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Acute and Chronic Morphine Administration in Swine

JACK M. RISDAHL,* CHUN CHAO,‡ MICHAEL P. MURTAUGH,†
PHILLIP K. PETERSON,‡ AND THOMAS W. MOLITOR*[1]

*Departments of *Clinical and Population Sciences and*
†Veterinary Pathobiology, University of Minnesota, St. Paul, MN 55108
‡Department of Medicine, Hennepin County Medical Center and
University of Minnesota Medical School, Minneapolis, MN 55108

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RISDAHL, J. M., C. CHAO, M. P. MURTAUGH, P. K. PETERSON AND T. W. MOLITOR. *Acute and chronic morphine administration in swine.* PHARMACOL BIOCHEM BEHAV 43(3) 799–806, 1992.—Functional responses to acute and chronic morphine administration in domestic swine were examined and correlated with pharmacokinetic profiles. Acute effects of morphine sulfate were monitored in pigs for 24 h and the chronic actions of morphine alkaloid were monitored for 21 days. Serum morphine levels, nociception, locomotor activity, respiratory rate, body temperature, and body weight were monitored during all studies. To assess nociception in a large laboratory animal, a portable thermal stimulating device was constructed. Morphine sulfate administered IV and SC had a half-life of approximately 1 h whereas delayed-release morphine alkaloid delivered SC had a half-life of 28 h. The degree of antinociception paralleled decline in blood morphine levels for both SC- and IV-administered animals. Tolerance occurred to both antinociception as well as weight gain despite morphine levels remaining constant over the 21-day period. Morphine dependence was demonstrated by precipitation of an abstinence syndrome using naloxone. Animals in withdrawal displayed consistent signs, including wet-dog shakes, posture changes, vocalization, and salivation. Collectively, these results indicate that swine may be reliably employed as a model to study the actions of morphine and opiate-like compounds.

Opiates Morphine Swine Nociception Dependence Withdrawal

THE study of in vivo functional alterations induced by abused substances requires a relevant animal model. Various animal models have been used to study biologic effects of abused substances. With the recent identification of drug abuse as a risk factor for acquired immunodeficiency syndrome (AIDS), the authors became interested in studying immune alterations induced by morphine. To develop an animal model for morphine abuse and study these alterations, certain criteria were required of the animal of study. A laboratory animal was required that was large enough to allow procedures that cannot be performed on rodents and other small laboratory animals. To avoid stress of handling as much as possible, it was preferable to use an animal easily trained to adapt to the procedures performed. And, finally, it was also necessary that the laboratory animal be similar in physiology and anatomy to humans to allow comparative interpretation. Classically, primates have fulfilled these requirements but in recent years have become expensive to procure and maintain. As an alternative, we hypothesized that swine would be a useful animal model to study the effects of short- and long-term exposure to morphine.

Swine offer many advantages as a laboratory animal. Swine are being used increasingly as animal models for biomedical research applications in such fields as cardiology, physiology, radiation, exercise, trauma, plastic surgery, pharmaceutical development, organ transplantation, and behavior (25). The gain of popularity of swine as biomedical models has occurred for several reasons. Swine share many similar biologic features in their anatomy and physiology with humans (18,24). Swine are large enough to allow for repeated blood sample collection, tissue biopsy, and other surgical procedures with little harm to animals. Swine are readily available at relatively low cost with modest housing requirements. Finally, swine can be trained through socialization with handlers and use of rewards can minimize the potential stress of procedures and sample collection (4,9,25).

Relatively little has been described about the effects of morphine administration to swine as compared to other laboratory animal species. In 1895, Dun described general clinical signs associated with morphine administration in the pig (11). In 1901, Hess characterized responses of swine to various doses of morphine and reported toxic drug levels (16). Several

[1] Requests for reprints should be addressed to: Dr. T. Molitor, Department of Clinical and Population Sciences, 1365 Gortner Avenue, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108.

studies have been performed to assess aspects of antinociception, behavior, or plasma protein binding of morphine in swine (3,8,10,11,14,15). Various swine anesthesia protocols have been developed that utilize morphine in combination with other drugs (5,17,21,22,26,29). Clinical reports on the use of morphine without combined agents are scarce due to their putative, unpredictable excitatory effects in swine (8, 11,14,28).

This article describes the development of a swine model for acute and chronic morphine delivery. To establish the model, studies were performed to: a) determine the half-life of morphine in the blood, b) characterize various functional responses to morphine including antinociception, and c) demonstrate evidence of dependence upon and tolerance to these drug effects. Definition of these features of the model are essential for further studies of the effects of opiates on the swine.

METHOD

Drug Preparation and Delivery

Morphine sulfate and morphine alkaloid were obtained from the National Institute on Drug Abuse (Bethesda, MD). Morphine sulfate was dissolved in 0.9% aqueous sodium chloride solution (pH 6.0) at various concentrations to limit delivery volume between 3 and 6 ml for ease of injection. The preparation was filter sterilized using 0.22 μm syringe filters (Gelman®, Ann Arbor, MI) and kept in sterile vials. Intravenous injections were administered in either the medial or lateral auricular vein. Subcutaneous injections were administered in the caudal dorsal pelvic region after modified surgical preparation of the area using povidone-iodine scrub followed by isopropyl alcohol rinse and povidone-iodine solution.

Both mineral oil emulsion and peanut oil suspension were tested for SC delivery of morphine alkaloid. Emulsions of morphine alkaloid were prepared as described previously; however, a higher concentration of morphine was used to keep delivery volume to a minimum (12). Briefly, morphine alkaloid was weighed to the desired amount and mixed with 10 ml of a 6 : 1 ratio of light mineral oil to mannide monooleate (Sigma Chemical Co., St. Louis, MO) to achieve a base concentration of approximately 280 mg/ml. Higher concentrations became too viscous to be delivered percutaneously. The preparation was autoclaved for 20 min, allowed to cool, and then emulsified by repeated mixing with an equal volume of sterile saline. Morphine alkaloid was also suspended in peanut oil (Spectrum Chemicals, Gardina, CA) at a concentration of 400 mg/kg and autoclaved. As opposed to mineral oil emulsion, this concentration of alkaloid in peanut oil allowed relatively easy delivery using syringe and needle. The morphine alkaloid preparations were delivered SC by injection in the caudal dorsal region of the animal. Controls received sterile peanut oil alone.

Animals

Outbred Yorkshire castrated male pigs 6–8 weeks of age and 20–40 kg of body weight (supplied by David Theis, Gibbon, MN) were housed in the University of Minnesota experimental animal holding facilities. All pigs were allowed at least a 2-week adaptation period before use in experiments. Typically, most animals received an additional 1–2 weeks of handling to achieve rapport with the investigators. Housing was organized by placing pigs' pens individually or in groups depending upon study requirements. Individually housed pigs had nose-to-nose contact with pigs in adjacent pens to allow for socialization. Pigs receiving morphine were housed in pens separate from pigs maintained as controls. All subjects were provided food and water ad lib. Environmental temperature was maintained between 18–25°C.

Experimental Design

The acute effects of morphine sulphate were tested in four experiments utilizing both IV and SC routes of administration. Doses of IV morphine sulphate were given at 1.5 mg/kg (n = 6) and 10 mg/kg (n = 18). Age-matched controls (n = 16) received equal volumes of sterile 0.9% saline. Behavioral and physiological parameters including antinociception, rectal temperature, and locomotor activity were measured at timed intervals up to 72 h after injection. Blood samples were obtained at the same time intervals to determine serum morphine concentration. Doses of 10 mg/kg (n = 5) and 30 mg/kg (n = 2) of morphine sulphate were administered SC. The same parameters measured for IV route were also measured after SC-administered morphine as described above.

Morphine base was delivered in either mineral oil or peanut oil as a carrier to achieve sustained drug release. Initial experiments in our study utilized the mineral oil emulsion carrier; however, due to intense inflammatory reaction the carrier was changed to peanut oil. Peanut oil has been shown to be degraded more rapidly in the animal and does not produce the degree of inflammation observed with the mineral oil emulsion. Peanut oil is approved by the U.S. Department of Agriculture for injection in swine (27,31). Initially, to evaluate drug dosage effects three concentrations—30 mg/kg (n = 3), 150 mg/kg (n = 5), and 180 mg/kg (n = 2)—were investigated. Thereafter, experiments were performed at 150 mg/kg (n = 10). In single-dose experiments, blood samples and observations were collected at timed intervals up to 168 h after injection. In chronic studies of morphine effects, swine were maintained on morphine with repeated dosages at 4-day intervals for up to 24 days. Parameters described above for morphine sulphate IV studies were measured daily for morphine base studies.

After 23 days of chronic morphine delivery, 5 pigs were given 0.02 mg/kg naloxone by IM injection to test their dependence upon opiates by precipitation of an abstinence syndrome. Four control pigs received the same dose of naloxone. Signs commonly associated with withdrawal, including wet-dog shakes, increased salivation, and increased vocalization, were monitored (6,19,30).

Morphine Blood Levels

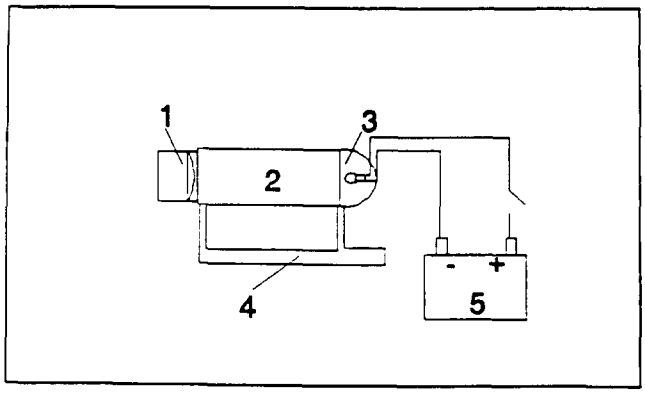
Serum samples collected at various times in each experiment were analyzed for morphine levels by radioimmunoassay (2,7). Serum was collected from individual animals and frozen until all samples from an individual experiment could be analyzed simultaneously. Results were plotted graphically to determine distribution and metabolism phases. Half-life was calculated using the Pharmacologic Calculation System computer program version 3.3 (Life Science Associates, Bayport, NY).

Antinociception Measurements

Exteroceptive sensation to either stimulation with a needle or heat from a portable thermal device were utilized to allow easy assessment of morphine-induced antinociception in swine in a pen situation while minimizing stress to the animal. Nee-

dle prick mechanical stimulation was modified from that previously described (15). Blind measurements were graded by at least two observers in every test. needle stimulation was used to elicit the cutaneous trunci reflex or head withdrawal (avoidance to noxious stimulus of the mandible). To elicit the cutaneous trunci reflex, a needle was gently touched to the dorsolateral skin and intensity of the reflex response was observed. The test was repeated at least twice and a clinical score assigned based upon degree of response. A score of 2 was assigned to a normal response. The normal cutaneous trunci response was brisk, easily elicited, and often resulted in a generalized response from the pig such as vocalization or turning to notice the stimulus. A score of 1 was assigned to a depressed cutaneous trunci response. Although pigs responded, a depressed cutaneous trunci response was more difficult to elicit and the degree of response was attenuated. No response received a score of 0. Head withdrawal to mandible stimulation was performed in a similar manner. A needle was gently touched to the angle of the jaw and the response was graded in a manner similar to that of the cutaneous trunci response.

Thermal stimulation of exteroceptive sensation to measure antinociception was tested utilizing a high-intensity light-powered heat source. The device used to elicit the response was similar to that described for guinea pigs (23) and is depicted diagrammatically in Fig. 1. Briefly, the heat source consisted of a tungsten halogen lamp powered by a 12-V battery. The intensity of the light emitted was 250,000 candle power. The light was concentrated by a planoconvex lens with a focal length of 120 mm. The lens was held in place by a metal housing and attached to the light source by a coffee can (used for its reflective inner surface). A distance probe extending from the distal end of the device was held in a manner so as not to touch the pig but approximate the distance of the focused spot. The temperature at the focal spot was measured to be 90°C. If a response did not occur in 40 s, the test was discontinued to prevent skin burns. The response tested was elicitation of the cutaneous trunci reflex. Cutaneous trunci was always measured in the same location on the pig by drawing an X from the angle of the last rib caudoventrally to the stifle joint and from the tuber coxae cranioventrally to the last costochondral junction. Response was measured in seconds using a stopwatch. Timing began from the moment of light impact to the time of observed response.



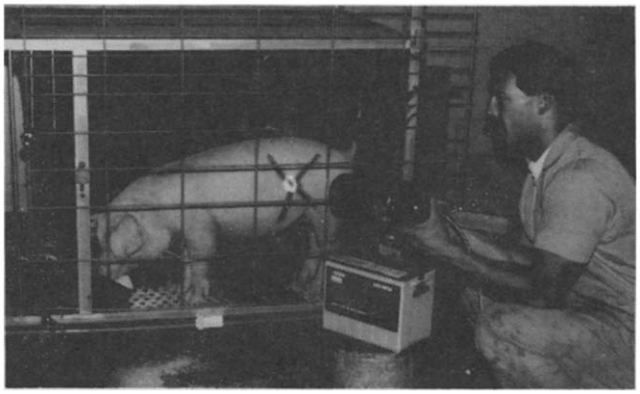


FIG. 1. Device for evaluating nociception in swine following morphine administration. Top: Diagram showing structural features of the device; the focussing lens is a planoconvex lens with a focal length of 120 mm and is contained in a disassembled camera lens housing (1). The body of the device is a coffee can used for its reflective inner surface (2). The light source is a tungsten halogen lamp used for off-road vehicles and gives off a light intensity of 250,000 candle power (3). The device has a handle that may be held by the observer or may be attached to a stand (4). The power source is a 12-V battery (5). Bottom: Photograph of light device in use. An "X" was painted on the flank of the pig to consistently direct the region tested.

Body Temperature, Weight, Activity, and Physical Parameters

Rectal temperature was monitored with a digital electronic thermometer (Agricultural Electronics Co., San Luis Obispo, CA). Body weight in kg was also measured at regular intervals during the chronic studies. Activity was determined by dividing each pen into 30 × 30-cm grids and counting the number of grids traversed by the thoracic limbs of the subject in a 1-min observation period. Animals were required to place both thoracic limbs on a given grid or to have completely passed their sternal thorax over a grid to be counted. The number of grids/min traversed were transformed to linear distance traveled in meters/min and compared between treated and controls animals. This transformation is subject to slight error when the subject passed over a grid diagonally; however, this error was negligible and observed significant differences did not change if calculated directly from grids or meters/min. Other physical parameters were monitored by observation during the experiments including respiratory rate, respiratory rhythm changes, abnormal micturition, abnormal defecation, vomiting, pulse, capillary refill time, pupil size, or other notable physical or behavioral abnormalities to determine any other generalized or toxic responses to morphine.

Statistical Analysis

In all cases, standard error was calculated within groups and is presented. For comparison to control groups, Student's *t*-tests for differences between means were performed. For multiple group comparisons, the significance of differences between the means of groups was determined by one-way analysis of variance using the Bonferroni or Scheffe's test.

RESULTS

Acute Morphine Administration

Serum morphine concentration. Serum concentration of morphine-like immunoreactivity after a 1.5-mg/kg IV injec-

tion rose to a peak level of 665 (±118) ng/ml at the 3-min sample following injection and declined to 3.0 (±0.45) ng/ml by 360 min (Fig. 2). The $t_{1/2}$ was calculated to be 50 min. After IV administration of 10 mg/kg morphine sulphate, serum morphine concentration reached a peak level of 5,900 (±800) ng/ml, declined to 3.0 (±0.67) ng/ml at the 24-h sample postinjection, and was not detected at 48 h postinjection (Fig. 2). The $t_{1/2}$ was also 50 min. Swine injected SC with 10 mg/kg morphine sulphate attained peak morphine levels of 4,500 (±1,300) ng/ml at the 10-min sample postinjection. The concentration declined to 0.9 (±0.6) ng/ml by 24 h; $t_{1/2}$ was calculated to be 65 min. Swine injected with 30 mg/kg morphine sulphate SC were not tested for serum concentration.

Antinociception. Following 10 mg/kg IV morphine sulfate, cutaneous trunci response to needle stimulation was abolished when measured 3 min postinjection, began to return by 4 h, and was normal at 24 h after morphine injection (Fig. 3). Head withdrawal to needle stimulation of the mandible did not begin to return until 10 h postinjection but was normal by 24 h. In this same experiment, antinociception was also measured by the thermal response. Cutaneous trunci response to thermal stimulation was abolished immediately following injection, began to return by 10 h postinjection, and was normal at 24 h postinjection (Fig. 4). Foot withdrawal to thermal stimulation also was refractory soon after injection, began to return by 50 h, and was normal at 24 h postinjection.

Body temperature. A rise in rectal temperature was seen at all doses tested. Following 1.5 IV morphine sulphate administration, temperature rose significantly ($p < 0.05$) to 40.4 (±0.17) compared to controls with an average rectal temperature of 39.4°C (±0.12). At the 10-mg/kg IV dose, rectal temperature rose significantly ($p < 0.05$) to 41.5°C (±0.2). These two doses were significantly different ($p < 0.05$), indicating a dose-dependent effect. The temperature increase was apparent at 3 min postinjection, reaching peak levels by 30 min, and remained elevated for 6 h after injection. In SC-administered swine, the rate of rise was slower than those given IV injections. Following 10 and 30 mg/kg SC, significant rise in temperature was apparent at 45 min following morphine sulphate administration. The peak temperature of 40.4°C (±0.3) was reached at 120 min and declined to a normal range by 10 h. No significant differences were observed between these two doses.

Activity. Rate of locomotor activity was found to be significantly greater ($p < 0.05$) in swine administered IV and SC 10 mg/kg morphine sulphate than in controls. Increased activity at higher IV and SC doses persisted for the first 4 and 7 h, respectively, after injection. Peak activity during acute morphine exposure was 1.7 (±0.2) m/min in the 1.5 mg/kg IV-administered, 5.4 (±0.6) m/min in the 10 mg/kg IV-administered swine, and 7.2 (±0.7) m/min in the 10 mg/

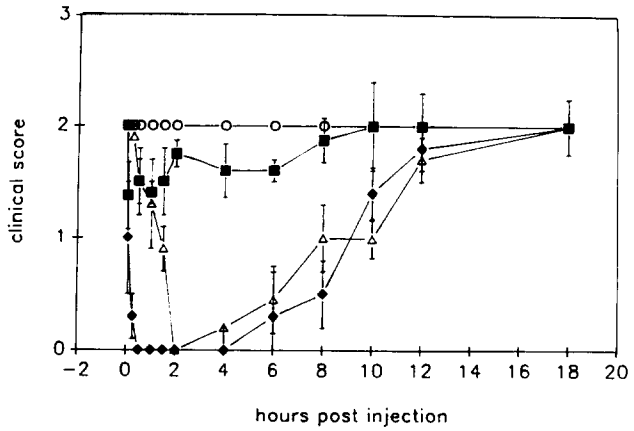


FIG. 3. Nociception response to pin-prick stimulation in swine administered a single injection of morphine sulfate at doses of 1.5 mg/kg IV (■-■), n = 6, 10 mg/kg IV (◆-◆), n = 10, and 10 mg/kg SC (△-△), n = 5. Control animals (○-○) received injections of IV or SC saline. A needle was used to induce the cutaneous trunci skin-twitch response in the test subjects. Responses were graded using a clinical score with 2 = normal response, 1 = depressed response, and 0 = no response (see the Method section).

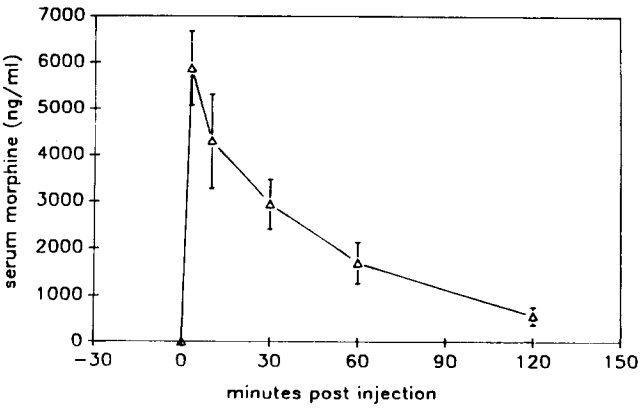


FIG. 2. Serum morphine concentrations in swine following single intravenous administration of morphine sulfate at 10 mg/kg. Blood samples were collected at 0, 10, 30, 60, 120, 240, and 360 min after morphine administration. Morphine concentration in sera were determined using radioimmunoassay (see the Method section). Data is expressed as mean ±SE of serum morphine concentration (in ng/ml), n = 4.

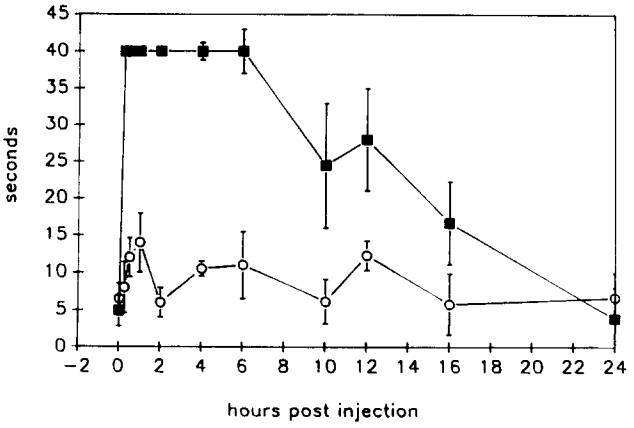


FIG. 4. Nociception response to thermal stimulation in swine administered a single injection of morphine sulfate at 10 mg/kg. Antinociception was measured by cutaneous trunci response to thermal stimulation. A stopwatch was used to measure the time to response. The test was discontinued if no response was detected by 40 s. Data is expressed as mean ±SE time to respond (in seconds); (■-■), morphine-treated animals, n = 5; (○-○), control animals, n = 12.

kg SC-administered and 7.0 (±0.8) m/min in the 30 mg/kg SC-administered swine, all significantly ($p < 0.05$) above the normal range of 0.9 (±0.3) m/min in controls.

Activity observed in swine following morphine included preoccupation with selected normal swine behavior such as rooting, vocalizing, chewing, and movement in pens. The gait of animals during this time was a cataleptic walk. Several swine displayed walking movement circling either clockwise or counterclockwise at higher doses lasting only several minutes in duration. No convulsions, running, or increased reflex activity were observed at the doses used.

Swine receiving morphine sulfate showed an elevated respiratory rate over controls during the period of increased activity. Swine receiving 10 mg/kg IV showed significant ($p < 0.05$) elevation in respiratory rate from normal 35 (±3.1) breaths per minute (bpm) to 75 (±7.4) bpm at 3 min postinjection. A peak respiratory rate of 100 bpm (panting) was attained in several animals at 10 min following injection. All animals recovered to normal respiratory rates by 10 h postinjection. In swine that received 1.5 mg/kg IV, respiratory rate significantly increased ($p < 0.05$) to 79.8 (±7.2) bpm at 20 min postinjection and remained near 60 bpm until return to normal at 6 h postinjection. Respiratory rate in swine administered SC morphine sulfate at 10 and 30 mg/kg showed a significant rise ($p < 0.05$) of 71.5 (±13.7) and 80 (±11.3), respectively, by 30 min, both doses reached peak levels of panting (approximately 90 bpm) at 90 min, and returned to normal by 10 h postinjection. No significant rise in respiratory rate was observed in animals receiving morphine alkaloid.

Chronic Morphine Administration

Serum morphine concentration. Swine administered 30 mg/kg morphine alkaloid in mineral oil emulsion showed a peak blood level at 24 h postinjection with a $t_{1/2}$ or 20 h (Fig. 5). These observations were similar for morphine alkaloid given at a dose of 150 mg/kg but with a $t_{1/2}$ of 28 h. Here, the peak levels occurred at 24 h measured at 690 (±320) ng/ml. In chronic morphine studies, swine were either repeatedly administered morphine emulsified in peanut oil or mineral oil carrier at a 4- to 5-day interval. Under this dosing scheme, using either carrier, morphine levels did not drop below 70 ng/ml.

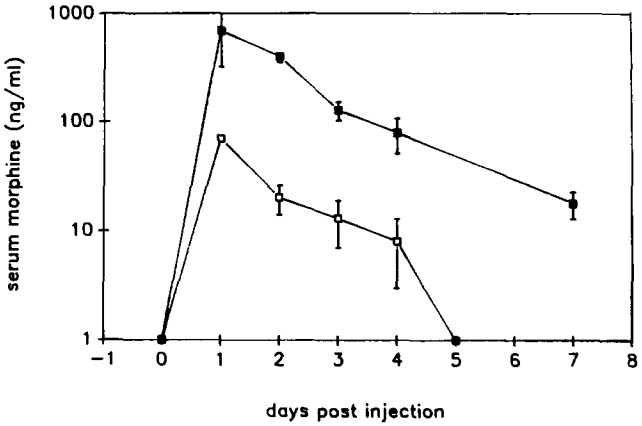


FIG. 5. Serum morphine concentration in swine administered drug in an oil emulsion. Morphine free base was administered at either 30 or 150 mg/kg subcutaneously. Values are expressed as mean ±SE for: (□-□) 30-mg/kg dose, $n = 3$; or for the (■-■) 150-mg/kg dose, $n = 6$. Blood samples were collected at 0, 6 h, and 1, 2, 3, 4, 5, and 7 days following morphine administration. Morphine levels were determined using radioimmunoassay (see the Method section).

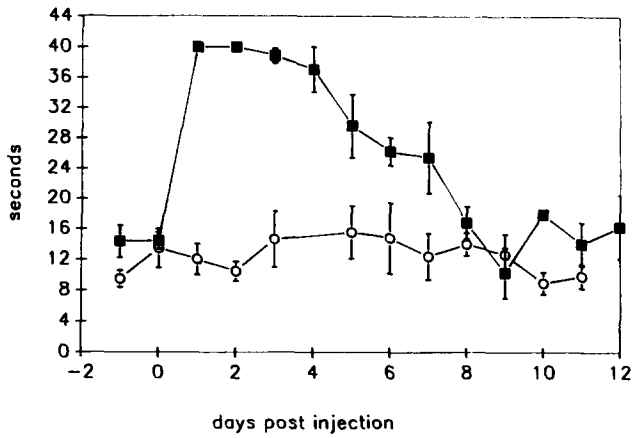


FIG. 6. Nociception response administered with a single injection of morphine alkaloid at 150 mg/kg SC. Antinociception response was measured by cutaneous trunci response to thermal stimulation. A stopwatch was used to measure the time to respond to stimulus. The test was discontinued if no response was detected by 40 s. Data is expressed as mean (±SE) of time to respond (in seconds); (■-■), morphine treated, $n = 10$; (○-○), control treated, $n = 15$.

Antinociception. The same studies of antinociception described for IV morphine delivery were tested for single- and multiple-dose studies with SC morphine base given at 150 mg/kg. Following a single SC injection of morphine alkaloid, cutaneous trunci responses to thermal stimulation were both refractory by 24 h postinjection and remained this way until day 6 after injection (Fig. 6). Head withdrawal to mandible needle prick stimulation was depressed by 6 h, began to return slightly by day 2, and returned to normal by day 6. Cutaneous

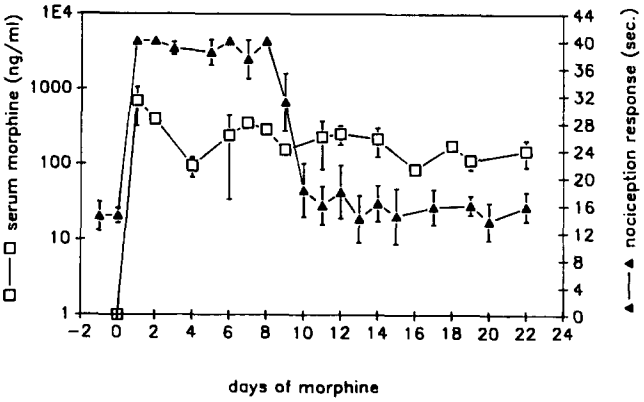


FIG. 7. Nociception response to thermal stimulation compared to serum morphine levels in swine chronically administered morphine. Swine were administered 150 mg/kg morphine in oil vehicle repeatedly on days 0, 4, 8, 12, 16, and 20. Blood samples were collected approximately every 2 days for measuring serum morphine levels. Morphine levels were determined using radioimmunoassay (see the Method section), $n = 3$. Nociception was measured using thermal stimulation and time to cutaneous trunci response recorded. The test was discontinued if no response was viewed at 40 s, $n = 15$. Data is expressed as mean values ±SE for both serum morphine and nociception.

trunci from needle stimulation also was depressed by 24 h and followed a return to normal similar to head withdrawal.

Antinociception results from chronic multiple-dose morphine alkaloid studies allowed interpretation of tolerance time course. With the thermal stimulation of nociception, swine displayed a gradual return to normal cutaneous trunci responses beginning at 8 days after morphine initiation and were within normal reaction times by 10 days despite blood levels of morphine remaining constantly above 70 ng/ml throughout the study (Fig. 7). Full return to normal cutaneous trunci responses was more gradual in the mechanically induced nociception test than the thermally induced test. Pin prick-induced responses began to show return to normal starting at day 7 but remained depressed until 12 days following initiation of chronic morphine (Fig. 8).

Body weight. Swine repeatedly administered morphine base displayed marked differences in body weights compared to carrier-treated swine (Fig. 9). Controls gained a mean of 0.63 kg/day throughout each study. Morphine recipients showed no significant weight gain until day 11, after which they gained an average of 0.58 kg/day. Following day 11, growth curves of morphine recipients and controls were essentially identical.

Body temperature. In contrast to the fever responses induced by IV or SC administration of morphine sulphate, no significant rise in temperature was observed with either single or multiple doses of subcutaneously administered morphine free base in an oil vehicle.

Activity. Swine receiving 150 mg/kg free base showed increased activity [peak value 1.4 (±0.34) m/min] between 10–20 h postinjection and then returned to normal activity after this time. No significant changes in activity were detected upon further injections. Behavior displayed during time of increased activity was similar to that described for morphine sulphate; however, no significant changes in respiratory rate were observed. Swine generally displayed a cataleptic gait until tolerance between 8–14 days.

Morphine dependence. To evaluate if chronic morphine induced a state of dependence, selected swine receiving multiple doses of morphine free base for 23 days were challenged with 0.02 mg/kg naloxone administered by intramuscular injection. Animals were observed for changes in behavior in response to naloxone. Upon injection of naloxone, all morphine recipient test subjects responded similarly. Within 5 min, swine showed wet-dog shakes occurring at 10- to 30-s intervals, crawling episodes where pigs would drag their abdomen along the ground for 3–5 s, and posture changes from standing to lying and return again to standing (Fig. 10). Other behavior changes included increased vocalization, copious salivation, increased defecation frequency and straining with occasional

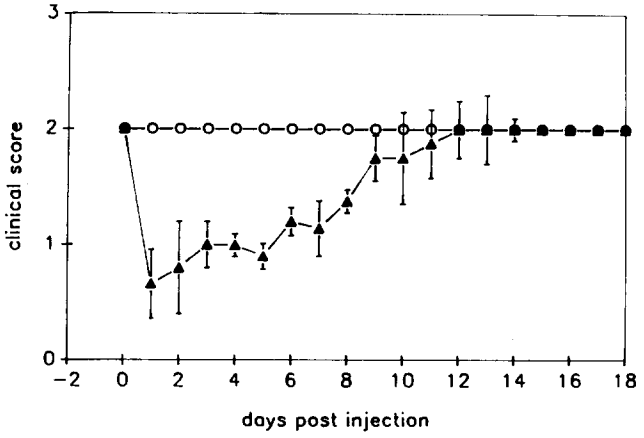


FIG. 8. Nociception response to pin-prick stimulation in swine chronically administered morphine. Swine were administered 150 mg/kg morphine alkaloid in an oil vehicle on days 0, 4, 12, 16, and 20. Nociception was measured using a needle to induce the cutaneous trunci response. The response was graded using a clinical score where 2 = normal response, 1 = depressed response, and 0 = no response.

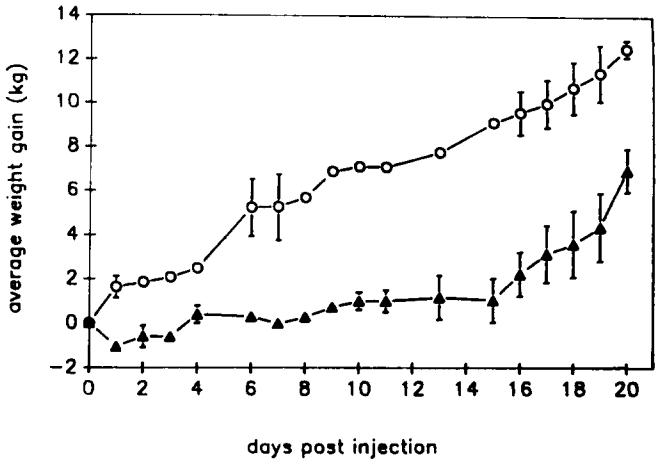


FIG. 9. Weight gain during chronic morphine administration. Morphine was repeatedly administered to swine over a 21-day interval. Control swine received oil carrier alone. Swine were weighed on a 1- to 2-day interval. Data are expressed as mean ±SE of cumulative weight gains; (■-■), morphine treated, n = 15; (○-○), control treated, n = 12.

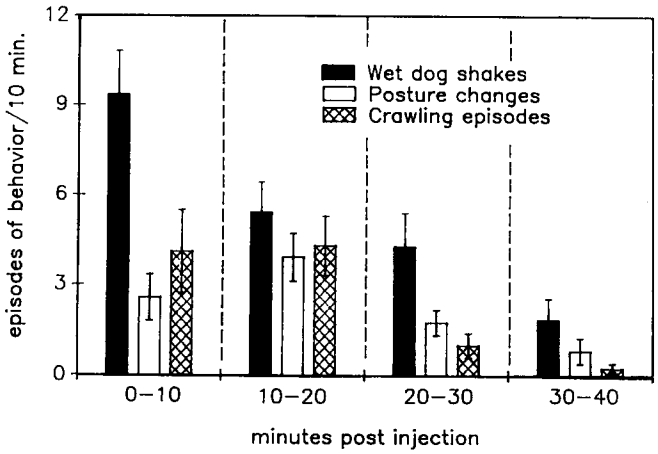


FIG. 10. Abstinence syndrome behavior frequency in swine administered naloxone following chronic morphine administration. Swine were repeatedly administered 150 mg/kg morphine alkaloid in an oil carrier on days 0, 4, 8, 16, and 20. On day 23, swine were administered 0.02 mg/kg naloxone IM and observed for behavioral changes. Number of episodes of wet-dog shakes, posture changes from standing to lying, and crawling episodes were counted in 10-min blocks. Data are expressed as mean episodes of each behavior observed/10 min following injection of naloxone, n = 5.

rectal prolapse, increased respiratory rate, and kicking of pelvic limbs caudally. These behaviors continued for approximately 45 min, after which pigs lay in lateral recumbency for 1–3 h, often sleeping. The greatest amount of withdrawal behavior occurred in the first 15 min following injection, slowly diminishing by 45 min. After this time, normal behavior was resumed. These signs were seen only in subjects that had received morphine. Control animals elicited no detectable changes after receiving the same dose of naloxone.

DISCUSSION

The goal of these studies was to develop a swine model with measurable parameters of responses to morphine sulfate and morphine base delivered in acute and chronic dosage forms, respectively. Our studies show that swine respond predictably to morphine and their responses may be monitored using several reliable indicators. We have shown that swine may be maintained for extended periods of time on morphine by subcutaneous delivery of morphine base. This method of morphine delivery offered the advantage of convenient administration at 4-day intervals with the avoidance of labor-intensive surgical implantation or chronic catheterization.

Antinociception was measured using both a needle stimulus and thermal stimulation. Antinociception in other animal models has been commonly measured using thermal stimulation to detect skin-twitch (cutaneous trunci) or tail-flick response (13,23). Antinociception resulting from morphine and other opiate derivatives has been characterized previously in swine using hot-plate or warm H_2O-induced tail withdrawal techniques (10,15). Here, we show it is also possible to measure skin-twitch responses in a large test subject using an appropriately designed thermal stimulating apparatus. Measurements taken with this device are objective and may be compared with blood level changes of morphine, similarly to that described for the mouse and rat (13). This device may also be useful for testing any drug for its antinociceptive induction properties not only in swine but other large animals species as well. Parameters tested by thermal stimulation, as well as needle stimulation, response resulted in consistent and repeatable antinociception detection following morphine administration. The thermal stimulation responses appear to be slightly more sensitive indicators of antinociception than needle stimulus based upon the longer duration of a refractory response in both acute and chronic single-dose trials. Thermal stimulation also resulted in earlier return to tolerance after chronic administration of morphine.

Tolerance to morphine was observed in tests of antinociception as well as normalization of growth rate of swine. Animals became completely tolerant to the thermal-induced antinociception testing by 10 days after initiation of chronic morphine base compared to 15 days when tested using the mechanical needle-induced responses. Similarly, animals began to show a growth rate comparable to controls by day 11. These data indicate occurrence of tolerance in swine between 10–15 days.

Dependence, displayed with classic withdrawal signs of an abstinence syndrome, was induced by a relatively small dose of naloxone in swine that were maintained on chronic morphine for 23 days. The clinical signs observed were typical of classic signs of withdrawal observed in humans and other animals (19,20). Although only tested at the termination of chronic administration, our purpose in pursuing withdrawal was to establish that a state of dependence to morphine existed in swine. We did not pursue further characterization of withdrawal but we feel evidence presented here argues that it occurs similarly to other well-characterized mammalian species.

Excitatory effects of morphine evidenced by increased rate of movement, hyperthermia, and increased respiratory rate were shown to be both drug and dose dependent. Excitatory effects were more severe in swine receiving morphine sulfate compared to morphine alkaloid. This observation was probably the result of higher blood levels of morphine after sulphate injection, as well as tolerance occurring while morphine was slowly released following free base injection. At the doses tested, the degree of excitation observed never caused swine any adverse traumatization and appears to be somewhat attenuated compared to other species that are excited by morphine (19). These findings are in agreement with those of Guinard, who found swine to be one of the least sensitive species of animals to excitatory effects by morphine (14).

A significant rise in rectal temperature was observed in animals receiving morphine sulphate but not morphine free base. Several theories as to the origin of the hyperthermic response to morphine have been described (1). The rapid onset of hyperthermia within 3 min following morphine sulphate injection in swine likely indicates a change in CNS set point. Swine did not appear to exert enough muscle activity with the displayed cataleptic walk in that early time period to explain the hyperthermic response by exercise-related effects. Hyperthermia was not observed after morphine free base administration. It is possible that the slow rise in morphine levels induced a state of tolerance hyperthermia before morphine concentration reached a critical point of induction. Another possibility for this observation may be route of administration or drug form.

In these studies, we examined both acute and chronic morphine administration in swine. Swine are gaining increased popularity in the study of many biologic systems. The information in the article will be useful in establishing dosing regimens of opiates and measurable parameters of response for the study of opiate drugs in swine.

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