Commentary

Thymic peptides and neuroendocrine-immune communication

G. Millington and J. C. Buckingham

Introduction

Communication between the neuroendocrine and immune systems is crucial to host defence in both health and disease for it provides a humoral means whereby the central nervous system may fine tune the immune system and thereby bring to bear the influence of a variety of physical, emotional and environmental factors. In the past decade, several lines of communication between the two systems have been identified. These include direct autonomic innervation of lymphoid tissues and humoral factors derived from immune cells (e.g. cytokines, eicosanoids, peptides) and peripheral endocrine glands (e.g. peptides, steroids). Central to this complex interplay are the thymic hormones, a heterogeneous family of polypeptides produced by the thymic epithelium whose members include thymosin \( \alpha_1 \), thymosin \( \beta_4 \), thymopoietin, thymulin, MB-35 and a number of less well-characterized peptides. These peptides possess a spectrum of immunoregulatory properties. In addition, they provide the basis of a significant humoral link between the thymus and the neuroendocrine system and themselves are subject to regulation by hormones derived from the pituitary gland and peripheral endocrine organs (Fabric, Mocchegiani, Muzzioli & Provinciali, 1989). This article will address the interrelationships of these immunoregulatory peptides with the neuroendocrine system with specific reference to thymulin.

Thymulin

Thymulin, a highly conserved nonapeptide, was originally characterized as facteur thymique serique from porcine serum and subsequently purified from human serum and calf thymus (Bach, Dardenne, Pleau & Rosa, 1977). The peptide, whose biological activity is Zn\(^{2+}\)-dependent (Dardenne, Pleau, Nabarra et al. 1982), is synthesized within the thymus by two discrete populations of epithelial cells located in the subcapsular/perivascular cortex and medulla respectively (Kendall, Safieh, Sareen et al. 1991). Its distribution within the thymus closely parallels that of thymosin \( \alpha_1 \) and thymopoietin II with which it may be co-localized (Savino & Dardenne, 1984) but differs markedly from that of other thymic peptides such as thymosin \( \beta_4 \) which is produced primarily by the cortical epithelia (Schulof, Naylor, Sztein & Goldstein, 1987). The gene encoding thymulin has not yet been isolated but several lines of evidence suggest that, like other polypeptide hormones, the peptide is formed by post-translational cleavage of a precursor molecule (Savino, Gastinel, Wolff & Dardenne, 1985), packaged and stored in membrane-bound vesicles (Savino & Dardenne, 1986) and released by a process of Ca\(^{2+}\)-dependent exocytosis (Buckingham, Safieh, Singh & Kendall, 1991). Thymulin is readily detectable in the blood of rats, man and a variety of other species by biological assay (Dardenne, Pleau, Nabarra et al. 1977) and by radio- or enzyme-linked immunoassays (Safieh, Kendall, Norman et al. 1990; Schulof et al. 1987). In line with thymic mass, the circulating levels of the peptide are invariably higher in neonates and very young subjects than in adults and are undetectable in plasma from nude mice or thymectomized animals (Safieh et al. 1990), suggesting that the circulating peptide is indeed derived primarily from the thymus.

The role of thymulin within the immune system is a focus of much current research. Several lines of evidence indicate that the peptide influences T-cell function by promoting the induction of differentiation markers on immature precursor cells (i.e aiding recruitment) and, dependent on dose, by modulating both the repertoire of mature T cells released into the periphery and the activity of T-suppressor and natural killer cells (Incsey, Mertelsmann, Yata et al. 1980; Bach, 1983; Dokhlar, Tursz, Dardenne & Bach, 1983; Mutchnik, Schaffner, Prieto et al. 1983; Sztein & Goldstein, 1986; Hadden, Galy, Chen et al. 1989;
Kendall, 1991; Kendall, Safieh, Buckingham & Ritter, 1992). It may also exhibit a number of other immunoregulatory properties and, for example, has recently been shown to modulate cytokine release from human mononuclear cells in vitro (B. Safieh, personal communication). Interestingly, plasma thymulin is decreased in patients with certain immunodeficiency syndromes (Bach & Dardenne, 1973; Inceý, Dardenne, Pahwa et al., 1977; Iwata, Inceý, Cunningham-Rundles et al., 1981; Bordigoni, Foure, Bene et al., 1982; Rubenstein, Novick, Sicklick et al., 1986; Schulof et al., 1987) including children with acquired immune deficiency syndrome (AIDS) and AIDS-related complex in whom the decline in circulating thymulin precedes the development of peripheral blood T-cell abnormalities (Rubenstein et al., 1986). Deficiencies in thymulin secretion have also been described in human subjects and experimental animals with various autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis (MS) and autoimmune diabetes (Bach, Dardenne & Solomon, 1973; Dixon, Theofilopoulos, Izui & McConahey, 1980; Calabrese, Bach, Currie et al., 1981; Amor, Dougados, Mery et al., 1984; Dardenne, Savino, Gastinel et al., 1983; Mocchegiani, Boemi, Fumelli & Fabris, 1989). The relationship between thymulin deficiency and the aetiology of the various disease states is as yet unclear. However, in limited clinical trials (not subject to randomized double-blind control), thymulin has produced apparently beneficial effects in subjects with rheumatoid arthritis (Amor et al., 1984) and in children with immunodeficiency syndromes (Bordigoni et al., 1982; Fauci, Macher, Longo et al., 1984). Furthermore, in experimental animals, the synthetic peptide has been shown to suppress the symptoms of acute experimental allergic encephalitis (an animal model of MS; Nagai, Osanai & Sakakibara, 1982; Kato & Nakamura, 1988). Similarly, the aberrant T-cell responses evident in NZB mice (a model of human autoimmune disease) are reduced by thymulin, although to our knowledge no long-term improvement in survival or clinical state has yet been reported (Bach, Bach, Bianot et al., 1978; Israel-Beit, Noel & Bach, 1983). Further studies in a variety of animal models of immunodysfunction may provide new insights into the pathophysiological role and clinical potential of thymulin and related compounds.

**Hormonal control of thymulin release**

Recent evidence suggests that the secretion of thymulin is regulated largely by blood-borne hormones derived from the pituitary gland and peripheral endocrine organs, a phenomenon which provides an effective means of relaying information concerning the pathophysiological, emotional and environmental status of the organism to the immune system. Local factors may serve to fine tune the secretory responses and, in addition, thymulin may negatively regulate its own release (Cohen, Berrín, Dardenne & Bach, 1981; Buckingham, Safieh, Singh et al., 1992).

Early evidence of a role for the pituitary gland was derived from studies which revealed that destructive lesions of the adenohypophysis attenuate thymulin production (Fabris et al., 1989). Both prolactin and growth hormone, pituitary hormones released in response to certain stressors, have been shown to be effective secretagogues. In the mouse, daily administration of prolactin causes significant increases in both the synthesis and release of thymulin while bromocriptine, which inhibits prolactin secretion, lowers the circulating levels of thymulin, an effect which is overcome readily by simultaneous administration of prolactin (Dardenne, Savino, Gagnérot et al., 1989). Similarly, in man hyperprolactinaemia is associated with an increase in plasma thymulin concentration (Timsit, Safieh, Gagnérot et al., 1990) while in vitro prolactin elicits the release of thymulin

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**Table 1. Thymic hormone preparations. Molecular weights are shown in parentheses.**

Several other peptides have been partially purified including thymosin α₁ (≈2200), thymosin α₁ (as thymosin α₁, plus an additional seven amino acids at the C terminus), thymopoietin I (differs from thymopoietin II by two amino acids).

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Chemistry</th>
<th>Sequenced</th>
</tr>
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<tbody>
<tr>
<td>Thymosin fraction V</td>
<td>Partially purified extract of calf thymus; &gt;40 peptides</td>
<td>—</td>
</tr>
<tr>
<td>Thymosin α₁</td>
<td>28 amino acids (3108)</td>
<td>±</td>
</tr>
<tr>
<td>Thymosin β₁</td>
<td>43 amino acids (4982)</td>
<td>±</td>
</tr>
<tr>
<td>Thymulin</td>
<td>9 amino acids (847)</td>
<td>±</td>
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<tr>
<td>Thymopoietin I</td>
<td>49 amino acids (5562)</td>
<td>±</td>
</tr>
<tr>
<td>MB-35</td>
<td>35 amino acids (3756)</td>
<td>±</td>
</tr>
<tr>
<td>Thymolymphotropin</td>
<td>Partially purified extract of bovine thymus; &gt;20 peptides</td>
<td>—</td>
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<tr>
<td>Thymic factor X</td>
<td>Partially purified extract, major component 4-2 kDa</td>
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from human and murine thymic epithelial cells maintained in culture (Dardenne et al. 1989). With respect to growth hormone, the secretion of thymulin is impaired in both dwarf mice (Fabris, Pierpaoli & Sorkin, 1971) and patients with pituitary-dependent disease (Fabris, Mocchegiani, Muzzioli & Imerbi, 1988). The deficiencies in thymulin production in the latter are overcome by administration of growth hormone (Fabris et al. 1989) as too is the decline in serum thymulin evident in ageing dogs (Goff, Roth, Arp & Incely, 1987). Some discrepancy exists as to the site and mode of action of growth hormone. On the basis of observations that human but not bovine or rat growth hormone elicits the release of thymulin from cultured murine and human epithelial cells, Dardenne et al. (1989) favoured an action at the thymic level and suggested that the responses to the human peptide may be mediated via prolactin receptors to which, unlike the bovine or rat peptides, it binds readily. Others, however, have suggested that the thymic response to growth hormone is mediated via insulin-like growth factor-I (Timsit et al. 1990), a concept which is supported by findings that the elevated plasma concentration of thymulin evident in acromegalic subjects is positively correlated not with the circulating levels of growth hormone but with those of the growth factor.

Deficiencies in thyrotrophin (TSH) release may also impair thymulin release but this appears to be secondary to the ensuing reduction in thyroid function. Thus, the hypothyroidism which follows surgical thyroidectomy is associated with a consistent reduction in circulating thymulin. By contrast, in hyperthyroidism due to diffuse nodular goitre, serum thymulin is elevated, particularly in elderly subjects in whom the resting secretion of thymulin is characteristically low (Fabris, Mocchegiani, Mariotti et al. 1986). Other in-vivo studies have led to similar conclusions (Savino, Wolff, Aratan-Spire & Dardenne, 1984) as too have in-vitro experiments which have demonstrated directly the ability of thyroid hormones, particularly tri-iodothyronine (T3), to stimulate both the synthesis and release of thymulin and to induce epithelial cell proliferation (Dardenne, Savino & Bach, 1988; Fabris et al. 1986, 1989).

Despite the wealth of evidence of interplay between the pituitary-adrenal axis and the immune system, until comparatively recently little attention has focussed on the potential role of hormones of this axis in the control of thymulin release. Initial studies indicated that adrenalectomy caused a transient decrease in serum thymulin which was accompanied by an increase in the number of thymulin-positive cells in the thymus (Dardenne, Savino, Duvel et al. 1986). The data were, however, difficult to interpret as the operation also elicited the secretion of a thymulin-inhibiting factor which effectively neutralized the activity of thymulin in the bioassay system employed. Several more recent lines of evidence suggest that corticotrophin (ACTH) and possibly other pro-opiomelanocortin (POMC)-derived peptides may exert a positive influence on thymulin release. First, in both the rat and man, thymulin exhibits a circadian periodicity which closely parallels that of the pituitary-adrenal axis (Buckingham et al. 1992; Safieh, Venn, Ritter et al. 1991). Secondly, thymulin, like ACTH and the glucocorticoids, is released in conditions of acute stress, a phenomenon which may provide a means whereby an acute stress may augment immunological activity (Buckingham et al. 1992). In-vivo and in-vitro experiments suggest that the driving factor is of pituitary origin. Thus chronic adrenalectomy, which effectively elevates the plasma ACTH concentration, precipitates a pronounced hypersecretion of immunoreactive thymulin which is readily reversed by replacement therapy with dexamethasone. Furthermore, both ACTH(1–39) (Buckingham et al. 1991, 1992) and β-endorphin (Savino, Gagnerault, Bach & Dardenne, 1990), which is co-released with ACTH, elicit concentration-dependent increases in the release of thymulin from thymic tissue in vitro. Interestingly, although glucocorticoids alone produce a small reduction in thymulin release in vitro, they potentiate markedly the thymulin-releasing activity of ACTH (Buckingham et al. 1991, 1992). The mechanisms underlying this striking synergistic effect are unknown but, if it occurs in vivo, it may be of considerable importance in relation to thymulin release and hence immune function in acute stress.

Several other hormones have been implicated in the regulation of thymulin release. These include peripheral hormones such as insulin (Fabris et al. 1989), and the gonadal steroids and local hormones released from either the thymic epithelium or the thymocytes. Several polypeptides traditionally associated with the neuroendocrine system have been localized in the thymus including oxytocin and vasopressin (Geenan, Legros, Franchimont et al. 1987), somatostatin (Fuller & Verrity, 1989), met-enkephalin and β-endorphin (Bhargara, Ramarao, Gulati et al. 1989). In addition, the thymus is rich in cytokines. We are unaware of any studies in which the influence of cytokines on the release of thymulin, or indeed any other thymic peptide, has been studied in depth. However, receptors for both gonadotrophin-releasing hormone (GnRH) (Marchetti, Guacrello, Morale et al. 1988a,b) and opioid peptides (Savino et al. 1990) have been described in the thymus and there is evidence that leu-enkephalin elicits the release of thymulin in vitro (Savino et al. 1990). Furthermore, we have observed significant increases in thymulin release following stimulation of thymic tissue in vitro.
with GnRH or vasopressin (G. Millington, P. Clift, S. Singh & J. C. Buckingham, unpublished observations). The significance of these findings in relation to the paracrine control of thymulin requires further study.

Thymic peptides and neuroendocrine function

The concept that humoral factors derived from the thymus may exert reciprocal regulatory effects within the neuroendocrine system originated from observations that congenitally athymic (nude) and surgically thymectomized animals invariably exhibit disturbances in pituitary function. For example, in the mouse, neonatal thymectomy produces progressive impairment of body growth (Fabris et al. 1989) coupled with degranulation of the somatotrophs (Bianchi, Pierpaoli & Sorkin, 1971). Similar observations have been made in nude mice which also exhibit marked degranulation of the lactotrophs and reductions in the content of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the pituitary gland and in the plasma concentrations of LH, FSH, growth hormone (GH), thyroxine and T3 (Pierpaoli & Sorkin, 1972; Pierpaoli, Kopp & Bianchi, 1976; Fabris & Piantanelli, 1982) together with a number of disorders of reproductive function (Marchetti, 1989). These abnormal hormonal profiles are corrected by implantation of thymic tissue at birth (Fabris et al. 1989). With regard to the pituitary-adrenal system, the data pertaining to thymic deficiency are conflicting. Both congenitally athymic and neonatally thymectomized mice exhibit histological abnormalities of the adrenal cortex coupled with transient increases in plasma corticosterone concentration (Fabris et al. 1989). By contrast, in the rat, neonatal thymectomy inhibits the release of ACTH (Fabris & Piantanelli, 1982). Similarly, surgical removal of the thymus in the prepubertal monkey precipitates a significant reduction in the secretion of ACTH, β-endorphin and glucocorticoids.

Early evidence that the thymic peptides may effect the influence of the thymus on neuroendocrine function was derived from studies utilizing partially purified protein extracts of the calf and bovine thymus, namely thymosin V and thymolymphophorin respectively, which include not only epithelial cell peptides but also factors (e.g. cytokines) derived from the thymocytes (Schulof et al. 1987; Kouttab, Prada & Brunetti, 1988). Such studies revealed that thymosin V stimulates the release of ACTH, β-endorphin and cortisol/corticosterone but fails to influence the production of the other anterior pituitary hormones, i.e. LH, FSH, prolactin, GH and TSH when given intravenously to prepubertal monkeys or rats (Healy, Hodgson, Schultz et al. 1983) while thymolymphophorin promotes the release of both prolactin and corticosterone in the rat (Travaglini, Moras, Prada & Cocchi, 1989). In-vitro thymosin fraction V has no effect on the secretion of the glucocorticoids but, in three different pituitary preparations, it elicits Ca2+-dependent, dexamethasone-reversible increases in the release of ACTH and β-endorphin and, like vasopressin, it potentiates the secretory response to the 41 amino acid corticotrophin-releasing factor (CRF-41) (McGillis, Hall & Goldstein, 1988). In addition, thymosin fraction V elicits the release of both GH and prolactin from anterior pituitary cells in vitro (Spangelo, Judd, Ross et al. 1987). However, it has no direct effect on the secretion of LH by pituitary tissue in vitro but appears to facilitate the release of GnRH from the isolated hypothalamus in vitro (Rebar, Miyake, Law & Goldstein, 1981).

There have been few attempts to delineate the active thymic factors responsible for these effects and, as can be seen from the ensuing discussion, relatively little is known of the role of thymulin in this context. With respect to the hypothalamo-pituitary (HPA) axis, studies utilizing synthetic, i.e. ‘pure’ peptides have revealed that unlike thymosin fraction V, thymulin has no discernable effects on the basal secretion of corticosterone in the rat (Buckingham et al. 1992), while neither thymosin α1 nor thymosin β1 influence the secretion of ACTH when given i.v. to prepubertal monkeys (Healy et al. 1983). Similarly, these and several other thymic peptides, namely thymosin 11, thymopoietin 5 and prothymosin do not affect the release of ACTH from cultured pituitary cells (McGillis et al. 1988). By contrast, thymopoietin and thymopentin (the active moiety of thymopoietin) stimulate the release of ACTH and other POMC-derived peptides from cultured pituitary cells (Farah, Hall, Bishop et al. 1987; Malaise, Hazee-Hagelstein, Reuter et al. 1981) but, unlike thymosin fraction V, they do not synergize with CRF-41, suggesting that other thymic peptides may contribute to the initiation of ACTH release evoked by the partially purified thymic extracts. Interestingly, thymosin α1 but not β1 stimulates the release of corticosterone in the rat when given intracerebroventricularly (i.e.v.) (Hall, McGillis, Spangelo et al. 1982); since both peptides are produced within the central nervous system, this may reflect a local control mechanism (Hall et al. 1982).

The thymic peptides effecting the thymosin fraction V- and thymolymphophorin-induced release of the other anterior pituitary hormone have not yet been identified. Thymopoietin 1 is without effect in vivo (Malaise et al. 1981). However, two peptides, MB-35 (Badamchian, Wang, Spangelo et al. 1990) and a larger peptide of molecular weight >10 kDa (Spangelo, Ross, Judd & Macleod, 1989), are effective GH and prolactin secretagogues in vitro. The latter peptide...
also promotes the release of LH in vitro (Spangelo et al. 1989) as too does thymulin (Zaidi, Kendall, Gillham & Jones, 1989); thymulin, however, has no effect on serum LH when given intraperitoneally to rats (Buckingham et al. 1992). Thymosin $\beta_4$ readily elicits the release of GnRH from medial basal hypothalami in vitro (Rebar et al. 1981) and causes a significant increase in LH secretion when injected i.c.v. (Hall et al. 1982). Since this peptide is present in the brain these properties, which are not shared by thymosin $\alpha_1$, may reflect local regulatory actions in the hypothalamus rather than influences of the thymus gland per se. Further studies in which the actions of not only the purified peptides but also antibodies and, when available, antagonists to them are examined in a variety of in-vivo and in-vitro models should throw more light on the role of the various thymic peptides within the neuroendocrine system.

Conclusions

In addition to being the primary lymphoid organ effecting cell-mediated immunity, the thymus is also a major endocrine gland being responsible for the secretion of a heterogeneous family of polypeptide hormones. These peptides not only exert important regulatory effects within both the immune and neuroendocrine systems but also are themselves subject to control by hormones derived from the hypothalamo-pituitary axis and other peripheral endocrine organs. They thus represent an important interface between the immune and neuroendocrine systems. A deeper understanding of the pathophysiology of the thymic peptides and of other factors effecting neuroendocrine-immune communication should provide new insights into the mechanisms of host defence and may ultimately open novel avenues for pharmacological intervention in certain disease states.

REFERENCES


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