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Body Temperature Effects of Opioids in Rats: Intracerebroventricular Administration

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GELLER, E. B., C. H. ROWAN AND M. W. ADLER. *Body temperature effects of opioids in rats: Intracerebroventricular administration.* PHARMACOL BIOCHEM BEHAV **24**(6) 1761–1765, 1986.—A variety of opioids were injected intracerebroventricularly into unrestrained rats, and rectal temperatures were monitored over several hours. Complete dose-response data for morphine, heroin, etorphine, *d*- and *l*-ethylketazocine, *d*- and *l*-pentazocine, and *d*- and *l*-N-allylnormetazocine revealed a predominant response of hyperthermia. Only etorphine in high doses caused a pronounced decrease in body temperature. These results differed considerably from those obtained previously with subcutaneously administered opioids but can be explained in terms of a dual-receptor theory of temperature control.

Body temperature Opiate receptors; mu, kappa Intracerebroventricular injection Route of administration Opioid-induced hyperthermia Opioid-induced hypothermia Opioids Rats

SINCE the discovery of the endogenous opiate-like substances about ten years ago, investigators have attempted to compare their actions to those of the much more familiar opiates and opioids. In the rush to characterize the newer compounds and their analogs and to find possible physiological functions for them and their older counterparts, it was largely ignored that the opioid peptides, because of problems of degradation and the difficulty in obtaining large quantities, were routinely administered into the ventricles of the brain while the standard opioids to which they were being compared were usually given by systemic routes. As a result of a series of studies conducted in our laboratory demonstrating route-dependent effects of certain opioids on seizure thresholds in rats [5, 12, 13], we thought it inappropriate to draw conclusions as to possible functions of different receptors based on such comparisons. We therefore undertook an investigation of body temperature effects in rats after ICV injection of several standard opioids which had elicited distinct response patterns after SC administration [6]. Although findings for morphine have been discussed in abbreviated form elsewhere [2], the present paper is the first presentation of complete temperature dose-response data for ICV morphine, heroin, etorphine, *d*- and *l*-ethylketazocine (EK), *d*- and *l*-pentazocine and *d*- and *l*-N-allylnormetazocine (SKF 10047) in this species. In addition, we compare the effects of the drugs between routes of administration and with endogenous opioids. Finally, we speculate further on the roles of opioid receptors in thermal responses.

METHOD

Male Sprague-Dawley (Zivic Miller) rats (225–275 g at time of testing) were housed 5 or 6 to a cage in an animal room maintained at 22±2°C and 50±10% relative humidity and on a 12 hr light/12 hr dark cycle. Food and water were available ad lib. One week prior to testing, a polyethylene cannula was implanted into the right lateral ventricle of each rat under ketamine anesthesia (100–150 mg/kg IP) [12]. Following surgery, animals were housed individually to prevent them from destroying the cannulae.

On the test day, the rats were weighed and placed into individual metal cages in an environmental room kept at 20±0.3°C and 52±2% relative humidity. After a 20-min acclimation period, a thermistor probe was inserted approximately 7 cm into the rectum. Measurements were read from a digital display thermometer. During the readings, the rat's tail was held gently between two fingers, and the animal was otherwise free to move about. The first three measurements were taken at 30-min intervals. To allow for adaptation to the procedure, the first reading was discarded and the subsequent two averaged to establish a baseline. Immediately following the third measurement, the rat was placed on a table and held firmly for 30 sec while the drug was injected into the cannula with a microliter syringe. Drugs were dissolved in pyrogen-free 0.9% saline and were injected in a volume of 5 μl followed by a 3-μl saline flush. Controls received 8 μl of saline.

Temperature measurements were made at 15, 30, 45, 60, 90, 120, 180, and, occasionally, 240 min after injection. When naloxone was administered, it was injected SC just prior to, and 60 min after, the ICV injection. A full dose range for each compound was tested, with 4–6 rats per dose. (Upper limits were determined either by the solubility of the drug or by signs of toxicity in the animals.) Each rat was

TABLE 1

MAXIMAL CHANGES IN BODY TEMPERATURE AFTER ICV OPIOIDS

Drug	Dose (μg)	N	Rise ± SEM (°C)	Time (min)	Fall ± SEM (°C)	Time (min)
Saline		40	0.40 ± 0.03	30	−0.39 ± 0.07	180
Morphine	0.1	4	0.33 ± 0.17	60	−0.38 ± 0.09	180
	0.5	6	1.08* ± 0.28	90		
	1	5	1.24* ± 0.25	60	−0.16 ± 0.20	180
	10	6	1.47* ± 0.28	180		
	30	6	2.45* ± 0.35	120		
	50	5	2.90* ± 0.60	180	−0.30* ± 0.24	15
	65	4	3.08* ± 0.26	180	−0.08 ± 0.25	15
Heroin	0.1	4	0.33 ± 0.10	45		
	1	6	0.68 ± 0.06	60	−0.10 ± 0.24	180
	10	6	0.88* ± 0.47	90		
	50	5	1.16* ± 0.43	120		
	100	5	1.48* ± 0.27	120		
Etorphine	0.01	4	1.05* ± 0.40	30	−0.83 ± 0.10	180
	0.1	5	1.58* ± 0.16	45	−0.18 ± 0.06	180
	1	6	0.13 ± 0.18	15	−0.60 ± 0.32	180
	10	6			−3.68* ± 0.65	120
l-Pentazocine	1	4	0.20 ± 0.07	15	−0.38 ± 0.27	180
	10	7	0.41 ± 0.16	30	−1.01 ± 0.26	240
	100	6	0.87 ± 0.26	45	−0.23 ± 0.40	180
	500	5	1.44* ± 0.19	60		
d-Pentazocine	1	5	0.20 ± 0.18	45	−0.62 ± 0.09	180
	10	4	0.53 ± 0.25	60	−0.65 ± 0.19	240
	100	6	0.45 ± 0.11	60	−0.62 ± 0.26	180
	500	6	0.50 ± 0.23	90	−0.23 ± 0.28	240
l-Ethylketazocine	1	5	0.10 ± 0.10	30	−0.20 ± 0.20	180
	10	5	0.58 ± 0.07	45		
	50	5	0.36 ± 0.18	45	−0.24 ± 0.15	120
	100	6	1.00* ± 0.16	60	−0.07 ± 0.30	180
d-Ethylketazocine	1	5	0.50 ± 0.13	60	−0.30 ± 0.05	180
	10	4	0.63 ± 0.21	30	−0.30 ± 0.27	180
	50	6	1.03* ± 0.27	30		
	100	5	1.22* ± 0.24	30		
	500	6	1.18* ± 0.25	30		
l-SKF 10047	10	5	0.34 ± 0.15	30	−0.72 ± 0.38	240
	100	6	0.55 ± 0.27	30	−0.78 ± 0.18	240
	500	6	0.93* ± 0.33	90		
	1000	7	0.63 ± 0.32	120	−0.69* ± 0.16	15
d-SKF 10047	10	4	0.50 ± 0.39	30	−0.58 ± 0.39	240
	100	6	0.53 ± 0.17	60	−0.58 ± 0.17	240
	500	6	0.52* ± 0.37	180	−0.90* ± 0.22	30
	750	4	0.95* ± 0.24	30		
	1000	6	1.37* ± 0.39	180	−0.37* ± 0.37	15

Mean group response>2 SD from saline mean at same time point.

used for only one experiment. Mean body temperature change from baseline was computed at each time point for each dose, and maximum effects were compared to effects in saline-treated animals at the same time point. For antagonism studies, peak responses from 0–120 min were compared to responses at the same time point from animals given both the opioid and naloxone (Student's *t*-test). The comparisons were restricted to the 0–120 min period to allow for the shorter half-life of naloxone.

At the conclusion of the experiment, each rat was lightly anesthetized with ether, injected with diluted India ink through the cannula, decapitated, and the brain sliced into coronal sections. Only data from animals with proper cannula placement in the lateral ventricle were included.

RESULTS

Table 1 shows the maximum mean changes from baseline temperature in each group of rats injected ICV with the various drugs, and the time at which the effects occurred. Based on results with 40 control rats given saline ICV, we define hyperthermia as an increase in temperature greater than 2

TABLE 2

EFFECT OF NALOXONE ON OPIOID-INDUCED CHANGES IN BODY TEMPERATURE

Naloxone Dose (mg/kg, SC)	Opioid/Dose (μg, ICV)	Max ΔT: 0–120 min Opioid Alone (°C ± SEM)	ΔT:Opioid + Naloxone*	Sig.
1	Morphine/10	1.17 ± 0.21	−0.44 ± 0.20	†
1	Morphine/50	1.06 ± 0.20	0.90 ± 0.30	NS
10	Morphine/50	1.06 ± 0.20	0.33 ± 0.21	†
1	Heroin/10	0.88 ± 0.47	−0.65 ± 0.16	†
1	Heroin/100	1.48 ± 0.27	0.28 ± 0.25	†
1	Etorphine/0.1	1.58 ± 0.16	−0.03 ± 0.23	†
1	Etorphine/10	−3.68 ± 0.65	0.25 ± 0.20	†
1	*l*-Pentaz/100	0.87 ± 0.26	0.20 ± 0.25	NS
1	*l*-Pentaz/500	1.44 ± 0.19	0.80 ± 0.47	NS
1	*d*-Pentaz/100	0.45 ± 0.11	−0.27 ± 0.15	†
1	*d*-Pentaz/500	0.50 ± 0.23	0.82 ± 0.32	NS
1	*l*-Ethylketaz/10	0.58 ± 0.07	0.10 ± 0.23	NS
1	*l*-Ethylketaz/100	1.00 ± 0.16	0.23 ± 0.11	†
1	*d*-Ethylketaz/10	0.63 ± 0.21	0.40 ± 0.23	NS
1	*d*-Ethylketaz/100	1.22 ± 0.24	0.10 ± 0.20	†
10	*l*-SKF 10047/100	0.55 ± 0.27	0.42 ± 0.21	NS
10	*l*-SKF 10047/500	0.93 ± 0.33	−0.10 ± 0.07	†
10	*d*-SKF 10047/100	0.53 ± 0.17	−0.15 ± 0.30	NS
10	*d*-SKF 10047/500	−0.90 ± 0.22	−0.75 ± 0.12	NS

*Compared at same time point.
†$p<0.05$, Student's *t*-test.

SD from the mean of saline-treated animals (at that time point) and hypothermia as a decrease of at least 2 SD below controls. For saline animals the peak rise was 0.4±0.22(SD)°C at 30 minutes and the maximum fall was −0.39±0.42(SD)°C at 180 minutes. In Table 2, the temperature effects produced by the opioids at representative doses are compared with and without the antagonist. It should be noted that in most cases where no statistically significant difference was found between groups receiving agonist and antagonist versus agonist alone, neither effect differed from that of control rats (e.g., *l*-pentazocine, 100 μg; *l*-EK, 10 μg).

Heroin and morphine caused dose-related increases in body temperature. (A small, transient drop in temperature occurred with the highest doses of morphine, but only at doses greater than 200 μg, which caused seizures and deaths in some of the animals, was any hypothermia seen with heroin.) The rise usually began 15 to 30 min post-drug for heroin, and 30 to 45 min for morphine, continuing for 2 to 4 hr. Etorphine produced a different dose relationship, the response changing from a hyperthermia at lower doses, to no effect at the middle dose, to hypothermia at the highest. Effects were observed by 15 min and lasted 1 to 3 hr, depending on dose. With each of these drugs, the degree of sedation and catatonia increased with dose. Naloxone, which has no effect itself on temperature in the doses used (1 or 10 mg/kg, SC), blocked the behavioral as well as the temperature effects. The hypothermia from high doses of etorphine reversed to hyperthermia at 30 to 60 min in the presence of 1 mg/kg naloxone, but this effect was no longer seen by 90 min after drug injection. Ten mg/kg of naloxone completely blocked any temperature response. Note that for morphine the peak effect used for comparison is not the actual maximum, which occurred at 180 min (Table 1). To compensate for the shorter half-life of naloxone, effects are not compared past 120 min, after which time the action of naloxone may be waning.

l-Pentazocine induced a dose-related increase in temperature which was significant only at the highest dose. Many animals exhibited periods of sedation interspersed with episodic darting about the cage. Naloxone diminished, but did not completely block, the temperature effect (Table 2). *d*-Pentazocine had no effect at doses up to 500 μg. Interestingly, when naloxone was given with *d*-pentazocine, an initial hypothermia (15–30 min) followed by a prolonged hyperthermia resulted. At low doses, *l*-EK and *d*-EK had no significant effect but at higher doses induced hyperthermia starting 15 to 30 min post-drug and lasting 90 to 120 min. With the *l* isomer, most rats were sedated or made catatonic by a dose of 50 μg or more. Naloxone blocked the temperature changes of both *l*- and *d*-EK as well as the behavioral effects of the *l* isomer. *d*-EK caused no sedative effects and, in fact, induced hyperactivity in some animals at the highest doses tested.

With both isomers of N-allylnormetazocine, 10 and 100 μg had no effect in most animals. A few were hyperthermic. At higher doses, about half the animals with the *l* form were hyperthermic. No changes in body temperature were observed when naloxone (10 mg/kg) was given with the *l* isomer except at the highest dose, at which hyperthermia appeared after several hours. Results with higher doses of the dextro isomer were unpredictable: some rats were hyperthermic, some hypothermic, some showed an initial decrease followed by an increase, and others were unaffected by this drug. Because of the variability of the response, effects with naloxone added are difficult to interpret. Animals given the higher doses of either isomer exhibited the typical behaviors

TABLE 3

COMPARISON OF TEMPERATURE EFFECTS OF OPIOIDS BY DIFFERENT ROUTES

		Temperature Change in Response to			
		Opioid		Naloxone* + Opioid	
Drug	Dose Range	SC	ICV	SC	ICV
Morphine	lower	↑	↑	0	0
	higher	↓	↑	0	↑
Heroin	lower	↑	↑	0	0
	higher	↓	↑	0	0
Etorphine	lower	↑	↑	0	0
	higher	↓	↓	0	↑
l-Ethylketazocine	lower	↓	0	0 or ↓†	0
	higher	↓	↑	0 or ↓	0
d-Ethylketazocine	lower	0	0	↓	0
	higher	0	↑	↓	0
l-Pentazocine	lower	↑	↑	0	0
	higher	↑	↑	0	↑
d-Pentazocine	lower	0	0	↓	0
	higher	0	0	↓	↓,↑‡
l-SKF 10047	lower	0	0	0	0
	higher	0	0	0	0 or ↓,↑
d-SKF 10047	lower	0	0	0	0
	higher	0	variable§	0	0 or ↓,↑

*One mg/kg, SC, except for SKF 10047, where dose was 10 mg/kg.
†Approximately 1/2 animals had each response.
‡Comma indicates sequential responses.
§More than two different responses observed in same group.

seen with this drug: ataxia, circling, and side-to-side head movements.

DISCUSSION

As we found with SC administration [6], the effects of ICV administered opioids on body temperature in rats at an ambient of 20°C fall into several patterns. The predominant response, however, is hyperthermia. In Table 3 we summarize and compare data from the opioids tested in our laboratory. (Some of this information appeared in a review [3].)

Etorphine evoked a dose-related, dual response by both routes. Its effect was like that seen with the opioid peptides β-endorphin and D-Ala^2-Met-enkephalinamide (DAME) [2], but the three differed in their interactions with naloxone. While the hyperthermic effect of a low dose of etorphine (0.1 μg) was blocked by 1 mg/kg of naloxone, the hypothermia of a high dose (10 μg) of etorphine was converted to a low-dose effect. A higher dose of naloxone blocked this effect as well. With β-endorphin, the low-dose hyperthermia could not be blocked by naloxone, and with DAME, both effects could be antagonized by 1 mg/kg of naloxone.

Like morphine [2], heroin produced a dose-related, naloxone-sensitive rise in temperature after ICV injection, although each had produced a dual effect (low-dose hyperthermia, high-dose hypothermia) when given SC. *l*-EK, which had lowered body temperature when given SC, caused an increase by the ICV route. Unexpectedly, the *d* isomer also produced an increase, despite having been inactive after SC administration. Since naloxone blocked both isomers, these actions are presumably at opiate receptors.

l-Pentazocine caused a dose-related increase in temperature by both routes. The SC effect was more sensitive to naloxone, however. Among the opioid peptides, D-Ala^2-D-Leu^5-enkephalin induced a similar thermal response [2]. *d*-Pentazocine, which had little effect of its own after SC administration, likewise had minimal activity ICV. The hypothermia which appeared on combination with naloxone was more pronounced SC, but an initial tendency in this direction was also noted after ICV injection. *L*- and *d*-SKF 10047 produced inconsistent or small changes by both routes. Although it is possible that the bizarre motor behaviors produced by these compounds may be affecting the temperature somewhat, it is unlikely that the sigma receptor plays a role in thermal effects in this species. Notably, none of the opioids studied produced solely hypothermia after ICV administration as had EK and several other opioids SC. Of the peptides previously tested, only dynorphin (1–17) induced a dose-related fall in temperature ICV [2].

A number of factors could be responsible for the qualitative differences observed between ICV and SC administered drugs. One of the first we considered was the isolated housing conditions dictated by the use of chronically implanted cannulae. On the basis of a small study in which we isolated animals for a week prior to SC injection of morphine or EK, however, we have no evidence that differential housing influenced the temperature effect. Because we factor out any effects of saline by our definition of hyperthermia, whatever part stress and endogenous opioids [4] may play is also excluded. However, in our hands this contribution is probably small, as the difference between animals receiving saline and those given naloxone amounts to changes of only several tenths of a degree. Although the ICV injection procedure involves a greater degree of restraint than SC dosing, it is not

known whether the interaction between the stress and the drug is significant. A recent report indicates that the increase in colonic temperature in rats receiving saline intracerebrally did not differ from those subjected to handling and temperature measurements without the injection [11].

A more likely explanation is that drug distribution and location of receptors are the critical determinants of the response. Among the many factors influencing drug distribution are the volume of injection, and the pH, osmolarity, electrovalance, lipid solubility, and molecular weight of the substance administered [9]. The rate of drug metabolism, coupled with the distance and barriers the drug must traverse to reach its receptors, governs the final effect [8]. Of possible importance is the order in which the drug penetrates specific brain areas. If receptors which mediate one response are activated first, it may be more difficult to obtain another response. Particularly in the case of late-onset responses and those seen only with high doses, the uptake of the ICV injected compound by the choroid plexus into the general circulation and its subsequent action on peripheral receptors could account for the observed effect.

Based on earlier work with SC administered opioids and ICV opioid peptides, we had postulated a two-receptor model, with one (possibly μ) mediating an increase in body temperature, and the other (possibly κ) responsible for hypothermic responses [2, 6, 7]. Further evidence of a hyperthermic role for mu receptors in the rat comes from a study of morphine and opioid peptides injected directly into the periaqueductal grey [14]. From the present work, it appears that the predominant response after ICV injection is hyperthermia. If our model is correct, then very few opioid receptors mediating hypothermia are being stimulated when these drugs are given into the ventricles. Our data indicate that hypothermic responses occur only when a compound is extremely lipophilic or is given in very high doses. These conditions allow for the removal of the drug from the brain and CSF into the general circulation for distribution throughout the body. In addition, with respect to peptides, at least, it appears that they can be rapidly transported in the cerebrospinal fluid [10]. Thus, it may be that receptors mediating the temperature decrease are located at sites far removed from the ventricles, perhaps even outside the brain.

Aside from confirming our suspicions that it may be erroneous in some cases to equate qualitative temperature data of opioids given peripherally with that of opioid peptides administered centrally, the results reported here lend support to our model for opioid receptors in thermoregulation. Based on these and more recent findings with the kappa-selective agonist, U-50,488H, which causes a dose-related hypothermia after SC administration but has no effect ICV [1], it is our current view that mu receptor activation in the brain produces hyperthermia, while hypothermia results when kappa receptors, primarily in the spinal cord and/or periphery, are stimulated. How the opiate receptor system operates in relation to other known or postulated modulators of thermal responses such as the biogenic amines remains to be determined.

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