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Chai T[1], Sun Y[1]
1. University of Maryland

EXTRACELLULAR ATP STIMULATES MORE ATP RELEASE FROM INTERSTITIAL CYSTITIS BLADDER UROTHELIAL CELLS COMPARED TO CONTROL CELLS

Aims of Study

Adenosine triphosphate (ATP) released by bladder urothelial cells (BUC) into the extracellular space can act as a nociceptive neurotransmitter by excitation of suburothelial sensory nerves. However, extracelluar ATP may also serve autocrine or paracrine function in affecting release of ATP by BUC. We determined the fate of exogenously ATP when added to confluent cell cultures. We investigated the effects of exogenous ATP on the release of intracellular ATP in primary human BUC cultures from both IC and control subjects. The findings from this study support the hypothesis that altered purinergic mechanisms in BUC could help to explain hypersensory dysfunction in interstitial cystitis patients.

Methods

BUC obtained from cystoscopic biopsies of 4 IC (as defined by NIH criteria) and 4 control subjects (no voiding complaints) were cultured according to established cell culture techniques. Once the cells were grown to confluence in media lacking ATP, exogenous ATP was added to cell culture media at concentrations between 10 – 40 µM. The supernatant was collected over a 3 hour period (0 hr – immediately after adding ATP, 1 hr, 2 hr and 3 hr after addition of ATP). ATP in the supernatant was measured using the luciferin-luciferase assay.

Results

Measured supernatant ATP was persistently higher with IC cells across all experimental conditions (Tables 1 and 2). When 30 µM ATP was added, there was a stimulation of significant more release of intracellular ATP release compared to control cells at 0 and 1 hour (Table 2). With IC cells, supernatant ATP levels at time 0 was greater than the amount of exogenous ATP added for the 30 and 40 µM experiments whereas it was not for the 10 and 20 µM experiments. Furthermore, the disappearance of supernatant ATP was slower in IC cells compared to control cells.

TABLE 1

	Measured Supernatant [ATP] (µM) 10µM exogenously added at time 0			Measured Supernatant [ATP] (µM) 20µM exogenously added time 0		
	IC cells	Control cells	p-value	IC cells	Control cells	p-value
0hr	3.66±0.60	0.89±0.26	**0.014**	12.32±2.77	5.12±1.52	**0.071**
1hr	0.24±0.13	0.04±0.010	0.22	3.24±0.54	2.86±0.80	0.71
2hr	0.024±0.0044	0.020±0.0028	0.44	1.50±1.01	0.63±0.46	0.48
3hr	0.021±0.0016	0.017±0.00073	**0.083**	0.034±0.012	0.019±0.0018	0.30

TABLE 2

	Measured Supernatant [ATP] (μM) 30μM exogenously added at time 0			Measured Supernatant [ATP] (μM) 40μM exogenously added at time 0		
	IC cells	Control cells	p-value	IC cells	Control cells	p-value
0hr	54.85±4.90	16.3±4.99	**0.0015**	108.92±47.00	53.08±20.14	0.34
1hr	22.1±4.25	9.89±3.15	**0.060**	97.45±37.75	43.71±19.38	0.27
2hr	2.56±1.44	0.92±0.39	0.35	31.64±11.92	26.34±13.78	0.78
3hr	0.29±0.24	0.60±0.36	0.51	35.18±13.89	5.70±2.59	0.13

Conclusions

Exogenous ATP stimulated significantly more release of ATP from IC BUC as compared to control BUC. Furthermore, with IC cells, there was a slower decay curve of extracellular ATP suggesting either diminished capacity to degrade extracellular ATP or a persistent release of intracellular ATP. The function of extracellular ATP may serve a paracrine role in the homeostasis of the bladder urothelium in addition to sensory neurotransmission. Persistence of extracellular ATP may play a role in the hypersensory state of IC. These findings suggest that purinergic activity in IC urothelial cells are altered and may be a therapeutic target in IC.