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Comparative analgesic efficacy of morphine sulfate and butorphanol tartrate in koi (*Cyprinus carpio*) undergoing unilateral gonadectomy

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Abstract

Objective—To identify pain-related behaviors and assess the effects of butorphanol tartrate and morphine sulfate in koi (*Cyprinus carpio*) undergoing unilateral gonadectomy.

Design—Prospective study.

Animals—90 adult male and female koi.

Procedures—Each fish received saline (0.9% NaCl) solution (which is physiologically compatible with fish) IM, butorphanol (10 mg/kg [4.5 mg/lb], IM), or morphine (5 mg/kg [2.3 mg/lb], IM) as an injection only (6 fish/treatment); an injection with anesthesia and surgery (12 fish/treatment); or an injection with anesthesia but without surgery (12 fish/treatment). Physiologic and behavioral data were recorded 12 hours before and at intervals after treatment.

Results—Compared with baseline values, the saline solution–surgery group had significantly decreased respiratory rates (at 12 to 24 hours), food consumption assessed as a percentage of floating pellets consumed (at 0 to 36 hours), and activity score (at 0 to 48 hours). Respiratory rate decreased in all butorphanol-treated fish; significant decreases were detected at fewer time points following morphine administration. In the butorphanol-surgery group, the value for food consumption initially decreased but returned to baseline values within 3 hours after treatment; food consumption did not change in the morphine-surgery group. Surgery resulted in decreased activity, regardless of treatment, with the most pronounced effect in the saline solution–surgery group. Changes in location in water column, interactive behavior, and hiding behavior were not significantly different among groups. Butorphanol and morphine administration was associated with temporary buoyancy problems and temporary bouts of excessive activity, respectively.

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Conclusions and Clinical Relevance—Butorphanol and morphine appeared to have an analgesic effect in koi, but morphine administration caused fewer deleterious adverse effects. Food consumption appeared to be a reliable indicator of pain in koi.

The demand for evidence-based fish medicine is growing, not only within zoo and aquaria medicine, but also within the aquaculture industry, fisheries management, companion and laboratory animal medicine, and conservation medicine.^{1–3} The lack of experimentally derived data for the use of analgesic drugs in fish is a major clinical disadvantage, particularly given the recent interest in fish surgical and diagnostic procedures.^{4–12} Despite the debate about whether fish are capable of feeling pain, teleost (bony) fish have the necessary neuroanatomic and neurophysiologic components and behavioral responses to meet the criteria for pain perception.^{3,13–19}

In response to stressors (eg, handling and noxious stimuli), fish develop avoidance and anomalous behaviors and develop pain-related physiologic responses (eg, changes in opercular rate, heart rate, cardiac output, food consumption, activity, and circulating concentrations of endogenous cortisol, glucose, electrolytes, and blood gases).^{3,15,17,19–26} Administration of morphine (a μ -opioid receptor agonist), tramadol (a μ -opioid receptor agonist and serotonin and norepinephrine reuptake inhibitor), and lidocaine (a local anesthetic agent) in fish attenuates some of these physiologic and behavioral changes in a dose-dependent manner.^{20,23,26–32} Although experiments involving morphine, tramadol, and lidocaine used chemical, thermal, or electrical methods to induce pain, a more relevant pain model involving surgery (ie, exploratory celiotomy) has been used to evaluate butorphanol and ketoprofen.²¹ In koi (*Cyprinus carpio*), intraoperative administration of butorphanol (a κ -opioid receptor agonist and partial μ -opioid receptor agonist and antagonist; 0.4 mg/kg [0.2 mg/lb], IM) resulted in only mild improvement in postsurgical behavior, compared with the effect of administration of saline solution.²¹ Butorphanol (0.1 to 0.4 mg/kg [0.05 to 0.2 mg/lb]) is used more commonly than other analgesic drugs in fish, but no information is available to substantiate that butorphanol is effective at these mammalian- or avian-derived doses.^{4,10} Similar to other ectotherms (ie, reptiles and amphibians) in which comparatively low body temperature and variable blood pH (compared with mammals) decrease opioid drug solubility and affect drug metabolism,^{33–35} fish may require relatively high doses of butorphanol to achieve an analgesic effect. Ketoprofen (an NSAID; 2.0 mg/kg [0.9 mg/lb], IM) had no analgesic effect in fish undergoing celiotomy.²¹

The present study was undertaken to investigate the efficacy of clinically relevant opioid drugs (morphine sulfate and butorphanol tartrate) administered IM in koi undergoing unilateral gonadectomy, a novel surgical model of pain in fish. The use of a surgical gonadectomy to assess pain is advantageous because it mimics a realistic, clinical situation and presumably causes more visceral pain than does celiotomy alone. Morphine and butorphanol were chosen because they are commonly used for analgesia in vertebrates and are considered the most effective drugs for controlling pain.³⁶ The doses of morphine (5.0 mg/kg [2.3 mg/lb]) and butorphanol (10 mg/kg [4.5 mg/lb]) evaluated were selected on the basis of previous reptile and fish studies.^{12,29,33} The objectives of the study reported here were to identify pain-related behaviors via surgical induction of pain, evaluate the effects of morphine and butorphanol on signs of postsurgical pain, and assess potential deleterious

adverse effects (eg, respiratory depression) of opioid drugs in koi. On the basis of results of previous reptile and amphibian studies,^{33,37} we hypothesized that morphine would be a more effective analgesic drug than butorphanol.

Materials and Methods

Animals

Ninety adult koi (23 males, 19 females, and 48 of unknown sex) were obtained from a commercial supplier.^a The mean $\pm$ SEM weight of the koi was 280 ± 6.7 g (0.62 ± 0.015 lb); they ranged in length from 22.9 to 33.0 cm. The koi were kept in 37.85-L ($50.8 \times 30.3 \times 25.4$ -cm) aquaria (1 fish/aquarium) in a stand-alone, flow-through system,^b with each of 12 aquaria equipped with an air stone. This was a semiclosed system with 10% automatic water exchange/d. Water temperature (20.0° to 23.9° C), pH (6.8 to 8.0), salinity (0 to 3 μ g/mL), ammonia concentration (total ammonia nitrogen concentration, 0.0 to 0.5 mg/L), and nitrate concentration (0.00 to 0.04 mg/L) were monitored and maintained at acceptable levels for koi. Light was provided for 14 h/d. Fish were fed a commercially available pelleted food^c and sinking food^d (1% of body weight/d). All housing and experimental procedures were approved by the Animal Care and Use Committee at the University of Wisconsin School of Veterinary Medicine.

Study design

A prospective experimental design was used to evaluate morphine- and butorphanol-dependent changes in physiologic and behavioral variables. One person who was both surgeon and observer made real-time observations and was unaware of which injection was administered. Equivalent volumes of physiologic saline (0.9% NaCl) solution, morphine sulfate, or butorphanol tartrate were administered IM to koi that were randomly assigned to 1 of 9 treatment groups: saline solution injection only (SS group; $n = 6$), saline solution with anesthesia and surgery (SS+ group; 12), saline solution with anesthesia but without surgery (SS- group; 12), butorphanol injection only (B group; 6), butorphanol with anesthesia and surgery (B+ group; 12), butorphanol with anesthesia but without surgery (B- group; 12), morphine injection only (M group; 6), morphine with anesthesia and surgery (M+ group; 12), or morphine with anesthesia but without surgery (M- group; 12). In the injection-only groups, saline solution, butorphanol (10 mg/kg, IM), or morphine (5.0 mg/kg, IM) was administered into the dorsal musculature just ventral to the dorsal fin of each fish. In the groups undergoing anesthesia, saline solution, butorphanol (10 mg/kg, IM), or morphine (5.0 mg/kg, IM) was administered into the dorsal musculature just ventral to the dorsal fin of each fish after bath induction of anesthesia. All fish undergoing anesthesia were anesthetized with buffered tricaine methanesulfonate (MS-222), which was prepared as a 10 mg/mL stock solution (10 g of MS-222 powder with 2.5 g of NaHCO_3 mixed in 1.0 L of distilled H_2O). For fish in the SS+, B+, and M+ groups, the surgical procedure performed was a unilateral gonadectomy.

^aHoffer's Pet Supply, Milwaukee, Wis.

^bAquatic Habitats, Apopka, Fla.

^cLaguna, Mansfield, Mass.

^dHikari, Hiakari, Japan.

Physiologic and behavioral data were recorded before (baseline) and at intervals after treatment. Variables of interest included respiratory rate (opercular beats/min), food consumption (assessed as percentage of 20 floating pellets consumed in a 20-minute period [after which the excess pellets were removed]), activity (scored on a scale of 0 to 10, based on the percentage of time fish performed swimming movements in a 2-minute period [0 = 0% of time or no movement, 1 = 10%, 2 = 20%, 3 = 30%, 4 = 40%, 5 = 50%, 6 = 60%, 7 = 70%, 8 = 80%, 9 = 90%, and 10 = 100% or constant spontaneous swimming movement]), location in aquarium water column (scored on a scale of 1 to 4 [1 = resting on bottom, 2 = located in bottom third, 3 = located in middle third, and 4 = located in top third]), interactive behavior (scored on a scale of 1 to 3 [1 = does not approach observer or sinking food pellet, 2 = approaches observer and sinking food pellet but does not eat pellet, and 3 = approaches observer and sinking food pellet and eats sinking food pellet]), and hiding behavior (scored on a scale of 1 to 2 [1 = not hiding and 2 = hiding behind drain pipe or with head facing back corner of aquarium]).

For all experiments, a physiologic variable (respiratory rate) was recorded 12 hours before and at 0, 3, 6, 9, 12, 18, 24, 36, 48, and 72 hours after treatment. For all experiments, values for measures of behavioral variables (food consumption, activity, water column location, interactive behavior, and hiding behavior) were recorded at baseline (means of values at 4 time points from the 24-hour period before treatment) and at 0, 3, 6, 9, 12, 18, 24, 36, 48, and 72 hours after treatment. In the injection-only groups (SS, B, and M), fish were immediately returned to the aquaria after injection to obtain data for the 0-hour time point. For all other treatment groups (SS+, SS−, B+, B−, M+, and M−), data for the 0-hour time point were obtained once the fish were returned to their aquaria approximately 15 minutes after recovery from anesthesia; for fish, the mean time to recovery from MS-222 anesthesia is approximately 15 minutes.³⁸

Anesthetic and surgical procedures

For the treatment groups that received anesthesia (SS+, SS−, B+, B−, M+, and M− groups), 1 fish undergoing anesthesia and surgery was paired with a fish undergoing anesthesia but no surgery. Fish were caught by netting. The paired fish were treated at the same time; anesthesia was maintained and, at completion of surgery in the assigned fish, both fish were allowed to recover from anesthesia simultaneously.

For perianesthetic monitoring, a blood sample from the caudal tail vein of each fish was collected (0.1 mL) in a 1-mL syringe containing lithium heparin with a 25-gauge needle prior to induction of anesthesia. The blood sample was immediately processed with a hand-held clinical analyzer^e and cartridge,^f which was used to measure blood lactate concentration (mmol/L), pH, P_{CO}₂ (mm Hg), and P_O₂ (mm Hg). Anesthesia was induced by placing each fish in a 37.85-L bucket containing 100 mg of buffered MS-222/L. Induction of anesthesia was considered to have occurred once the fish's righting reflex was lost. Each anesthetized fish was then administered the appropriate analgesic drug or saline solution injection and placed in dorsal recumbency on a foam pad placed atop a recirculating fish

^ei-STAT, Abbott Laboratories, Princeton, NJ.

^fCG4+ handheld clinical analyzer cartridge, Heska Corp, East Windsor, NJ.

anesthesia delivery system (supplied with room air and a maintenance concentration of 60 mg of MS-222/L).

For each fish undergoing surgery, the ventral midline was prepared with sterile saline solution, and the surgical site was draped. Approximately 10 to 15 minutes elapsed between drug or saline solution administration and the initial scalpel incision. A No. 15 scalpel blade was used to incise along the ventral midline through the skin and coelomic membrane; the incision extended from immediately cranial to the cloacal opening to the cartilaginous pectoral girdle, which was incised with scissors. The coelomic cavity was exposed, and the gonads were identified lateral to the gastrointestinal tract; the left gonad was selected for removal. Cautery and traction were used to dissect the gonadal tissue and prevent hemorrhage. Hemoclips were used to transfix the testicular or ovarian artery and vein. After the left gonad was removed, the coelom and skin were closed in a single, continuous layer by use of 3-0 polyglyconate suture in a Ford interlocking pattern, and povidone-iodine antiseptic ointment was applied to the closed incision. Before recovery from anesthesia, a second blood sample (0.1 mL) was collected from fish that did or did not undergo surgery, and all fish were administered oxytetracycline (20 mg/kg [9.1 mg/lb], IM). Mean duration of anesthesia and surgery was 53 and 28 minutes, respectively. Each fish was allowed to recover from anesthesia and was monitored for approximately 15 minutes in a 37.85-L bucket containing saline solution (2 µg/mL) dissolved in aquarium water. Once fully recovered, each fish was returned to its home aquarium.

The analgesic rescue protocol involved removal from the study and administration of morphine (5.0 mg/kg, IM) and appropriate palliative care if an individual koi from any of the treatment groups appeared markedly distressed (eg, not breathing or persistent inactivity for > 24 hours). Euthanasia was to be considered in cases with severe, nonresponsive conditions; however, no fish in the study required postoperative euthanasia.

Data analysis

For respiratory rate; values for measures of location in the water column, food consumption, and activity; and blood variables, a Kruskal-Wallis ANOVA on ranks was calculated for comparisons among multiple treatment groups. Post hoc comparisons were made via the Dunn test. A Mann-Whitney rank sum test was calculated for comparisons between 2 treatment groups. A Wilcoxon matched pairs test was calculated for comparisons with baseline values within treatment groups. For interactive and hiding behavior scores, a 2-tailed Fisher exact test was performed. All data were analyzed with the aid of commercially available software.[§] All data are expressed as mean ± SEM. Values of $P < 0.05$ were considered significant.

Results

Because of nonsignificant variability in baseline respiratory rates (Table 1), data for this variable were normalized for individuals given the same treatment and means calculated and graphed as changes with respect to baseline values to improve clarity (Figure 1). Actual data

[§]Sigma Stat, version 2.03, Jandel Scientific Software, San Rafael, Calif.

for food consumption were graphed (Figure 2), whereas data for location in the water column, interactive behavior, and hiding behavior were not graphed. Similarly, because of nonsignificant variability in baseline activity scores, data for this variable were normalized for individuals given the same treatment and means calculated and graphed as changes with respect to baseline values to improve clarity (Figure 3).

Respiratory rate

Among the injection-only groups (S, B, and M groups; Figure 1), respiratory rate remained unchanged from baseline value for those in the SS group. Fish in the B group had significantly ($P < 0.05$) decreased respiratory rate, compared with the baseline value, at 3, 9, 12, and 18 hours after treatment; these fish had significantly ($P = 0.034$) decreased respiratory rate, compared with that for the SS group, at 3 hours after treatment. Respiratory rate for fish in the M group did not differ from baseline value or from the finding for the SS group at any time point. A significant ($P = 0.039$) difference in respiratory rate between the B and M groups was evident only at 0 hours after treatment.

For fish undergoing anesthesia without surgery (SS-, B-, and M- groups; Figure 1), respiratory rate was significantly ($P = 0.04$) increased, compared with baseline value, in the SS- group at 0 hours after treatment; however, respiratory rate was decreased, compared with baseline value, in the SS- group at 18 hours after treatment. Compared with the SS group, respiratory rate was significantly ($P < 0.05$) decreased in the SS- group at 3, 24, and 48 hours after treatment. The fish in the B- group had significantly ($P < 0.05$) decreased respiratory rates, compared with baseline value, at 3 to 18 hours after treatment except at the 9-hour time point ($P = 0.220$). The B- group also had significantly decreased respiratory rates, compared with those for the SS- group, at 0 ($P = 0.039$) and 6 ($P = 0.018$) hours after treatment. No significant differences in respiratory rate were observed for the M- group, compared with baseline value or findings for the SS- or B- group.

For fish undergoing anesthesia and surgery (SS+, B+, and M+ groups; Figure 1), respiratory rate was significantly ($P < 0.05$) decreased, compared with baseline value, in the SS+ group at 12 to 24 hours after treatment. Compared with the SS- group, respiratory rate was significantly ($P < 0.05$) decreased in the SS+ group at 9 to 18 hours after treatment. In the B+ group, respiratory rates were significantly ($P < 0.05$) decreased, compared with baseline value, from 0 to 72 hours after treatment. Fish in the M+ group had significantly ($P < 0.05$) decreased respiratory rates, compared with baseline value, at 0 to 18 hours after treatment, except at the 9-hour time point ($P = 0.345$). Respiratory rates in the M+ group were significantly ($P = 0.036$) decreased, compared with findings in the M- group, at 6 hours after treatment. Thus, during the experimental period, significant decreases in respiratory rate were observed in the SS+ and M+ groups and all butorphanol-treated fish.

Food consumption

Baseline values for the percentage of 20 floating food pellets consumed in 20 minutes was 100% for all fish in all treatment groups (Figure 2). For the injection-only groups (SS, B, and M groups), food consumption did not differ significantly from baseline value or among groups at any time point. Among the fish undergoing anesthesia without surgery (SS-, B-,

and M– groups), food consumption in the SS– group was significantly ($P = 0.027$) decreased, compared with the findings for the SS group, at 0 hours after treatment; no other significant differences in food consumption were detected within or between these 2 groups. Among the fish undergoing anesthesia and surgery (SS+, B+, and M+ groups), significantly ($P < 0.05$) decreased value for food consumption, compared with baseline value, was detected in the SS+ group at 0 to 36 hours after treatment. Compared with the SS– group, food consumption was significantly ($P < 0.05$) decreased in the SS+ group at 12 to 36 hours after treatment. Fish in the B+ group had significantly ($P = 0.006$) decreased food consumption, compared with baseline, at the 0-hour time point. Food consumption value in the M+ group did not differ significantly from baseline value or from findings for the M– or B+ groups at any time point. Thus, surgery alone without analgesic drugs resulted in a prolonged decrease in feeding behavior, whereas surgery with administration of butorphanol briefly decreased feeding behavior.

Activity

Mean baseline activity scores ranged from 4.5 to 7.2 (Table 1), with no significant differences among baseline values. Activity score was significantly ($P = 0.002$) decreased, compared with the baseline value, in the SS group at the 0-hour time point (Figure 3). For fish in the B group, activity score was significantly ($P < 0.05$) decreased, compared with baseline value, at 0 to 18 hours after treatment. Compared with findings for the SS group, activity score was significantly ($P = 0.009$) decreased in the B group at 6 and 9 hours after treatment. In the M group, activity score did not differ significantly from baseline value at any time point; however, there was a significant ($P = 0.026$) decrease in activity score, compared with the score for the SS group, at 6 hours after treatment.

For fish undergoing anesthesia without surgery (SS–, B–, and M– groups; Figure 3), significantly ($P < 0.05$) decreased activity scores, compared with the baseline value, were detected in the SS– group at 0, 3, and 12 hours after treatment. Compared with the score for the SS group, fish in the SS– group had significantly ($P < 0.05$) decreased activity scores at 3 to 12 hours after treatment. At 0 to 6 hours after treatment, activity score in the B– group was significantly ($P < 0.05$) decreased, compared with the baseline value. In the M– group, activity score was decreased, compared with the baseline value, at 0 ($P = 0.005$) and 3 ($P = 0.012$) hours after treatment. However, fish in the M– group had increased activity score, compared with the score for the SS– group, at 18 ($P = 0.038$) and 24 ($P = 0.033$) hours after treatment.

For fish undergoing anesthesia and surgery (SS+, B+, and M+ groups; Figure 3), activity score was significantly ($P < 0.05$) decreased, compared with the baseline value, in the SS+ group at 0 to 48 hours after treatment. Compared with findings for the SS– group, activity score was significantly ($P < 0.05$) decreased in the SS+ group at 18 ($P = 0.033$) and 36 ($P = 0.021$) hours after treatment. In the B+ group, activity score was decreased, compared with the baseline value, at 0 to 18 and at 36 hours after treatment. In the M+ group, activity score was significantly ($P < 0.05$) decreased, compared with the baseline value, at 0 to 72 hours after treatment, except at the 24-hour time point ($P = 0.179$). Compared with findings for the M– group, larger decreases in activity score in the M+ group were evident at all time points

($P < 0.05$) except at 0 ($P = 0.166$) and 24 ($P = 0.175$) hours. Among the 30 fish that received morphine, regardless of treatment group, 13 (43%) fish displayed intermittent hyperactivity, characterized by staying in 1 location while rapidly undulating near the top of the water column. Thus, activity in koi was decreased by administration of butorphanol alone and also by anesthesia and surgery, given that there were significant decreases in activity score detected in the SS-, SS+, B-, B+, M-, and M+ groups.

Water column location

Data for location of fish in the aquarium water column were not graphed because no consistent changes were observed following any treatment. Mean baseline location scores ranged from 2.0 to 2.6 (Table 1), and scores for the injection-only groups (SS, B, and M groups) did not differ significantly from their respective baseline values. For fish undergoing anesthesia without surgery (SS-, B-, and M- groups), significant changes from baseline value were evident at only 2 time points. In the M- group, the score for location of fish in the water column was higher at 24 (2.6 ± 0.1 ; $P = 0.013$) and 48 (2.5 ± 0.2 ; $P = 0.027$) hours after treatment, compared with baseline value (2.1 ± 0.1). For fish undergoing anesthesia and surgery (SS+, B+, and M+ groups), scores for location in water column did not differ significantly among those treatment groups and did not differ from their respective baseline values over time, except in the SS+ group, in which score for location in the water column was lower than baseline value (2.4 ± 0.1) at 6 (2.1 ± 0.1 ; $P = 0.016$) and 36 (2.1 ± 0.2 ; $P = 0.041$) hours after treatment. Among the 30 fish that received butorphanol, regardless of treatment group, 28 (93%) fish developed temporary buoyancy problems, characterized by positioning of the head near the tank bottom and caudal fin near the surface of the tank. This positioning was maintained even during swimming and resolved within 48 hours after butorphanol administration, with no further complications. Thus, assessment of the location of fish within the aquarium water column was not useful for distinguishing among the treatment groups.

Interactive behavior

Data for interactive behavior were not graphed because no consistent changes were observed following any treatment. Baseline values for all fish in all treatment groups were each 3.0 (ie, approached observer and ate sinking food pellet). No significant differences in interactive behavior were observed within or among the injection-only groups (SS, B, and M). For fish undergoing anesthesia without surgery (SS-, B-, and M- groups), the only significant ($P = 0.037$) changes were decreased interactive behavior score in the SS- group at 0 hours after treatment (2.1 ± 0.3), compared with baseline value and compared with findings for the B- group at that time point (2.9 ± 0.1). In the SS+ group, significantly ($P = 0.019$) decreased interactive behavior score (2.0 ± 0.3), compared with baseline value, was detected at 0 hours after treatment. No other significant differences in interactive behavior score were observed within or among the SS+, B+, and M+ groups. Thus, changes in interactive behavior of the fish were sporadic and not useful for distinguishing among the treatment groups.

Hiding behavior

Baseline hiding behavior score was 1.0 (ie, not hiding) for all fish in all treatment groups. Hiding was not observed in the SS, SS-, B, B-, M, and M- groups; however, hiding was observed in 3 of 12 of fish in the SS+, B+, and M+ groups, regardless of treatment. Among the fish that displayed hiding behavior, the duration of hiding was 42.0 ± 11.4 hours in the SS+ group, 10.0 ± 6.4 hours in the B+ group, and 9.0 ± 1.7 hours in the M+ group. However, no significant differences in hiding behavior were observed within or among treatment groups. Thus, hiding behavior of the fish was not useful for distinguishing among the treatment groups.

Discussion

To our knowledge, this is the first study to use gonadectomy as a pain model to evaluate the efficacy of analgesic drugs in fish. Results of the present study suggested that both morphine and butorphanol have an analgesic effect on koi undergoing unilateral gonadectomy. Koi undergoing this surgical procedure without analgesic medication (receiving treatment with saline solution only) had responses associated with pain, including decreases in respiratory rate, food consumption, and activity. Food consumption appeared to be the most reliable indicator of pain in koi. In contrast, location of the fish in the water column and their interactive or hiding behavior were not sensitive indicators of pain.

The most striking finding was that in koi that received butorphanol in the B and B- groups, respiratory rates decreased, compared with baseline values, for approximately 3 to 18 hours after treatment. In a previous study²¹ in koi, decreased respiratory rate following butorphanol administration was not detected, but a much lower drug dose (0.4 mg/kg, IM) was used, compared with the dose of 10 mg/kg IM used in the present study. In the present study, no respiratory depression was observed in the injection-only and anesthesia without surgery groups that were administered morphine. Decreased respiratory rates were observed for koi after surgery, regardless of treatment group, but respiratory rates in fish that were administered morphine returned most quickly to baseline values (within 24 hours after treatment). For fish that were administered saline solution or butorphanol and underwent surgery, respiratory rates did not return to baseline value until 36 and 72 hours after treatment, respectively. Although respiratory depression appeared to be an adverse effect of butorphanol administration in koi, there was a prolonged decrease in respiratory rate in B+ group, compared with findings for the B and B- groups. It is unclear whether pain contributed to the prolonged respiratory depression in the B+ group or to the initial decrease in respiratory rate in the M+ group, but similar pre- and postsurgery food consumption data in both groups suggested an analgesic effect with either opioid drug.

Interestingly, respiratory rates in the fish in the SS- and SS+ groups were initially increased immediately after treatment, but then decreased for up to 24 hours after treatment. An initial increase in respiratory rate and a subsequent decreasing respiratory rate starting several hours after surgery are both consistent responses in fish, as described in other reports.^{17,21-23,26,39,40} The initial increased respiratory rate may be indicative of a secondary (compensatory) stress phase that results from activation of the hypothalamic-pituitary-interrenal axis and subsequent cortisol release,⁵ whereas the prolonged decrease in

respiratory rate that follows may be indicative of a tertiary (decompensatory or maladaptive) stress phase.⁴¹

Although the effect of respiratory depression and potential compensatory mechanisms in fish are not yet known, hypoxemia has been implicated in causing the release of stress hormones.^{5,42} Therefore, it seems reasonable to suggest benefits in the clinical use of a drug with less respiratory compromise. Even though morphine induced intermittent hyperactivity in koi at the dose administered in the present study, it appeared to be the superior choice over butorphanol (at the doses studied) for providing analgesia to koi while minimizing potentially deleterious adverse effects. In one of the authors' experience, hyperactivity is observed in a variety of reptile species after morphine is administered at higher (> 10 mg/kg) doses. Interestingly, results of a recent dose-response study²⁹ in rainbow trout (*Onchoryhncus mykiss*) confirmed that the dose of morphine that is pharmacologically effective for 50% of the population exposed to it (6.7 ± 0.8 mg/kg [3.0 ± 0.4 mg/lb]) is higher for fish than for mammals and approximates the morphine dose used in the present study.

Fish in the SS+ group had a prolonged decrease in food consumption through the 36-hour time point in the present study. Administration of morphine or butorphanol greatly attenuated the decrease in food consumption in the SS+ group. Food consumption did not decrease in fish administered morphine, and the food consumption value returned to baseline value within 3 hours after treatment in fish administered butorphanol, suggesting that both opioid drugs provided analgesia. Similarly, decreased food consumption was also observed in rainbow trout after injections of bee venom or acetic acid into the lip, which was also attenuated by morphine or lidocaine administration.^{6,26} The most reliable indicator of pain evaluated in the present study was food consumption because in the SS+ group, the food consumption value decreased, compared with baseline value and compared with findings for the M+, B+, SS- groups.

Decreased activity was observed after surgery, regardless of treatment group, but the SS+ group had the most pronounced decrease in activity. The prolonged decrease in activity through the 48-hour time point in this group was striking but not surprising, given that brown trout (*Salmo trutta*) required up to 7 days to return to baseline activity patterns after only 5 minutes of restraint.²² The decrease in activity score from baseline value in the M+ group (from 0 to 72 hours after treatment) was unexpected because decreased activity was not observed in the corresponding injection-only group and was observed only transiently in the M- group at 0 and 3 hours after treatment. In fact, activity was increased in the M- group, compared with that in both the B- and SS- groups. Anesthesia with MS-222 may have contributed to the decreased activity observed in all anesthesia without surgery groups. Similar to the findings for food consumption, activity was decreased in the butorphanol injection-only and anesthesia without surgery groups, but the decrease was even more prolonged in the B+ group. It is unclear whether the prolonged decrease in activity in the M+ and B+ groups was a result of inadequate analgesia. Alternative explanations for these results are that activity was an inadequate indicator of pain in koi or, more likely, that the measure of activity used was not sufficiently sensitive to detect changes. If the latter is true,

assessment of caudal fin beat rate²¹ or use of a bioelectronic monitoring system²² may have provided a more rigorous method for measuring overall activity level.

The development of adverse effects in fish that were administered morphine or butorphanol was unexpected. Morphine was associated with intermittent hyperactivity, similar to opioid-induced hyperactivity observed in other species, such as cats, mice, horses, and humans.^{43–45} This adverse effect was observed previously following administration of doses of morphine as low as 2.0 mg/kg.^{28,46} Those researchers described the behavior as hyperactive, manic, and erratic with “extremely quick and intense, jerky, zigzag movement without orientation.”⁴⁶ Following butorphanol administration, most fish developed buoyancy problems, which has not been previously described, to our knowledge. Butorphanol administration was also associated with decreased respiratory rate and food consumption (in both the B and B– groups), which was not the case in the morphine treatment groups. Overall, morphine administration caused fewer important adverse effects, compared with butorphanol administration. This is an interesting finding because butorphanol efficacy was previously evaluated and is more commonly administered empirically to fish for procedures or conditions that are considered painful.^{10,21,47}

For the fish that underwent anesthesia and unilateral gonadectomy, there was no significant change in location within the water column, except at 2 time points for the SS+ group. In a previous study,²¹ koi were observed to swim lower in the water column after surgery. Thus, location in water column was not a useful indicator of pain in the fish of the present study.

Despite an apparent decrease in response to interactive stimuli and increase in hiding behavior in the SS+ group in the present study, these findings were not significant. It is possible that hiding behavior would become a more useful indicator of pain in fish if the tank were equipped with a structure behind or within which the fish could hide.

Fish species not only respond to pain differently than mammals,⁴⁸ but opioids can also affect fish species differently.⁴⁹ With approximately 30,000 identified fish species, determining behavioral responses to pain and analgesic efficacy in fish is a daunting task. Koi were used in the present study because they belong to the cyprinid family, which includes freshwater fish species that are commercially and scientifically important worldwide. Koi are commonly used in displays and are an internationally important ornamental and aquaria species; also, a variety of carp species are commonly used as human food sources in Europe and Asia.^{50,51} The considerable numbers of cyprinids being used or displayed in research projects, aquaria, aquaculture, and zoos and maintained by hobbyists highlight the need to understand pain mechanisms and responses and analgesic efficacy in these species.

Knowledge of species-typical behavior and the ability to discriminate behavior indicative of pain are crucial to the study of analgesia in humans and other animals. Few methods have been used for assessing pain in fish. To quantify behavioral responses to noxious stimuli in fish, it is imperative to develop an ethogram, which operationally defines normal behaviors and behaviors associated with pain. Once a species-specific ethogram is refined for koi, the efficacy of commonly used clinical analgesic agents can be systematically evaluated. These ethograms could be practically applied across clinical veterinary medicine, laboratory

animal medicine, and aquacultural settings. Although plasma cortisol concentration does not appear to be a predictor of pain in koi,²¹ the measurement of plasma cortisol and blood glucose concentrations may provide an additional tool for assessing pain responses in some fish species.^{29,52}

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Figure 1.

Mean $\pm$ SEM change in respiratory rate from the baseline value (12 hours before treatment) in koi that were administered saline (0.9% NaCl) solution (A), butorphanol tartrate (B), or morphine sulfate (C) following random assignment to 1 of 9 treatment groups: saline (0.9% NaCl) solution only (SS group; $n = 6$), saline solution with anesthesia and surgery (SS+ group; 12), saline solution with anesthesia but without surgery (SS- group; 12), butorphanol only (B group; 6), butorphanol with anesthesia and surgery (B+ group; 12), butorphanol with anesthesia but without surgery (B- group; 12), morphine only (M group; 6), morphine with anesthesia and surgery (M+ group; 12), or morphine with anesthesia but without surgery (M- group; 12). A single dose of saline solution (equivalent volume to opioid injection), butorphanol (10 mg/kg [4.5 mg/lb]), or morphine (5 mg/kg [2.3 mg/lb]) was injected IM in each fish. In the injection-only groups (SS, B, and M), fish were immediately returned to the aquaria after injection to obtain data for the 0-hour time point. For all other treatment groups (SS+, SS-, B+, B-, M+, and M-), data for the 0-hour time point were obtained once the fish were returned to their aquaria approximately 15 minutes after recovery from anesthesia. Black bars indicate values that are significantly ($P < 0.05$) different from the respective baseline value. *Change in respiratory rate is significantly ($P < 0.05$) different from the value for the respective injection-only group. †Change in respiratory rate is significantly ($P < 0.05$) different from the value for the respective anesthesia without surgery group. ‡Change in respiratory rate is significantly ($P < 0.05$) different from the value for the respective saline solution treatment group.

Figure 2.

Mean $\pm$ SEM food consumption, assessed as the percentage of 20 floating food pellets that were consumed within 20 minutes after feeding, in the koi included in the treatment groups in Figure 1. Baseline data were obtained 12 hours before treatment (B). See Figure 1 for remainder of key.

Figure 3.

Mean $\pm$ SEM change in activity score from the baseline value (12 hours before treatment) in the koi included in the treatment groups in Figure 1. Activity was scored on a scale from 1 to 10. See Figure 1 for remainder of key.

Table 1

Mean $\pm$ SEM, median, and interquartile range (25th to 75th percentile) baseline values (-12 hours before treatment) for respiratory rate, score for location in the water column, and activity score in koi that were randomly assigned to 1 of 9 treatment groups: saline (0.9% NaCl) solution only (SS group; $n = 6$), saline solution with anesthesia and surgery (SS+ group; 12), saline solution with anesthesia but without surgery (SS- group; 12), butorphanol tartrate only (B group; 6), butorphanol with anesthesia and surgery (B+ group; 12), butorphanol with anesthesia but without surgery (B- group; 12), morphine sulfate only (M group; 6), morphine with anesthesia and surgery (M+ group; 12), or morphine with anesthesia but without surgery (M- group; 12).

Variable	Treatment group								
	SS	SS-	SS+	B	B-	B+	M	M-	M+
Respiratory rate (opercular movements/min)									
Mean $\pm$ SEM	49.0 $\pm$ 7.5	37.3 $\pm$ 3.8	52.6 $\pm$ 5.1	39.9 $\pm$ 4.8	39.0 $\pm$ 6.4	47.6 $\pm$ 5.2	48.1 $\pm$ 6.5	41.1 $\pm$ 3.6	52.0 $\pm$ 5.0
Median	48.3	34.5	53.0	41.8	28.8	45.0	45.8	41.3	49.5
Interquartile range	36.6–61.4	28.1–42.4	39.6–68.4	32.3–46.4	24.3–45.0	31.4–62.9	43.4–48.5	33.5–53.3	42.3–65.3
Location in water column score									
Mean $\pm$ SEM	2.6 $\pm$ 0.2	2.2 $\pm$ 0.1	2.4 $\pm$ 0.1	2.0 $\pm$ 0.1	2.0 $\pm$ 0.1	2.2 $\pm$ 0.1	2.3 $\pm$ 0.2	2.1 $\pm$ 0.1	2.2 $\pm$ 0.1
Median	2.5	2.3	2.5	2.0	2.1	2.1	2.1	2.0	2.0
Interquartile range	2.3–2.7	2.0–2.3	2.2–2.6	2.0–2.2	2.0–2.3	2.0–2.3	2.0–2.6	2.0–2.3	2.0–2.5
Activity score									
Mean $\pm$ SEM	6.4 $\pm$ 0.7	5.0 $\pm$ 0.5	7.2 $\pm$ 0.4	4.6 $\pm$ 0.8	4.5 $\pm$ 0.5	5.6 $\pm$ 0.6	6.0 $\pm$ 0.7	5.9 $\pm$ 0.5	6.8 $\pm$ 0.5
Median	6.1	5.0	7.3	4.1	4.7	5.8	6.0	5.6	6.3
Interquartile range	5.1–7.8	3.8–5.4	6.7–8.2	3.2–5.7	3.1–5.0	4.2–7.0	5.2–7.1	5.0–6.9	5.6–7.7

Location in aquarium water column was scored on scale of 1 to 4 (1 = resting on bottom, 2 = located in bottom third, 3 = located in middle third, and 4 = located in top third). Activity was scored on a scale of 0 to 10, based on the percentage of time fish performed swimming movements in a 2-minute period (0 = no movement [0% of time], 1 = 10%, 2 = 20%, 3 = 30%, 4 = 40%, 5 = 50%, 6 = 60%, 7 = 70%, 8 = 80%, 9 = 90%, and 10 = 100% or constant spontaneous swimming movement).