Tyrosine Hydroxylase in Vasopressinergic Axons of the Pituitary Posterior Lobe of Rats Under Salt-Loading as a Manifestation of Neurochemical Plasticity

Marina Abramova,^{1,2} André Calas,² Jean Thibault³ and Michael Ugrumov^{1,2†}

¹Laboratory of Hormonal Regulations, Institute of Developmental Biology, Russian Academy of Sciences, 26 Vavilov str., Moscow 117808, Russia; ²Département de Cytologie, Institut des Neurosciences, CNRS UMR 7624, Université P. et M. Curie, 7 quai St. Bernard, F-75252 Paris Cedex 05, France; ³Laboratoire de Neurobiologie moléculaire, Faculté des Sciences et Technologie, Université Paris 12-Val de Marne, 61, avenue de Général de Gaulle, 94010 Créteil, France; [§]Laboratory of Neurohistology, Institute of Normal Physiology, Russian Academy of Medical Sciences, 8 Baltiiskaya str, Moscow, Russia

SUMMARY

In this study, we attempted to test whether tyrosine hydroxylase (TH), the first rate-limiting enzyme of catecholamine synthesis, is confined to the perikarya of activated magnocellular vasopressinergic (VPergic) neurons or is also present in their distal axons in the pituitary posterior lobe (PL). In addition, we evaluated the possible correlation between TH and VP turnover in the axons of rats drinking 2% NaCl for 1, 2, and 3 weeks. To this aim, we examined the large swellings of VPergic axons, the so-called Herring bodies, using the double-immunofluorescent technique and the avidin-biotin technique, combined with image analysis. Here we have demonstrated for the first time a colocalization of TH and VP in Herring bodies, which is a strong argument in favor of TH transport from the perikarya of VPergic neurons via axons toward their terminals. TH-immunoreactive (IR) and VP-IR materials were

distributed in Herring bodies with seeming zonality. The number of VP-IR Herring bodies decreased by a factor of four over the first week of osmotic stimulation, remaining at almost the same low level until the end of the experiment. Conversely, the content of the VP-IR material within the individual Herring bodies fell gradually during the three weeks of salt-loading. The results suggest that VP depletion from Herring bodies prevails in its transport into these structures during the whole period of osmotic stimulation. In contrast to VP-IR Herring bodies, the number of TH-IR Herring bodies and the content of TH-IR material within the individual Herring bodies increased progressively during the entire experiment. The synchronization of the VP depletion and TH accumulation in Herring bodies during long-term osmotic stimulation raised the question about a possible functional interaction between both substances.

KEY WORDS

Herring bodies, hypothalamo-posthypophyseal system, salt-loading

[†]Corresponding author:

Tel: +7 095 135 88 42

Fax: +7 095 135 80 12

e-mail: michael-ugrumov@mail.ru

INTRODUCTION

Over the past two decades, many investigators (Brownstein & Mezey, 1986; Hökfelt et al., 1987; Verney et al., 1988; Seil et al., 1992; Nagatsu et al., 1997) have demonstrated that some non-catecholaminergic neurons, mainly peptidergic neurons, express tyrosine hydroxylase (TH), the first rate-limiting enzyme of catecholamine synthesis. Vaso-pressinergic (VPergic) neurons of the supra-optic nucleus, projecting their axons to the posterior lobe (PL) of the pituitary, are considered one of the most promising cell models for studying this phenomenon. TH is expressed in VPergic neurons under functional stimulation (Kiss & Mezey, 1986; Young et al., 1987; Meister et al., 1990a,b; Marsais & Calas, 1999) following the perturbations of neural afferents (Kiss & Mezey, 1986), during aging (Marsais et al., unpublished), and in pathology (Fetissov et al., 1997).

Taking into account that TH is a cytosolic non-secreted protein, one might expect its accumulation in the perikarya of VPergic neurons during long-term osmotic stimulation, followed by its possible transport toward the axon terminals. However, a previous attempt to detect differences in TH-immunoreactivity (TH-IR) in the PL of intact and salt-loaded animals was unsuccessful (Meister, 1990b). Certain difficulties in detecting TH in VPergic axons should be related to a minor TH expression in VPergic neurons in intact animals and, conversely, to the rapid depletion of the axons from VP under salt-loading. Moreover, differentiating TH-containing catecholaminergic and VPergic axons with a low VP level—undetectable with immunocytochemistry—is quite difficult or even impossible. Indeed, the PL is innervated by dopaminergic axons (Saavedra et al., 1975; Saavedra, 1985) belonging to the neurons of the periventricular (Kawano & Daikoku, 1987) nucleus, as well as by noradrenergic fibers issued from the superior cervical ganglia (Saavedra, 1985; Vanhatalo et al., 1995).

The difficulties in detecting both substances in VPergic axons most probably could be overcome by studying Herring bodies, the large axon swellings that are generally considered a specific attribute of VPergic axons, but not of catecholaminergic axons (Dellmann & Rodriguez, 1970; Polenov & Garlov, 1971). In contrast to the relatively small swellings of the VPergic axons, Herring bodies partly retain VP under osmotic stimulation (Ugrumov & Belenkii, 1974), namely, after the onset of TH synthesis in VPergic neurons (Kiss & Mezey, 1986).

The present study attempted to test whether TH is an attribute not only of the cell bodies but also of the axons of magnocellular VPergic neurons, subsequently evaluating a possible correlation between TH and VP dynamic in Herring bodies during long-term salt-loading of the rats.

EXPERIMENTAL PROCEDURES

Animals and Processing of Materials

Adult male Wistar rats, weighing 200 to 300 g each, were used in this study. The animals were maintained at 21°C to 23°C in a light-dark cycle (light period between 8:00 and 20:00). Control (intact) rats had free access to food and tap water (nine animals), whereas the experimental rats were given 2% NaCl instead of tap water for either 1 week (nine animals), 2 weeks (three animals), or 3 weeks (three animals). The rats were anesthetized with Pentobarbital (50 mg/kg body wt) and perfused via the heart—first with saline for 10 min at 37°C, and then with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.3) for 15 min at 4°C. Thereafter, the pituitaries were postfixed by immersion in the same fixative for 2 h at 4°C, rinsed for 1 h in 0.02 M phosphate buffer with 0.9% NaCl (PBS, pH 7.2 to 7.4) at 4°C, immersed overnight in 15% sucrose PBS at 4°C,

and then frozen in isopentane at -45°C .

Immunohistochemistry and Quantification

In the first series of immunocytochemical studies, the pituitaries of intact rats and of rats that were salt-loaded for 7 d (3 rats in each group) were used for TH and VP double-immunolabeling on the same cryostat sections, 14 μm or 30 μm thick. The sections were incubated overnight at 4°C in PBS containing a mixture of either (a) mouse monoclonal antibodies to TH (diluted 1:1000) (Sigma, USA) and rabbit polyclonal antibodies to VP (diluted 1:4000) (Chemicon, USA) or (b) mouse monoclonal anti-bodies to VP (diluted 1:20000) (Robert et al., 1985) and rabbit polyclonal antibodies to TH (diluted 1:2000), 1% normal goat serum, and 0.1% Triton X-100. The sections were then incubated for 2 h at room temperature with a mixture of the secondary antibodies, rhodamine-conjugated goat anti-rabbit (diluted 1:40) (DAKO, Denmark) and fluorescein-conjugated goat anti-mouse (diluted 1:100) (Pasteur, France). The sections were rinsed twice in PBS, embedded in a mounting medium for fluorescence (Vectorlab, USA), coverslipped, and examined using either a fluorescence microscope (Leitz Diaplan, Germany) or a confocal microscope (Leica TCS 4D, Germany). The fluorescence microscope was equipped with filters for fluorescein-induced fluorescence (450–490 nm excitation filter, 520–560 nm barrier filter, and 520–550 nm extra barrier filter) and for rhodamine-induced fluorescence (546/10 nm excitation filter and 610 nm barrier filter). The confocal microscope was equipped with an argon/krypton laser 25 mW adjusted at 488 nm and 568 nm wavelengths for the respective excitations, as well as with filter sets for the selective detection of fluorescein (488 nm, green) and rhodamine (568 nm, red) fluorescence. The 30

μm -thick sections were x/y scanned along the z-axis with 1–1.5 μm step. Each section was scanned twice: at first with oil immersion 63 $\times$ objectives for mapping Herring body distribution and then with an oil immersion 63 $\times$ with zoom 2 $\times$ objective for evaluating the fine structure of the Herring bodies. Vertical scanning planes were within the whole depth of the section.

The specificity of the antiserum to TH was determined previously (Arluison et al., 1984). Additional controls for the specificity of antibodies to VP and TH consisted of either omitting the primary antibodies or preadsorbing them with antigens. The possible cross-reactivity between primary and secondary antibodies was excluded by incubating for each antigen the corresponding mouse primary antibodies with goat anti-rabbit secondary antibodies or, conversely, rabbit primary antibodies with goat anti-mouse secondary antibodies, which resulted in no labeling. The intermediate lobe of the pituitary, being free of VP-immunoreactive (VP-IR) structures, served as an additional physiological control.

In the second series of the immunocytochemical studies, the pituitaries of intact rats, as well as those of rats that were salt-loaded for 1, 2, or 3 weeks (three animals in each group), were cut with a cryostat microtome for serial sections, 14 μm thick. Adjacent sections were mounted on two sets of slides and processed separately for either VP or TH immunocytochemistry. Each slide displayed the sections of an intact animal, as well as those of animals at any period of salt-loading (six sections per animal). The sections were incubated successively with the following: (a) goat normal serum (1%) for 30 min at room temperature; (b) rabbit polyclonal antibodies to TH (diluted 1:2000) or to VP (diluted 1:4000, Chemicon, USA) overnight at 4°C ; (c) biotinylated goat anti-rabbit antibodies (diluted 1:200, Vector, USA) for 2 h at room temperature; and (d) an avidin-biotin complex (diluted 1:100, Vector, USA) for 40 min

at room temperature. The normal serum and primary and secondary antibodies were diluted in PBS with 0.1% Triton X-100 (Sigma, USA). The sections were rinsed with PBS after each incubation, except the first. Finally, the sections were rinsed with 0.05 M Tris-HCl buffer (pH 7.6), and the peroxidase of the avidin-biotin complex was revealed in the same buffer containing 0.05% 3,3'-diaminobenzidine tetra-hydrochloride (Sigma, USA) and 0.01% H₂O₂. The developing time was constant for each antigen. The sections were dehydrated, mounted in Permount, coverslipped, and then examined with a light microscope.

Carto[®] software (IMSTAR, France) was used to carry out a quantitative and semi-quantitative examination of VP-IR and TH-IR large axon swellings ($\geq 6 \mu\text{m}$ in size), Herring bodies, in intact and salt-loaded rats. According to this approach, the relative concentrations of VP and TH could be accurately assessed, and the results obtained at different periods of osmotic stimulation could easily be compared in-between and with those in control animals. Indeed, all preparative procedures of the sections were performed under standardized conditions that allowed us to apply meaningful quantitative and semi-quantitative analysis. Each section in the entire series of the PL was reviewed under a light microscope using a 25 $\times$ objective, followed by transferring the image via video camera (CCD) to a computer monitor (IBM PC Pentium 60) and recording.

The relative concentrations of VP-IR and TH-IR materials were measured as the 'gray level' (GL), related to the optical density (OD) of the specimen, as follows (Conn, 1990):

$$\text{OD}_{\text{Specimen}} - \text{OD}_{\text{Background}} = \log(\text{GL}_{\text{Background}}) - \log(\text{GL}_{\text{Specimen}})$$

The GL of the background was measured in the vicinity of the PL, namely, in the anterior lobe with no specific immunostaining. The following average parameters of Herring bodies were estimated in intact animals, as well as in each experimental

group: (a) OD of VP-IR or TH-IR material in Herring bodies, (b) number of VP-IR or TH-IR Herring bodies per section, (c) area of the individual VP-IR or TH-IR Herring bodies, (d) relative amount of VP-IR or TH-IR material per Herring body (area $\times$ OD) as a percentage of its level in the control (intact) animals (100%). The profiles of individual Herring bodies were outlined with a light pen to obtain a morphometric mask.

Statistical analysis was carried out using descriptive statistics and the non-parametric Wilcoxon matched pair test.

RESULTS

Intact Rats

The PL of intact rats contained an abundant network of VP-IR fibers having transient and terminal swellings. A minor portion of the swellings was represented by Herring bodies, more than $6 \mu\text{m}$ in diameter (Fig. 1a). The Herring bodies were either scattered throughout the PL or concentrated in its proximal portion, as well as along the border with the intermediate lobe. In the individual Herring bodies, VP-IR material was distributed either homogeneously or mainly at the periphery, thereby showing weak immunostaining of the central portion.

The PL of intact animals was also occupied by a network of TH-IR fibers (Fig. 1b), being particularly dense along the intermediate lobe and in the most proximal region of the PL. In contrast to VP-IR fibers, TH-IR fibers possessed swellings that were usually no more than $2 \mu\text{m}$ in size. Nevertheless, occasional, relatively large and intensely immunostained TH-IR swellings were also observed (Figs. 2 to 4). No Herring bodies with colocalization of VP-IR and TH-IR materials were detected in the PL of intact animals when using immunofluorescence double-labeling.

Fig. 1: Vasopressin-immunoreactive (a, c) and tyrosine hydroxylase-immunoreactive (b, d) Herring bodies (arrows) in the posterior lobe (PL) of intact rats (a, b) and following the osmotic stimulation for a week (c, d). Avidin-biotin technique. IL - intermediate lobe. Scale bar = 20 μ m for a, c, d; 25 μ m for b.

Rats Salt-Loaded for 1 Week

Herring bodies, immunoreactive for both VP and TH, were distributed in the same fashion as in the PL of intact rats (Fig. 1c, 1d). They were usually seen in close vicinity of the intermediate lobe and the hypophysial stalk. According to the avidin-

biotin immunocytochemical study, the VP-IR Herring bodies decreased dramatically in number (by fourfold) (Fig. 2) and slightly but significantly in size (Fig. 3). Although no change in the OD of VP-IR Herring bodies was observed (Fig. 4), the relative content of VP-IR material per Herring body slightly decreased (Fig. 5).

Fig. 2: Number of the vasopressin-immunoreactive (VP-IR) and tyrosine hydroxylase-immunoreactive (TH-IR) Herring bodies in the posterior lobe per section in control (intact) rats (c) and in rats salt-loaded (2% NaCl) for 1, 2 and 3 weeks (w). * $P < 0.01$, compared to the control. ** $P < 0.01$, comparison between selected groups.

Fig. 3: Area of vasopressin-immunoreactive (VP-IR) and tyrosine hydroxylase-immunoreactive (TH-IR) Herring bodies in the posterior lobe in control (intact) rats (c) and in rats salt-loaded (2% NaCl) for 1, 2 and 3 weeks (w). * $P < 0.01$, compared with the control. ** $P < 0.01$, comparison between selected groups. NS, non significant.

Fig. 4: Optical density of vasopressin-immunoreactive (VP-IR) and tyrosine hydroxylase-immunoreactive (TH-IR) Herring bodies in the posterior lobe in control (intact) rats (c) and in rats salt-loaded (2% NaCl) for 1, 2 and 3 weeks (w). * $P < 0.01$, compared to the control. ** $P < 0.01$, comparison between selected groups.

Fig. 5: Relative amount of vasopressin (VP) and tyrosine hydroxylase (TH) per Herring body in control rats and rats salt-loaded for 1, 2 and 3 weeks (w). The results are presented as percentage of the control level (100%).

Fig. 6: Herring bodies (a, b, c, d) and small axon swellings (e) immunoreactive for vasopressin (rhodamine, red for b; fluorescein, green for c, d, e), tyrosine hydroxylase (fluorescein, green for a; rhodamine, red for c, d, e) as well as for both vasopressin and tyrosine hydroxylase (yellow for c, d, e) in the posterior lobe (PL) of rats salt-loaded for a week. a, b, fluorescent microscopy; c-e, confocal microscopy. IL, intermediate lobe. Arrows, vasopressin- and/or tyrosine hydroxylase-immunoreactive Herring bodies; arrowheads, double-labeled Herring bodies with zonality in the distribution of the immunoreactive material. Scale bar = 20 μm for a, b; 6 μm for c and 8 μm for d, e.

In contrast to the VP-IR Herring bodies, the number (Fig. 2) and the average area (Fig. 3) of the TH-IR Herring bodies increased considerably, which was accompanied by an increase of the amount of the TH-IR material per Herring body (Fig. 5).

The double-immunofluorescence study showed that numerous VP-IR Herring bodies contained TH-IR material (Fig. 6a, b), although mono-labeled VP-IR (Fig. 6c), and TH-IR were also observed. The distribution of VP-IR and TH-IR materials in some Herring bodies was not homogeneous (Fig. 6d). The VP-IR material was often distributed at the periphery of Herring bodies. In addition to Herring bodies, double-immunolabeled thin fibers with small swellings ($<1.5\ \mu\text{m}$) were regularly seen in a confocal microscope (Fig. 6e).

Rats Salt-Loaded for Two Weeks

Over the second week of osmotic stimulation, no change in the number and size of the VP-IR Herring bodies was observed (Figs. 2, 3), whereas their OD and the content of the VP-IR material per Herring body slightly decreased (Figs. 4, 5). In contrast to the VP-IR Herring bodies, the number (Fig. 2) and OD (Fig. 4) of TH-IR Herring bodies, as well as the content of the TH-IR material per Herring body (Fig. 5), increased significantly. The average area of the TH-IR Herring bodies did not change during the second week of osmotic stimulation (Fig. 3).

Rats Salt-Loaded for Three Weeks

During the third week of osmotic stimulation, the number, size, and OD of the VP-IR Herring bodies did not change (Figs. 2 to 4), whereas the content of the VP-IR material per Herring body slightly decreased. The same was true for the

average area of the TH-IR Herring bodies (Fig. 3), whereas their numbers (Fig. 2), OD (Fig. 4), and content of TH-IR material in the individual Herring bodies increased considerably.

DISCUSSION

Technical Remarks

In this study, a double-immunofluorescence technique was applied to determine whether TH is an attribute not only of the perikarya but also of the distal axons of stimulated magnocellular VPergic neurons. Moreover, an image analysis made it possible to specify VP-IR and TH-IR Herring bodies in rats, under normal laboratory conditions and following 1, 2, or 3 weeks of salt-loading, by measuring the number of Herring bodies, their individual area, and the OD of IR material. We have emphasized (see Experimental Methods) that the PL of intact rats and of those at any stage of salt-loading were processed under standardized conditions (fixation, cutting, immunostaining of the sections mounted on the same slides) for quantitative studies and particularly for the semi-quantitative analysis of TH-IR and VP-IR materials. The validity of this approach was proved in earlier technical studies (Conn, 1990) and then confirmed by concrete applications (Beltramo et al., 1994, 1997). Still, it should be taken into account that we could compare only the relative intracellular amounts of the IR materials in the animals of different experimental groups, but separately for TH-IR and VP-IR materials. In other words, this technique failed to evaluate the absolute content of the IR material and to compare the OD or the relative amounts of TH-IR and VP-IR materials in Herring bodies.

This is the first study attempting to compare the dynamics of the TH and VP turnover in the axons of VPergic neurons of the supraoptic

nucleus during long-term osmotic stimulation by using avidin-biotin immunocytochemistry and image analysis. When evaluating the intra-axonal dynamics of TH and VP, it should be taken into account that these substances differ considerably in some specific properties. Thus, TH is a non-releasable cytosolic protein that is degraded by cytosolic peptidases (Fernandez & Craviso, 1999). Conversely, VP is a secreted peptide that is stored in secretory granules and protected from enzymatic degradation by the granule membrane (Castel et al., 1984). From the above comments follows that the relative average concentration (OD) or the content (OD $\times$ area of a Herring body) of TH-IR material per Herring body is proportional to the difference between the TH transport from the cell bodies of VPergic neurons and the enzymatic degradation of TH in Herring bodies. In turn, the relative average concentration (OD) or content (OD $\times$ area of a Herring body) of the VP-IR material per Herring body is proportional to the difference between VP transport from the cell bodies of VPergic neurons and VP depletion from these structures. The relative contents of polypeptides (IR materials) per Herring body appeared to be a more informative index for their turnover than their relative concentrations were because the Herring body size changed during the course of the experiment. For instance, the relative concentration of the VP-IR material (OD) in Herring bodies did not change for the first week of osmotic stimulation, whereas the relative content of the VP-IR material per Herring body decreased over the same period (see Results).

Colocalization of TH and VP in Herring Bodies

In this study, we have demonstrated for the first time a colocalization of TH and VP in Herring bodies in the PL by using an immunofluorescence double-labeling technique. In addition to Herring

bodies, small double-labeled axon swellings were seen regularly in the PL of the rats that were salt-loaded for a week when using confocal microscopy. The data strongly suggest that TH is transported from the cell bodies located in the supraoptic nucleus toward the axon terminals in the PL. Although TH mRNA has been detected before in the VPergic axons in the median eminence on sections (Skutella et al., 1994) and in the homogenate of the 'neurointermediate' (PL + intermediate lobe) lobe (Melia et al., 1994), the authors are skeptical about the possible translation of TH mRNA in the axon terminals because of the absence of translational machinery in this neuronal compartment.

Although both intact rats and those that were salt-loaded for a week were tested for colocalization of TH and VP, double-labeled Herring bodies were detected only in the salt-loaded animals. Apparently, this result is accounted for by the extremely low frequency of TH-containing Herring bodies in intact rats, whereas their number significantly increased for the first week of osmotic stimulation (see Results). The same was also true for VPergic cell bodies expressing TH gene and synthesis in rats during the first week of salt-loading (Abramova et al., unpublished).

Surprisingly, some double-labeled Herring bodies did not show full superposition of the TH-IR and VP-IR profiles. Moreover, VP-immuno-staining of some Herring bodies was stronger at the periphery than in the center. These findings appear to agree with earlier electron microscopic observations of the zonality in the distribution of subcellular structures in Herring bodies—the VP-containing secretory granules occupied the periphery, whereas the central area was filled with microtubules (Dellmann & Rodriguez, 1970; Polenov & Garlov, 1971; Ugrumov & Belenkii, 1974).

It is noteworthy that VP-IR Herring bodies decreased in size for the first week of osmotic stimulation, while TH-IR Herring bodies increased

in size during the same period, becoming as large as VP-IR Herring bodies. After that, the Herring bodies immunostained for TH or VP did not change in size until the end of the experiment. These observations might be a result of two synchronous processes: (1) a sharp depletion of the peripheral area of Herring bodies from the VP-containing secretory granules that perhaps was followed by the shrinkage of Herring bodies, and (2) a spread of the TH-IR material from the center to the periphery of Herring bodies, due to its diffusion either through axoplasm or via the hypertrophied microtubular system. Indeed, the concentration of intra-axonal microtubules increases under functional stimulation of the VP magnocellular system (Knowles & Vollrath, 1974).

TH and VP Turnover in Herring Bodies

In the present study, peculiar attention was paid to VP and TH turnover in Herring bodies during the long-term osmotic stimulation of VPergic neurons. The number of VP-IR Herring bodies decreased fourfold over the first week of osmotic stimulation, remaining almost at the same low level until the end of the experiment. In other words, the depletion of VP from Herring bodies appeared to occur at a higher rate when compared with the depletion of the VP axons in the PL as a whole (Abramova et al., unpublished), which contradicts earlier qualitative studies (Ugrumov & Belenkii, 1974). The relative content of VP-IR material within individual Herring bodies fell gradually over the entire period of osmotic stimulation studied here, whereas its concentration decreased only for the second and the third week. This result suggests that the VP depletion from Herring bodies prevailed during its transport from the cell bodies for the whole experiment. In contrast to VP-IR Herring bodies, the number of TH-IR Herring bodies progressively increased during the 3 weeks of salt-loading. This

phenomenon is apparently a consequence of the gradually increasing number of VPergic neurons co-expressing TH (Abramova et al., unpublished) and finally, of the transport of TH in these neurons from the perikarya toward axon terminals.

The functional significance of TH in magnocellular VPergic neurons remains unclear. The VPergic cell bodies in the supraoptic nucleus lacked the specific markers of TH enzymatic activity: guanosine triphosphate-cyclohydrolase, the enzyme catalyzing the TH co-factor production (BH_4), and L-DOPA, the immediate precursor of dopamine (Marsais & Calas, 1999). Moreover, one cannot exclude the superfluous gene expression of TH as that of tryptophan hydroxylase in the neurons of the zona incerta (Vanhatalo & Soinila, 1996). Still, the synchronization of gradual VP depletion and TH accumulation in Herring bodies during the entire period of long-term osmotic stimulation studied here raises the question of the functional interaction between VP and TH in axon terminals.

Thus, the VPergic axons of osmotically stimulated neurons contained TH. The VP release from Herring bodies gradually increased during long-term osmotic stimulation and was synchronized with TH accumulation, at least for the second and third weeks. This correlation suggests functional interactions between VP and TH in axon terminals.

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REFERENCES

- Arluison M, Dietl M, Thibault J. Ultrastructural morphology of dopaminergic nerve terminals and synapses in the striatum of the rat using tyrosine hydroxylase immunocytochemistry: A topographical study. *Brain Res Bull* 1984; 13: 169–185.
- Beltramo M, Calas A, Chernigovskaya E, Borisova N, Polenova O, Tillet Y, et al. Postnatal development of the suprachiasmatic nucleus in the rat. Morpho-functional characteristics and time course of tyrosine hydroxylase immunopositive fibers. *Neuroscience* 1994; 63: 603–610.
- Beltramo M, Calas A, Chernigovskaya E, Thibault J, Ugrumov M. Long-lasting effect of catecholamine deficiency on differentiating vasopressin and oxytocin neurons in the rat supraoptic nucleus. *Neuroscience* 1997; 79: 555–561.
- Brownstein MJ, Mezey E. Multiple chemical messengers in hypothalamic magnocellular neurons. *Prog Brain Res* 1986; 68: 161–168.
- Castel M, Gainer H, Dellmann HD. Neuronal secretory systems. *Int Rev Cytol* 1984; 88: 303–458.
- Conn PM, ed. *Methods in Neurosciences. Quantitative and Qualitative Microscopy; Image Analytic Techniques for Quantification of Immunohistochemical Staining in the Nervous System*. San Diego, California, USA: Academic Press, 1990.
- Dellmann HD, Rodriguez EM. Herring bodies: an electron microscopic study of local degeneration and regeneration of neurosecretory axons. *Z Zellforsch* 1970; 111: 293–315.
- Fernandez E, Craviso GL. Protein synthesis blockade differentially affects the degradation of constitutive and nicotinic receptor-induced tyrosine hydroxylase protein level in isolated bovine chromaffin cells. *J Neurochem* 1999; 73: 169–178.
- Fetissov S, Marsais F, Nicolaïdis S, Calas A. Expression of tyrosine hydroxylase in magnocellular hypothalamic neurons of obese (fa/fa) and lean heterozygous (Fa/fa) Zucker rats. *Mol Brain Res* 1997; 50: 314–318.
- Hökfelt T, Meister B, Melander T, Everitt B. Co-existence of Classical Transmitters and Peptides with Special Reference to the Arcuate Nucleus-Median Eminence Complex. *Hypothalamic Dysfunction in Neuropsychiatric Disorders*. New York, NY, USA: Raven Press, 1987.
- Kawano H, Daikoku S. Functional topography of the rat hypothalamic dopamine neuron systems: retrograde tracing and immunohistochemical study. *J Comp Neurol* 1987; 265: 242–253.
- Kiss JZ, Mezey E. Tyrosine hydroxylase in magnocellular neurosecretory neurons. *Neuroendocrinology* 1986; 43: 519–525.
- Knowles F, Vollrath L, ed. *Neurosecretion—The Final Neuroendocrine Pathway; Overactivity of the Hypothalamo-Neurohypophyseal Neurosecretory System and the Problem of Mechanisms of Transporting Neurosecretory Material*. Berlin, Germany: Springer, 1974.
- Marsais F, Calas A. Ectopic expression of non-catecholaminergic tyrosine hydroxylase in rat hypothalamic magnocellular neurons. *Neuroscience* 1999; 94: 151–161.
- Meister B, Villar MJ, Ceccatelli S, Hökfelt T. Localization of chemical messengers in magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei: An immunohistochemical study using experimental manipulations. *Neuroscience* 1990a; 37: 603–633.
- Meister B, Cortes R, Villar MJ, Schauling M, Hökfelt T. Peptides and transmitter enzymes in hypothalamic magnocellular neurons after administration of hyperosmotic stimuli: Comparison between messenger RNA and peptide-protein levels. *Cell Tissue Res* 1990b; 260: 279–298.
- Melia KR, Trembleau A, Oddi R, Sanna PP, Bloom FE. Detection and regulation of tyrosine hydroxylase mRNA in catecholaminergic terminals fields: Possible axonal compartmentalization. *Exp Neurol* 1994; 130: 394–406.
- Nagatsu I, Sakai M, Takeuchi T, Arai R, Karasawa N, Yamada K, Nagatsu T. Tyrosine hydroxylase (TH)-only-immunoreactive non-catecholaminergic neurons in the brain of wild mice or the human TH transgenic mice do not contain GTP cyclohydrolase I. *Neurosci Lett* 1997; 228: 55–57.
- Polenov AL, Garlov PE. The hypothalamo-hypophyseal system in Acipenseridae. I. Ultrastructural organization of large neurosecretory terminals (Herring bodies) and axoventricular contacts. *Z Zellforsch* 1971; 116: 349–374.
- Robert FR, Leon-Henri BP, Chapleur-Chateau MM, Girr MN, Burlet AJ. Comparison of three immunoassays in the screening and characterization of monoclonal antibodies against arginine-vasopressin. *J Neuroimmunol* 1985; 9 (3-4): 205–20.
- Saavedra JM, Palkovits M, Kizer JS, Brownstein M, Zivin JA. Distribution of biogenic amines and

- related enzymes in the rat pituitary gland. *J Neurochem* 1975; 25: 257–260.
- Saavedra JM. Central and peripheral catecholamine innervation of the rat intermediate and posterior pituitary lobes. *Neuroendocrinology* 1985; 40: 281–284.
- Skutella T, Probst JS, Blanco E, Jirikowski GF. Localization of tyrosine hydroxylase mRNA in the axons of the hypothalamo-neurohypophyseal system. *Mol Brain Res* 1994; 23: 179–184.
- Seil FJ, Johnson ML, Nishi R, Nilaver G. Tyrosine hydroxylase expression in non-catecholaminergic cells in cerebellar cultures. *Brain Res* 1992; 569: 164–168.
- Ugrumov MV, Belenkii MA. Electron microscope investigation of the large swelling of neurosecretory fibres (the Herring bodies) in the posterior pituitary of chronically dehydrated albino rats. *Tsytologia* 1974; 16: 281–286 (in Russian).
- Vanhatalo S, Soinila S, Kaartinen K, Bäck N. Co-localization of dopamine and serotonin in the rat pituitary gland and in the nuclei innervating it. *Brain Res* 1995; 669: 275–284.
- Vanhatalo S, Soinila S. Superfluous expression of tryptophan hydroxylase in the zona incerta dopaminergic neurones. *Neuroreport* 1996; 7: 2889–2892.
- Verney C, Gaspar P, Febvret A, Berger B. Transient tyrosine hydroxylase-like immunoreactive neurons contain somatostatin and substance P in the developing amygdala and bed nucleus of the stria terminalis of the rat. *Brain Res* 1988; 470: 45–58.
- Young WS, Warden M, Mezey E. Tyrosine hydroxylase mRNA is increased by hyperosmotic stimuli in the paraventricular and supraoptic nuclei. *Neuroendocrinology* 1987; 46: 439–444.