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Supporting Text

Models of Acute Somatic Pain

Acute Mechanical Nociception. The response to noxious mechanical stimulation was determined by measuring withdrawal threshold to paw pressure by using the Ugo Basile (Comerio, Italy) analgesymeter. The animals were gently restrained, and steadily increasing pressure was applied to the dorsal surface of a hind paw via a dome-shaped plastic tip (diameter = 1 mm). The pressure required to elicit paw withdrawal was determined. Two measurements were taken, and the mean was calculated.

Acute Thermal Nociception. The response to acute thermal stimulation was determined by using a commercially available paw thermal stimulator (UARDG, University of California, San Diego; ref. 1), modeled after that described by Hargreaves *et al*. (2). Rats were placed individually in Plexiglas cubicles mounted on a glass surface maintained at 30°C and allowed a 30-min habituation period. A thermal stimulus, in the form of radiant heat emitted from a focused projection bulb, was then applied to the plantar surface of each hindpaw. In each test session, each rat was tested in three sequential trials at approximately 5 min intervals. Paw withdrawal latencies were calculated as the mean of the two shortest latencies.

Hotplate Assay. Analgesia was measured by using an automated hotplate analgesia monitor (model AHP16AN, Omnitech Electronics, Columbus, OH) using methodology as described by Kowaluk *et al*. (3). Mice were placed in individual plastic enclosures on the hotplate, and the latency until the 10th jump was recorded by disruption of a photocell beam located 12.5 cm above the surface of the hotplate. Mice were removed from the

hotplate after either 10 jumps were made or 180 sec (test termination) had elapsed, whichever occurred first. The latency until the 10th jump was used for statistical analysis.

Capsaicin-Induced Nociception. After a 30-min acclimation period to individual observation cages, 10 μg/10 μl of capsaicin solution was injected s.c. into the dorsal aspect of the right hindpaw and the rats were then returned to the clear observation cages, which were suspended above mirrors. Rats were observed for a continuous period of 5 min. Nociceptive behaviors recorded included flinching, licking, or biting the injected paw. Both the number of nocifensive behaviors and the time spent having these behaviors were recorded.

Models of Inflammatory Somatic Pain

Formalin Test. After a 30-min acclimation period to individual observation cages, 50 μl of a 5% formalin solution was injected (s.c.) into the dorsal aspect of the right hindpaw and the rats were then returned to the clear observation cages. Rats were observed for periods of time corresponding to phase 1 (0-10 min) and phase 2 (30-50 min) of the formalin test (4). Nociceptive behaviors were recorded from animals during the session by observing each animal for one 60-s observation period during each 5-min interval. Nociceptive behaviors recorded included flinching, licking, or biting the injected paw.

Carrageenan- and CFA-Induced Thermal Hyperalgesia. Unilateral inflammation was induced by injecting 100 μl of a 1% solution of λ-carrageenan or 150 μl of a 50% solution of CFA (Sigma) in physiological saline into the plantar surface of the right hindpaw of the rat. The hyperalgesia to thermal stimulation was determined 2 h or 48 h after carrageenan or CFA injections, respectively, using the same apparatus as described above for the noxious acute thermal assay.

Models of Neuropathic Somatic Pain

Spinal Nerve (L5/L6) Ligation Model. As described in detail by Kim and Chung (5), a 1.5-cm incision was made dorsal to the lumbosacral plexus. The paraspinal muscles (left side) were separated from the spinous processes, and the L5 and L6 spinal nerves were isolated and tightly ligated with 3-0 silk thread. After hemostasis, the wound was sutured and coated with antibiotic ointment. The rats were allowed to recover and then placed in a cage with soft bedding for 7 days before behavioral testing for mechanical allodynia.

Sciatic Nerve Ligation Model. As described in detail by Bennett and Xie (6), a 1.5-cm incision was made 0.5 cm below the pelvis, and the biceps femoris and the gluteous superficialis (right side) were separated. The sciatic nerve was exposed and isolated, and four loose ligatures (5-0 chromic catgut) with 1-mm spacing were placed around it. The rats were allowed to recover and then placed in a cage with soft bedding for 14 days before behavioral testing for mechanical allodynia. Thermal hyperalgesia was evaluated as described above for the noxious acute thermal assay.

Mechanical Allodynia. Mechanical (tactile) allodynia was measured by using calibrated von Frey filaments (Stoelting). Briefly, rats were placed into individual Plexiglas containers and allowed to acclimate for 15-20 min before testing. Withdrawal threshold was determined by increasing and decreasing stimulus intensity and estimated by using a Dixon nonparametric test (7). Only rats with threshold scores $\leq$4.5 g were considered allodynic and used in further testing.

Model of Postoperative Somatic Pain: Plantar Incision Model

As described by Brennan *et al*. (8), a 1-cm longitudinal incision was made through the skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The underlying muscle was elevated and incised longitudinally with origin and insertion of the muscle remaining intact. The skin was then closed with two mattress sutures of 5-0 nylon. After surgery, the animals were allowed to

recover and housed individually with soft bedding. Animals were tested for mechanical allodynia by using von Frey hairs as described above 2, 24, and 48 h after surgery.

Models of Visceral Pain

Abdominal Constriction Assay. The test used was a modification of the abdominal constriction test described by Collier *et al*. (9). Each animal received an i.p. injection of 0.3 ml of 0.6% acetic acid in normal saline to evoke writhing. Abdominal constriction was defined as a mild constriction and elongation passing caudally along the abdominal wall, accompanied by a slight twisting of the trunk and followed by bilateral extension of the hind limbs. The total number of abdominal constrictions was recorded from 5 to 20 min after acetic acid injection.

Noxious Colonic Distension of the Normal or Inflamed Colon. Contraction of the abdominal musculature during colorectal distension (CRD) in conscious rats was measured as described (10). On the day of testing, a 7-cm-long latex balloon tied to Tygon tubing was lubricated with Surgilube (E. Fougera and Co., Melville, NY) and inserted intra-anally until the end of the balloon was 1 cm inside the rectum (total insertion distance of 8 cm). Materials and methods for CRD and recording and analysis of the electromyogram signal have been fully described (10, 11). Each distension trial lasted 40 s, and electromyogram activity was quantified during the 10 s before distension, 20 s of distension, and 10 s after distension.

To assess visceral hyperalgesia, three phasic distentions (80 mmHg, 20 s), at 4-min intervals, were given to establish a baseline response magnitude. The animals were then anesthetized with halothane, and zymosan (1 ml, 25 mg/ml in 30% ethanol, Sigma) was instilled into the distal colon by using a 7-cm, 16-gauge stainless steel feeding needle connected to a 1-ml syringe. Three hours or 3 days after the installation of zymosan, three phasic distentions were repeated to evaluate hyperalgesia. Immediately after the third

distension, drug (or vehicle) was administered s.c. to the hip area. Thirty minutes after drug administration, the three phasic distensions were repeated to evaluate drug effects.

Motor Performance and General CNS Function

Locomotor activity was measured in an open field by using photobeam activity monitors (AccuScan Instruments, Columbus, OH), and rotorod performance was measured by using an accelerating rotorod apparatus (Omnitech Electronics) as described (12). For the rotorod assay, rats were allowed a 30-min acclimation period in the testing room and then placed on a 9-cm diameter rod that increased in speed from 0 to 20 rpm over a 60-s period. The time required for the rat to fall from the rod was recorded, with a maximum score of 60 s. Each rat was given three training sessions. Latencies to fall from the rotorod were determined 30, 60, and 120 min after compound injection.

After s.c. administration, A-317491 (10-1,000 μmol/kg) was also assessed in a battery of standardized CNS functional assays including the Irwin test, barbital- and ethanol-induced sleep induction, effects on rectal temperature, and two seizure models, pentylenetetrazole and electroconvulsive shock (Porsolt & Partners Pharmacology, Boulogne-Billancourt, France).

Heart Rate and Blood Pressure

Hemodynamic parameters were assessed by using the LabPro telemetry system (Dataquest, Data Sciences International). Approximately 1.5 weeks before the study and under aseptic conditions, male Sprague-Dawley rats were implanted with indwelling telemetry transmitters connected to a small gel-filled catheter that was secured nonocclusively in the abdominal aorta. Arterial pressure and heart rate data were sampled at 5-min intervals and serial 10-min averages were determined. A 2-day washout period was allowed between experiments. A-317491 (n = 6 per dose; 10, 30, 100, and 300

μmol/kg or vehicle group) was administered s.c.. Mean arterial pressure and heart rate were measured beginning 1 h before and continuing for 6 h after treatment.

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