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Thermal Threshold Testing for Evaluation of Analgesics in New Zealand White Rabbits

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We adapted a thermal analgesiometric device developed for cats for use in unrestrained rabbits. A probe composed of an electrical element and temperature sensor was held against shaved skin by using an elasticized band placed circumferentially around the thorax. An inflated bladder located between the probe and elastic maintained constant contact between probe and skin. The probe was heated until the rabbit displayed a behavioral reaction or the safety cutoff of 55 °C was reached. Threshold temperatures in unmedicated rabbits were stable over a 5-h period provided that tests were 15 min or more apart. Careful acclimation and testing resulted in no false-negative responses, and sham testing did not produce false-positive results. When compared with baseline values, thermal thresholds were significantly increased from 30 to 240 min, but not 300 min, after the administration of morphine at 3 mg/kg. Administration of equivalent volumes of saline via the same route had no effect on thermal threshold. This device may be suitable for investigating analgesic pharmacology in rabbits.

Rabbits are widely used in biomedical research and are a common pet species in many countries. Therefore, experimental or clinical surgical procedures are likely to be performed with increasing frequency and may be accompanied by acute postoperative pain. Currently, little objective information has been published regarding the efficacy or duration of analgesics in rabbits. Pain is difficult to assess in any animal species. Many physiologic and biochemical markers of pain are unreliable due to their multifactorial etiology. Some of the most reliable indicators of pain are physical signs such as changes in behavior. However, reliable detection of behavioral changes in a stress-prone species such as rabbits may be difficult in clinical and uncontrolled laboratory settings. Therefore, analgesic dose recommendations based on subjective observations made in such settings may be inaccurate.

Objective assessment of analgesic efficacy and duration in rabbits is needed, and to obtain such data repeatable and ethical testing methods are required. A thermal threshold testing device, developed for use in unrestrained cats, may meet these criteria. This device allows for repeated testing, without injury, in the same animal with little to no interference to normal behavior.[2] This device has been used in multiple studies investigating the pharmacology of analgesics in cats—but not in rabbits to date.[6,9,11]

Our goal was to adapt this device for use in rabbits and evaluate it as a means for assessment of analgesic drug efficacy in that species. We hypothesized that administration of the μ-opioid–receptor agonist morphine would increase thermal thresholds in rabbits.

Materials and Methods

Adult female New Zealand White rabbits (Harlan Laboratories, Indianapolis, IN; $n = 9$; weight, 3.68 ± 0.10 kg [mean ± 1 SD]) were used in this study, which was approved by the IACUC of the University of California–Davis. Rabbits were maintained by an AAALAC-accredited facility and deemed to be in good health based on normal physical examination. Rabbits were housed individually in either stainless-steel wire cages measuring 63 × 76 × 40 cm (Suburban Surgical, Wheeling, IL) or galvanized wire cages measuring 58 × 66 × 36 cm (Country Cages, Pengrove, CA). Mesh-style rubber matting covered a smaller area of the cage floor to provide more stable footing but still allow for passage of feces and urine to the collection pan below the cage. Pans were changed twice weekly and cage racks every other week. Cages were in a room that housed only rabbits and that was on a 12:12-h light:dark cycle and maintained at a temperature of 21 ± 1 °C and humidity of between 45% and 55%. Animals were fed 5 oz pelleted rabbit chow (High Fiber Rabbit Diet no. 5326, PMI Nutrition International, Brentwood, MI) daily with free access to water.

For acclimation and testing, rabbits were transported in pet carriers (44 × 38 × 35 cm; Petco Animal Supplies, San Diego, CA) to the laboratory located in the same building. In the laboratory, rabbits were housed individually in galvanized cages measuring 64 × 68 × 61cm under the same environmental conditions as described earlier. Rabbits were allowed free access to timothy hay (Oxbow Animal Health, Murdock, NE) and water and received part of their daily allowance of pellets. Rabbits were acclimated to the laboratory environment and personnel over a 4-wk period. Environmental enrichment was provided in the form of hutches, hay balls, and chew toys when active testing was not underway.

Testing device. The testing device developed by Topcat Metrology (Ely, Cambridgeshire, UK) has been previously described.[2] In brief, a 10 × 5-mm probe containing a heating element and temperature sensor encased in conductive epoxy was attached to a pressure bladder, which was held against the shaved thorax of the rabbits by using an elasticized band (Figure 1 A). The pressure bladder was inflated manually to 100 mm Hg by using an air-filled syringe as measured by a manometer connected intermittently to the cuff. The temperature probe was connected to a control unit by ribbon cable, which was encased in a lightweight plastic cover for protection during

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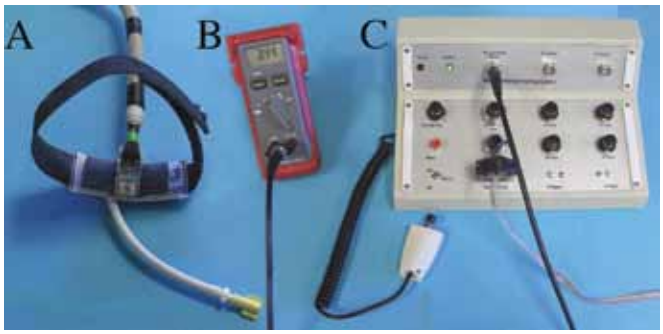

Figure 1. (A) The thermal probe was connected to a covered cable and positioned over an inflatable bladder inside an elasticized band. This band was placed circumferentially around the hairless rabbit thorax. (B) A voltmeter connected to the control box provided the temperature reading. (C) The control box for the thermal-threshold testing device, with activation toggle switch connected.

the study (Figure 1 C). The rate of temperature rise for the heating element is 0.5 °C per second, with an automatic cutoff at 55 °C to prevent thermal injury. The control unit was connected to a voltmeter, which had a hold function; temperature readings were obtained from the voltmeter (Figure 1 B). A handheld toggle switch connected to the control unit activated and deactivated the heating element (Figure 1 C). The probe was calibrated before each use and the reported temperatures have been adjusted to reflect this calibration.

Testing protocol. On the day of testing, rabbits were shaved by using electric clippers followed by a safety razor. The rabbits then were allowed at least 60 min for exploratory behavior before being fitted with the testing device. A minimum of 15 min was allowed after application of the device for the probe to reach skin temperature before testing. Because the rabbits chewed on it, the cable connecting the probe to the control unit was not left attached but instead was attached to the probe just prior to testing and at random intervals between to avoid rabbits developing learned behavior. At the time of cable attachment, skin temperature was recorded and visual inspection of the bladder confirmed inflation.

To test thermal threshold, the handheld toggle switch was depressed to initiate probe heating. When the rabbit displayed a behavioral reaction to the stimulus, the hold button on the voltmeter and the toggle switch were depressed simultaneously, providing the threshold temperature and ending probe heating. Typical behavioral reactions included turning the head toward the probe, biting at the probe, and hoping away from the probe.

Testing interval, reproducibility, and sham testing. In each of 5 rabbits, we performed a series of 4 thermal-threshold evaluations at each testing interval (5, 10, and 15 min); different intervals were tested on different days. The purpose of this experiment was to evaluate the effect of testing interval on thermal excursion (thermal threshold minus skin temperature) and to determine the minimal testing interval for future trials.

To assess reproducibility of the test, thermal thresholds at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 min were evaluated in the 4 rabbits that had not been tested previously. Skin temperature, thermal threshold, and thermal excursion were evaluated by using repeated-measures ANOVA (Prism 5, GraphPad Software, San Diego, CA). In these rabbits, a sham test was performed during each intertest interval from 60 to 300 min. This testing involved connecting the cable to the probe, mimicking performance of a thermal-threshold test without turning on the heating element, and observing the rabbits for any behavioral reaction.

Observed behavior	Score
Asleep or unconscious	0
Awake but immobile	1
Awake; head up	2
Moving slowly about cage	3
Alert; moving about cage; investigating	4

Figure 2. Activity scoring system for rabbits, based on the system described in reference 4.

Analgesiometry. We assessed thermal thresholds in rabbits that had received 2 treatments, morphine sulfate (3 mg/kg IM; Baxter Healthcare, Deerfield, IL) and an equivalent volume of 0.9% saline administered intramuscularly. Each of the 9 rabbits received both treatments in random order at least 7 d apart. Thermal threshold was determined at the start of the experiment (baseline) followed by treatment administration (time 0). Thermal-threshold evaluation then was repeated at 30, 60, 90, 180, 240, and 300 min after treatment by a blinded assessor. The activity level of the rabbits was assessed at each time point according to a previously published scoring system that we adapted for use in this scenario (Figure 2).[4]

Thermal threshold data initially were examined by using 2-way repeated-measures ANOVA for effects of time and treatment. This analysis was followed by repeated-measures ANOVA within each treatment group (saline or morphine) to assess the effect of treatment over time on skin temperature, thermal threshold, and thermal excursion. Where significant effects were detected, pairwise comparisons were made between values at each time point and baseline. A sequentially rejective Bonferroni technique was used to correct for multiple comparisons, with an α level of 0.05.[5]

Daily after testing, rabbits were observed for skin lesions due to thermal-threshold testing and to ensure return of appetite, urination, and defecation. Any rabbit that had more than slight skin erythema or that reacted to palpation of the probe placement area would have received a single dose of meloxicam (0.5 mg/kg SC) and buprenorphine (0.05 mg/kg SC) as required, to a maximum of every 6 h.

Results

After acclimation, rabbits were comfortable in the laboratory, as displayed by their normal eating, drinking, and other behaviors. Rabbits did not interfere with the probe or elasticized band but did chew on the cable that connected the probe and control box. Disconnecting the cable between tests minimized interference with the study. Minor skin lesions were noted in some rabbits with testing after morphine treatment. These consisted of slightly raised and mildly erythematous areas of skin that did not elicit reaction on palpation, healed within a few days, and did not require analgesic administration.

Testing interval, reproducibility, and sham testing. Testing at 5- or 10-min intervals resulted in thermal excursions that tended to increase over time. This effect was not evident when 15-min intervals were allowed between tests (Figure 3). In the 4 untreated rabbits that were tested at intervals over 5 h, there was no significant effect of time on skin temperature, thermal threshold, or thermal excursion. Sham testing did not produce any false-positive reactions.

Analgesiometry. Both time and treatment had significant effects on thermal threshold. The saline-treated rabbits showed no significant change in skin temperature or thermal threshold over time (Figure 4). Skin temperature in the morphine-treated

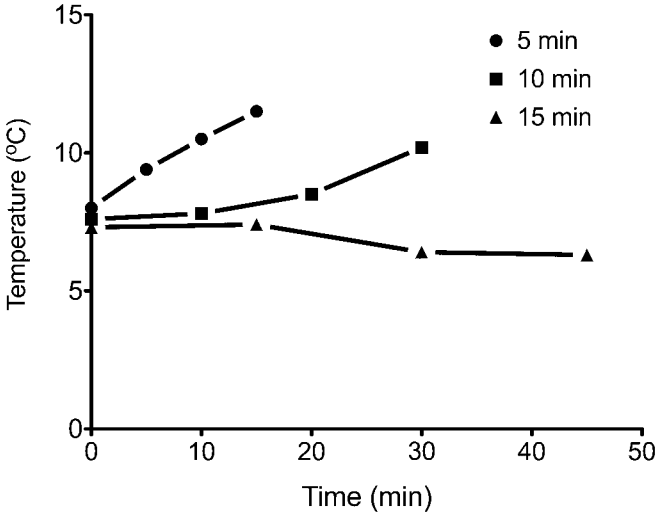

Figure 3. Effect of testing interval on thermal excursion (thermal threshold minus skin temperature). Data displayed are from a representative rabbit after 4 successive tests at intervals of 5, 10, or 15 min.

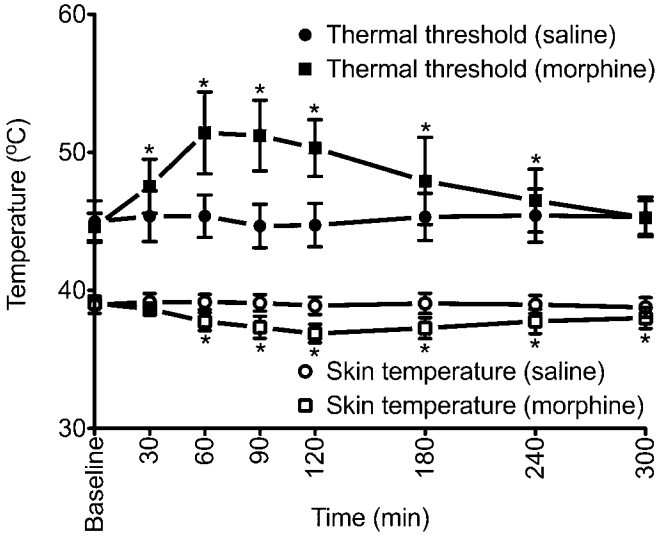

Figure 4. Skin temperature and thermal threshold (mean ± 1 SD) in 9 rabbits after intramuscular administration of 0.9% saline or morphine (3 mg/kg) at time 0. *, Value significantly ($P \leq 0.05$) different from baseline value.

rabbits was significantly ($P < 0.05$) lower than baseline at 60 min and all subsequent time points, and thermal threshold was significantly ($P < 0.05$) higher than baseline from 30 to 240 min (Figure 4). Thermal excursion did not differ from baseline after saline administration but was significantly ($P < 0.05$) greater than baseline from 30 to 240 min after morphine administration (Figure 5).

Activity levels. Subjectively, rabbits had decreased activity levels by 30 min after morphine administration. Some rabbits had returned to normal activity levels by 180 min, but others took 300 min to return to normal (Figure 6).

Discussion

Our findings support the use of this thermal threshold testing device for objective assessment of analgesic pharmacology in rabbits. The device was well tolerated and was used repeatedly on the same rabbit without harm. Consistent with published data in cats, thermal-threshold responses were stable over time,

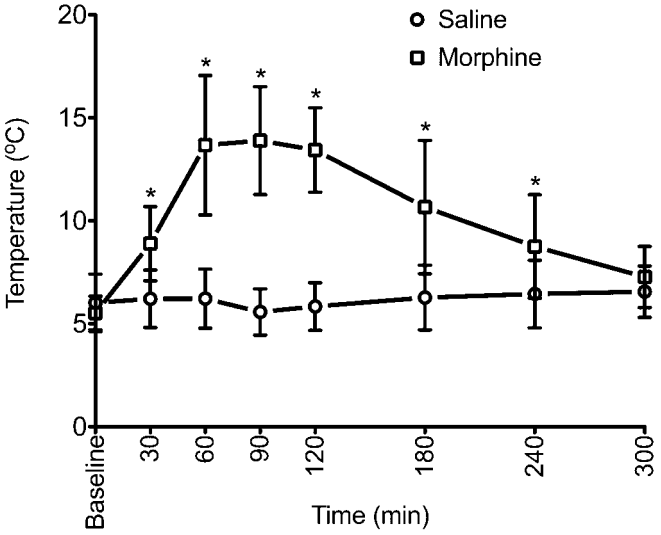

Figure 5. Thermal excursion (skin temperature minus thermal threshold; mean ± 1 SD) in 9 rabbits after administration of morphine (3 mg/kg IM) or equivalent volume of 0.9% saline at time 0. *, Value significantly ($P \leq 0.05$) different from baseline value.

and testing did not evoke a learned response.[2] Importantly, evaluation of thermal threshold provided quantitative data that were sensitive to the administration of a μ-opioid–receptor agonist, as is also the case in cats.[2,9,11]

Clinically, pain is a multidimentional, unpleasant, sensory, and emotional experience, making it difficult to model in the laboratory setting. Heat is just one form of noxious stimulation that can be used in experimental pain models. A single noxious stimulus is not likely to approximate all aspects of clinical pain; however, any methods used for evaluation of analgesic efficacy in the laboratory setting must be repeatable and ethical. The thermal stimulus applied in this model can be terminated rapidly and creates minimal to no tissue damage, allowing repeated testing of an individual rabbit and thus facilitating drug and dose comparisons.

Thermal stimuli have been used previously for the investigation of analgesic efficacy in rabbits.[4,12] This earlier system involved the application of a focused heat source to the dorsum of an animal in a carrier box and measured latency to skin twitch. Restraint induces stress and may divert the animals' attention from the nociceptive stimulus and potentially alter their response to antinociceptive testing. Attention levels in people have been shown to alter their responses to thermal stimulation.[1]

Like many other laboratory antinociceptive models, thermal pain may not be directly comparable to acute postsurgical pain. However, studies in cats that have used the thermal threshold testing device that we describe here document opioid-induced thermal antinociception that appears to correlate well with clinical analgesic efficacy.[7-11]

Similar to findings in cats, the minimal interval between tests in rabbits appears to be between 10 and 15 min.[2] At intervals of 5 min, both skin temperature and thermal excursion tended to increase with repeated testing. This effect was less evident at 10 min and not evident at 15 min. This situation complicates the use of the current system for the assessment of analgesia after the administration of a single dose of a short-acting drug.

We selected morphine for use in the current study because it is the prototypical analgesic for the control of acute clinical pain and a drug with which other opioids are commonly compared. The dose of morphine, 3 mg/kg IM, was selected because of

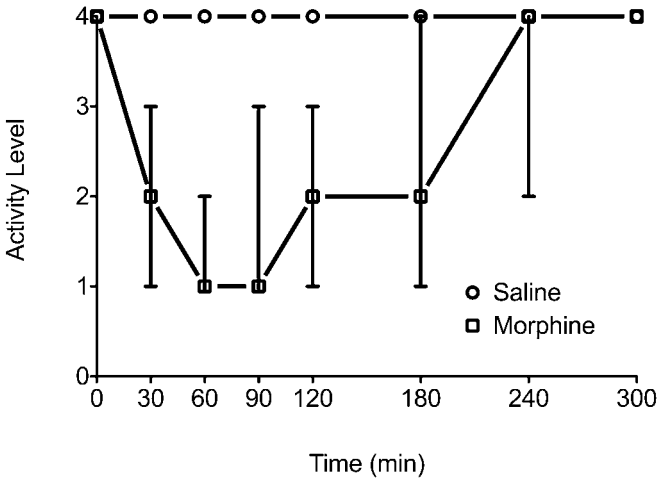

Figure 6. Median activity levels (bars, range) in 9 rabbits after administration of morphine (3 mg/kg IM) or equivalent volume of 0.9% saline at time 0. A score of 0 represents an asleep or unconscious rabbit, with increasing scores representing increasing activity. A score of 4 indicates a normal level of activity.

its large effect, according to clinical experience and published reports.[3] This dose produced analgesia for approximately 4 h, as evidenced by the significant elevation of the thermal threshold from 30 to 240 min. It also reduced the activity level of the rabbits for the same period, although some animals had returned to normal activity levels by 180 min after administration.

Inherent in the use of behavioral responses to noxious stimulation for evaluation of analgesics is the assumption that the drug administered influences only the neural circuitry underlying the response that is specific to the pain pathway. For example, we surmise that if a drug produces severe cognitive impairment, in the extreme unconsciousness, behavioral responses to noxious stimulation could be abolished in the absence of a true antinociceptive drug effect. The interpretation of data in analgesia trials of drugs with less extreme effects on cognitive function is less clear. Many analgesics have behavior-modifying effects, and it is difficult to separate those effects from analgesic effects. Future studies to investigate the effects of sedative nonantinociceptive drugs on thermal threshold in rabbits would be valuable.

After acclimation, rabbits tolerated this thermal-threshold testing device well, and it produced repeatable results without injury to the animals. Morphine treatment resulted in significant increases in thermal threshold consistent with an analgesic effect of approximately 4-h duration. This device holds promise for evaluating the pharmacology of analgesics in rabbits.

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