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Short communication

Serotonergic modulation of cat bladder function before and after spinal transection

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Abstract

Micturition was evoked in conscious cats by infusing saline into the bladder at a physiological rate. Drugs were administered intrathecally. Micturition volume threshold was increased by 5-hydroxytryptamine (5-HT, serotonin) and decreased by zatosetron, a 5-HT_3 receptor antagonist, in spinally intact cats. Thus 5-HT_3 receptors inhibit micturition. After complete spinal transection, serotonin reduced volume threshold in 3 of 4 cats, indicating an alteration in serotonergic control. However, 2-methyl-5-HT, a 5-HT_3 receptor agonist, increased volume threshold. Thus 5-HT_3 receptor-mediated inhibition of bladder function remains after spinal transection. We conclude that some, but not all, serotonergic modulation of bladder function is altered after spinal transection.

Keywords: Micturition; Spinal cord; 5-HT_3 receptor; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

Urinary tract function is dramatically altered following spinal injury. Bladder contractions produce high intravesical pressures but are not sustained and do not produce bladder emptying. A compound that would reduce bladder activity between catheterizations would be useful to protect the kidneys against pressure-induced damage as high pressure episodes in the bladder would be minimized (Gerridzen et al., 1992). Serotonin (5-hydroxytryptamine, 5-HT) may be inhibitory to micturition at a spinal level as methysergide, a non-selective 5-HT receptor antagonist, decreases the volume at which micturition occurs in conscious, spinally intact cats (Espey et al., 1992). Serotonergic pathways, and thus perhaps an inhibitory modulator of micturition, are lost after spinal transection. Replacement of 5-HT may therefore ameliorate bladder hyperactivity in spinally transected cats. Exogenous 5-HT was administered to spinally intact cats to determine if exogenous 5-HT is inhibitory to micturition. Spinally intact cats were treated with a 5-HT_3 receptor antagonist (zatosetron) to determine the involvement of 5-HT_3 receptors in the actions of endogenous 5-HT. The actions of 5-HT and a 5-HT_3 receptor agonist, 2-methyl-5-HT, were then examined in spinally transected cats to determine if these compounds retained their inhibitory actions following spinal transection.

2. Materials and methods

Ten adult male mongrel cats weighing between 3.3 and 4.6 kg were used in these studies. The experiments were performed in accordance with guidelines established by the Canadian Council on Animal Care and with the approval of the local University Committee on Laboratory Animals.

After a conditioning period of 3 weeks, the first group of 6 cats underwent aseptic surgery in which an intrathecal (i.t.) 24-gauge Teflon cannula terminating at the level of the lumbosacral cord was implanted. In a second surgery, 1–3 weeks later, a 20-gauge Silastic tubing cannula with a fenestrated end was implanted in the bladder lumen and a 20-gauge Silastic cannula with

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a balloon end fitting was implanted in the peritoneal space. All three cannulae were tunnelled subcutaneously to the head and attached to screw-capped plastic fittings. The fittings were secured with dental acrylic to screws anchored in the skull. These cats were used prior to spinal transection for testing of i.t. drugs on bladder function. Spinal cord transection at T11–T12 was performed, under anaesthesia and aseptic conditions, 8–16 weeks after bladder line implantation.

The second group of 4 cats was prepared as above but did not receive an intrathecal cannula and was not used for intrathecal testing of drugs prior to spinal cord transection. The cats were spinally transected as for the first group after a recovery period of one week.

Both groups of cats were used in investigations of causative factors responsible for the emergence of distension-evoked bladder contractions (M.J. Espey, J.W. Downie and J.B. Gajewski, unpublished observations). Bladder drainage was accomplished by urethral catheterization or by drainage via the indwelling bladder cannula with the addition of tactile perineal stimulation. All cats used in this study demonstrated reflex bladder activity after spinal cord transection.

Bladder function was tested before and after spinal cord transection in i.t. implanted cats and after transection in non-implanted cats. For bladder function testing, conscious cats were placed in a cage measuring $3 \times 2 \times 3$ feet. The bladder cannula was connected at the headcap through a fluid swivel to an infusion pump. The bladder was infused with sterile saline at a physiological rate of 10 hourly diuresis units (1 hourly diuresis unit = 1.1 ml/kg/h) (Klevmark, 1974). Bladder pressure was monitored during bladder filling (cystometrogram) from a transducer on a sidearm of the bladder filling line. The following parameters were measured: volume of first contraction greater than 15 cm H_2O (V_T), residual volume left after contraction (V_R), maximum pressure amplitude during contraction (P_C) and contraction duration (T_C). Intrathecal injection of serotonergic compounds was accomplished by indwelling intrathecal cannula in the first group and by lumbar puncture by a qualified anaesthetist in the second group.

Serotonin hydrochloride, 2-methyl-5-HT maleate (Research Biochemicals International) and zatosetron maleate (Lilly Research Labs) were dissolved in 0.9% saline just prior to injection. Drugs were injected in a volume of 0.1 ml followed by a flush of 0.15 ml of saline. The cystometrogram was begun 10 min after drug injection.

3. Results

In spinally intact cats, bladder filling proceeded apparently unnoticed by the animals until the volume threshold was approached. The cats then moved to the litterbox, adopted a typical posture and voided as described previously (Espey et al., 1992). In the cats implanted with an intrathecal cannula, saline was injected intrathecally to serve as a control for injection. If saline did not produce a change of more than 20% of the volume threshold before injection, the active drug was administered intrathecally. In cats administered drug via lumbar puncture, drug administration was performed if two preceding volume thresholds did not vary more than 20%. Initially, a dose of 1100 nmol 5-HT was tested in 2 spinally intact cats but only one cat showed a response. Five spinally intact cats given 2350 nmol 5-HT showed an average increase in volume threshold to 201% of control (range 143–300%, Fig. 1) while the other bladder function parameters were not affected. Zatosetron (10 nmol, $n = 3$) decreased volume threshold to 65% of control (range 57–71%) without altering other parameters of micturition (Fig. 2).

Beginning 1–4 weeks after spinal cord transection,

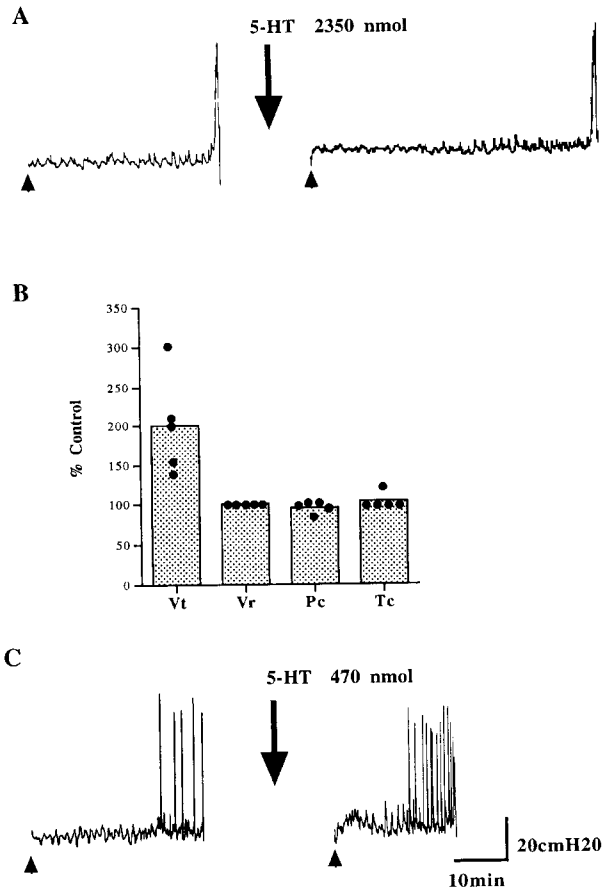

Fig. 1. Cystometrograms carried out before and 10 min after intrathecal injection of 5-HT in a spinal intact cat (A) and in a cat 21 days after spinalization (C). Upward arrowheads indicate start of cystometrograms. Calibration bars apply to both A and C. Panel B shows the effect of 5-HT (2350 nmol) on bladder function parameters in five spinally intact cats. Bars represent mean for group, black dots represent individual animals.

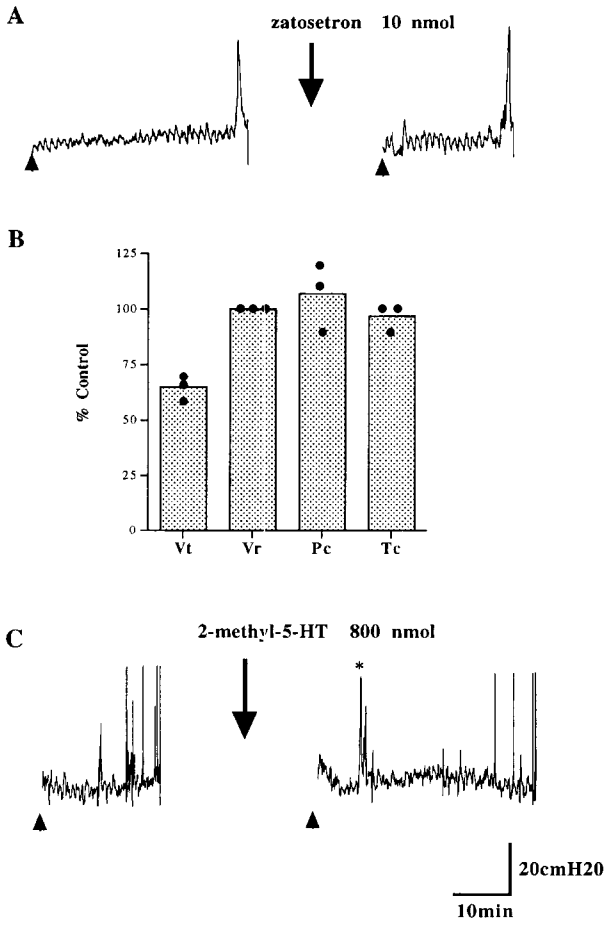

Fig. 2. Cystometrograms carried out before and 10 min after intrathecal injection of zatosetron (10 nmol) in a spinally intact cat (A) and 2-methyl-5-HT (800 nmol) in a cat 86 days after spinalization (C). Asterisk denotes a defecation episode. Upward arrowheads indicate start of cystometrograms. Calibration bars apply to both A and C. Panel B shows the effect of zatosetron (10 nmol) on bladder functional parameters in 3 spinally intact cats. Bars represent mean for group, black dots represent individual animals.

bladder filling induced large (> 15 cm H_2O), brief, repetitive contractions which did not completely empty the bladder. The volume at which these contractions appeared was considered to be the volume threshold after spinalization. Due to concerns relating to 5-HT receptor supersensitivity (Sawynok and Reid, 1994), 5-HT dosing in 4 spinally transected cats was begun at 20% of the dose used before transection. In 3 cats, the volume threshold was reduced by 470 nmol 5-HT to an average of 59% of control (range 54–67%, Fig. 1). The fourth cat was unaffected by 5-HT (106% of control) but demonstrated transient hind limb spasticity. Therefore a higher dose of 5-HT was not tested. No other side effects of 5-HT were seen. The two cats that received 5-HT by lumbar puncture demonstrated extensor spasms in the hind limbs whereas the two cats injected with 5-HT via the i.t. cannula did not.

In chronic spinal cats, intrathecal 2-methyl-5-HT (800 nmol, $n = 4$) increased the volume threshold to an average of 141% of control (range 122–160%, Fig. 2). In two cats administered 2-methyl-5-HT by lumbar puncture, the hind limbs became spastic transiently. This did not occur in those cats receiving the drug through the implanted intrathecal cannula. One animal receiving 2-methyl-5-HT by lumbar puncture defecated shortly after drug injection (Fig. 2).

No consistent relationship was seen between the method of draining the bladder in spinalized cats and the results of drug testing. Catheterized cats responded to both 5-HT and 2-methyl-5-HT. The chronic spinal cat which did not respond to 5-HT by lumbar puncture was managed by drainage via an indwelling cannula (Espey et al., 1992). Testing a higher dose was not considered feasible because of the hindlimb extensor spasms which accompanied the drug injection.

4. Discussion

In the present experiments, the actions of serotonergic ligands on distension-evoked bladder contractions were examined before and after spinal cord transection. Intrathecal administration of 5-HT in spinally intact cats increased volume threshold whereas in cats spinally transected at T11–T12, a decrease in volume threshold was observed. Supersensitivity to the effect of 5-HT was also encountered. Zatosetron, a 5-HT_3 antagonist, decreased volume threshold in spinally intact cats. 2-Methyl-5-HT, a 5-HT_3 receptor agonist, increased volume threshold after spinal transection. These results imply that spinal 5-HT_3 receptors are tonically activated to produce an inhibitory action on bladder activity in spinally intact cat and can be activated to produce a similar effect after chronic spinal transection. As the effects of 5-HT and 2-methyl-5-HT are in opposite directions in spinally transected cats, 5-HT_3 receptors do not mediate the actions of 5-HT after spinal transection.

The observation that 5-HT was inhibitory to bladder function in spinally intact cats is consistent with our previous results demonstrating a reduction of volume threshold in conscious, spinally intact cats with intrathecal methysergide, a 5-$HT_{1D/2A/2C}$ receptor antagonist (Espey et al., 1992). As well, spontaneous bladder contractions are inhibited by stimulation of the raphe nucleus (the source of serotonergic innervation of the spinal cord, McMahon and Spillane, 1982) and by application of high doses of 5-methoxy-*N*,*N*-dimethyltryptamine, a serotonergic agonist (Thor et al., 1990). In the present experiments, zatosetron, a 5-HT_3 receptor antagonist, decreased volume threshold suggesting that endogenous 5-HT, through activation of 5-HT_3 receptors, inhibits micturition. Coupled with our previ-

ous observations with methysergide, these results suggest that 5-HT is inhibitory to micturition at the spinal level in spinally intact cats and that 5-$HT_{1D/2A/2C}$ and 5-HT_3 receptors are involved in this action.

Spinal cord transection interrupts the spino-bulbospinal reflex pathway that subserves micturition and thus distension-evoked bladder contractions are eliminated. Reflex bladder contraction in response to bladder distension appeared in spinally transected cats after 1–4 weeks in our study. This is comparable to the 1–2 weeks reported by De Groat et al. (1981). These reflex contractions were strong but were not sustained and did not empty the bladder. The underlying organization of this reflex activity differs from that in intact cats (De Groat et al., 1981).

Intrathecal administration of 5-HT in three of four cats resulted in an effect opposite to that demonstrated before spinal cord transection. The reason for this difference is not clear but a number of possibilities can be raised: (a) Removal of descending pathways may allow a spinal mechanism of action to operate which is masked in the spinally intact animal. A similar situation has been suggested for pain transmission wherein two populations of dorsal horn nociceptive units have been proposed: one mediating spinal nociceptive reflexes and facilitated by 5-HT, the other projecting to the brain and inhibited by 5-HT (Zemlan, 1983). This would allow protective spinal reflexes to be maintained while diminishing the perception of pain. Data consistent with this hypothesis have been obtained from experiments comparing the effects of serotonergic compounds on ascending activity and spinally mediated reflexes related to bladder function (M.J. Espey, H.J. Du and J.W. Downie, unpublished observations). (b) Neuronal plasticity due to spinal cord injury may lead to a reversal of 5-HT effects. After spinal cord injury, the afferent path of reflex bladder contractions changes form Aδ-fibres in intact animals to C-fibres (De Groat et al., 1981). This alteration in the pathway subserving distension-evoked bladder contractions could be accompanied by changes in pharmacological characteristics. (c) Differential supersensitivity among 5-HT receptor subtypes following spinal cord transection could lead to a reversal of 5-HT effect. It has been demonstrated that following depletion of spinal 5-HT with 5,7-dihydroxytryptamine, antinociceptive effects of 5-HT and analogs acting on 5-HT_1 receptors are enhanced whereas the effects of 5-HT_2 and 5-HT_3 agonists are not (Sawynok and Reid, 1994). If an analogous selective supersensitivity occurred among receptors concerned with bladder control, a reversal of 5-HT action might result as spinal 5-HT_{1A} receptors appear to facilitate bladder contractions (Lecci et al., 1992; Espey, Du and Downie, unpublished observations).

Intrathecal administration of 2-methyl-5-HT increased volume threshold in spinally transected cats. Although 2-methyl-5-HT is not highly selective for 5-HT_3 receptors in binding studies (Van Wijngaarden et al., 1990; Hoyer et al., 1994), the results are consistent with the results obtained with zatosetron in spinally intact cats. We also have demonstrated that the depressant effect of 2-methyl-5-HT on ascending activity evoked by pelvic nerve stimulation is antagonized by zatosetron (Espey, Du and Downie, unpublished observations). Therefore, it is probable that spinal cord injury does not alter the action of 5-HT_3 receptors on bladder function. The opposite effects of 5-HT and 2-methyl-5-HT imply that 5-HT_3 receptors may not be responsible for the action of 5-HT after spinal cord injury.

Although some cats were managed post-spinal transection by intermittent catheterization and others were drained through an implanted bladder cannula and received tactile stimulation of the perineal region, all cats used in this study demonstrated bladder contractions in excess of 15 cm H_2O on filling. The spinalized group tested with 5-HT contained three cats with bladder emptying accomplished by catheterization and one managed by drainage through the indwelling cannula. The latter did not respond to the drug. However, we feel that this may be a problem related to dosing rather than method of management, as cats representing both methods of management were responsive to 2-methyl-5-HT.

In 3 of 4 spinally transected cats, 5-HT reduced volume threshold. The unaffected cat received 5-HT via lumbar puncture. Both cats receiving drug by lumbar puncture demonstrated stiffening of the hindquarters. Motor spasticity due to spinal injury can be reduced by administration of a serotonergic antagonist, cyproheptadine (Wainberg et al., 1986, 1990), implying that 5-HT can evoke spasticity. Motoneuron activity is facilitated by serotonin (White and Neuman, 1983) through 5-$HT_{2A/2C}$ receptors (Yamazaki et al., 1992). As the cat that did not demonstrate a change in volume threshold did show hind limb spasticity, this would suggest that 5-HT was reaching the ventral horn of the spinal cord. The cat with the unaltered volume threshold had its bladder drained through the implanted cannula with the addition of perineal stimulation while the other cats received urethral catheterization. It is possible that the pharmacology of the spinal pathways mediating bladder contractions after spinal cord injury differs between animals drained by headcap with the addition of perineal stimulation and those drained by urethral catheterization. However, there is no reason to suspect this from any previous work.

In conclusion, in spinally intact cats, 5-HT and 5-HT_3 receptors are inhibitory to bladder function at a spinal level. However, the action of 5-HT is reversed by spinal cord injury. Activation of 5-HT_3 receptors still appears to cause inhibition of bladder activity after spinal cord

transection but the precise site of action of 2-methyl-5-HT requires further investigation. The present results suggest that some aspects of serotonergic modulation of bladder contractions are altered by spinal cord injury. Thus, simple replacement of 5-HT would not alleviate high bladder pressures and consequent renal damage seen after spinal cord injury (Gerridzen et al., 1992). However, further study of the actions of 5-HT_3 receptor agonists is indicated by the inhibitory effect of 2-methyl-5-HT after spinal cord injury.

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References

De Groat, W.C., I. Nadelhaft, R.J. Milne, A.M. Booth, C. Morgan and K. Thor, 1981, Organization of the sacral parasympathetic reflex pathways to the urinary bladder and large intestine, J. Auton. Nerv. Syst. 3, 135.

Espey, M.J., J.W. Downie and A. Fine, 1992, Effect of 5-HT receptor and adrenoceptor antagonists on micturition in conscious cats, Eur. J. Pharmacol. 221, 167.

Gerridzen, R.G., A.M. Thijssen and E. Dehoux, 1992, Risk factors for upper tract deterioration in chronic spinal cord injury patients, J. Urol. 147, 416.

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena and P.P.A. Humphrey, 1994, International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46, 157.

Klevmark, B., 1974, Motility of the urinary bladder in cats during filling at physiological rates. I. Intravesical pressure patterns studied by a new method of cystometry, Acta Physiol. Scand. 90, 565.

Lecci, A., S. Giuliani, P. Santicioli and C.A. Maggi, 1992, Involvement of 5-hydroxytryptamine$_{1A}$ receptors in the modulation of micturition reflexes in the anesthetized rat, J. Pharmacol. Exp. Ther. 262, 181.

McMahon, S.B. and K. Spillane, 1982, Brain stem influences on the parasympathetic supply to the urinary bladder of the cat, Brain Res. 234, 237.

Sawynok, J. and A. Reid, 1994, Spinal supersensitivity to 5-HT_1, 5-HT_2 and 5-HT_3 receptor agonists following 5,7-dihydroxytryptamine, Eur. J. Pharmacol. 264, 249.

Thor, K.B., T. Hisamitsu and W.C. De Groat, 1990, Unmasking of a neonatal somatovesical reflex in adult cats by the serotonin autoreceptor agonist 5-methoxy-*N*,*N*-dimethyltryptamine, Dev. Brain Res. 54, 35.

Van Wijngaarden, I., M.Th.M. Tulp and W. Soudijn, 1990, The concept of selectivity in 5-HT receptor research, Eur. J. Pharmacol. Mol. Pharmacol. 188, 301.

Wainberg, M., H. Barbeau and S. Gauthier, 1986, Quantitative assessment of the effect of cyproheptadine on spastic paretic gait: a preliminary study, J. Neurol. 233, 311.

Wainberg, M., H. Barbeau and S. Gauthier, 1990, The effects of cyproheptadine on locomotion and on spasticity in spinal cord injured patients, J. Neurol. Neurosurg. Psychiatry 53, 754.

White, S.R. and R.S. Neuman, 1983, Pharmacological antagonism of facilitatory but not inhibitory effects of serotonin and norepinephrine on excitability of spinal motorneurons, Neuropharmacology 22, 489.

Yamazaki, J., H. Fukuda, T. Nagao and H. Ono, 1992, 5-HT_2/5-HT_{1C} receptor-mediated facilitatory action on unit activity of ventral horn cells in rat spinal cord slices, Eur. J. Pharmacol. 220, 237.

Zemlan, F.P., L.-M. Kow and D.W. Pfaff, 1983, Spinal serotonin (5-HT) receptor subtypes and nociception, J. Pharmacol. Exp. Ther. 226, 477.