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Thymic Factors Influence on Behavior in Rodents

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IURATO, M. P., A. CHIARENZA, N. BARBERA, G. CANTARELLA, L. LEMPEREUR, F. DRAGO, U. SCAPAGNINI AND R. BERNARDINI. *Thymic factors influence on behavior in rodents.* PHARMACOL BIOCHEM BEHAV 44(3) 665-671, 1993. – We studied the effect of thymopentin, a synthetic thymic peptide, on spontaneous behavior and stress models in BALB/c mice in which a WEHI 164 clone 13 murine fibrosarcoma had been implanted, as well as in the intact Sprague-Dawley rat. In untreated animals with tumors, spontaneous behavior was significantly inhibited. Resistance to swimming in cold water was also decreased in untreated animals. Thymopentin (10, 100, 1,000, and 5,000 μg/kg body weight, IP, 20 min before the test) enhanced spontaneous behavior in tumor-implanted mice. In addition, thymopentin partially restored floating capability of tumor bearers in either freely moving or animals on which an additional weight had been applied. In the latter test, plasma corticotropin and corticosterone levels were relatedly modified according to treatment. Rats treated with thymopentin showed a decreased sensitivity to painful stimuli. The effect of thymopentin was comparable to acetylsalicilate. Finally, thymic factors appeared capable of restoring the diminished behavioral activity of animals bearing tumors, as well as of increasing resistance to stressful stimuli and pain.

Thymopentin Behavior Hypothalamic-pituitary-adrenal axis Pain

GROWING evidence demonstrates that the neuroendocrine and immune systems mutually influence their own function (7). In this line, attention has been focused on the effects of mediators of the inflammatory/immune response on the CNS (3-5,20,22). The finding that many mediators of the immune response, including thymic hormones, possess central effects (15) has led to extensive investigation about their neurochemical and behavioral actions. In this line, it has been reported that cytokines are able to induce centrally mediated anorexia and sleepiness (18), as well as electroencephalographic changes (19) and activation of neuroendocrine secretion (3,5).

In this line, thymic hormones have been shown to activate the hypothalamic-pituitary-adrenal (HPA) axis (14,23), a system regulatory of response to stressful stimuli that also plays a key role in behavioral adaptation (13). HPA axis-mediated behavioral changes also occur in the course of chronic diseases, such as cancerous tumors (9,17).

Among thymic peptides, thymopentin (TP) is a synthetic pentapeptide, corresponding to the 32-36 amino acid sequence of thymopoietin (12), that modulates the immune response at different levels (10). No reports exist describing a direct antitumor activity of TP, although it has been shown that TP may activate immunocytes clones, such as natural killer cells, involved in tumor growth inhibition (16).

It seemed of interest to us to evaluate the effects of TP, which possesses effects identical to natural thymic peptides (12), on behavior in BALB/c mice bearing a murine fibrosarcoma. In the same animals, we measured plasma corticotropin (ACTH) and corticosterone levels.

Finally, we extended the study of behavioral activity of TP to its possible role in modulation of pain in the adult, male Sprague-Dawley rat.

METHOD

Animals

Adult, male BALB/c mice (20–22 g) and Sprague-Dawley rats (200–225 g) from Charles River (Calco, Italy) were housed in standard conditions (22 ± 2°C, 12 L : 12 D cycle, commercial rat chow and tapwater available ad lib). Animals studies were conducted in accord with the highest standards of care. Animals were divided into groups of eight.

Tumor Implantation

Murine fibrosarcoma WEHI 164 clone 13 cells [5×10^5, suspended in phosphate-buffeted saline (PBS), pH 7.4] were injected SC in the back, about 2 cm below the neck line. Seven

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days later, a tumor of approximately 5 mm of diameter had developed in the injection site of each animal. All animals were used for behavioral tests on day 8.

Drugs

Injectable, lyophilized TP (kindly supplied by Cilag, Milan, Italy) was reconstituted in sterile PBS at an initial concentration of 50 mg/ml. The drug was then further diluted in PBS to obtain the following concentrations: 10, 100, 1,000, and 5,000 μg/kg. The zero dose was PBS alone. All solutions were prepared immediately before injection. TP was injected IP 20 min before each test except in pain sensitivity tests (see the appropriate section below).

Behavioral Techniques

Open field. A circular box, the floor of which was divided into 19 equal sections, was used to measure spontaneous motor activity. Each animal was initially placed at the center of the field and allowed to move freely for 5 min. During the test, the following parameters were scored: ambulation (number of sections explored); number of rears toward the center; number of wall rears; time spent grooming; number of fecal boluses deposited.

Swimming and forced swimming tests. Animals were individually placed into a water bath containing cold water (temperature: 15–16°C) and allowed to float until they were no longer able to float (immersion of head). In the forced swimming test, a similar procedure was followed after application of a standard 10-g lead weight safely secured to the animal.

In both tests, the time of floating for each animal was recorded. At the end of the experiment, all animals were sacrificed by decapitation. Trunk blood was collected in prechilled heparinized tubes and plasma separated and frozen at −80°C until assayed for ACTH and corticosterone.

The above tests were carried out in both tumor bearers and intact animals.

Pain sensitivity as assessed by the hot-plate test. Rats were placed onto a hot plate (50°C) and the latency time to the appearance of behavioral reactions to heat (jumping, squeaking) was scored. The effects of various treatments were calculated as percent change in sensitivity compared to untreated controls. The latter were taken as 100% of sensitivity. Change in pain sensitivity was calculated as the ratio between the time spent on the hot plate by untreated controls compared to that spent by treated animals. Drugs administered were TP (10, 100, and 1,000 μg/kg IP, 30 min prior to the test), acetylsalicilic acid (ASA, 25 mg/kg per os 1 h prior to the test), or morphine (MOR, 10 mg/kg, SC, 30 min prior to the test).

Plasma Hormone Assays

For measurement of plasma ACTH, 150-μl aliquots of plasma were passed through a C18 Sep-Pak cartridge (Waters

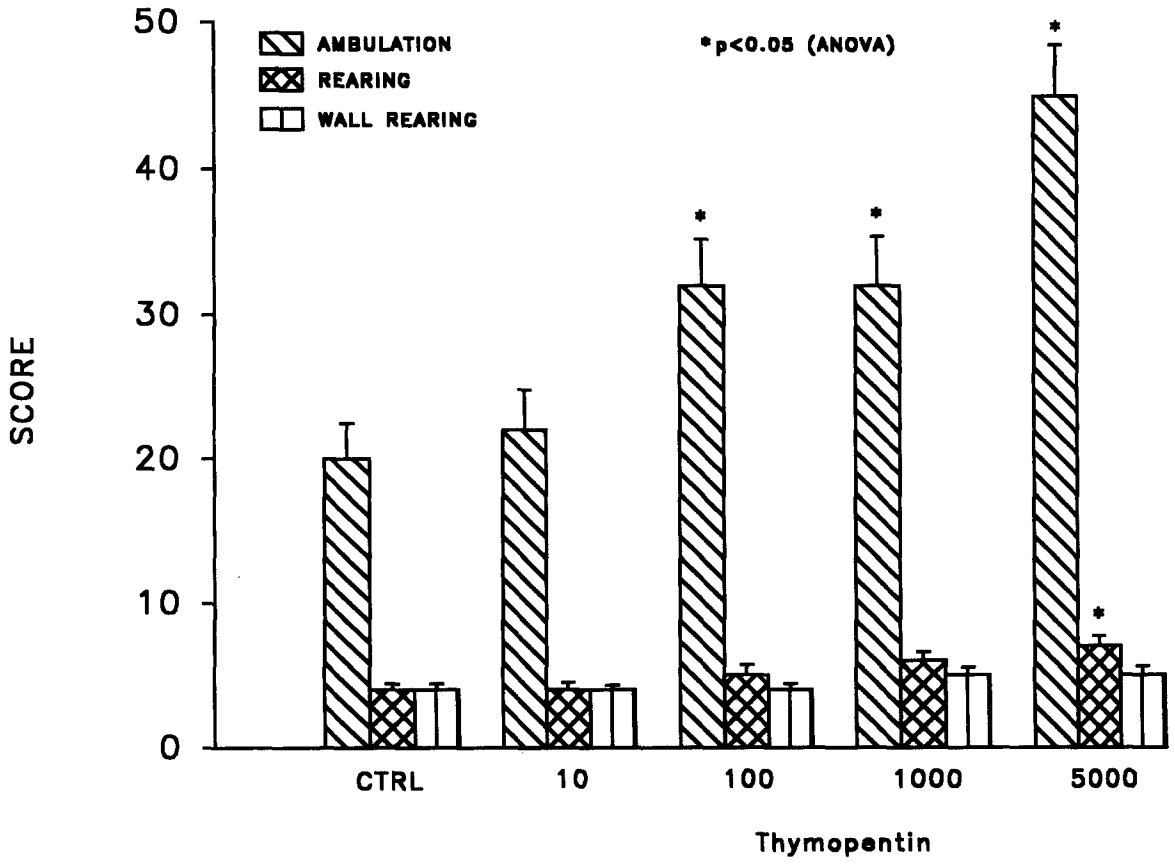

FIG. 1. Dose-related effects on spontaneous behavior parameters in intact BALB/c mice treated with thymopentin (IP, 20 min prior to the test); vertical bars are means ± SE; $n = 8$ animals/group.

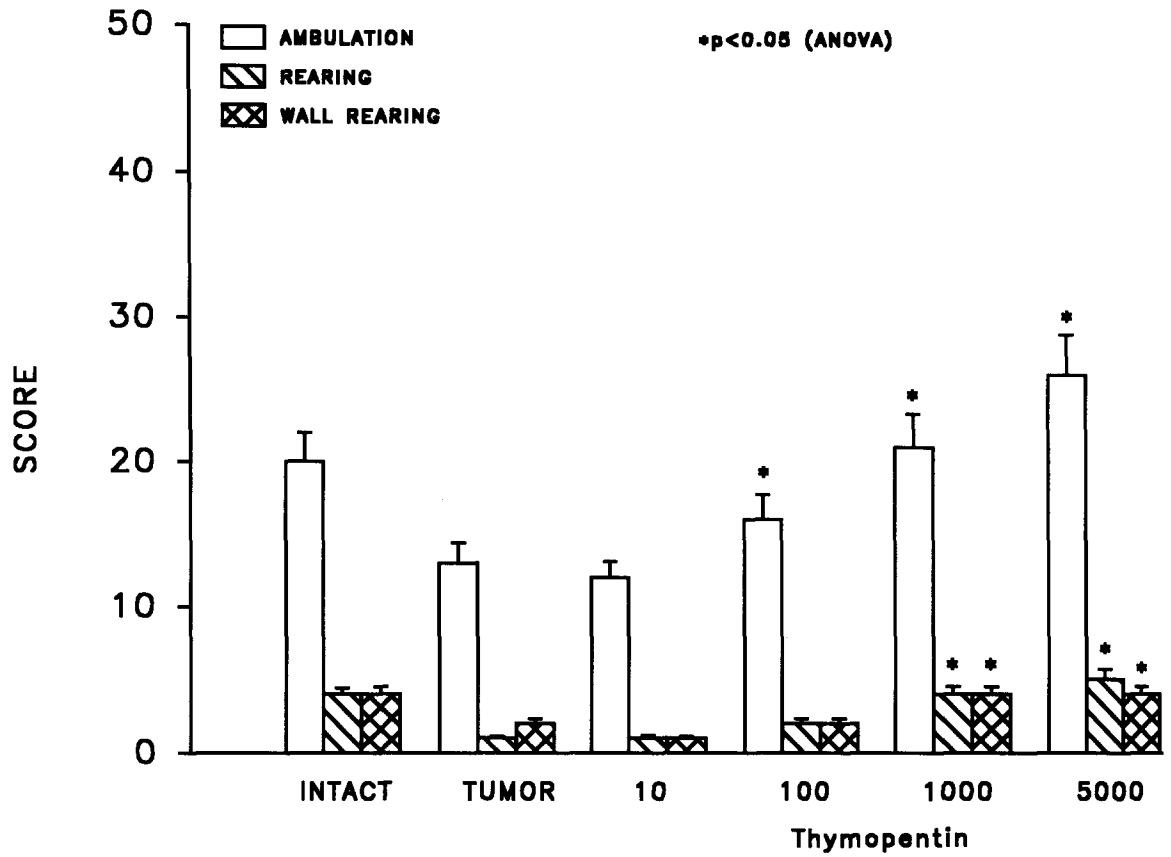

FIG. 2. Dose-related effects on spontaneous behavior in BALB/c mice bearing a murine fibrosarcoma (WEHI 164 clone 13) treated IP with thymopentin 20 min prior to the test; vertical bars are means ± SE; n = 8 animals/group.

Assoc., Milford, MA), washed with 0.1% trifluoroacetic acid (TFA) buffer, and ACTH eluted in 5 ml of a 6:4 (v:v) acetonitrile:TFA mixture. Solvent was then evaporated in a lyophilizer and samples reconstituted in assay buffer. Samples were assayed for ACTH in duplicate by means of a specific radioimmunoassay (RIA). Anti-ACTH antibody (IgG Corp., Nashville, TN) was used at a final dilution of 1 : 21,000 (assay volume: 300 μl). Inter- and intrassay coefficients of variation (CV) were 6.2 and 9.1%, respectively.

Plasma corticosterone was measured directly, without extraction, by RIA using a [^{125}I]corticosterone kit supplied by Radioimmunoassay System Laboratories, Inc. (Carson, CA). Inter- and intrassay CV were 8 and 11%, respectively.

Analysis of Results

All data were subject to statistical analysis using one-way analysis of variance (ANOVA), followed by the Duncan multiple-range test.

RESULTS

TP injection resulted in a significant increase in ambulation in intact animals. The effect of TP was dose dependent. Rearing was significantly increased only at the highest dose of TP used (5,000 μg/kg) (Fig. 1).

Ambulation of untreated tumor bearers was significantly decreased in comparison to intact controls. However, treatment with TP resulted in an increase in the ambulation of tumor bearers although the ambulation score did not reach the values measured in intact animals. Similarly, rearing and wall rearing were also increased in TP-treated tumor bearers compared to untreated tumor bearers (Fig. 2).

The grooming score was increased in mice with implanted tumors whereas it was significantly decreased in animals treated with TP. The effect of TP was dose dependent (Fig. 3). Number of boluses deposited followed a pattern similar to that observed for grooming (Fig. 4).

Resistance to swimming in cold water was significantly increased in tumor bearers treated with TP (Fig. 5A). Parallel results were obtained when a lead weight was applied to animals. In the forced swimming test, effects of TP were significant only at the dose of 1,000 μg/kg (Fig. 5B).

Plasma ACTH levels of animals that had undergone the forced swimming test were significantly increased in tumor bearers compared to intact animals. Treatment with TP significantly reduced plasma ACTH levels in tumor bearers. However, plasma ACTH levels did not return to basal levels (Fig. 6). Plasma corticosterone levels increased in parallel to plasma ACTH levels (data not shown).

TP injected prior to the hot-plate test showed an analgesic effect similar to that of ASA. However, the effect was not comparable to the practically complete analgesia obtained with morphine. The maximum analgesic effect occurred 10 min after administration of TP (Fig. 7).

In additional experiments, aimed to proof the effectiveness of TP in intact animals, we observed that the thymic peptide

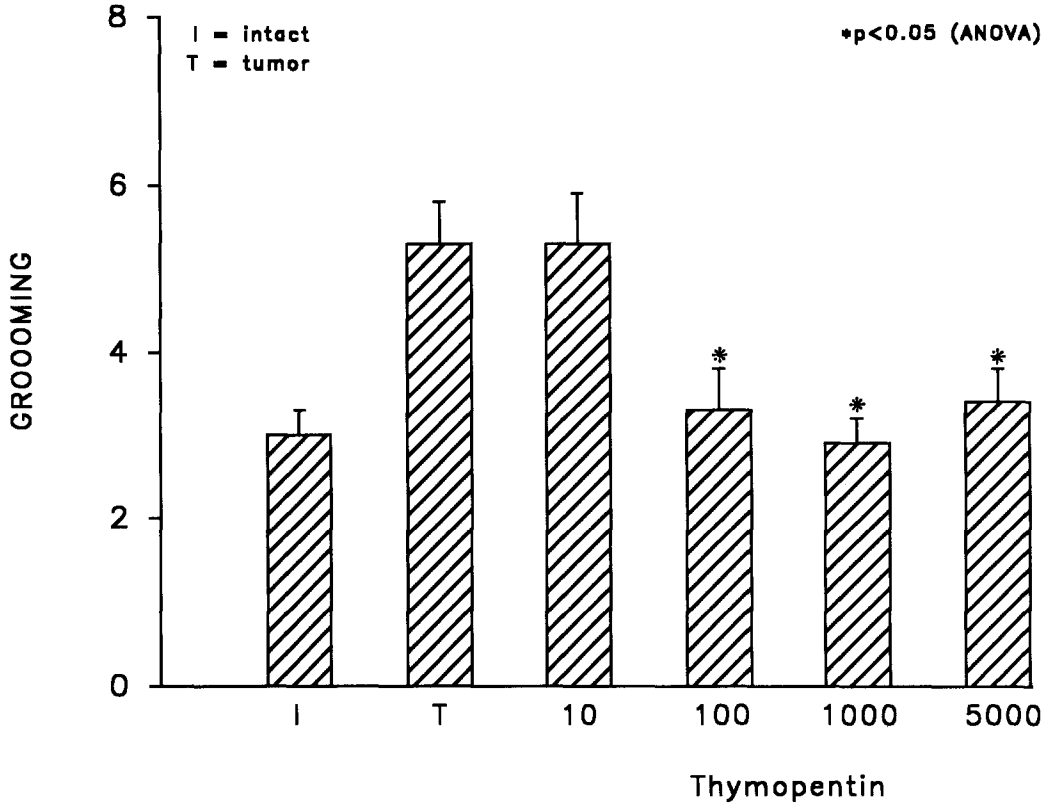

FIG. 3. Effects of thymopentin (IP injection 20 min prior to the test) on grooming in BALB/c mice bearing a murine fibrosarcoma (WEHI 164 clone 13); vertical bars are means ± SE; n = 8 animals/group.

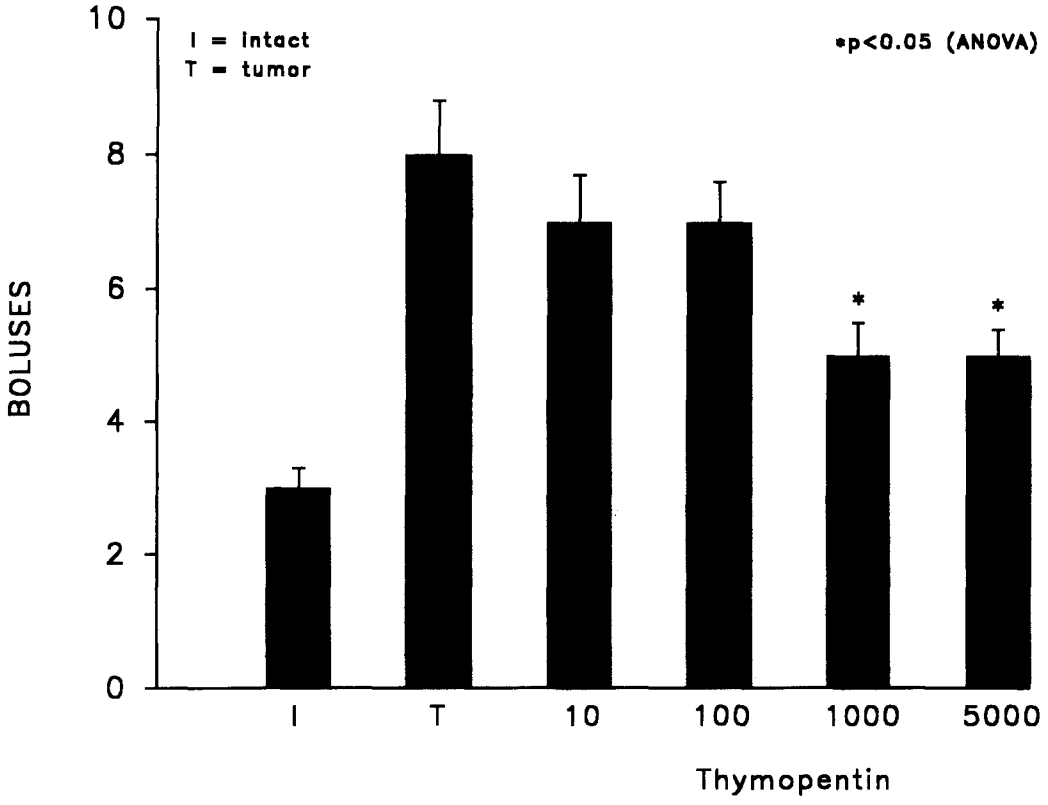

FIG. 4. Effects of thymopentin (IP injection 20 min prior to the test) on boluses deposition in BALB/c mice bearing a murine fibrosarcoma (WEHI 164 clone 13); vertical bars are means ± SE; n = 8 animals/group.

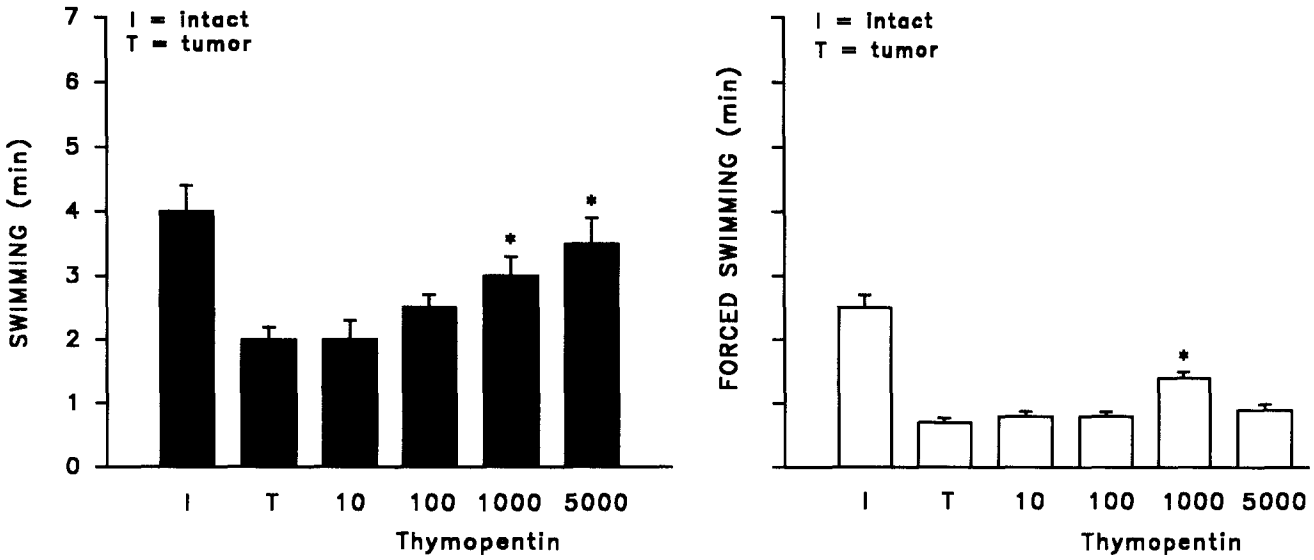

FIG. 5. Effects of thymopentin (IP injection 20 min prior to the test) on (A) swimming and (B) forced swimming in cold water, in tumor-bearing BALB/c mice; vertical bars are means ± SE; n = 8 animals/group.

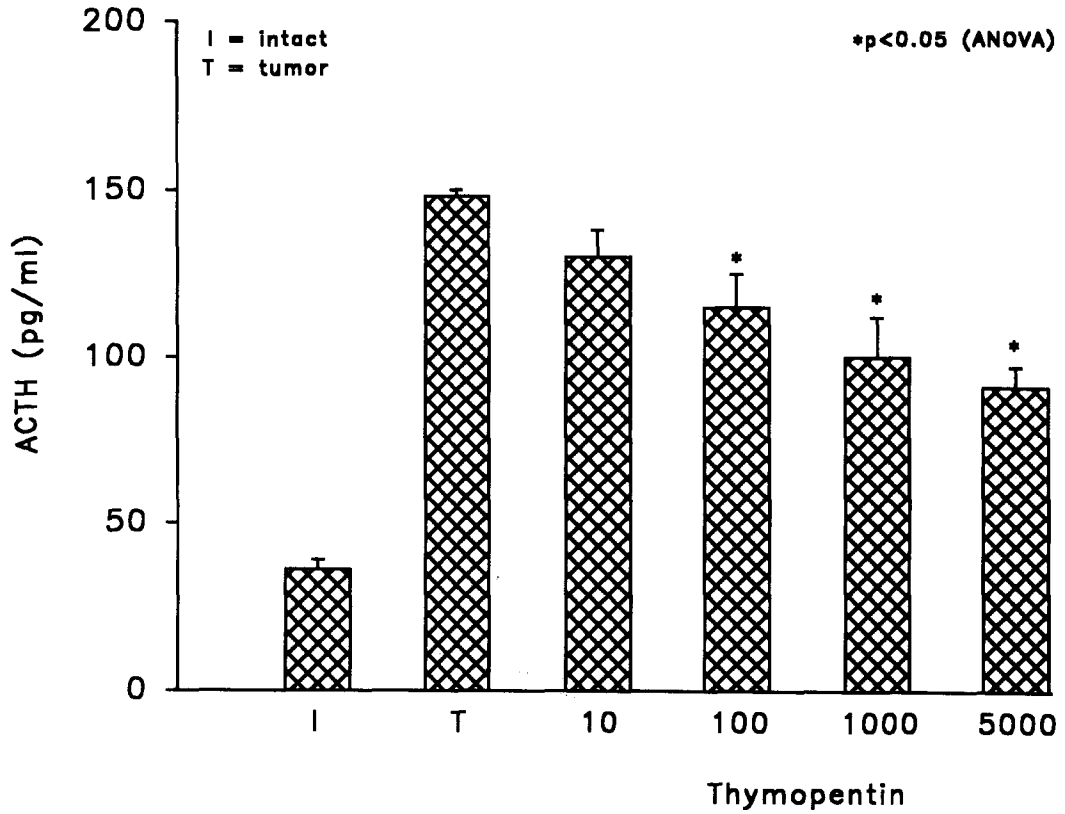

FIG. 6. Effects of thymopentin (IP injection 20 min prior to the test) on plasma corticotropin (ACTH) levels in tumor-bearing BALB/c mice that have undergone the forced swimming in cold water test; vertical bars are means ± SE; n = 8 animals/group.

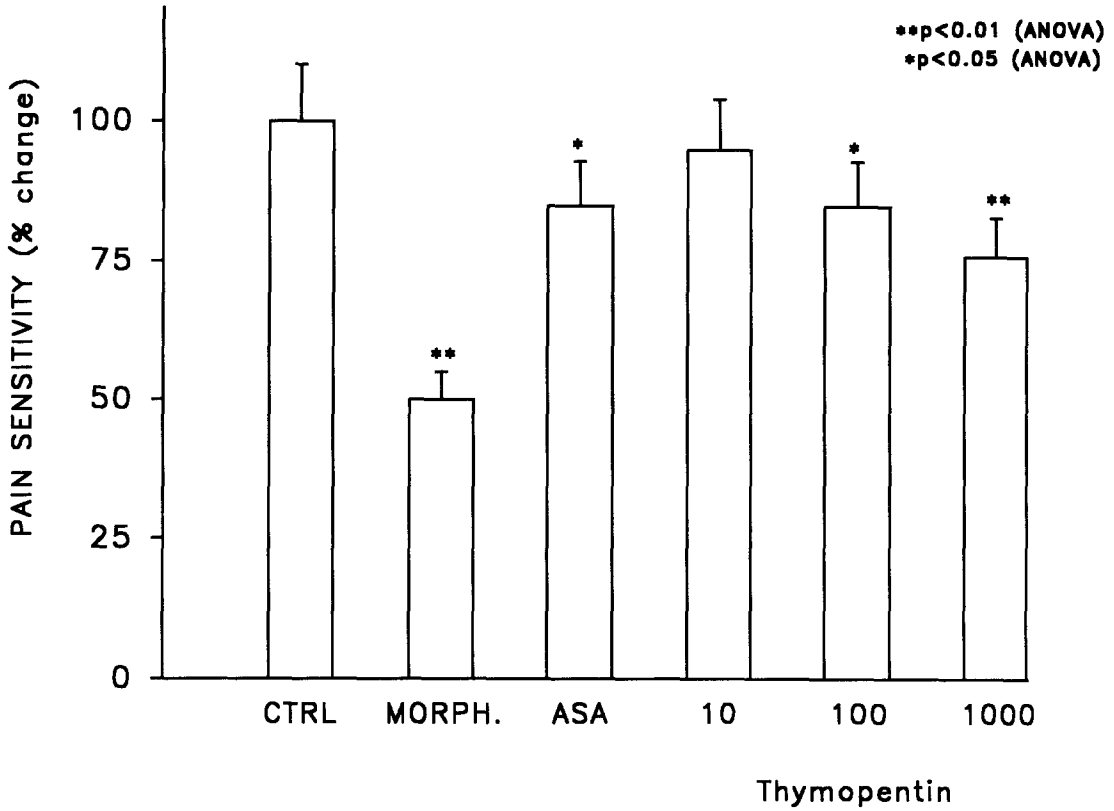

FIG. 7. Effects of thymopentin (IP injection 10 min prior to the test) on pain sensitivity in Sprague-Dawley rats as assessed by the hot-plate test; vertical bars are means ± SE; n = 8 animals/group; MORPH., morphine; ASA, acetylsalicilic acid.

was able to increase behavioral performances in those animals. Tests performed were identical to those performed with neoplastic animals (data not shown).

DISCUSSION

TP increased behavioral parameters such as ambulation, rearing, and wall rearing in mice bearing a WEHI 164 clone 13 murine fibrosarcoma compared to untreated mice bearing the same tumor. It has been reported that other mediators of the immune response may modify behavioral parameters in the intact animal (18). In this line, we also provide evidence that TP, injected in intact, freely moving mice, increases ambulation and rearing. Behavioral parameters related to the anxiety status of animals, such as grooming and boluses deposited, are decreased in response to TP injection. We have also shown that TP treatment is associated with prolongation of swimming in cold water, another parameter that parallels the increased ambulation and decrease of grooming, which is, in turn, negatively correlated with ambulation scores. In addition, we observed a decrease in plasma ACTH levels related to the increased resistance to forced swimming. In the latter test, only the single dose of 1,000 μg/kg was effective. TP at higher doses was not able to influence the test. It may be suggested that at high doses receptor desensitization occurs, with subsequent hyporesponsiveness to TP. In summary, our results indicate that TP is able to enhance spontaneous behavior and regulatory responses to stress in mice. It is plausible to hypothesize that these effects of TP are related to an interference with neuropeptide and/or peptide hormone secretion. In this line, cytokines and thymic factors have been shown to interfere with hypothalamic corticotropin-releasing hormone (CRH) secretion and/or production in the rat (2,23). The latter peptide is regarded as a major factor implicated in adaptation as it mediates behavioral responses in humans (11) and experimental species (8,22).

Second, the behavioral effects of TP may be direct as it has been shown that other mediators of the immune response act directly at the level of discrete areas of the CNS (19). In addition, binding sites for cytokines have been identified in the CNS of rodents (13).

Thus, our results provide additional evidence that thymic factors may influence behavior either directly or via interference with other mediators of the immune response and/or neuropeptides. In our example, the resulting homeostatic adjustment appears to influence positively the naturally occurring decrease in spontaneous behavior in tumor-bearing animals. Such adjustments produced by thymic factors may also be associated with increased resistance to stressful stimuli, as shown by modified responses to spontaneous and forced swimming tests.

Finally, we have shown that thymic factors interact with pain sensitivity by increasing resistance to painful stimuli. Classically, mediation of pain is possibly the result of neuropeptide effects within the CNS (1), including fragments of ACTH (6). It seems reasonable to hypothesize that the analgesic effect of TP may originate from the relative decrease in secretion of pituitary ACTH. The hyperalgesic effect of

ACTH 1-39 has been described (6) along with the analgesic effect of some of its fragments, that is, ACTH 1-9, 5-9, and 7-9 (6). In fact, secretion of such fragments parallels secretion of pituitary and central ACTH 1-39, stimulated by thymic factors (14,23).

Finally, it is plausible that thymic peptides could interfere with central production of endogenous opioids, which, similarly to ACTH, are tightly related to stress response (21), although this action would involve different sites of production.

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