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

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## INTRODUCTION

- 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).
- It is unknown whether mGluRIII plays a role in the regulation of neural mechanisms controlling the micturition reflex.

## OBJECTIVES

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.

## METHODS

Adult female Spraque-Dawley rats (weighing 238-261 g) were used. Rats were maintained under standard laboratory conditions with a 12-h light/12-h dark cycle and free access to food pellets and tap water.

### Drugs

L-AP4, a selective mGluRIII agonist, was dissolved in saline.

### Intrathecal administration of L-AP4

- Rats were anesthetized with isoflurane followed by urethane (1.2 g/kg subcutaneously).
- A midline abdominal incision was made, and a transvesical catheter (PE-60) with a fire-flared tip was inserted into the dome of the bladder and secured with silk thread for bladder filling and pressure recording. A 3-way stopcock was connected to the transvesical catheter to monitor the bladder pressure.
- 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.
- L-AP4 (1, 3 and 10 µg, n=8 per dose) was administered intrathecally to evaluate changes in bladder activity.
- PE-10 intrathecal 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 volume of 2 µl, followed by a 6 µl flush with saline.
- Cystometric parameters were recorded and compared before and after drug administration.

### Intracerebroventricular administration of L-AP4

- 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 volume of 2 µl during 2 minutes.
- Cystometric parameters were recorded and compared before and after drug administration.

### Statistics

- Wilcoxon signed rank test was used to compare cystometric variables before and after treatment.

## RESULTS

- Intracerebroventricular administration of L-AP4 at doses of 1, 3 and 10 µg (n=8 per dose) increased intercontraction intervals (ICI) in dose dependent fashion, but did not affect maximum pressure (MP), basal pressure (BP), post void residual (PVR) at any doses tested.
- Intrathecal administration of L-AP4 at doses of 1, 3 and 10 µg (n=8 per dose) also increased ICI in dose dependent fashion, but did not affect MP, BP, PVR at any doses tested.
- Intracerebroventricular or intrathecal administration of L-AP4 also increased threshold pressure (TP) in dose dependent fashion.

## DISCUSSION

- 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 ICI and TP.
- We postulate that the site of action may be the supraspinal and spinal sites.

**Table 1.** Changes in cystometric parameters after intracerebroventricular L-AP4 administration in rats

| Variable | Vehicle | L-AP4 (1 µg) | L-AP4 (3 µg) | L-AP4 (10 µg) |
|---|---|---|---|---|
| Number of rats | 8 | 8 | 8 | 8 |
| Mean ± SD | | | | |
| ICI, mins | | | | |
| before | 10.1 ± 2.31 | 10.7 ± 3.18 | 10.5 ± 1.19 | 11.7 ± 3.15 |
| after | 10.5 ± 1.18 | 12.4 ± 4.16*† | 13.8 ± 2.17*† | 15.9 ± 5.51*† |
| %ICI, % | | | | |
| | 103.9 ± 9.7 | 117.1 ± 12.3† | 132.5 ± 10.5† | 137.1 ± 15.6† |
| BP, $cmH_2O$ | | | | |
| before | 4.18 ± 3.21 | 5.18 ± 1.52 | 4.61 ± 1.02 | 3.99 ± 1.27 |
| after | 4.00 ± 1.15 | 4.15 ± 1.26 | 5.10 ± 2.16 | 3.67 ± 1.38 |
| TP, $cmH_2O$ | | | | |
| before | 7.67 ± 1.23 | 6.78 ± 1.01 | 7.27 ± 0.09 | 8.45 ± 2.15 |
| after | 7.56 ± 1.15 | 10.5 ± 1.11*† | 14.8 ± 2.18*† | 16.8 ± 3.61*† |
| MP, $cmH_2O$ | | | | |
| before | 30.6 ± 4.17 | 31.8 ± 2.67 | 33.5 ± 4.16 | 27.8 ± 6.78 |
| after | 28.6 ± 3.61 | 28.5 ± 2.56 | 36.1 ± 8.56 | 30.6 ± 7.16 |
| PVR, ml | | | | |
| before | 0.05 ± 0.01 | 0.15 ± 0.04 | 0.14 ± 0.02 | 0.09 ± 0.01 |
| after | 0.08 ± 0.02 | 0.09 ± 0.06 | 0.09 ± 0.03 | 0.12 ± 0.06 |

* P <0.05 (*paired t-test*); † P <0.05 vs vehicle injection (Dunnett's multiple comparison test)

**Table 2.** Changes in cystometric parameters after intrathecal L-AP4 administration in rats

| Variable | Vehicle | L-AP4 (1 µg) | L-AP4 (3 µg) | L-AP4 (10 µg) |
|---|---|---|---|---|
| Number of rats | 8 | 8 | 8 | 8 |
| Mean ± SD | | | | |
| ICI, mins | | | | |
| before | 10.7 ± 1.67 | 10.1 ± 2.10 | 11.6 ± 2.35 | 10.2 ± 1.01 |
| after | 10.8 ± 1.32 | 12.7 ± 3.56*† | 15.7 ± 3.11*† | 14.7 ± 5.17*† |
| %ICI, % | | | | |
| | 100.9 ± 1.5 | 125.3 ± 8.2† | 136.9 ± 7.1† | 142.7 ± 12.6† |
| BP, $cmH_2O$ | | | | |
| before | 3.28 ± 1.10 | 4.78 ± 1.01 | 3.89 ± 0.89 | 4.19 ± 1.15 |
| after | 4.01 ± 1.14 | 4.04 ± 0.89 | 4.11 ± 1.16 | 4.51 ± 2.15 |
| TP, $cmH_2O$ | | | | |
| before | 9.56 ± 2.56 | 8.92 ± 1.91 | 7.87 ± 1.14 | 9.18 ± 2.01 |
| after | 8.72 ± 1.02 | 12.3 ± 2.78*† | 13.9 ± 3.21*† | 17.4 ± 5.10*† |
| MP, $cmH_2O$ | | | | |
| before | 28.6 ± 3.67 | 25.4 ± 3.01 | 29.5 ± 5.19 | 26.6 ± 4.89 |
| after | 29.9 ± 4.61 | 28.7 ± 5.01 | 31.4 ± 7.15 | 31.6 ± 6.17 |
| PVR, ml | | | | |
| before | 0.15 ± 0.02 | 0.11 ± 0.03 | 0.09 ± 0.05 | 0.10 ± 0.02 |
| after | 0.17 ± 0.05 | 0.09 ± 0.04 | 0.13 ± 0.07 | 0.12 ± 0.05 |

* P <0.05 (*paired t-test*); † P <0.05 vs vehicle injection (Dunnett's multiple comparison test)

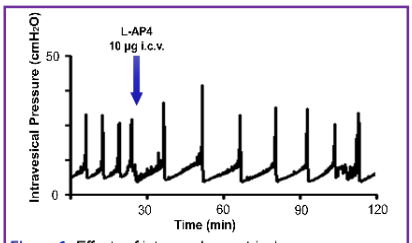


**Figure 1** Effects of intracerebroventricular administration of L-AP4 on bladder activity in rats.

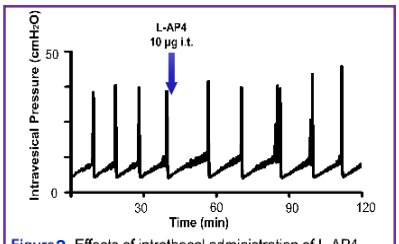


**Figure 2** Effects of intrathecal administration of L-AP4 on bladder activity in rats.

## CONCLUSIONS

- 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.

## REFERENCES

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## Disclosures Statement

No disclaimers or financial support to declare and no conflict of interests.