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EFFECTS OF GROUP III METABOTROPIC GLUTAMATE RECEPTOR AGONIST ON THE MICTURITION REFLEX IN URETHANE-ANESTHETIZED RATS

Hypothesis / aims of study

The modulatory actions of glutamate, the main excitatory neurotransmitter in the central nervous system, are exerted via activation of metabotropic glutamate receptors (mGluRs) [1]. Eight distinct mGluRs (mGluR1−8) have been classified into three groups (I−III) based on their sequence homology [2]. Group III mGluRs (mGluRIII; mGluR4, mGluR6, mGluR7 and mGluR8) are widely distributed throughout the central nervous system [3]. However, the role of mGluRIII in the regulation of neural mechanisms controlling the micturition reflex is unknown. The aim of this study is to investigate supraspinal and spinal effects of L-(+)-2-amino-4-phosphonobutyric acid (L-AP4), a selective mGluRIII agonist, on the micturition reflex in urethane-anesthetized rats.

Study design, materials and methods

A total of 48 adult female Sprague-Dawley rats weighing 238−261 g were used in this study. Rats were anesthetized with isoflurane followed by urethane (1.2 g/kg, administered subcutaneously). Next, the abdomen was opened by a midline incision and a PE-60 polyethylene catheter was implanted into the bladder through the bladder dome. The catheter was connected via a three-way stopcock to a pressure transducer and a pump for continuous saline infusion. Continuous cystometrograms (0.04 ml/min) were performed in two groups of urethane-anesthetized rats. Twenty-four rats were administered intrathecal L-AP4 via PE-10 intrathecal catheters, which were implanted at Th11 via an incision in the dura under isoflurane anesthesia 3 days before the experiments. For the experiment, saline was first continuously infused for 2 hours to evaluate bladder activity during a control period. Then, L-AP4 (1, 3 and 10 μg, n=8 per dose) was administered intrathecally to evaluate changes in bladder activity. The catheter was directed caudally into 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 2 μl volume, followed by a 6 μl saline flush. In the other group of 24 rats, L-AP4 (1, 3 and 10 μg, n=8 per dose) was administered intracerebroventricularly. Using a stereotaxic micro-injector, a 30-gauge needle attached to a 10 μl Hamilton syringe was inserted into the lateral ventricle, and single doses of drugs were administered in a 2 μl volume over a 2 minute period. 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. Wilcoxon's signed rank test was used to compare cystometric variables before and after treatment. For all statistical tests, $p<0.05$ was considered significant.

Results

Intracerebroventricular administration of L-AP4 at doses of 1, 3 and 10 μg (n=8 per dose) increased intercontraction intervals in a dose-dependent fashion to 117.1 ± 12.3%, 132.5 ± 10.5% and 137.1 ± 15.6% of the control value, respectively ($p <0.01$), but did not affect maximum pressure, basal pressure or post void residual at any of the doses tested. Intrathecal administration of L-AP4 at doses of 1, 3 and 10 μg (n=8 per dose) also increased intercontraction intervals in a dose-dependent fashion to 125.3 ± 8.2%, 136.9 ± 7.1% and 142.7 ± 12.6% of the control value, respectively ($p <0.01$), but did not affect maximum pressure, basal pressure or post void residual at any of the doses tested. Intracerebroventricular or intrathecal administration of L-AP4 at 1, 3 and 10 μg also increased threshold pressure in a dose-dependent fashion.

Interpretation of results

In urethane-anesthetized rats, intracerebroventricular or intrathecal administered L-AP4 has an inhibitory effect on the micturition reflex, as shown by the observed increases in intercontraction intervals and threshold pressure. The main function of L-AP4 seems to be mediated by modulation of afferent activity, rather than efferent or smooth muscle activity, because L-AP4 induced increases in intercontraction intervals and threshold pressure without affecting maximum pressure or basal pressure. We postulate that the site of action may be the supraspinal and spinal sites.

Concluding message

The results of our study indicate that in urethane-anesthetized rats activation of mGluRIII can inhibit the micturition reflex at supraspinal and spinal sites. Thus, mGluRIII could be a potential target for the treatment of bladder dysfunction such as overactive bladder.

References

1. Nicoletti F, Bockaert J, Collingridge GL et al. Metabotropic glutamate receptors: from the workbench to the bedside. Neuropharmacology 2011; 60:1017-1041.
2. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol 2010; 50:295-322.
3. Messenger MJ, Dawson LG, Duty S. Changes in metabotropic glutamate receptor 1-8 gene expression in the rodent basal ganglia motor loop following lesion of the nigrostriatal tract. Neuropharmacology 2002; 43:261-271.

Disclosures

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