μ-1 Opioid Receptor Stimulation Decreases Body Temperature in Conscious, Unrestrained Neonatal Rats

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The influence of μ-selective opioid agonists on neonatal thermoregulatory mechanisms has received little attention. Opioid treatment in adult subjects can cause either hyper- or hypothermia, depending on the experimental conditions, the strain of rat used, and the dose and route of administration of the drug. The present study assessed the effect of two μ opioid agonists on body temperature in neonatal Wistar rats aged 2 to 13 days. Rat pups were administered either saline or one of the two μ-selective opioid agonists, dermorphin (0.4 mg/kg) or fentanyl (0.06 mg/kg), by subcutaneous injection. Continuous rectal temperatures were measured both prior to and following drug or saline injection in freely moving, conscious animals. Ambient temperature in a plethysmograph chamber was maintained within or close to the thermoneutral zone for pups (32°C). To distinguish between μ-1 and μ-2 effects, all animals received either saline or 10 mg/kg of the irreversible μ-1 antagonist naloxonazine (NALZ) 1 day prior to agonist administration. NALZ on its own had no effect on body temperature. Dermorphin and fentanyl both caused a fall in body temperature in pups of all age groups. The temperature decreases ranged from 0.8°-2.2°C. These opioid-induced changes were inhibited by NALZ pretreatment. Although there was no evidence for endogenous μ-1 opioid activity, this study indicated that stimulation of μ-1 opioid receptors causes a decrease in body temperature in conscious, unrestrained neonatal rats under or close to thermoneutral conditions. [Exp Biol Med Vol. 227(6):377–381, 2002]

Key words: dermorphin; fentanyl; μ-1; naloxonazine; neonate; opioid; rat; thermoregulation

Opioid drugs have effects on body temperature, although the direction and magnitude of the effects depend on many factors, including species, age, dose, route of administration, ambient temperature, and the presence of physical restraint during tests (1, 2). In adult rats, morphine generally causes hyperthermia at low doses and hypothermia at high doses (3). Restraint often reduces or prevents opioid-induced hyperthermia and enhances hypothermic responses (1, 4). High ambient temperatures favor opioid-induced hyperthermia, whereas low ambient temperatures favor hypothermia (5–7). At least three classes of opioid receptors exist: μ, δ, and κ. Both μ and κ receptors appear to play a role in thermoregulation (4, 6, 8–13) and are expressed within the preoptic area of the hypothalamus (14, 15), an important site for temperature control. In brain slices from the hypothalamus of a rat, μ agonists block responses of temperature-sensitive neurons of the preoptic/anterior hypothalamic area (16). It has been suggested that the hyperthermic response to opioids is mediated by μ receptors, whereas the hypothermic response is mediated by κ receptors (8–10, 12, 13). It seems doubtful, however, that the system is as simple as this because μ-selective agonists such as dermorphin or fentanyl can, under some conditions, cause hypothermia in a number of species, including rats (5, 6), mice (17), rabbits (18), and humans (11, 19, 20), and this hypothermia has even been reported under thermoneutral conditions when hyperthermia is more commonly observed (6). The mechanisms by which opioids affect thermoregulation are not completely known, but opioid pathways appear to be involved in the thermoregulatory effects of non-opioid compounds, including hormones such as cholecystokinin-8 (CCK-8), somatostatin (SST) (9, 21), and thyroid stimulating hormone (TSH) (22), as well as neuronal histamine (17). CCK-8 and SST at low doses cause a hyperthermia that is blocked by μ opioid antagonism, whereas SST at a high dose causes a hypothermia via κ-opioid receptor action (9). There are no reports for adult mammals on the role of μ-1 and μ-2 opioid receptor subtypes in thermoregulation, although one study in rats showed that μ-1 receptors were not involved in morphine-induced hyperthermia (23).

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Most studies on opioid effects on thermoregulation have been carried out in adults, with opioid modulation of body temperature in neonates receiving relatively little attention. In three studies on human infants, fentanyl anesthesia was shown to prevent non-shivering thermogenesis (20) and increase the susceptibility to post-operative hypothermia (19), possibly via impairment of hepatic oxidative metabolism (24). In the present study using unrestrained, conscious neonatal rats, we examined the effects of two μ opioid agonists on body temperature under or close to thermoneutral conditions and determined the contribution of the μ-1 opioid receptor subclass to the observed temperature changes.

Materials and Methods

Animals. Wistar rats bred within the Victoria University Animal Facility were used in this study. Rat pups of either sex aged between 2 and 13 days postpartum (P2-13) were selected for the opioid tests. Litters were standardized within 1 day of birth to a size of eight to 12 pups. Water and feed (Diet 86 nuts, Carterton, NZ) were available *ad libitum*. A 12:12-hr light:dark cycle was maintained in the animal holding rooms, and all experiments were carried out during light hours. All experimental procedures and treatments were approved by the Animal Ethics Committee of Victoria University of Wellington.

Continuous body temperature measurements (T_b) were recorded in 118 neonatal rats aged 2 to 13 days. The same animals were used in a simultaneous study on the effects of opioids on ventilation (25). Conscious, unrestrained subjects were placed on their own inside a Perspex chamber (220-ml volume) through which prewarmed, humidified air flowed at a rate of approximately 1.5 liters/min. Chamber temperature (T_{ch}) was held near 32°C, a temperature within the thermoneutral zone for the older age pups (26–28). Because respiratory tidal volume was being measured along with T_b by barometric plethysmography, the T_{ch} could not be set to 34° to 35°C, the thermoneutral zone for younger pups (less than 8 days postpartum), without interfering with the tidal volume measurements because the magnitude of the difference between T_{ch} and T_b is important in barometric plethysmography. After a 30-min acclimatization in the chamber, the subjects were briefly removed and injected with either dermorphin (0.4 mg/kg s.c.), fentanyl (0.06 mg/kg s.c.), or saline, and were then returned to the chamber. The injection volumes were 5 ml/kg. T_b was recorded immediately prior to drug injection, and every 5 to 10 min for 50 to 60 min afterward.

Body Temperature Measurements. Rectal temperature was continuously recorded in pups using a fine nylon-coated thermistor catheter probe (0.7-mm diameter, YSI 555; Cole-Parmer Instrument Co., Vernon Hills, IL) inserted 1 to 1.5 cm and fixed in place by carefully taping the shaft of the probe to the tail. The soft, flexible shaft of the probe was introduced into the Perspex chamber via an airtight port. The length of loose probe between the rat and the port was sufficient to allow the pup to move freely within the chamber. Because the wires of the probe were extremely fragile, the entire length of the probe (with the exception of the tip) was encased in soft Silastic tubing to reduce the frequency of breakages and protect the shaft from being gnawed by the older pups. A similar but more sturdy Teflon-coated probe (1.0-mm diameter, YSI 554; Cole-Parmer) proved to be too inflexible for temperature measurements in unrestrained rat pups.

For the data of Table I on temperature changes with age, in addition to the P2-13 pups, T_b was measured in four P17-21 pups and 11 adult male rats. For adults, temperature was measured using telemetry thermistor capsules surgically implanted in the peritoneal cavity as previously described (25).

Experimental Design. On the day of experimentation, pups in the dermorphin group each received two injections: an initial saline injection, followed later in the day by dermorphin. Because of the long-lasting effects of dermorphin, the order of injection was never reversed. Pups were returned to the litter for approximately 2 hr between sessions. The T_b response to dermorphin injection was compared with the earlier response to saline; thus, each subject served as its own control. A group of control pups received two injections of saline ($n = 37$) to test the consistency of T_b responses to repeated testing. Because the response to saline on its own had already been tested in the dermorphin group, pups in the fentanyl group were only given a single injection of the drug without a prior saline control. One day before the administration of opioid agonist, all subjects were pretreated with either the selective, irreversible μ-1 antagonist naloxonazine (10 mg/kg s.c.) or saline. T_b was not measured immediately before or after these pretreatment injections because selective μ-1 antagonism requires approximately 24 hr to become established.

Drugs. Dermorphin was purchased from Sigma Chemical Co. (St. Louis, MO). The μ-1 antagonist naloxonazine-2HCl (NALZ) was purchased from Research Biochemicals International (Natick, MA). Fentanyl (in 2-ml ampoules) was obtained from Janssen-Cilag (New South Wales, Australia). Dermorphin and NALZ were dissolved in saline (0.9% NaCl) and were stored at −20°C until needed, and fentanyl was stored at room temperature. As previously reported (25), NALZ was administered at 10 mg/kg s.c., 23 to 28 hr prior to testing of agonist responses in order to ensure that the antagonist was selective against μ-1 receptors. The dose used was chosen to give a maximal effect with a minimal loss of selectivity. The rationales for the particular doses given in this study have been previously discussed (25). No attempt was made to determine the dose-response effects of the μ agonists on thermoregulation.

Data Analysis. All data are presented as the mean ± SEM. Statistical differences between groups were analyzed

Table I. Body Temperature of Neonatal and Adult Rats

	Age				Adult
	P2-3	P6-8	P11-13	P17-21	
Temp (°C)	35.7 ± 0.2	35.8 ± 0.2	36.1 ± 0.1	37.0 ± 0.1	37.1 ± 0.1
n	14	16	15	4	11

Note. Body temperature of conscious, unrestrained neonatal and adult rats after a 30-min isolation in a warmed, Perspex chamber. Ambient chamber temperature (32°C for pups, 29°C for adults) was within or close to thermoneutral.

using the Wilcoxon signed-rank non-parametric test for paired data or the Mann-Whitney non-parametric test for unpaired data.

Results

Effect of Age on Body Temperature. Changes in body temperature occurred with increasing postnatal age as shown in Table I. Mean T_b increased by 1.4°C between P2-3 pups and adult rats.

Effect of Subcutaneous Saline or NALZ Injection on Body Temperature in Pups. Subcutaneous injection of 5 ml/kg prewarmed saline caused a small drop of about 0.5°C in the rectal temperature of P2-3 pups (Fig. 1) that was not prevented by pretreatment with the μ-1 antagonist NALZ (data not presented). Pups aged 6 to 8 days and older showed no temperature change following saline injection (Fig. 1).

NALZ pretreatment had no effect on body temperature

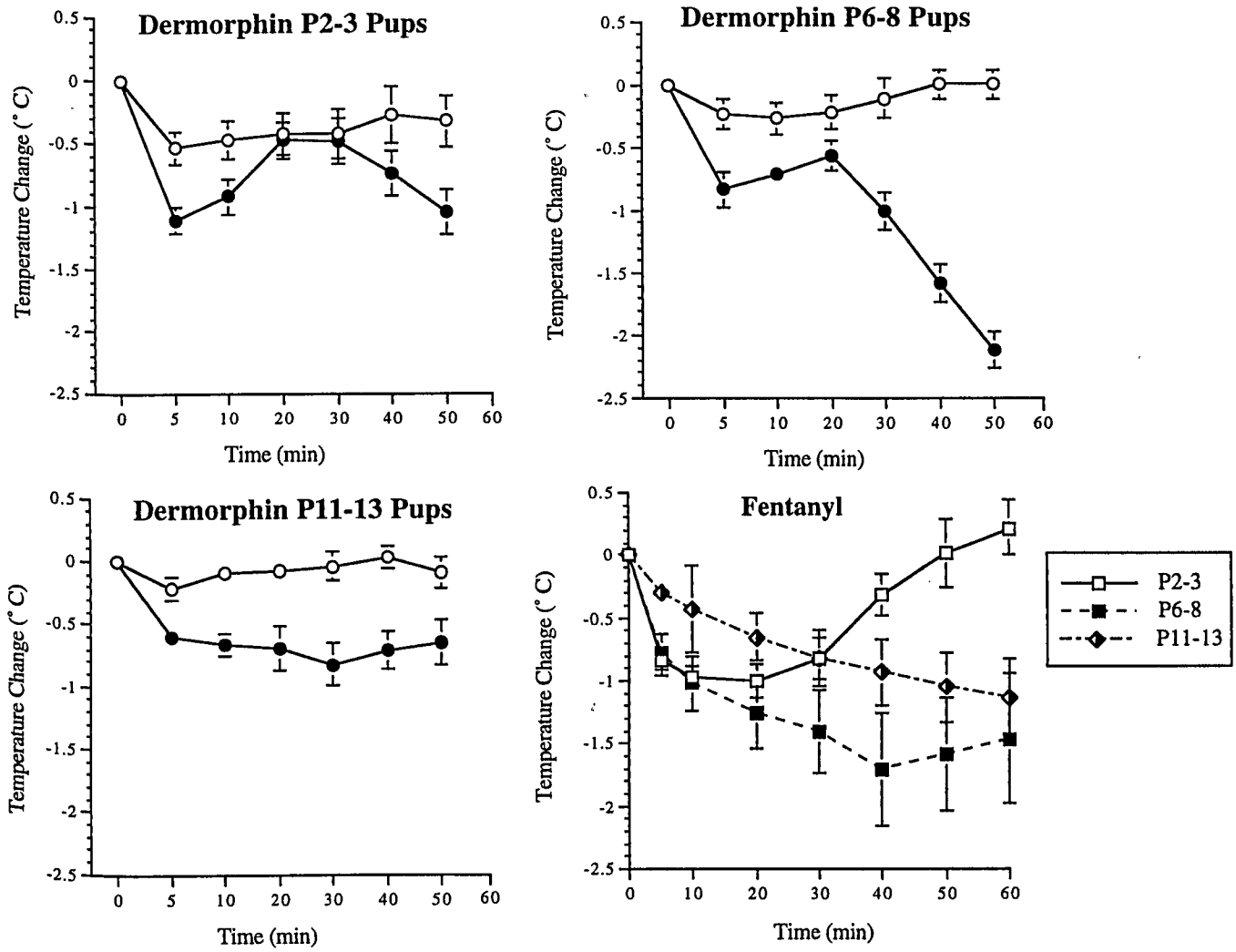

Figure 1. Time course of temperature changes following opioid administration in rat pups. Saline, dermorphin (0.4 mg/kg s.c.), or fentanyl (0.06 mg/kg s.c.) were administered to rat pups at t = 0, and rectal temperature was continuously monitored for up to 60 min. Animals were equilibrated in a Perspex chamber at 32°C for 30 min prior to injection. For dermorphin, responses to saline (○) were monitored in the same animals prior to measuring dermorphin responses (●). The number of animals (*n*) was 14 for P2-3, 16 for P6-8, and 13 for P11-13. Data are presented as the mean ± SEM.

measured 24 hr later in the day that the effects of opioids were tested, thus ruling out a role in neonatal thermoregulation of endogenous opioids acting on μ-1 receptors. Body temperatures measured 1 day after pretreatment were 35.9° ± 0.2°C for NALZ pretreatment (n = 13) versus 35.7° ± 0.2°C for saline pretreatment (n = 14) in P2-3 pups; 36.0° ± 0.2°C (n = 14) versus 35.8° ± 0.2°C, respectively, in P6-8 pups (n = 16); and 36.2° ± 0.2°C (n = 13) versus 36.1° ± 0.1°C, respectively, in P6-8 pups (n = 13).

Temperature Effects of μ Opioids in Pups. Dermorphin treatment consistently reduced T_b in pups. In a time-course study (Fig. 1), changes in T_b following dermorphin or fentanyl administration were monitored for 50 to 60 min. This time course was chosen in line with the ventilatory responses of the pups to opioids (25), and may not have been optimal for the effects on temperature, particularly for the P6-8 pups, whose temperature was still falling 1 hour following dermorphin administration. The dermorphin effect on temperature at 50 min was also examined in the presence of NALZ (Fig. 2). Dermorphin significantly decreased T_b compared with saline in pups of all ages. NALZ pretreatment in P2-3 pups and P6-8 pups blocked the temperature decrease caused by dermorphin, but had no effect on the dermorphin-induced fall in T_b in P11-13 pups.

Fentanyl administration also caused a significant decrease in T_b in pups of all ages, reaching its maximal effect at different times depending on the age of the pups (Fig. 1). The fentanyl-induced decrease in T_b was prevented by NALZ in all age groups, including P11-13 pups (Fig. 3).

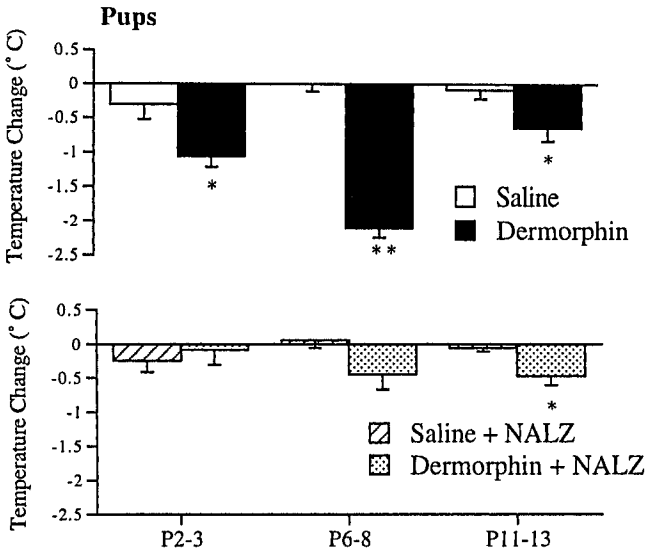

Figure 2. NALZ sensitivity of the dermorphin temperature response in rat neonates. The effect of saline (5 ml/kg s.c.) or dermorphin (0.4 mg/kg s.c.) on rectal temperature in rat pups after pretreatment with saline (top graph) or NALZ (10 mg/kg s.c.; bottom graph) is shown. In the top graph, the saline- and dermorphin-treated values represent the 50-min time course values of Figure 1. For the NALZ-treated group, temperatures were also measured at 50 min, and the numbers of animals were 13 for P2-3, 14 for P6-8, and 13 for P11-13. Data are presented as the mean ± SEM. *P < 0.05; **P < 0.01 when compared with saline values (Wilcoxon signed-rank).

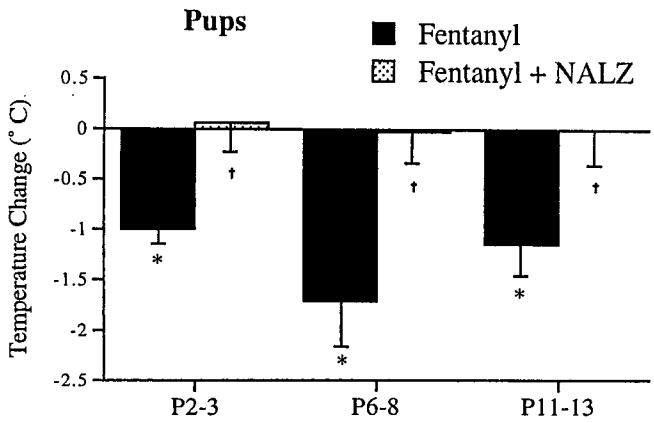

Figure 3. NALZ sensitivity of the fentanyl temperature response in rat neonates. Effect of fentanyl (0.06 mg/kg s.c.) on rectal temperature in rat pups after pretreatment with saline or NALZ (10 mg/kg s.c.). For P2-3 pups, n = 7 and 6 (saline and NALZ pretreated animals, respectively). For P6-8 and P11-13 pups, n = 6 and 5. *P < 0.05 when compared with pre-fentanyl values (Wilcoxon signed-rank test). † indicates significant blockade of the fentanyl effect by NALZ (Mann-Whitney test; P < 0.05).Temperatures were measured at the time of peak fentanyl effect, occurring 20 min postinjection in P2-3 pups, 40 min postinjection in P6-8 pups, and 60 min postinjection in P11-13 pups. Data are presented as the mean ± SEM.

The T_b changes following fentanyl administration were similar to those seen with dermorphin.

Discussion

Resting T_b in rat pups at an ambient temperature of 32°C increased from 35.7° to 37.0°C between P2-3 and P17-21, values that are similar to those reported for pups in a typical nest (26). The increase in T_b with postnatal age is consistent with other reports that demonstrate marked maturational improvements in thermoregulation during the postnatal period in rats (28–30). This improvement is likely to be due to a combination of an increased capacity for non-shivering thermogenesis (29) and improved thermal insulation (28).

Inhibition of μ-1 opioid receptor activity by NALZ in the absence of exogenous opioid agonists had no effect on T_b in neonatal rat pups, indicating that endogenous μ-1 opioid activity is not present in conscious, unrestrained animals. Addition of a μ opioid agonist, however, caused a significant decrease in T_b that was sensitive to inhibition by the μ-1 antagonist. Thus, a μ-1 opioid receptor system is present that could affect thermoregulation in neonatal rats. In adult rats, physical restraint has been shown to enhance hypothermic responses (1, 4), and based on the results of the present study, it is possible that μ-1 opioid pathways are involved in the effects of restraint. It is unclear why the dermorphin-induced fall in T_b in P11-13 pups was insensitive to naloxonazine, because the fentanyl-induced fall in T_b in this same age group was reversed by the μ-1 antagonist. In adult studies, temperature responses have been shown to vary with dose and route of administration (2, 3). The dermorphin and fentanyl doses chosen for this study were selected on the basis of their ventilatory effects (25), not their temperature effects. Changing the dose might alter the mag-

nitude and duration of the responses, and could even lead to temperature changes in the opposite direction to those observed in our study.

The magnitudes of the temperature changes varied between age groups, with the P6-8 pups showing the largest decreases in temperature. In most cases, T_b had stabilized by 60 min or earlier; however, a longer duration of recording would have helped ensure that equilibrium had been reached. The opioid system affected in this study could have been located either centrally or peripherally; thus, the balance between central and peripheral effects could have determined the net response. Some of the differences in the duration and magnitude of the response between age groups may have arisen from differences in rates of subcutaneous absorption of drug or maturation of blood-brain barrier function (2, 3). Interpretation of the results is also complicated by the fact that the younger pups were tested at an ambient temperature that was 2° to 3°C below their thermoneutral optimum, and ambient temperature is known to affect the direction and magnitude of responses to opioids (6, 7). Despite these problems of interpretation of the physiological and clinical significance of the opioid-induced changes, it is clear that the μ-1 opioid pathways affecting thermoregulation in rat pups are present at the earliest stages of postnatal development (2–3 days postpartum).

In conclusion, although there was no evidence for endogenous μ-1 opioid effects on thermoregulation in neonatal rat pups, stimulation of the μ-1 subclass of opioid receptor caused a decrease in body temperature in conscious, unrestrained neonates under or close to thermoneutral conditions. This is the first study that we are aware of to test the role of the μ-1 opioid receptor subclass in thermoregulation in a mammalian neonate.

1. Adler MW, Geller EB. Physiological functions of opioids: temperature regulation. In: Herz A, Ed. Opioids II. Berlin: Springer-Verlag, pp205–238, 1993,
2. Adler MW, Geller EB, Rosow CE, Cochin J. The opioid system and temperature regulation. Annu Rev Pharmacol Toxicol **28**:429–449, 1988.
3. Geller EB, Hawk C, Keinath SH, Tallarida RJ, Adler MW. Subclasses of opioids based on body temperature change in rats: acute subcutaneous administration. J Pharmacol Exp Ther **225**:391–398, 1983.
4. Spencer RL, Hruby VJ, Burks TF. Body temperature response profiles for selective μ, delta and kappa opioid agonists in restrained and unrestrained rats. J Pharmacol Exp Ther **246**:92–101, 1988.
5. Broccardo, M. Effect of dermorphin on body temperature in rats. Pharmacol Res Commun **19**:713–721, 1987.
6. Emel'yanova TG, Usenko AB, Deigin VI, Yarova EP, Kamensky AA. Effect of dermorphin on thermoregulation in rats at selected ambient temperatures. Peptides **17**:241–245, 1996.
7. Handler CM, Piliero TC, Geller EB, Adler MW. Effect of ambient temperature on the ability of μ-, κ- and δ-selective opioid agonists to modulate thermoregulatory mechanisms in the rat. J Pharmacol Exp Ther **268**:847–855, 1994.
8. Chen X, Geller EB, DeRiel JK, Liu-Chen L, Adler MW. Antisense confirmation of μ- and κ-opioid receptor mediation of morphine's effects on body temperature in rats. Drug Alcohol Depend **43**:119–124, 1996.
9. Ghosh S, Geller EB, Adler MW. Interaction of cholecystokinin and somatostatin with a selective μ-opioid agonist and μ- and κ-antagonists in thermoregulation. Brain Res **745**:152–157, 1997.
10. Handler CM, Geller EB, Adler MW. Effect of μ-, κ-, and δ-selective opioid agonists on thermoregulation in the rat. Pharmacol Biochem Behav **43**:1209–1216, 1992.
11. Ikeda T, Kurz A, Sessler DI, Go J, Kurz M, Belani K, Larson M, Bjorksten AR, Dechert M, Christensen R. The effect of opioids on thermoregulatory responses in humans and the special antishivering action of meperidine. Ann NY Acad Sci **813**:792–798, 1997.
12. Spencer RL, Hruby VJ, Burks TF. Alteration of thermoregulatory set point with opioid agonists. J Pharmacol Exp Ther **252**:696–705, 1990.
13. Xin L, Geller EB, Adler MW. Body temperature and analgesic effects of selective μ and κ opioid receptor agonists microdialyzed into rat brain. J Pharmacol Exp Ther **281**:499–507, 1997.
14. Mansour A, Fox, CA Burke S, Meng F, Thompson RC, Akil H, Watson SJ. μ, δ and κ Opioid receptor mRNA expression in the rat CNS: an *in situ* hybridization study. J Comp Neurol **350**:412–438, 1994.
15. Moriwaki A, Wang J-B, Svingos A, Van Bockstaele E, Cheng P, Pickel V, Uhl GR. μ Opiate receptor immunoreactivity in rat central nervous system. Neurochem Res **21**:1315–1331, 1996.
16. Yakimova KS, Sann H, Pierau FK. Neuronal basis for the hyperthermic effect of μ-opioid agonists in rats: decrease in temperature sensitivity of warm-sensitive hypothalamic neurons. Neurosci Lett **218**:115–118, 1996.
17. Ulugol A, Karadag HC, Dokmeci D, Baldik Y, Dokmeci I. The role of histamine H_1 receptors in the thermoregulatory effect of morphine in mice. Eur J Pharmacol **308**:49–52, 1996.
18. Albanese CT, Nour BMK, Rowe MI. Anesthesia blocks nonshivering thermogenesis in the neonatal rabbit. J Pediatr Surg **29**:983–986, 1994.
19. Okada Y, Powis M, McEwan A, Pierro A. Fentanyl analgesia increases the incidence of postoperative hypothermia in neonates. Pediatr Surg Int **13**:508–511, 1998.
20. Plattner O, Semsroth M, Sessler DI, Papousek A, Klasen C, Wagner O. Lack of nonshivering thermogenesis in infants anesthetized with fentanyl and propofol. Anesthesiology **86**:772–777, 1997.
21. Ghosh S, Handler CM, Geller EB, Adler MW. Effect of a μ-selective opioid antagonist on CCK-8-induced changes in thermoregulation in the rat. Pharmacol Biochem Behav **59**:261–264, 1998.
22. Ignar DM, Kuhn CM. Relative ontogeny of opioid and catecholaminergic regulation of thryrotropin secretion in the rat. Endocrinology **123**:567–571, 1988.
23. Piepponen TP, Kivastik T, Katajamaki J, Zharkovsky A, Ahtee L. Involvement of opioid μ-1 receptors in morphine-induced conditioned place preference in rats. Pharmacol Biochem Behav **58**:275–279, 1997.
24. Zamparelli M, Eaton S, Quant PA, McEwan A, Spitz L, Pierro A, Analgesic doses of fentanyl impair oxidative metabolism of neonatal hepatocytes. J Pediatr Surg **34**:260–263, 1999.
25. Colman, AS, Miller, JH, Modulation of breathing by μ_1 and μ_2 opioid receptor stimulation in neonatal and adult rats. Respir Physiol **127**:157–172, 2001.
26. Gordon CJ. Thermal biology of the laboratory rat. Physiol Behav **47**:963–991, 1990.
27. Nedergaard J, Connolly E, Cannon B. Brown adipose tissue in the mammalian neonate. In: Trayhurn P, Nicholls DG, Eds. Brown Adipose Tissue.: London, UK, Edward Arnold, pp152–213, 1986.
28. Spiers DE, Adair ER. Ontogeny of homeothermy in the immature rat: metabolic and thermal responses. J Appl Physiol **60**:1190–1197, 1986.
29. Bertin R, De Marco F, Mouroux I, Portet R. Postnatal development of nonshivering thermogenesis in rats: effects of rearing temperature. J Dev Physiol **19**:9–15, 1993.
30. Pinkerton CR. Development of thermal stability in the neonate rat. J Physiol **263**:259P–260P, 1976.