



## Research Article

### KCNQ potassium channel expression in the caprine ureter and inhibition of the contractility of isolated distal caprine ureter by KCNQ channel opener Retigabine

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#### ABSTRACT

Voltage gated potassium channels i.e. KCNQ potassium channels are expressed in several types of smooth muscles and may possibly mediate physiological responses in the tissues where they are expressed. Flupirtine and Retigabine is two smooth muscle relaxant agents that act by opening KCNQ (Kv7) potassium channels. This study was conducted to ascertain the ability of retigabine to inhibit the bethanechol induced contractility of isolated distal caprine (goat) ureter. The study suggests the presence of KCNQ potassium channels in the isolated caprine ureter based on the results obtained. The ability of 5, 10, 50, and 100  $\mu$ M concentrations of retigabine to inhibit the bethanechol induced contractility of isolated distal goat ureter was studied. The ability of the non-specific potassium channel blocker 4-aminopyridine (1 mM) and the specific KCNQ channel blocker Linopirdine dihydrochloride (50  $\mu$ M) to reverse the inhibitory effect of retigabine on ureteric contractility was also evaluated. The presence of KCNQ channels in isolated goat ureter was investigated using immunofluorescent microscopy. At the concentrations of 10, 50 and 100  $\mu$ M concentrations, retigabine significantly inhibited the spontaneous contractility of the isolated goat ureter. At 10  $\mu$ M Retigabine showed a reduction of 19.2 % for a contact period of 10 minutes. The inhibitory effect of retigabine on ureteric contractility was significantly reversed by 4-aminopyridine and Linopirdine. Immunofluorescent microscopy revealed the presence of KCNQ channels in the goat ureter. Retigabine inhibits the spontaneous contractility of the isolated goat ureter by opening KCNQ channels. Our study presents evidence of KCNQ channel expression in the goat ureter.

**Keywords:** Contractility, retigabine, isolated, KCNQ channel, ureter

#### INTRODUCTION

KCNQ channels contribute to the regulation of smooth muscle contractility and are expressed in the urinary bladder and ureter. Flupirtine a KCNQ channel opener is a centrally acting analgesic available in many European countries for treating a variety of painful states (1,2). On the other hand in the recent years, evidence have arisen, from studies on rat (3) guinea pig (4), pig (5) and from clinical trials with KCNQ activator retigabine (6), that Kv7 channels are key regulators of urinary bladder contractility. More specifically, it has been shown that retigabine increases micturition volume in rats, reduces tone and contractility of rat and pig detrusor strips (7), and enhances Kv7

potassium currents in isolated detrusor smooth muscle cells (8). These channels are voltage-gated, and are 6 transmembrane channels which open or close depending on membrane potential and are involved in controlling cell excitability (9). In addition to acting as a potassium KCNQ channel opener in neurons, retigabine also opens KCNQ channels in other body tissues like smooth muscles. In this context it has been shown that a KCNQ channels are expressed *in vitro* on isolated smooth muscle like rat basilar artery, (10) rat pulmonary artery, (11) guinea pig detrusor (12) and human detrusor (13). There are few studies only reported on the ability of retigabine to inhibit the contractility of the isolated ureter. The objective of this study was to test the inhibitory effect of

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retigabine on the spontaneous contractility of isolated caprine (goat) ureter. It was also felt that if retigabine is found to inhibit the spontaneous contractility of the goat ureter, the presence of KCNQ channels in the isolated goat ureter should be investigated using immunofluorescence techniques, there by immunofluorescence studies were conducted using the polyclonal antibodies.

### Materials and Methods:

This study was conducted on caprine ureter obtained after extirpation and held in physiological salt solution. During the first part of the study, the inhibitory effect of retigabine on isolated distal goat ureter was studied. The tissue specimens were obtained from a local slaughter house and transported to the Pharmacology Laboratory in oxygenated mammalian Ringer solution at room temperature. The tissue specimen was dissected and the distal portion of the ureter was identified. Thereafter, it was separated from the bladder and a 8 mm long specimen just above the vesico-ureteric junction was used for the experimental work after removing fat around the tissue. It was divided into strips measuring 1.8 to 2.0 mm long and the lumen was cut open longitudinally before mounting as previously described in our laboratory (14). The tissue was mounted in a 20 ml organ bath filled with mammalian Ringer solution, adequately aerated with oxygen and maintained at a temperature of 37°C. The study was approved by the Institutional Ethics and Research Review Board.

### Chemicals and reagents

Retigabine was obtained from Santa Cruz Biotechnology, Dallas, TX, USA. The inhibitory agents 4-aminopyridine (4-AP) and Linopirdine were obtained from Santa Cruz Biotechnology, Dallas, TX, USA and Tocris Bioscience, UK respectively. The reversal agents were both dissolved in double distilled water and DMSO respectively. The vehicle used for dissolving retigabine was 60% ethanol to yield a clear solution and the maximum volume did not exceed 0.1 ml to avoid a significant vehicle effect. The composition of mammalian Ringer solution was as follows: NaCl: 154 mM; KCl: 5.6 mM; NaHCO<sub>3</sub>: 0.595 mM; dextrose: 5.5 mM; anhydrous CaCl<sub>2</sub>: 2.2 mM, per liter of double distilled water. These salts were obtained from Qualigens, Mumbai, India. The concentrations of

retigabine used in the study were 5, 10, 50, and 100 μM at a volume not exceeding 0.1 ml. Concentrations of 4-AP and linopirdine used were 1mM and 50 μM, respectively. Rabbit polyclonal KCNQ 1/2/3/4/5 primary antibodies were obtained from Santa Cruz Biotechnology, Dallas, TX, USA.

### Experimental procedure

#### Experiments involving the study of the inhibitory effect of retigabine on isolated caprine ureter:

A maximum of one hour was given for each tissue to allow spontaneous contractions. Tissues which failed to show spontaneous contractions within 1 hour were not used for the experimental study. Once the pattern of contractions became fairly stable, control tracings of contractions were recorded in two time periods. After this the tissues were precontracted with 5μM bethanechol. The first time period was 0 to 5 minutes and the next time period was 5 to 10 min. The reason for this two staged observation was to ensure that there was no significant change in the activity before treatment with the test drug. After establishing this fairly stable pattern, logarithmic doses of retigabine were added to the organ bath.

A contact period of 10 minutes was given for observing drug effects on tissue activity. The mechanism of action of retigabine was studied using the reversal agents, Linopirdine (50 μM) and 4-aminopyridine (1mM). The reversal agent was added at the end of 10 minute contact period with retigabine. The tissue activity was observed for 15 min after adding the reversal agent.

#### Experiments involving the study of the presence of KCNQ channels in the isolated caprine ureter:

In the first phase of the study, it was found that retigabine inhibits the contractility of isolated goat ureter, therefore we conducted a second study phase to determine if the receptor for retigabine, the KCNQ channel, was present in the isolated goat ureter. For this, 3 to 6 mm lengths of caprine ureter were obtained. Using primary polyclonal antibodies against KCNQ channel subtypes 7.1, 7.2, 7.3, 7.4, and 7.5 immunohistochemical staining was done using standard protocols. Secondary antibodies (goat anti-rabbit IgG FITC, fluorescein-conjugated) were used to tag the KCNQ primary antibodies as per standard protocols for conducting immunofluorescence microscopy.

Polyclonal antibodies bind multiple epitopes with enhanced affinity and thus provide more robust results.

### Statistical analysis

The activity score, the product of average height of contractions over a specified period of time and the number of contractions during this period, was determined at each five minute intervals for the total study duration of 35 min. Log transformed activity scores, before and after treatment with retigabine and the reversal agents were compared using repeated measures analysis of variance test for each dose including vehicle effect. The dose response relationship was determined using the DRC package in R version 3.2.2. The student's 't' test and ANOVA were used to evaluate the significance of the results obtained. 'P' values less than 0.05 were considered statistically significant.

### Results:

Table 1 shows the effects of the retigabine on the spontaneous contractility of the isolated goat

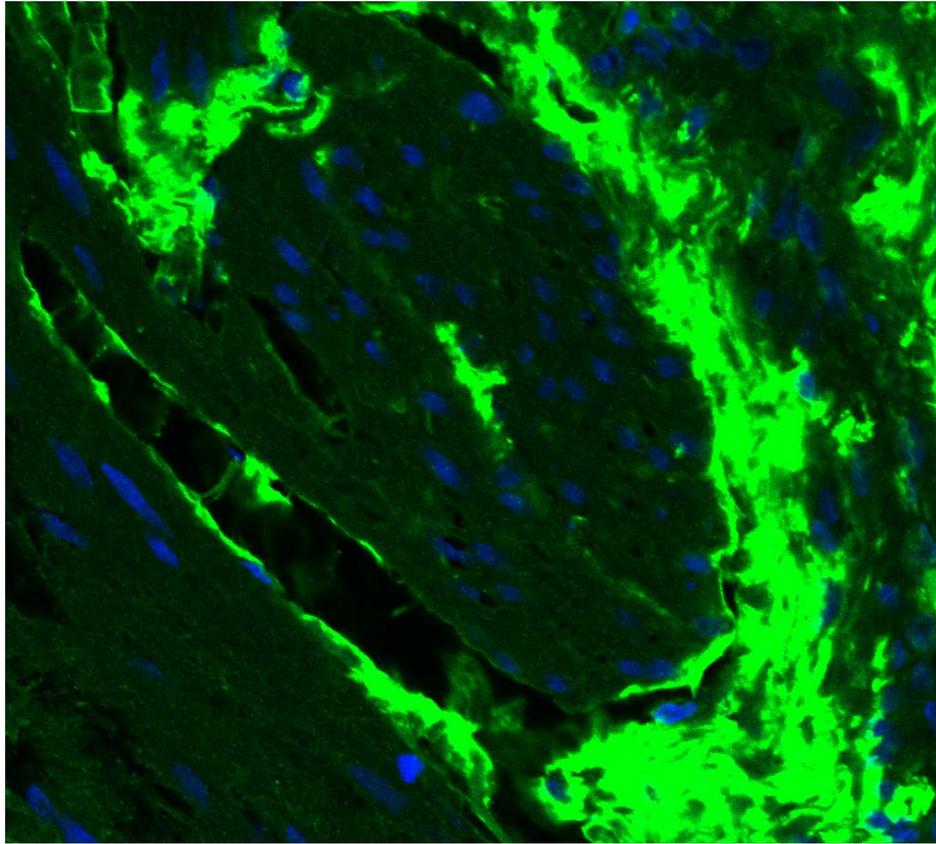
ureter. As shown, the control values depict the precontracted ureter strips with bethanechol (5 $\mu$ M). Retigabine at a concentration of 1  $\mu$ M also did not appreciably inhibit ureteric contractility, but at concentrations of 5, 10, 50, and 100 $\mu$ M significantly inhibited ureteric contractility in a dose-dependent manner. The most effective response of retigabine for a contact period of 10 minutes was observed at 50 $\mu$ M i.e a reduction of 26.9 % from the basal precontracted values. As shown in Table 2, the non-specific potassium channel blocker 4-AP and the specific KCNQ channel blocker linopirdine reversed the inhibitory effect of retigabine on the contractility of the isolated goat ureter. Sample tracings of the inhibitory effect of retigabine on the contractility of the isolated goat ureter and the reversal effect of 4-AP and Linopirdine were used to calculate the percentage change from the control values and are shown in Table 2. Figure 1 shows the presence of KCNQ channels in the isolated goat ureter as demonstrated by immunofluorescent staining.

**Table 1: Inhibitory effect of retigabine on spontaneous contractility of isolated distal caprine ureter (n = 7 for each drug administration)**

Treatment	After test drug (0-5 minutes) % change from control (A)	After test drug (5-10 minutes) % change from control (B)	P Values
Vehicle (Bethanechol 5 $\mu$ M) precontracted	100 %	100 %	---
Retigabine 5 $\mu$ M	11.2	13.4	P>0.05
Retigabine 10 $\mu$ M	16.6	19.2	P<0.05 (B)
Retigabine 50 $\mu$ M	24.2	26.9	P<0.05
Retigabine 100 $\mu$ M	29.7	31.1	P<0.05

**Table 2: Effect of KCNQ blockers 4-AP and Linopirdine on inhibitory effect of retigabine on contractility of isolated caprine ureter (n=7 for each drug administration)**

Treatment	After test drug (0-5 minutes) % change from control (A)	After test drug (5-10 minutes) % change from control (B)	P Values
Control (bethanechol 5 $\mu$ M)	100	100	
Retigabine 10 $\mu$ M	16.6	19.2	P<0.05
4-AP 1mM + Retigabine 10 $\mu$ M	5.2	6.1	P>0.05
Linopirdine 50 $\mu$ M + Retigabine 10 $\mu$ M	3.2	4.1	P<0.05



**Figure 1: Immunofluorescent staining of isolated goat ureter demonstrating the presence of KCNQ potassium channels using polyclonal antibodies**

### Discussion:

This study has shown that the KCNQ channel opener retigabine inhibits the contractility of the isolated goat ureter. Retigabine at concentrations ranging from 10 to 100  $\mu\text{M}$  significantly inhibited ureteric contractility (Table 1). The inhibition by retigabine was appreciably reversed by the non-specific potassium channel blocker 4-AP and the KCNQ-specific channel blocker linopirdine (Table 2). These results suggest that KCNQ channels are present in the ureter and that retigabine exerts its inhibitory effect on spontaneous contractility of the ureter by opening KCNQ channels and it seems that retigabine has specific stimulatory actions on the activation of type 7 voltage-dependent  $\text{K}^+$  ( $\text{Kv7}$ ) channels encoded by the KCNQ genes (15). Similar results have been found in studies on other isolated smooth muscles from the urinary tract. Thus, KCNQ openers have been found to inhibit the contractility of the isolated urinary bladder of guinea pigs, and humans, with subsequent reversal by Linopirdine. A similar trend in results were also found in the renal vascular

artery in rats (16). Linopirdine is an interesting agent as the administration of linopirdine is well tolerated in humans (17-18), as well as in animals (19). During the second phase of the study it was found by immunofluorescent microscopy that KCNQ channels are present in the goat ureter (Figure 1). This finding supports the idea of the first phase of the study which showed that KCNQ openers inhibited the contractility of goat ureter by opening KCNQ channels. It appears based on the immunostaining with the polyclonal antibodies that there is anatomical and functional presence of KCNQ channels in the ureter. The existence of  $\text{Kv7}$  channels has been shown by previous studies in the smooth muscles therefore these results are supportive of the previous studies (20).

Ureteric calculi are an important problem seen in clinical practice. One form of therapy for this is the use of medical expulsion therapy (MET), the use of drugs to decrease the associated pain and aid in the expulsion of calculi. Drugs that are used for MET include calcium channel blockers, alpha adrenergic receptor blockers, and non-steroidal

anti-inflammatory drugs. Pickard and colleagues(21) recently conducted a randomized, multicenter placebo-controlled trial of the alpha adrenergic receptor blocker tamsulosin (400 µg) and the calcium channel blocker nifedipine(300 mg) for the treatment of urinary stones. The authors found that these drugs were not very effective in decreasing the need for further treatment to achieve stone clearance in 4 weeks. Hence they suggested that other drugs must be investigated for the treatment of urinary stones. The results of the current study suggest that one such drug that can be tried for MET is retigabine or a similarly acting drug i.e.flupirtine.

### Conclusions:

This study has shown that the potassium channel opener retigabine inhibits the spontaneous contractility of the isolated caprine ureter. The inhibitory effect was reversed by the non-specific potassium channel blocker 4-AP and the specific KCNQ channel blocker linopirdine suggesting that retigabine inhibited ureteric contractility by opening KCNQ potassium channels. Immunofluorescent microscopy has demonstrated that KCNQ channels are present in the goat ureter, supporting the results of the inhibitory effect of retigabine on isolated caprine ureter.

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