11

Ukimura O, Iwata T, Inaba M, Honjo H, Kojima M, Miki T
Kyoto Prefectural University of Medicine

ZONAL DIFFERENCE IN ALPHA-1-ADRENOCEPTOR SUBTYPE DISTRIBUTION IN THE HUMAN PROSTATE WITH SPECIAL REFERENCE TO THE ANTERIOR FIBROMUSCULAR STROMA

Aims of Study

According to McNeal's zonal anatomy, the prostate has an area known as the anterior fibromuscular stroma (AFMS). Although the AFMS comprises up to one-third of the total bulk of the prostate, its physiological function remains unknown. In our previous study, we reported the possible functional contribution of the AFMS to micturition (1). Additionally, using immunohistochemistry, we observed significantly different innervation in the AFMS, compared with the other glandular tissue of the prostate, which suggested that the AFMS could have a specific function different from the other zones of the prostate (2). It has been reported (3) that prostatic smooth muscle tension is mediated by the α1-adrenoceptor, and that 98% of the α1-adrenoceptor in the prostate is associated with the stromal elements of the human prostate. However, the zonal difference in distribution of the α1-adrenoceptor subtypes, with special reference to the AFMS remains unknown. The aim of this study is to reveal, at the mRNA level, the difference in expression ofα1-adrenoceptor subtypes between the AFMS and the glandular zones.

Methods

Four whole human prostate specimens were obtained from male subjects (mean age: 62 years old) with unilateral low volume prostate cancer undergoing radical prostatectomy, or with bladder cancer undergoing cysto-prostatectomy. The Institutional Board of Research Associates at our institution approved these research procedures involving human tissue. According to McNeal's zonal anatomy, tissue from the AFMS, the transition zone (TZ), and the peripheral zone (PZ) was dissected into cubes <5mm in dimension, and quickly stored in liquid nitrogen at –80□. The forward primers, reverse primers and TaqMan probes ofα1-adrenoceptor subtypes (α1-a,α1-b, andα1-d) were designed to lie in adjacent exons. Real-time quantitative polymerase chain reaction (RT-PCR) analysis using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) was performed according to the reported method. Briefly, fluorescent signal from each PCR reaction was collected as peak-normalized values plotted versus the cycle number. Reactions were characterized by comparing threshold cycle (CT) values. The CT value was a unitless value defined as the fraction cycle number at which the sample fluorescent signal passed a fixed threshold above the baseline. Samples with a high starting copy number show an increase in fluorescence early in the PCR process, resulting in a low CT number, whereas a low starting copy number resulted in higher CT numbers. The exact amount of total RNA and its quality (level of degradation) had to be determined for each sample. Therefore, a transcript of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was quantified as the endogenous control, with each unknown sample normalized to GAPDH content. For each unknown sample, the relative or absolute value was determined using linear regression analysis from the respective standard curves, as was obtained from standard samples comprises in five known absolute amounts of human prostatic cDNA molecules. A relative value for each α1-adrenoceptor subtype expression was then obtained by divising its value by the GAPDH value.

Results

In all 4 males, analysis of the relative values of α1-adrenoceptor subtype expression (α1/GAPDH) at the mRNA level demonstrated that the AFMS had significantly more α1-adrenoceptors than both the TZ and the PZ in the human prostate: 40-140 times more than the PZ and 10-35 times more than the TZ.

Table: Zonal differences of α1a-adrenoceptor subtype expression (α1-subtype/GAPDH) mean±S.D. and p value (vs .AFMS)

		α1-a	α1-b	α1-d
Case1				
	AFMS	65061±8231	32562±6355	2342±195
	TZ	2840±385 (p<0.01)	777±83 (p<0.05)	106±41(p<0.01)
	PZ	610±391 (p<0.01)	50±33 (p<0.05)	7±5(p<0.01)
Case 2				
	AFMS	5906±1179	2521±274	375±53
	TZ	371±50 (p<0.01)	67±59 (p<0.01)	18±4(p<0.05)
	PZ	587±65 (p<0.01)	133±17 (p<0.01)	33±6(p<0.05)
Case 3				
	AFMS	11087±2301	12538±2505	3625±719
	TZ	2050±436 (p<0.05)	1696±441 (p<0.05)	479±85(p<0.05)
	PZ	1686±371 (p<0.05)	1050±216 (p<0.05)	276±94(p<0.05)
Case 4				
	AFMS	25115±3513	10941±1574	1511±258
	TZ	188±40 (p<0.05)	23±1 (p<0.05)	8±2(p<0.05)
	PZ	427±126 (p<0.05)	118±22 (p<0.05)	7±1(p<0.05)

Conclusions

All of the three subtypes of α1-adrenoceptor (α1-a,α1-b, andα1-d) at the mRNA level are significantly more expressed in the AFMS than in the TZ and the PZ in the human prostate. The AFMS might be a therapeutic target of the α1-adrenoceptor antagonist or agonist. Its physiological function and possible clinical significance should be determined in further research,

References

1 Neuro Urody 17: 377, 1998
2 Prostate 48:242-247, 2001
3 J Urol 154: 2096-2099, 1995