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EFFECT OF SOMATOSTATIN ON THE PROLIFERATION OF MOUSE SPLEEN LYMPHOCYTES IN VITRO

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The effect of somatostatin on the spontaneous proliferation of mouse spleen lymphocytes was investigated in vitro. The rate of ^{3}H-thymidine incorporation was used as an index of lymphocyte proliferation. Somatostatin in a concentration of 10^{-7} M enhanced the lymphocyte proliferation and abolished the antiproliferative effect of rat hypothalamic extract. Lower concentrations of somatostatin slightly decreased the lymphocyte DNA synthesis.

Our knowledge of the neuroendocrine control of the immune system is incomplete. The central nervous system can influence the immune system indirectly, through the hypophysial hormones, but the possibility of a direct effect of neuropeptides must be also considered. Recently, it has been shown that beta-endorphin (1) and enkephalins (2) enhance the proliferation of lymphocytes. Several other neuropeptides such as vasopressin, oxytocin, TRH, LH-RH, neurotensin did not influence the spontaneous proliferation of the mouse spleen lymphocytes in vitro (3). However, a crude extract of rat hypothalami was found to suppress the lymphocyte proliferation (3). Since the hypothalamic extracts contain somatostatin, among many other substances, and lymphocytes possess somatostatin receptors (4), it seemed interesting to investigate whether this neuropeptide influences the lymphocyte proliferation. The results of our study are reported therein.

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MATERIALS AND METHODS

Animals. Male intact BALB/c mice, approximately 5-6 weeks of age were used as spleen donors.

Preparation of cultures of spleen lymphocytes. The cell culture system has been described in detail previously (5). Briefly, the spleens were aseptically removed and immediately transferred to MEM-medium (Gibco, Grand Island, N.Y) buffered with 10 mM Hepes to pH = 7.42. A suspension of spleen cells was obtained by enzymatic digestion with 0.1% Collagenase (Millipore Corp., N.Y.). After a 15 min exposure to collagenase, the cells were centrifuged at 250 x g for 10 min and then washed three times with the same medium. This procedure yielded a population containing more than 95% viable cells, as estimated by exclusion test with 0.5% Trypan blue. After a 30 min preincubation, the cells were counted and resuspended in RPMI-1640 medium (Gibco), supplemented with 15% fetal calf serum, 25 mM Hepes buffer, penicillin (100 U/ml) and streptomycin (100 µg/ml). The hypothalamic extract and/or somatostatin were dissolved in 100 µl of RPMI-1640 medium at the desired concentrations and added to the cell suspension containing 1 x 10^6 mononuclear cells per tube. Triplicate tubes were incubated at 37°C in humidified atmosphere of 95% air and 5% CO_2. After 4 hours of incubation, 2 µCi of ^{3}H-thymidine, specific activity 20 Ci/mMol (New England Nuclear Co., Boston, Mass.) were added in 50 µl of RPMI-1640 medium. Twenty hours later the incubation was terminated and the cells were washed with 3 ml of cold 0.9% NaCl. The precipitation of DNA and determination of radioactivity were done according to previously published methods (5,6). The results were expressed as the mean counts per minute (c.p.m.) of three cultures ± SEM. The Student's test was used to determine statistical significance.

Preparation of the hypothalamic extracts. Rat hypothalami were collected and homogenized in 0.1 M acetic acid at 4°C. The homogenate was centrifuged at 4500 r.p.m. for 45 min. The supernatant was lyophilized and the resultant powder was dissolved in RPMI-1640 medium pH 7.35 just before adding to the culture media.

RESULTS

Hypothalamic extract (HE) and lower concentrations of somatostatin (10^{-9} and 10^{-8}M) decreased ^{3}H-thymidine incorporation into lymphocytes (table 1). However, a higher somatostatin concentration (10^{-7}) produced an increase in the lymphocyte DNA synthesis, although this increase was not significant statistically. Somatostatin in the concentration of 10^{-7}M also totally abolished the antiproliferative effects of HE.

DISCUSSION

We have reported previously that rat hypothalamic extracts exert an antiproliferative effect on the mouse spleen lymphocyte proliferation in vitro (3). The factor responsible for this effect remains unknown. However, this factor may represent the porcine antimitogenic

Table 1

Incorporation of ^{3}H-Thymidine into the Mouse Spleen Lymphocytes

Additions to the Culture Medium	Concentration	cpm/culture Mean ± SEM	Significance vs Control
None (control)	-	1181.3 ± 56.4	-
Hypothalamic Extract (HE)	1 equiv/ml *	673.7 ± 61.6	$p < 0.01$
Somatostatin	10^{-9}M	846.3 ± 23.6	$p < 0.02$
Somatostatin	10^{-8}M	835.8 ± 51.1	$p < 0.02$
Somatostatin	10^{-7}M	2271.8 ± 518.7	NS
Somatostatin + HE	10^{-7}M + 1 equiv/ml *	1226.0 ± 177.1	NS

* Hypothalamic Equivalent

hypothalamic peptide, reported by Redding and Schally (7). Somatostatin in the concentration of 10^{-7}M reversed the antiproliferative effect of the HE. However, lower concentrations of somatostatin exerted some antiproliferative effect. Thus, somatostatin may have a dual influence on lymphocyte proliferation: inhibitory at lower concentrations and stimulatory at higher doses. Wagner et al. (8) have shown that when somatostatin is given systemically, it inhibits the endotoxin-induced leukocytosis in men and rats. It has been also found that somatostatin inhibits the mitogenic effects of TRH on the anterior pituitary (9) and of epidermal growth factor on gerbil fibroma cells and HeLa cells (10). The inhibitory effect of lower concentrations of somatostatin on lymphocyte proliferation seems to be an example of its more generalized antimitogenic action. At present it is difficult to explain why higher somatostatin concentrations exert a stimulatory effect. It is necessary to emphasize that the population of lymphocytes studied was not homogenous and that different lymphocyte subpopulations may react to

somatostatin in different ways. Nevertheless, the present study indicates a possible involvement of somatostatin in the neuroendocrine control of the lymphocyte.

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