Spinal Cord (2009) 47, 561–564

www.nature.com/sc

ORIGINAL ARTICLE

Expression of purinergic P_2X_2-receptors in neurogenic bladder dysfunction due to spinal cord injury: a preliminary immunohistochemical study

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Study design: Retrospective descriptive study.
Objective: Although muscarinic receptors are the main targets for the treatment of detrusor overactivity today, anticholinergic therapy is not satisfying in a substantial percentage of patients. Recently, overexpression of P_2X_2 receptors in patients with idiopathic overactive bladder was demonstrated, indicating that purinergic innervation may play an important role in the pathophysiology of detrusor overactivity. We evaluated the expression of P_2X_2 receptors in patients with spinal cord lesions.
Setting: German university hospital.
Methods: By immunohistochemical staining, the frequency and intensity of P_2X_2 expression in bladder specimens from 15 patients with suprasacral spinal cord lesion were compared to those from 11 patients with bladder disorders not related to spinal cord injury (overactive bladder: $n=6$; chronic non-obstructive retention: $n=2$; bladder tumour: $n=3$).
Results: Specimens (12/15) from patients with spinal cord lesions and specimens (8/11) without spinal cord lesions demonstrated staining for P_2X_2 receptors in the detrusor muscle and the urothelium. There was a tendency towards a stronger staining in specimens from patients with spinal cord lesion.
Conclusion: Our pilot study gives a first hint that the P_2X_2 expression in patients with suprasacral spinal cord injury seems to be comparable to the expression in patients with idiopathic overactive bladder. Therefore, P_2X_2 receptors in detrusor tissue may be a future target for the treatment of detrusor overactivity.
Spinal Cord (2009) **47**, 561–564; doi:10.1038/sc.2008.165; published online 23 December 2008

Keywords: neurogenic lower urinary tract dysfunction; spinal cord injury; purinergic receptors; innervation

Introduction

Spinal cord lesions are well known to cause neurogenic bladder dysfunctions.[1] Suprasacral spinal cord lesions lead to neurogenic detrusor overactivity. An elevated storage pressure is the major risk factor for renal deterioration.[2] Therefore, the primary goal of bladder management in these patients is to achieve low pressure urine storage.[3] Today, detrusor relaxation by oral anticholinergic treatment along with intermittent catheterization is regarded as the standard first line treatment in patients with neurogenic detrusor overactivity.[4]

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Received 25 August 2008; revised 10 November 2008; accepted 22 November 2008; published online 23 December 2008

Although cholinergic innervation is predominant in normal bladder tissue,[5] P_2X receptor expression can be detected in a certain extent in normal bladder tissue as well. Although the exact function of P_2X receptors in bladder physiology is not completely clarified, there is a growing body of literature indicating that these receptors play a significant role in urinary tract disease.[6] The homomeric P_2X_3 receptor and the heteromeric $P_2X_{2/3}$ receptor seem to be involved in bladder sensory dysfunction and in bladder pain, for example, in interstitial cystitis.[7]

Moreover, P_2X receptors play a role in detrusor motor function as well. In patients with idiopathic overactive bladder (OAB), an increased expression of the P_2X_2 subtype protein[8] and a loss of P_2X_3 and P_2X_5 proteins have been described.[9] Thus, it seems that an abnormal expression of P_2X receptors may lead to a disturbance of the purinergic inhibitory control of the parasympathetic release of

acetylcholine.[9] As, however, ATPase activity is also altered in the tissue of overactive detrusor, P_2X receptor expression and abnormalities in ATPase activity may both influence purinergic signalling in detrusor overactivity.[10]

Studies evaluating the expression of P_2X receptors in neurogenic detrusor overactivity are rare. Brady *et al*. found an increased expression of P_2X_3 protein,[11] and successful treatment of detrusor overactivity in these patients with Botulinum-A-toxin correlated with a decrease of P_2X_3 receptors.[12] Bayliss *et al*.[13] demonstrated that specimens from patients with neurogenic detrusor overactivity did not show atropine resistance, whereas atropine resistant contractions were found in specimens from overactive detrusor tissue. The latter findings imply that there is a difference between the purinergic pathways in idiopathic detrusor overactivity and in neurogenic detrusor overactivity.

The P_2X_2 receptor subtype, which expression was demonstrated to be significantly increased in patients with idiopathic detrusor instability by about 50%,[8] has not been studied in neurogenic bladder dysfunction yet. This receptor has some unique features. It is not expressed at all in fetal bladders[14] and its expression is not altered by bladder outlet obstruction, although the expression of all other known P_2X receptors has changed in this condition, indicating a plasticity in the purinergic innervation of the bladder when obstruction occurs.[15] Also, in patients with interstitial cystitis, P_2X_2 receptor expression was unchanged when compared to normal detrusor tissue, whereas P_2X_3 receptor expression was decreased.[7] Thus, the only condition altering P_2X_2 receptor expression was idiopathic detrusor overactivity. Comparing expression in detrusor overactivity and in bladder dysfunction caused by suprasacral spinal cord injury (SCI) may, therefore, aid in answering the question if the P_2X_2 receptors are predominantly interacting with bladder control on neuronal or muscular level. Thus, we studied the P_2X_2 receptor expression in neurogenic detrusor overactivity.

Materials and methods

For immunohistochemical staining, 26 individual detrusor tissue specimens were evaluated. Specimens from 15 patients with suprasacral spinal cord lesion, 6 patients with idiopathic OAB and 5 patients without detrusor overactivity (2 patients, chronic non-obstructive retention; 3 patients, no bladder dysfunction; cystectomy for urothelial cancer of the bladder) were included. The tissue was isolated from cystectomy specimens ($n=14$), from patients receiving bladder augmentation ($n=9$) or from transurethral full thickness bladder resection ($n=3$). In all specimens, histologic evidence of cancer was excluded by a pathologist. The mean age of the 8 men and 18 women was 39.4 years (range 16–65 years).

All patients with voiding disorders underwent urodynamic evaluation before surgery. In all patients with suprasacral SCI and in four of the six patients with idiopathic OAB, detrusor overactivity could be confirmed. The two patients with chronic retention presented with underactive detrusors.

Before the tissue specimens were taken, 4 of the 6 patients with idiopathic OAB and 12 of the 15 patients with suprasacral SCI had received at least one Botulinum-A-toxin injection. Time between the most recent injection and harvesting of the detrusor specimens was at least 10 months (range 10–18 months).

For all immunohistochemical staining procedures, formalin-fixed, paraffin-embedded tissue was used. The tissue was cut in 5 μm sections, deparaffinized in xylene and rehydrated. The sections were washed with Tris-buffered saline and were incubated with 5% normal goat serum for 30 min. After washing with Tris-buffered saline, the slides were incubated with a polyclonal rabbit anti-P_2X_2 antibody (Alomone Labs, Jerusalem, Israel) at a dilution of 1:200 for 4 h at room temperature. Peroxidase was detected by adding AEC (3-amino-9-ethylcarbazol). The slides were counterstained with hematoxylin. Normal human bladder tissue served as a positive control. Omission of the primary antibody was used as a negative control.

Evaluation of the staining results

All slides were independently reviewed by two researchers blinded for the results of each other. At least 10 different section fields were evaluated by light microscopy. Staining was graded on a scale from 0 to 3 where 0 indicated no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. In controversial cases, the specimens were classified according to the lower result. Moreover, the location of staining (urothelium/suburothelial layer/detrusor muscle) was noted.

Statistical analysis

For statistical analyses, a statistics and graphics management system (STATA, Santa Monica, California, CA, USA) was used. All data were analysed for statistical significance using Student's *t*-test. A value of $P<0.05$ was considered statistically significant.

Results

The interobserver variation was 7.7% (discrepancies between the two observers: $n=2$). In controversial cases, the specimens were finally classified according to the lower reading result. The strongest immunoreactivity was observed in the detrusor muscle, but weak urothelial staining was detectable in all specimens with positive staining (Figure 1). No significant staining was detected in the suburothelial layer. Positive immunostaining for P_2X_2 was found in 12/15 (80%) specimens of the SCI patients. In 7 patients, staining was weak. Moderate staining was detected in 5 patients. In the specimens of 3 SCI patients, no staining was found.

In the specimens of patients with idiopathic OAB, five of the six patients demonstrated weak staining. In this cohort, all 4 patients with detrusor overactivity demonstrated staining. In three of the five patients with either retention or without any bladder dysfunction, weak staining was found. Although there was a trend towards a more intense staining in the specimens from patients with neurogenic

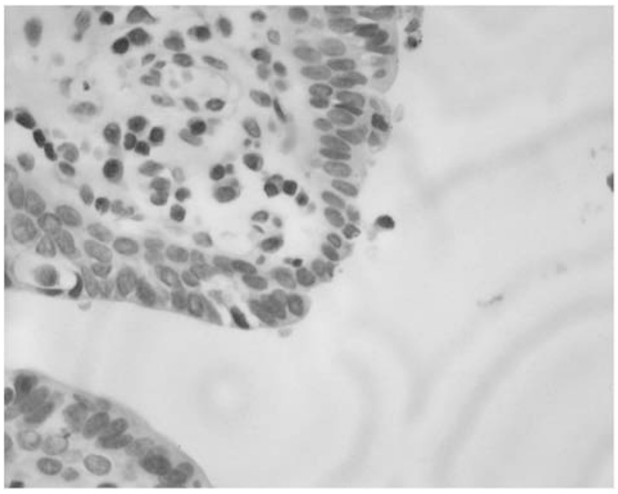

Figure 1 Bladder urothelium, 400-fold magnification: weak urothelial staining for P_2X_2.

Table 1 Staining characteristics in relation to underlying pathology

Staining	*SCI (n = 15)*	*No SCI (n = 11)*	P *values*
Negative (%)	3 (20)	3 (27.3)	0.685
Positive (%)	12 (80)	8 (72.7)	0.65
Weak (%)	7 (46.7)	8 (72.7)	0.192
Moderate (%)	5 (33.3)	0 (0)	0.019

Abbreviation: SCI, suprasacral spinal cord injury.

bladder dysfunction, this trend did not reach statistical significance regarding the entire group. Moderate staining, however, was significantly more frequently found in the SCI patient group. Subdividing the idiopathic OAB group in patients with proven detrusor overactivity ($n = 4$) and patients with OAB symptoms did not lead to different results (data not shown).

In summary, 80% of the specimens from patients with SCI and 72.7% of those from patients without suprasacral SCI (idiopathic OAB: 83%; retention/no bladder dysfunction: 60%) demonstrated staining for P_2X_2. Although none of the specimens from patients without suprasacral SCI showed more than weak staining, in 5/15 (33%) specimens from SCI patients moderate staining was observed. Strong staining was not detected in any of the specimens. The degree of staining is depicted in Table 1.

Discussion

Extracellular adenosine triphosphate binds to purinergic receptors of the P2 class including the transmembrane domain—containing P2Y receptors and the ligand-gated ion-conducting P_2X receptors, of which 7 receptor subunits have been described (P_2X_1–P_2X_7). [6] The expression of P_2X receptors seems to differ between fetal and adult human bladder tissue. P_2X_1 appears to be the main purinoreceptor, and its expression increases significantly in adulthood.[14] Therefore, a role of the P_2X receptors in maturation of the lower urinary tract is discussed.[14] Furthermore, there is a growing body of literature indicating that these receptors play a significant role in urinary tract physiology and disease.[6,16]

Our pilot study is the first to evaluate the expression of P_2X_2 receptors in neurogenic detrusor overactivity. P_2X_2 receptors were detected in detrusor smooth muscle and urothelium. This finding confirms previous results from localization studies in animals[17] and humans.[7,8]

The data presented here demonstrate that P_2X_2 receptors are frequently expressed in neurogenic detrusor overactivity. Moreover, we found P_2X_2 receptor expression in idiopathic detrusor overactivity in 72% of the specimens, confirming previous findings that this receptor subtype is frequently expressed in tissues from overactive detrusors. Although there was no statistically significant difference regarding the incidence of P_2X_2 receptor immunoreactivity between specimens from bladders with neurogenic detrusor overactivity specimens and the overactive detrusor group or normal controls, more pronounced staining was significantly more frequent in specimens from neurogenic detrusor tissue demonstrating detrusor overactivity. Thus, P_2X_2 receptors seem to play a role in the purinergic control of neurogenic bladder dysfunction. Based on our results and previously published data, either changes in bladder function correlating with P_2X_2 receptor expression may be more specifically linked to the detrusor muscle than to the neural supply, as P_2X_2 receptor expression in idiopathic detrusor overactivity and suprasacral SCI, also leading to detrusor overactivity, are comparable. On the other hand, however, bladder obstruction and interstitial cystitis both can well lead to detrusor overactivity, but previous studies could not detect a change in P_2X_2 receptor expression in these disorders. Therefore, it is also possible, or even more likely, that 'idiopathic' detrusor overactivity may be related to alterations in neural bladder control, which are too subtle to be readily diagnosed by our diagnostic means. In this case, the common P_2X_2 receptor expression may indicate that both forms of bladder dysfunction are neurogenic, whereas bladder dysfunction due to interstitial cystitis or obstruction clearly is not.

Our pilot study has several drawbacks. The majority of the neurogenic bladder and idiopathic detrusor overactivity specimens were derived from patients undergoing urinary diversion or augmentation, indicating that these patients suffered from severe bladder dysfunctions, refractory to conservative means as well as to Botulinum-A-toxin injections. It would be of interest to study specimens from patients with less pronounced lower urinary tract dysfunction. Furthermore, in merely four of the six patients with OAB symptoms, detrusor overactivity has been proven by standard urodynamioc evaluation. Studies using ambulatory urodynamics, however, have demonstrated that a significant proportion of patients without detrusor overactivity at standard evaluation do have detrusor overactivity detected by this examination.[18] As subdividing the results of the idiopathic OAB group in patients with and without proven detrusor overactivity did not alter the results, we decided to present the data of patients with idiopathic OAB including

the patients with proven detrusor overactivity as a single group. Second, the number of patients included in out study is rather small. Moreover, the coexpression of the other P_2X receptor subtypes may have been of interest. On the other hand, the main goal of our study was to evaluate the P_2X_2 receptor expression in bladder specimens from patients with suprasacral SCI. Our study gives a first hint that P_2X_2 receptors are at least as frequently expressed in this patient group as in idiopathic detrusor overactivity. Therefore, it may be awaited that both patient groups will profit from treatment with a selective P_2X_2 antagonist.

In summary, the P_2X_2 expression in detrusors specimens from patients with suprasacral SCI seems to be comparable to the expression in detrusor tissues from patients with idiopathic OAB. Therefore, P_2X_2 receptors in detrusor tissue may be a future target for the treatment of detrusor overactivity.

This option may not be far away anymore, although today merely preclinical data with selective P_2X receptor antagonists exist. For example, intravenous administration of a P_2X_3–$P_2X_{2/3}$ receptor antagonist in chronic suprasacral spinal cord injured rats decreased the number of non-voiding bladder contractions and increased the interval between voids.[19] As oral application seems possible, this compound may become a new treatment option for neurogenic detrusor overactivity in the future.

References

1 de Groat WC, Kawatani M, Hisamitsu T, Cheng CL, Ma CP, Thor K *et al*. Mechanisms underlying the recovery of urinary bladder function following spinal cord injury. *J Auton Nerv Syst* 1990; **30** (Suppl): 71–77.
2 Gerridzen RG, Thijssen AM, Dehoux E. Risk factors for upper tract deterioration in chronic spinal cord injured patients. *J Urol* 1992; **147**: 416–418.
3 Perkash I. Long-term urologic management of the patient with spinal cord injury. *Urol Clin North Am* 1993; **20**: 423–434.
4 Nosseir M, Hinkel A, Pannek J. Clinical usefulness of urodynamic assessment for maintenance of bladder function in patients with spinal cord injury. *Neurourol Urodyn* 2007; **26**: 228–233.
5 Inoue R, Brading AF. Human, pig and guinea-pig bladder smooth muscle cells generate similar inward currents in response to purinoceptor activation. *Br J Pharmacol* 1991; **103**: 1840–1841.
6 Rapp DE, Lyon MB, Bales GT, Cook SP. A role for the P2X receptor in urinary tract physiology and in the pathophysiology of urinary dysfunction. *Eur Urol* 2005; **48**: 303–308.
7 Tempest HV, Dixon AK, Turner WH, Elneil S, Sellers LA, Ferguson DR. P2X and P2X receptor expression in human bladder urothelium and changes in interstitial cystitis. *BJU Int* 2004; **93**: 1344–1348.
8 O'Reilly BA, Kosaka AH, Knight GF, Chang K, Ford APDW, Rymer JM *et al*. P2X receptors and their role in female idiopathic detrusor instability. *J Urol* 2002; **167**: 157–164.
9 Moore KH, Ray FR, Barden JA. Loss of purinergic P2X(3) and P2X(5) receptor innervation in human detrusor from adults with urge incontinence. *J Neurosci* 2001; **21**: 17–22.
10 Harvey RA, Skennerton DE, Newgreen D, Fry CH. The contractile potency of adenosine triphosphate and ecto-adenosinetriphosphatase activity in guinea pig detrusor and detrusor from patients with a stable, unstable or obstructed bladder. *J Urol* 2002; **168**: 1235–1239.
11 Brady CM, Apostolidis A, Yiangou Y, Baecker PA, Ford AP, Freeman A *et al*. P2X3-immunoreactive nerve fibres in neurogenic detrusor overactivity and the effect of intravesical resiniferatoxin. *Eur Urol* 2004; **46**: 247–253.
12 Apostolidis A, Popat R, Yiangou Y, Cockayne D, Ford AP, Davis JB *et al*. Decreased sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity. *J Urol* 2005; **174**: 977–982.
13 Bayliss M, Wu C, Newgreen D, Mundy AR, Fry CH. A quantitative study of atropine-resistant contractile responses in human detrusor smooth muscle, from stable, unstable and obstructed bladders. *J Urol* 1999; **162**: 1833–1839.
14 O'Reilly BA, Kosaka AH, Chang TK, Ford AP, Popert R, Rymer JM *et al*. A quantitative analysis of purinoceptor expression in human fetal and adult bladders. *J Urol* 2001; **165**: 1730–1734.
15 O'Reilly BA, Kosaka AH, Chang TK, Ford AP, Popert R, McMahon SB. A quantitative analysis of purinoceptor expression in the bladders of patients with symptomatic outlet obstruction. *BJU Int* 2001; **87**: 617–622.
16 Ford APDW, Gever JR, Nunn PA, Zhong Y, Cefalu JS, Dillon MP *et al*. Purinoceptors as therapeutic targets for lower urinary tract dysfunction. *Br J Pharmacol* 2006; **147**: S132–S143.
17 Lee HY, Bardini M, Burnstock G. Distribution of P2X receptors in the urinary bladder and the ureter of the rat. *J Urol* 2000; **163**: 2002–2007.
18 Radley SC, Rosario DJ, Chapple CR, Farkas AG. Conventional and ambulatory urodynamic findings in women with symptoms suggestive of bladder overactivity. *J Urol* 2001; **166**: 2253–2258.
19 Lu SH, Groat WC, Lin AT, Chen KK, Chang LS. Evaluation of purinergic mechanism for the treatment of voiding dysfunction: a study in conscious spinal cord-injured rats. *J Chin Med Assoc* 2007; **70**: 439–444.