GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effect of Gonadotropin-Releasing Hormone Analogue on Thermal Nociception in Mice

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Intraperitoneal treatment with an analogue of gonadotropin-releasing hormone in doses of 0.004-450 μg/kg produced an analgesic effect on male mice in the hot plate test. Castration significantly elevated the nociceptive thresholds. In castrated mice the effects of the test peptide were less pronounced and had an algesic nature. Our results indicate that these effects depend on functional activity of the hypothalamic-pituitary-gonadal axis.

Key words: *gonadotropin-releasing hormone analogue; Surfagon; pain; castration*

Peptidergic mechanisms play an important role in the pain response. Regulatory peptides can produce both algesic and analgesic effect [4]. However, physiological role of gonadotropin-releasing hormone (GnRH) and hypothalamic-pituitary-gonadal axis in these processes remains unknown. Much attention to this problem is related to extensive use of synthetic GnRH analogues. Our previous studies of animal's behavior during unavoidable electric shock showed that GnRH analogue Surfagon modifies the nociceptive threshold in aggression-defense reaction [3,6]. However, under these experimental conditions the response threshold could depend on emotional nociceptive stress, affective aggressiveness, and individual reaction to electrical shock [4]. The role of Surfagon in pain response was studied on the model of thermal nociceptive stimulation.

MATERIALS AND METHODS

Experiments were performed on 250 male BALB/c mice weighing 22-25 g. The animals were divided into groups of 10 specimens each. GnRH agonist Surfagon (pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-ethylamide) was synthesized at Laboratory of Peptide Synthesis (Russian Cardiology Research-and-Production Center, Russian Ministry of Health). The test drug was dissolved in physiological saline and injected intraperitoneally in doses of 0.004, 0.02, 0.1, 0.5, 1.5, 5, 15, 50, 150 and 450 μg/kg 12 min before the start of the study. Control animals received an equivalent volume of physiological saline (1 ml/kg). The study was conducted on intact and castrated animals. Castration was performed through midsection of the scrotum under hexenal anesthesia (70 mg/kg intraperitoneally). The study was performed 12 days after surgery.

For evaluation of thermal nociception, intact mice were placed on a plate surrounded with a glass cylinder and heated to 50°C. Castrated mice demonstrated no jumping-out reaction at this temperature and the plate was heated to 60°C. The animals underwent four 1-min trials at 1-min intervals. Nociception was estimated by the latency of paw-licking (sec, trials 1 and 2) and jumping-out (trials 3 and 4) [8]. The results were analyzed by Student's *t* test.

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RESULTS

In intact mice during trial 1 the paw-licking latency significantly increased only after administration of Surfagon in the maximum dose (Table 1). A significant decrease in this index (by 18-23%) during trial 2 was revealed upon treatment with the peptide in low doses (0.004-0.5 μg/kg). The test peptide in higher doses produced minor effect.

During trial 3, the peptide had an analgesic effect in most mice. This effect was most significant after treatment with the peptide in low doses. The latency increased most significantly after administration of the test drug in a dose of 0.004 μg/kg (by 115 %, $p<0.01$). Surfagon in doses of 0.5-5.0 μg/kg was nearly ineffective. The analgesic effect became more pronounced with increasing the dose of Surfagon to 15-450 μg/kg. The dose-response dependence reflects the existence of U-shaped association typical of regulatory peptides. During trial 4, Surfagon in various doses produced a significant analgesic effect.

Castration significantly increased the nociceptive threshold (Table 2). Castrated mice demonstrated longer paw-lick latency in trials 1 and 2 at 50°C compared to intact animals (by 39 and 35%, respectively, $p<0.05$). Castrated mice did not exhibit the jumping-out reaction in all trials. Therefore, castration produced a significant antinociceptive effect.

Surfagon mainly reduced the paw-lick latency in castrated mice during trial 1 (Table 3). This effect was most pronounced after treatment with the peptide in doses of 0.1 and 15 μg/kg (by 32 and 33%, respectively, $p<0.05$). The nociceptive effect of Surfagon was preserved in trial 2. However, the latency tended to increase in animals receiving Surfagon in a dose of 5 μg/kg. This effect of Surfagon was more significant during trial 3, which contributed to a considerable increase in the nociceptive threshold (61%, $p<0.05$). The peptide in other doses was ineffective. Surfagon in doses of 0.1, 15 and 50 μg/kg had a nociceptive effect during trial 4.

Our results show that Surfagon in various doses had an antinociceptive effect on intact animals during trials 3 and 4. However, during trials 1 and 2 the peptide produced both algesic and analgesic effect. In some groups, the algesic effect of Surfagon changed to analgesic during trial 2. The test peptide mainly decreased the response threshold in castrated animals characterized by lower initial sensitivity compared to intact mice. The range of effective doses of Surfagon decreased after castration.

We previously revealed a significant effect of Surfagon on orientation and exploratory activity and grooming in the glass-field test (similar to a restrictive cylinder in the hot plate test) [3,6]. The same behavioral reaction was observed at the beginning of the nociceptive test, which could affect manifestations of the pain response. Moreover, behavioral reactions were probably determined by the influence of Surfagon on acquisition of the conditioned response [7].

It is unlikely that the effects of this peptide are mediated by sex steroids and hypophyseal gonadotropins, since the period between Surfagon administration and end of study is insufficient for modulating the concentration of these compounds in the

TABLE 1. Latency of Nociceptive Response in Intact Mice (sec, $M \pm m$)

Group	Paw licking		Jumping-out	
	Trial 1	Trial 2	Trial 3	Trial 4
Control	18.6±1.6	29±3	25.8±3.2	17.1±2.1
Surfagon, μg/kg				
0.004	17.0±1.5	23.6±2.1*	55.5±0.5**	37.0±12.0*
0.02	18.4±1.5	23.4±2.3*	49.7±3.3**	27.8±4.1
0.1	20.1±1.3	22.2±2.3*	48.3±7.4*	28.0±2.3**
0.5	18.3±1.6	23.4±2.5*	20±2	24.5±9.4
1.5	19.6±1.4	26.9±3.1	30±4	33.0±8.7*
5	20.4±1.4	28.6±2.6	30.0±6.5	20.8±3.6
15	18.4±1.9	26.7±2.6	46.5±8.5*	43.0±7.3**
50	22.3±2.1	24.1±2.1	43.4±4.9*	35.3±6.8
150	18.5±1.0	25.4±2.7	45.0±5.7*	39.0±6.4**
450	31.1±4.1**	30.8±3.9	41.2±3.6	23.0±2.2*

Note. *$p<0.05$ and **$p<0.01$ compared to the control.

TABLE 2. Latency of Nociceptive Response in Intact and Castrated Mice (sec, *M*±*m*)

Group	Paw licking		Jumping-out	
	Trial 1	Trial 2	Trial 3	Trial 4
Intact	20.3±1.8	19.0±1.2	40.8±5.7	29.7±4.5
Castrated	28.3±3.1*	25.6±2.4*		

Note. *p<0.05 compared to intact mice.

TABLE 3. Latency of Nociceptive Response in Castrated Mice (sec, *M*±*m*)

Group	Paw licking		Jumping-out	
	Trial 1	Trial 2	Trial 3	Trial 4
Control 1	9.3±0.8	21.9±1.8	15.3±3.6	11.3±2.3
Surfagon, μg/kg				
0.02	7.1±0.6*	13.2±1.2*	17.9±2.5	8.9±1.4
0.1	6.3±0.6*	13.8±1.1*	14.1±1.8	6.0±0.7*
0.5	8.2±0.7	20.8±2.0	17.0±1.4	10.4±1.7
1.5	7.9±0.9	15.0±2.2*	13.1±2.4	9.7±2.8
5	9.2±1.0	25.5±2.6	24.7±5.1*	16.6±4.7
150	7.9±0.7	16.4±1.7*	21.5±4.9	10.6±3.0
450	7.5±0.4*	17.6±2.0*	21.0±3.5	15.3±3.9
Control 2	10.2±1.2	18.1±3.3	23.4±3.1	16.7±2.6
Surfagon, μg/kg				
0.004	8.9±0.9	13.7±1.5	22.8±2.8	15.7±3.0
15	6.8±0.6*	15.0±1.2	19.2±2.9	10.2±1.1*
50	9.1±0.9	16.3±1.8	22.1±5.3	9.4±1.6*

Note. *p<0.05 compared to the control.

body [5]. The observed algesic and analgesic effects of Surfagon can result from its direct neurotropic influence. Published data show that GnRH and its analogues bind to specific receptors in the gray matter of the midbrain [10], activate monoaminergic neurotransmission in CNS, and stimulate the endogenous opioid system [11,14]. Naloxone abolished the analgesic effect of GnRH and its analogue 6-Tre-GnRH in the hot-plate test. Moreover, GnRH *in vivo* stimulates the release of β-endorphin from rat pituitary [9].

The analgesic effect of castration is mainly due to activation of the serotoninergic system in the spinal cord [12], which plays a role in local regulation of nociception [4]. Changes in serotoninergic activity could modulate the opposite effect of Surfagon in castrated mice. Studying the mechanisms for these effects should take into account a variety of castration-induced changes in activity of the opioidergic and dopaminergic systems [13].

The doses of Surfagon that most significantly modified thermal nociception did not differ from those used in previous studies [1-3,7].

Our findings indicate that Surfagon in a wide range of specified doses modifies thermal nociception in mice. The degree and directionality of changes depend on functional activity of the hypothalamic-pituitary-gonadal axis.

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