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Extreme Hyperthermia Induced in Cats by the Enkephalin Analog FK 33-824[1]

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CLARK, W. G., G. L. BERNARDINI AND S. W. PONDER. *Extreme hyperthermia induced in cats by the enkephalin analog FK 33-824.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 989–993, 1982.—FK 33-824 [H_2N-Tyr-D-Ala-Gly-MePhe-Met(O)-OH] was injected into the third cerebral ventricle of unrestrained cats. Doses of 0.25–4 μg induced dose-related increases in body temperature. Hyperthermic responses to 1 μg of the peptide were greater the warmer the environment. Naloxone given intraventricularly 1 hr after FK 33-824 (1 μg) reduced the hyperthermia. In 12 tests with six cats FK 33-824 (1–25 μg) increased temperature 4.2–4.7°C. These marked responses were also inhibited by naloxone, but two cats died when administration of antagonist was delayed for 80 min to 3 hr after attainment of maximal body temperature. Larger doses of FK 33-824 (50–250 μg) evoked little increase in temperature, indicative of a separate action to depress thermoregulation. Although responses to FK 33-824 were antagonized by naloxone, this peptide, like another enkephalin analog D-Ala²-Met-enkephalinamide, must act on receptors which are not affected by morphine since (1) the hyperthermic response to FK 33-824 varied with environmental temperature, whereas the response to morphine does not, and (2) high doses of FK 33-824 depressed thermoregulation, an activity not shared by morphine in the cat. Furthermore, the maximal increases in temperature after FK 33-824 injection were greater than those evoked by either morphine or D-Ala²-Met-enkephalinamide. This observation provides evidence for an additional subset of naloxone-sensitive, ν, receptors, stimulation of which can influence thermoregulation in the cat.

FK 33-824 Hyperthermia Naloxone Opioid peptides Opioid receptors Thermoregulation

RESULTS of previous studies with the cat indicate that stimulation of as many as five distinct receptors by centrally injected peptide and non-peptide opioids can alter body temperature in this species [6]. The criteria for distinguishing among these receptors have included (1) the direction of change in body temperature, i.e., hyperthermia or hypothermia, induced by the opioid under usual laboratory conditions, (2) the relative ability of naloxone to inhibit the response and (3) the pattern of change in body temperature when the animals were tested while housed over a range of environmental temperatures. Met-enkephalin is a weakly hyperthermic peptide which is resistant to antagonism by naloxone [3,11]. In the present experiments FK 33-824 [H_2N-Tyr-D-Ala-Gly-MePhe-Met(O)-OH], a potent agonist in analgesic assays [19], was studied in an attempt to find a more stable and potent, naloxone-resistant analog for further studies of enkephalin-induced thermoregulatory changes. FK 33-824 given centrally increased, with lower doses, or decreased body temperature of rats [2]. Injected peripherally, it induced hypothermia in dogs [13] but did not alter the temperature of humans [22]. Our results indicate that FK 33-824, instead of mimicking Met-enkephalin in the cat, acts on naloxone-sensitive receptors, much like the D-Ala²- Met-enkephalinamide analog, to elicit increases in body temperature which are greater the warmer the environment and to induce, with higher doses, some depression of thermoregulation as well. However, unlike any of the other opioids, FK 33-824 was able to increase the temperature of cats to lethal levels, indicative of an additional action not shared with related agents.

METHOD

Sixteen unrestrained cats, weighing from 2.5–4.9 kg were used. They were kept in an environmental chamber maintained at 22±1°C except for specified studies in which the chamber temperature was increased to 34±1°C or for studies in the cold which were performed in another chamber maintained at 4±2°C. Procedures for care and feeding, for automatically recording core temperature from the retroperitoneal space, for implantation of cannulas for injections into the third cerebral ventricle, for sterilizing glassware and avoiding pyrogenic contamination and for calculating thermal response indexes (TRIs) have been described previously [4,18]. TRIs are estimates of the area between a response curve and base-line body temperature, determined by averaging temperatures 10, 20 and 30 min before the initial injection, calculated so that 1 unit of TRI represents a 1°C change lasting for 1 hr. Both TRIs and maximal increases in body temperature were used in the Friedman two-way analysis of variance or the Wilcoxon matched-pairs signed-ranks test to evaluate differences among treatments [21]. Results in the

[1]A preliminary report of these studies [10] was presented at the 65th Annual Meeting of the Federation of American Societies for Experimental Biology.

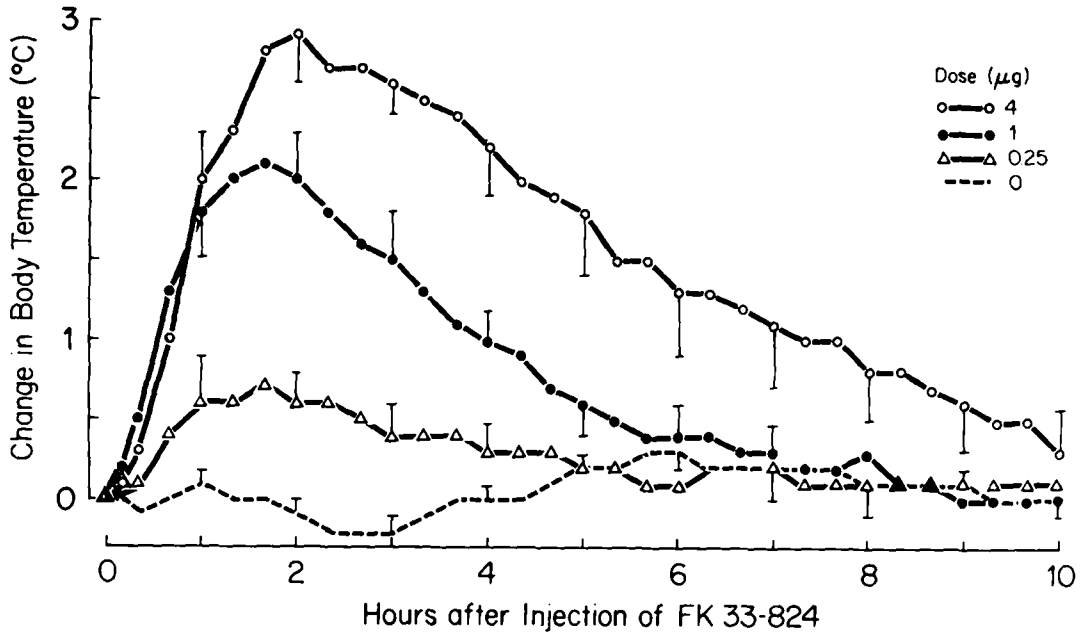

FIG. 1. Responses of eight cats to injection of FK 33-824 or vehicle into the third cerebral ventricle. $p<0.001$, Friedman test.

figures are expressed as mean±SE. With the exception of individual experiments in which hyperthermias of over 4°C were induced, each animal in each experiment received all treatments in randomized order. Prior to surgery, cats were given 2.2 mg/kg xylazine (Haver-Lockhart) SC. After they were sedated, 15–20 mg/kg pentobarbital sodium (Abbott Laboratories) was injected IV.

Stock solutions of FK 33-824 (Boehringer-Mannheim), naloxone hydrochloride and naltrexone hydrochloride in 0.9% NaCl solution were stored at 4°C. Doses of the antagonists refer to these salts. All agents were injected into the ventricular cannula in a volume of 0.05 ml. The cannula was flushed with 0.1 ml saline solution after recovery from hyperthermia or 24 hr after the initial injection. Peptide injections were given at 10:00 a.m. ± 5 min and were spaced at least 48 hr apart to avoid tolerance development.

RESULTS

FK 33-824 induced dose-related increases in body temperature over the dose range of 0.25–4 μg (Fig. 1). All doses elicited prolonged mydriasis, and initially all but the lowest dose evoked forward licking, vomiting and hyperactivity which was characterized by pacing or running around the cage. Shivering was apparent in some but not all tests. Even with doses as low as 1 μg, some animals responded with temperature increases greater than 4°C (Table 1). Such marked rises in temperature were more consistently induced by doses of 10–25 μg. No obvious compensatory increase in respiratory rate was noted during development of hyperthermia, but naloxone injection at the time of maximal hyperthermia almost immediately evoked panting and effectively, but transiently, reduced body temperature (Fig. 2, Table 1). When antagonist administration to cats 2 and 5 was delayed until 3 hr or 80 min, respectively, after attainment of maximal hyperthermia, body temperature decreased rapidly to normal, but the animals nevertheless died within the next 3 hr, presumably as a result of the more prolonged increase in temperature. Doses of 50–250 μg FK 33-824 induced little or no hyperthermia (maximum increase=1.3°C) in four cats. However, when two of these were subsequently given naloxone (Fig. 3), marked increases in temperature occurred. In a cross-over study, 20 μg naloxone effectively reduced the hyperthermic response to a small dose of peptide (Fig. 4). Administration of FK 33-824 elicited hyperthermia over a

TABLE 1

MAXIMUM INDIVIDUAL RESPONSES TO FK 33-824

	FK 33-824			Subsequent Response to naloxone	
		Maximum increase			
Cat No.	Dose (μg)	(°C)	Time (min)	Dose (μg)	Maximum decrease °C)
1	1	4.7	70	—	—
	2	4.5	70	—	—
	4	4.2	100	—	—
	4	4.6	90	—	—
	10	4.5	100	—	—
2	1*	4.7	100	100	4.7
3	2	4.2	110	—	—
	25	4.5	170	500	5.5
4	10	4.4	100	10	0.5
5	10	4.4	90	50	1.0
	20*	4.6	60	50†	5.4
6‡	25	4.4	150	250	4.3

*Lethal.
†Naltrexone.
‡Illustrated in Fig. 2.

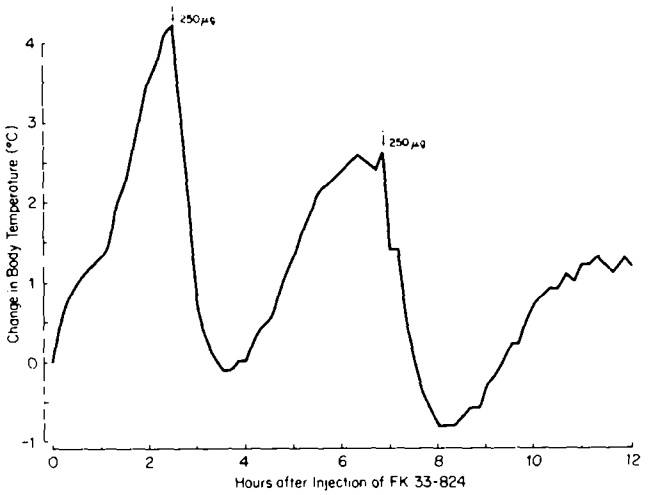

FIG. 2. Hyperthermic response of one cat to intraventricular injection of 25 μg FK 33-824. Naloxone was given at times indicated by the arrows.

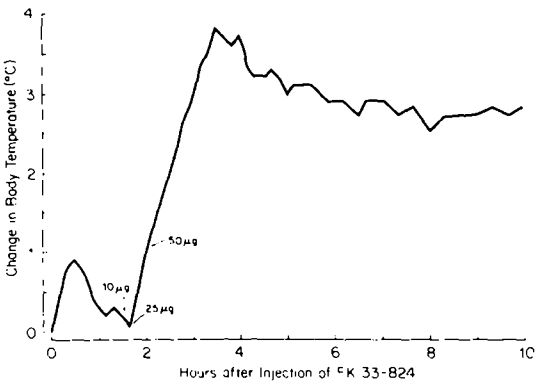

FIG. 3. Response of the same cat as in Fig. 2 to 100 μg FK 33-824. The marked hyperthermic action became apparent only after the initial peptide-induced thermoregulatory depression was reduced by naloxone, given as indicated by the arrows.

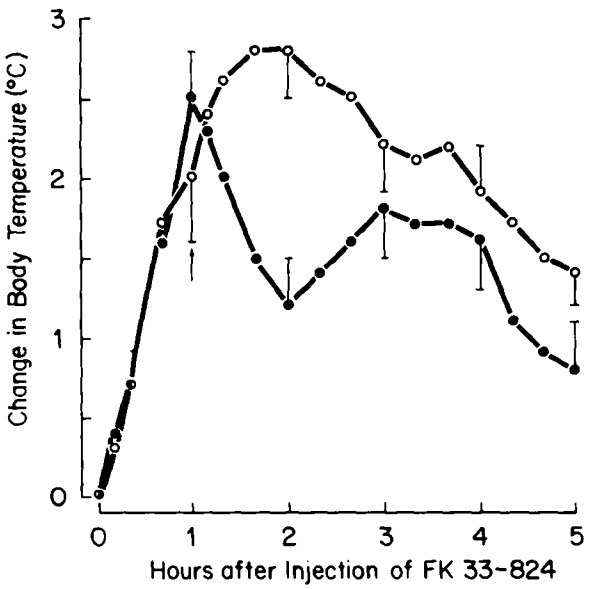

FIG. 4. Reduction of FK 33-824-induced hyperthermia by naloxone. Naloxone (●——●, 20 μg) or vehicle (○——○) was given to six cats 1 hr after FK 33-824 (1 μg). $p \leq 0.05$, Wilcoxon test.

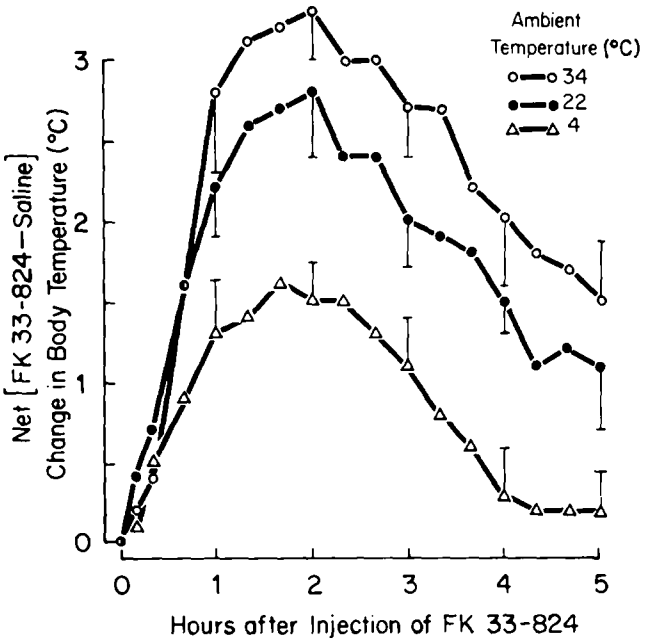

FIG. 5. Effect of environmental temperature on responses of six cats to third ventricular injection of 1 μg FK 33-824. The response of each animal after vehicle was subtracted from its response to FK 33-824 at the same ambient temperature to obtain the net change in body temperature. $p = 0.029$ (maximum difference), 0.0055 (TRI difference), Friedman test. Base-line body temperatures did not differ among treatments. Maximum mean increases in body temperature after saline vehicle administration were 0.4, 0.4 and 0.7°C for ambient temperatures of 4, 22 and 34°C respectively.

wide range of environmental temperatures, but the responses were enhanced in the warmer environments (Fig. 5).

DISCUSSION

FK 33-824 is a very potent hyperthermic agent. If the doses required to evoke comparable increases in temperature of 1.2–2.0°C in the cat are compared, it is about 20 times more potent than morphine and about 4000 times more potent than Met-enkephalin. Our study with D-Ala²-Met-enkephalinamide [9] and the present results with FK 33-824 indicate that, although the effects of these analogs on thermoregulation are inhibited by naloxone, they both cause responses which are not elicited by morphine. For instance, relatively large doses of D-Ala²-Met-enkephalinamide induce hypothermia in the cold and hyperthermia in a hot environment, a pattern indicative of depression of thermoregulation [5]. Even very large doses of morphine do not depress thermoregulation in the cat [8], and thus it is not likely that this lack of depression is due to an inability of intraventricularly

TABLE 2

CLASSIFICATION OF NALOXONE-SENSITIVE RECEPTOR SUBTYPES, STIMULATION OF WHICH CAN INFLUENCE THERMOREGULATION IN THE CAT

Agonist	Dose* (μg)	Receptor designation	Result
Morphine	10	ν_1	Body temperature regulated at higher level: independent of ambient temperature; No depression up to 1250 μg.
D-Ala^2-Met-enkephalinamide	12.5	ν_2	Body temperature regulated at higher level: varies with ambient temperature.
	50–400	ν_3	Thermoregulation is depressed.
FK 33-824	1	ν_4 &/or ν_2	Body temperature regulated at higher level: varies with ambient temperature.
	1–25	ν_4 + ν_2	Lethal hyperthermia can develop.
	50–250	ν_3	Thermoregulation is depressed.

*Injected into the third cerebral ventricle.

injected morphine to penetrate to anatomical sites reached by D-Ala^2-Met-enkephalinamide. Therefore, excitation of multiple types of naloxone-sensitive opioid receptor, of which the morphine receptor is only one, can apparently influence thermoregulation in the cat. A classification of these receptors is proposed in Table 2. Activation of the ν_1 receptor, the μ receptor of Martin and co-workers [14,17], by morphine increases the level about which body temperature is regulated [8], as indicated by a coordinated thermoregulatory response which raises body temperature; that is, shivering and increased locomotor activity are unopposed by compensatory heat-loss effector activities such as panting during development of the hyperthermia. The increase in body temperature does not vary with ambient temperature, a pattern that is consistent with an increase in the thermoregulatory set-point [5]. However, a similar pattern was obtained when morphine was injected into the spinal subarachnoid region of the rat [20]. This result would seem to rule out the necessity for a direct action on the thermostat, at least in the rat, since the thermostat is generally thought to derive from the hypothalamus [1]. An alternative explanation, which would account equally well for the temperature pattern after morphine, is that a central action on ν_1 receptors alters the afferent input from thermosensors [5]; i.e., decreases input from core warmth sensors and/or enhances input from core cold sensors. Core sensors are specified because deep body temperature remains relatively constant over a wide range of environmental temperatures, and so a given change in input from these sensors would be expected to cause comparable increases in body temperature, regardless of ambient temperature. Let us assume that morphine enhances input from core cold sensors.

D-Ala^2-Met-enkephalinamide also induces a regulated increase in the level of temperature regulation [9]. Unlike the response to morphine, however, this increase is enhanced in warmer environments. This pattern could occur if D-Ala^2-Met-enkephalinamide activates a different naloxone-sensitive receptor, ν_2, and thereby enhances input from peripheral cold sensors, either in addition to or instead of stimulating core cold sensors. In the coldest environment input from these peripheral sensors would be relatively high before peptide administration, and further sensor pathway activation and, therefore, the increase in body temperature would be limited. In the warmer environments input from the peripheral cold sensors would be less initially and could be enhanced more effectively by the peptide leading to greater increases in body temperature.

A low dose of FK 33-824 in the present study produced a pattern of change in body temperature in different environments that was similar to the pattern after a low dose of D-Ala^2-Met-enkephalinamide. In contrast, intermediate and, in some cats, even low doses of FK 33-824 induced very large, and in two cases lethal, increases in temperature which are not evoked by either morphine or D-Ala^2-Met-enkephalinamide. These marked responses to FK 33-824 could be completely inhibited by naloxone. Hyperthermic responses to this peptide might occur in either of two ways: (1) sufficiently low doses may primarily stimulate ν_2 receptors, and activation of another naloxone-sensitive receptor, ν_4, may account for the additional increase in temperature over that induced by ν_2 receptor activation alone or (2) ν_4 receptor activation may be entirely responsible for hyperthermic responses to FK 33-824. An action of FK 33-824 or D-Ala^2-Met-enkephalinamide on the ν_1 receptor is unlikely because morphine-tolerant cats do not exhibit cross-tolerance to these peptides [7]. Ordinarily body temperature is regulated within fairly narrow limits and even in fever seldom exceeds a level compatible with survival, about 41°C in the human [12] and squirrel monkey [16]. DuBois [12] proposed the existence of an "emergency regulatory mechanism" to protect against further increases in temperature. The mechanism of such a protective barrier is not understood, but a possibility with regard to the opioids is that the increase in body temperature induced by activation of ν_1 or ν_2 receptors activates warmth sensors and that this enhanced sensor input eventually opposes further elevation of temperature. A new equilibrium would be reached as the drives to heat production and conservation lessen and the drive to heat loss increases. FK 33-824 could breach this barrier if with intermediate doses it continued to enhance input from cold sensors (ν_4 and/or ν_2) and also began to reduce input from warmth sensors (ν_4), thereby preventing attainment of equilibrium. Still larger doses of FK 33-824, like large doses of D-Ala^2-Met-enkephalinamide, depressed thermoregulation in that they prevented development of the hyperthermia characteristically evoked by smaller doses. Since the depressant effects of both peptide analogs were also

inhibited by naloxone (Fig. 3; [9]) and morphine does not depress thermoregulation in the cat [8], another naloxone-sensitive receptor, ν_3, must be involved. The peptides might induce poikilothermia either by inhibiting input from both warmth and cold sensors or by a general depression of thermoregulatory coordination mechanisms.

Central administration of 250 μg naloxone reduced the hyperthermic potency of Met-enkephalin about 50% in one study [3], whereas 3 mg/kg given SC did not antagonize this peptide in another study with the cat [11]. Hence Met-enkephalin is much more resistant to antagonism by naloxone than is morphine [4] and acts at least partially on naloxone-resistant receptors. We originally examined FK 33-824 with the idea that it might provide a naloxone-resistant analog which could be more easily studied than the parent enkephalin. However, the hyperthermic effect of FK 33-824 was readily inhibited by low doses of naloxone. Thus the modification of Met-enkephalin structure in this analog shifted the affinity toward receptors which are sensitive to naloxone. This same shift has recently been noted by other [15,23].

ACKNOWLEDGEMENTS

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