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NEUROPEPTIDE Y Y1 RECEPTOR-MEDIATED REGULATION OF MICTURITION REFLEX IN URETHANE-ANESTHETIZED RATS

Hypothesis / aims of study

Neuropeptide Y (NPY) is a peptide consisting of 36 amino acids that was originally isolated from porcine brain (1). It is one of the most abundant peptides in the mammalian central nervous system and five Y receptors (Y1, Y2, Y4, Y5 and Y6) are known to mediate the action of NPY (1). Recent studies have demonstrated that Y1 and Y2 receptors are located in a subpopulation of dorsal root ganglia and these receptors are involved in nociceptive responses (2). Also, changes in the expression level of NPY are shown in severe bladder dysfunction pathologies (3). However, it is unknown whether NPY or Y receptors play a role in modulation of the micturition reflex. Therefore, this study was performed to elucidate the urodynamic effects of activation of Y1 or Y2 receptor on the micturition reflex in rats.

Study design, materials and methods

Adult female Sprague-Dawley rats weighing 236 to 255 g were used. Rats were anesthetized with isoflurane followed by urethane (1.2 g/kg subcutaneously). Thereafter the abdomen was opened through a midline incision and a PE-60 polyethylene catheter connected to a pressure transducer and amplifier was implanted into the bladder through the bladder dome. This catheter was used to fill the bladder by continuous infusion of saline and record intravesical pressure during cystometry. After intravesical catheter insertion, saline was continuously infused into the bladder for 2 hours at a rate of 0.04 ml per minute to record cystometrograms during a control period. [Leu^{31}, Pro^{34}]-NPY (0.03, 0.1, 0.3 and 1.0 μg, n=6 per dose) or NPY-(13-16) (0.03, 0.1, 0.3 and 1.0 μg, n=6 per dose), selective agonists of Y1 or Y2 receptors, respectively, was administered intrathecally and changes in bladder activity were monitored. Intrathecal administrations were made through a catheter (PE-10) implanted via a small incision of the dura at the Th11 vertebra under isoflurane anesthesia 3 days before the experiments. The intrathecal catheter was directed caudal in the spinal subarachnoid space and positioned at the level of the L6-S1 spinal cord. The volume of fluid in the catheter was kept constant at 6 μl. Single doses of drugs were then administered in a volume of 2 μl, followed by a 7 μl flush with saline. In another group of animals, [Leu^{31}, Pro^{34}]-NPY (1.0 μg) or NPY-(13-16) (1.0 μg) was administered intrathecally when the first bladder contraction was observed after intrathecal administration of BVD10, a selective Y1 antagonist (10 μg, n=6) or BIIE0246, a selective Y2 antagonist (3 μg, n=6) to examine the effects of NPY receptor antagonists. Cystometric parameters were recorded and compared before and after drug administration. All data values are expressed as the mean ± standard deviation. A one-way ANOVA followed by Dunnett's multiple comparison test was used for the statistical analysis between the vehicle and drug-treated groups. Student's paired *t*-test was used to compare cystometric variables before and after treatment, with $p < 0.05$ considered to indicate statistical significance.

Results

Intrathecal administration of [Leu^{31}, Pro^{34}]-NPY at 0.03, 0.1, 0.3 and 1.0 μg (n=6 per dose) significantly increased intercontraction interval at doses of 0.1 μg or higher in a dose-dependent fashion to 102.4 ± 3.2%, 110.5 ± 7.2%, 131.9 ± 9.2% and 139.3 ± 12.1% of the control value, respectively (at 0.1, 0.3 and 1.0 μg, $p < 0.01$). Intrathecal administration of [Leu^{31}, Pro^{34}]-NPY at 0.03, 0.1, 0.3 and 1.0 μg also increased threshold pressure in dose dependent fashion to 4.82 ± 0.84 cmH_2O, 9.51 ± 1.45 cmH_2O, 12.6 ± 1.69 cmH_2O and 13.8 ± 2.56 cmH_2O, respectively, from the control value of 4.67 ± 1.21 cmH_2O ($p<0.01$). These inhibitory effects were observed immediately after administration. There were no significant changes in basal pressure or maximum pressure at any doses tested. On the other hand, intrathecal administration of NPY-(13-16) (0.03-1.0 μg, n=6 per dose) did not affect any cystometric parameters investigated. When BVD10 (10μg, n=6) was administered one voiding cycle before [Leu^{31}, Pro^{34}]-NPY (1.0 μg, n=6) administration, the increases in intercontraction intervals and threshold pressure induced by [Leu^{31}, Pro^{34}]-NPY administration alone were not seen. In contrast, when BIIE0246 (3 μg, n=6) was administered before [Leu^{31}, Pro^{34}]-NPY (1.0 μg, n=6) administration, increases in intercontraction intervals and threshold pressure were still observed, as they were after [Leu^{31}, Pro^{34}]-NPY alone. BVD10 (10 μg, n=6) or BIIE0246 (3 μg, n=6) itself did not cause any significant changes in cystometric parameters.

Interpretation of results

In the present study, [Leu^{31}, Pro^{34}]-NPY administered intrathecally to urethane-anesthetized rats, increased intercontraction intervals and threshold pressure. Moreover, the inhibitory effects of [Leu^{31}, Pro^{34}]-NPY were antagonized by intrathecal administration of BVD10, but not by BIIE0246. These findings indicate that activation of NPY Y1 receptor by intrathecally administered [Leu^{31}, Pro^{34}]-NPY has an inhibitory action on the micturition reflex in urethane-anesthetized rats. The main function of [Leu^{31}, Pro^{34}]-NPY seems to be mediated by modulation of afferent activity, rather than efferent or smooth muscle activity, because [Leu^{31}, Pro^{34}]-NPY induced increases in intercontraction intervals and threshold pressure without affecting maximum pressure or basal pressure.

Concluding message

These results in this study suggest that NPY Y1 receptors located in the spinal cord have a inhibitory role in the modulation of the micturition reflex in urethane-anesthetized rats. Thus, spinal NPY Y1 receptors could be a potential target for the treatment of bladder dysfunction such as overactive bladder.

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Disclosures
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