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Buprenorphine Given After Surgery Does Not Alter Renal Ischemia/Reperfusion Injury

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Background and Purpose: **Potential drugs for human acute renal failure are often tested in an animal model of renal ischemia/reperfusion injury. Analgesics are often not given after surgery because of concerns that they would alter renal function. Therefore, we tested whether postoperative analgesia would alter animal health or affect the degree of renal injury.**

Methods: **Mice were subjected to either 32 or 37 minutes of renal ischemia, given two or six doses of buprenorphine or vehicle at 12-hour intervals, and followed for 72 hours. In some animals, we measured body temperature and physical activity by use of telemetry.**

Results: **Animals treated with buprenorphine recovered more rapidly from surgery based on postoperative activity, and had a small but not significant tendency for faster restoration of normal body temperature. Animals treated with buprenorphine had less weight loss after 37 minutes of ischemia. Buprenorphine given after surgery did not influence the degree of renal injury after ischemia/reperfusion.**

Conclusions: **Buprenorphine should be given after renal ischemia-reperfusion surgery because administration of the proper analgesic improved animal health without interfering with the renal ischemia/reperfusion model. Analgesic treatment at the time of the operation and 12 hours after was sufficient. Buprenorphine may reduce the post-surgical stress response, and thus potentially improve the specificity of testing for drugs that reduce or treat renal injury.**

Acute renal failure (ARF) is a disease of high morbidity and mortality that has not improved markedly over the past 30 years (1). This disease also develops after renal transplantation, and may increase the risk of acute rejection and chronic allograft dysfunction (2). Drugs that might be helpful in treating human ARF are often tested in an animal model of renal ischemia/reperfusion injury (3). This experimental form of injury results in rapid necrosis of the proximal straight tubule in the outer stripe of the outer portion of the medulla by six hours. There is an increase in serum creatinine concentration, a marker of renal dysfunction, to approximately 2 to 3.5 mg/dl within 24 hours. Recovery of renal function begins at approximately 24 to 48 hours, and is indicated by a gradual decrease of serum creatinine concentration. The cellular events include migration of neutrophils, T cells, and macrophages into the outer portion of the renal medulla, and accumulation of erythrocytes in the capillaries and interstitium of the outer and inner portions of the medulla (4, 5).

The experimental model consists of making a midline abdominal incision and placing clamps on the renal arteries for 30 to 60 minutes. The clamps are then removed and the abdomen is closed in several layers. After this major surgery, animals may take four to six hours to recover (i.e., return to upright position and begin walking). This model is known to be sensitive to the temperature and fluid volume status of the animals. Lowering body temperature (e.g., as caused by cooling during surgery) protects against renal injury, whereas coexisting fluid volume depletion causes more injury (6). Thus, human kidneys are harvested and placed on ice to prevent ischemic damage. To allow study of warm ischemic injury and to make the model as constant as possible, surgery is performed on a warming table to keep body temperature constant. Volume depletion is controlled by administering 1 ml of normal saline intraperitoneally after surgery. In previous studies, we found that placing animals in a 29°C incubator for 4 hours after the operation, similar to a warming blanket in a clinical recovery room, allowed the animals to recover more rapidly from anesthesia and surgery (unpublished observations, 7).

The renal ischemia/reperfusion animal model is performed without use of postoperative analgesia because of the concern that analgesic agents might adversely influence the course of the renal injury, either by direct drug-induced toxicosis, or by altering one of the hemodynamic, inflammatory, or regenerative pathways that affect renal injury (1, 8). For example, naloxone (a broad opioid antagonist [9]) decreased renal injury in dogs subjected to warm ischemia with contralateral nephrectomy (10). A combination of morphine (a mu agonist [9]) and naloxone inhibited the ischemia-induced increase in superoxide anion generated by zymosan-stimulated phagocytes in renal venous blood (11). Thus, there is suggestion that opioid antagonists or mu agonists could be beneficial, although this has not been well studied. Most patients with acute renal failure are not in pain, and analgesics are ineffective in treating symptoms of moderate uremia. The mouse model is a good model of acute renal failure after renal transplantation in humans, a setting where analgesics are carefully administered. There is accumulating evidence that animals experience pain (12, 13). Non-verbalizing animals cannot vocalize pain; however, the presence of pain can be inferred by physical signs, such as altered heart rate or altered breathing patterns. However, lesser degrees of pain induce less profound changes and can only be manifested by changes in posture or patterns of sleeping, eating, or elimination (12–14).

Because of the concern that analgesic medications that shorten postoperative recovery from anesthesia might alter renal recovery,

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we tested whether buprenorphine (a mixed partial opioid mu agonist and kappa antagonist [15, 16]) given after surgery would influence the degree of renal injury. Our initial observations indicated that buprenorphine-treated animals recovered from anesthesia more rapidly. Therefore, we also measured the effects of buprenorphine given after surgery on activity, body temperature, and daily weight gain.

Material and Methods

Animals: Healthy female BALB/CAnNCr mice, 7 to 8 weeks old (20 to 22 g), were obtained from National Institutes of Health, Division of Cancer Treatment Animal Production Area (DCT-APA), Frederick, MD. The mice tested negative for cilia-associated respiratory bacillus, *Mycoplasma pulmonis*, mouse adenovirus, ectromelia, lymphocytic choriomeningitis, minute virus of mice, mouse cytomegalovirus, mouse encephalomyelitis virus, mouse hepatitis virus, mouse rotovirus, mouse parvovirus, pneumonia virus of mice, reovirus 3, Sendai virus, polyoma virus, *Salmonella* spp, and endoparasites and ectoparasites. All animals had ad libitum access to water and food (NIH-07 Rodent Chow, Zeigler Bros., Inc., Gardners, PA). Animal care followed the criteria published in the *Guide for the Care and Use of Laboratory Animals* (17), and under a protocol approved by the NIDDK Animal Care and Use Committee. The animal room was environmentally controlled to provide a temperature between 22-23°C and 30 and 70% relative humidity, with a 12:12-hour light: dark cycle and a minimum of 15 complete air exchanges/h.

Chemicals: Buprenorphine was purchased from Reckitt & Colman Pharmaceuticals, Inc. (Richmond, VA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Groups of animals: Four groups of animals were studied. Animals in each group received either vehicle or buprenorphine in random manner. Group-1 animals were subjected to 37 minutes of ischemia, placed in 29°C incubator for 4 hours, then returned to room temperature. Buprenorphine was administered every 12 hours for 3 days. Group-2 animals were treated similarly to group-1 animals except that the former were subjected to 32 minutes of ischemia. Group-3 animals were subjected to 32 minutes of ischemia, and body temperature and activity were measured for 3 days while animals were housed at 30°C. Buprenorphine was given at 0 and 16 hours. Treatment of group-4 animals was similar to that of group-3 animals except that buprenorphine was given 0, 8, 20, 32, 44, 56, and 68 hours.

Surgery and experimental protocol: Mice were anesthetized by intramuscular administration of ketamine (100 mg/kg of body weight), xylazine (10 mg/kg), and acepromazine (KXA: 1 mg/kg). The hair over the abdomen was shaved, and skin was scrubbed three times with a Betadine surgical scrub, followed by wiping of the surgical site with alcohol. The animals were placed on a heating table kept at 39°C to maintain constant body temperature. Blood (100 μl) was collected through a tail vein. A midline incision was made, and both renal pedicles were cross-clamped for 32 or 37 minutes. Both kidneys were inspected for ischemia after 2 minutes. To help maintain thermoregulation during surgery, the abdominal contents were replaced, and the abdomen was temporarily closed with several sutures. The abdomen was re-opened, and the clamps were removed. The kidneys were again inspected for restoration of blood flow, and 1 ml of pre-warmed (37°C) normal saline was instilled into the abdominal cavity. The abdomen was closed in two layers.

Immediately after the operation, mice were treated with buprenorphine (0.5 mg/kg) or vehicle (0.3 ml of normal saline) given subcutaneously. Although the published dosage of buprenorphine in mice varies, we chose 0.5 mg/kg which is slightly higher than the recommended dosage. This dose provided adequate analgesia without complications. To promote recovery from anesthesia and surgery, animals were placed for four hours in a transparent lucite-walled animal incubator kept at 29°C. The temperature was controlled by a proportional solid-state feedback controller, and fresh air was constantly introduced into the incubator and vented into the building exhaust system. Additional buprenorphine was given. Blood samples were obtained from a tail vein every 24 hours. The animals were euthanized at 72 hours. At the time of euthanasia with KXA, blood was collected for measurement of plasma BUN and creatinine concentrations. Both kidneys were harvested for histologic examination.

Measurements: Renal function in humans is typically measured by changes in serum or plasma creatinine concentration, since the steady-state value accurately reflects glomerular filtration rate. Higher creatinine concentration is indicative of more intense renal injury. Therefore, we estimated renal function, using plasma creatinine concentration, as in previous studies (7, 18). Creatinine was measured by use of an Astra 8 autoanalyzer (Beckman Instruments, Inc., Fullerton, CA). Semi-qualitative renal histologic examination was performed on formalin-fixed, hematoxylin and eosin-stained sections (7). We measured the axial percentage of the outer stripe of the outer portion of medulla that contained necrotic outer medullary thick ascending limbs, and the axial percentage of the inner stripe of the outer portion of the medulla that contained erythrocyte aggregation.

Body temperature and physical activity: In two groups of animals, core body temperature and physical activity were measured in conscious unrestrained animals, using a VitalView system (MiniMitter, Sunriver, OR). During renal ischemia reperfusion surgery, a temperature-sensitive transponder (PDT-4000) was inserted into the peritoneal cavity. The signal emitted by the transponder is converted into temperature by the VitalView software. Activity was measured by use of biotelemetry in a cage containing multiple zones. Movement of an animal from one zone to the next zone counts as one unit of activity. Temperature and activity were recorded by biotelemetry every second, and averaged over 1-hour intervals. Instead of placing the animals in a 29°C incubator, the animals were housed in standard individual barrier plastic cages maintained at 30 ± 1°C. This allowed the animal's temperature to be determined as soon as possible after surgery. This temperature is within the thermoneutral zone (30 to 34°C) for mice (19). The thermoneutral zone is defined as the temperature range at which the animal has minimal metabolic rate (19, 20). Mice housed at room temperature (24 to 25°C) experience some degree of cold stress (20).

Statistical analysis: All data were expressed as mean ± SEM. Different treatments were compared, using *t*-tests (two groups) or analysis of variance techniques (multiple groups; completely randomized design) followed by Dunnett's test for individual comparisons between group means. The null hypothesis was rejected when $P < 0.05$.

Results

Body weight: Mice were subjected to 32 (group 1) or 37 (group 2) minutes of ischemia, and given either buprenorphine or vehicle

after surgery, at 0, 6, 16 hours after the operation and every 12 hours for 3 days. Animals were weighed daily. There were no significant differences in weight (Figure 1) in the animals subjected to 32 minutes of ischemia; however, animals treated with buprenorphine after 37 minutes of ischemia had significantly less weight loss than did untreated animals.

Renal injury: Figure 2 shows the plasma creatinine concentration measured daily in the same group of animals subjected to 32 or 37 minutes of ischemia and treated with buprenorphine or vehicle. There was no effect of buprenorphine on renal injury or renal recovery in either group. Buprenorphine also did not significantly alter the semi-quantitative measurements of histologic damage (outer stripe necrosis, inner stripe hemorrhage; Figure 3).

Body temperature and activity: We noticed that the buprenorphine-treated animals were standing on all four limbs and moving around the cage sooner after surgery than did the vehicle-treated animals. Therefore, we formally measured activity and body temperature in two additional groups of animals. Transponders that report core body temperature and activity were implanted into the peritoneal cavity at the time of the renal pedicle clamping. Some animals (group 3) were subjected to 32 minutes of ischemia and were given vehicle or buprenorphine immediately after surgery, and again 16 hours later (Figure 4). The buprenorphine-treated animals recovered normal body temperature and diurnal rhythm faster than did untreated mice (Figure 4, top). The buprenorphine-treated animals were more active at the end of the first postoperative day (Figure 4, bottom). Buprenorphine did not significantly alter the creatinine concentration measured at 24 hours (72-hour samples not obtained; data not shown).

The final group of animals (group 4) was subjected to 32 minutes of ischemia, and treated with vehicle or buprenorphine starting immediately after surgery and approximately every 12 hours for 3 days. The buprenorphine-treated mice had some tendency to recover body temperature faster than did untreated mice (Figure 5, top). The buprenorphine-treated mice also were more active than the untreated mice during the first postoperative day (Figure 5, bottom). Buprenorphine did not significantly alter the creatinine concentration measured at 24, 48, or 72 hours (data not shown).

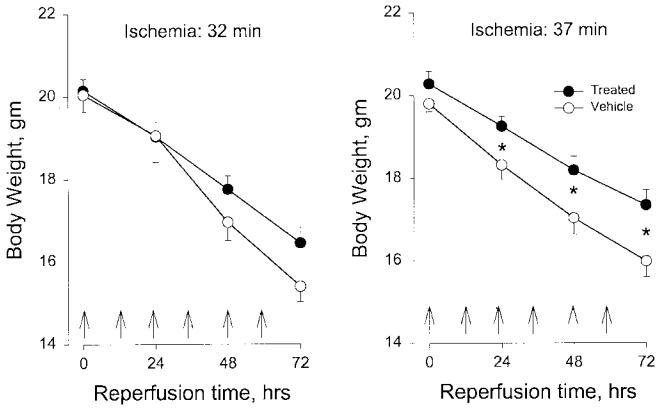

Figure 1. Effect of buprenorphine given after surgery on body weight. Mice were subjected to 32 (left) or 37 (right) minutes of bilateral renal ischemia. Buprenorphine or saline vehicle was started immediately after the operation and given every 12 hours (arrows). Body weight was measured before surgery and daily. N = 8/group for 32 minutes of ischemia, and N = 16/group for the 37 minutes of ischemia experiment. One animal of the 32 minutes untreated group and two animals of the untreated 37 minutes group died. The animals presumably died of renal failure, as we have seen in previous studies (7). * $P < 0.05$.

Discussion

Postoperative pain management in animal research involves understanding of pain in animals, and close cooperation between the investigator, investigator's staff, surgeon, and veterinary care staff. In the study reported here, the investigators consulted with the veterinarian and Animal Care and Use Committee on the proper selection of analgesics so as not to compromise the study objective, but still follow animal welfare regulations and guidelines. Buprenorphine hydrochloride was chosen since it appears to have minimal effects on renal function in renal disease, compared with nonsteroidal anti-inflammatory drugs (21). Buprenorphine is a combined opiod agonist-antagonist that provides pain relief by binding to mu, kappa, and delta opioid receptors. It is a partial mu-agonist (22) that binds mu_1 receptors (23), and a kappa antagonist (15). Some (24) but not all (23) studies have indicated that it is a delta agonist. Buprenorphine is useful for postoperative analgesia because it is a strong analgesic that has pharmacologic effects that occur within 15 minutes after intramuscular administration, with prolonged duration of action; a single dose of 0.3 mg/kg may provide analgesia for approximately 6 to 12 hours (25). The danger of respiratory depression is low, and can be reversed by administration of naloxone. The buprenorphine dose commonly used varies depending on route of administration and species (16, 22, 26). The published dose of buprenorphine in mice varies greatly. The normal accepted and recommended range of buprenorphine dosing is 0.05 to 0.1 mg/kg given intraperitoneally, although doses as high as 50 mg/kg have been published (22, 27–29). It is recommended that buprenorphine be used with caution in animals with severe hepatic, pulmonary, or renal dysfunction. We selected a mid-range dose of buprenorphine (0.5 mg/kg) to ensure adequate analgesia and to simplify testing for an interaction of buprenorphine and the renal injury model. We observed that buprenorphine-treated animals had more locomotion activity in

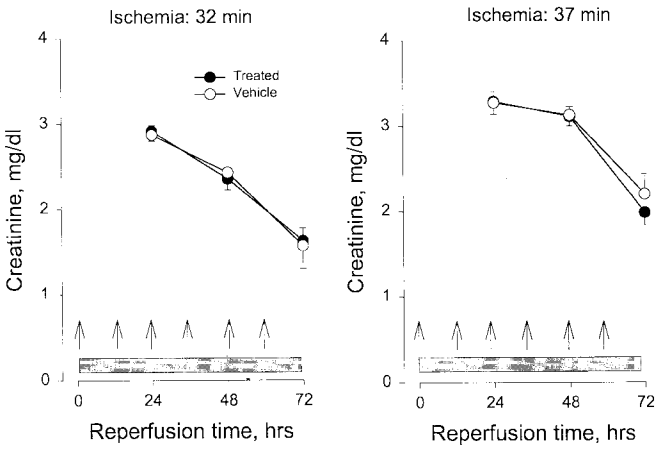

Figure 2. Effect of buprenorphine on renal function. Mice were treated as indicated in Figure 1. Tail vein blood was obtained every 24 hours. N = 8/group for 32 minutes of ischemia, and N = 16/group for the 37 minutes of ischemia experiment. Shaded area indicates normal creatinine concentration in separate group of mice.

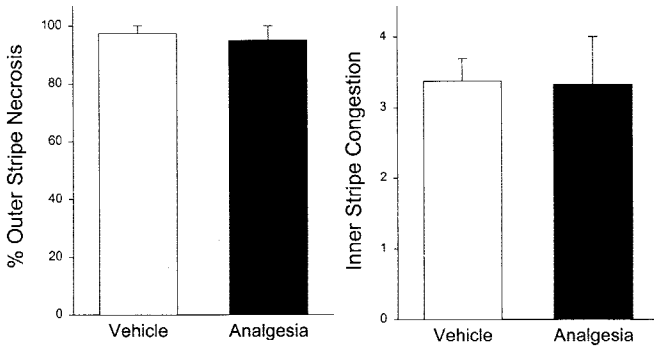

Figure 3. Effect of buprenorphine on renal histologic changes. Mice were treated as indicated in Figure 1. Renal histologic changes at day 3 were measured as described in Materials and Methods. N = 8/group (only the last 8 of the animals subjected to 37 minutes of ischemia were studied).

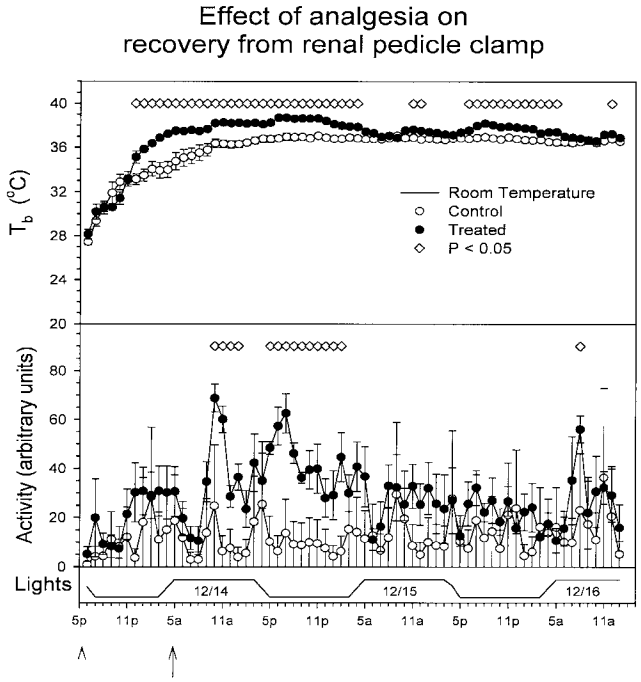

Figure 4. Effect of a short course of buprenorphine on body temperature and activity. After 32 minutes of bilateral renal ischemia, a transponder was implanted in the peritoneal cavity. Buprenorphine was given immediately after the operation and 16 hours later. N = 8/group.

the immediate postoperative period; the animals were up on their feet faster. Formal testing was performed, using telemetry to measure activity. Mice were housed at 30°C, within the thermoneutral zone to minimize temperature stress (19, 20). Buprenorphine caused small but significant improvements in body weight and activity, and a tendency for more normal body temperature control. The reasons for the different patterns of body temperature and activity between animals of groups 3 and 4 (Figures 3 and 4) during the first 24 hours are not known. However, the temperature and activity measurements are subject to wide variation; hence, it is important to simultaneously measure responses in a control (vehicle-treated) group as was done in these experiments.

In contrast, buprenorphine did not have any significant ef-

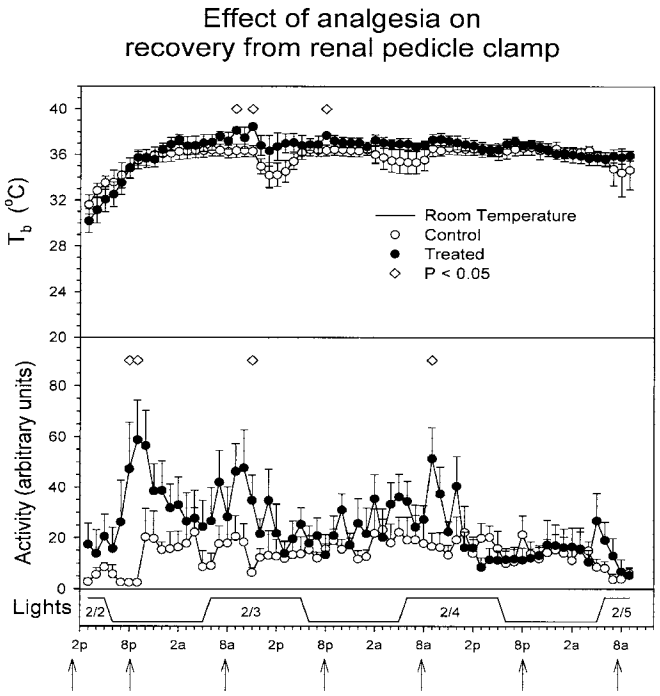

Figure 5. Effect of a long course of buprenorphine on body temperature and activity. Mice were treated as in Figure 4 except that buprenorphine was given approximately every 12 hours for 3 days. N = 8/group.

fects on renal injury. The increase in creatinine concentration from day 0 to day 1 was identical in the 32 and 37 minutes of ischemia groups. Furthermore, the recovery of renal function from day 1 to days 2 and 3 was also unaltered by buprenorphine. Finally, buprenorphine treatment did not affect the degree of renal damage assessed by histologic changes, using measures of renal injury in the outer and inner stripes of the outer portion of the medulla. Taken together, these observations suggest that animals were in pain after abdominal surgery, and that treatment of pain improved the general health of the animal without altering the degree of renal injury.

Pain management should include observation of the animal during and after the surgical procedure with close cooperation between the investigator, investigator's staff, surgeon, and veterinary care staff. Other factors to consider include proper selection of analgesics and effective dosages, ensuring individuals performing procedures and administrating analgesics are properly trained and are following established veterinary medical surgical and postoperative care procedures. Personnel training is key for proper aseptic surgical techniques, recognition and assessment of pain, and administrating analgesics at the proper dose and frequency. It is difficult to assess pain and distress in rodents, but analgesics should not be avoided because pain cannot be quantified. Objective assessment for pain can be done by observing changes in food and water intake, body weight, and activity patterns (30, 31).

We conclude that buprenorphine should be given after renal ischemia-reperfusion surgery since administration of the proper analgesia improved animal health without interfering with the renal ischemia/reperfusion model. Analgesic treatment at the time of the operation and 12 hours after was sufficient. Proper analgesia may reduce the postsurgical stress response, and thus

potentially improve the specificity of testing for drugs that reduce or treat renal injury.

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