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VASOPRESSIN AND OXYTOCIN : THYMIC SIGNALS AND RECEPTORS IN T CELL ONTOGENY

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INTRODUCTION

In recent years, different peripheral sites for the synthesis of the neuropeptides oxytocin (OT) and arginine vasopressin (AVP) have been described (reviewed in 1). The debate was first opened in 1982 with the dicovery of immunoreactive (ir) OT in the ovary of various species (2,3). Cultured bovine luteal and granulosa cells were found to secrete ir OT and ir neurophysin I (NP I), the associated part of the hypothalamic precursor prooxyphysin (4,5). The demonstration of a peripheral synthesis was established by the discovery of OT gene expression in bovine corpus luteum (6), while AVP gene was shown to be expressed in Sprague-Dawley, Long-Evans, and Brattleboro rat ovary (7,8). The presence of ir OT and AVP was also reported in the testis (9,10) and in adrenals (11-13). Simultaneous detection of OT- and AVP-associated NPs in these organs constituted a first indirect evidence for a local synthesis, which was confirmed by the characterization of mRNAs in rat testicular and adrenal extracts (8). Even if AVP- and OT-like peptides from peripheral sources have not been fully sequenced, the concept of an extra-hypothalamic synthesis of these neuropeptides is now largely substantiated.

As early as 1910, Ott and Scott published the existence in the thymus, as in luteal extracts, of a factor causing milk ejection after administration to the goat (14). The thymus gland is the major site for T cell differentiation in many species. During this process, bone marrow precursors migrate into the thymus and engage in a complex programme of maturation involving the sequential acquisition of functional surface markers, the T cell repertoire, and the rearrangement of the genes coding for the T cell receptor for the antigen (reviewed in 15). At the end of the process, mature T cells leave the thymus with two separate phenotypes, the "helper" and "cytotoxic" phenotypes. Two additional major properties of the immune cellular system seem to be acquired within the thymus : the major histocompatibility complex (MHC) restriction, which means that one T cell can recognize an antigen only if it is presented in association with MHC proteins ; and the induction of "self" tolerance, which results from the clonal deletion of T cell clones highly reactive for "self" MHC molecules (16). Rather than being a pure automatic genetically-programmed process, T cell differentiation seems to be controlled and probably induced by the thymus environment. At this level could intervene both direct cell-to-cell receptor-mediated contacts between immature T cells

and thymic stromal cells (macrophages, epithelial and dendritic cells), as well as chemical paracrine messengers from the environment like thymic hormones (17) or interleukine-1. Besides these paracrine mechanisms, autocrine processes may also occur since some thymocytes subsets can produce interleukine-2 and express interleukine-2 receptor (18).

In this chapter, we should like to present our studies on the synthesis of AVP- and OT-like peptides in the thymus, and the possible intervention of these neuropeptides in the control of T cell early activation and differentiation.

IDENTIFICATION OF THYMIC OT AND AVP

High equimolar concentrations of ir OT and total NP were detected in human thymic extracts, and were found to decrease with aging. G-75 chromatography revealed the correspondence between the elution sites of thymic ir peptides and the standard preparations (purified bovine NP I and synthetic OT). On HPLC analysis, a peak of ir OT appeared with the same retention time as synthetic OT. The biological activity of one thymic extract was also tested, and a characteristic uterine contraction was evidenced with a quantified bioactivity close to what could be expected from the radioimmunoassay (19). In the normal human thymus, intratissular concentrations ranged from 1.0 to 18.4 ng/g for ir OT, and from 19 to 142 ng/g for total ir NP; higher ir OT concentrations were measured in the thymus from a myasthenic 26 yr-old man (35.5 ng/g), but without parallel increase of thymic ir NP (52 ng/g). In a second series of experiments, using octadecasilyl-silica columns extraction of human thymic samples, we found similar concentrations of ir OT (1.6 - 6.5 ng/g), whereas concentrations of ir AVP were rather lower (0.06 - 0.3 ng/g) (unpublished results). Ir AVP has also been characterized in the thymus from Sprague-Dawley, Long-Evans, Brattleboro rats and BALB/c mice, with concentrations ranging from 0.46 to 2.23 ng/g (20). In these species, thymic AVP content seemed to be dependent on mineralocorticoid function. More recently, the presence of ir OT, AVP and vasotocin has been reported in ovine fetal and neonatal thymic glands (21), with a predominance of vasotocin upon the other peptides during the fetal period. The intrathymic synthesis of OT and AVP in the human species was further confirmed by the observation in immunocytochemistry of ir NP, AVP and OT-containing thymic epithelial cells, and the simultaneous detection within the same specimens of positive hybridization in dot blot assays with OT and AVP cDNA probes (22).

CONFIGURATION OF THE THYMIC NEUROENDOCRINE MICROENVIRONMENT

Thymic neuroendocrine cells were identified by immunocytochemistry with specific polyclonal and monoclonal antibodies against OT, AVP and NP (22-24; Robert et al., in preparation). Two ir cellular subpopulations could be cleraly distin-

guished using different procedures (indirect immunofluorescence, alkaline phosphatase-antialkaline phosphatase and peroxydase-antiperoxydase techniques) : a strongly ir band of cells in the subcapsular cortex, and scattered cells in the medulla, mainly around and at the close vicinity of Hassall's corpuscles in human thymus. The same pattern of immunoreactivity was evidenced in the thymus from C57BL/Ka mice. The specificity of the procedure was confirmed by the absence of immunostaining after preincubation of the antibodies with their respective antigens. By double immunofluorescence cytochemistry, a close correspondence was observed between ir NP-containing cells and those labelled with the monoclonal A2B5. This latter antibody was previously shown to recognize a complex membrane ganglioside expressed on neuroendocrine cell types in peripheral organs (25) and some thymic epithelial subsets (26). The epithelial nature of ir neuropeptide-containing cells was established by their labelling with a monoclonal antibody against cytokeratin (24). The eventuality of a cross-reaction with some determinants of cytokeratin was discarded by different controls. Anti-OT and anti-NP did not exhibit any immunoreactivity with skin epithelium and squamous cell carcinomas, while anti-AVP showed only a slight reaction with rare basal cells and the horny layer of the skin; preabsorption of anti-OT and anti-AVP on normal skin did not alter the immunoreactivity observed in the human thymus (24).

A striking example of a neuroendocrine-immune microenvironment was given by the "thymic nurse cells" (TNCs). These large epithelial cells are located in the subcapsular cortex and the outer cortex of the thymus from different species, including man. They are characterized by their capacity of enclosing 20-200 actively dividing immature T cells within special caveoles delineated by membrane leaflets (27,28). The epithelial component of these complexes, but not the engulfed thymocytes, expresses different neuroendocrine markers (A2B5, neuron-specific enolase) and contains ir NP, OT and AVP (23). From these observations, we have postulated that TNCs are a component of the diffuse neuroendocrine system (29) or "paraneurons" (30), and we think that they represent an interesting morphological basis for the study of close intercellular communications between a neuroendocrine element, the TNC itself, and the immature TNC-engulfed thymocytes.

At this point, it is noteworthy to mention the common occurrence of paraneoplasic syndromes associated with epithelial thymomas and carcinomas. Most frequent of these are the syndromes of ectopic ACTH and of inappropriate AVP secretion (31-33). Therefore, in some pathologies, an oversecretion of thymic peptides into the bloodstream could be observed. In the light of our findings, it is conceivable that some of these thymomas could emerge from the clonal proliferation of thymic neuroendocrine cells.

PHYSIOLOGICAL SIGNIFICANCE OF THYMIC OT AND AVP

The mitogenic properties of AVP upon thymocytes have already been recognized in 1970 (34). This observation is highly relevant with regard to the high mitotic index of TNC-engulfed thymocytes (27) and could indicate a role for thymic AVP in the positive selection of thymocytes within TNCs.

The presence of ir AVP and OT in thymic epithelial medullary cells, however, supports the concept that thymic neuropeptides could also intervene at a further stage of T cell differentiation. Interestingly enough, AVP and OT were shown to replace interleukine-2 requirement for the production of gamma-interferon by cytotoxic T cells (35), an action which seems to involve V_1-type AVP receptors (36). Therefore, some (co)mitogenic or differentiative paracrine properties of thymic AVP and OT are rationale hypotheses which deserve to be further investigated.

BINDING CHARACTERISTICS OF AVP ON A MURINE THYMIC LYMPHOID CELL LINE

To characterize the molecular aspects of AVP interaction with T cells, the parameters of ^{3}H-AVP (NEN-DuPont de Nemours; specific activity = 67.0 Ci/mmol) were investigated on human thymocytes and a murine thymic lymphoma cell line. While specific binding sites could be detected at low levels on human thymocytes, the murine lymphoid cell line RL12-NP, obtained after X-ray irradiation of C57BL/Ka mice (37), was found to express high levels of ^{3}H-AVP specific binding sites. The binding of radioactivity on RL12-NP cells was time- and temperature-dependent and equilibrium at 37°C was reached within 90 min (Fig. 1).

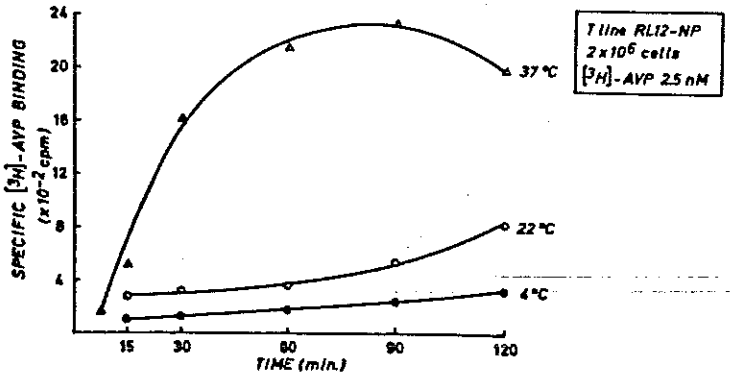

Fig. 1. Time- and temperature-dependence of the specific binding of ^{3}H-AVP(2.5nM) to RL12-NP cells (2x10^6 cells per assay).

Specific binding of tritiated AVP increased linearly with the number of RL12-NP cells present in the incubation medium (PBS, Flow Laboratories).

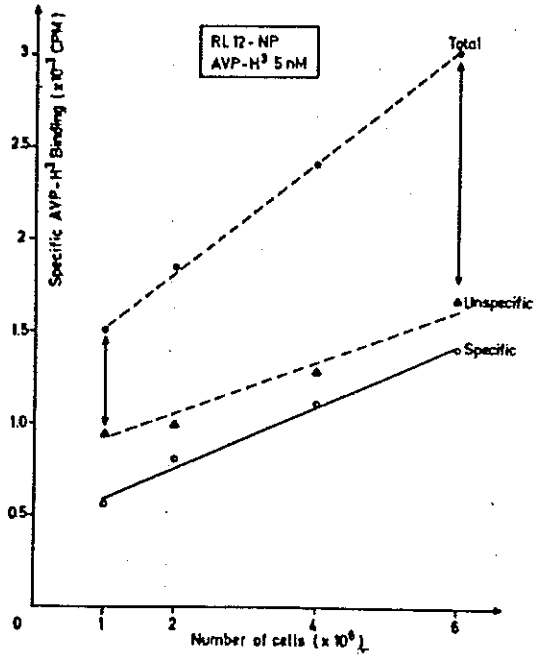

Fig. 2. Relationship between the amount of ^{3}H-AVP bound and the number of RL12-NP cells in the assay. Incubation was performed for 90 min at 37°C.

Scatchard analysis of concentration-dependent binding suggested some heterogeneity of ^{3}H-AVP binding sites, with one class of high affinity (K_D = ± 1 nM), and another one with low affinity (K_D = ± 40 nM) but very high capacity (B max = ± 180,000 sites per cell). The analysis of binding displacement curves in presence of different AVP analogues revealed that AVP and a V_1 agonist (Phe^2Orn^8Vasotocin) competed similarly, while OT and V_{1a} antagonist were much less effective. The V_2 antagonist $d(CH_2)_5$(D-Ile^2, Abu^4)AVP had no effect on the binding of ^{3}H-AVP on RL12-NP cells (Fig. 3). These preliminary data suggest that RL12-NP cells possess specific ^{3}H-AVP binding sites, which could be related to the V_{1b}-subtype with a ligand affinity analogue to the antehypophyseal AVP receptor (38). If this first conclusion remains to be further evaluated, however, it is in accordance with a previous study showing the synergistic action of AVP upon CRF-induction of ACTH-like peptides by peripheral mononuclear cells (39). Another point to consider is the nature and phenotype of murine RL12-NP cells. This cell line is, as

indicated above, derived from a radiation-induced C57BL/Ka mouse thymic lymphoid tumor; cultured cells are lymphoblastic and express high levels of Thy 1.2, Ly 1.2 and Ly 2.2, a phenotype indicative of an immature T lymphocyte population (37). The expression of AVP receptor could therefore be a new marker of the immature T cell phenotype and, from a speculative point of view, overexpression or excessive activation of AVP receptor could play a role in the induction or the maintenance of RL12-NP thymic lymphoid tumor.

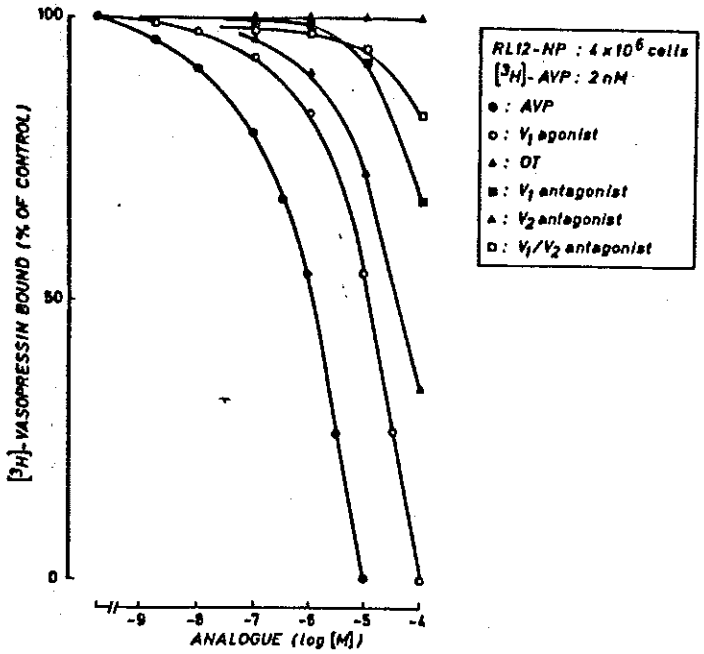

Fig. 3. Displacement of ^{3}H-AVP bound to murine RL12-NP cells by different AVP analogues. Note the poor efficiency of the V_1 antagonist d(CH_2)$_5$Tyr(Me)AVP, and the parallel displacement curves observed with AVP and the V_1 agonist.

CONCLUSIONS AND PERSPECTIVES

We have presented different experimental arguments supporting the intrathymic synthesis of the neuropeptides AVP and OT. We have described an original neuroendocrine-immune microenvironment, where AVP and OT could act as local growth or differentiative factors for immature T cells. Preliminary experiments suggest the presence of specific AVP binding sites on a murine thymic lymphoid cell line, with a ligand affinity analogue to the V_{1b}-subtype AVP receptor. We think that thymic AVP and OT could act as early activation signals for the T cell differen-

tiative programme. The intervention of this neuroendocrine thymo-lymphoid axis could be determinant in some pathological states and, consequently, new therapeutic strategies for the control of T cell function could be designed from the study of the receptor activation as well as the intracellular pathways mobilized by neuropeptide agonists and/or antagonists.

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