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# EFFECTS OF INTRATHECAL OF SEROTONIN ON BLADDER ACTIVITY, URETHRAL ACTIVITY, AND AMINO ACID LEVELS IN THE LUMBOSACRAL CORD OF RAT

Hypothesis / aims of study

Serotonin (5-hydroxytryptamine: 5-HT) has been involved in the central control of lower urinary tract function. The cell bodies of 5-HT neurons are located in the raphe nuclei of the brainstem and project their axons widely throughout the brain and spinal cord. Electrical stimulation of the raphe nuclei inhibits the micturition reflex,[1] suggesting that 5-HT neurons influence urine storage. On the other hand, amino acid neurons, such as glutamate, γ-aminobutyric acid (GABA), and glycine, are also known to be involved in the central nervous mechanism of micturition and urine storage.[2] However, the relationship between 5-HT neurons and amino acid neurons in micturition reflex is unclear. In order to clarify this relationship and the role of serotonergic mechanisms in the central nervous control of micturition, the effects of intrathecal injection of 5-HT on bladder and urethral activity, as well as on amino acids levels in the lumbosacral cord, were examined in intact rats and rats with hypogastric nerve transection (HGNT rats).

Study design, materials and methods

A total of 30 female Sprague-Dawley rats weighing 200 to 250 g were used in this study.
(1) Isovolumetric cystometry and measurement of urethral pressure before and after intrathecal injection of 5-HT (n=16). In 8 rats, under urethane anesthesia (1.2 g/kg), the bilateral ureters were transected and the distal ends were ligated, after which the bladder neck was ligated to produce isovolumetric conditions. A catheter was inserted into the bladder through the dome for cystometry, and another catheter was inserted into the urethra through the external meatus for measurement of the urethral pressure. Laminectomy was performed at L3, and a catheter was inserted into the subarachnoid space and was advanced to the level of the sacral cord. Isovolumetric cystometry and measurement of the urethral pressure were performed as follows. The bladder was filled with physiological saline (0.05 ml/min) to above the threshold volume in order to induce rhythmic isovolumetric contractions. After bladder contractions had been stable for at least 30 min, 5-HT (0.1 ng-1 μg) was injected intrathecally, and the changes of bladder and urethral activity were recorded. In another 8 rats, the bilateral hypogastric nerves were transected (HGNT rats) to block sympathetic activity and then preparations for cystometry and measurement of urethral pressure were done in the same way as mentioned above. 5-HT (1ng) was injected intrathecally, and the changes of bladder and urethral activity were recorded.
(2) Measurement of amino acid levels in the whole lumbosacral cord after intrathecal injection of 5-HT in intact rats (n=12). In 6 rats, 5-HT (1 μg) was injected intrathecally at the sacral level under urethane anesthesia (1.2 g/kg). At 7 min after drug injection, the rats were sacrificed and the lumbosacral cord was immediately removed. Levels of four amino acids (glutamic acid, aspartic acid, GABA and glycine) in homogenates of lumbosacral cord tissue were measured by a capillary electrophoresis system.In another 6 rats (control), amino acid levels in the lumbosacral cord were measured after intrathecal injection of physiological saline.
Results are reported as the mean ± standard deviation.

Results

(1) In intact rats, the interval between bladder contractions (1.5 ± 0.4 min), the maximal contraction pressure (40.5 ± 10.5 cm $H_2O$), the intravesical baseline pressure (14.0 ± 3.6 cm $H_2O$), and the urethral baseline pressure (16.1 ± 5.2 cm $H_2O$) were all stable before drug administration. Intravesical pressure decreased during bladder contraction. Rhythmic bladder contractions were abolished immediately after the intrathecal injection of 5-HT (10 ng-1 μg), and the time until the reappearance of contractions was 3-18 min. However, the interval between the re-established bladder contractions was not changed by intrathecal injection of 5-HT (0.1 ng-1 μg). After intrathecal injection of 5-HT at a higher dose (1 μg), the intravesical baseline pressure increased significantly (17.3 ± 4.2 cm $H_2O$, a 23% increase, P=0.016). The maximal contraction pressure of the re-established bladder contractions after intrathecal injection of 5-HT (1ng-1 μg) was significantly decreased (38.5 ± 11.2 cm $H_2O$, a 12% decrease at 100 ng of 5-HT, P=0.01). The urethral baseline pressure measured after intrathecal injection of 5-HT (1 ng-1 μg) showed a significant increase (19.6 ± 7.5 cm $H_2O$, a 21% increase at 1 μg of 5-HT, P=0.017). In HGNT rats, the interval between contractions (1.5 ± 0.4 min), the maximal contraction pressure (37.1 ± 5.1 cm $H_2O$), the intravesical baseline pressure (14.9 ± 4.7 cm $H_2O$), and the urethral baseline pressure (18.7 ± 7.5 cm $H_2O$) also became stable before drug administration. There were no significant differences of these parameters between intact rats and HGNT rats. Intravesical pressure was decreased during bladder contraction. Rhythmic bladder contractions were abolished immediately after the intrathecal injection of 5-HT (1 μg), and the time until the reappearance of contractions was 2-16 min. The urethral baseline pressure was significantly increased (20.4 ± 5.02 cm $H_2O$, a 9% increase, $P$ = 0.013) after intrathecal injection of 5-HT (1 μg). However, the increase of urethral baseline pressure after intrathecal injection of 5-HT was smaller in HGNT rats than in intact rats. Intrathecal injection of 5-HT (1 μg) did not affect the interval between re-established bladder contractions, the maximal contraction pressure, or the intravesical baseline pressure.
(2) Among the four amino acids measured in the lumbosacral cord, only the glycine level was significantly lower after intrathecal injection of 5-HT than in the control group (control: 31.7 ± 1.8 mg/L, 5-HT: 28.4 ± 2.7 mg/L, a 10% decrease, $P$ = 0.015).

Interpretation of results

In this study, there were no significant differences in the parameters of bladder and urethral activity before the intrathecal injection of 5-HT between intact and HGNT rats. Rhythmic bladder contractions were transiently abolished after intrathecal injection of 5-HT in both intact and HGNT rats, suggesting that intrathecal 5-HT inhibited the afferent limb of the micturition reflex pathway via the pelvic nerves. In intact rats, the maximal contraction pressure of re-established bladder contractions was lower than before 5-HT injection, while the intravesical baseline pressure and urethral baseline pressure were increased after intrathecal injection of 5-HT. These results suggest that intrathecal injection of 5-HT also affected the efferent limb of the micturition reflex pathway. In HGNT rats, rhythmic bladder contractions were abolished immediately after intrathecal injection of 5-HT and urethral baseline pressure was increased. If the effects of 5-HT on HGNT rats are subtracted from its effects on intact rats, the influence of intrathecal 5-HT on the hypogastric nerves leads to a decrease of the maximal pressure of re-established bladder contractions and an increase of the intravesical baseline pressure. These effects may be due to an increment of sympathetic activity.
The glycine level of the lumbosacral cord was lower after intrathecal injection of 5-HT than in the control group. Glycinergic neurons are reported to project their axons to the sacral cord and to motoneurons in Onuf's nucleus.[3] Therefore, serotonergic inhibition of

glycinergic neurons may activate Onuf's nucleus lead to an increment of the urethral baseline pressure. However, inhibition of bladder activity after the intrathecal injection of 5-HT may not be related to amino acid neurons.

Concluding message

The results obtained during this study indicate that the serotonergic system in the spinal cord may be involved in the following: 1) blocking the afferent pathway of the micturition reflex via the pelvic nerves, 2) increasing sympathetic activity via the hypogastric nerves innervating the bladder and urethra, and 3) secondary promotion of urethral contraction through inhibition of glycinergic neurons in the lumbosacral cord. On the basis of these findings, serotonergic neurons may be involved in the regulation of urine storage.

References

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