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HYPERTHERMIC RESPONSES TO CENTRAL AND PERIPHERAL INJECTIONS OF MORPHINE SULPHATE IN THE CAT

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1 The effect of morphine on body temperature was studied in conscious, unrestrained cats provided with implanted third or lateral cerebral ventricular cannulae, jugular venous catheters and retroperitoneal thermocouples.

2 Intraventricular injections of 2.5–50 μg and intravenous injections of 1–10 mg/kg morphine sulphate produced dose-related hyperthermic responses. Similar mean increases in body temperature after administration of a given dose were elicited in cats which had not previously received morphine and, provided that tolerance was avoided by spacing injections at least 72 h apart, in cats which received a series of injections of morphine. Morphine was at least 850 times more potent when injected into the third ventricle than when given intravenously. Increasing the dose of morphine sulphate injected into the third ventricle to 1250 μg only prolonged the hyperthermia. Morphine did not produce hypothermia at any dose tested.

3 Injection of 10 μg morphine sulphate into the third ventricle produced similar hyperthermias at ambient temperatures (*t*as) of 4–6, 21–23 and 33–36°C. The increase in body temperature was associated with shivering at the lower *t*as. At the highest *t*a, shivering was not evoked, but respiratory rate decreased after morphine if it was initially elevated. These results suggest that morphine increased the level at which body temperature was regulated.

4 Neither metiamide nor indomethacin antagonized morphine so histamine and prostaglandins were apparently not required for the hyperthermic effect.

Introduction

It has long been known that morphine causes hyperthermia in cats, as well as in horses and apparently cows and goats, while a number of other species generally respond with hypothermia or biphasic temperature changes (Krueger, Eddy & Sumwalt, 1941; Lotti, 1973). The changes in thermoregulatory mechanisms which lead to hyperthermia after morphine have not been extensively studied. Stewart & Rogoff (1922) noted that increased body temperature after morphine in the cat was not necessarily associated with excitement, while McCrum (1953) reported that dihydro-β-erythroidine prevented the hyperthermia, suggesting that increased muscular activity was required. Recently Cox, Ary, Chesarek & Lomax (1976) studied the hyperthermic action of low doses of morphine given intraperitoneally to the rat. When the animals were put into an enclosure in which they were free to move away from a heat lamp, they waited longer before escaping from the heat after administration of morphine, thereby facilitating the morphine-induced increase in body temperature. This was interpreted as indicating an increase in the thermoregulatory set-point. The primary purpose of our study was to examine the mechanism of thermoregulatory action of morphine in the cat, primarily by determining the effect of morphine on body temperature at a variety of environmental temperatures, in accordance with a scheme proposed by Borison & Clark (1967). Since no systematic determination of the dose-hyperthermic response relationship has been reported in unanaesthetized cats, this relationship was first studied with both intraventricular and intravenous administration. Finally, possible roles of histamine and prostaglandins in morphine-induced hyperthermia were examined with appropriate antagonists.

Methods

Thirty unanaesthetized cats weighing between 2.6 and 5.9 kg were used. Procedures for care and feeding of the animals, for recording body temperature auto-

matically from the retroperitoneal space, for implanting lateral or third cerebral ventricular cannulae or intravenous cathethers, for sterilization of glassware and for otherwise avoiding pyrogenic contamination have been described previously (McCarthy & Borison, 1966; Clark & Moyer, 1972). The animals were housed and most experiments were done in a chamber maintained at an ambient temperature (*ta*) of 22 ± 1°C. In specified experiments the temperature of the chamber was raised to 33–36°C. For studies at lower *ta*s, individual animals were temporarily transferred to another room maintained at 4–6°C.

Cats were exposed to the experimental *ta* for at least 1 h before drug injection to allow body temperature to stabilize. The average of retroperitoneal temperature readings at 0, 15 and 30 min before the initial injection on any given day was used as the base line from which changes were measured. Deviations of body temperature from base line were tabulated at 15 min intervals. A 'thermal response index' (TRI) was calculated for each response. This is equivalent to the area between the response curve and the base line if the response is plotted so that 1 unit on the ordinate represents a change of 1°C and 1 unit on the abscissa equals 1 hour. One unit of TRI is, therefore, equivalent to a 1°C change lasting for 1 hour. Unless otherwise specified, TRIs after morphine were determined from the time of injection until body temperature had fallen back to base line or until 20 h after injection if the hyperthermia was more prolonged. TRIs after saline (0.9% w/v NaCl solution) injection were determined for the same period of time as for the corresponding morphine test in the same animal. Statistical analyses were performed with the *t* test for paired data or analysis of variance.

Volumes of 0.10 and 0.05 ml were used for injections into the lateral and third ventricles respectively. Cannulae were routinely flushed with 0.10 ml saline solution about 24 h after drug injections. Intravenous injections were immediately flushed from the catheters with 1.0 ml saline.

Dose–response relationships were determined in two types of experiments. In one type, cats which had not previously received morphine were given an intraventricular injection of morphine and a control injection of saline solution on separate occasions. A table of random numbers was used to allocate doses of morphine to specific cats and to determine the order of administration of morphine and saline. The effect of morphine given intravenously was evaluated similarly, with the exception that some of these cats had previously received morphine. Tolerance to morphine was not likely to influence the results since none of these animals had received morphine for at least 11 days. Because the cats used in these assays had either had no prior exposure to morphine or had not had morphine for a prolonged period, they are termed 'novice' animals. In the second type of assay, each cat received several doses of morphine and also a control injection of saline solution. The term 'cross-over' is used to designate such studies in which each cat received each of the treatments including control vehicle injection in randomly assigned order.

Morphine sulphate (Mallinckrodt) was injected at 10 h 00 min ± 5 min. All doses refer to the sulphate. Except in experiments on tolerance, morphine injections were spaced at least 72 h apart. Stock solutions of morphine in saline solution were stored at 4°C. Naloxone hydrochloride (Endo Laboratories) was dissolved in saline shortly before use. Metiamide was prepared just before each injection by dissolving it in acidified saline which was then adjusted to pH 7 with NaOH. Indomethacin (Merck, Sharp & Dohme), dissolved in 95% ethanol, was given slowly intravenously in a volume of 0.10 ml/kg.

Results

In the initial experiments, morphine was injected into the lateral ventricle. Novice cats were assigned saline vehicle and one of four doses of morphine (Table 1). Each dose caused relatively rapid development of hyperthermia accompanied by shivering, which began within 15 min after injection, and mydriasis. Emesis, usually preceded by forward licking and vocalization, occurred 1–15 min after injection of the larger (50–100 μg) doses. Some animals became restless and paced about their cages but seldom were so active as to break the wires leading from the top of the cage to their harness. Doses lower than 10 μg had no effect on body temperature in pilot experiments. A number of cats were then given a series of injections of morphine to determine an interval at which tolerance to the hyperthermic action did not develop (Figure 1). Partial tolerance occurred when morphine was given nearly every day as in the upper two examples. When morphine was given approximately every other day, more consistent responses were obtained, particularly when the maximum increases were compared. Little or no tolerance developed if injections were spaced about 3 days apart as in the lower three examples. An interval of at least 72 h was, therefore, maintained between injections of morphine in later cross-over studies.

To avoid cortical effects of centrally injected morphine, we decided to inject it into the third ventricle in subsequent studies. By this route we were able to give quite large doses without the animals becoming active enough to break the thermocouple wires. Otherwise, the effects after doses of 40 μg or greater were similar to those seen after the larger lateral ventricular injections. After doses of 20 μg or less, emesis and mydriasis were less consistently observed. Similar

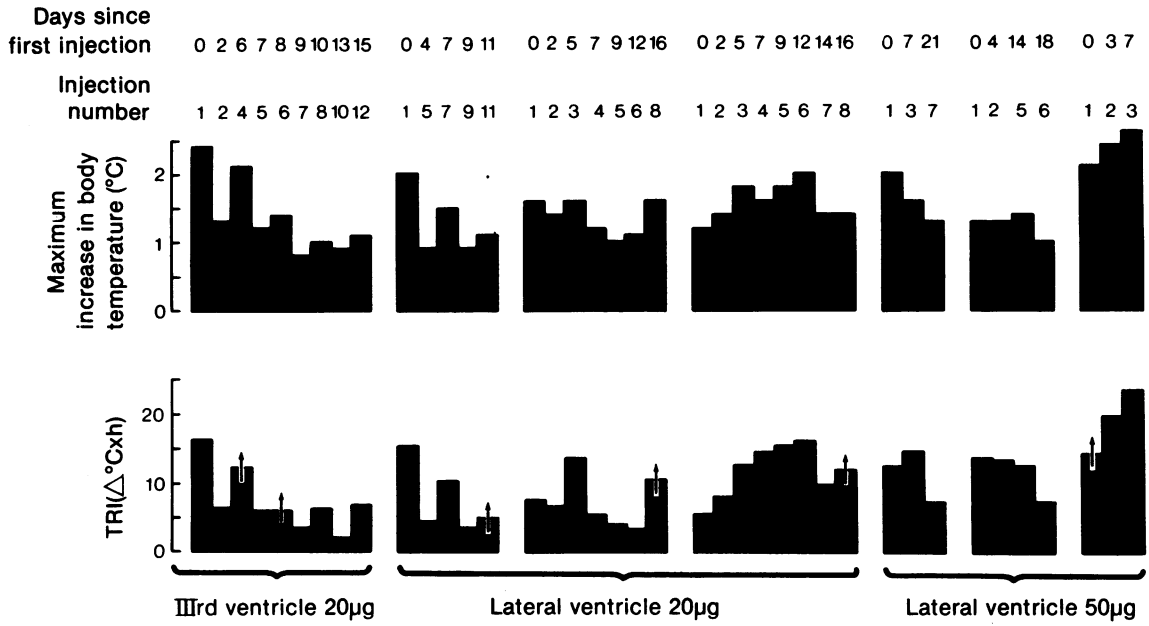

Figure 1 Each pair of histograms indicates hyperthermic responses at ambient temperature of 22°C of a single cat to repeated intraventricular injections of the dose of morphine sulphate indicated below. Comparison of the injection number with the days since first injection, above, allows an approximation of the average interval between injections. Arrows indicate that body temperature was not recorded until full recovery because of technical problems and that the total response was actually greater than indicated by the bar. Injections which are not shown were given at *t*as other than 22°C. TRI = thermal response index (see text).

dose–response relationships were obtained (Table 1) whether morphine was tested in novice cats or in a cross-over study (Figure 2). Analysis of variance of responses to 2.5–20 μg doses in the cross-over study indicated highly significant regressions ($P < 0.001$) for both maximum increase and TRI. After completion of the cross-over study, the animals were given larger doses of morphine sulphate, from 80–1250 μg. In no instance was the maximum increase lessened at higher doses. Hypothermia was not caused by any dose of morphine tested. Administration of naloxone hydrochloride (100–250 μg) to four cats during hyperthermic responses to morphine transiently lowered body temperature (Figure 3).

Morphine was also given intravenously (Table 1) to obtain an indication of potency relative to intraventricular administration. Again the only change in temperature was hyperthermia, and the mean responses to each dose were similar in both the cross-over comparison and in the novice animals. Analysis of variance of maximum increases and TRIs in the cross-over assay indicated significant regressions ($P < 0.002$). The time courses of these responses were similar to those after third ventricular injections of equi-effective doses. Injection of the larger doses intravenously was often followed by mydriasis, salivation, vocalization and hyperexcitability, indicated by continuous or intermittent pacing and by startle reactions to a sudden noise. Vomiting occurred in only two animals after intravenous morphine injections.

The hyperthermic response to third ventricular injection of morphine was studied at three *t*as in six cats (Figure 4). A Latin square design was used in which one cat received morphine in each possible sequence of *t*as on consecutive weeks. The order of administration of morphine and saline was randomized so that one of the cats being tested at a given *t*a during the week received morphine first, then saline, and the other cat received saline first. The interval between morphine injections ranged from 5 to 9 days. Since responses after vehicle differed considerably at different *t*as, the results in Table 2 were obtained by tabulating the differences between the morphine and saline tests for each cat at each *t*a. Analyses of variance indicated a significant difference ($P < 0.05$) between the cats but no significant differ-

Table 1 Morphine sulphate dose–hyperthermic response relationships

		Novice animals			*Cross-over*	
		Maximum increase	*TRI*		*Maximum increase*	*TRI*
Route	*Dose*	n	*(°C)*	*(Δ°C × h)*	n	*(°C)*	*(Δ°C × h)*
Lateral ventricle	100 μg	4	1.8 (1.4–2.2)	13.9 (10.7–17.1)			
	50 μg	4	2.3 (1.9–2.7)	17.7 (11.8–23.6)			
	25 μg	3	1.6 (0.0–4.1)	8.1 (3.9–12.3)			
	10 μg	2	1.4 (0.0–>5)	8.7 (0.0–30.7)			
	0 μg	13	0.2 (0.0–0.4)	2.9 (0.7–5.1)			
Third ventricle	80 μg					2.2 (1.8–2.6)	15.4 (11.5–19.3)
	40 μg					1.9 (1.6–2.2)	13.3 (11.0–15.6)
	20 μg	3	2.0 (0.9–3.1)	15.1 (3.0–27.2)		1.9 (1.6–2.2)	12.1 (9.0–15.2)
	10 μg	3	1.5 (0.1–2.9)	7.2 (0.0–15.6)	8	1.4 (1.1–1.7)	8.8 (5.5–12.1)
	5 μg	3	1.2 (0.2–2.2)	8.4 (0.0–23.0)		1.1 (0.8–1.4)	7.2 (3.5–10.9)
	2.5 μg	3	0.3 (0.0–1.2)	3.9 (0.0–20.0)		0.6 (0.3–0.9)	2.4 (0.1–4.7)
	0 μg	12	0.2 (0.1–0.3)	1.1 (−0.5–2.7)		0.2 (0.1–0.3)	
Intravenous	10 mg/kg	3	2.4 (1.6–3.2)	27.1 (16.1–38.1)		2.5 (1.9–3.1)	25.2 (21.4–29.0)
	5 mg/kg	3	2.5 (0.0–>5)	19.2 (0.0–37.5)		2.0 (1.6–2.4)	15.9 (8.4–23.4)
	2.5 mg/kg	3	0.8 (0.0–1.6)	6.0 (0.0–20.8)	6	1.0 (0.5–1.5)	5.9 (1.5–10.3)
	1 mg/kg	3	0.8 (0.0–2.3)	3.7 (0.0–12.0)		0.6 (0.4–0.8)	2.8 (1.3–4.3)
	0 mg/kg	12	0.3 (0.2–0.4)	0.2 (−1.8–2.2)		0.3 (0.0–0.6)	

Means are listed with their 95% confidence limits in parentheses.

Table 2 Cross-over comparison of the hyperthermic effect of 10 μg morphine sulphate injected into the third ventricle at three ambient temperatures (*ta*s)

ta	*4–6°C*	*21–23°C*	*33–36°C*
Maximum difference (°C)	1.4 (0.7–2.1)	1.6 (1.2–2.0)	1.4 (0.7–2.1)
TRI (Δ°C × h)	7.7 (3.3–12.1)	6.6 (3.3–9.9)	6.9 (2.0–11.8)

Mean differences between response to morphine and response to vehicle at each *ta* are listed with their 95% confidence limits in parentheses. TRIs were determined from the time of injection until the response to morphine equalled the response to vehicle or until 12 h after injection if full recovery had not occurred. TRI = thermal response index (see text).

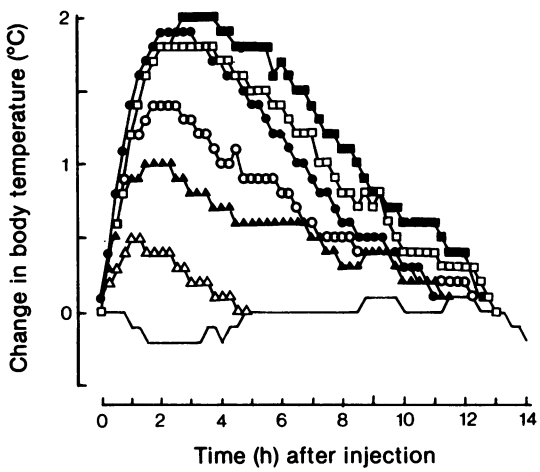

Figure 2 Cross-over determination of the mean responses of eight cats to morphine sulphate (0–40 μg) injected into the third ventricle. (—) Control; (△) 2.5 μg; (▲) 5.0 μg; (○) 10 μg; (●) 20 μg; (□) 40 μg; (■) 80 μg. The 80 μg dose was given after the conclusion of the cross-over series.

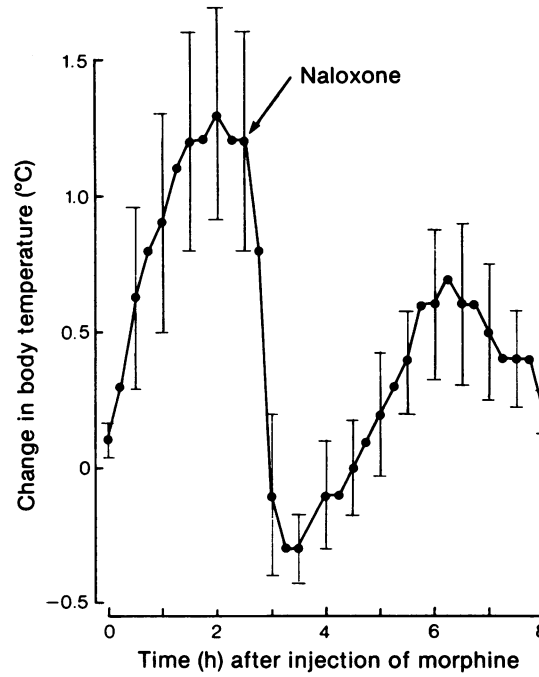

Figure 3 Antagonism of morphine by naloxone. Mean response of three cats to naloxone hydrochloride (250 μg) injected into the third ventricle 2.5 h after intraventricular administration of morphine. Vertical lines show s.e. means.

ence between body temperature changes at the three *t*as. In the coldest and intermediate environments the increase in body temperature after morphine was accompanied by shivering, indicative of an increase in heat production. At the hot *t*a, shivering was not seen. Instead, in the three cats in which respiratory rate was somewhat elevated at the time of injection, the respiratory rate decreased, indicative of a reduction in heat loss.

The hyperthermic response to intraventricular morphine was not antagonized by prior administration of metiamide (Table 3a) or by a large dose of indomethacin (Table 3b).

Discussion

Our major finding was that morphine caused similar changes in body temperature at *t*as both above and below the thermoneutral temperature of the cat. Furthermore, the effectors involved in the rise of body

Table 3 Cross-over studies of the interactions of metiamide and indomethacin with morphine

	Dose	*Maximum increase (°C)*	*TRI (Δ°C × h)*
a. Metiamide[1]	0.5 mg	0.8 (0.3–1.3)	3.6 (1.4–5.8)
	0.0 mg	0.9 (0.5–1.3)	3.5 (1.5–5.5)
b. Indomethacin[2]	2.0 mg/kg	1.3 (1.0–1.6)	6.3 (4.9–7.7)
	0.0 mg/kg	1.5 (1.3–1.7)	7.8 (5.0–10.6)

[1] Injected into the third ventricle 30–60 min before morphine sulphate (10 μg). [2] Given intravenously 1.5 h after morphine sulphate (10–80 μg).
Means are listed with their 95% confidence limits in parentheses. Five cats were used for each study.

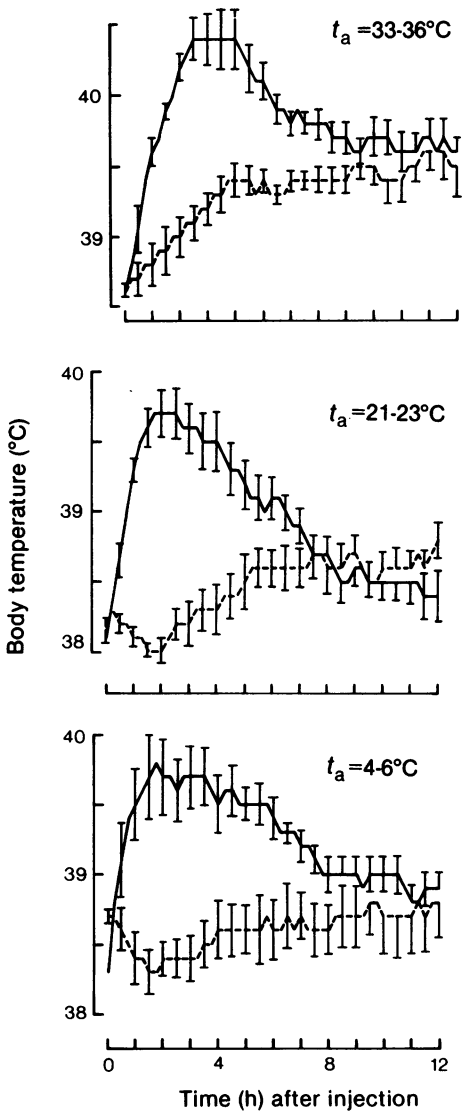

Figure 4 Mean responses of six cats to 10 μg morphine sulphate (continuous line) and saline (dashed line) at three ambient temperatures (*ta*s). Each cat in this cross-over study received each of the six possible combinations of treatment. Vertical lines show s.e. means. See Table 2 for tabulation of differences between mean response to morphine and to vehicle at each *ta*.

temperature varied with *t*a. These results are comparable to those obtained after pyrogen and prostaglandin administration (Palmes & Park, 1965; Hales, Bennett, Baird & Fawcett, 1973; Stitt, 1973; Crawshaw & Stitt, 1975; Veale & Whishaw, 1976) and are indicative of an increase in the level at which body temperature is regulated. This could be due to an increase in the thermoregulatory set-point, as Cox *et al.* (1976) have proposed as the mechanism for the increase in temperature produced by small doses of morphine in the rat, or to an excitatory influence on a pathway between cold sensors and thermoregulatory effectors which increase heat production and/or decrease heat loss, as pointed out by Hales *et al.* (1973).

Recently, methionine-enkephalin was shown to evoke emesis and transient hyperthermic responses in cats (Clark, 1977). These responses to enkephalin and the hyperthermic response to morphine were inhibited by prior administration of naloxone. When given after morphine in the present study, naloxone was also effective in interrupting the hyperthermia. Thus the response to morphine was mediated by an action at specific opiate receptors. Conceivably endogenous opioid peptides such as the enkephalins might mediate certain hyperthermias via these receptors.

The response to 100 μg morphine sulphate after lateral ventricular injections was less than after 50 μg, but this difference may well have been due to random allocation of the 50 μg dose to more sensitive animals. Alternatively, this difference might indicate that 100 μg was beginning to produce a general CNS depressant effect which secondarily impaired thermoregulation. When direct cortical effects were avoided by giving injections into the third ventricle, the dose of morphine could be increased greatly without any tendency for the hyperthermic response to diminish. Examination of the data in Table 1 suggests that, with regard to the maximum increase in temperature, 5 and 2.5 mg/kg doses given intravenously were approximately equivalent to 20 and 5 μg respectively given via the third ventricle. If the average weight of a cat is taken as 3.4 kg, morphine was about 850–1700 times more potent intraventricularly than after intravenous injection.

Of interest is determination of mediators of the hyperthermic response. Morphine has been shown to release histamine peripherally (Feldberg & Paton, 1951) and centrally (Tanaka & Lin, 1969) in the cat. Injections of histamine into the lateral (Clark & Cumby, 1976) or third (W.G. Clark & H.R. Cumby, unpublished results) ventricles of cats caused a delayed hyperthermia which was prevented by metiamide. Pretreatment with metiamide had no effect on the response to morphine so that it is unlikely that histamine release was responsible for the hyperthermia.

Other possible mediators of hyperthermia after morphine administration are prostaglandins, some of which cause a rapid, marked rise in body temperature after central administration (Feldberg & Milton,

1973). Morphine has been reported to increase prostaglandin synthesis by rabbit brain homogenates (Collier, McDonald-Gibson & Saeed, 1974). However, a large dose of indomethacin, sufficient to reduce greatly hyperthermic responses to sodium arachidonate (W.G. Clark & H.R. Cumby, unpublished results) presumably by inhibiting its conversion to prostaglandin E_2, did not alter the response to morphine. Thus prostaglandins are apparently not required for morphine-induced hyperthermic responses.

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