, both opioid- and nonopioid-mediated, is activated by various kinds of stressors in laboratory animals. The first scientific observation of stress-induced analgesia in humans has been credited to Beecher,1 who in 1946 reported diminished analgesic requirements in wounded soldiers. However, to the best of our knowledge, analgesia activated by surgery has not been reported in humans or in animals. That surgical procedures evoke a stress response is well established. It seemed reasonable that surgical stress could activate endogenous analgesic systems. Endogenous enkephalins have been implicated in stress-induced analgesia.2,3 Drugs such as SCH 32615, by preventing the destruction of enkephalins by the enzyme enkephalinase, lead to increased amounts of enkephalins and detectable analgesia.4 When endogenous enkephalin activity is increased, these agents, by preventing metabolism of enkephalins, would be expected to produce significant analgesia.

We tested the analgesic effect of surgery and its enhancement by SCH 32615 with the hot-plate test, an established test of analgesia. We evaluated the following hypotheses: mice have longer hot-plate latencies (HPL) after surgery; SCH 32615 enhances this surgery-activated analgesia; naloxone inhibits both surgery-evoked analgesia and its enhancement by SCH 32615; anesthesia alone would not evoke analgesia; and SCH 32615 is not analgesic in the absence of antecedent surgery.

Methods and Materials

Approval was obtained from the Animal Care Committee at the Oregon Health Sciences University. Fe-
male Swiss-Webster mice (25–35 g) were studied. Animals were allowed food and water ad libitum.

Analgesia was assessed on a hot plate. This consisted of a heated stainless steel surface (31 × 19 cm) maintained at 55 ± 0.5°C and enclosed on its perimeter by plexiglass. Animals were placed unrestrained on the hot plate, and the time to hind-paw licking or jumping off the hot surface with both hind limbs was recorded as the HPL. Animals not showing such aversive behavior were removed at 120 s to prevent injury. Only animals with a baseline HPL of 10–20 s were studied further. After baseline observations of HPL, animals were anesthetized and operated on or not as detailed below. Either the study drug, or its vehicle, or naloxone alone or in combination with SCH 32615 was administered subcutaneously at the end of the procedure in a volume of 1 ml. Recordings of HPL were made at hourly intervals thereafter for a total of 6 h and, in some instances, recorded again at 24 h. In all cases, the person recording HPL was blinded to the nature of the injection.

Surgical Procedure

Anesthesia was induced by placing the animal in an airight box containing 5% halothane in air and oxygen. After induction, the animal was placed supine and allowed to breathe through a specially constructed mask, with anesthesia maintained with 2% halothane in oxygen-enriched air. The surgery was standardized and performed through a 2.54-cm incision made on the anterior abdominal wall. Through this incision, the abdominal aorta was palpated and compressed against the vertebral column for 1 s at a time, for a total of three times, at 5-s intervals. This was done to increase the stress associated with the stimulus. Visible bleeding or extrusion of the viscera led to exclusion from the study. After this, the abdomen was closed in one layer with silk sutures. Duration of the procedure was 10 ± 0.5 min. The animal then was allowed to recover, and the time at which recovery of the righting reflex occurred was noted in each instance. This usually occurred within 2 min of conclusion of the procedure.

Animals were tested in the following groups: (1) surgery only (n = 15): in this group, three animals were returned to their cages at the end of surgery and were then retested at 24 h after surgery; (2) surgery plus 5 mg/kg naloxone (n = 15); (3) surgery plus 0.9% methylcellulose (vehicle; n = 15); three animals in this group were retested at 24 h; (4) surgery plus 150 mg/kg SCH 32615 (n = 14): three animals in this group were retested at 24 h; this dose of SCH 32615 was chosen because an earlier study had revealed it to have maximal effect; and (5) surgery plus 150 mg/kg SCH 32615 plus 5 mg/kg naloxone (n = 15). Two further groups were included to control for effects of anesthesia: anesthesia only for 10 min, no surgery, plus 0.9% methylcellulose (n = 15), and anesthesia only for 10 min, no surgery, plus 150 mg/kg SCH 32615 (n = 12). To avoid undue suffering of animals, a technician was assigned to closely observe those animals that were returned to their cages and retested after 24 h. Signs that were taken to suggest suffering were (1) absence of normal feeding and drinking, (2) absence of normal grooming behavior, (3) absence of spontaneous movement and adoption of a particular posture, e.g., head down and limbs tucked under the body, for any length of time. None of the animals exhibited these signs.

Test of Motor Ability and Alertness

Animals anesthetized only (without surgery, n = 5), with anesthesia plus surgery (n = 5), or with anesthesia, surgery, and SCH 32615 (n = 5) were tested further to ensure there was no motor disability or sedation resulting from either the surgical procedure or the anesthesia. These tests were conducted before treatment and repeated every hour for 6 h.

The tests were as follows: (1) 80° inclined plane (rectangular piece of fine wire mesh, 40 × 15 cm, on a wooden frame): normally, a mouse placed on the inclined plane will climb to the top, turn around, and descend; (2) grasping a metal pole (diameter 17 mm): normally, mice can firmly grip and hold on to such metal pole without falling off; and (3) corneal reflex. In each case, the normal response was scored as 1 and an abnormal or absent response as 0.

Drugs Used

SCH 32615 was a gift from Dr. Allen Barnett (Schering Plough, Bloomfield, NJ). Methylcellulose was obtained from our hospital pharmacy. Naloxone was purchased from Sigma Chemical Co. (St. Louis, MO). Halothane U.S.P. was manufactured by Halocarbon Laboratories (River Edge, NJ).

Data Analysis

Data are expressed as mean ± SEM. For comparison between treatment groups, HPL was converted to percentage of maximal possible effect (%MPE), where %MPE = (post-drug latency – pre-drug latency)/cutoff time (120 s – pre-drug latency) × 100. Analysis of variance was used to analyze data with post hoc analysis.
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by Student-Newman-Keuls test. To analyze the analgesic effect of surgery and its antagonism by naloxone, a repeated-measures model with 1 between factor with two levels and 1 within factor with eight repeated measures, was set up. Post hoc analysis was done with Dunnett's test (to assess postsurgical HPL in relation to baseline) and unpaired t tests (to assess difference between the surgery only and naloxone plus surgery group).

Results

Surgery evoked an increase in HPL that was significantly different from baseline at 4 and 5 h after surgery (fig. 1). By 24 h, this analgesia had subsided. This analgesic effect of surgery was inhibited by the administration of the opioid antagonist naloxone (5 mg/kg; fig. 1).

Administration of 150 mg/kg subcutaneous SCH 32615 to animals undergoing anesthesia and surgery resulted in the development of significant analgesia at 4, 5, and 6 h after surgery (fig. 2). Coadministration of 5 mg/kg naloxone with SCH 32615 (150 mg/kg) prevented this analgesia from developing (fig. 2). The SCH 32615 vehicle (0.9% methylcellulose) was not analgesic (fig. 2). Anesthesia alone did not evoke analgesia, and SCH 32615 150 mg/kg was not analgesic under these circumstances (fig. 2).

In the 15 animals tested for motor ability and alertness (see methods), the maximum possible score was obtained both before and for each hour till 6 h after the procedure, thus indicating that lack of the ability to move or sedation was not responsible for the increase in HPL.

Discussion

Our results indicate that surgery activates an endogenous analgesic effect. Naloxone prevented this analgesia from developing, indicating that it is likely to be opioid in nature. Using the hot-plate test, we have shown that this analgesia is enhanced by SCH 32615, an enkephalinase inhibitor. This suggests that opioids metabolized by enkephalinase are responsible for this analgesia. In common with other enzymes, enkephalinase has many substrates, but the only endogenous opioids it metabolizes to any great extent are enkephalins. Thus, under the conditions of our study, it is highly probable that surgery activated endogenous enkephalins.

Endogenous analgesia that develops in response to stress may be opioid or nonopioid in nature. Opioid analgesia is distinguished by its reversal by specific antagonists, cross-tolerance with exogenous opioids, such as morphine, and the development of tolerance on re-
peated exposure to the stressful stimulus. We restricted our study to the use of a specific antagonist, naloxone, because we considered it neither humane nor germane to our study to repeatedly subject animals to surgery. Naloxone completely reversed surgical stress-evoked analgesia and completely suppressed the analgesic action of SCH 32615. This indicates, under the circumstances of our study, the underlying opioid mechanism of both the analgesia that developed after surgery and its enhancement by SCH 32615.

Our findings, implicating endogenous opioids in analgesia after injury, support earlier studies in humans and in rats. However, we believe that our study more closely mimics the stress associated with surgery and that this is the first report of analgesia activated by surgery. Also, in those earlier papers, analgesia was associated with increased plasma levels of β endorphin. The systemic administration of SCH 32615 increases cerebrospinal fluid but not plasma levels of enkephalins. Yaksh et al. comment that this lack of effect on plasma levels of enkephalin by enkephalimase inhibitors may lead to analgesia without the deleterious systemic effects associated with circulating enkephalins. Analgesia evoked by trauma, whether burns or surgery, may be the conjoint expression of many endogenous opioid systems. However, as mentioned earlier, stress-induced analgesia may be nonopioid as well.

The stress of anesthesia has been reported to produce transient analgesia that was partly potentiated by an enkephalimase inhibitor. In our study, we allowed the animals to recover their righting reflex and then waited an hour before testing for analgesia. Also, the nature of our anesthetic procedure (not involving laryngoscopy and intubation or painful injections) was not stressful. As a result of these factors, we did not see analgesia develop in animals anesthetized without surgery, including those also given SCH 32615.

Injury has been shown to have a hyperalgesic effect. Preventing this effect from developing has been shown to reduce postoperative analgesic requirements. On the other hand, we have shown analgesia developing with injury. Several reasons may account for this. Our protocol called for minimal injury by way of dissection or handling of the abdominal wall or viscera (visible bleeding or extrusion of the viscera led to exclusion from the study). The hot-plate test involves complex organized behavior, perhaps more amenable to modification by endogenous opioids. Certainly, inhibitors of enkephalin-metabolizing enzymes are more effective as analgesics in tests such as the hot-plate jump test than in tests of reflexive behavior, such as the tail-flick test. Also, enkephalins may not be restricted to inhibition of pain transmission. It has been suggested that they may be involved physiologically in pain perception by establishing a contrast between excited and inhibited neurons. Our study was designed more to detect analgesia: it may be that hyperalgesia develops in consonance with the activation of endogenous analgesia, perhaps accounting for the inadequacy of this analgesia in the clinical setting. Finally, in support of our findings, other studies have demonstrated decreased analgesic requirements in children after burns and decreasing requirements of isoflurane after prolonged surgery.

In conclusion, we demonstrated analgesia activated by surgery in mice, using the hot-plate test. Also, we showed enhancement of this analgesia by SCH 32615, an enkephalimase inhibitor. Lastly, naloxone inhibited surgery-activated analgesia and antagonized the effect of SCH 32615, suggesting an opioid mechanism for both.

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