HUMAN β-ENDORPHIN: DEVELOPMENT OF TOLERANCE AND BEHAVIORAL ACTIVITY IN RATS

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SUMMARY: Human β-endorphin produced a potent antinociceptive response as estimated by the tail-flick test in rats after intraventricular injection. On a molar basis, the peptide was 21 times more potent than morphine and in addition, the peptide produced morphine-like catatonia and hypothermia. These responses were blocked by naloxone. Repeated injections of the peptide induced tolerance to analgesic response, catatonia and hypothermia. Cross tolerance to morphine was also observed.

The in vivo analgesic activity of the pentapeptide, methionine-enkephalin, has been reported to be very weak (1, 2). In contrast, β-endorphin, an untriakontapeptide isolated from pituitary glands of camel or humans, produced in mice a potent naloxone-reversible analgesic response in vivo (3-6). We now report the analgesic and behavioral effects of, and development of tolerance to, β-endorphin, as well as cross tolerance to morphine in rats.

Experimental

Human β-endorphin (β_h-endorphin) was synthesized as previously described (6). Morphine sulfate was purchased from Mallinckrodt Chemical Works (St. Louis, Mo.). Naloxone HCl was a gift from Endo Laboratories (Garden City, N.Y.). Morphine pellets containing 75 mg of morphine base were formulated according to Gibson and Tingstad (7).

Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif.), weighing 180 to 250 gm, were used. For the intraventricular injection, rat brains were stereotaxically implanted with 20 guage stainless steel guide cannulae 3 days before the drug injection or morphine pellet implantation. β_h-Endorphin and morphine were injected unilaterally in a volume of 20 μl. Naloxone HCl was injected intraperitoneally.

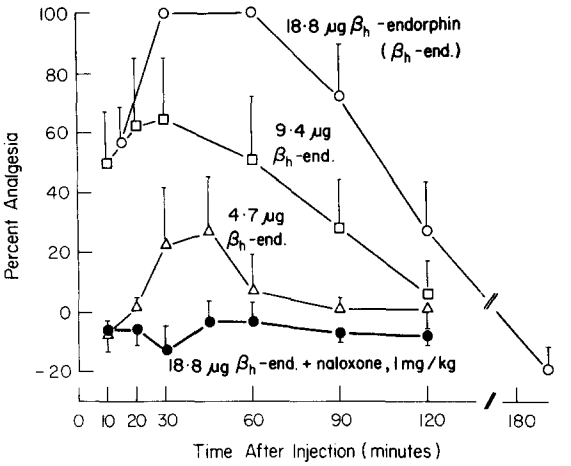

Fig. 1 Inhibitory effect on tail-flick response following the intraventricular injection of β_h-endorphin and its blockade by naloxone. Various doses of β_h-endorphin were injected at 0 times. Naloxone HCl (1 mg/kg S.C.) was injected 10 min before the injection of β_h-endorphin, 18.8 μg per rat. N=5-6 rats per group. The vertical bars indicate the S.E.M.

The analgesic properties of β_h-endorphin and morphine were assessed by the tail-flick method (8). To evaluate these responses, a control latency (T_0) was obtained from the mean of two latencies determined prior to drug injection, and the latencies (T_1) were determined at various times after injection for each animal. "Percent analgesia" was calculated as $[(T_1-T_0)/(T_2-T_0)] \times 100$, where the cut-off time (T_2) was 12 seconds. With a 2-fold increase in latency of reaction time as a quantal index of inhibition, the median antinociceptive dose (AD_{50}) and 95% confidence limits were calculated according to the methods of Litchfield and Wilcoxon (9). At least 6 to 8 rats were tested at each dose with three to four dose levels used for determining the AD_{50}.

The core temperature was measured with a thermistor probe inserted at least 6 cm into the rectum and connected to a telethermometer. The room temperature was 23 to 24° C. For assessing catatonia, rats were placed with their forelegs on a bar (15 cm height) and if catatonia was present, this position was maintained for at least 20 seconds.

For studies of cross tolerance of β_h-endorphin to morphine, rats were rendered tolerant to morphine by subcutaneous implantation of one morphine pellet and 2 more pellets 8 hrs later. The pellets were removed 70 hrs after the first pellet implantation. Five hrs after the pellets' removal, rats were injected intraventricularly with different doses of β_h-endorphin.

Results and Discussion

β_h-Endorphin, at doses of 4.7, 9.4, and 18.8 μg per rat administered intraventricularly, showed a dose-related inhibition of the tail-flick response in

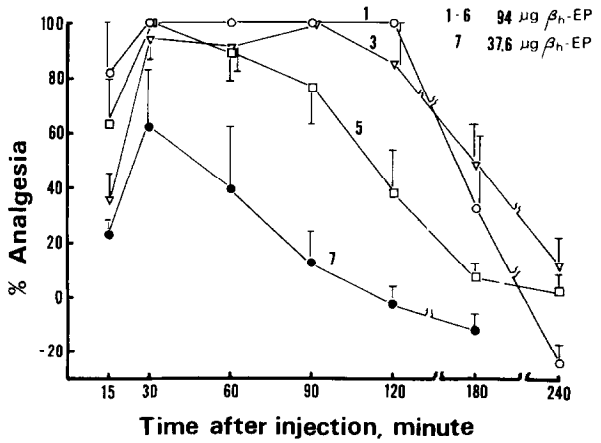

Fig. 2 Development of tolerance of antinociceptive response to β_h-endorphin and to morphine.

Rats were injected intraventricularly with 94 μg of β_h-endorphin (β_h-EP) per rat twice a day for 3 days. On the 4th day, rats were injected intraventricularly with 37.6 μg of β_h-endorphin. Seven rats in 1 to 6 injections and 4 rats in the 7th injection. 37.6 μg of β_h-endorphin only caused slight inhibition of tail-flick response whereas 18.8 μg of β_h-endorphin caused 100% inhibition of tail-flick response for 60 to 90 min in naive rats (see Fig. 1).

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both intensity of the analgesic response and duration of action (Fig. 1). The AD_{50} and the 95% confidence limits of β_h-endorphin and morphine sulfate were calculated to be 7.1 (5.4 - 9.5) and 15.0 (12.2 - 18.5) μg per rat, respectively. On a molar basis, β_h-endorphin was 21.8 times more potent than morphine suflate. In addition to the antinociceptive response, β_h-endorphin (9.4 μg to 94 μg per rat), like morphine, produced a catatonic response in rats. There was a strong rigidity of the whole body. The animals assumed a waxy inflexibility and could be hung on a steel bar for a long time. They lost the righting reflex and could be placed on their backs. The inhibition of tail-flick response and production of catatonia were completely blocked by pretreatment with naloxone (1 mg/kg , i.p.) 10 min before the injection of β_h -endorphin.

β_h-Endorphin was injected intraventricularly twice a day (at 9:00 A.M.

Table 1

Development of Tolerance of Catatonic Response to β-Endorphin* and Morphine Sulfate

No. of injections	Dose μg	Min after intraventricular injection (catatonia/rats)						
		15	30	60	90	120	180	240
	β_h-Endorphin							
1	94	7/7	7/7	7/7	7/7	7/7	4/7	0/7
3	94	7/7	7/7	7/7	7/7	7/7	2/7	0/7
5	94	6/7	7/7	7/7	7/7	1/7	0/7	---
7	37.6	0/4	1/4	1/4	0/4	0/4	---	---
	Morphine sulfate							
1	40	5/5	5/5	5/5	5/5	5/5	1/5	0/5
3	40	---	5/5	5/5	5/5	4/5	0/5	0/5
5	40	0/5	4/5	4/5	4/5	2/5	1/5	0/5

* The animals are the same as in Fig. 2.

and 4:30 P.M.) at a dose of 94 μg per rat. Rats showed antinociceptive effect to heat stimuli and catatonia for about 150 to 180 min after the first injection of 94 μg per rat of the peptide. The durations of antinociceptive response and catatonia were gradually reduced after multiple injections of the same dose. On the 4th day, after six injections of 94 μg per rat of β_h-endorphin, rats showed only slight analgesic response and catatonia after injection of 37.6 μg per rat of the peptide (Fig. 2, Table 1). 18.8 μg per rat was shown to produce 100% antinociceptive response and strong catatonia for more than 1 hr in naive animals (Fig. 1, Table 2). Similar results were observed when morphine sulfate, 40 μg, was injected (Table 1).

β_h-Endorphin, 94 μg per rat, caused hypothermia following the first

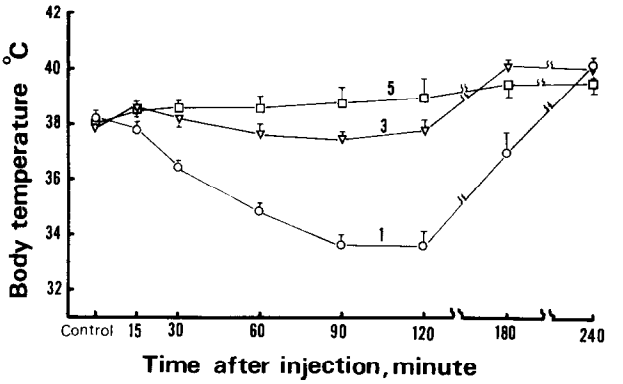

Fig. 3 Development of tolerance of hypothermic response to β_h-endorphin. The animals are the same as in Fig. 2. The vertical bars indicate the S.E.M.

injection. The body temperature decreased at 30 min after injection and reached maximum (4 ° C lower than preinjection) at 90 to 120 min and produced hyperthermia at 240 min after injection. Tolerance to the hypothermic effect of β_h-endorphin rapidly developed after multiple injections of β_h-endorphin and no hypothermia was found at the 3rd and 5th injection of the peptide (Fig. 3). Similar results were observed when morphine sulfate, 40 μg, was injected.

β_h-Endorphin at a dose of 18.8 μg per rat produced 100% analgesia and strong catatonia in naive rats. The same dose or double the dose of the peptide produced much less inhibition of tail-flick response to nociceptive stimuli and less catatonia in rats rendered tolerant to morphine-pellet-implantation for 3 days (Fig. 4, Table 2). 18.8 μg of β_h-endorphin caused hypothermia in naive animals. The body temperature decreased at 30 min after injection and reached maximum at 60 min (2.4° C lower than preinjection), and produced hyperthermia at 120 and 240 min after injection. No hypothermia was found after injection of 18.8 and 37.6 μg per rat of β_h-endorphin in morphine-pellet-implanted rats.

We have previously demonstrated that synthetic human β-endorphin, like

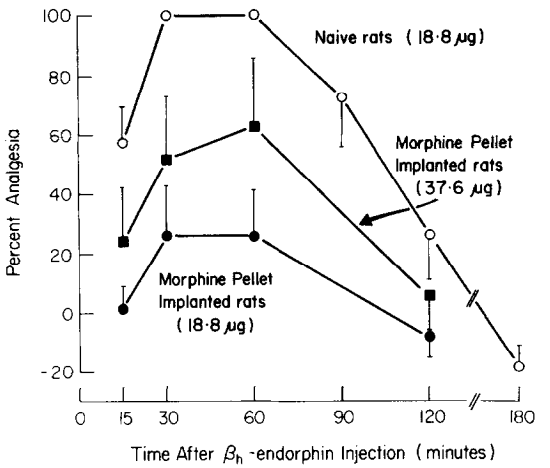

Fig. 4 Antinociceptive effects following intraventricular injection of β_h-endorphin in naive and morphine-pellet-implanted rats. The antinociceptive effect was measured by the tail-flick test. Rats, 6 per group, were rendered tolerant to morphine by implanting 3 morphine pellets for 3 days. The pellets were removed after 70 hrs. Five hrs later, rats were injected with β-endorphin, 18.8 µg and 37.6 µg per rat at 0 time. The vertical bars indicate the S.E.M.

Table 2

Catatonia Induced by β_h-Endorphin in Naive and Morphine-Pellet-Implanted Rats*

Animals	Dose µg	Min after intraventricular injection (catatonia/rats)				
		15	30	60	90	120
Naive	18.8	6/6	6/6	6/6	4/6	0/6
Morphine-pellet-implanted rats	18.8	1/6	1/6	1/6	---	0/6
	37.6	2/5	4/5	2/5	---	0/5

*Animals are the same as in Fig. 4.

camel β-endorphin, produced potent antinociceptive response in mice (4,5). Others also reported that β-endorphin from pig or camel pituitary glands produced potent analgesic response in cats and rats (10-14). In a preliminary note, Jacquet et al. (11) reported that camel β-endorphin caused catatonia in the rat.

In present studies we show that human β-endorphin produced catatonia and hypothermia in rats. The responses were blocked by a low dose of naloxone. We demonstrate for the first time herein that β_h-endorphin was able to develop tolerance by repeated injections of the peptide. Furthermore, β_h-endorphin showed cross tolerance to morphine as evidenced by the reduced responsiveness to the peptide in morphine-pellet-implanted rats. The cross tolerance of camel β-endorphin to morphine has also been reported in mice (12).

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