tyrosine kinase, then IFN-γ and GM-CSF would have different pathways of action.

The regulation of macrophage activation by GM-CSF provides a mechanism whereby T lymphocytes, in response to antigen, may regulate a nonspecific effector function of macrophages in the absence of any requirement for an exogenous signal provided by bacterial products. This may therefore represent an important pathway of antitumor defense.

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The Neuroendocrine Thymus: Coexistence of Oxytocin and Neurophysin in the Human Thymus

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Immunoreactive oxytocin and neurophysin were identified and measured by radioimmunoassay in human thymus extracts. Serial dilutions of extracts paralleled the appropriate standard curves. Thymus-extracted oxytocin and neurophysin eluted in the same positions as reference preparations on Sephadex G-75. Authenticity of oxytocin was confirmed by biological assay and high-performance liquid chromatography analysis. In most instances, thymus contents of oxytocin and neurophysin were far greater than those expected from known circulating concentrations and declined with increasing age. The molar ratio of oxytocin to neurophysin in thymus was similar to that found in the hypothalamo-neurohypophyseal system, which strongly suggested with the other data a local synthesis of oxytocin. These findings indicate the presence of neurohypophyseal peptides in the human thymus and further support the concept of a neuroendocrine function integrated in an immune structure.

RECENT STUDIES HAVE PROVIDED evidence of reciprocal interactions between the neuroendocrine and immune systems (*1*). In this respect, thymic hormones, besides their known immunological properties, have been found to modulate some important hypothalamo-hypophyseal functions (*2*). In the thymus, distinct cell populations have been identified through the use of monoclonal antibody A_2B_5, which recognizes a complex ganglioside expressed on the membrane of neurons and neural crest-derived and neuropeptide-secreting endocrine cells (*3*). The thymus contains mesenchymal cells, probably of neural crest origin; the development of thymic epithelium may depend on induction by neural crest mesenchyme (*4*). To our knowledge, the presence of neurohormones in the thymus has not been systematically investigated until now.

Oxytocin is a 1000-dalton nonapeptide synthesized in hypothalamic magnocellular neurons as a large molecular weight precursor which is then cleaved during axonal transport into oxytocin and a 10,000-dalton oxytocin-related carrier protein, neurophysin (*5*). The main physiological actions of oxytocin in mammals are the stimulation of milk ejection and uterus contractions. Oxytocin and neurophysin are widely distributed in the central nervous system (*6*) and in peripheral organs such as ovary, testis, and adrenal medulla (*7*). These data led us to investigate the presence of oxytocin and neurophysin in the human thymus.

Thymus tissues were obtained from six patients undergoing cardiovascular or thoracic surgery for different reasons (Table 1): therapeutic thymectomy for myasthenia gravis (patient 1), coronary bypass (patients 2 and 3), anterior mediastinal mass (patient 4), and congenital heart disease (patient 5). One thymus was excised at autopsy of a newborn who died after acute respiratory distress (patient 6). Care was taken to obtain thymuses from patients in early childhood to advanced adult age; no other selection method was used. In most cases, thymic fragments were histologically analyzed (Table 1). The anterior mediastinal mass was found to be a thymoma (patient 4). In patient 1, further routine immunohistological analyses revealed a normal distribution of lymphoid phenotypes Leu-1 to Leu-4.

Thymic specimens were weighed, finely minced in a dish on crushed ice, and homogenized by sonication in 0.4*M* acetic acid at 4°C (1 ml per 100 mg of tissue). Homogenates were centrifuged at 13,000*g* for 20 minutes at 4°C; then supernatants were separated and filtered to remove insoluble particles. When mentioned further, the term "crude extract" will refer to this preparation step. An additional extraction procedure was carried out before we measured immunoreactive or biological activities and subjected the extract to high-performance liquid

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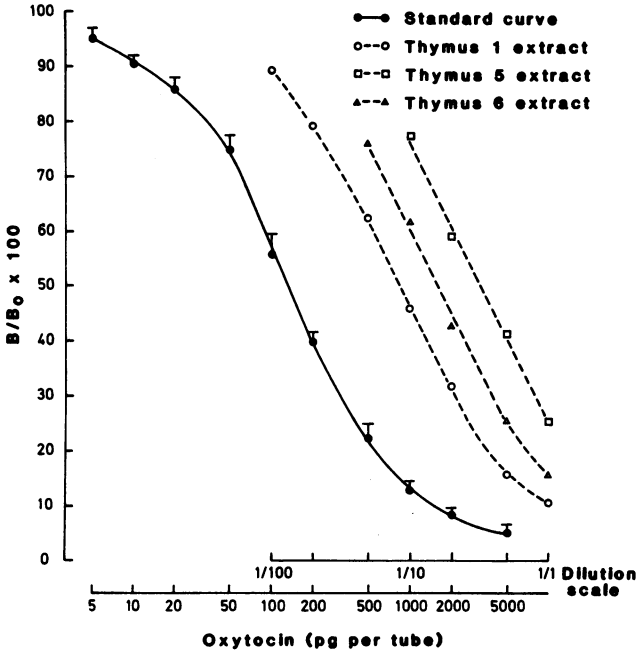

Fig. 1. Oxytocin immunoreactivity of serial dilutions of human thymus extracts compared with the oxytocin standard curve. The ordinate shows bound ^{125}I-labeled oxytocin (B) expressed as the percentage of ^{125}I-labeled oxytocin bound in the absence of synthetic oxytocin (B_o). The points on the standard curve are given as mean ± 1 SEM of triplicate incubations.

chromatography (HPLC): 2-ml portions of crude extract equivalent to 0.2 g of tissue were subjected to gel filtration on a Sephadex G-25 column (0.9 × 30 cm) eluted at 4°C in 0.1*M* ammonium acetate at *p*H 6.8. Fractions of 1 ml corresponding to the elution volume of synthetic oxytocin (fractions 13 to 20) were pooled, lyophilized, and stored at −20°C before the next analyses.

Table 1 shows the amounts of immunoreactive oxytocin and neurophysin detected in human thymus extracts by specific radioimmunoassays (*8, 9*). Thymic oxytocin content was particularly high in patient 1 when compared with the other patients; in general the oxytocin concentration of the thymus declined with increasing age of the patient. In thymus extracts 2 to 6, the mean molar ratio of oxytocin to neurophysin was 1.1 ± 0.2 (SEM). This ratio was clearly higher in the thymus extract 1, so it was excluded from the global mean.

Figure 1 illustrates the parallelism observed between the oxytocin standard curve and the displacement curves produced by serial dilutions of three separate thymus extracts. Similar curves were obtained with the extracted neurophysin immunoreactivity.

An extract of thymus 1 was tested for oxytocin biological activity by an assay with isolated rat uterus (*10*). A uterine contraction that was typical of oxytocin occurred with an estimated oxytocin-like bioactivity of 4.5 mIU (corresponding to 7.7 ng of the Fourth International Oxytocin Standard used as reference). This value was in quantitative agreement with the amount detected by radioimmunoassay (7.1 ng per 0.2 g of extracted tissue).

Figure 2 shows the results from Sephadex G-75 chromatography of the thymus crude extracts 5 and 6. In both cases, separate peaks of oxytocin and neurophysin immunoreactivities were evidenced in the same positions as their respective reference preparations. A discrete peak of immunoreactive oxytocin was also coeluted in fractions of the ascending part of the neurophysin peaks.

Further characterization of the thymic oxytocin-like peptide was determined by HPLC analysis of thymus extracts 3 and 6 (Fig. 3). In each case, a single peak of oxytocin immunoreactivity was observed

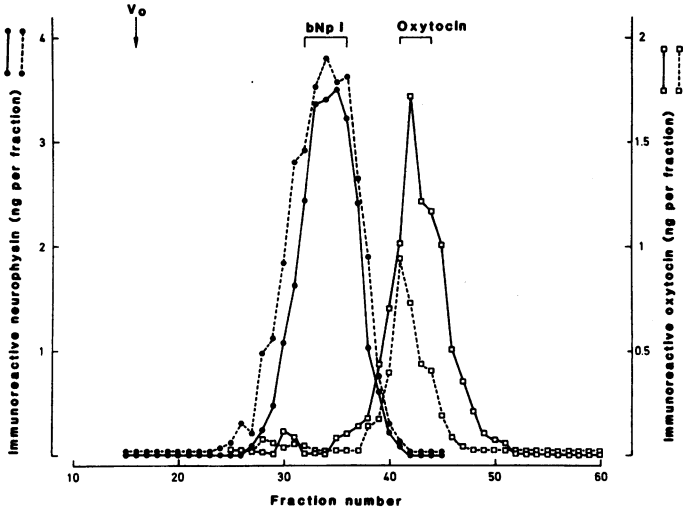

Fig. 2 (left). Sephadex G-75 elution profiles of neurophysin (●) and oxytocin (□) immunoreactivities of two separate thymus crude extracts. Four milliliters of samples 5 (---) and 6 (—) were applied to a column of Sephadex G-75 (medium, Pharmacia, 0.9 by 60 cm) and eluted at 4°C in 0.1*M* formic acid with a flow rate of 15 ml/hour. Fractions of 1 ml were collected, lyophilized, and assessed for oxytocin and neurophysin immunoreactivities. Synthetic oxytocin and purified bovine oxytocin-associated neurophysin (bNp I) were used as reference preparations and chromatographed in identical conditions.

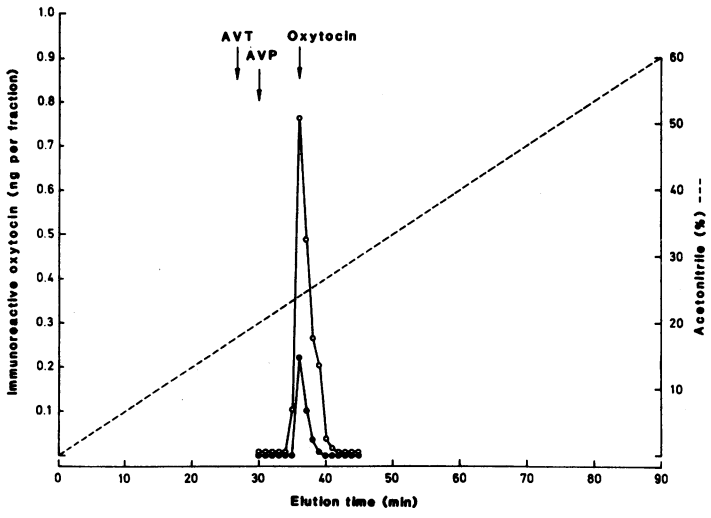

Fig. 3 (right). HPLC elution profiles of oxytocin immunoreactivity of thymus extracts 3 (●) and 6 (○) after preliminary Sephadex G-25 filtration. HPLC system (Waters Associates) consisted of a solvent delivery system (model 6000 A), a microprocessor-based system controller (model 720) and a variable wavelength detector. The column used was a 10-μm Bondapak C-18 (0.39 × 30 cm). Elution was performed in 1% trifluoroacetic acid, and a linear gradient from 0 to 60% acetonitrile was applied for 90 minutes with a flow rate of 1 ml/min. Fractions of 1 ml were collected, lyophilized, and assayed for oxytocin immunoreactivity. The positions of standard peptides oxytocin, arginine-vasopressin (AVP), and arginine-vasotocin (AVT) were determined by means of ultraviolet detection after elution of thymus extracts to avoid HPLC shadowing artifacts.

Table 1. Clinical characteristics, thymus histological analyses, and immunoreactive oxytocin and neurophysin contents. Reproducibility of the assays was tested for samples 1 and 5; mean coefficients of variation were in the range of values previously reported for oxytocin and neurophysin assays (*8, 9*)—14.7 and 16.8%, respectively. Mean molar ratio of oxytocin:neurophysin for patients 2 to 6 was 1.1 ± 0.2. Human blood concentrations reported for oxytocin and neurophysin are 0.3 to 6.7 pg/ml (*11*) and 0.1 to 7.5 ng/ml (*9*), respectively.

Patient				Thymus			
Number	Age (years)	Sex	Diagnosis	Histology	Oxytocin (ng/g wet weight)	Neurophysin (ng/g wet weight)	Oxytocin: neurophysin molar ratio
1	26	M	Myasthenia gravis	Normal	35.5	52	6.8
2	51	M	Coronary bypass	Normal (adipose involution)	2.2	24	0.9
3	31	M	Coronary bypass	Normal (partial adipose involution)	3.4	27	1.2
4	52	F	Anterior mediastinal mass	Thymoma	1.5	19	0.8
5	8	M	Ductus arteriosus	Normal	10.5	142	0.7
6	1	M	Acute respiratory distress	Not examined	18.4	94	1.9

running in the same elution site as authentic oxytocin.

Altogether, these data show the presence in the human thymus of a peptide sharing biological, immunological, and physicochemical properties with authentic oxytocin. A neurophysin immunoreactivity was also reported, usually with a molar ratio of oxytocin:neurophysin analogous to that found (1:1) in the hypothalamus. Thymic contents of oxytocin and neurophysin were considerably higher than those expected from the previously reported human blood concentrations: 0.3 to 6.7 pg/ml for oxytocin (*11*) and 0.1 to 7.5 ng/ml for neurophysin (*9*). The coexistence of high equimolar amounts of oxytocin and neurophysin in the human thymus strongly suggests a local biosynthesis by cleavage from a common precursor as demonstrated in the hypothalamus (*5*). Also supporting this hypothesis was the coelution of some oxytocin immunoreactivity at the start of the neurophysin peaks during Sephadex G-75 chromatography (Fig. 2), which may indirectly reflect the presence of such a larger precursor in the thymus. In fact, the high concentrations of immunoreactive neurophysin in the thymic gland would be difficult to explain if this organ were just a target for the circulating neurohypophyseal peptides since peripheral receptors to neurophysin have not been described. Moreover, such a peripheral biosynthesis of oxytocin has already been demonstrated in the bovine corpus luteum (*12*).

The myasthenic patient constituted an exception, with a molar ratio of oxytocin to neurophysin far greater than the theoretical one. The intrathymic oxytocin content was particularly high in this patient compared with other patients, but we do not know the physiopathological reason for this finding. Numerous abnormalities have been described in thymuses of myasthenic patients, including thymoma (*13*) (which was not detected in our patient), activation of thymic B lymphocytes (*14*), and an excess of myoid cells bearing acetylcholine receptors (*15*). Related or not, the high oxytocin content in the thymus of a myasthenic patient raises several questions for further investigations.

The nature of oxytocin-producing cells in thymus is still to be determined, but some hypotheses may be advanced on the basis of recent reports. Lymphocytes produce adrenocorticotropin- and endorphin-like substances, two polypeptides also coordinately synthesized in the anterior pituitary as a large molecular weight precursor, as well as immunoreactive thyroid-stimulating hormone and human chorionic gonadotropin (*16*). Thus, the production of oxytocin and associated neurophysin by thymocytes must be regarded as plausible. Another possibility is that some thymic stromal cells derived from the neural crest are the oxytocin- and neurophysin-secreting cells. This suggestion would be more appropriate with regard to a proper neuroendocrine function of the thymus. Immunohistological studies with the use of antibodies to oxytocin and monoclonal A_2B_5 antibodies used conjunctively are needed to clarify this point.

The physiological meaning of the presence of oxytocin-related peptides in human thymus is still unknown. It is tempting to speculate that thymocytes could be the targets for oxytocin. Both vasopressin and oxytocin have replaced interleukin-2 (the T-cell growth factor) for γ-interferon production by Lyt 2+ mouse lymphocytes (*17*). Although the neurohypophyseal peptides are not directly mitogenic for peripheral lymphocytes [(*17*) and personal observations], vasopressin stimulates DNA synthesis in bone marrow cells (*18*). Recently the role of several neuropeptides in the control of cell proliferation has been suggested (*19*). Finally, oxytocin was reported to stimulate glucose oxydation in rat thymocytes (*20*). Therefore, some comitogenic, inductive, or repressive actions of thymic oxytocin during thymocyte differentiation are attractive speculations deserving of future investigation.

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Receptor-Associated Resistance to Growth Hormone–Releasing Factor in Dwarf "Little" Mice

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Anterior pituitaries from the dwarf mouse strain "little" did not release growth hormone or accumulate adenosine 3',5'- monophosphate (cyclic AMP) in response to human and rat growth hormone–releasing factor (GRF). Dibutyryl cyclic AMP, as well as the adenylate cyclase stimulators forskolin and cholera toxin, markedly stimulated growth hormone (GH) release. The basis of the GH deficiency in the little mouse may therefore be a defect in an early stage of GRF-stimulated GH release related either to receptor binding or to the function of the hormone-receptor complex.

MOST CASES OF CONGENITAL HUman growth hormone (GH) deficiency are idiopathic; that is, no organic lesion or other etiological factor can be identified. The disorder may involve only GH (isolated GH deficiency) or may be associated with deficiencies of other pituitary hormones (*1*). Since histological and ultrastructural studies of pituitaries from patients with isolated GH deficiency have revealed somatotrophs capable of GH synthesis (*2*), it has been suggested that stimulation of the pituitary by hypothalamic GH-releasing factor (GRF) is defective in many of these patients (*1*). Such a defect could be attributed to impaired GRF synthesis, release, or transport or to insensitivity of the somatotrophs to GRF.

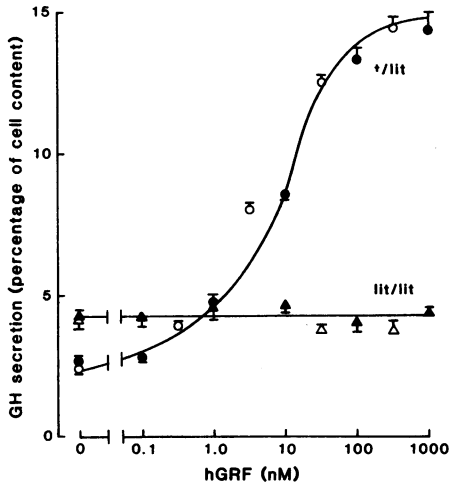

Fig. 1. Effect of hGRF on GH release from dwarf *lit/lit* and normal +/*lit* pituitary cells during a 4-hour incubation. Each point represents the mean ± SEM of four to eight observations. The dose-response curve of +/*lit* mice was analyzed by the ALLFIT program (*22*). Results are pooled data from two separate experiments. Filled and open symbols represent experiments 1 and 2, respectively.

The recessive autosomal little (*lit*) mutation in mice results in decreased growth and partial GH deficiency. It has been suggested that the little mouse represents a useful model for isolated human GH deficiency, especially the inherited isolated GH deficiency type I (*3*). We now report that pituitaries of little mice are completely insensitive to GRF, and we address the cellular basis for this phenomenon.

Homozygous 60- to 80-day-old female little mice (*lit/lit*) were compared with their heterozygous litter mates (+/*lit*), which are phenotypically identical to normal C57 BL/6J (+/+) mice. Anterior pituitaries were dissociated (*4*) and cultured for 3 days before being used. The yield from *lit/lit* and +/*lit* mice ranged from 0.45 to 0.5 × 10^6 and 1.2 to 1.4 × 10^6 cells per pituitary, respectively. Mouse GH was measured by a rat GH radioimmunoassay (RIA) (*5*).

The little mouse (*lit/lit*) pituitary contained only 9 percent of the normal concentration of GH in heterozygotes [902 ± 25 ng (mean ± SEM) compared with 9748 ± 298 ng per 10^5 cells], confirming previous observations (*3*). Incubation of +/*lit* pituitary cells with human GRF (1-40)-OH (hGRF) at 300 to 1000 n*M* for 4 hours stimulated GH secretion by a factor of six (Fig. 1). The half-maximal stimulatory concentration (EC_{50}) of hGRF, was 6 n*M*. In contrast, pituitary cells from *lit/lit* mice did not respond to hGFR, even at a concentration (1000 n*M*) that was more than 100 times that of the EC_{50} for +/*lit* pituitaries. These results are supported by the observation that anesthetized little mice do not release GH in response to the intravenous injection of a GRF fragment (*6*).

Exposure of +/*lit* pituitary cells to hGRF for 24 hours also caused a dose-dependent stimulation of GH release (Table 1), whereas GH secretion from *lit/lit* somatotrophs remained unaffected. Human GRF increased total GH (medium + cells) in cultures of +/*lit* but not *lit/lit* somatotrophs. These results suggest that hGRF-stimulated transcription of the GH gene and subsequent GH synthesis (*7*) does not occur in *lit/lit* pituitaries.

We next examined the cellular mechanisms responsible for the absence of GRF responsiveness in *lit/lit* pituitaries. GRF induces a rapid increase in intracellular adenosine 3',5'-monophosphate (cyclic AMP) concentrations, which seems to mediate the hormone's effects on GH synthesis and release (*8, 9*). Human GRF had a pronounced dose-related effect on cyclic AMP accumulation in pituitary cells from +/*lit* but not *lit/lit* mice (Fig. 2). The absence of increased

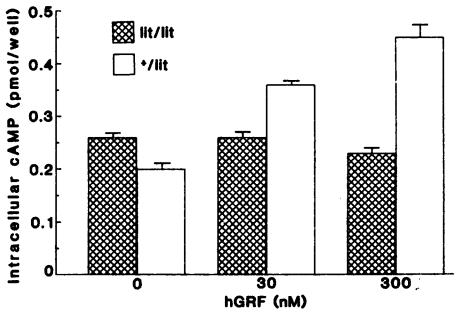

Fig. 2. Effect of hGRF on intracellular cAMP levels as measured by RIA (*23*) in pituitary cells from +/*lit* and *lit/lit* mice. Incubations were of 4 hours' duration. Results are the mean ± SEM of four observations.

cyclic AMP concentrations in *lit/lit* somatotrophs may therefore constitute an etiological factor in the lack of GH response to GRF. To test this possibility, we investigated the effects of a cyclic AMP analog and stimulators of the adenylate cyclase (AC)–cyclic AMP system other than GRF. Dibutyryl cyclic AMP induced a dose-related increase in GH secretion from +/*lit* as well as *lit/lit* pituitaries (Fig. 3). Forskolin, a plant diterpene capable of stimulating the catalytic subunit (C) of the AC system in the absence of the regulatory subunit (G_S) (*10*), stimulated GH secretion in *lit/lit* and +/*lit* cultures. Cholera toxin, an agent that in-

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