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Original Research Article

Vasodilator effect of 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea is predominantly mediated through activation of voltage-dependent K+ channels

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Abstract

Purpose: *To determine the mechanism of vasorelaxant effect of 1-trifluoromethoxyphenyl – 3 -(1-propionylpiperidin–4-yl) urea (TPPU) in cardiovascular diseases, including hypertension.*

Methods: *Isolated rat thoracic aortic tissue preparations were mounted in an organ bath set up integrated with isometric transducer and a Power Lab assembly. TPPU (0.3 - 100 µM) was tested for vasorelaxant effect against low K^+ (25 mM) and high K^+ (80 mM)-induced contractions and its mechanism was determined in the presence of different antagonists (glibenclamide, 4- aminopyridine and tetraethyl ammonium).*

Results: *In rat aortic preparations, TPPU showed a concentration-dependent (0.3 – 100 µM) and significant ($p < 0.001$) inhibition of low K^+ induced contractions with complete inhibition obtained at 100 µM. TPPU produced significant ($p < 0.05$) inhibition of high K^+ induced contractions with maximum relaxation of 15.36 ± 1.95 % and 15.85 ± 3.35 % at 30 and 100 µM, respectively. Glibenclamide (Gb,10 µM) pretreatment partially inhibited the vasorelaxant effect of TPPU against low K^+ in a concentration range of 1 - 30 µM. 4-Aminopyridine (4-AP, 1 mM) and tetraethyl ammonium (TEA, 10 mM), markedly inhibited the vasorelexant effect of TPPU against low K^+ induced contractions with maximum relaxation of 20.09 ± 2.40 and 21.67 ± 0.88 %, respectively, at 100 µM.*

Conclusion: *TPPU possesses marked vasorelaxant properties which provides sound pharmacological evidence for its use as a potential drug candidate in the management of hypertension.*

Keywords: *1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea, Hypertension, vasodilator, K^+-channel activation, Ca^+- channel antagonist*

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INTRODUCTION

Soluble epoxide hydrolase (sEH) has been a recent focus of research, is an indigenous enzyme which metabolizes epoxyeicosatrienoic acids (EETs) to functionally less active produces, the dihydroxyeicosatrienoic acids DHETs [1]. In endothelial cells, cytochrome P450

epoxygenase metabolizes arachidonic acid to produce EET. EETs are well known for diverse biological activities including vasodilation, anti-inflammatory, platelet aggregation inhibitory, analgesic and cardioprotection [2,3].

sEH is an indigenous enzyme which transforms EETs to inactive dihydroxyeicosatrienoic acids DHETs [2]. The potential biological activities of DHETs such as vasodilatation and anti-inflammatory effects are less compared to EETs [4]. sEH is widely distributed in intestine, liver, kidney, vascular smooth muscles, neuronal cells and astrocytes [5]. Inhibition of sEH enzyme activity causes elevated levels of EETs in biological fluid and tissues, thus promoting beneficial pharmacological actions of EETs in the body. These findings suggest that sEH inhibition could be a potential therapeutic target for cardiovascular disease and pain and inflammatory conditions [6].

Inhibition of sEH enzyme prevents biodegradation of EETs and enhances their beneficial actions. Growing body of literature revealed that deletion of sEH or over activity of CYP epoxygenase lowered blood pressure in animal model of hypertension [7]. Large number of studies have revealed anti-inflammatory, vasodilator, antihypertensive, cardiac and renal protective effects of sEH inhibitors [6,8,9].

1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin -4-yl) urea (TPPU) is novel among the sEH inhibitors with relatively better pharmacokinetic and biological activity profile [10]. Human and animals studies have also shown TPPU as a potent inhibiter of sEH [11]. Though TPPU has been widely studied for its diverse biological activities, however, its vasorelaxant effect in intact vascular tissues is yet to be explored. The present study explored the possible vasodilator activity of TPPU, mediated predominantly through activation of voltage-dependent K^+ channels, which may explain the potential therapeutic role of this compound in the management of hypertension.

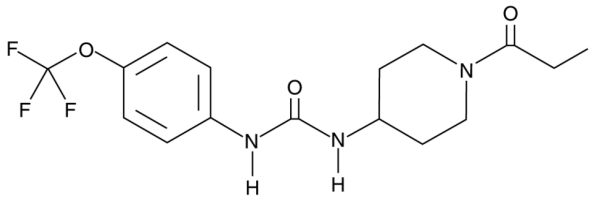

Figure 1: Chemical structure of soluble epoxide hydrolase inhibitor, TPPU

EXPERIMENTAL

Chemicals

Different K^+ channel antagonists, tetraethyl ammonium (TEA), 4-aminopyridine (4-AP) and glibenclamide (Gb) were acquired from Sigma Chemicals Company (St Louis, MO, USA). TPPU was procured from Synthia laboratories Davis, California, USA. Other chemicals used in the study such as Potassium chloride, calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate and sodium chloride were obtained from E. Merck, Darmstadt, Germany. All chemicals used were of the analytical grade.

EXPERIMENTAL ANIMALS

Sprague–Dawley rats of either sex and weighing 180 – 200 g were maintained at the Animal House facility of Aga Khan University Medical College at 23 - 25 °C. Animals had free access to tap water, *ad libitum.* Animal were provided standard diet which consists of (g/kg): flour 380, fiber 380, molasses 12, NaCl 5.8, nutrivet L. 2.5, powdered milk 150, vegetable oil 38, potassium metabisulfate 1.2 and fish meal 170. Water was withdrawn from rats for 12 - 14 h prior to anesthesia. The animals were euthanized following deep anesthesia with isoflurane (2 - 5 % v/w) by inhalation in a closed chamber. After the achievement of deep anesthesia that was confirmed by absence of touch and corneal reflexes of the animals, thoracotomy was performed followed by cardiac puncture/heart excision to euthanize the animals.

Experiments were conducted and compiled to the guidelines of Institutional ethics committee and the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996) [12]. This research work was the part of the PhD dissertation of Mr. Shafiq Ali Shah, which was reviewed and approved by the Board of Studies and Research at University of Malakand, KPK, Pakistan and Aga Khan University Medical College Karachi Pakistan, with approval no. 60-ECACU-BBS-15.

Animal studies

Rats were euthanized after they were fully anesthetized with isoflurane by inhalation. Isoflurane was used 2-5 % v/w, till achievement of deep anesthesia. Once deep anesthesia was achieved and confirmed by absence of touch and corneal reflexes, thoracotomy was performed followed by cardiac puncture/heart excision to euthanize the animals. Afterwards, the thoracic

aorta was isolated, cleaned of fatty tissues, cut into small rings and mounted individually in a tissue bath (5 mL) filled with Kreb's solution at 37 ºC and aerated with carbogen [13]. A resting tension of 2 g was gradually applied to mounted aortic tissue preparation. The tissues were initially incubated for 30 min and finally equilibrated for 1 h prior to addition of any chemical agent. K^+ at low (25 mM) and high (80 mM) concentration was used to stabilize the respective tissue preparations until achievement of constant responses usually after 2 – 3 times application followed by washing of the tissue with fresh Kreb's solution [14]. After stabilization state, low and high K^+ was administered to the tissue to induce sustained contractions, respectively. The relaxant effect of TPPU at 0.1 - 100 µM was assessed against low and high K^+-induced contractions, respectively. Isometric responses of the vessels were measured employing isometric transducer 50-7996 (Harvard Apparatus, Holliston, MA, USA), connected to PowerLab assembly.

To explore the participation of K^+ channel opening and/or Ca^{++} channel antagonist properties [15], the vasorelexant effect of TPPU was further studied against low K^+ (25 mM) and high K^+ (80 mM)-induced contractions, respectively. After achieving sustained contractions of vessels to K^+, TPPU (0.1 - 100 µM) was added in a cumulative fashion to the vessels to obtain its concentration-dependent inhibitory responses. The relaxation of the tissue preparation was expressed as percentage of the control contraction mediated by K^+.

To characterize and confirm the type of K^+-channels involved in the vasodilating effect, the inhibitory effect of TPPU was reproduced in the aortic preparation pretreated with glibenclamide (Gb, 10 µM), an ATP-dependent K^+ channel antagonist [16], tetraethyl ammonium (TEA, 10 mM), a nonselective antagonist of the K^+ channels [17] and 4 - aminopyridine (4-AP, 1 mM), a voltage-dependent K^+ channel blocker add [14].

Statistical analysis

Data is presented as mean ± standard error of mean (s.e.m, n = 4 - 6) and the median effective concentrations (EC_{50}) with 95 % confidence intervals (CI). Data was considered statistically significant at $p < 0.05$. The inhibitory effects of various treatments were statistically analyzed by non-linear regression employing GraphPad program (GraphPad, San Diego, CA, USA).

RESULTS

Vasodilating effect of TPPU in isolated rat vascular tissues

When tested on isolated rat aortic tissue, TPPU caused significant ($p < 0.001$) inhibition of low K^+-induced contractions in a concentration dependent manner with an EC_{50} value of 6.72 µM (6.32 - 7.02, 95 % CI, n = 4), while it has produced complete inhibition (100% relaxation) at highest tested concentration of 100 µM (Figure 2). TPPU produced mild but significant ($p < 0.05$) relaxation of high K^+ (80 mM)-induced contractions with resultant values of 15.36 ± 1.95and 15.85 ± 3.35 % at higher test concentrations of 30 and 100 µM, respectively (Figure 2).

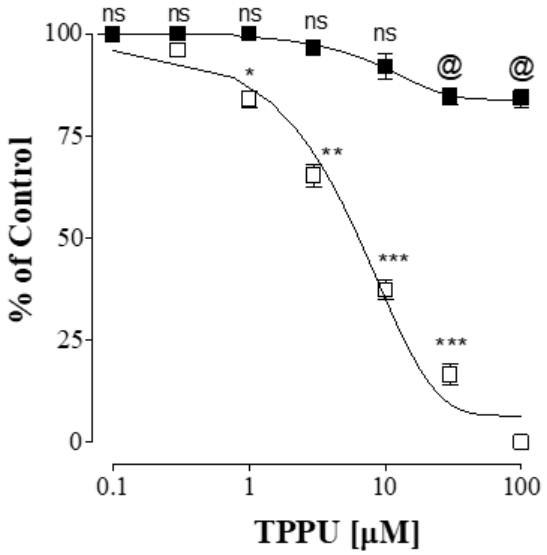

Figure 2. Inhibitory effect of TPPU on "□" low K^+ (25 mM) and "■"high K^+ (80 mM)-induced contractions in isolated rat aortic rings. Values shown are mean ±S.E.M, n = 4-6. "ns" represents non-significant, $^{*/@}p < 0.05$, $^{**}p < 0.0$, $^{***}p < 0.001$, * shows comparison of the relaxant effect of TPPU on low K^+ (25 mM) -induced contractions vs. 100 % control contractile response in that tissue.@ shows comparison of the relaxant effect of TPPU on high K^+ (80 mM)-induced contractions vs. 100 % control contractile response in that tissue and ns = non-significant

Insight into mechanisms mediating vasodilating effect of TPPU

To explore the subtype of K^+ channels involved in the observed vasodilator effect of TPPU, the tissues were pre-incubated with different K^+ channel blockers. As shown in Figure 3 pre-treatment of tissue with 4-aminopyridine (4 - AP, 1 mM), suppressed the relaxant effect of TPPU against low K^+-induced contractions ($p < 0.001$) with remaining resultant relaxation of 12.9±2.46

% (n = 5) at 100 µM compared to control vessels showing 100 % relaxation in the absence of 4 - AP observed at the same concentration. Similarly pretreatment of blood vessels with TEA (10 mM) caused significant ($p < 0.001$) attenuation of the relaxant effect of TPPU against low K^+ induced contractions with maximum relaxation of 13.5 ± 3.50 % versus 100 % in control vessels in the absence of TEA (Figure 3). Pre-incubation of the blood vessels with glibenclamide (10 µM) caused partial ($p < 0.01$) antagonism of the inhibitory effect of TPPU (Figure 3).

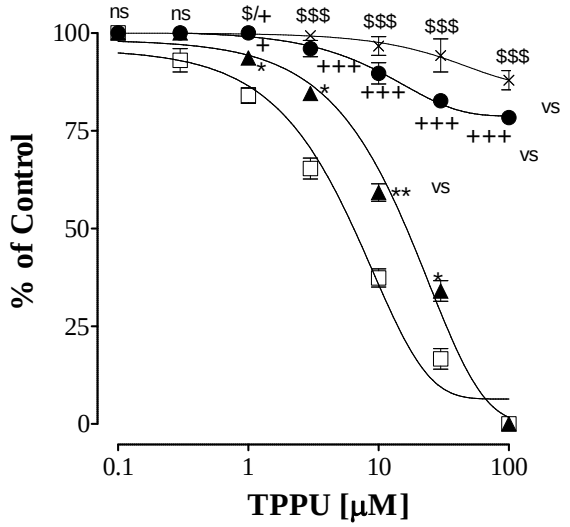

Figure 3. Inhibitory effect of TPPU on "□" low K^+-induced contractions in the absence and presence of "▲" glibenclamide (Gb, 10 µM), "●"tetraethyl ammonium (TEA, 10 mM) and "×" 4-aminopyridine (4 - AP, 1 mM) in isolated rat aortic preparations. Values shown are mean ± S.E.M, n = 4 - 6. "ns" represents non-significant, $*/+/\$ p < 0.05$, $**/++/\$\$ p < 0.01$, $+++/\$\$\$ p < 0.001$, "*"shows comparison of the relaxant effect of TPPU on low K^+ (25 mM)-induced in the presence vs. absence of Gb. "+" shows comparison of the relaxant effect of TPPU on low K^+ (25 mM) -induced in the presence vs. absence of TEA. "$"shows comparison of the relaxant effect of TPPU on low K^+ (25 mM)-induced in the presence vs. absence of 4-AP

DISCUSSION

Recently TPPU has got much research attention for its efficacy as a potential therapeutic agent in cardiovascular disorders [18]. It has been shown to have antihypertensive effects by enhancing EETs level in the body via sEH inhibition. EETs are well recognized for their potent vasodilator effect.

The objective of this investigation was to explore the vasodilatory effect of TPPU and its possible mechanism in isolated rat aortic preparations. Previous studies have shown that vasodilator response of vessel is usually mediated through K^+ channel opening or Ca^{++} channel blockade [15]. In order to characterize, whether the vaso-relaxant effect of TPPU was mediated via similar pathways, its relaxant efficacy was tested against low K^+(25 mM) and high K^+ (80 mM)-induced contractions [19]. Interestingly, TPPU caused statistically significant ($p < 0.001$) and dose dependent inhibition of low K^+-induced contractions with partial but significant ($p < 0.05$) inhibition against high K^+-induced contractions only at 30 and 100 µM. The efficacy of TPPU against low K^+ (25 mM)-induced contractions suggesting K^+ channel opening activity of this compound [20]. It has been shown that compounds that selectively inhibit the low K^+ (< 30 mM) induced contractions are considered K^+ channel opener, while Ca^{++}channel blockers are efficacious against both low and high K^+-induced contractions [13, 21].

To elucidate the type of K^+ channels implicated in the vasorelaxant activity of TPPU, its effect was assessed against low K^+-induced contraction in tissue pretreated with glibenclamide, a ATP-dependent $K^+(K_{ATP})$ channels antagonist [16], TEA, a non-specific K^+ channel blocker [17] and 4-Aminopyridine, a voltage dependent K^+ channels blocker [22]. Glibenclamide had partial inhibitory influence, while 4-AP and TEA caused marked inhibition of low K^+-induced contractions suggesting that the vasorelaxant effect of TPPU is most likely mediated via activation voltage-dependent K^+ channels (Kv channels) and non-specific K^+ channels. Moreover, these findings indicate the additional role of K_{ATP} channel activation and a weak Ca^{++} antagonist-like effect [23] as possible vasorelaxant mechanism(s) of TPPU in part, though additional mechanism(s) cannot be ruled out.

Among varied types of K^+ channels, Kv neutralize the depolarization of the membrane potential via K^+ efflux [17]. Studies have also expressed Kv channels in aortic smooth muscles [24]. K^+ channel openers are potential new class of drugs with diverse therapeutic potential in hypertension, asthma, and gastrointestinal problems [25]. These compounds cause membrane hyperpolarization by opening K^+ channels and increase in K^+ efflux, decreasing intracellular free Ca^{++} leading to smooth muscle relaxation [17].

In conclusion, this study demonstrates that TPPU possesses profound vasorelaxant properties. These findings provide sound evidence for TPPU as a potential antihypertensive agent. Future studies are warranted to explore the mechanism

of TPPU as potential vasodilator agent in the management of cardiovascular disorders.

DECLARATIONS

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Conflict of interest

The authors declare that they have no competing interests with regard to this work.

Authors' contributions

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Malik Hassan Mehmood and Ishfaq Ali Bukhari Ishfaq designed the project, Malik Hassan Mehmood and Anwarul Hassan Gilani supervised the study and drafted final manuscript. Shafiq Ali Shah carried out literature search, experimental work and data analysis. Anwar-ul-Hassan Gilani and Munasib Khan supported in data analysis and review of the manuscript. All authors reviewed and approved the final manuscript for publication.

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