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TEMPORAL PROFILE OF PROTEIN KINASE C ISOZYMES IN THE UROTHELIUM AND DETRUSOR OF RAT

Aims of Study

Protein Kinase C (PKC) modulates the effects of cholinergic and adrenergic neurotransmission in many tissues/cells and mediates the contractility of the smooth muscle in the cardiovascular and gasterointestinal organs. Studies of PKC profile on other tissues have suggested alteration in the profile of PKC isozymes during the stages of development. Our objective was to assess the temporal profile of the PKC in the bladder tissues of rat.

Methods

Sprague Dawley rats were used for the experiments. After anesthesia, the Detrusor muscle and urothelium were removed by in-vivo dissection using dissecting microscope. The brain was also removed for internal control of isozymes. The tissues were weighted, snap frozen and stored at -80°C. The frozen tissue was grinded and homogenized in TrizolTM. Standard molecular isolation methods were used to precipitate and dissolve the rat RNA in specialized H2O. The RNA concentration was determined by using a UV spectrophotometer. RT-PCR was conducted according to the Invitrogen Superscript TM RT-PCR product instructions. The amplified RT-PCR products were run on a gel and photographed. The pictures were computer scanned and the computer software program ImageQuantTM was used to estimate the intensity of the bands, which generates a value corresponding to the amount of PKC isozyme nucleic acids expressed. Chi-Square and t-test were used for analysis of dichotomous and continuous variables; with 95% confidence intervals reported for all estimates; and 80% power with 0.05 significance.

Results

18 rats were used for these experiments that were equally divided into young (5 weeks old) and old (25-31 weeks old) groups. Table below shows the difference among the amount of RNA for the PKC isozymes of alpha, Beta I, Beta II, Epsilon, Zeta, and Lambda detected in bladder urothelium (BU) and bladder muscle (BM) of the animals. PKC gamma, delta, eta, theta, and mu were not expressed in the young and old urothelium and detrusor muscle of the rat bladder compared to brain.

Table 1. PKC isozymes profile of the bladder urothelium and detrusor muscle.

	Young animals	Old animals		Young animals
PKC isozyme	Urothelium	Detrusor muscle	PKC isozyme	Urothelium
Alpha	Increased	Decreased	Alpha	Increased
Beta 1	Increased	Same	Beta 1	Increased
Beta 2	Increased	Decreased	Beta 2	Increased
Epsilon	Increased	Decreased	Epsilon	Increased
Zeta	Increased	Increased	Zeta	Increased
Lambda	Increased	Same	Lambda	Increased

Increased = levels that were higher ($p<0.05$) in one age group compared to other
Decreased = levels that were lower ($p<0.05$) in one age group compared to other
Same = no differences among the groups.

Conclusions

These findings indicate an age-dependent distribution of PKC isozymes in both urothelium and the Detrusor muscle. Correlation of these finding with contractility study of the bladder will indicate whether such age-dependent profile of PKC translates into age-dependent alteration in the contractility of the Detrusor.