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Seasonal Difference in Thermoregulatory Responses to Opiates in a Mammalian Hibernator

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WANG, L C H , T F LEE AND M L JOURDAN *Seasonal difference in thermoregulatory responses to opiates in a mammalian hibernator* PHARMACOL BIOCHEM BEHAV **26**(3) 565–571, 1987 —Accumulated evidence suggests that increased endogenous opioid activities may facilitate the onset of hibernation The present study investigated the change in thermoregulatory responses following ICV infusion of morphine or [D-Ala²]-Met enkephalinamide (EK) in unanesthetized, unrestrained Columbian ground squirrels (*Spermophilus columbianus*) during its annual hibernation cycle In the non-hibernating phase, low doses of either morphine (<160 μg) or EK (<400 μg) elicited a dose-related hyperthermia and an increase in heat production, whereas a higher dose of opiates caused hypothermia and a decrease in metabolic rate Naloxone (5 mg/kg, SC) pretreatment reduced or reversed both the hyper- and hypothermic responses to opiates Lower ambient temperature (5°C) enhanced the hypothermic response and attenuated the hyperthermic response. In the hibernating phase, euthermic ground squirrels exhibited a reduced responsiveness to exogenous opiates. the hyperthermic response to low dose of morphine (10 μg) was significantly reduced and hyperthermia, rather than hypothermia was observed at the highest dose of morphine (160 μg) The reduced responsiveness to opiates observed during the hibernating phase seems to suggest a reduction in opiate receptor efficacy which is in agreement with the contention that an increase in endogenous opioid activities may be incumbent with the commencement of hibernation

Opioid systems Morphine Enkephalin Hyperthermia Hypothermia Body temperature Oxygen consumption Circannual rhythm of hibernation

SINCE the discovery of the wide distribution of endogenous opioid-containing neurons within the central nervous system, these opioid peptides have been suggested to have multi-functional roles in various physiological and behavioral responses (for review see [1, 12, 25]). Recent studies have suggested that opioids may be involved in the process of hibernation. Evidence to date has shown that (a) administration of opioid antagonist to hibernating animals either reduces the duration of hibernation bout [4] or induces premature arousal from hibernation [17,20]; (b) an increase in brain levels of Met- and Leu-enkephalins [16] and Met-enkephalin immunoreactivity in specific hypothalamic areas [22] have been reported in hibernating animals; (c) the hibernating ground squirrel is resistant in developing physical dependence to morphine [6]; and (d) the "hibernation trigger"-induced hypothermia, hypophagia and bradycardia in monkey can be reversed by opioid antagonists [23]. Taken together, these results lead to the suggestion that endogenous opioid is involved in maintaining the state of hibernation [30].

In experimental animals, exogenous opiates induce different temperature responses which depend on the species tested, the dose, route of administration, ambient temperature and the degree of restraint used during testing (for review see [7,8]). These variations could be due to the presence of varying endogenous opioid activities associated with the different physiological and behavioral states prior to the test of opiates on thermoregulation. Since increase in endogenous opioid activity appears to be evident during hibernation (see above), it is possible that a differential thermoregulatory response to exogenous opiates may be predicted during the annual hibernation cycle. To test this possibility, the thermoregulatory changes of a hibernator, the Columbian ground squirrel (*Spermophilus columbianus*), to centrally administered (ICV) opiates were measured during both the active and hibernating phases of the annual hibernation cycle The results from the present study may provide more information on the possible role of central opioid systems in their participation of regulation of hibernation.

METHOD

Animals

Mature female Columbian ground squirrels were used in this study. They were live-trapped in the foothills of the Rocky mountains north of Hinton, Alberta, between May and August, and kept individually at an ambient temperature of 22°C with 12 L:12 D photoperiod. Food and water were available ad lib. Weekly measurements of weight were made to aid determination of the endogenous phase of the annual

hibernation cycle. The hibernation phase was characterized by a rapid weight gain followed by a weight plateau and anorexia. The completion of transition to the hibernation phase from the non-hibernation phase was further verified by the exhibition of hibernation when the animal was placed in cold (5°C) and dark without food in a walk-in environmental chamber. Animals were used after having completed at least two hibernation bouts and then tested in euthermia following spontaneous or disturbed arousal from hibernation (by transferring the animal to 22°C room temperature and uncurling the hibernating posture). The non-hibernating phase was evident when the animals showed no weekly weight increase and did not hibernate when placed in cold and dark without food for up to 7 days

Stereotaxic Procedure

Prior to being transferred to the cold room, a guide cannula (23-gauge stainless steel tubing) was implanted unilaterally into each ground squirrel under halothane anesthesia, and utilizing the following stereotaxic coordinates: AP=7.0 mm, L=2.0 mm, H=2 0 mm below the dura matter (using earbars as zero point). The tip of each guide tube was beveled and positioned 1.0 mm above the lateral ventricle in order to minimize damage to the actual infusion site. A 27-guage indwelling stylet was always kept in the cannula to prevent its occlusion. After completion of the experiments, the precise anatomical location of the injector cannula (27 gauge) was subsequently verified histologically according to standard histological procedures.

Body Temperature and Oxygen Consumption Measurements

On the day of experiment, the animal was transferred to a circular, Plexiglas water-jacketed metabolic chamber (20×20 cm, diameter × height) in which the ambient temperature could be controlled accurately at either 5 or 22°C The core temperature (Tb) of the ground squirrel was recorded continuously with a pre-calibrated temperature-sensitive radiotransmitter (Model T-M, Mini-Mitter Co. Inc., Sunriver, OR), which was implanted in the peritoneal cavity under halothane anesthesia at the same time as the ICV cannula implantation. This transmitter has a squegging oscillator circuit such that the frequency of the oscillator is linear to its surrounding temperature. The transmitter's signal was received by the FM receiver and displayed on an oscilloscope so that frequency of the signal could be verified accurately Using a calibration curve for each transmitter prepared prior to implantation, the frequency was converted into temperature (°C) and stored on-line by an IBM personal computer

Dried room air was drawn into the animal chamber at a flowrate of 1500 ml/min STP, which was regulated by a calibrated electronic mass flow controller (Matheson model 8240 controller + 8142 transducer) A fraction of the exhaust gas from the animal chamber was passed through tubes containing Ascarite and Drierite for CO_2 and water removal before it was measured by an oxygen analyzer (Applied Electrochemistry S-3A). The output from the analyzer was recorded by a chart recorder, and synchronously computed and integrated at once per second by an IBM personal computer

Once placed in the test chamber, the animal was not handled throughout the test session At least 1 5 hr elapsed before the drugs were injected in order to insure that Tb and O_2 consumption attained stable baseline upon which the drug

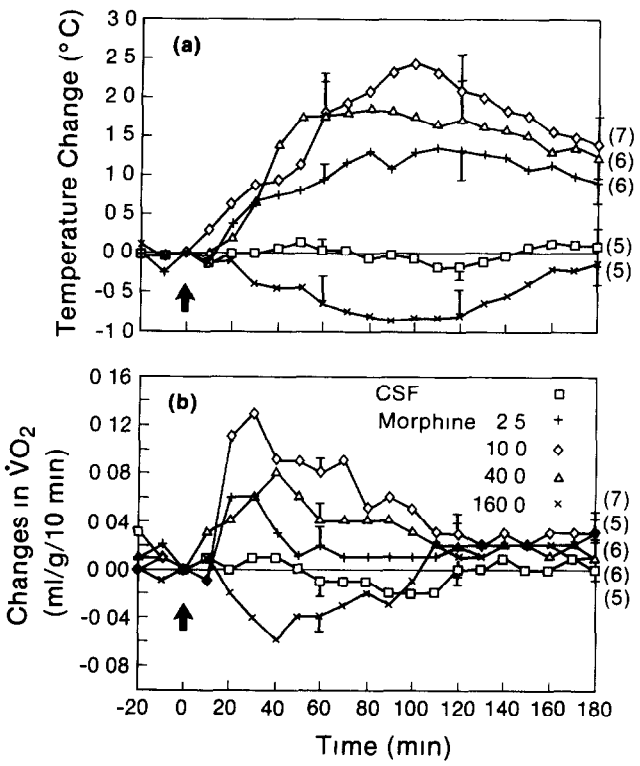

FIG 1 Time course of changes in (a) core temperature and (b) oxygen consumption after ICV infusion of CSF (□), morphine 2 5 (+), 10 (◇), 40 (△) or 160 μg (×) in non-hibernating Columbian ground squirrels tested at 22°C Each point represents the mean change from the number of squirrels shown beside each line The s e is represented by vertical bars at 60, 120 and 180 min Arrow indicates time of infusion

effects could be assessed accurately. Behavioral observations were also noted throughout the experimental period.

ICV Administration of Opiates

One week after surgery, each squirrel was tested with a standard dose of norepinephrine (20 μg/5 μl) given ICV by gravity flow [21]. Only those animals which responded to NE with a fall in Tb of more than 0 75°C were included for further study.

The following compounds were used· [D-Ala²]-Met-enkephalinamide (EK) acetate (Sigma), morphine sulphate (BDH) and naloxone hydrochloride (Sigma) Each drug was prepared immediately before an experiment began in a pyrogen-free artificial CSF. Each test solution, except EK, was always passed through a 0 22 μm Swinnex Millipore filter and then infused into the lateral ventricle in a volume of 5 μl by gravity flow The doses in the Results section are expressed in terms of μg of the salt In order to minimize the possibility of tolerance development, animals were tested with at least a 3-day rest between experiments.

Statistical Analysis

Comparisons between groups were made by two-tailed Student's *t*-test, and unless otherwise stated, a significant

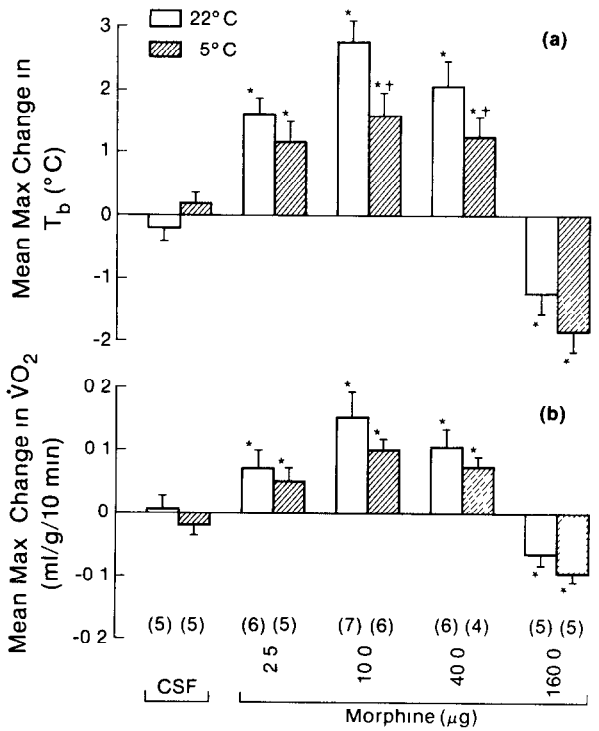

FIG 2 Mean maximum changes in (a) core temperature (Tb) and (b) oxygen consumption (VO_2) after ICV infusion of CSF or different doses of morphine in non-hibernating Columbian ground squirrels maintained at either 22°C (open colums) or 5°C (striped columns) Vertical bars indicate s e *Significantly different from the same ambient temperature CSF control group, $p<0.05$ †Significantly different from 22°C-exposed animal group receiving the same dose, $p<0$ 05

difference between groups was taken as $p<0.05$ For each comparison, the mean±s e. was presented for the response.

RESULTS

Effects of Morphine on Thermoregulation in the Non-Hibernating Phase

Control ground squirrels exposed to 22°C had an initial mean Tb of 37.2±0.22°C and basal oxygen consumption (VO_2) of 0.17±0 03 ml/g/10 min (n=9), these did not change significantly after ICV infusion of CSF (Fig. 1). Low doses of morphine (2 5 to 40 μg) caused a rapid rise in Tb which persisted for more than 2 hr after infusion. In all cases an increase in VO_2 always preceded the rise in Tb (Fig. 1). Increase in activity was also observed after morphine infusion. A lower dose of morphine (2 5 μg) generally induced grooming and exploratory behaviour whereas higher doses (10 and 40 μg) elicited a marked increase in locomotor activity, and on some occasions continuous tail chasing. However, these behavioral changes occurred with varying latencies and time courses and were not coincided with changes in Tb and VO_2. In contrast, the highest dose of morphine (160 μg) caused a decrease in VO_2 and hypothermia. In most cases, the animal appeared to have been sedated and lay spread out on the floor of the metabolic chamber for about 30 to 45 min. Thereafter, the animal returned to normal posture and alertness and showed a slight increase in locomotor activity at the end of the test session.

Figure 2 summarizes the group results after ICV infusion of morphine at both 5 and 22°C At 22°C, low doses of morphine (up to 40 μg) significantly elicited an increase in VO_2 and a rise in Tb. On the other hand, significant decreases in both the mean maximum VO_2 and Tb were observed after a high dose of morphine (160 μg). At 5°C, the VO_2 (0 31±0.09 ml/g/10 min, n=7) prior to ICV infusion was significantly higher than that observed in animals exposed to 22°C, but the Tb (37.5±0.32°C) was the same Infusion of low doses of morphine (up to 40 μg) in animals kept at 5°C caused an elevation of VO_2 and hyperthermia, both of which were significantly greater than the CSF-infused controls. Comparing the thermoregulatory change at 22 and 5°C, a significant reduction in mean maximum Tb was observed at 5°C, after 10 and 40 μg morphine However, the corresponding reductions of VO_2 at these doses were not significant (Fig. 2). Although the decreases in mean maximum VO_2 and Tb were greater at 5°C after 160 μg morphine infusion, these changes were not significantly different to those observed at 22°C (Fig 2) After morphine, the behavioral changes of the 5°C-exposed ground squirrels were more or less the same as those observed at 22°C

Effects of Met-Enkephalinamide (Met-EK) on Thermoregulation in the Non-Hibernating Phase

Similar to that observed after morphine administrations, ICV infusion with low doses of Met-EK (25 and 100 μg) caused a dose-related increase in Tb which was preceded by an increase in VO_2 (Fig. 3). Also, a decrease in both VO_2 and Tb was seen after a high dose of Met-EK (400 μg) In all cases, the duration of the response to Met-EK was shorter than that observed with morphine. The behavioral changes elicited by Met-EK were about the same as those observed with morphine

Figure 4 shows the effect of Met-EK on thermoregulatory changes in ground squirrels exposed to both 22 and 5°C. The thermoregulatory patterns of the 5°C-exposed ground squirrels were quite similar to that observed at 22°C Even though the hyperthermic responses were slightly decreased and the hypothermic responses slightly enhanced at 5°C, only the mean maximum change in Tb after Met-EK at 100 μg was significantly lower than that for 22°C

Effects of Subcutaneous Pretreatment of Naloxone on Opiate-Induced Thermoregulatory Changes in the Non-Hibernating Phase

At 22°C, subcutaneous injection of naloxone (5 mg/kg) had no significant effect on changes in Tb (−0 12±0 1°C, n=5) and VO_2 (−0.02±0.01 ml/g/10 min). Further, no noticeable change in the behaviour of the animal was observed. However, pretreatment with the same dose of naloxone 5 min prior to ICV infusion of opiates not only significantly reduced the hyperthermic response after a moderate dose of opiates (10 μg morphine or 100 μg Met-EK) but also reversed the hypothermic responses after a high dose of opiates (160 μg morphine or 400 μg Met-EK) (Fig. 5). After naloxone pretreatment, the changes in behavioral activities typically induced by a low dose of opiates were not seen and the typical sedative effect of a high dose of opiates was also completely abolished.

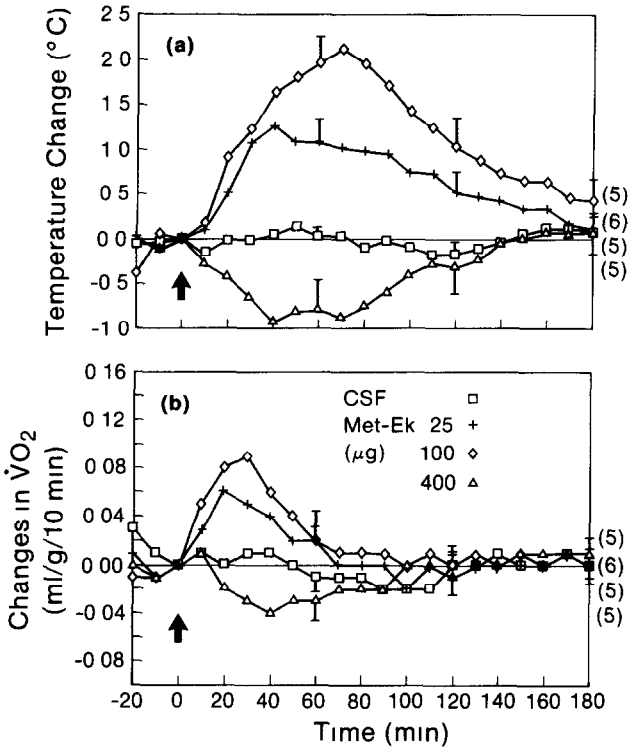

FIG 3 Time course of changes in (a) core temperature and (b) oxygen consumption after ICV infusion of CSF (□), Met-enkephalinamide 25 (+), 100 (◇) or 400 μg (△) in non-hibernating Columbian ground squirrels maintained at room temperature Each point represents the mean change from the number of squirrels shown besides each line The s e is represented by vertical bars at 60, 120 and 180 min Arrow indicates time of infusion

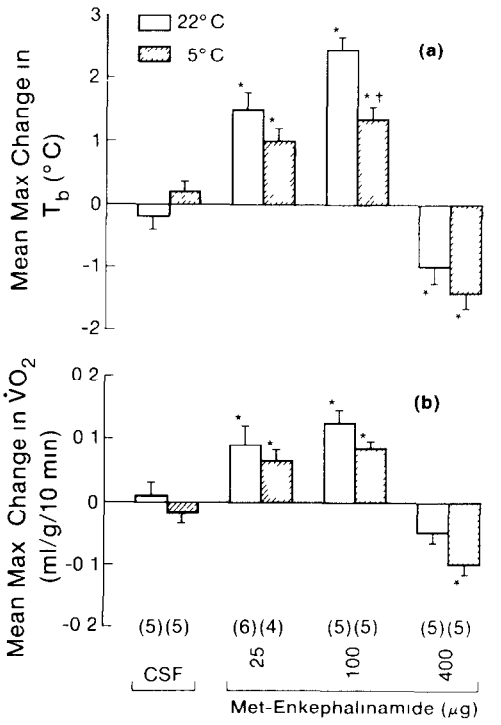

FIG 4 Mean maximum changes in (a) core temperature and (b) oxygen consumption after ICV infusion of CSF or different doses of Met-enkephalinamide in non-hibernating Columbian ground squirrels maintained at either 22°C (open columns) or 5°C (striped columns) Vertical bars indicate s e *Significantly different from the same ambient temperature CSF control group, $p<0.05$ †Significantly different from 22°C-exposed animal group receiving the same dose, $p<0.05$

Effects of Morphine on Thermoregulation in the Hibernating Phase

Compared with squirrels in the non-hibernating phase exposed to 5°C, squirrels in the hibernating phase showed a significantly lower initial VO_2 (0.21±0.03 ml/g/10 min, n=5) but not Tb (36.9±0.56°C) Figure 6 shows the thermoregulatory responses to ICV infusion of either 10 or 160 μg morphine Low dose of morphine caused only a slight increase in Tb (mean maximum change=0.59±0.21°C), which was significantly ($p<0.05$) lower than that observed in the non-hibernating animals (mean maximum change=1.63±0.33°C). Even though the change in VO_2 (0.076±0.027 ml/g/10 min) induced by low dose of morphine in the hibernating phase animal was less than that of the non-hibernating phase ground squirrel (mean maximum change=0.103±0.024 ml/g/10 min), this difference was not statistically significant. In contrast to the hypothermic responses observed in the non-hibernating phase, a significant increase in both VO_2 and Tb was seen in the hibernating phase after 160 μg morphine. Further, rather than a sedative effect, an increase in locomotor activity was noted after this dose

DISCUSSION

Other than the difference in duration, ICV infusion with morphine and Met-EK at 22°C caused a similar dual dose-related changes in Tb in squirrels in their non-hibernating phase· lower doses elicited hyperthermia whereas higher doses elicited hypothermia Subcutaneous pretreatment with naloxone was able to reduce or reverse these changes suggesting that the thermoregulatory effects of morphine and Met-EK were by activating central mu-receptor This pattern of thermoregulatory changes is similar to that observed in other species after ICV infusion of morphine or EK [9, 11, 15]. The course of hyperthermia evoked by relatively low doses of opiates was always preceded by an increase in VO_2 The exact mechanisms for the increased VO_2 are not known Even though increase in locomotor activity was generally observed after opiate infusion, this may not be the main reason for the increased VO_2 because the time course of VO_2 change did not always parallel that of locomotor activity and in some cases, the increase in activity occurred only intermittently Further, it has been shown recently that morphine-induced hyperthermia in rats is not brought about by an increased locomotor activity [29]. Whether the causes of acute hyperthermia observed in ground squirrels after opiates share a similar increase in vasomotor tone [3,10] and/or skeletal muscle tonus [28] as have been shown for other species also remains to be investigated In contrast to that observed with lower doses, the highest dose of opiate suppressed the VO_2 and resulted in a fall of Tb. Since a

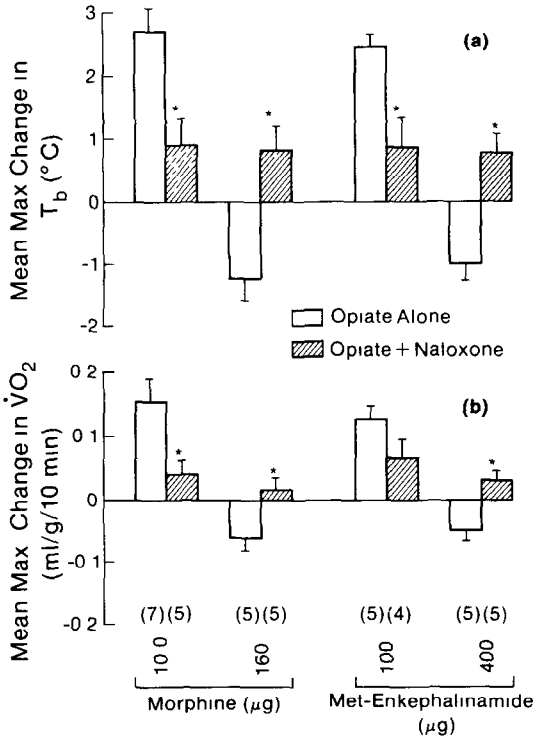

FIG 5 Effects of subcutaneous pretreatment with naloxone (5 mg/kg) on opiate-induced (a) core temperature and (b) oxygen consumption responses in non-hibernating ground squirrels kept at room temperature Vertical bars indicate s e *Significantly different from appropriate agonist control, $p<0$ 05

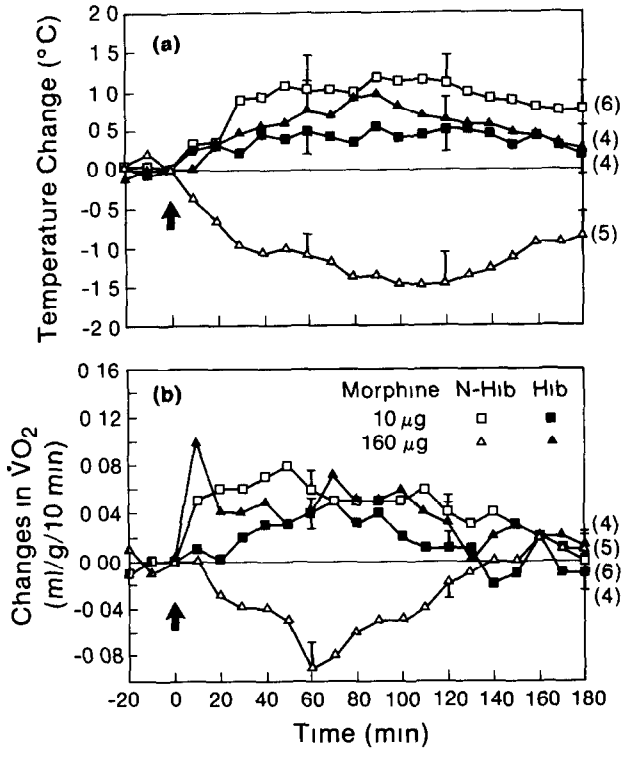

FIG 6 Comparison of mean changes in (a) core temperature and (b) oxygen consumption after ICV infusion of morphine 10 μg (□) or 160 μg (△) into non-hibernating (open symbols) and hibernating phase (closed symbols) ground squirrels kept at 5°C Each point represents the mean change from the number of squirrels shown besides each line. The s.e is represented by vertical bars at 60, 120 and 180 min Arrow indicates time of infusion

decrease in VO_2 and sedation of the animal were typically observed, it is quite possible that the hypothermic response to the highest dose of opiate is consequent to a transient depression of CNS function.

The biphasic nature of the dose-response relationship indicates that opiates may have more than one action on central thermoregulatory pathways. A most common method to evaluate the drug effect on thermoregulatory function is to determine drug-induced Tb changes over a range of ambient termperatures. When ground squirrels were exposed to cooler temperature, the magnitude of hyperthermia induced by lower doses of opiates was attenuated. This finding agrees with previous reports that the degree of hyperthermia is greater in warmer ambient temperature [9,26]. The increase in neuronal activity of hypothalamic cold-sensitive cells after iontophoretic application of morphine [19] suggests that the hyperthermia induced by low doses of opiate can be brought about by increasing the activity of central cold-sensor pathways. Such an action would be expected to depend on the initial activity within the pathway and should be more effective in the warmer environment when the activity along the pathway was low to begin with. This suggestion is supported by the fact that a smaller net increase in VO_2, even though not significantly different from that recorded at 22°C, was consistently observed in animals kept at 5°C after ICV opiates However, the possibility that a greater rate of heat loss in the cold environment may have caused the smaller hyperthermic response to the same dose of opiate is also a tenable explanation. Similar to other previous studies [18,24], the hypothermic response to the highest dose of opiate was enhanced when the ground squirrel was exposed to the cold. This is likely due to a suppression of heat production as observed in the present study and an increase in heat loss as observed previously in rabbits [18], both of which would contribute to the greater decrease of Tb in the cold than that at 22°C.

The most striking finding of the present study is the reduction of thermoregulatory responsiveness to exogenous opiates in the ground squirrels during their hibernating phase Not only was significant reduction in hyperthermic response to low dose of morphine (10 μg) observed but more evidently, the highest dose of morphine (160 μg) caused hyperthermia rather than hypothermia. It is as if the opiate-Tb dose response curve has been shifted to the right when the animal undergoes its physiological transition from the non-hibernating to the hibernating phase. The reduced sensitivity to opiates does not seem to be due to a general depression of CNS thermoregulatory function since the animal in the hibernating phase still responded to standard doses of ICV NE with the same degree of fall in Tb. Further, it has been shown previously that both an increase in thermoregulatory response to serotonin [13] and a constant responsive-

ness to NE [13] and prostaglandins [14] are typical in ground squirrels during their hibernating phase as compared to their non-hibernating phase. The seasonal difference in thermoregulatory sensitivity to exogenous morphine is also unlikely due to cold-acclimation in the hibernating animals since either no change [27] or increased thermoregulatory responses [31] to exogenous opiates has been reported in cold-acclimated rats

The differential thermoregulatory response to exogenous opiate in ground squirrels during different phases of the hibernating cycle may indicate an endogenous change in the central opioid pathways To date, there has been much evidence indicating an increase in central opioid activity during the hibernating phase (see the Introduction section) It is quite possible that the reduced response to opiates observed during the hibernating phase may be due to a reduction in opiate receptor efficacy resulted by an increase in endogenous opioid activity during this part of the annual cycle This suggestion is further supported by recent findings that the overall brain opioid binding efficacy is decreased in the hibernating ground squirrel [2,5] In view of the fact that numerous brain sites have been implicated in the thermoregulatory effect of opiates (for review see [7,8]) and the multiplicity of areas affected after ICV infusion, the results from the present study are corroborative in nature However, taking previous findings into consideration, our results strongly suggest that an altered CNS opioid activity for thermoregulation occurs in consonance with the annual cycle for hibernation

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REFERENCES

1 Akil, H , S J Watson, E Young, M E Lewis, H Khachaturian and J M Walker Endogenous opioids Biology and function *Annu Rev Neurosci* **7**: 223–255, 1984
2 Aloia, R C , B J Vasquez and S L Tucker Opioid receptors in the brain of active and hibernating ground squirrels, *Spermophilus lateralis* Proceedings of the International Symposium "Living in the Cold," Fallen Leaf Lake, CA, p 211, 1985
3 Ary, M and P Lomax Temperature changes during morphine dependence and withdrawal in the rat In *Drugs, Biogenic Amines and Body Temperature*, edited by K E Cooper, P Lomax and E Schonbaum Basel Karger, 1977, pp 188–195
4 Beckman, A L and C Llados-Eckman Antagonism of brain opioid peptide action reduced hibernation bout duration *Brain Res* **328**: 201–205, 1985
5 Beckman, A L , C Llados-Eckman, S K Salzman and T L Stanton Modulation of opioid physical dependence during the hibernation cycle In *Mechanisms of Tolerance and Dependence*, edited by C W Sharp Washington, DC U S Government Printing Office, 1984, pp 209–238
6 Beckman, A L , C Llados-Eckman, T L Stanton and M W Adler Physical dependence on morphine fails to develop during the hibernating state *Science* **212**: 1527–1529, 1981
7 Clark, W G Effects of opioid peptides on thermoregulation *Fed Proc* **40**: 2754–2759, 1981
8 Clark, W G and J M Lipton Brain and pituitary peptides in thermoregulation *Pharmacol Ther* **22**: 249–297, 1983
9 Clark, W G and S W Ponder Thermoregulatory effects of (*D*-ala²)-methionine-enkephalinamide in the cat Evidence for multiple naloxone-sensitive opioid receptors *Brain Res Bull* **5**: 415–420, 1980
10 Cox, B , M Ary, W Chesarek and P Lomax Morphine hyperthermia in the rat an action on the central thermostats *Eur J Pharmacol* **36**: 33–39, 1976
11 Ferri, S , R Arrigo Reina, A Santagostino, G M Scoto and C Spadaro Effects of met-enkephalin on body temperature of normal and morphine-tolerant rats *Psychopharmacology (Berlin)* **58**: 277–281, 1978
12 Frederickson, R C A and L E Geary Endogenous opioid peptides Review of physiological, pharmacological and chemical aspects *Prog Neurobiol* **19**: 19–69, 1982
13 Glass, J D and L C H Wang Thermoregulatory effects of intracerebroventricular injection of serotonin and a monoamine oxidase inhibitor in a hibernator, *Spermophilus richardsonii* *J Thermal Biol* **4**: 149–156, 1979
14 Hartner, W C and C J Young Role of prostaglandins in mammalian hibernator temperature regulation *Cryobiology* **18**: 90–91, 1981
15 Kandasamy, S B and B A Williams Peptide and non-peptide opioid induced hyperthermia in rabbits *Brain Res* **265**: 63–71, 1983
16 Kramarova, L I , S H Kolaeva, R Y Yukhananov and V V Rozhanets Content of DSIP, enkephalins, and ACTH in some tissues of active and hibernating ground squirrels *(Citellus suslicus) Comp Biochem Physiol* **74C**: 31–33, 1983
17 Kromer, W Naltrexone influence on hibernation *Experientia* **36**: 581–582, 1980
18 Lin, M T and C Y Su Metabolic, respiratory, vasomotor and body temperature responses to beta-endorphin and morphine in rabbits *J Physiol (Lond)* **295**: 179–189, 1979
19 Lin, M T , W N Uang and H K Chan Hypothalamic neuronal responses to iontophoretic application of morphine in rats *Neuropharmacology* **23**: 591–594, 1984
20 Margules, D L , B Goldman and A Finck Hibernation An opioid-dependent state? *Brain Res Bull* **4**: 721–724, 1979
21 Myers, R D and T L Taksh Feeding and temperature responses in unrestrained rat after injections of cholinergic and aminergic substances into the cerebral ventricles *Physiol Behav* **3**: 917–928, 1968
22 Nurnberger, F Der Hypothalamus des Igels (*Erinaceus europaeus* L) unter besonderer Berucksinchtigung des Winterschlafes Cytoarchitektonische und Immuncytochemische Studien PhD thesis, Phillips-Universitaet Marburg, 1983
23 Oeltgen, P R , J W Walsh, S R Hamann, D C Randall, W A Spurrier and R D Myers Hibernation "trigger" Opioid-like inhibitory action on brain function of the monkey *Pharmacol Biochem Behav* **17**: 1271–1274, 1982
24 Paolino, R M and B K Bernard Environmental temperature effects on the thermoregulatory response to systemic and hypothalamic administration of morphine *Life Sci* **7**: 857–863, 1968
25 Schick, R and V Schusdziarra Physiological, pathophysiological and pharmacological aspects of exogenous and endogenous opiates *Clin Physiol Biochem* **3**: 43–60, 1985
26 Thornhill, J A , K E Cooper and W L Veale Effects of restraint and ambient temperature on core temperature responses to morphine in the rat In *Thermoregulatory Mechanisms and Their Therapeutic Implications*, edited by B Cox, P Lomax, A S Milton and E Schonbaum Basel Karger, 1980, 159–162
27 Thornhill, J A and M Desautels Is acute morphine hyperthermia of unrestrained rats due to selective activation of brown adipose tissue thermogenesis? *J Pharmacol Exp Ther* **231**: 422–429, 1984

28 Thornhill, J A , J Saucier and K Powell-Jones Acute morphine induced hyperthermia in unrestrained rats is caused by skeletal muscle thermogenesis In *Homeostasis and Thermal Stress Experimental and Therapeutic Advances*, edited by K E Cooper, P Lomax, E Schonbaum and W L Veale Basel Karger, 1985, pp 156–160

29 Vezina, P and J Stewart Hyperthermia induced by morphine administration to the VTA of the rat brain. An effect dissociable from morphine-induced reward and hyperactivity *Life Sci* **36:** 1095–1105, 1985

30 Wang, L C H Life at low temperature mammalian hibernation *Cryo Lett* **6:** 257–274, 1985

31 Wong, T M and S Y H Tse Cold acclimation increases physiological responsiveness to intraventricular injection of β-endorphin in pentobarbital anesthetized rats II Metabolic function. *Int J Pept Protein Res* **24:** 74–78, 1984