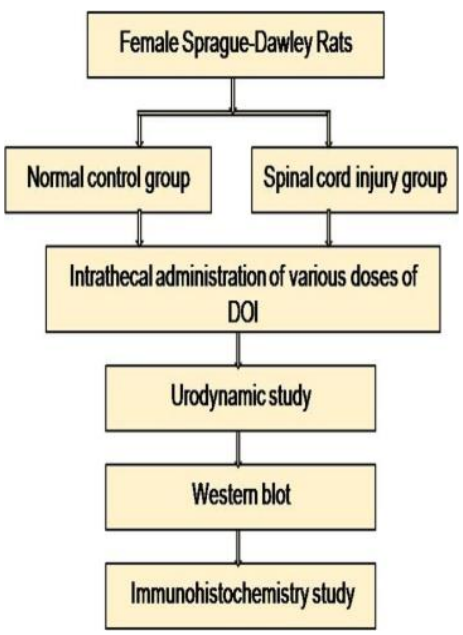
Chronic spinal cord injury causes up-regulation of serotonin (5-HT) 2A and 2C receptors in lumbosacral cord motoneurons

Cao N[1] , Gu B[1].
1.Shanghai sixth people's hospital, Shanghai Jiaotong University

Aims of study

Spinal cord injury can cause voiding dysfunction and intravenous administration of the 5-HT2A/2C receptor agonist, DOI has been demonstrated to improve urinary function in spinal cord injury rats, while this mechanism has not been well studied. So our objective was to discuss whether the main mechanism ascribe to serotonin 2A and 2C receptors up-regulation in lumbosacral cord motoneurons after chronic spinal cord injury.

Materials & methods



Results

Table 1. Urodynamic parameters in NC and SCI rats

Parameters	NC rats (n=10)	SCI rats (n=8)	P-value
Body mass(g)	276.2 ± 16.2	245.4 ± 11.3	0.044
Bladder mass(mg)	188.2 ± 17.7	485.3 ± 9.9	<0.001
Bladder capacity (ml)	0.38 ± 0.02	2.72 ± 0.16	<0.001
Micturition volume (ml)	0.3 ± 0.02	0.98 ± 0.22	<0.001
Residual volume (ml)	0.08 ±0.01	1.74 ± 0.25	<0.001
Voiding efficiency (%)	78.6 ± 2.93	23.34 ± 2.27	<0.001
Non-voiding contraction	1.0 ±0.53	11.63 ± 2.45	<0.001
The number of HFOs	21.9 ± 1.37	2.75 ± 1.49	<0.001
Peak Bladder Pressure(cmH2O)	22.95 ± 0.93	34.26 ± 2.40	0.012
Basal pressure(cmH2O)	6.18 ± 0.64	9.21 ± 1.23	0.082
Pressure threshold (cmH2O)	9.91 ± 0.66	13.25 ± 2.21	0.034

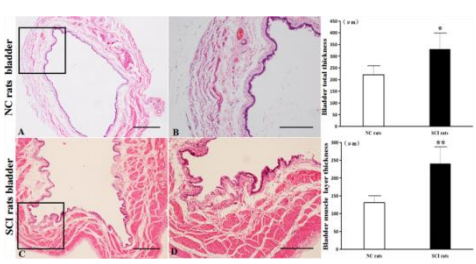


Fig.1 Representative H&E staining (muscle: red) of bladder in NC and SCI rats. (A) and (C) were original magnification (40×). (B) and (D) were original magnification (100×). Histogram showing the obviously hypertrophic changes of the bladder detrusor muscles in SCI rats .*P< 0.05, ** P<0.01 vs. corresponding NC group. Scale bar, 100μm.

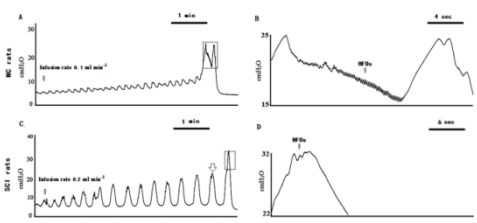


Fig.2 IVP activity recorded in urethane anesthetized NC and SCI rats. It is clearly that multiple NVC waves are increased and simultaneously the numbers of HFOs are reduced during per micturiton in SCI rats.

Results

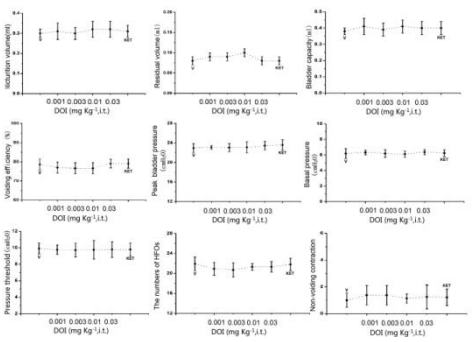


Fig.3 Dose-response curves of intrathecal DOI effects on CMG variables in NC rats. None of the urodynamic parameters are significantly affected. KET = ketanserin 0.01 mg/kg, i.t.

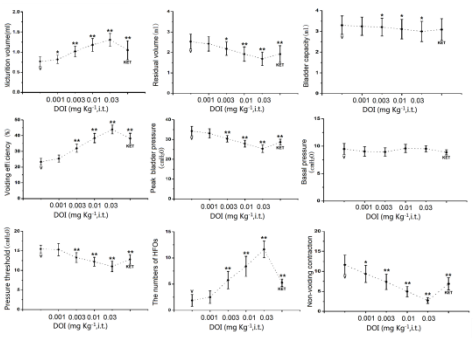


Fig.4 Dose–response curves for the effects of DOI on cystometric variables in SCI rats. *P<0.05, **P<0.01 indicate significant increases in micturition volume, voiding efficiency, the numbers of HFOs, and decreases in residual volume, bladder capacity, peak bladder pressure, pressure threshold and non-voiding contraction relative to vehicle values (V) or Ketanserin(KET) (0.01mg/kg, i.t.) treatment.

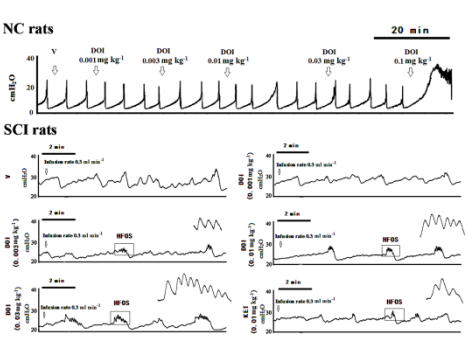


Fig.5 The effects of the DOI by intrathecal administration on the lumbosacral cord controlling micturition in NC rats and SCI rats. After intrathecal injection of DOI (0.1 mg kg-1), there was an increased peak bladder pressure and an inhibition of micturition in NC rats. Moreover, a clear dose-related response of intrathecal injection of DOI by increasing of the number of HFOs per micturition and reversal by Ketanserin (KET) is evident in SCI rats.

Results

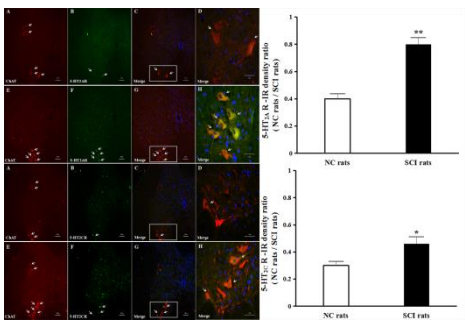


Fig.6 Photomicrographs from a lumbosacral cord segment double-stained with 5-HT2AR or 5-HT2CR (B, green) and ChAT (A, red) showing the 5-HT2AR and 5-HT2CR immunolabeling pattern from NC rats. (C) Merged images of (A) and (B). (D) Magnification of the boxes of C. DAPI staining was used to observe cell nuclei (blue). From the merged image it is clear that most, especially the smaller, ChAT-labeled neurons in the ventral horn were 5-HT2AR-positive or 5-HT2CR-positive (arrows). Histogram showing the differences in 5-HT2AR-IR and 5-HT2CR-IR densities in the motoneuron region between the NC rats and SCI rats. *, **: indicate the difference is statistically significant with t-test or Mann–Whitney rank sum tests (U-tests) (* P<0.05; ** P<0.01). Scale bar, 100μm.

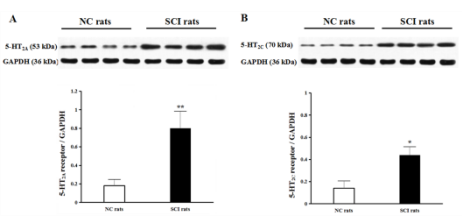


Fig.7 Expression of 5-HT2A receptors (A) and 5-HT2C receptors (B) in lumbosacral cord were analyzed by Western blotting. Representative gels obtained from 4 NC rats and 4 SCI rats are illustrated in A and B, respectively. Histogram showing the increased expression of 5-HT2A and 5-HT2C receptors in the ipsilateral ventral spinal cord at lumbosacral cord after spinal cord injury. *P< 0.05, ** P<0.01 vs. corresponding NC group.

Compared to controls, spinal cord injured rats had higher bladder capacity and post-void residual urine volume, and lower voiding efficiency. DOI could improve voiding efficiency via affecting external urethral sphincter activity after spinal cord injury. Furthermore, immunohistochemical staining and Western blot showed that serotonin 2A and 2C receptors were up-regulated in lumbosacral cord motoneurons after chronic spinal cord injury. .

CONCLUSION

DOI can improve voiding efficiency, and this may be due to serotonin 2A and 2C receptors up-regulation in lumbosacral cord motoneurons in controlling external urethral sphincter activity after chronic spinal cord injury.

Disclosures

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Clinical Trail: No **Subjects**: Animal(Rats)

Ethics Committee: The animal care and use committee of Shanghai Sixth People's Hospital