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Enhancement of the In Vitro Antibody Response by Thyrotropin

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The pituitary hormone thyrotropin (TSH) has been shown to enhance in a dose dependent manner the in vitro antibody response. Highly purified preparations of bovine and human TSH enhanced up to 375% the number of cells producing antibody to sheep erythrocytes. TSH had to be present prior to 24-48h of the initiation of culture for enhancement of the antibody response. An analogy is discussed between TSH and B lymphocyte growth and differentiation factors.

Cells of the immune system have been shown to produce and to be acted upon by the neuroendocrine peptide hormones, corticotropin (ACTH) and endorphins (1-4). Immunoregulatory actions of these hormones included suppression of in vitro antibody (5) and IFN-γ production (6) plus enhancement of T cell mitogenesis (7) and natural killer cell cytotoxicity (8). Recently, we have shown that the T cell mitogen, staphylococcal enterotoxin A (SEA), induced human peripheral leukocyte production of a thyrotropin (TSH)-like molecule (9). In light of the previous finding with ACTH and endorphins, a possible immunoregulatory function for TSH was suggested. In the present report, we show that TSH is a potent enhancer of the in vitro antibody response. These data

Abbreviations used in this paper: ACTH, corticotropin; IFN, interferon; PFC, plaque forming cell; SEA, staphylococcal enterotoxin A; SRBC, sheep red blood cells; TSH, thyrotropin.

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provide further evidence for a complete regulatory circuit between the immune and neuroendocrine systems which may involve common peptide hormones.

MATERIALS AND METHODS

Mice. C57BL/6 female mice, 8 to 12 weeks old, were obtained from The Jackson Laboratories, Bar Harbor, ME.

Hormones. Purified bovine TSH was obtained from Armour (Phoenix, AZ) and Sigma Chemical Company (0.5 to 1 I.U./mg, St. Louis, MO). Highly purified human TSH was obtained from Boehringer Mannheim (>5 IU/mg, New York, NY).

Antibody production. Except for the omission of 2-mercaptoethanol dissociated mouse spleen cells were cultured for in vitro plaque forming cell (PFC) responses to sheep red blood cells (SRBC) as previously described (5). Cultures consisted of 1.5×10^7 cells in 1 ml. All PFC responses were determined on day 5 and are expressed as the mean of duplicate or triplicate cultures.

RESULTS

We first determined the effect of TSH on the in vitro PFC response of mouse spleen cells to SRBC antigen. Figure 1 shows that TSH in a dose dependent manner enhanced this response. The enhancement was evident at the lowest TSH dose tested in these experiments (3mU/ml) and

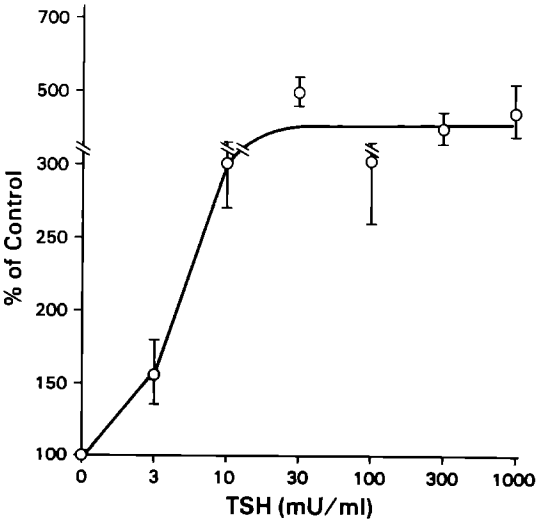

Figure 1. Dose-response of TSH enhancement of the mouse spleen cell anti-SRBC PFC response. TSH and SRBC were added to cultures at the same time. The data represent the mean ± SEM of six experiments.

was maximal by about 10mU/ml. In other experiments, we have frequently observed a significant increase with levels as low as 0.1mU/ml (data not shown). Since the specific activity of pure TSH is approximately 30 U/mg and the hormone has a molecular weight of about 28,000 d, one m U/ml of TSH represents 1nM (10). Thus TSH is a potent enhancer of the PFC response to SRBC which is active at nanomolar concentrations.

Since the previous experiments were performed primarily with bovine TSH from a single source (Armour), we were interested in the relative potency of TSH from different sources as well as from a different species. Table 1 shows that regardless of the source of TSH, the enhancement of the PFC response to SRBC by 10mU/ml was essentially the same for each. Furthermore, both bovine and human TSH were effective. This finding is consistent with the high degree of structural homology between these molecules (11) as well as the lack of species specificity of their action in classic thyroid cell assays (10).

Table 1. Effect of different sources and species of TSH on the mouse spleen anti-SRBC PFC response[a]

Experiment #	Addition	PFC/culture ±S.D.	% of control
1	Bovine TSH (Armour)	1600±174	375
	Bovine TSH (Sigma)	1307±180	306
	Control	427±122	100
2	Human TSH (Boehringer Mannheim)	1545±318	219
	Control	705±147	100
3	Human TSH (Boehringer Mannheim)	555± 21	308
	Control	180± 84	100

[a]TSH (10mU/ml) and SRBC were added to mouse spleen cell cultures on day 0.

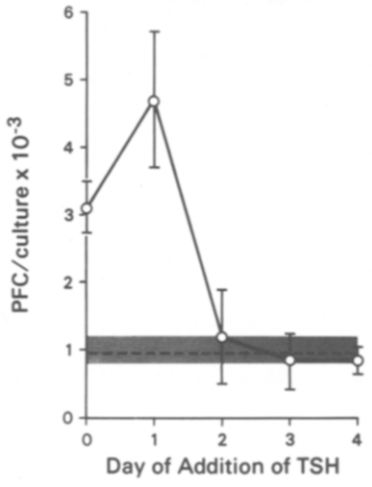

Figure 2. Kinetics of TSH enhancement of the mouse spleen cell anti-SRBC PFC response. SRBC were added to cultures on day 0 and TSH (100mU/ml) was added at the indicated times. Data are presented as the mean ± S.D. Dashed line indicates the control response in the absence of TSH ± S.D. (hatched area).

Figure 2 shows the effect of the addition of TSH to spleen cells simultaneously with or at times following exposure to SRBC. Addition of TSH could only be delayed one day in order to realize a maximal enhancement of the response. Following day 1 there was a loss of the hormone's ability to increase the PFC response. Thus it would appear that TSH enhanced the PFC response by affecting some critical early event in cell activation for antibody production.

DISCUSSION

This report describes the second situation in which a neuroendocrine peptide hormone that is produced by cells of the immune system also acts upon them. In these two instances, different stimuli, virus infection and a T cell mitogen, elicited the production of different hormones, ACTH and TSH, respectively (1-3,9). Interestingly, the two pituitary hormones in turn caused different lymphocyte responses. ACTH suppressed an early event during the antibody response while TSH enhanced the number of antibody producing cells. Thus it is tempting to speculate that in addition to possible signalling between

the immune and neuroendocrine systems, the same set of hormones may be used for intra immune system regulation. In this regard, it is interesting to note that TSH essentially behaves as a B-cell growth (BCGF) or differentiation factor (BCDF). For instance, TSH, BCGF and BCDF are all required early in the immunoglobulin secretion pathway (12). Whether or not the ir TSH induced by T-cell mitogens in lymphocytes (9) is a BCGF or BCDF remains to be determined. However, it is provocative that while the exact number of species and composition of BCGF is still clouded, some species have similar molecular weights to the α or β chain of TSH (for review see 12). Taken together, these findings seem to strengthen the notion that the immune and neuroendocrine systems may in fact represent a totally integrated circuit which shares sensory and immunoregulatory functions (13).

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REFERENCES

1. Blalock, J.E. and Smith, E.M. (1980) Proc. Natl. Acad. Sci. USA 77:5972-5974.
2. Smith, E.M. and Blalock, J.E. (1981) Proc. Natl. Acad. Sci. USA 78:7530-7534.
3. Smith, E.M., Meyer, W.J. and Blalock, J.E. (1982) Science 218:1311-1312.
4. Lolait, S.J., Lim, A.T.W., Toh, B.H., and Funder, J.W. (1984) J. Clin. Invest. 73:277-280.
5. Johnson, H.M., Smith, E.M., Torres, B.A., and Blalock, J.E. (1982) Proc. Natl. Acad. Sci. USA 79:4171-4174.
6. Johnson, H.M., Torres, B.A., Smith, E.M., Dion, L.D. and Blalock, J.E. (1984) J. Immunol 132:246-250.
7. Gilman, S.C., Schwartz, J.M., Milner, R.J., Bloom, F.E. and Feldman, J.D. (1982) Proc. Natl. Acad. Sci. USA 79:4226-4230.
8. Matthews, P.M., Froelich, C.J., Sibbit, W.L. and Bankhurst, A.D. (1983) J. Immunol. 30:1658-1662.
9. Smith, E.M., Phan, M., Coppenhaver, D., Kruger, T.E. and Blalock, J.E. (1983) Proc. Natl. Acad. Sci. USA 80:6010-6013.
10. Rapoport, B. and Adams, R.J. (1978) Metabolism 27:1732-1742.
11. Sariam, M.R. and Li, C.H. (1973) Biochem. Biophys. Res. Comm. 54:426-431.
12. Howard, M. and Paul, W.E. (1983) Ann. Rev. Immunol. 1:307-333.
13. Blalock, J.E. 1984. The immune system as a sensory organ. J. Immunol. 132:1067-1070.