PHYSIOLOGY

Potentiating Effect of Thyrotropin-Releasing Hormone and of Its Synthetic Analog Digipramine on Morphine-Induced Analgesia

A. A. Guseva, I. E. Gurskaya, P. Ya. Romanovskis, and I. P. Ashmarin

UDC 615.357:577.175.829].015.4.07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 8, pp. 117-119, August, 1994
Original article submitted December 6, 1993

Thyrotropin-releasing hormone (TRH) and its synthetic analog digipramine (DP) shortened the latency of the tail-flick response in mice when injected intraperitoneally in a dose of 5 mg/kg and to potentiate significantly the analgesic action of morphine. Possible mechanisms of these two effects and prospects for their medical use are discussed.

Key Words: *thyrotropin-releasing hormone; morphine; TRH analog; nociception*

Thyrotropin-releasing hormone (TRH) is widely distributed in the central nervous system [14] and has been shown to produce a wide range of effects not associated with its hypophyseotropic functions [10]. One such manifestation of its central action is its antagonism to endogenous and exogenous opiates [12,13], which is so powerful that TRH is often referred to as a "physiological antagonist" of these [11]. TRH has been found to abolish opiate-induced catalepsy [12] and hypothermia [13] and to restore the activity of the respiratory center [13].

The reported data on how TRH influences the antinociceptive effects of opiates are contradictory. Whereas some authors report a potentiating effect [7], others have not found any change in opiate-induced analgesia [5]. Highly conflicting results also came from studies in which TRH itself was examined for its influence on the perception of nociceptive stimuli: some workers stated that TRH does possess antinociceptive activity [7], whereas others concluded that it fails to exhibit any analgesic properties [5,12]. The most contradictory data were obtained after peripheral administration of TRH.

In the present study on mice we examined TRH, injected intraperitoneally, for its analgesic activity and its influence on morphine-induced analgesia in the tail-flick reaction, which is the most frequently used test involving thermal nociceptive stimulation.

In addition, since the half-life of TRH in plasma is short, we deemed it useful to compare its effects with those of its synthetic analog digipramine (DP), which is more resistant to peptidases and produces less marked endocrine effects than TRH [4].

MATERIALS AND METHODS

The experiments were carried out on male (CBA×C57Bl/6)F_1 mice weighing 20-25 g. The antinociceptive effect was evaluated by the tail-flick response on a standard apparatus (Hugo Sachs Electronik, Germany) using an adaptation of the met-

Chair of Human and Animal Physiology, Department of Biology, Moscow State University.

0007-4888/94/0008-0803$12.50 ©1995 Plenum Publishing Corporation

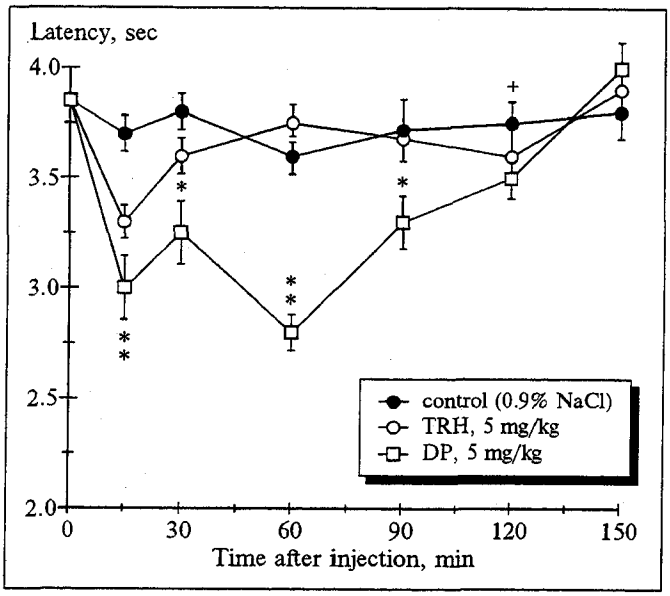

Fig. 1. Effects of TRH and DP on the latency of the tail-flick response. One and two asterisks: significant differences at $p<0.05$ and $p<0.01$, respectively, between DP-injected and control mice; crosses: significant differences at $p<0.05$ between TRH-injected and control mice.

hod described by D'Amour and Smith [9]. Mice were placed in a Plexiglas chamber and a beam of light was focused on a site 1-2 cm from the tip of the tail. The intensity of the beam was so chosen that the initial latency of the tail-flick response was 4-5 sec. The maximal exposure time was 20 sec. There were 10 mice in each group.

Morphine (4 mg/kg), TRH (5 mg/kg), and DP (5 mg/kg) were each dissolved in physiological saline and on the same day injected intraperitoneally in an amount of 0.1 ml per 10 g body weight. Each of them was injected from a separate syringe. Control mice received physiological saline by the same route in the equivalent volume. Animals were tested before injection and then at 15, 30, 60, 90, and 120 min postinjection, followed by further tests at hourly intervals if the analgesic effect persisted.

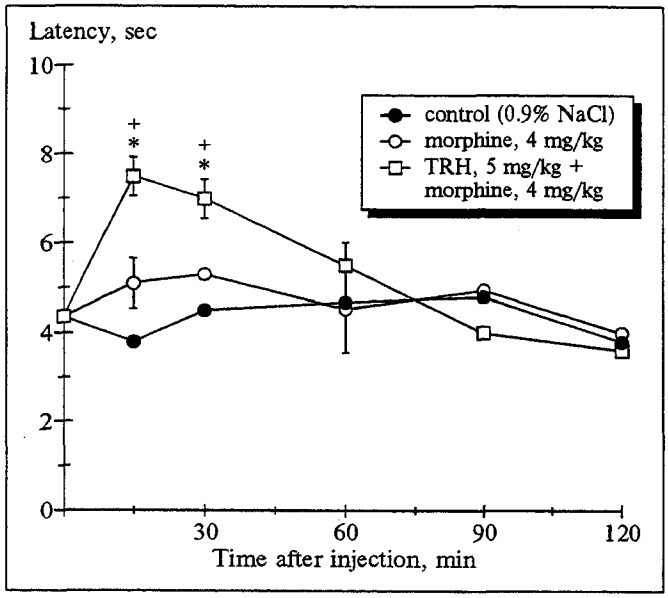

Fig. 2. Latencies of the tail-flick response in mice injected with both TRH and morphine. Asterisks: significant differences at $p<0.05$ between mice given TRH + morphine and those given morphine alone; crosses: significant differences at $p<0.01$ between TRH + morphine-injected and control mice.

The significance of differences between the groups of mice was evaluated by Student's test for nonpaired samples.

RESULTS

In the dose used (5 mg/kg), both TRH and DP significantly shortened the latency of the tail-flick response (Fig. 1). The effect of TRH reached its peak 15-20 min postinjection and then declined rapidly to become insignificant by minute 30. The effect of its analog on this response was more strongly marked and remained significant until minute 90 postinjection.

The morphine dose used (4 mg/kg) led to a slight and, as a rule, insignificant prolongation of the reaction latency. Mice injected with morphine together with TRH (Fig. 2) or its analog (Fig. 3) exhibited a significant increase in the pain sensitivity threshold. In the group given morphine plus TRH, the reaction latency 15 min postinjection was 7.1 ± 0.8 sec and differed significantly from the values both in the control group (3.9 ± 0.1 sec; $p<0.01$) and in the group given morphine alone (5.0 ± 0.5 sec; $p<0.05$). DP, in addition to enhancing the antinociceptive effect of morphine, also prolonged it to 120 min.

Our findings presented above agree with those of Vlasov *et al.* [2], who reported a potentiating effect of TRH on morphine analgesia in the hot-plate test; the latter authors did not, however, provide any data on how TRH itself influenced the reaction time in this test.

Although the mechanism by which morphine analgesia is enhanced in the presence of TRH remains to be ascertained, a highly plausible hypothesis can be formulated on the basis of the evidence presented by Balashov and Shurin [1]: they found that TRH, without interacting itself with opiate receptors, increases the binding of opiate ligands with these receptors within a particular concentration range.

This, however, does not explain why the latency of the tail-flick response was found to decrease in the presence of TRH or DP. One possible explanation may lie in the fact that the back-

ground quantity of endogenous opiates in unstressed animals is small so that their analgesic effect is only slightly potentiated by TRH and is undetectable in a test involving acute pain. The decrease in reaction latency may be attributed to other central effects of TRH such as the facilitating effect on spinal reflexes [8] and the hyperthermal effect [6]. A rise of temperature in the tail skin of an animal has been shown to result in a shortened latency of the tail-flick response [15].

This hypothesis agrees well with the observed potentiation by TRH of the antinociceptive effect of morphine, for the latter, too, inhibits spinal reflexes and lowers skin temperature in animals, which possibly enables TRH to produce its potentiating effect.

For a thorough verification of this hypothesis, further experiments are necessary to study TRH effects in models with nonthermal pain stimuli.

Thus, as this study indicates, the antagonizing action of TRH on opiates does not extend to the analgesic effect of morphine; on the contrary, this latter effect is strongly enhanced rather than weakened in its presence. Moreover, there are reasons to regard tests using thermal pain stimuli as being inadequate for evaluating the analgesic properties of TRH.

Possible medical aspects of the present study arise from the fact that narcotic analgesics still remain the most efficient pain-relieving agent in use, even though they are known to have serious side-effects limiting their applicability, such as the development of tolerance, inhibition of respiratory activity and of mental alertness, and impairment of many autonomic functions [3]. One method of mitigating these effects in clinical settings is to use analgesics in combination with drugs from other groups. Combined use of opiate analgesics with TRH or its analogs appears to be a promising approach both to reducing the doses of analgesics and to preventing or minimizing their adverse effects such as inhibition of respiratory activity and alertness.

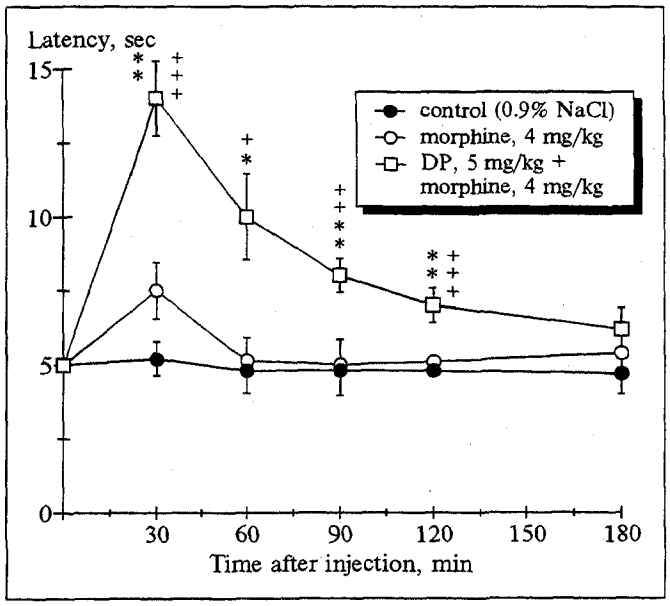

Fig. 3. Latencies of the tail-flick response in mice injected with both DP and morphine. One and two asterisks: significant differences at $p<0.05$ and $p<0.01$, respectively, between mice given DP + morphine and those given morphine alone; one; two, and three crosses: significant differences at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, between mice given DP + morphine and control mice.

REFERENCES

1. A. M. Balashov and M. R. Shurin, *Byull. Eksp. Biol. Med.*, **102**, № 8, 174 (1986).
2. G. P. Vlasov, E. K. Georgianova, N.Yu. Kozhevnikova, *et al.*, in: *Neuropeptides: Their Role in Physiological and Pathological States (Abstracts of papers)* [in Russian], Tomsk (1985).
3. N. A. Osipova, *Assessing the Effect of Narcotics, Analgesics, and Psychotropic Agents in Clinical Anesthesiology* [in Russian], Leningrad (1988).
4. P. Ya. Romanovskis, Sh. V. Svirskis, and G. I. Chipens, Author's Certificate № 892870, *Byulleten' Izobretenii*, № 47, 296 (1981).
5. H. N. Bhargava, *Life Sci.*, **29**, 1015 (1981).
6. G. Boschi and R. Rips, *IRCS Med. Sci.*, **9**, 200 (1981).
7. G. Boschi, M. Desiles, V. Reny, *et al.*, *Brit. J. Pharmacol.*, **79**, 85 (1983).
8. R. W. Clarke, T. W. Ford, S. M. Harris, and J. S. Tailor, *Neuropharmacology*, **27**, № 12, 1279 (1988).
9. F. E. D'Amour and D. L. Smith, *J. Pharmacol. Exp. Ther.*, **72**, 74 (1941).
10. E. C. Griffiths, *Psychoneuroendocrinology*, **10**, 225 (1985).
11. J. W. Holaday, R. J. D'Amato, B. A. Ruvio, and A. Faen, in: *Thyrotropin-Releasing Hormone*, edited by E. Costa and M. Trabucchi, New York (1982), p. 353.
12. J. W. Holaday, G.-F. Tseng, H. H. Loh, and C. H. Li, *Life Sci.*, **22**, 1537-1544 (1978).
13. A. Horita, M. A. Carno, and R. M. Chestnut, *Psychopharmacology*, **49**, 57-62 (1976).
14. C. Oliver, R. L. Eskay, N. Ben-Jonathan, and J.C. Porter, *Endocrinology*, **96**, 540 (1974).
15. A. Tjolsen, A. Lund, P. K. Eide, *et al.*, *Pharmacol. Biochem. Behav.*, **32**, № 3, 601 (1989).