

## **A QUANTITATIVE ANALYSIS OF mRNA EXPRESSION OF $\alpha_1$ - AND $\beta$ -ADRENOCEPTOR SUBTYPES AND THEIR FUNCTIONAL ROLES IN HUMAN NORMAL AND OBSTRUCTED BLADDERS.**

### **Aims of Study:**

Irritative bladder symptoms are frequently associated with bladder outlet obstruction (BOO) derived from benign prostatic hyperplasia (BPH). Because of the usefulness of  $\alpha_1$ -AR antagonists for treating these symptoms, attention has recently focused on the possibility of  $\alpha_1$ -AR subtype ( $\alpha_{1D}$ -AR) in the bladder that may concern the mechanisms for detrusor instability and related irritative symptoms. However, in order to elucidate the above mechanisms, it is important to investigate the regulation of the  $\beta$ -AR and its subtypes in obstructed bladder because relaxation-mediating  $\beta$ -ARs predominate over contraction-mediating  $\alpha_1$ -ARs in detrusor muscle. In the present study, we therefore compared the expression level of  $\alpha_1$ -AR subtype mRNAs to that of  $\beta$ -AR subtype mRNAs in the control and obstructed human bladders. In addition, an isometric contraction study was also performed to determine whether the  $\alpha_1$ -AR - mediated contraction and  $\beta$ -AR - mediated relaxation of human detrusor muscle are altered by BOO.

### **Methods:**

Muscle specimens from the bladder dome were obtained from 7 BPH patients (mean 67.7 years) with BOO and 10 male patients (mean 65.3 years) without BOO undergoing open pelvic surgery. BOO was judged by pressure / flow studies before the surgery.

For mRNA analysis, total RNA was extracted from each detrusor muscle specimen. Real-time quantitative reverse transcription (RT) -polymerase chain reaction (PCR) based method was used to quantify  $\alpha_1$ -AR and  $\beta$ -AR subtypes mRNA expressions.

For functional studies, detrusor muscle strips were mounted in a 25ml organ bath containing Krebs solution at 37°C oxygenated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The tension of the strips was measured isometrically. The contractile response to phenylephrine ( $\alpha_1$ -AR agonist) was examined by a cumulative addition of the drug to the bathing fluid. When the relaxant effect of  $\beta$ -AR agonists was evaluated, the detrusor muscle contraction was induced by 10<sup>-7</sup> M carbachol. After this pre-contraction reached plateau,  $\beta$ -AR agonist was added into the bath cumulatively.

### **Results:**

In control bladders,  $\alpha_{1a}$ -,  $\alpha_{1b}$ -,  $\alpha_{1d}$ -,  $\beta_1$ - and  $\beta_2$ -AR mRNAs were expressed at very low levels (less than 10 copies per ng. total RNA) while  $\beta_3$ -AR was the most highly expressed subtype at the mRNA level (mean 390.2 copies per ng. total RNA), representing 94.4% of overall  $\alpha_1$ - and  $\beta$ -ARs messages. In obstructed bladders, the expressions of  $\alpha_{1a}$ -,  $\alpha_{1d}$ -,  $\beta_2$ - and  $\beta_3$ -AR mRNAs were increased while  $\alpha_{1b}$ - and  $\beta_1$ -AR mRNAs expressions were decreased. However, these changes were not significant. Thus, the distribution of all the subtype mRNAs in human bladder was not altered by BOO. The predominant expression of  $\beta_3$ -AR mRNA was retained in obstructed bladders, accounting for 95.7% of overall  $\alpha_1$ - and  $\beta$ -ARs messages.

Phenylephrine at concentrations up to 10<sup>-5</sup> M produced no response in the detrusor muscles from both control and obstructed bladder. The contractile responses to 10<sup>-4</sup> M phenylephrine were only 4.4±1.4% in the group and 5.2±1.4% in the obstructed group, respectively of 10<sup>-7</sup> M carbachol-induced contraction. The contractile responses to phenylephrine were not significantly increased in obstructed bladder.

Isoproterenol and L755,507 ( $\beta_3$ -AR selective agonist) relaxed human detrusor muscle in a concentration-dependent manner (10<sup>-9</sup> to 10<sup>-4</sup> M). The relaxing effects of these agonists in the control group were not significantly different from those in the obstructed group. However, dobutamine ( $\beta_1/\beta_2$  -AR agonist) and clenbuterol ( $\beta_2$ -AR selective agonist) did not produced relaxation at concentrations from 10<sup>-9</sup> to 10<sup>-5</sup> M in the two groups.

### **Conclusions:**

These findings indicate that neither an upregulation of  $\alpha_1$ -ARs nor a downregulation of  $\beta$ -ARs occur and relaxation-mediating  $\beta_3$ -ARs are by far predominant in human obstructed bladder. Therefore, it is not likely that bladder  $\alpha_1$ -ARs ( $\alpha_{1D}$ -AR) are responsible for detrusor instability (irritative symptoms) in patients with benign prostatic obstruction.

	$\alpha_{1a}$	$\alpha_{1b}$	$\alpha_{1d}$	$\beta_1$	$\beta_2$	$\beta_3$
Control	$\pm 0.8$	$\pm 1.5$	$\pm 0.2$	$\pm 1.2$	$\pm 0.6$	$\pm 61.1$
	$\text{total } \alpha_1 \ 11.5 \pm 2.1$			$\beta \ 401. \pm 61.2$		
Obstruction	$\pm 1.1$	$\pm 1.5$	$\pm 0.6$	$\pm 1.8$	$\pm 1.2$	$\pm 69.3$
	$\text{total } \alpha_1 \ 13.3 \pm 2.9$			$\beta \ 586. \pm 70.0$		

Table

Subtype specific and total  $\alpha_1$ - and  $\beta$ -AR mRNA levels (copies per ng. total RNA  $\pm$  S.E) in control and obstructed human detrusor.