Effect Of Some Adenosine Compounds On Smooth Muscles Of The Sheep Bladder *Osteius Ovis L.*

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Summary:

Backgraound: *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: *Preperations were prepreated with adenosine or ATP befor agonist exposure. It was found that adenosine inhibited K- induced contracture and enhanced Ach- induced contracture. These actions were blocked by Pi antagonist theophylline. The results also show that ATP potentiated both KCl and ACh induced contracture. Theses actions antagonized by P_2 receptor antagonist Quinidine. These results suggest that blader smoth muscle may have Aj, A_2 and P_{2x} receptors.*

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

Introduction:

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

The effect of these neurons through their neurotransmitters (adenosine & ATP) was found to be variable on the different tissues & organs of different species [1,5,6]. These differences were explained in part by the type of receptor that they posses, since pharmacological & biochemical studies showed that there are two main types of receptors namely Pj (Adenosine) and P_2 (ATP) receptors [7]. Pi receptors are subclassified into two types Ai, which inhibit cAMP adenylate cyclase at P site, and A2 that stimulate cAMP adenylate cyclase at R site. While P2 receptor are subclassified into two classes namely P_2x which contract smooth muscle and P_2y that relaxes smooth muscle .

Later on a newer classification were proposed in which PI receptors were divided into at least four (Al, A2A, A2B, A3) all of them are associated with different G proteins. While P2 receptors were further subdivided into P2u, P2z, P2y, P2x, and P2t depending on the relative potency of agonist, selective potency of antagonist and the effect on adenylate cyclase[9].

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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[10]. In addition, Adenosine has a relaxation effect on these muscles in guinea pig [11] These effects were suggested to be as a result of reduction of intracellular cAMP due to the activation of A1 receptors .

On the other hand ATP causes contraction of bladder smooth muscle in most of the mammalian animals as well as human urinary bladder [13].This effect is suggested to be as a result of opening Ca^{+2} channels due to the reaction between ATP and P2x receptors [14].

This study was designed to study the effect of adenosine and ATP on the contraction induced by (KC1 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 Kreb's solution containing in (mM): NaCl 120.7, KC1 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaHPO4 1.2, $NaHCO_3$ 15.5 and glucose 11.5. The saline was adjusted to pH 7.3 and continuously aerated with 95% O_2 and 5%

CO2 and kept at 37°C.

Preparations were cleaned, cut into strips of 1 cm-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 lg 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 KC1 or ACh induced contraction, bladder strips were firstly incubated in Kreb'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 Kreb's solution containing 10^{-4} M theophylline or quinidine for 2 minutes.

Then different concentrations (10" - 10 ") of Adenosine or ATP were added and then after 5 minutes the contraction was induced either by 100 mMKClorlO"^{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 Kreb's solution. These spontaneous activities were enhanced by the effect of KC1, ACh, and ATP and reduced by the addition of theophyline, quinidine, and adenosine (Fig.l).

KCL and ACh induced contraction:

KC1 and ACh induces both phasic and tonic contraction (biphasic contraction) of bladder smooth muscles in a concentration dependent manner. The maximum response induced by KC1 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 KC1 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 ^ M) of adenosine (Fig. 3 a).

While ATP potentiate the effect of KC1 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 KC1 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 KC1 induced contraction:

Quinidine showed significantly lower phasic and tonic response ($p<0.05$) not only in comparison to the effect of KC1 and ATP alone but also in the comparison to the control group (KC1 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. Whle on the tonic phase adenosine reduces the contraction also in a concentration dependent manner. A significant effect was observed in the concentrations between 10" - 10" 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 ~ 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 [13] and that of human [15]. KCl and ACh showed a potentiation effect on these spontaneous activities that may be explained by the opening of L-type Ca^{2+} channels by KCl and the depolarization of smooth muscles by ACh that facilitates such type of contraction [16]. 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[17]. 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[18]and that ofHuddart[19].

The inhibitory effect of adenosine on KCl induced contraction support the previous suggestion which mentioned that the bladder smooth muscles contain Al receptors that relax them due to the reduction of the intracellular calcium ions in the smooth muscle cells [20,21]. 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[22], while the inhibitory effect on the tonic response may be explained by the blocking of the L-type calcium ion channels[11].

Theophylline is a selective antagonist for adenosine on PI receptors [2], so it reduces the contraction induced by adenosine which support our previous conclusion that the bladder smooth muscles have Al 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 [23].

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 [14].

Quinidine is a selective antagonist to ATP on P2 receptors [22,23]. 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[24].

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