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Depression of Learned Thermoregulatory Behavior by Central Injection of Opioids in Cats

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CLARK, W. G. AND G. L. BERNARDINI. *Depression of learned thermoregulatory behavior by central injection of opioids in cats.* PHARMAC. BIOCHEM. BEHAV. 16(6) 983–988, 1982.—Third cerebral ventricular administration of morphine (20 μg), pentazocine (1 mg) or the synthetic opioid peptides D-Ala²-Met-enkephalinamide (25 μg) and D-Ala²-D-Leu⁵-enkephalin (10 μg) decreased heat-reinforcement activity and either reduced or did not increase heat-escape activity of cats trained to control thermal stimuli through behavior. If the behavioral changes had been coupled to the primary effects of these opioids on thermoregulation, one would have expected an inverse relationship between changes in heat-reinforcement and heat-escape activity. Motor incapacitation appeared not to have contributed to the reduction in responding except briefly after pentazocine injection. The opioids may have distracted the animals or otherwise reduced their reaction to thermal stimuli until body temperature increased to a potentially dangerous level.

Thermoregulatory behavior Opioids Central injection Morphine Pentazocine

THE functional points within the thermoregulatory system at which drugs act can be assessed through the use of two approaches. The more versatile of these involves determination of the pattern of changes in temperature produced by a drug in subjects exposed to a series of environmental temperatures ranging from below to above the thermoneutral temperature of the species [1,3–5]. This approach will usually allow conclusions as to whether the drug (1) causes coordinated changes in the level at which body temperature is regulated, (2) depresses thermoregulatory coordination or control mechanisms or (3) affects thermoeffector pathways. The second approach utilizes thermoregulatory behavior and primarily allows a decision as to whether a drug (1) alters the level at which body temperature is regulated or (2) acts in some other way [14, 19, 20]. A change in the level of regulation causes an "inappropriate" thermoregulatory response in the sense that thermoeffector activities, though still coordinated, are shifted to drive body temperature away from the initial value. In this case, behavior is predicted to facilitate development of the drug-induced change. In contrast, if a drug depresses thermoregulation, allowing body temperature to drift passively in response to changes in environmental temperature, or directly affects thermoregulatory effector pathways, the subject can correctly sense the resulting change in body temperature and will behave appropriately to oppose the drug-induced temperature change. For conclusions of specific thermoregulatory changes based on behavioral studies to be valid, the drug must not impair the subjects' ability to respond by causing anesthesia, paralysis, etc. Hence, studies of this sort are often designed so that an increase in responding is demonstrated, since a decrease in responding may be non-specific. The converse is also possible, that drug-induced stimulation enhances responding nonspecifically, and, as emphasized by Satinoff [19], it is best to study behavioral responses of animals trained for both heat-reinforcement and heat-escape.

The effect of morphine on thermoregulatory behavior has been studied only in untrained rats [13]. In these experiments hyperthermic doses of the drug delayed departure from a heat source. This delay was considered a thermoregulatory response rather than evidence of a less specific depression of locomotor activity because the animals did not appear sedated or catatonic. The purpose of the present experiments was to examine the effects of morphine on learned thermoregulatory behavior; namely, bar-pressing by cats to obtain or to escape heat. In addition, changes in body temperature and behavior after pentazocine and a synthetic opioid peptide, D-Ala²-Met-enkephalinamide, neither of which acts on morphine-sensitive receptors [6–8], were also determined. Previous studies with central administration of these opioids to cats exposed to different ambient temperatures provided evidence that morphine [9] and D-Ala²-Met-enkephalinamide [11] increase the level at which temperature is regulated. Behavioral responses would, therefore, be predicted to facilitate the rise in body temperature. Pentazocine initially depresses thermoregulation [12], and so behavioral responses should oppose the fall in body temperature in a cold environment. Hence, all three agents were expected to increase responding by cats trained to press a lever to obtain heat and, conversely, to decrease heat-escape responding.

Contrary to these expectations, both types of response were depressed by all three agents, indicative of a non-specific decrease in learned thermoregulatory behavior.

METHOD

Nine adult cats, weighing from 2.5–5.4 kg, were housed between tests at an environmental temperature of 22±2°C. Procedures for their care and feeding, for recording body temperature automatically from the retroperitoneal space, for implantation of cannulas for injections into the third cerebral ventricle (ICV), for avoiding pyrogenic contamination and for calculating thermal response indexes (TRIs) have been described previously [6,18]. TRIs, estimates of the area between a response curve and the base-line temperature determined by averaging temperatures 10, 20 and 30 min before opioid or saline injection, were calculated so that 1 unit is equivalent to a 1°C change lasting for 1 hr.

The apparatus in which the animals were trained and tested was a stainless steel cage, pictured elsewhere [10], over which a battery of infra-red lamps and a small fan were suspended. In heat-reinforcement (HR) trials, two lamps suspended over the front end of the cage were activated and the fan was turned off for as long as a lever, which protruded through the front of the cage, was depressed. The fan was on and the lamps off if the lever was not depressed. In heat-escape (HE) trials, six lamps were mounted over the cage, and the conditions were reversed; i.e., depressing the lever turned the lamps off and activated the fan. In control (C) trials, the lever was inoperative and neither the lamps nor the fan were on. All studies were done in a cold-room maintained at 4±2°C. Initially the animals were trained for HR by placing them for a period of 2–5 hr in the cage with the fan on. In all, 19 cats were tested. Those which were used in this study were those which spontaneously began to bar press for heat within two to four sessions. Training was then continued in additional sessions during which the height of the lamp was adjusted individually for each animal until a height was established at which the subject depressed the lever approximately 50% of the time. Once training was completed, the cat was given a series of four trials, in randomly determined order, of all combinations of saline vehicle or opioid injection with or without HR behavior. The animal was placed in the cage shortly before 9 a.m. Opioid or vehicle was injected at 10:00 a.m. ±5 min, and the prior 30-min period was used to determine both base-line body temperature and the base-line level of bar pressing. Trials continued for 5 hr. The amount of time the animal was on the bar was tabulated from a cumulative-time meter at 5-min intervals for the first 90 min after injection and at 30-min intervals thereafter. The animals were also observed, through a small window in the door of the cold-room, for changes in general locomotor activity, posture, etc. To minimize distraction and other interference with the animals' behavior, however, observations were intermittent, and although previously reported, non-thermoregulatory responses such as mydriasis, emesis, etc. often occurred, no detailed compilation of their incidence was attempted. Trials were spaced at least 48 hr apart to avoid acclimatization to the cold or development of tolerance to the opioids. As necessary, after completion of a set of trials with one agent, cats were assigned to a series with another opioid. After an animal had completed HR experiments, it was retrained for HE trials, usually within one session, simply by placing it in the cage which had been modified for such behavior. After further sessions to ensure consistent HE responding and to adjust the height of the lamps, the effect of the opioids on HE responding was then tested.

Mean values±SE are indicated in the table and figures The Newman-Keuls multiple range test [22] was used for multiple comparisons of TRIs and maximal changes in body temperature. The paired t-test was used to evaluate differences in thermoregulatory behavior between opioid and saline trials [21], based on the change in time on the bar after injection relative to the time on the bar during the 30-min base-line period before injection.

Stock solutions of morphine sulfate (Mallinckrodt), D-Ala^2-Met-enkephalinamide (Calbiochem), D-Ala^2-D-Leu^5-enkephalin (Peninsula) and pentazocine (Winthrop) were stored in plastic, disposable vials in 0.9% NaCl solution at 4°C. Doses refer to these entities. Doses were chosen which had consistently altered body temperature in previous studies. All opioid and control injections were made into the third cerebral ventricle in a volume of 0.05 ml, and residual opioid was flushed from the cannula with 0.1 ml saline solution shortly after completion of each trial.

RESULTS

Morphine

Heat-reinforcement. Morphine consistently enhanced locomotor activity, as previously reported [9]. This opiate induced essentially the same increase in body temperature in both control and HR trials, but it markedly reduced the amount of time spent working for heat during the entire 5-hr period of the trial as compared to HR activity after administration of saline vehicle alone (Fig. 1, Table 1). The decrease in bar pressing was apparent in all cats within 10 min after injection, and behavior had increased only slightly after 5 hr when body temperature had nearly returned to the control level.

Heat-escape. Mean HE responding decreased, but not significantly, during the initial 90-min period after morphine injection (Fig. 2, Table 1). Although morphine increased body temperature to a significantly higher level during the first 3 hr of the behavioral trial than in the control session, none of the animals increased their level of responding to prevent the rise in temperature. However, when a maximal increase of 1.5–3.1°C was reached, the level of responding returned to base-line levels and was thereafter maintained near the control level of behavior.

D-Ala^2-Met-enkephalinamide

Heat-reinforcement. Locomotor activity was not increased by D-Ala^2-Met-enkephalinamide, but neither did the animals appear depressed or distressed. Hyperthermic responses to the peptide lasted about 3 hr and, as with morphine, comparable hyperthermias were evoked in both control and HR trials (Fig. 3, Table 1). Although, in this particular set of cats, HR responding declined after saline solution, it was reduced to a greater extent after injection of D-Ala^2-Met-enkephalinamide, beginning within 5 min of administration.

Heat-escape. D-Ala^2-Met-enkephalinamide decreased mean HE responding within 5 min of injection, but the decrease lasted only for the first hour after administration (Fig. 4, Table 1). During the initial hour when HE responding was depressed and the animals were, therefore, exposed to an increased amount of heat, body temperature increased about

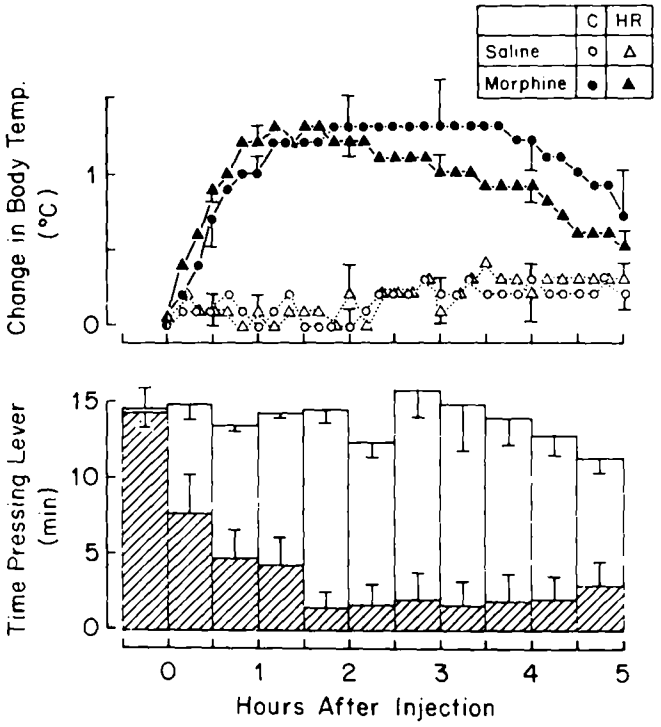

FIG. 1. Mean changes in core temperature of six cats after ICV administration of saline vehicle or morphine sulfate (20 μg) with (HR) or without (C) learned heat-reinforcement (HR) activity (above). The concomitant amount of time spent on the lever during the behavioral trials after saline (open bars) or morphine (hatched bars) is indicated below.

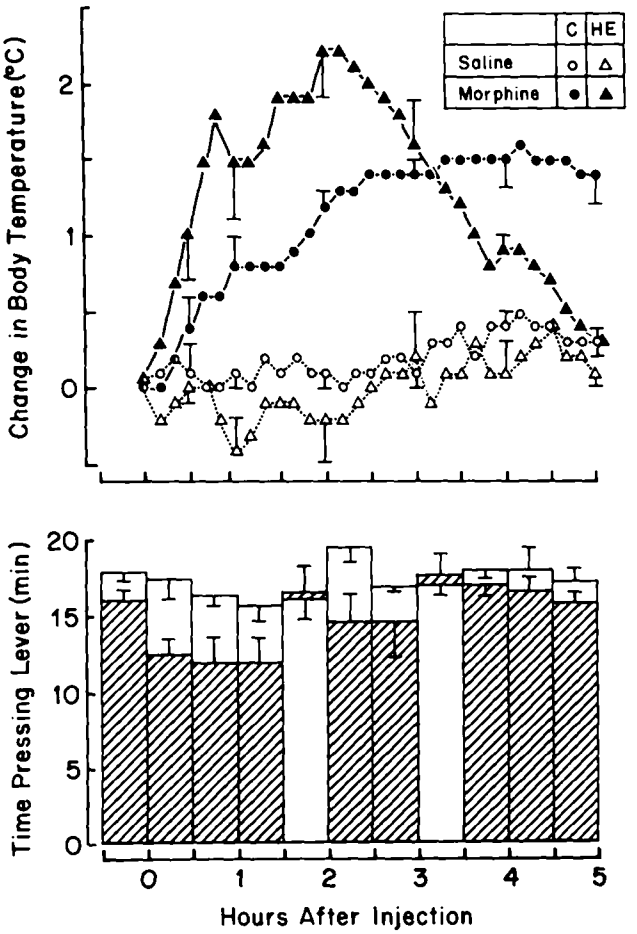

FIG. 2. Changes in temperature and behavior of six cats after saline or morphine (20 μg) injection with (HE) or without (C) learned heat-escape (HE) activity. See legend of Fig. 1 for further details.

TABLE 1

RESPONSES TO OPIOID ADMINISTRATION*

			Maximum change in body temperature (°C)					Thermal response index (Δ°C×hr)				Behavioral depression‡	
Opioid	Behavior	N	Vehicle	Vehicle + behavior	Opioid	Opioid + behavior	(hr)†	Vehicle	Vehicle + behavior	Opioid	Opioid + behavior	(hr)	p
Morphine	HR	6	0.0±0.2	0.4±0.3	1.4±0.2	1.4±0.1	5	0.6±0.5	0.8±0.4	5.4±1.0	4.8±0.5	0–5	<0.005
	HE	6	0.6±0.1	−0.4±0.5	1.7±0.2	2.6±0.3	5	1.0±0.2	−0.1±0.8	5.7±0.5	6.6±0.8	0–1.5	<0.2
							3	0.3±0.2	−0.4±0.5	2.7±0.4	4.9±0.8		
DAME	HR	4	−0.2±0.3	0.2±0.2	1.2±0.1	1.3±0.1	3	0.3±0.4	0.1±0.1	2.4±0.4	1.9±0.4	0–3	<0.1
	HE	4	0.4±0.3	0.0±0.3	1.4±0.2	2.9±0.2	3	0.7±0.3	0.3±0.4	2.7±0.4	4.4±0.7	0–1	<0.1
Pentazocine	HR	4	−0.1±0.3	−0.1±0.5	−3.1±0.4	−2.3±0.6	4	0.2±0.3	0.5±1.1	−6.0±1.3	−3.6±1.8	0–4	<0.025
	HE	4	−0.1±0.3	−0.1±0.3	−3.0±0.7	1.9±0.2	4	0.7±0.4	−0.4±0.6	−3.1±0.7	−0.2±1.7	0–0.5	<0.005
							0.5	0.0±0.1	−0.1±0.1	−1.0±0.2	0.7±0.1		

*Mean values underscored by the same line are not statistically different ($p>0.05$, Newman-Keuls multiple comparisons).
†Period after injection for which TRI was calculated.
‡Duration of reduced level pressing; opioid+behavior vs. vehicle+behavior (paired t-test).

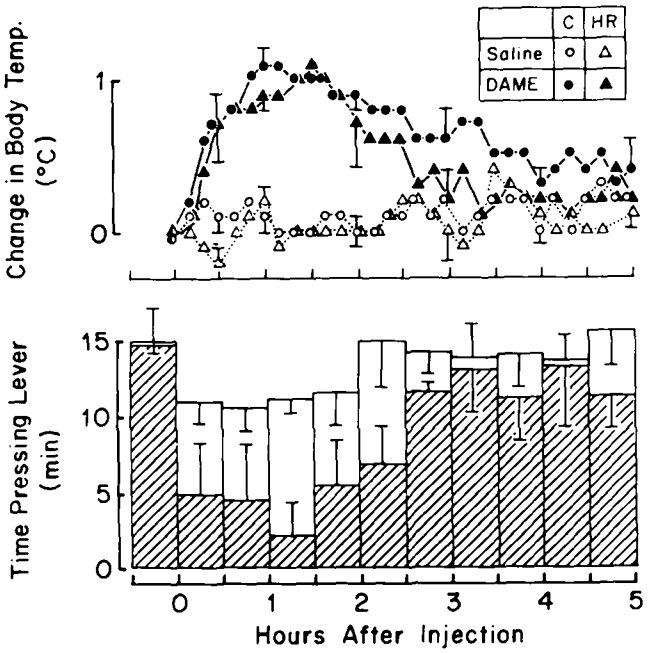

FIG. 3. Changes in temperature and behavior of four cats after saline or D-Ala²-Met-enkephalinamide (DAME, 25 μg) injection with (HR) or without (C) learned HR activity.

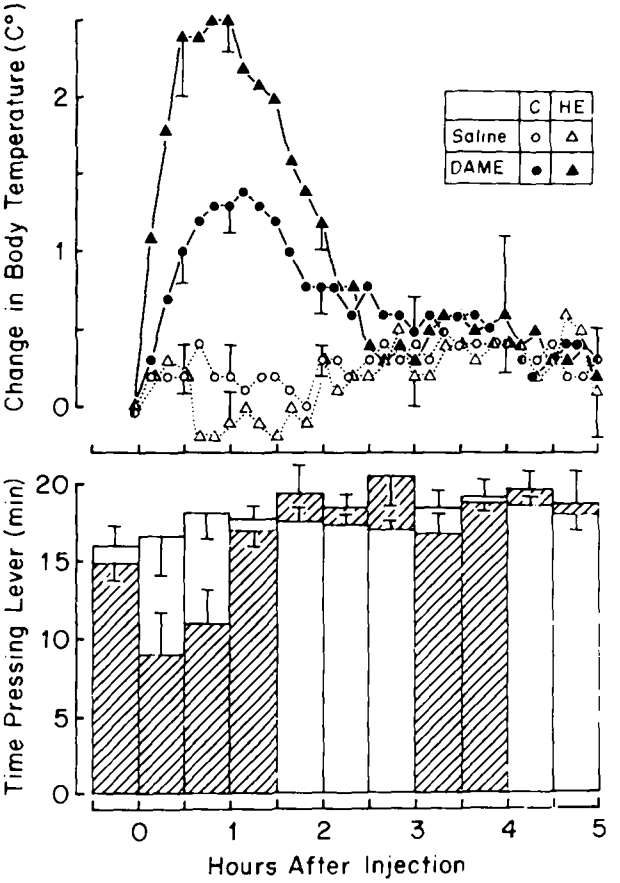

FIG. 4. Changes in temperature and behavior of four cats after saline or D-Ala²-Met-enkephalinamide (25 μg) injection with (HE) or without (C) learned HE activity.

twice as much as after D-Ala²-Met-enkephalinamide alone. As HE responding returned to control levels during the second hour, body temperature declined nearly to normal. When tested in two cats D-Ala²-D-Leu⁵-enkephalin (10 μg), another synthetic peptide which increases body temperature in the cat (Clark, W. G. and I.-H. Pang, unpublished data), nearly abolished HR activity for 2 hr but did not appreciably alter HE responding.

Pentazocine

Heat-reinforcement. For about the first 30 min after administration of this drug, the animals were either sprawled on the floor of the cage or, though sitting, were slumped over as though exhausted. Although the mean initial hypothermia after pentazocine was less during HR trials than in control trials, the responses were quite variable (Fig. 5, Table 1) and not significantly different. There was, however, a reduction in HR responding for about 4 hr with a total cessation of bar pressing within 5 min of injection in all four animals.

Heat-escape. Pentazocine significantly reduced HE responding only during the first 30 min during which period body temperature rose rapidly (Fig. 6, Table 1), in sharp contrast to the usual hypothermia after pentazocine injection. Lever pressing by all four cats ceased by 5–15 min after injection. During the second 30-min period the animals resumed responding and increased the level of responding to above control levels ($p<0.01$) while body temperature rapidly fell to or below the base-line level. This latter response was attained when the animals crawled or pulled themselves over to the bar and laid down on it, as the cats were still unable to sit up or locomote normally until about 1 hr after injection.

DISCUSSION

In previous studies morphine [9], D-Ala²-Met-enkephalinamide [11] and D-Ala²-D-Leu⁵-enkephalin (Clark, W. G. and I.-H. Pang, unpublished data) increased body temperature of cats whether they were exposed to hot or cold environments. There was no associated tachypnea that would indicate a compensatory increase in heat-loss mechanisms. Hence, these opioids appear to increase the level of temperature regulation, by raising the set-point or by altering input from thermosensors [5], so that effector activities are adjusted as though the animals are cold. Body temperature decreased initially after pentazocine administration, and the cats were, in fact, cold in this case. If drug-induced changes in behavior are due to an action directly on the thermoregulatory system, a reciprocal relationship between HR and HE behavior should result [19,20]. If the subject feels cold, it should seek heat; i.e., increase HR behavior or decrease HE behavior. Although HE responding did decrease, or at least did not increase, HR was also inhibited by the opioids. Because of this lack of reciprocal effects of the opioids on thermoregulatory behavior, our conclusion is that the opioids reduced learned thermoregulatory behavior by some mechanism not directly coupled to their primary thermoregulatory actions. This conclusion is supported by the temporal dissociation between body tempera-

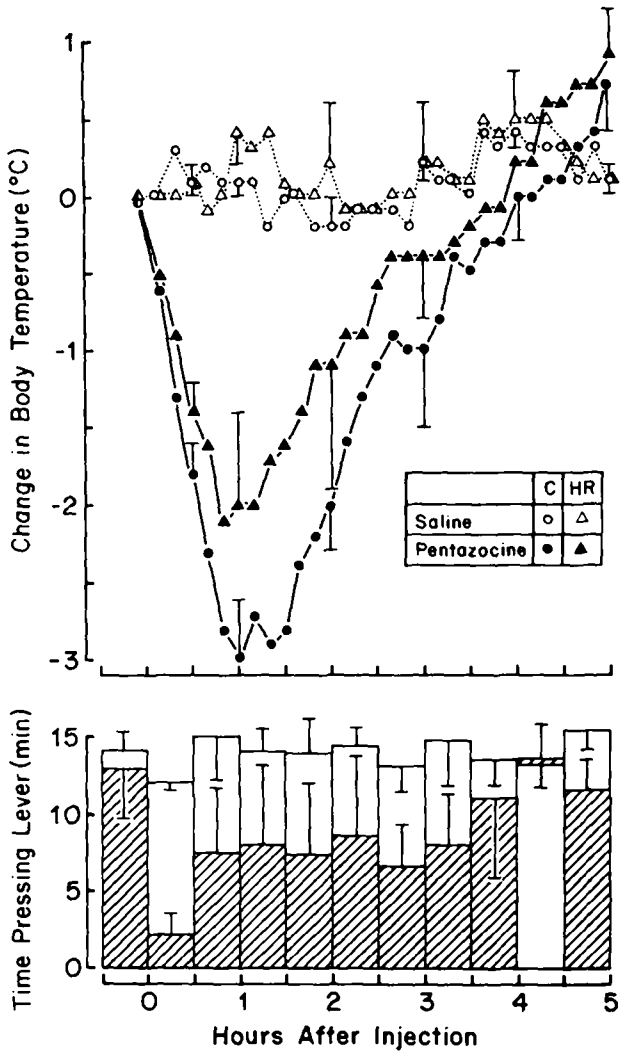

FIG. 5. Changes in temperature and behavior of four cats after saline or pentazocine (1 mg) injection with (HR) or without (C) learned HR activity.

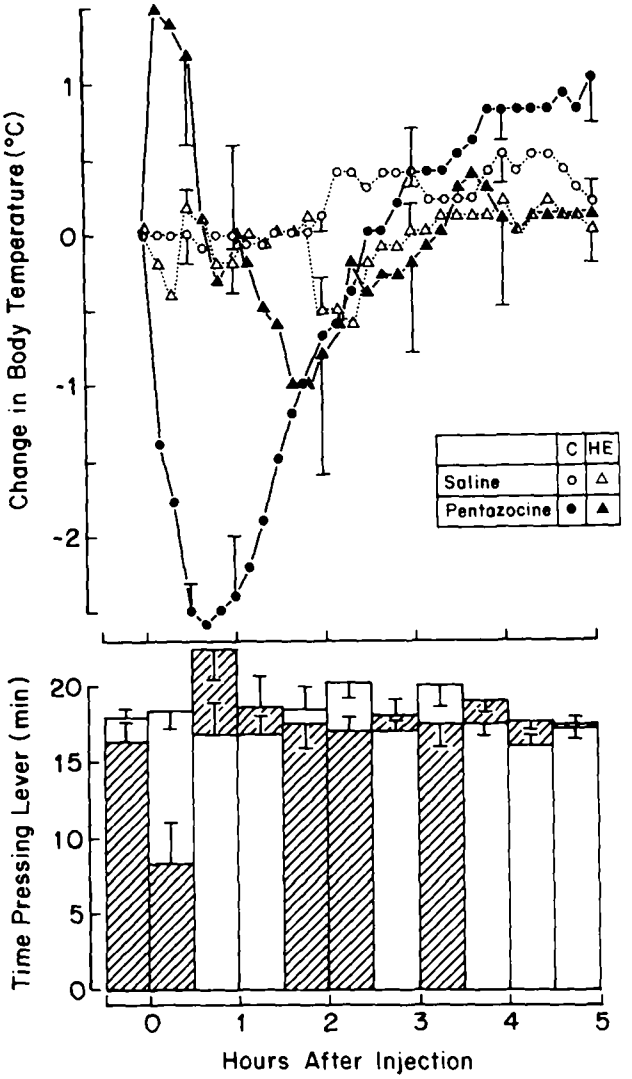

FIG. 6. Changes in temperature and behavior of four cats after saline or pentazocine (1 mg) injection with (HE) or without (C) learned HE activity.

ture change and behavioral depression in response to morphine. The depression of HR responding was still considerable and significant 5 hr after injection when the hyperthermic response was nearly complete. Morphine decreased escape of rats from heat [13] and, while it did not appear to impair motor activity, a non-specific depression of thermoregulatory behavior similar to that seen with the cat was not ruled out by a counter experiment demonstrating an increase in HR activity.

It is likely that the initial decrease in responding, whether for HR or HE, after pentazocine administration was due to impairment of locomotor activity. However, the prolonged depression of HR behavior, up to about 4 hr after administration, cannot be attributed solely to motor impairment since the animals were able to respond, though with difficulty, for HE beginning about 30 min after injection. During the initial 30-min period when they did not respond adequately for HE, body temperature increased rapidly. During the subsequent half-hour, the animals were able to depress the lever more than during the control period, and body temperature rapidly declined to base line. This latter period was the only one in this study in which there was a significant increase in learned thermoregulatory behavior.

Motor incapacitation does not seem to have contributed to depressed thermoregulatory behavior after morphine, D-Ala^2-Met-enkephalinamide or D-Ala^2-D-Leu^5-enkephalin administration, since morphine, as is usual in the cat [9,17], and D-Ala^2-D-Leu^5-enkephalin increased locomotor activity and D-Ala^2-Met-enkephalinamide did not appear to stimulate or depress general activity. Furthermore, all the opioids caused greater and more prolonged depression of HR than of HE behavior. Cats can maintain their body temperature quite well in the cold-room for at least 12 hr [9,12] without an external heat source. Thus, exposure to this environment for a prolonged period is not life-threatening. Furthermore, if a cat does not respond at all in the HR situation, it is then exposed to essentially the control situation without any input from the lamps, except that the fan remains on. In contrast, increased exposure to the heat from the lamps can quickly

raise body temperature to dangerous levels as indicated by the rapid increase in temperature after pentazocine and by enhanced hyperthermic responses to D-Ala^2-Met-enkephalinamide and morphine when HE behavior was depressed. After the latter two agents, the return of HE behavior to control levels coincided with the start of recovery from peak hyperthermia, whereas HR behavior did not return to control levels before hyperthermia had ended. Thus, with the exception of the first 30 min after pentazocine injection, the general appearance of the animals and the earlier reestablishment of control levels of HE responding indicate that they were able, if stressed sufficiently, to perform physically the learned thermoregulatory behaviors. Clearly, the animals could also sense an excessive heat load. Opiates have long been thought to produce their analgesic effect partially by altering the so-called "reaction component" so that patients who can feel pain report that it no longer bothers them [2]. Likewise, an inattention to other stimuli may contribute to opioid-induced constipation and urinary retention [16]. It may be that, again with the exception of pentazocine initially, the opioids studied in these experiments distract or otherwise reduce the reaction of cats to thermal stimuli until body temperature raises to a potentially dangerous level. This possibility is in accord with Hardy's conclusion [15] that the thermoregulatory system is geared more to protect against overheating than against overcooling.

ACKNOWLEDGEMENTS

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