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ZINC-ENRICHED NEURONS (ZEN) OF THE BLADDER – A NEW PLAYER EMERGING IN THE FIELD?

Hypothesis / aims of study

Intrinsic ganglia of the urinary bladder trigone play a crucial role in the maintenance of bladder function, both during the storage and voiding phases. Although many of biologically active substances have been found in these neurons so far, and a plethora of them have been shown to fulfill the criterion of being transmitter of these perikarya, we are far from completing the list of potential modulators of bladder intrinsic neurons. Quite recently, Zn^{2+} ions have been implicated to be possible modulators of glutamatergic ZEN (i.e., zinc-enriched) neurons in the brain, where they were co-sequestered and co-released with glutamate during the synaptic transmission. Furthermore, zinc-enriched peripheral neurons have more recently been described in the mouse sympathetic ganglia (superior cervical ganglion and the ganglia of the lumbar sympathetic chain), thus opening a new chapter in the possible regulatory mechanisms in the control of peripheral organs. In the pig, although ZEN were not found in the sympathetic ganglia, we have quite recently demonstrated that a fair population of intrinsic enteric neurons belonged to this neural cells population. As the second organ being under continuous control of intrinsic parasympathetic ganglia is the urinary bladder, the present study was aimed at unraveling the possibility of co-incidence of ZnT_3 (a transporter, responsible for transporting zinc ions from the cytoplasm into the synaptic vesicles) and the most important peptidergic neurotransmitter molecules in intramural neurons of the porcine urinary bladder.

Study design, materials and methods

Tissue blocks from urinary bladder trigone of 6 immature female pigs were collected after transcardial perfusion of animals with freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer. After repetitive washes in phosphate buffered saline (PBS; 0.1 M, pH 7.2; twice a day through 3 days), tissue blocks were transferred into 18% sucrose and stored at 4 °C until sectioning. Routine double-immunofluorescence labellings were performed on 10-µm-thick cryostat sections of the trigone by means of antisera raised in different species. In order to reduce the non-specific background staining, the sections were incubated for 1 hour in a "blocking" mixture containing 1% Triton-X-100, 0.1% bovine serum albumin 0.05% thimerosal and 10% normal donkey serum. Well-characterized rabbit anti-ZnT3 antiserum (1:4000) was used in combination with either mouse anti-VIP (1:1000), mouse anti-NOS (1:1000), rat anti-NPY (1:300) or rat anti-SOM (1:300) antibody. Antigen-antibody complexes were visualized by appropriate secondary antisera (donkey anti-rabbit, -mouse or –rat $F(ab')_2$ fragments) coupled to FITC or CY3. Labelled sections were evaluated under the Olympus BX51 microscope and microphotographs were captured by a CCD camera under control of AnalySIS software (ver. 3.2, Soft Imaging System, FRG). Pictures were then printed on a wax printer (Tektronix, USA).

Results

ZnT_3-like immunoreactivity (ZnT_3-LI) was found in approximately 40% of all intramural bladder neurons. However, such coded neurons were unequally distributed within the urinary trigone ganglia: in the same section, we have found either ganglia, where virtually all of neurons were ZnT_3-LI, as well as such ones, where all of the perikarya lacked studied transporter. It should be stressed however, that the vast majority of ganglia contained a mixed population of nerve cells, approximately half of which were ZnT_3-LI. Of all ZnT_3 60% contained NPY, 30% exhibited SOM and 20% were simultaneously ZnT_3/VIP-LI. All of ZnT_3-LI neurons were supplied by SP-IR terminals, but none of these cells simultaneously contained this tachykinine.

Interpretation of results

It appears possible that in similarity to the situation observed in the hippocampus of mouse and monkey, the presence of ZnT_3 in intramural neurons of the porcine bladder may be indicative for a neuromodulatory role of the Zn^{2+} ions, both in presumably excitatory (e.g. SOM-IR), as well as inhibitory neurons (e.g. VIP-IR). As of now, there is a great need to combine the ZnT3-LI with those of dopamine-β-hydroxylase- (a marker for noradrenergic sympathetic neurons) or vesicular acetylcholine transporter-LI (as a marker of cholinergic, parasympathetic neurons) in order to answer in detail the question of the sympathetic vs. parasympathetic trait of urinary bladder ZEN. Furthermore, as the exact physiological role of Zn^{2+} ions in the control of the urinary bladder function(s) remains still obscure yet, further physiological studies are needed to clarify this issue..

Concluding message

This study for the first time provide evidence for the presence of yet unknown population of intrinsic urinary bladder ganglia neurons that most probably are able to store and release Zn2+ ions as a kind or neuromodulatory substance during excitation. Thus, this broaden our knowledge dealing with the complexity of peripheral nervous pathways involved in the control of the urinary bladder function(s).