Higher Environmental Temperature Potentiates Cataleptic Effect of Fentanyl in Rats

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Received September 14, 1998 Accepted October 27, 1998

ABSTRACT—The influence of higher environmental temperature (HET = 30±1°C) on fentanyl-induced behavior was studied in unrestrained rats. Subacute exposure (3 days) of rats to HET significantly (P < 0.01) increased the cataleptic effect of fentanyl citrate (0.5 mg/kg), in comparison to the corresponding exposure to normal environmental temperature (NET = 22±1°C). Also, the hyperthermic response of rats to a low dose of fentanyl citrate (0.2—0.5 mg/kg) was significantly (P < 0.01) potentiated, and the hypothermic response to a high dose of fentanyl citrate (1.5 mg/kg) was significantly (P < 0.05) attenuated after exposure to HET. Fentanyl-induced hyperexcitability, loss of righting reflex, loss of corneal reflex and analgesia were not significantly affected by HET. This study provides the first evidence on the influence of environmental temperature on drug-induced catalepsy. HET-induced potentiation of the cataleptic response to fentanyl could be the result of an interference with behavioral thermoregulation.

Keywords: Fentanyl, Behavior, Environmental temperature

It is known that the environmental temperature modifies the action of a number of drugs, neurotransmitters and neuromodulators (1—4). For example, the effects of opioids on the body temperature have been shown to be complex and dependent on many factors (species, strain, age, dose, route of administration, receptor specificity, degree of restraint, handling and environmental temperature) (5, 6). Using a large range of opioids, Rosow et al. showed that an environmental temperature above 25°C favors opioid-induced hyperthermia in mice (7). In addition, Ghosh and Poddar reported that the hyperthermic response in rats induced by morphine at 28°C is potentiated when the environmental temperature was increased to 40°C (4). Handler et al. have demonstrated that at an environmental temperature of 30°C, a μ-selective agonist (PL-017) caused a significant hyperthermia in rats (8).

It has already been shown that at normal environmental temperature (NET = 22±1°C), a single i.p. administration of the μ-selective opioid agonist fentanyl to unrestrained rats evoked analgesia and behavioral effects such as catalepsy, hyperexcitability, loss of righting reflex and loss of corneal reflex along with the changes in body temperature (9, 10). Although many thermoregulatory studies with opioids have been done in the past (4, 8, 11, 12), there is still little evidence on the influence of higher environmental temperature on temperature response and behavioral effects of opioids, in particular. Thus, the purpose of the present work was to study the effect of subacute exposure to higher environmental temperature (HET = 30±1°C) on fentanyl-induced changes in the body temperature, catalepsy, hyperexcitability, loss of righting reflex, loss of corneal reflex and analgesia in unrestrained rats.

In these experiments, rats (150—250 g) of both sexes were used. Before the experimental procedure, the animals were divided into two groups balancing both the body weights and the sex. The animals of the experimental group were housed in an animal room maintained at HET (30±1°C), and the corresponding control groups of animals were kept at NET (22±1°C) under identical conditions (with natural light-dark cycles). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National
Institute of Health (NIH Publication No. 85-23, revised 1985). Access to food and water was unrestricted in both groups. After exposure of rats to HET and NET for three days, all experiments were performed under the corresponding environmental temperature, HET and NET, respectively. Prior to each experiment, the animals were habituated to the handling and experimental procedures. The experiments began at 8 a.m. to avoid changes due to circadian rhythms. The rat was restrained (within a hemicylindrical plastic cage) only during the time that rectal temperature was measured. Each temperature measurement took approximately 20 sec, after which the probe was withdrawn. Between temperature measurements, the rats were unrestrained and kept in cages (four rats per cage). The rats were treated with intraperitoneal injection of fentanyl citrate (0.06 – 1.5 mg/kg; ICN Yugoslavia, Belgrade) dissolved in saline in volumes from 0.15 to 0.5 ml. Each rat in the control group was treated with the corresponding volume of saline (0.15 – 0.5 ml, i.p.).

Catalepsy was defined as the failure of the animal to move within 30 sec from a position in which the forepaws and hindpaws were placed on bars 10 cm from the floor (9).

Hyperexcitability was examined on digital touching and/or hand-clapping.

The righting reflex was measured by placing the animal onto its back and measuring how long it took to regain an upright position. The righting reflex was considered absent when all four limbs remained off the table surface for at least 30 sec.

Touching the cornea of both eyes assessed loss of corneal reflex.

Testing for catalepsy, hyperexcitability, loss of righting reflex and loss of corneal reflex were done before and at 5, 20, 40, 60, 90, 120, 180 and 240 min after the injection of fentanyl citrate.

Body temperature was measured by the insertion of a Model 46 Tele-thermometer probe (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA), about 6 cm into the colon (6). Temperature was measured before and at 10, 30, 60, 90, 120, 180 and 240 min after the injection of fentanyl citrate.

Noctiception was assessed using a "tail immersion" test, as described by Janssen et al. (13).

The percentage of animals with catalepsy, hyperexcitability, loss of righting reflex, and loss of corneal reflex, for each dose in both treated groups was determined. Basic data for colonic temperature were also converted to percentage of animals responding with hyperthermia or hypothermia, using criteria of four times the standard deviation of the baseline values (11). Mean body temperature change from baseline was computed at each time point for each dose and compared to the effect of saline. Antinociceptive potency was calculated by first transforming the "tail immersion" data to percent maximum possible effects (%MPE), where %MPE = (post-drug latency – predrug latency) / (cutoff time – predrug latency) × 100. The ED50 values with 95% confidence limits were then calculated and compared (14). Statistical analyses of the data were performed using the Fisher test, ANOVA followed by the least significance difference test and Student's t-test for grouped data (14). A P value of less than 0.05 was considered statistically significant.

The subacute exposure of rats to HET (30±1°C) significantly increased the incidence and duration of fentanyl-induced catalepsy, in comparison to NET (22±1°C) (Fig. 1: A and B). Also, at HET, catalepsy occurred at lower doses of fentanyl citrate (0.2 mg/kg) in comparison to NET (0.5 mg/kg). Only at the dose of 0.5 mg/kg, catalepsy was observed within 5 min after i.p. injection of fentanyl, developed its maximum 20 min post injection and lasted up to 40 and 90 min in the NET and HET groups, respectively (Fig. 1: A and B). In comparison to the thermogenic response of fentanyl, catalepsy occurred a few minutes before the changes in body temperature in both groups of animals.

Injection of fentanyl citrate (0.06 to 0.5 mg/kg, i.p.) produced a dose-dependent rise in colonic temperature of about 0.3°C to 1.2°C in rats kept at 22±1°C and approximately 0.5°C to 2.0°C in rats kept at 30±1°C. The increase in body temperature at 0.06 mg/kg was significantly higher (P<0.05) in comparison to the saline control levels in only HET-exposed rats (not shown). Hyperthermia developed 10 min after i.p. injection of fentanyl citrate (0.2–0.5 mg/kg), reached its maximum 30 min and 30–60 min after the injection, and remained elevated for the next 30 and 60–90 min approximately, at 22°C and 30°C, respectively (Fig. 1: C and D). However, when fentanyl citrate was injected in higher doses (1.5 mg/kg, i.p.) hypothermia occurred in all tested animals at NET (in 8 of 8 rats). At HET, the same dose of fentanyl citrate (1.5 mg/kg, i.p.) significantly decreased the incidence of hypothermia (2 of 6 rats, P<0.05) and significantly increased the incidence of hyperthermia (4 of 6 rats, P<0.05) (Table 1), in comparison to NET. Subacute exposure to HET did not alter the baseline body temperature. Mean baseline body temperature was 37.8±0.04 (n=32) and 38.0±0.08 (n=28) in the NET and HET group, respectively. Also, the injection of saline had no significant effect on the animal’s body temperature.

There were no differences in the incidence of loss of righting reflex, loss of corneal reflex and hyperexcitability on touching and/or hand-clapping between two groups (Table 1). Based on the ED50 values, the antinociceptive potency of fentanyl did not differ between
subjects placed at 30°C (0.02 mg/kg, range of 0.013–0.04 mg/kg) and those kept at 22°C (0.017 mg/kg, range of 0.011–0.027 mg/kg). Also 3-day exposure to HET did not alter the control (predrug) response latency to tail immersion (2.4 sec, n=24 at HET and 2.2 sec, n=25 at NET). The injection of corresponding volumes of saline did not produce catalepsy, loss of righting reflex, loss of corneal reflex, hyperexcitability and analgesia in rats (not shown).

In the present study, it was observed that fentanyl produces a low-dose hyperthermia and a high-dose hypothermia in rats. Fentanyl citrate at 0.5 mg/kg, i.p. significantly elevated the baseline body temperature of the rats for about 1.2±0.2°C (n=8). However, fentanyl

![Graphs showing percentage of rats with catalepsy](image)

**Fig. 1.** Fentanyl-induced catalepsy and rise in body temperature in rats exposed to normal (NET=22±1°C) and higher (HET=30±1°C) environmental temperature. The incidence of catalepsy (panels A and B) and the mean deviation from baseline colonic temperature (panels C and D) at 22±1°C (□) and 30±1°C (●) are plotted as a function of time after the i.p. injection of 0.05 mg/kg (○), 0.2 mg/kg (■) and 0.5 mg/kg (△) of fentanyl citrate. Each point represents the mean response±S.E.M. of 6–8 rats. *P<0.05 and **P<0.01 indicate a significant difference of the responses at environmental temperature of 30±1°C to the control of 22±1°C.
citrate at three times larger dose (1.5 mg/kg) showed the opposite effect in that the drug significantly decreased body temperature by 4.2±0.9°C (n=8). This dual pattern in temperature response is in accordance with previous results obtained with other predominantly μ-selective agonists such as β-endorphine (1.1–8.5 μg, intracerebrally) (15), morphine (2.5–20 mg/kg, s.c.) (16) and DAGO, [d-Ala²,MePhe⁴,Gly⁵-ol] enkephalin (0.3–3 μg, i.c.v.) (17). Thermoregulatory studies carried out at different environmental temperature demonstrated that hyperthermic effects of morphine (4) and the mostly selective μ-agonist PL-017 (8) are potentiated in rats exposed to higher environmental temperature.

Our results, which are in agreement with these findings, indicate that subacute exposure of rats to higher environmental temperature significantly potentiated a low-dose hyperthermic effect of fentanyl as well. Moreover, we showed that the higher environmental temperature significantly attenuated the high-dose hyperthermic effect of fentanyl. This finding is consistent with the previous one and may be explained as a direct consequence of the HET-induced potentiation of the hyperthermic response to fentanyl.

Also, the present experiments showed that higher environmental temperature, in addition to the hyperthermic effect, potentiated the cataleptic effect of fentanyl. Catalepsy is characterized by a behavioral immobility associated with varying degrees of enhanced muscular rigidity and/or "waxy flexibility" (18). Although it is already known that acute morphine (19), β-endorphine (15) or fentanyl (10) administration to rats evokes catalepsy, there is no evidence about the influence of environmental temperature on the opioid-induced catalepsy.

Poole and Stephenson reported that in rats, within an environmental temperature range of 28–32°C, behavioral thermoregulation includes inactivity as a response to heat stress (20). They suggested that the inactivity is responsible for the reduced metabolic rate under higher environmental temperature. Furthermore, it has been observed that when exposed to hot environments, rats assume an extended posture to enhance heat loss through an increase in the surface area: body mass ratio (21). Therefore, according to present knowledge, we can only suggest that at least part of the reason behind the HET-induced alteration in cataleptic response to fentanyl could be an interference with HET-induced postural changes.

It has been previously reported that fentanyl produces hyperexcitability on touching and/or hand-clapping, loss of righting reflex and loss of corneal reflex in rats (9, 10). In the present study, we demonstrated that HET did not modify and of these behavioral effects. Also, the control "tail immersion" latency and the analgesic potency of fentanyl were not affected by the exposure to HET. Similar to our finding, Lichtman et al. observed that placing mice in heated cages (38°C) did not reduce tail-flick latency and the analgesic potency of morphine (22).

Ghosh and Poddar demonstrated that exposure of rats to environmental temperature of 40°C for 2 hr elevated baseline body temperature (12). Contrary to this, in our study, exposure of animals to 30°C for 3 days had no effect on the baseline body temperature of the rats. This is probably due to the differences in the degree of environmental temperature to which rats were exposed, as well as the duration of the exposition (23).
In conclusion, this study provides the first evidence that environmental temperature influences drug-induced catalepsy. However, studies on the mechanism(s) of this effect are still lacking. The next study will aim to define the probable mechanisms of HET-induced changes of both catalepsy and hyperthermic response to fentanyl. The understanding of these mechanisms will increase our basic understanding of brain function in a hot environment, as well as behavioral effects of opioids per se.

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