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INHIBITORY EFFECT OF KETOTIFEN ON CONTRACTIONS OF RAT ISOLATED BLADDER

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ABSTRACT

Ketotifen is a benzocycloheptathiophene with a range of pharmacological activities. The present study was carried out to evaluate the action of ketotifen on isolated rat bladder contractions induced by KCl and acetylcholine, compared with the effects of other drugs. Ketotifen (5 μM) reduced the response to acetylcholine on rat isolated bladder without altering the maximum response and shifted the acetylcholine concentration-response curve to the right 16 - fold. Ketotifen also reduced the KCl response, while atropine only inhibited the response to acetylcholine. Diazoxide inhibited bladder - induced contraction only at high concentration (500 μM). This study shows that ketotifen is a relaxant of isolated rat bladder. As the inhibition of contractile overactivity of the bladder is the basis of treatment of bladder instability, provided that a similar effect will be seen *in vivo*, then ketotifen may have clinical benefits for treatment of this condition.

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Keywords: Bladder, Ketotifen, Atropine, Diazoxide, Smooth Muscle.

INTRODUCTION

Ketotifen is a benzocycloheptathiophene which was initially designed as an antiasthmatic drug (for review see ref. 1). However, this drug has a wide spectrum of other pharmacological activities, including antagonistic effect on muscarinic receptors,[2] anti-inflammatory effect in the stomach and large intestine,[3] prevention of gastric damage by non-steroidal anti-inflammatory drugs,[4] as well as inhibitory effect on guinea-pig intestinal smooth muscle,[5] vas deferens [2] and rat uterus.[6] The exact mechanism of action of ketotifen is unknown, nevertheless its mast cell stabilizer effect could explain its anti-inflammatory action and it is thought to act like sodium cromolyn.[1] On the other hand, ketotifen can stimulate nitric oxide synthase activity,[7] which may explain some other actions of this drug. It seems that the effect of ketotifen is more than antagonistic on serotonergic, histaminergic or cholinergic receptors and its inhibitory effect on smooth muscles is relatively non-selective. In this study, the effect of ketotifen on isolated rat bladder contractions induced by acetylcholine and KCl was examined to see if it could inhibit contractions of this tissue and look for possible clinical applications of ketotifen in bladder incontinence.

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MATERIALS AND METHODS

Experimental procedure

Male Wistar rats (200 - 250 g) bred in Isfahan, were killed by a blow on the head followed by exsanguination. The whole bladder was removed and placed in oxygenated Tyrode's solution (see solutions) at room temperature. The connective issue was carefully trimmed and then suspended in Tyrode's solution at 37°C and bubbled with oxygen. From a resting tension of 1g, isotonic contractions, elicited by KCl and acetylcholine, were recorded using a Harvard isotonic transducer and displayed on a Harvard Universal Oscillograph pen recorder device. Drugs were added directly to the organ bath in volumes usually not exceeding 5% of bath volume (20 mL organ bath). The spasmogen, KCl, was added cumulatively at 5 min intervals. The effect of acetylcholine was studied using a single dose regimen with a contact time of 30 s and time cycle of 4 min. Four consecutive full concentration-effect curves were obtained in absence, vehicle-treated and then in the presence of two concentrations of testing drugs (ketotifen 500 nM and 5 μM; diazoxide 50 and 500 μM and atropine 5 and 50 nM). Each drug concentration was in contact with the tissue at least 10 min before their effects were evaluated.

Measurements and statistical analysis

Contractions were measured as maximum changes in tension from pre-drug baseline within the contact time or as area under the curve produced by tissue contraction at 5 min intervals just before addition of the next concentration of the test drug and expressed as percentage of control or maximum induced response for each tissue. Mean and standard error of mean (S.E.M.) values were calculated for each group of results and significance of differences between the means was calculated by two-tailed paired Student's t-test or by one way analysis of variance (ANOVA). Differences were considered statistically significant when $p \leq 0.05$. Origin computer program was used for fitting non-linear curve.

Drugs and solutions

Tyrode's solution composed of (mM): NaCl, 139.9; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.42 and glucose 5.55 made up in double distilled water and bubbled with CO_2 until the pH was adjusted to 7.4, thereafter the pH remained constant. KCl was made up as 2M solution in double distilled water.

The following drugs were used for the experiments: acetylcholine chloride; ketotifen fumarate; diazoxide; atropine sulphate. Acetylcholine was made up as 100 mM stock solution in double distilled water and acidified with acetic acid, dilution being made in distilled water or Tyrode's solution as appropriate. Ketotifen was made up as 100 mM stock solution in double distilled water; further dilution was made in Tyrode's solution. Diazoxide was made up in 0.015% sodium hydroxide as 50 mM stock solution, dilution being made in double distilled water (final concentration of solvent in the bath was without effect). Drugs were purchased from Sigma, ketotifen was from Sandoz and chemicals were from Merck.

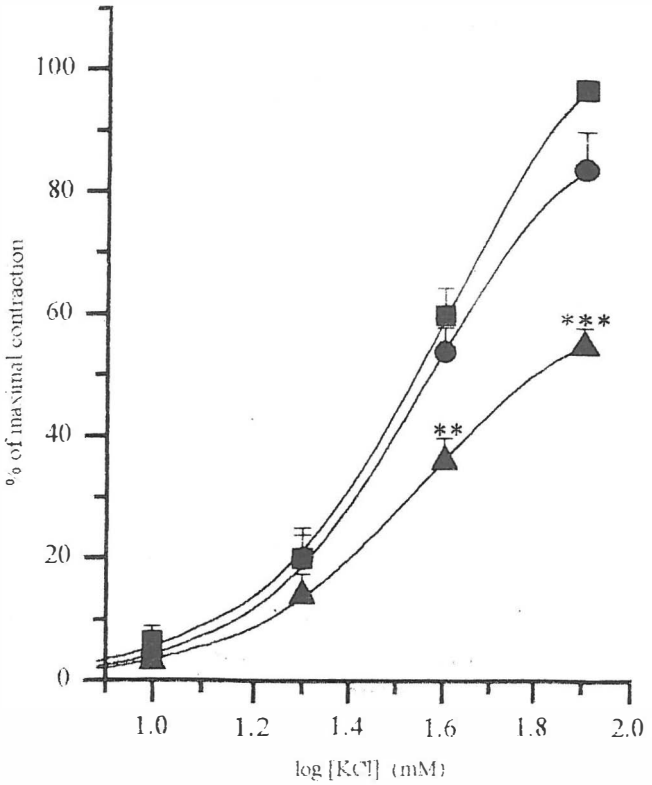

Fig. 1. Effect of ketotifen on tension development to KCl (10, 20, 40 and 80 mM) in isolated bladder of rats. Sigmoidal curve fitted through the points in the absence (square) and presence of ketotifen 500 nM (circle) and 5 μM (triangle). Ordinate scale: response expressed as a % of the maximum response to KCl in each tissue. Abscissa scale: $\log_{10}$ concentration of KCl. The points are mean and the vertical bars show the S.E.M. (n=6). $^{**}p<0.01$, $^{***}p<0.001$ compared with corresponding KCl concentration in control (t-test).

RESULTS

Rat bladder suspended in Tyrode's solution occasionally showed small spontaneous contractile activity and KCl (10, 20, 40 and 80 mM) produced a concentration-dependent tonic contraction with slight fluctuations. Acetylcholine casued a concentration-dependent contraction of rat bladder, reaching a maximum within 30 s of contact. Ketotifen at 5 μM bath concentration had a statistically significant inhibitory effect on the KCl concentration-response curve in rat bladder and reduced the maximum induced contraction (Fig. 1), while the muscarinic receptor antagonist atropine had no effect on the KCl concentration-response curve in this tissue (n=4). When ketotifen was washed out, normal response to KCl was restored again. However, the inhibitory

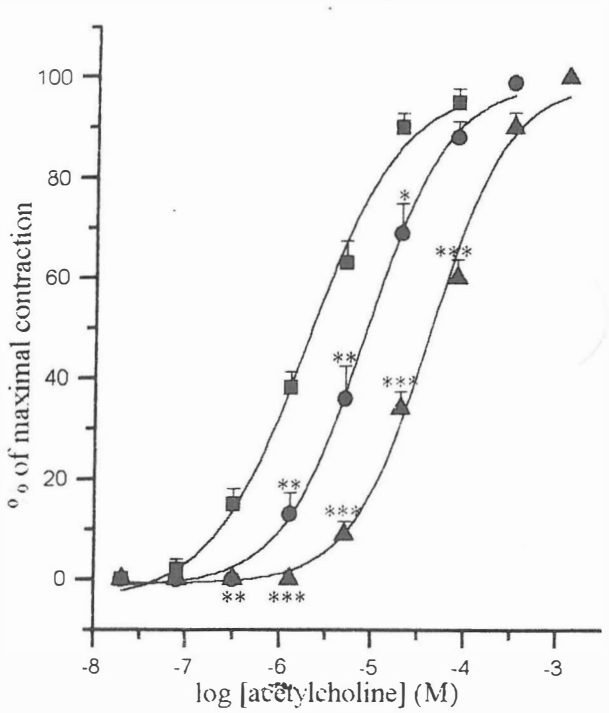

Fig. 2. Effect of ketotifen on tension development to aceytlcholine in isolated bladder of rats. Sigmoidal curve fitted through the points in the absence (square) and presence of ketotifen 500 nM (circle) and 5 μM (triangle). Ordinate scale: response expressed as a % of the maximum response to acetylcholine for each tissue. Abscissascale: $\log_{10}$ concentration of acetylcholine. The points are mean and the vertical bars show the S.E.M. (n=6). *p<0.05, **p<0.01, ***p<0.001 compared with corresponding acetylcholine concentration in control (t-test).

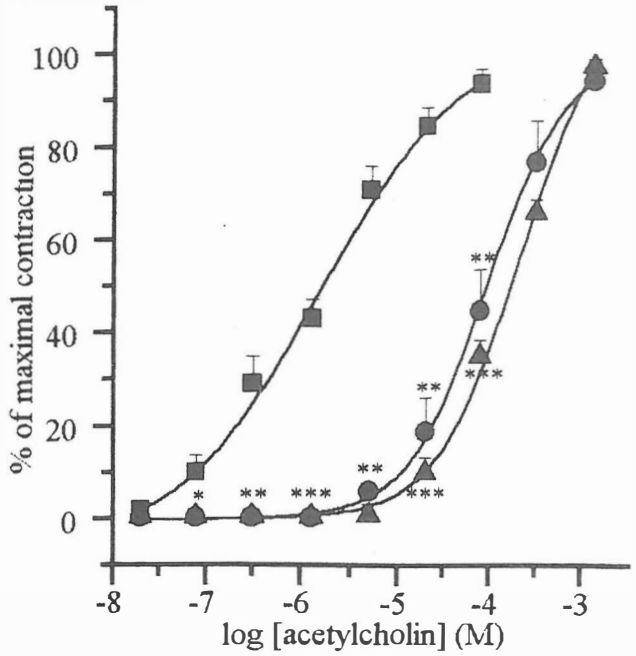

Fig. 3. Effect of atropine on tension development to acetylcholine in isolated bladder of rats. Sigmoidal curve fitted through the points in the absence (square) and presence of atropine 5 nM (circle) and 50 nM (triangle). Ordinate scale: responseexpressed as a % of the maximum response to acetylcholine for each tissue. Abscissa scale: $\log_{10}$ concentration of acetylcholine. The points are mean and the vertical bars show the S.E.M. (n=6). *p<0.05, **p<0.01, ***p<0.001 compared with corresponding acetylcholine concentration in control (t-test).

effect of ketotifen on rat bladder was not complete and at 5 μM bath concentration, 45 ± 3% of the response to 80 mM KCl still remained (n=6). Ketotifen reversibly antagonized the response to acetylcholine without altering the maximum response. In the presence of ketotifen (500 nM and 5 μM) there were 4- and 16-fold rightward shifts in the concentration-response curves of acetylcholine, respectively (see Fig. 2). Atropine at 50 nM concentration reduced the acetylcholine response and shifted the concentration-response curve to the right 65-fold (Fig. 3). This antagonist of muscarinic receptors was used for comparison and our results are comparable to the effect of atropine on the carbachol concentration-response curve in the rat bladder (see ref. 8).

Diazoxide at 50 μM concentration had no significant effect on the response of rat bladder to acetylcholine, but at 500 μM bath concentration caused a three-fold shift of the curve to the right without affecting the maximum response (Fig. 4). Diazoxide mainly affected the contractions to lower concentrations of KCl and response to 20 mM KCl was reduced from 43 ± 3.8 to 31± 3.5 and 14 ± 2.2 with 50 and 500 μM bath concentrations, respectively (Fig. 5, n = 6). Similarly, the response to 10 mM KCl was reduced from 13 ± 2.5 to 7 ± 1.4 with 50 μM bath concentration ($p \leq 0.01$). With 500 μM diazoxide in the bath, total inhibition was achieved ($p \leq 0.001$, n= 6). There were no significant changes in response of the tissues treated with the vehicle.

DISCUSSION

The objective of this work was to study the action of ketotifen on contractile activity induced by two different spasmogens on rat bladder in comparison with two other agents to see if there is a possibility for the beneficial use of this drug in pathological conditions like bladder instability, which is a common problem especially in old age. Current therapy for this disturbance is directed towards inhibition of smooth muscle contractions. Antagonists of muscarinic receptors are used for control of bladder instability; however, they have undesired adverse effects. Thus, there is a need to investigate other mechanisms by which inhibition of bladder contractions can be achieved.

Bladder incontinence may arise from involuntary contractions of the detrusor muscle. The ideal drug for treatment of detrusor overactivity should abolish involuntary contractions without altering normal emptying of the bladder.

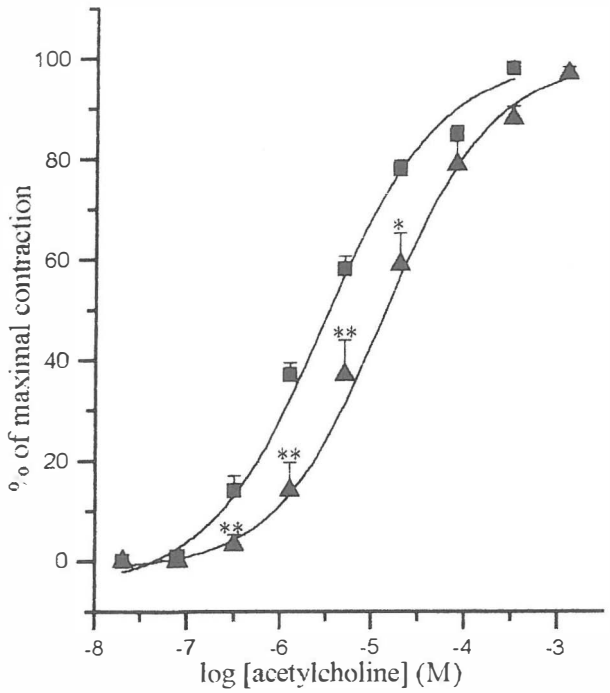

Fig. 4. Effect of diazoxide on tension development to acetylcholine in isolated bladder of rats. Sigmoidal curve fitted through the points in the absence (square) and presence of diazoxide 500 μM (circle). Ordinate scale: response expressed as % of the maximum response to acetylcholine for each tissue. Abscissa scale: $\log_{10}$ concentration of acetylcholine. The points are mean and the vertical bars show the S.E.M. (n=6). *p<0.05, **p<0.01 compared with corresponding acetylcholine concentration in control (t-test).

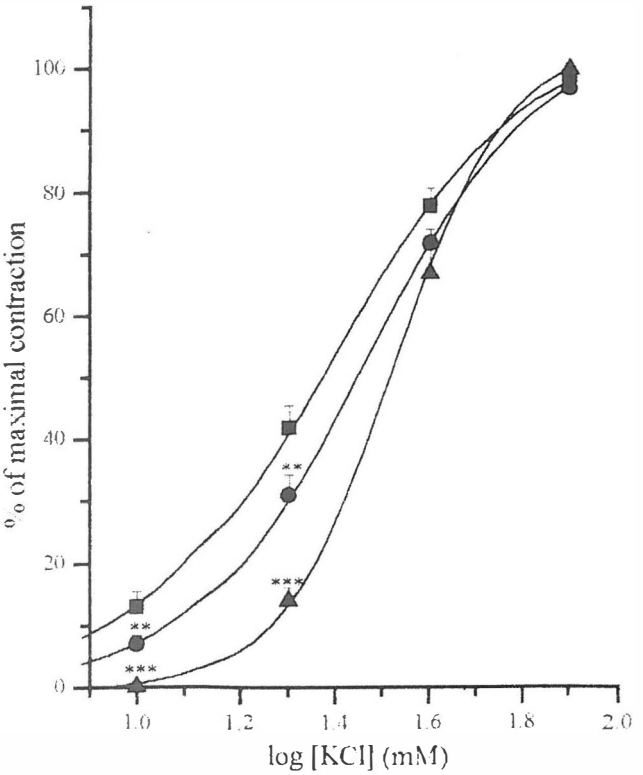

Fig. 5. Effect of diazoxide on tension development to KCl (10, 20, 40 and 80 mM) in isolated bladder of rats. Sigmoidal curve fitted through the points in the absence (square) and presence of diazoxide 50 μM (circle) and 500 μM (triangle). Ordinate scale: response expressed as a % of the maximum response to KCl in each tissue. Abscissa scale: $\log_{10}$ concentration of KCl. The points are mean and the vertical bars show the S.E.M. (n=6). **p<0.01, ***p<0.001 compared with corresponding acetylcholine concentration in control (t-test).

No existing drug has this profile and the agents which are currently used in the treatment of incontinence, mainly antimuscarinic and smooth muscle relaxants, have undesirable side effects (for review see ref. 9). The antagonists of muscarinic receptors such as atropine increase the capacity and reduce the frequency of urinary bladder contractions by antagonizing the parasympathetic control of this organ and for this purpose they have been used for control of enuresis in children.[9] Although the antimuscarinic agents generally produce significant improvement in patients with involuntary bladder contraction, only a partial inhibition results. Oxybutinin which has relatively less anticholinergic and greater antispasmodic activity than atropine, is more effective in the treatment of a range of unstable bladder conditions.[10] The potassium channel opener cromakalim at 3-30 μM concentration inhibits the response to carbachol in rat bladder,[8] however cromakalim is approximately 5-10 times more potent on vascular than on bladder smooth muscle.[11] The potency of diazoxide for inhibiting bladder contraction is far less than cromakalim and its cardiovascular effects are very pronounced.[12,13]

In this experiment we have shown that ketotifen is a relaxant of rat bladder smooth muscle, partially due to its ability to antagonize muscarinic receptors,[2] but this is not all and this drug was also able to reduce the response to non-specific spasmogens like KCl. Ketotifen has been used clinically for the treatment of asthma and is free of many adverse effects of typical anticholinergic agents, although drowsiness is occasionally observed. Ketotifen has no significant effect on the cardiovascular system at clinical doses[1] and these are the advantages of this agent over existing drugs.

There is no doubt that regardless of the kind of induced contraction, ketotifen is a potent relaxant of rat bladder *in vitro* but the remaining question is how the relaxant effect of ketotifen is brough about. Ketotifen was introduced as a prophylactic drug for asthmatic patients and thought to have similar actions to cromolyn sodium.[1] The relaxant effects of ketotifen on bladder smooth muscle ought to be different since the onset of action is relatively quick (less than 10 min). There is also a report suggesting that ketotifen has some inhibitory effect on phosphodiesterase and increases cAMP concentration.[14] This may explain the

inhibitory effect of ketotifen on KCl response.

In conclusion, we can definitely state that ketotifen is a novel and potent relaxant of rat bladder, relaxing the tissue contraction induced by two different spasmogens. In this study, the effect of ketotifen on bladder was far better than antagonists of muscarinic receptors and it may have an advantage over them in the treatment of bladder contractile disturbances. Unlike antagonists of muscarinic receptors, ketotifen inhibits the response to both KCl and acetylcholine and it shows that the inhibitory effect of ketotifen is through mechanism(s) other than antagonism of muscarinic receptors, probably involving the second messenger system.

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