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Munch E[1], Munoz A[1], Boone T[2], Smith C[1], Somogyi G[1]
1. *Baylor College of Medicine,* ***2.*** *The Methodist Hospital*

RELEASE OF ATP AND NO FROM RAT PROSTATE: AN IN VITRO AND IN VIVO ASSESSMENT OF ADRENERGIC RECEPTOR STIMULATION

Hypothesis / aims of study

Benign prostatic hyperplasia (BPH) and concomitant lower urinary tract symptoms (LUTS) affect millions of men worldwide. Recently, there has been an increasing effort to understand neurotransmitter mechanisms related to voiding dysfunction symptoms in BPH. Several studies have suggested changes in ATP and nitric oxide (NO) release in models of voiding dysfunction. The current literature suggests that ATP can often lead to overactivity in the genitourinary tract, and NO leads to relaxation. Our experiments aim to explore the relationship between ATP and NO release in prostate tissue when alpha-1 adrenergic receptors are activated with the agonist phenylephrine. We do this using both an *in vitro* organ bath system measuring stromal and capsular release, as well as an *in vivo* system which uses a microdialysis probe to isolate and sample the stromal contribution.

Study design, materials and methods

Prostatic tissue from male Sprague-Dawley rats was tested *in vitro* and *in vivo*. Each rat was anesthetized with urethane, and after a midline abdominal incision and sharp dissection, the lateral part of the left prostate lobe was isolated using 5-0 silk suture and removed for *in vitro* studies. The microdialysis probe was then placed in the right prostate lobe for *in vivo* studies. *In vitro*: The tissue was divided in two strips: one strip was left intact and is referred to as "intact prostate." With the other strip, the capsular component of collagenous tissue and smooth muscle was carefully dissected off the stroma and glandular epithelium and is referred to as "capsule." Both tissue strips were bathed in continuously flowing oxygenated Krebs solution. After an initial equilibrium period and baseline measurements, the tissue strips were challenged with phenylephrine-containing Krebs (1uM) for 1 minute. 1-minute effluents were collected thereafter for a total of 10 minutes. *In vivo*: a commercially available microdialysis probe, containing a 5mm diffusion window, was thread through a 27-gauge needle and placed through the right lateral lobe of the prostate. The probe was perfused at a rate of 5uL/min with Krebs solution. After an initial equilibrium period and baseline measurements, the probe was perfused with Krebs solution containing phenylephrine (50nmol) for 15 minutes. 15-minute effluents were collected thereafter for a total of 150 minutes. ATP and NO release were measured in the effluents from both systems: ATP measured using HPLC and NO measured using an NO analyzer. Comparable groups were statistically analyzed using the unpaired t-test.

Results

In vitro:

Capsular and intact-prostate release of ATP and NO are shown in Figure 1. ATP and NO release, per mg tissue, were significantly higher from the capsule as compared to the intact prostate. (ATP: $p = 0.021$; NO: $p = 0.005$).

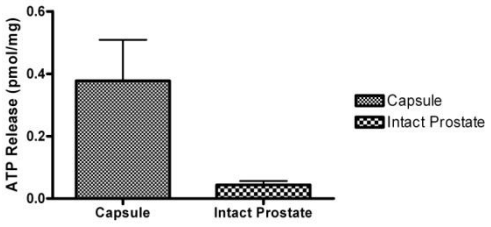
In vivo:

ATP release from prostatic stromal tissue, *in vivo*, averaged 1014000 ± 46155 pmol over baseline. NO release averaged 52.61 ± 5.289 pmol over baseline.

Comparison of ATP/NO ratio between systems:

Because the results of the *in vitro* studies (evoked release in pmol/mg tissue) were not directly comparable to the *in vivo* studies (total pmol released from prostate), the ratios of ATP/NO release for each system (*in vitro* and *in vivo*) were compared. ATP/NO ratio was consistently above 1 for the *in vivo* system (21675 ± 3299) and consistently less than 1 for the *in vitro* system (0.08376 ± 0.02626).

1a.



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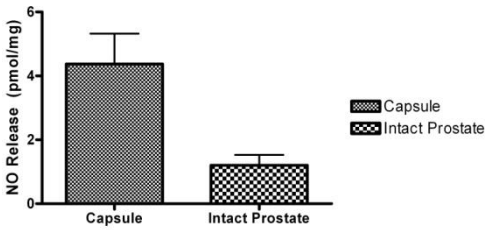

Figure 1. This figure shows *in vitro* neurotransmitter release in prostate tissue per milligram. (a) *In vitro* ATP release (n = 10), and (b) *in vitro* NO release (n = 11). Both panels demonstrate that, per milligram tissue, capsular release of neurotransmitter was higher than that in the intact prostate (ATP: $p = 0.021$; NO: $p = 0.005$).

Interpretation of results

The major findings of are study are that (1) the evoked response of ATP and NO, per mg tissue, to phenylephrine was higher in capsular prostate tissue than for the whole intact prostate, and (2) the ATP/NO ratio is high (greater than 1) in the *in vivo* system (where stroma is isolated), and low (less than 1) in the *in vitro* system (where both stroma and capsule contribute). These findings suggest hypotheses for further study:

1) The prostatic capsule, and release of ATP and NO, may play a role in both sensory and motor nerve pathways.

In our *in vitro* experiments, ATP and NO were released in higher quantities, per mg tissue, from the capsule compared to the intact prostate. This suggests that either the capsule is the main contributor to ATP and NO release, or alternatively that there might be mechanisms within the prostate stroma that modulate or inhibit release of these neurotransmitters. It is known that the capsule contains nerve endings, and these might be important contributors to sensory pathways of the prostate.

2) The capsule might be primarily responsible for the release of NO.

Our results suggest that the capsule may contribute, in greater proportion than the stroma, to the prostatic release of NO. In our *in vivo* system, the microdialysis probe was placed such that it isolated and sampled the neurotransmitters present in the

stromal environment. The *in vitro* system sampled neurotransmitters released from all parts of the intact prostate, including capsule, stroma, and glandular epithelium. Evaluating the proportions of ATP and NO release for each system, we found that the molar release of ATP was much higher than NO (by a factor greater than 20,000x) in the *in vivo* system, a system that isolating stroma and glandular epithelium from the capsule. Conversely, we found the release of ATP was much lower than NO (by a factor less than 0.1x) in the *in vitro* system, a model which includes the capsule as well as stroma and epithelium. This may suggest that the capsule is primarily responsible for NO release (or, alternatively, that the stroma has an inhibitory effect on NO release).

Concluding message

We found that ATP and NO were released in significantly higher quantities from the prostatic capsule than from the intact prostate, and that the ATP/NO ratio in the *in vivo* system (a model of stroma and epithelium only) was higher than the ATP/NO ratio in the *in vitro* system (a model including stroma, epithelium, and capsule). This study provides important insight into the purinergic and nitrergic signaling pathways using alpha adrenergic receptor activation. Understanding the release mechanisms for ATP and NO might further our knowledge about prostate pathophysiology and LUTS in benign prostatic hyperplasia.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee of Baylor College of Medicine**