Abstract

Context: Kv7 potassium channels are expressed in several types of smooth muscles and could mediate physiological responses in the tissues expressed. Flupirtine is an analgesic that acts by opening Kv7 potassium channels. It has been shown to inhibit the contractility of several types of isolated smooth muscle. Aims: This study investigated the ability of flupirtine to inhibit the spontaneous contractility of isolated distal caprine (goat) ureter. Settings and Design: Spontaneous contractility of the isolated goat ureter was recorded using a physiograph. Materials and Methods: The ability of 1, 3, 10, 30, and 90 μM concentrations of flupirtine maleate to inhibit the spontaneous contractility of isolated distal goat ureter was investigated. The ability of the nonspecific potassium channel blocker 4-aminopyridine (4-AP; 1 mM) and the specific Kv7 channel blocker XE-991 (100 μM) to reverse the inhibitory effect of flupirtine on ureteric contractility was also investigated. Statistical Analysis Used: Both parametric and nonparametric statistical tests were used. Results: At 10, 30, and 90 μM concentrations, flupirtine significantly inhibited the spontaneous contractility of the isolated goat ureter. The EC₅₀ of flupirtine for a contact period of 10 min was 17.7 μM. The inhibitory effect of flupirtine on ureteric contractility was significantly reversed by 4-AP and XE-991. Conclusions: Flupirtine inhibits the spontaneous contractility of the isolated goat ureter by opening Kv7 channels.

Keywords: Contractility, flupirtine, isolated, Kv7 channel, ureter

Introduction

Flupirtine is a centrally acting analgesic available in many countries in Europe for treating various painful states. It is an unusual analgesic in that it is thought to have a novel mechanism of action: opening of Kv7 potassium channels in neurons. These channels were formally called KCNQ channels. These channels are voltage-gated, 6-transmembrane channels involved in controlling cell excitability. Flupirtine, in addition to acting as a Kv7 channel opener in neurons, also opens Kv7 channels in other body tissues such as smooth muscles. In this context, it has been shown to act as a Kv7 channel opener in vitro on isolated smooth muscles such as rat basilar artery, rat pulmonary artery, guinea pig detrusor, and human detrusor. To date, the ability of flupirtine to inhibit the contractility of the isolated ureter has not been studied. We recently showed that the calcium channel blocker (CCB) benidipine inhibits the contractility of the isolated caprine (goat) ureter. Goat ureter is thought to be a suitable substitute for the human ureter for in vitro studies of the ureter. The aim of this study was to investigate the inhibitory effect of flupirtine on the spontaneous contractility of isolated goat ureter, using a similar methodology as we used previously.

Materials and Methods

Tissue preparation

Ureteric tissues of the goat were obtained from a local slaughterhouse and transported to the Departmental Laboratory in oxygenated mammalian Ringer solution maintained at room temperature. The tissue specimen was dissected and the distal portion of the ureter was determined. Then, it was separated from the urinary bladder, and a 7-mm long specimen just above the vesicoeureteric junction was used for the experiments after removing fat around the tissue. It was divided into strips 1.5–1.8 mm long, and the lumen was longitudinally cut open before mounting the strip as done previously. The tissue was mounted in a 20 ml organ bath containing mammalian physiological responses in the tissues expressed.
Ringer solution, adequately aerated with oxygen and kept at a temperature of 37°C. The Institutional Ethics and Research Review Board approved the study (IRB minute number: 7752, dated February 6, 2012).

**Drugs and chemicals used**

Flupirtine maleate was obtained from Santa Cruz Biotechnology, Dallas, TX, USA. The reversal agents, i.e., 4-aminopyridine (4-AP) and XE-991 were obtained from Santa Cruz Biotechnology, Dallas, TX, USA and Sigma Aldrich, Mumbai, India, respectively. The reversal agents were both dissolved in double distilled water. The vehicle used for dissolving flupirtine was 60% methanol to yield a clear solution and it was made sure that the maximum volume administered to the organ bath did not exceed 0.1 ml to avoid a vehicle effect. The composition of mammalian Ringer solution was as follows: NaCl: 154 mM; KCl: 5.6 mM; NaHCO₃ 0.595 mM; dextrose: 5.5 mM; and anhydrous CaCl₂: 2.2 mM per liter of double distilled water. These salts were obtained from Qualigen, Mumbai, India. The concentrations of flupirtine used in the study were 1, 3, 10, 30, and 90 μM. The concentrations of 4-AP and XE-991 that were used were 1 mM and 100 μM, respectively.

**Experimental procedure**

A maximum of 60 min was allowed for each tissue to exhibit spontaneous contractility. Tissues which failed to show spontaneous contractility within 60 min were discarded. Once the pattern of contractility was stable, control tracings of contractility were observed during two time periods. The first was 0–5 min and the second was 5–10 min. We validated this procedure.[13] Based on the validation, we found that tissues whose contractility were similar during the two time periods had consistent contractility for the entire study duration. After establishing this stable pattern, logarithmic doses of flupirtine were added to the organ bath. A contact period of 10 min was allowed to observe drug effects on tissue activity. The mechanism of action of flupirtine was studied using the reversal agents, 4-AP (1 mM) and XE-991 (100 μM). The reversal agent was added at the end of a 10-min incubation period with flupirtine. The tissue activity was observed for 15 min after adding the reversal agent. The concentrations of reversal agents were those used in previous studies.[13,14]

**Analysis of data**

The tissue activity score, the product of the average height of contractility and the number of contractions over a specified period of time,[10,15] was determined at each 5-min interval for the total study time of 35 min. Log-transformed activity scores, before and after flupirtine treatment, and the reversal agents were compared using repeated measures analysis of variance for each drug treatment including the vehicle. Greenhouse–Geisser correction was used to reduce the probability of type 1 error, if the Mauchly’s test of sphericity was violated. $P < 0.05$ was considered statistically significant. The dose-response relationship was determined using the DRC package in R version 3.2.2. R Foundation for Statistical Computing, Vienna, Austria. For this, the percent inhibition of tissue activity score was used. The log-logistic model, Weibull type 1, and Weibull type 2 models were checked for fitting the data. Nonlinear regression was used to model and fit the dose-response curve for percent inhibition of tissue activity score and percent tissue activity score. The package DRC was used to perform this fit using R version 2.12.2. Weibull model 2.4 with lower and upper asymptote fixed at 0 and 100 produced the best fit for percent inhibition of the tissue activity score.

**Results**

Table 1 shows the effects of the vehicle used in the study (60% methanol) and flupirtine on the spontaneous contractility of the isolated goat ureter. The vehicle did not significantly inhibit ureteric contractility. Flupirtine at concentrations of 1 and 3 μM also did not significantly inhibit ureteric contractility. However, at concentrations of 10, 30, and 90 μM, flupirtine significantly inhibited ureteric contractility in a dose-dependent manner. The EC₅₀ of flupirtine for a contact period of 10 min was 17.7 μM. As shown in Table 2, the nonspecific potassium channel blocker 4-AP and the Kv7 channel blocker XE-991 significantly reversed the inhibitory effect of flupirtine on the contractility of the isolated ureter. A representative tracing of the reversal by XE-991 of the inhibition by flupirtine of the spontaneous contractility of the ureter is shown in Figure 1.

**Discussion**

For the first time, this study has demonstrated that the Kv7 channel opener flupirtine inhibits the contractility of the isolated goat ureter. Flupirtine at concentrations ranging from 10 to 90 μM significantly inhibited spontaneous ureteric contractility [Table 1]. The inhibition by flupirtine

| Drug treatment | Tissue activity score/time period | Before test drug (0-5 min) | After test drug 0-5 min | After test drug 5-10 min | P* |
|----------------|----------------------------------|--------------------------|------------------------|-------------------------|----|
| Vehicle        | 201.7                            | 212.3                    | 198.9                  | 0.855                   |    |
| 1 μM flupirtine| 518                              | 518                      | 507.8                  | 0.857                   |    |
| 3 μM flupirtine| 201.9                            | 207.7                    | 201.9                  | 0.348                   |    |
| 10 μM flupirtine| 448                            | 386.5                    | 386.8                  | 0.011                   |    |
| 30 μM flupirtine| 485.4                          | 196.6                    | 4.8                    | <0.001                  |    |
| 90 μM flupirtine| 516.5                           | 129.3                    | 1                      | <0.001                  |    |

*For differences between the three time periods for each drug treatment, based on repeated measures ANOVA. ANOVA: Analysis of variance
was significantly reversed by the nonspecific potassium channel blocker 4-AP and the Kv7-specific channel blocker XE-991 [Table 2]. These results suggest that Kv7 channels are present in the ureter and that flupirtine exerts its inhibitory effect on spontaneous contractility of the ureter by opening Kv7 channels. Similar results have been found in studies on other isolated smooth muscles from the urinary tract. Thus, flupirtine has been found to inhibit the contractility of the isolated urinary bladder of guinea pigs [8,16] and humans, [9] with subsequent reversal by XE-991. [9] 4-AP and XE991 when administered to isolated smooth muscle alone in the absence of an agonist are known to have a stimulant (contractile) effect. Thus, Huang [17] showed that at concentrations of 1-10 mM, 4-AP dose-dependently induced contractility of the isolated rat vas deferens. Yeung and Greenwood [18] showed that 10 μM XE-991 increased the spontaneous contractility of the isolated mouse portal vein.

An important problem seen in clinical urological practice is ureteric calculi. One type of therapy for ureteric calculi is the use of medical expulsion therapy (MET) - the use of drugs to reduce the associated pain and help expel the calculi. Drugs used for MET include CCB and α-adrenergic receptor blockers. [19] The aim of MET is to facilitate the spontaneous passage of ureteric stones. Meta-analyses have shown that patients with ureteric calculi treated with α-adrenergic receptor blockers and CCB are more likely to pass calculi with fewer colic episodes than those who do not receive MET. [19] The results of the current study suggest that potassium channel openers such as flupirtine can also be investigated for use in MET. To the best of our knowledge, to date, potassium channel openers have not been investigated in clinical trials for this purpose. Flupirtine is currently being used in a number of European countries for pain relief and is not known to have any major adverse effects. [20]

Conclusions

The potassium channel opener flupirtine inhibits the spontaneous contractility of the isolated goat ureter. The inhibitory effect was reversed by the nonspecific potassium channel blocker 4-AP and the specific Kv7 channel blocker XE-991, suggesting that flupirtine inhibited ureteric contractility by opening Kv7 channels. Flupirtine could be investigated for clinical use as part of MET.

Acknowledgment

The authors acknowledge Drs A. Devasia, T. S. Vijayakumar, and S. M. Amirtham for their help.

Financial support and sponsorship

This study was funded by an intramural research grant, Christian Medical College, Vellore.

Conflicts of interest

There are no conflicts of interest.

References

1. Devulder J. Flupirtine in pain management: Pharmacological properties and clinical use. CNS Drugs 2010;24:867-81.
2. Brown DA, Passmore GM. Neural KCNQ (Kv7) channels. Br J Pharmacol 2009;156:1185-95.
3. Szelenyi I. Flupirtine, a re-discovered drug, revisited. Inflamm Res 2013;62:251-8.
4. Alexander SP, Mathie A, Peters JA. Guide to receptors and channels (GRAC), 5th edition. Br J Pharmacol 2011;164 Suppl 1:S1-324.
5. Rivera-Arcos J, Vicente-Baz J, Lopez-Garcia JA. Targeting Kv7 channels in pain pathways. Oncotarget 2017;8:12554-5.
6. Mani BK, Brueggemann LI, Cribs LL, Byron KL. Activation of vascular KCNQ (Kv7) potassium channels reverses spasmogen-induced constrictor responses in rat basilar artery. Br J Pharmacol 2011;164:237-49.
7. Joshi S, Sedivy V, Hodyc D, Herget J, Gurney AM. KCNQ
modulators reveal a key role for KCNQ potassium channels in regulating the tone of rat pulmonary artery smooth muscle. J Pharmacol Exp Ther 2009;329:368-76.

8. Anderson UA, Carson C, Johnston L, Joshi S, Gurney AM, McCloskey KD, et al. Functional expression of KCNQ (Kv7) channels in guinea pig bladder smooth muscle and their contribution to spontaneous activity. Br J Pharmacol 2013;169:1290-304.

9. Bientinesi R, Mancuso C, Martire M, Bassi PF, Sacco E, Curró D, et al. Kv7 channels in the human detrusor: Channel modulator effects and gene and protein expression. Naunyn Schmiedebergs Arch Pharmacol 2017;390:127-37.

10. Mathew SK, Naik GS, Peedicayil J. Inhibition by benidipine of contractility of isolated proximal and distal caprine ureter. Int J Appl Basic Med Res 2017;7:155-9.

11. Smita K, Sushil Kumar V, Premendran J, Sharma ML. Goat ureter – An alternative model for measuring ureteral peristalsis. J Smooth Muscle Res 2006;42:117-30.

12. Naik GS. Effect of Flupirtine Maleate, a Kv7 Channel Opener on the Spontaneous Contractility of Isolated Distal Caprine Ureter. Thesis Submitted to the Tamil Nadu Dr MGR Medical University, in Partial Fulfillment for the MD Pharmacology Degree; April, 2014.

13. Aaronson PI, Sarwar U, Gin S, Rockenhauch U, Connolly M, Tillet A, et al. A role for voltage-gated, but not Ca2+-activated, K+ channels in regulating spontaneous contractile activity in myometrium from virgin and pregnant rats. Br J Pharmacol 2006;147:815-24.

14. Macvinish LJ, Guo Y, Dixon AK, Murrell-Lagnado RD, Cuthbert AW. XE991 reveals differences in Kv7 channels regulating chloride secretion in murine airway and colonic epithelium. J Pharmacol Exp Ther 2001;60:753-60.

15. Parker A, Rush FN, Dennis KJ. The spontaneous motility of the human fallopian tube. J Reprod Fertil 1974;39:425-7.

16. Takagi H, Hashitani H. Effects of K(+) channel openers on spontaneous action potentials in detrusor smooth muscle of the guinea-pig urinary bladder. Eur J Pharmacol 2016;789:179-86.

17. Huang Y. BaCl2- and 4-aminopyridine-evoked phasic contractions in the rat vas deferens. Br J Pharmacol 1995;115:845-51.

18. Yeung SY, Greenwood IA. Electrophysiological and functional effects of the KCNQ channel blocker XE991 on murine portal vein smooth muscle cells. Br J Pharmacol 2005;146:585-95.

19. Türk C, Petřík A, Sarica K, Seitz C, Skolarikos A, Straub M, et al. EAU guidelines on diagnosis and conservative management of urolithiasis. Eur Urol 2016;69:468-74.

20. Treudler R, Pohle K, Simon JC. Flupirtine is a safe alternative drug in patients with hypersensitivity to NSAIDs. Eur J Clin Pharmacol 2011;67:961-3.