## Research line No. 4

## Cellular biochemistry of peptidergic neurons

**Programs**

4.1 Regulation of hypothalamic peptide metabolism.

4.2 Release and inactivation mechanisms of TRH in rodent's nervous system.

4.3 Studies on the expression of genes which code for neuropeptides in the X organ of *Procambarus bouvieri*.

**Program 4.1** Regulation of hypothalamic peptide metabolism.

We are interested in undersanding the intracellular mechanisms that participate in neuroendocrine integration. As a tripeptide released from the hypothalamus that controls the release and biosynthesis of Thyrotropin and Prolactin. Complex regulation exists by target tissue hormones and neurotrasmitters but how this effect is exered at hypothalamic level is still poorly understood. We therefore study the cellular biochemistry of the peptidergic neuron: biosynthesis, degradation and release of TRH and how these events are regulated.

To search physiological conditions where TRH gene expression is modified, TRH mRNA levels are quantified in several rodent brain regions in various physiological circunstances which include: hypo or hyperthyroid state, estrous cycle, cold exposure and lactation. In order to identify the responsible effectors, whether neural or hormonal, a hypothalamic cell dispersed culture system is being developed where the effects on biosynthesis will be studied (by measuring mRNA levels and 3H-Pro incorporation into TRH).

TRH inactivation is also studied in diverse paradigms. Results demonstrate regulation of some TRH peptidases by thyroid hormones in a tissue specific manner suggesting their involvement on feedback events.

These findings will be compared with TRH tissue levels and release in order to fully determine the intracellular steps which are under regulation.

**Specific projects**

Studies on TRH and SRIF biosynthesis and regulation in cell culture.
C. Cruz, M.L. Covarrubias, J.L. Charli and P. Joseph
1985/P/S/DBP/UB

Ontogenesis of TRH mRNA circadian rhythms in rats.
L. Covarrubias, R.M. Uribe, J.L. Charli and P. Joseph
1986/I/S/DBP/UB

Level of TRH mRNA during lactancy, in response to thyroid hormones and durling the estrous cycle.
R.M. Uribe, L. Covarrubias, P. Joseph and J.L. Charli
1986/I/S/DBP/UB

Phylogeny of the TRH gene.
L. Covarrubias, M. Rodríguez, J.L. Charli and P. Joseph
1987/I/S/DBP

**Program 4.2** Release and inactivation mechanisms of TRH in rodent's nervous system.

In this regard our research focuses on the processes involved in TRH release as well as its inactivation inside the nerve terminal and once released into the synaptic space. We explore three possible inactivating mechanisms: uptake, degradation due to soluble soluble or membrane-bound peptidases and modification. Once these phenomena are characterized, we will try to define their physiological relevance.

We have been able to: 1) demonstrate the presence of an accumulation process for TRH in brain; 2) characterize a membrane peptidase (pyroglutamate aminopeptidase II, - PGAII) with high specificity for TRH which is localized in the external site of the synaptosomal plasma membrane and has heterogeneus regional distribution in brain. We are

currently trying to determinate if this enzyme is the principal agent responsible for TRH inactivation in the synaptic cleft. We are also pursing studies on its cellular localization, regional and organ distribution and developmental control. The process of intracellular TRH degradation and the role of soluble enzymes is being studied with the use of specific inhibitors of these enzymes.

**Specific projects**

Distribution of the PGA-II in rat brain, spinal chord and peripheral organs.
M.A. Vargas, M. Cisneros, M. Méndez, P. Joseph and J.L. Charli
1984/P/S/DBP/UB

TRH degradation in slices of rat brain: effects of its inhibition on TRH release.
M. Méndez, M.A. Vargas, M. Cisneros P. Joseph and J.L. Charli
1984/P/S/DBP/UB/URIA

Effect of endocrine feedback on TRH degradation.
G. Ponce, J.L. Charli, J. Pastén, F. Mena, M.A. Vargas, C. Valverde and P. Joseph
1986/I/S/DBP/UB

Cellular localization of TRH degrading enzymes in cell culture.
C.Cruz, M.A. Vargas, J.L. Charli and P. Joseph
1986/I/S/DBP/UB

**Program 4.3**. Studies on the expression of genes which code for neuropetides in the X organ of *Procambarus bouvieri*.

The crustacean's X organ contains a series of neurosecretory cells which secrete various neuropeptides with hormonal function. Two of these are the pigment concentrating hormone (PCH) and the pigment dispersing hormone (PDH) which together control pigment concentration in the chromatophores. PCH and PDH molecular structures are known in some species, as well as some of their functions and part of their regulation. Therefore, because of the simplicity of crustacean nervous system, and the minimal interactions, they are being used as a model for answering the following question: Does nervous transmission participate and how in neuropeptide biosynthesis regulation?. The research strategy involves the isolation of the cDNA to their messenger RNAs and their use *in vivo* in various paradigms in order to delineate the regulatory events.

**Specific projects**

Search for the PCH gene through synthetic oligonucleotides.
Y. Fuchs, E. Calva, L. Covarrubias, P. Joseph and J.L. Charli.
1985/P/DBP/DGBM

Characterization of the gene and cDNA which code for DPLH.
L. Covarrubias, J. Santaolalla, H. Aréchiga and P. Joseph
1986/I/DBP/USQM