

## ALTERATIONS IN MEDIATOR RELEASE FROM THE UROTHELIUM IN A RAT MODEL OF CEREBRAL INFARCTION: EFFECT OF THE B3-AGONIST BRL37344

### Hypothesis / aims of study

Cerebral infarction (CI) impairs the suprapontine regulatory system for the micturition reflex and consequently causes neurogenic detrusor overactivity (DO). However, there is evidence that DO induced by CI in the rat is suppressed by an  $\alpha$ 1-blocker silodosin that hardly penetrates the central nervous system [1].  $\alpha$ 1-blockers are known to decrease the release of adenosine triphosphate (ATP) from the urothelium, and we have recently reported that another  $\alpha$ 1-blocker naftopidil attenuates non-voiding contractions on cystometrogram and decreases the intravesical release of not only ATP but also prostaglandin (PG) E2 and nerve growth factor (NGF) in a rat model of bladder outlet obstruction. These findings suggest that there may be an alteration in mediator release from the urothelium in neurogenic DO induced by CI and that it may contribute to the development of DO. In our previous study the amount of ATP but not PGE2 in the bladder tissue was found out to be increased in the rat with CI [2]. However, alterations in the intravesical release of mediators from the urothelium have not been examined in a rat model of CI *in vivo*.

The inhibition of the release of ATP, PGE2 or NGF from the urothelium may inhibit DO because these mediators are considered to be involved in the afferent pathway of the micturition reflex in pathological conditions of the bladder. A  $\beta$ 3-agonist is now a therapeutic option for overactive bladder syndrome.  $\beta$ 3-agonists have been speculated to modulate mediator release from the urothelium like  $\alpha$ 1-blockers or antimuscarinics do so. However, the effect of  $\beta$ 3-agonists on mediator release from the urothelium remains largely to be elucidated.

The aim of the present study was to examine the alterations in mediator release from the urothelium in neurogenic DO induced by CI and the effect of the  $\beta$ 3-agonist BRL37344 on the DO and mediator release from the urothelium *in vivo*.

### Study design, materials and methods

Female Sprague-Dawley rats (9 weeks old) were used for the experiment (n=8). A polyethylene catheter for cystometry was implanted through the bladder dome. One week later cystometry was performed with physiological saline in the awaken condition. The cystometric parameters for each rat were determined with repeated cystometry at least three times. Post-void residual was drained via the cystometry catheter and measured after three consecutive micturitions. After the bladder capacity (BC) was determined, the bladder was distended to 80% BC twice. The instilled perfusate was collected for the measurement of the amounts of ATP, NGF and PGE2. The left middle cerebral artery was occluded using 4-0 monofilament nylon thread to create CI. After the creation of CI the measurement of cystometric parameters, the determination of the BC and the collection of perfusate at 80% BC were repeated before and after the intravenous administration of BRL37344 ( $10^{-3}$  ml/kg). ATP was measured with the luciferin-luciferase assay, and PGE2 and NGF were measured with the enzyme immunoassay. To confirm cerebral ischemic status, TCC (2% 2,3,5-triphenyl chloride) staining of sliced forebrain was performed in each rat. Data are expressed as mean  $\pm$  SEM.

### Results

TCC staining confirmed the presence of CI in all rats. The main results are shown in the Table.

The BC was significantly decreased by CI, and BRL37344 partially restored the decreased BC with a statistical significance. Post-void residual volume was small at baseline, after CI and after the administration of BRL37344 ( $0.07 \pm 0.03$ ,  $0.250 \pm 0.04$  and  $0.20 \pm 0.07$  ml, respectively). The micturition pressure was not changed by CI and by the BRL37344 administration. BRL37344 improved DO without impairing the voiding function.

The intravesical concentrations of ATP and NGF at 80% BC were increased by CI. BRL37344 reduced the NGF concentration. The ATP concentration was not significantly reduced by BRL37344, but it was reduced in six of the eight rats with CI. The PGE2 concentration was not influenced by CI and the administration of BRL37344.

Table. The changes in cystometric parameters and intravesical concentrations of ATP, NGF and PGE2 at 80% bladder capacity by CI and the BRL37344 administration ( $10^{-3}$  ml/kg)

	After CI	BRL37344 administration
Bladder capacity	$51.3 \pm 7.3^{\dagger\dagger}$	$72.5 \pm 7.5^{\dagger,**}$
Threshold pressure	$104.3 \pm 8.6$	$97.5 \pm 12.0$
Micturition pressure	$102.9 \pm 7.5$	$94.2 \pm 7.3$
ATP concentration	$191.9 \pm 38.6^{\dagger\dagger}$	$183.6 \pm 55.2$
NGF concentration	$177.4 \pm 33.0$	$140.6 \pm 29.1^*$
PGE2 concentration	$94.8 \pm 17.9$	$88.2 \pm 21.5$

The values are expressed as % of the baseline values (n=8).  $^{\dagger}$   $p < 0.05$ ,  $^{\dagger\dagger}$   $p < 0.01$  compared to baseline: \*  $p < 0.05$ , \*\*  $p < 0.01$  compared to after CI

### Interpretation of results

We found out that the intravesical concentrations of ATP and NGF were increased in rats with CI. It is possible that ATP and NGF from the urothelium contribute to the development of DO induced by CI since the DO was reported to be inhibited by the administration of  $\alpha$ 1-blocker silodosin or naftopidil that may reduce the ATP release from the urothelium. However, there is no direct evidence for a possible contribution of the increased release of ATP or NGF from the urothelium to the development of DO induced by CI. This deserves further study.

Improvement of the DO by the  $\beta 3$  agonist BRL37344 was accompanied by an alteration in intravesical mediator concentration. The intravesical NGF concentration was significantly reduced by BRL37344 and the ATP concentration was also reduced in six of the eight rats with CI although the change in the mean ATP concentration did not reach the statistical significance. These findings suggest that the modulation of NGF or ATP release from the urothelium by  $\beta 3$  agonists is involved in the mechanism underlying the improvement of the DO by  $\beta 3$  agonists.

In matters of the PGE2 concentration, there was no obvious change by CI. This is in accordance with the finding in our previous study that the amount of PGE2 in the bladder tissue was not changed by CI [2]. In addition, the PGE2 concentration was not changed along with the improvement of the DO by BRL37344. Therefore, PGE2 may not be associated with the development of DO induced by CI and the improvement of the DO by  $\beta 3$  agonists.

#### Concluding message

The intravesical release of ATP and NGF from the urothelium was increased in rats with neurogenic DO induced by CI. The  $\beta 3$  agonist BRL37344 improved the DO and decreased the NGF release and probably the ATP release from the urothelium in the setting of the present study. The PGE2 release from the urothelium was not altered by CI and the BRL37344 administration. The ATP or NGF release from the urothelium may be associated with the development of DO induced by CI and the improvement of the DO by BRL37344.

#### References

1. Yokoyama O, et al. World J Urol 2010.
2. Zha X, et al. ICS 2010 abstract #340.

#### Disclosures

**Funding:** none **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** The Institutional Animal Care and Use Committee of the University of Fukui