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EVIDENCE OF A HYPOCRETIN SYSTEM IN THE RAT BLADDER

Aims of Study

Hypocretin is a recently discovered neuropeptide produced in the lateral hypothalamus. Hypocretin-containing neurons project throughout the central nervous system, suggesting that this peptide is involved in multiple regulatory processes. Although a hypocretin system has recently been described in the gut, the role of hypocretin outside of the central nervous system is unclear. Many neurotransmitters and neuropeptides initially described in the brain and enteric nervous system have subsequently been identified in the lower urinary tract. Moreover, the overlapping innervation pathways of visceral organs support the activity of common neurotransmitters and neuropeptides in the regulation of organ function. These factors have prompted our investigation aimed to localize the hypocretin-1 ligand and its receptor within the bladder, to identify its relationship with the autonomic innervation of the bladder and to determine the effect of hypocretin on bladder contractility.

Methods

The presence of a hypcretin system in the rat bladder was determined by RT/PCR. Total RNA was extracted from adult male rat bladders and reverse transcribed using SuperScript II and the appropriate gene specific primer. The reverse transcription mixture (5Φl) was amplified using AmpliTaq Gold. After one 95^{o}C incubation to activate the Amplitaq Gold, the following cycling conditions were used: 95^{o}C-1 min, 55^{o}C-1.5 min, and 72^{o}C-1.5 min (total of 35 cycles). A final incubation at 72^{o}C for 10 min completed the amplification. mRNA was visualized by ethidium bromide staining of RT/PCR products.

To determine the distribution of hypocretin and its receptor and to assess their co-localization with autonomic innervation, immunofluorescence imaging was used. Sections of rat bladder (10 μm) were fixed in cold acetone for fifteen minutes, washed in phosphate buffered solution (PBS), and blocked with PBS containing 0.1% Triton X-100 and 10% normal serum for thirty minutes. Sections were then incubated in primary antibodies raised against hypocretin 1 or hypocretin receptor-1 for 18 hours at 4°C. After washing, sections were incubated at room temperature for two hours with host-specific fluorescent-labeled secondary antibody. Parallel sections were processed as above, omitting primary antibodies. Antibodies used for the second labeling procedure included a neural tissue specific marker (protein gene product 9.5), a marker protein for cholinergic neurons (vesicular acetylcholine transporter), and a marker protein for noradrenergic processes (dopamine beta-hydroxylase). Immunolabeled samples were imaged using BioRad MRC 1024ES multi-photon/confocal imaging system. Images were collected using internal detectors at a pixel resolution of 0.240-0.484 μm with a Kalman 3 collection filter, and were reconstructed using the BioRad LaserSharp software.

To determine the functional effect of hypocretin, the urinary bladder and proximal urethra were removed from anesthetized adult male rats and placed in cold, oxygenated Kreb's solution. A catheter was inserted into the bladder transurethrally, fixed in place with a silk ligature around the bladder neck and attached to a pressure transducer to monitor bladder pressure. The bladder was suspended in an organ bath (37º), filled with 0.3 ml Kreb's solution and allowed to equilibrate for sixty minutes. Spontaneous bladder contractions were then monitored for fifteen minutes. Hypocretin-1 was administered to the bath in increasing concentrations (1 nM – 1 μM) every fifteen minutes. The amplitude of spontaneous contractions was determined during baseline and ten minutes after administration of each hypocretin concentration.

Results

Hypocretin receptor-1 mRNA was detected in the base and body of the bladder. Prominent immunoreactive fibers were detected in the bladder, traversing throughout all smooth muscle layers. Staining was not detected in control sections processed without the primary antibody. Immunoreactive fibers containing hypocretin, were also found in the smooth muscle layers of the bladder. Hypocretin receptor-1 Immunostaining completely colocalized with PGP9.5, confirming that hypocretin receptors were located on neural tissue. However, hypocretin receptor-1 did not co-localize with vanilloid receptor-1 (marker for capsaicin-sensitive sensory C-fiber afferents). Furthermore, vesicular acetylcholine transporter and hypocretin receptor-1 did not co-localize. In contrast, hypocretin receptor-1 immunoreactivity completely co-localized were dopamine beta-hydroxylase.

After the equilibration period, all whole bladders exhibited marked, consistent spontaneous contractions. Exposure of the bladder to exogenous hypocretin-1 produced an increase in the amplitude of

spontaneous contractions without a significant change in the frequency of contractions. The dose-dependent increase in spontaneous contractions was greater than both the baseline contraction amplitude and the amplitude of control bladders calculated during the same time period. The amplitude of spontaneous contractions in bladders that were not exposed to hypocretin-1 was unchanged throughout the experimental protocol.

Conclusions

The expression of hypocretin receptor mRNA and the distribution of hypocretin and its receptor suggest that a hypocretin system is present in the rat bladder. Moreover, hypocretin and its receptor are localized in sympathetic fibers in the bladder. The dose-dependent increase in spontaneous bladder contractions induced by hypocretin suggests that the hypocretin system may play a role in the neural modulation of bladder function. Therefore, inhibition of this system may provide a novel pharmacologic approach to the treatment of bladder overactivity.