

# Effect of some adenosine compounds on smooth muscles of the sheep bladder *Osteius ovis L.*

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## Summary:

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**Background:** Purines have widespread and specific extracellular signalling actions in the regulation of a variety of functions in many tissues of both invertebrates and vertebrates.

**Material and Methods:** The effect of some adenosine compound on sheep bladder smooth muscle contraction induced by KCl and ACh was investigated invitro.

**Results and Conclusions:** Preparations were preprepared with adenosine or ATP before agonist exposure. It was found that adenosine inhibited K- induced contracture and enhanced ACh-induced contracture. These actions were blocked by P<sub>1</sub> antagonist theophylline. The results also show that ATP potentiated both KCl and ACh induced contracture. These actions antagonized by P<sub>2</sub> receptor antagonist Quinidine. These results suggest that bladder smooth muscle may have A<sub>1</sub>, A<sub>2</sub> and P<sub>2x</sub> receptors.

**Key words:** Bladder smooth muscles, Purinergic receptors, Adenosine, Theophylline, Quinidine.

## Introduction:

Evidences of the non-adrenergic non-cholinergic nerve supply to the visceral organs had been proposed long ago<sup>1</sup>. Later on the neurotransmitters for these nerves were specified to be adenosine & ATP, accordingly they were called as purinergic nerves<sup>2</sup>.

The effect of these neurons through their neurotransmitters (adenosine & ATP) was found to be variable on the different tissues & organs of different species<sup>3,4,5,6</sup>. These differences were explained in part by the type of receptor that they possess, since pharmacological & biochemical studies showed that there are two main types of receptors namely P<sub>1</sub> (Adenosine) and P<sub>2</sub> (ATP) receptors<sup>7</sup>. P<sub>1</sub> receptors are subclassified into two types A<sub>1</sub>, which inhibit cAMP adenylate cyclase at P site, and A<sub>2</sub> that stimulate cAMP adenylate cyclase at R site. While P<sub>2</sub> receptor are subclassified into two classes namely P<sub>2x</sub> which contract smooth muscle and P<sub>2y</sub> that relaxes smooth muscle<sup>8</sup>.

Later on a newer classification were proposed in which P<sub>1</sub> receptors were divided into at least four (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, A<sub>3</sub>) all of them are associated with different G proteins. While P<sub>2</sub> receptors were further subdivided into P<sub>2u</sub>, P<sub>2z</sub>, P<sub>2y</sub>, P<sub>2x</sub>, and P<sub>2t</sub> depending on the relative potency of agonist, selective potency of antagonist and the effect on adenylate cyclase<sup>9</sup>.

Studies on adenosine and ATP showed that these substances exhibit variable effects on the smooth muscles of urinary system including; bladder and urethra in different species. Some studies showed that adenosine reduces smooth muscle tone and rate of spontaneous activity of the

smooth muscle of the bladder<sup>10</sup>. In addition, Adenosine has a relaxation effect on these muscles in guinea pig<sup>11</sup>. These effects were suggested to be as a result of reduction of intracellular cAMP due to the activation of A<sub>1</sub> receptors<sup>12</sup>.

On the other hand ATP causes contraction of bladder smooth muscle in most of the mammalian animals as well as human urinary bladder<sup>13</sup>. This effect is suggested to be as a result of opening Ca<sup>+2</sup> channels due to the reaction between ATP and P<sub>2x</sub> receptors<sup>14</sup>.

This study was designed to study the effect of adenosine and ATP on the contraction induced by (KCl and ACh) of the smooth muscles in sheep urinary bladder, using different antagonizing agents (theophylline, quinidine) aiming to highlight some points in regard to the purinergic receptors that might be present in the smooth muscles of the bladder and its sensitivity.

## MATERIALS AND METHODS:

Sheep (*Osteius ovis L.*) bladders were obtained from Al-Shulla slaughterhouse. The preparations were maintained in normal Krebs's solution containing in (mM): NaCl 120.7, KCl 5.9, CaCl<sub>2</sub> 2.5, MgCl<sub>2</sub> 1.2, NaHPO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 15.5 and glucose 11.5. The saline was adjusted to pH 7.3 and continuously aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and kept at 37°C.

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Preparations were cleaned, cut into strips of 1cm-length and 0.5 cm-width. Then each strip was ligated with monofilament nylon and suspended in (20 ml) Jacket organ baths between J-shape hook and Grass FTO3 force displacement transducers. Four channel Grass 79-model polygraph was used to display and record tension generated by bladder smooth muscle. Standardization by preset baseline tension of 1g weight was used before each experiment.

All drugs used in this study (adenosine, ATP, theophylline and quinidine) were added with digital adjust pipettes to the organ-baths from a highly concentrated stock solutions freshly prepared at the day of experiment. The calculated concentration was done in such a way that the final concentration is reached in the 20ml organ bath.

In the experiment designed to see the effect of adenosine or ATP on KCl or ACh induced contraction, bladder strips were firstly incubated in Krebs's solution containing different concentrations ( $10^{-8}$ – $10^{-4}$  M) of adenosine or ATP for 5 minutes before induction of contraction. While for the experiment of theophylline and quinidine role on the effect adenosine and ATP on the induced contractions, the preparation were incubated in Krebs's solution containing  $10^{-4}$  M theophylline or quinidine for 2 minutes.

Then different concentrations ( $10^{-8}$ – $10^{-4}$ ) of Adenosine or ATP were added and then after 5 minutes the contraction was induced either by 100 mM KCl or  $10^{-4}$  ACh.

For statistical analysis, t – test was used and a probability of  $<0.05$  was regarded to be significant.

## RESULTS:

### Spontaneous activity:

Most of sheep bladder smooth muscles showed irregular spontaneous phasic contraction when they were incubated in Krebs's solution. These spontaneous activities were enhanced by the effect of KCl, ACh, and ATP and reduced by the addition of theophylline, quinidine, and adenosine (Fig.1).

### KCL and ACh induced contraction:

KCl and ACh induces both phasic and tonic contraction (biphasic contraction) of bladder smooth muscles in a concentration dependent manner. The maximum response induced by KCl was reached at a concentration of 100 mM, while for the ACh the maximum response was reached at a concentration of  $10^{-4}$ M (Fig. 2 a, b).

### Effect of adenosine and ATP on KCl-induced contraction:

Adenosine attenuated the effect of KCl on bladder smooth muscles (both phasic and tonic contraction). This effect was concentration dependent and it was significant ( $p<0.05$ ) in a concentration range of ( $10^{-7}$  –  $10^{-4}$  M) of adenosine (Fig. 3 a).

While ATP potentiate the effect of KCl on bladder smooth muscles (both phasic and tonic contractions). This potentiation although was increased with the increment of ATP concentration, but it was significant ( $p<0.05$ ) only at a concentration of  $10^{-4}$ M ATP (Fig.3 b).

### Effect of theophylline and adenosine (different concentrations) on KCl-induced contraction.

Theophylline showed an incomplete antagonizing effect to adenosine compound, since it increases the phasic and tonic response significantly ( $p<0.05$ ) in comparison to the experiment of KCl and adenosine, but it does not completely remove the effect of adenosine. (Fig.4 a,b).

### Effect of quinidine and ATP at different concentration on KCl induced contraction:

Quinidine showed significantly lower phasic and tonic response ( $p<0.05$ ) not only in comparison to the effect of KCl and ATP alone but also in the comparison to the control group (KCl induced contraction without ATP). Which means that quinidine has a relaxant effect in addition to the antagonizing effect to ATP. (Fig.5 a,b).

### Effect of ATP and adenosine on ACh induced contraction:

Adenosine also potentates the phasic phase of ACh induced contraction, in a concentration dependent manner. While on the tonic phase adenosine reduces the contraction also in a concentration dependent manner. A significant effect was observed in the concentrations between  $10^{-8}$  –  $10^{-5}$  for both phasic and tonic responses (Fig.6 a, b).

The effect of ATP on ACh induced contraction showed a potentiation effect on both phasic and tonic responses. The tonic contraction was significantly ( $p<0.05$ ) potentates at a low concentration of ATP ( $10^{-8}$  M), in contrast to the phasic contraction which needed a higher concentration of ATP ( $10^{-4}$  M) to get significant difference (Fig. 7 a.b.).

### Effect of theophylline and different concentration of adenosine on ACh induced contraction:

Theophylline antagonizes the effect of adenosine on the phasic phase of contraction in a concentration dependent manner and this effect was significant at a concentration of adenosine equal to  $10^{-5}$  M in comparison to that of adenosine and ACh. While, on the tonic phase of contraction, theophylline not only abolishes the effect of adenosine at all concentrations significantly but in addition it induces a relaxant effect in comparison to the control group. (Fig. 8 a. b.).

### Effect of quinidine and different concentrations of ATP on ACh induced contraction:

Quinidine antagonized the effect of ATP on the phasic contraction in a concentration dependent manner, which was significant only when the concentration of ATP was  $10^{-4}$ M. While

in regard to the tonic phase of contraction quinidine not only inhibits the effect of ATP but in addition it causes a relaxant effect on the smooth muscle in comparison to the control group. (Fig. 9 a, b).

#### DISCUSSION:

Detrusor muscle of the sheep bladder showed spontaneous activities similar to that of guinea pig<sup>13</sup> and that of human<sup>15</sup>. KCl and ACh showed a potentiation effect on these spontaneous activities that may be explained by the opening of L-type Ca<sup>2+</sup> channels by KCl and the depolarization of smooth muscles by ACh that facilitates such type of contraction<sup>16</sup>. While the addition of KCl or ACh in the presence of adenosine does not showed the same potentiation effect as a result of the reduction in the intracellular calcium ion by adenosine<sup>17</sup>. On the other hand, ATP that increases the intracellular calcium ions showed a reversed effect to that of adenosine on the spontaneous activity. Theophylline and quinidine showed a relaxant effect, since they terminate these spontaneous activities. These findings support that of McKenzie<sup>18</sup> and that of Huddart<sup>19</sup>.

The inhibitory effect of adenosine on KCl induced contraction support the previous suggestion which mentioned that the bladder smooth muscles contain A1 receptors that relax them due to the reduction of the intracellular calcium ions in the smooth muscle cells<sup>20,21</sup>. On the other hand, adenosine potentate the phasic response and suppresses the tonic response induced by ACh indicating that these cells may posse in addition A2 receptors also. Since the potentiation of the phasic response occurs through the activation of the adenylate cyclase system<sup>22</sup>, while the inhibitory effect on the tonic response may be explained by the blocking of the L-type calcium ion channels<sup>11</sup>.

Theophylline is a selective antagonist for adenosine on P1 receptors<sup>2</sup>, so it reduces the contraction induced by adenosine which support our previous conclusion that the bladder smooth muscles have A1 receptors. The present work showed that the inhibition of adenosine was incomplete suggesting that these muscles have in addition A2 receptors which is furtherly supported in the experiments of ACh induced contraction in which theophylline antagonized completely the effect of adenosine<sup>23</sup>.

ATP potentate the phasic and tonic contraction induced by both KCl and ACh, which suggest that bladder smooth muscles have P2x receptors that increase calcium ion influx to the bladder smooth muscles. The increase in the phasic response was more evident than that of the tonic response indicating that there may be opening of L-type calcium channels for a longer period<sup>14</sup>.

Quinidine is a selective antagonist to ATP on P2 receptors<sup>22,23</sup>. The inhibitory effect of quinidine on KCl and ACh induced contraction support the

suggestion of the presence of P2x receptors in bladder smooth muscles, and the extra relaxation that we observe may be explained by its inhibitory effect on calcium release from their internal stores<sup>24</sup>.

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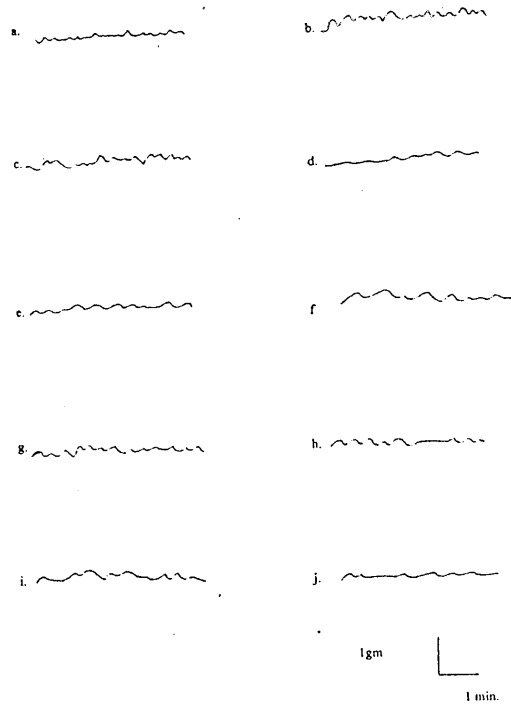


Figure 1. Spontaneous phasic contraction of the bladder smooth muscles; a. in Kreb's solution. b. after stimulation by Ach or KCl. c. before the addition of adenosine. d. after the addition of adenosine. e. before the addition of ATP. f. After the addition of ATP. g. before the addition of theophylline. h. after the addition of theophyllin. i. before the addition of quinidine. j. after the addition of quinidine.

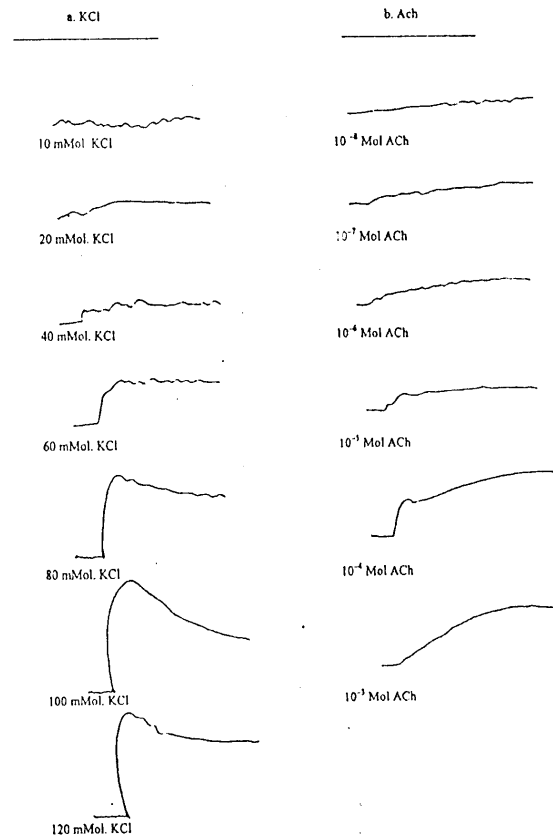


Figure 2. The response of bladder smooth muscles for different concentration of a. KCl b. Ach

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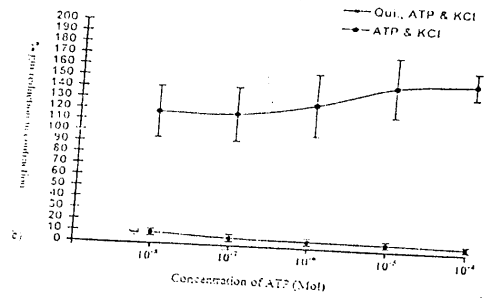
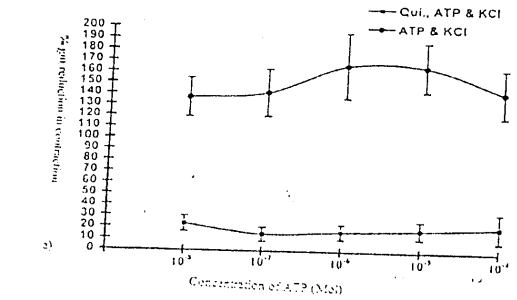
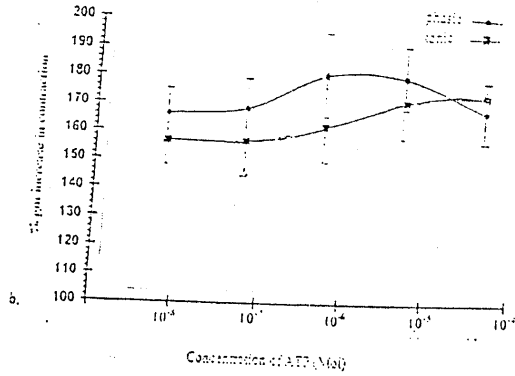
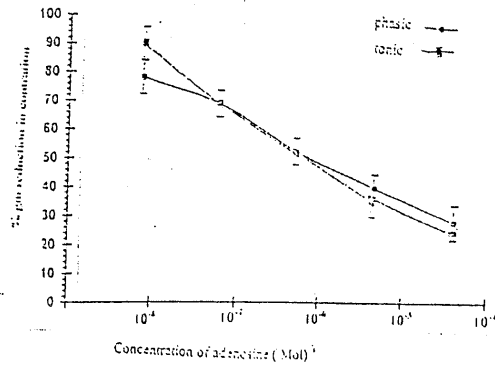


Figure 5. The effect of 10<sup>-4</sup> M quinidine and the different concentration of ATP on the a) phasic and b) tonic contraction of bladder smooth muscles induced by 100 mM KCl.

Figure 3. 1. The effect of different concentrations of adenosine on the KCl induced contraction (phasic and tonic) of bladder smooth muscle. 2. The effect of different concentrations of ATP on the KCl induced contraction (phasic and tonic) of bladder smooth muscle.

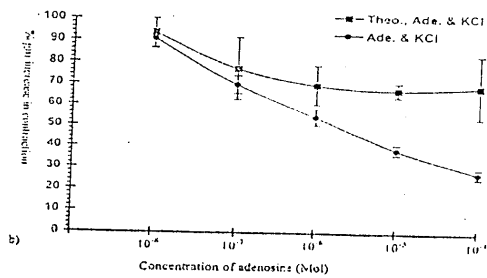
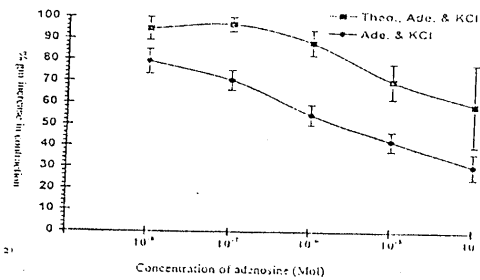


Figure 4. The effect of 10<sup>-4</sup> Mol theophylline and the different concentration of adenosine on the a) phasic and b) tonic contraction of bladder smooth muscles induced by 100 mMol KCl.

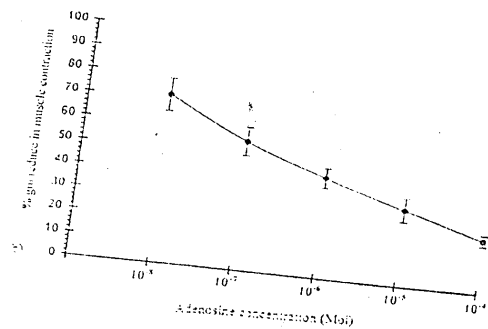
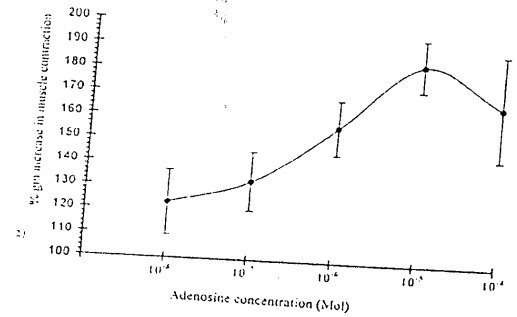


Figure 6. a) The effect of different concentration of adenosine on ACh induced contraction phasic and tonic of bladder smooth muscle.

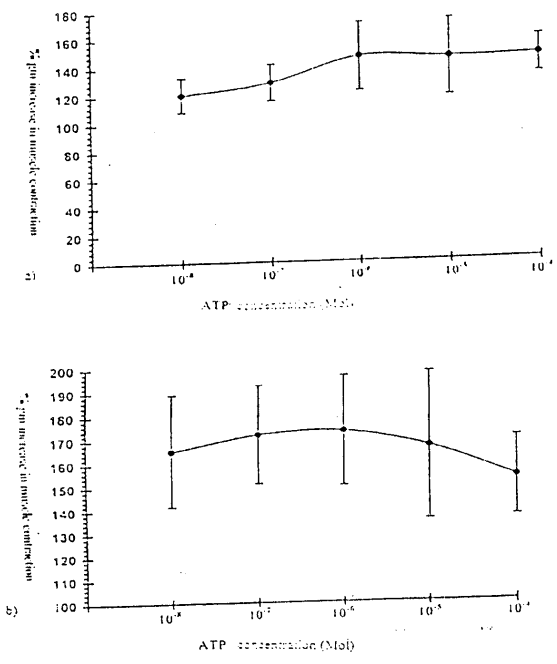


Figure 7. The effect of different concentration of ATP on Ach induced contraction a) phasic and b) tonic of bladder smooth muscle.

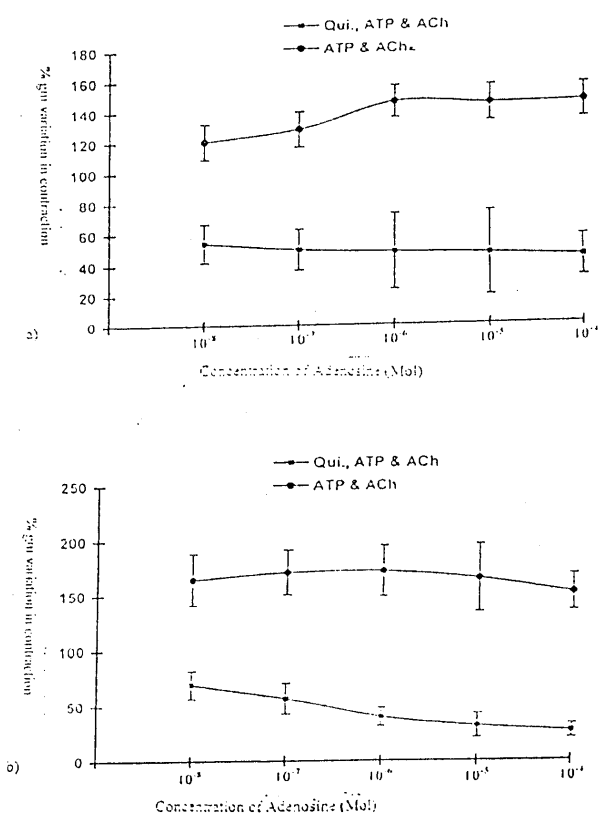


Figure 9. The effect of  $10^{-4}$  M quinidine and the different concentration of adenosine on the a) phasic and b) tonic contraction of bladder smooth muscles induced by  $10^{-6}$  M Ach.

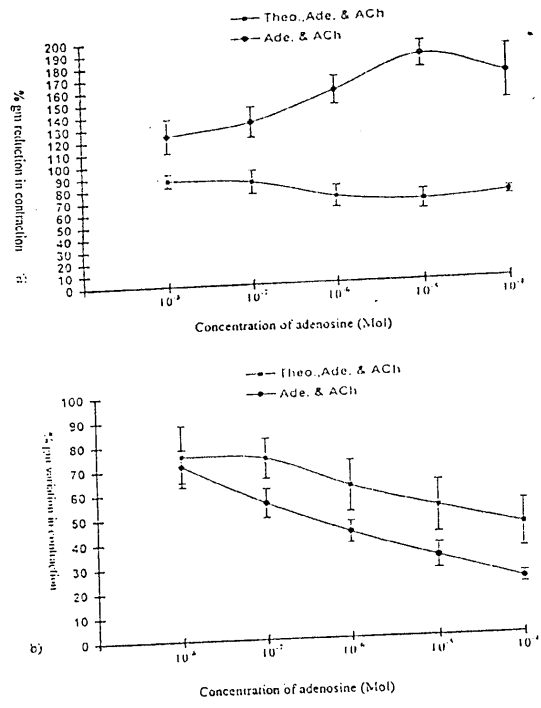


Figure 8. The effect of  $10^{-4}$  M theophylline and the different concentration of adenosine on the a) phasic and b) tonic contraction of bladder smooth muscles induced by  $10^{-6}$  M Ach.