Changes in Hyperthermic Effect of Morphine After Long-Time Application: Relationships with Hypothalamic Serotonin Level

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Abstract. It has been shown long time ago that morphine causes hyperthermia in rats in low doses, as well as hypothermia in high doses. The present study has investigated effect of morphine on body temperature in rats after chronic administration, and changes of the serotonin level in rat hypothalamus after acute and chronic administration of morphine. Body temperature was measured with thermistor probes (TX8) inserted rectally, and monitored on multichannel recorder Thermex 16. Single administration of morphine (3 mg/kg, i.p.) produced hyperthermia in rats with maximum between 90 min and 120 min after drug application. Repeated injection of morphine (3 mg/kg/day, i.p.) over a 9-week period caused similar hyperthermic reaction in rats, but maximal effect was found between 60 min and 90 min after drug application (the time to appearance of the maximal hyperthermal effect was significantly shortened). These data are in correspondence with the changes of hypothalamic serotonin levels observed. After chronic administration of morphine the serotonin level in rat hypothalamus was increased.

Keywords: Morphine, serotonin, hyperthermia, tolerance, rats.

1. INTRODUCTION

The interactions of several neurotransmitters and neuromodulators including norepinephrine, dopamine, 5-hydroxytryptamine (serotonin), acetylcholine, prostaglandin E1, GABA, and opioids are involved in the regulation of body temperature at the level of central temperature controller.

The opioid system is involved in the control of thermoregulation in mammals. Endogenous opioid peptides and opioid drugs, such as morphine can influence body temperature. Opioids produce their effects through stimulation of membrane bond opioid receptor: μ (mu), κ (kappa), and δ (delta). The specific effect of morphine on body temperature is dependent of various factors, such as species of animal, dose and route of administration of the drug, environmental temperature, circadian rhythms, age and degree of restraint of the animal [1]. Hyper- and hypothermia produced by the opioid agonists are opioid receptor effects because both effects can be blocked by the opiate antagonists and tolerance and cross-tolerance can be developed to both effects as well [2]. At laboratory temperature administration of morphine in low doses produced hyperthermia in rats by activating of μ-opioid receptors, while administration of morphine in high doses caused hyperthermia by stimulating of κ-opioid receptors [3-6]. Low doses of morphine caused an increase in thermoregulatory set point, as well as increased oxygen consumption, increased peripheral vasoconstriction and behavioral heat seeking [7, 8].

Experimental studies suggest a central neurochemical mechanism in morphine induced hyperthermia in rats. Pretreatment of the animals with hemicholinium delayed hyperthermic effect of morphine [9]. Experimental data demonstrated attenuation of morphine-induced hyperthermic reaction by administration of GABA and drugs with GABA-enhancing action, such as diazepam, sodium valproate and vigabatrin [10].

It has been found that tolerance develops to the hypothermic effect of morphine, while little or no tolerance develops to the morphine produced hyperthermia in rats after chronic administration [11, 12].

The aim of this study was to examine the changes of the hyperthermic effect on body temperature after 9 week administration of morphine in rats and interactions with the changes in hypothalamic level of serotonin (5-hydroxytryptamine, 5-HT).

2. MATERIAL AND METHODS

2.1. Substances and Design of In Vivo Experiments

Morphine hydrochloride was obtained from Sopharma (Bulgaria) and injected to the rats intraperitoneally (i. p.) in a volume 0.5 ml/100 g.

In acute experiments morphine was injected in a dose of 3 mg/kg i.p. after determination of initial
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Temperature. The control rats were injected with saline (0.9% NaCl) in a volume 0.5 ml/100 g.

In chronic experiments morphine was injected in a dose of 3 mg/kg/day i.p. for 9 weeks. The control rats were injected with saline (0.9% NaCl) in a volume 0.5 ml/100 g/day for 9 weeks.

2.2. Animals

In the present study male Wistar rats weighing between 200 and 220 g were used. They were housed in group cages of six animals each and kept at 22 °C. Food and water were available ad libitum. Animal procedures were conducted in accordance with the International Guiding Principles For Biomedical Research Involving Animals.

2.3. Body Temperature Experiments

All experiments were conducted at ambient temperature of 22±1 °C. Body temperature was measured with thermistor probes (TX8) and monitored on multichannel recorder Iso-Thermex 16 (Columbus Instruments, USA). The thermistor probes were lubricated and inserted rectally to a depth of 6 cm. Before drug administration the initial temperature of the animals was determined, and then checked at 30, 60, 90, 120 and 180 min.

2.4. Fluorimetric Determination of Serotonin Concentration in Rat Hypothalamus

At termination of the 9-week period, the animals were sacrificed by decapitation and removal of the hypothalami, assessment of the quantities of serotonin (after the method of Atack and Lindquist, 1973) was performed [13]. The obtained results were compared with the ones recorded in animals undergoing 9-week treatment with saline, as well as with the results in acute experiments made (after single administration of morphine).

2.5. Data Analysis

The results were expressed as delta (Δ) values (average changes in temperature compared to the initial one) (mean Δ values ± S.E.M.) and analyzed with two-way analysis of variance. For statistical significance a Student’s t-test was used.

3. RESULTS

3.1. Effect of Morphine on Core Body Temperature of Rats After Acute Administration

Single injection of morphine (3 mg/kg, i.p.) produced hyperthermia in rats. Maximal hyperthermal effect of morphine is found between 90 min and 120 min after drug application (Figure 1).

3.2. Effect of Morphine on Core Body Temperature of Rats After Chronic Administration

Following morphine injection (3 mg/kg/day, i.p., over a 9-week period), it was established hyperthermal effect with maximum between 60 min and 90 min after drug application (Figure 2).

![Figure 1: Effect of morphine on core body temperature of rats after acute administration.](image_url)

Average changes in temperature (Δ t °C ± S.E.M.) of rats following single injection of morphine hydrochloride (3 mg/kg, i.p.) (1) and saline (0.9% NaCl, i.p.) (2). Maximal hyperthermal effect of morphine is found between 90 min and 120 min after drug application.

Significant values: *P < 0.05; **P < 0.01; ***P < 0.001.
Comparison between hyperthermic effect of morphine after acute and chronic administration was established that the intensity of morphine-induced hyperthermal effects was not significantly altered, while the time to appearance of its maximal hyperthermal effect was significantly shortened after chronic (9-week) application (P<0.05) (Figure 3).

3.4. Hypothalamic Concentration of Serotonin After Acute and Chronic Administration of Morphine

Hypothalamic concentration of serotonin significantly increased in rats with chronic administration of morphine (3 mg/kg/day, i.p., over a 9-week period) in comparison with control rats (treated with saline), as well as those with single injection of morphine (P<0.05) (Figure 4).

Figure 2: Effect of morphine on core body temperature of rats after chronic administration.
Average changes in temperature (Δt °C ± S.E.M.) of rats following morphine hydrochloride (3 mg/kg, i.p.) (1) and saline (0.9% NaCl, i.p.) (2) injection over a 9-week period. Maximal hyperthermal effect of morphine is found between 60 min and 90 min after drug application.
Significant values: *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 3: Comparison between acute and chronic effects of morphine on body temperature of rats.
Average changes in temperature (Δt °C ± S.E.M.) of rats following single (1) and chronic (2) injection of morphine hydrochloride (3 mg/kg, i.p.). It was established that the intensity of morphine-induced hyperthermal effects was not significantly altered, while the time to appearance of its maximal hyperthermal effect was significantly shortened after chronic (9-week) application.
4. DISCUSSION

Our results suggest that after both acute and chronic administration of morphine hyperthermic effect was occurred, while the time to appearance of maximal hyperthermal effect was significantly shortened after chronic application. These results indicate that tolerance did not develop to the grade of hyperthermic response of morphine, but it was developed to the time of appearance of the maximal hyperthermic reaction, after long time application of morphine. Hypothalamic concentration of serotonin was increased after chronic administration of morphine. After systemic morphine administration increases in extracellular serotonin were observed in the nucleus accumbens, amygdala, frontal cortex, striatum, thalamus, hypothalamus and ventral hippocampus [14].

Serotonin plays a role in regulation of body temperature. Ghosh and Poddar [15] suggest involvement of serotonergic regulation in the opioidergic-cholinergic interaction via GABA system in higher environmental temperature-induced increase in BT. Paul and Phillips [16] observed that pirenperone, a serotonin antagonist with a preferential affinity for the 5-HT\textsubscript{2} receptor, attenuates morphine-produced analgesia. Nemmani and Mogil [17] demonstrated the interaction between serotonergic and gamma-aminobutyric acid-ergic systems in the modulation of analgesia from morphine, a mu-opioid agonist, and U50488, a kappa-opioid agonist.

Serotonin receptor (5-HT\textsubscript{1A} and 5-HT\textsubscript{1B/1D}) agonist dihydroergotamine significantly increased the analgesic effect of morphine but did not reduce the expression of morphine tolerance, while serotonin/norepinephrine reuptake inhibitors (amitriptyline, venlafaxine) significantly increased the analgesic effect of morphine and attenuated the expression of morphine tolerance [18]. Fluoxetine, a selective serotonin reuptake inhibitor reduced the ED\textsubscript{50} for morphine and extended the duration of morphine analgesia [19].

Animal studies suggest involvement of 5-hydroxytryptamine in morphine-, pethidine-, and methadone- induced hypothermia in rats at low ambient and room temperature [20].

CONCLUSION

The intensity of morphine-induced hyperthermal effects was not significantly altered, while the time to appearance of its maximal hyperthermal effect was significantly shortened after chronic (9-week) application. The quantitative changes in serotonin in rat hypothalamus is synchronous with the development of tolerance to the hyperthermal effect of morphine upon continued administration.

REFERENCES


