Kynurenine causes vasodilation and hypotension induced by activation of KCNQ-encoded voltage-dependent K⁺ channels

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A B S T R A C T

Kynurenine is a potential contributor to hypotension in animal and human sepsis. The present study was designed to examine whether the voltage-dependent K⁺ channels encoded by the KCNQ gene family (Kv7 channels) mediate vasodilator effects of kynurenine and whether modulation of these channels ameliorates hypotension caused by this compound. Rat aortas and mesenteric arteries or human omental arteries without endothelium were used. Some rings were incubated with the selective Kv7 channel inhibitor linopirdine (10 μM). L-Kynurenine (10 μM–1 mM) induced concentration-dependent relaxation in rat aortas and mesenteric arteries as well as human omental arteries, whereas linopirdine abolished the relaxation. L-Kynurenine (1 mM) produced hyperpolarization of vascular smooth muscle, which was reversed by linopirdine (10 μM). Wistar rats received L-kynurenine (1 mM) iv and subsequent linopirdine (10 μM) iv under 3% sevoflurane inhalation. L-Kynurenine iv caused hypotension, whereas linopirdine iv partially reversed it. In conclusion, kynurenine dilates arteries from rats as well as humans via Kv7 channels in the vascular smooth muscle. In rats, this tryptophan metabolite causes hypotension, which is partly counteracted by Kv7 channel inhibition. These results suggest that modulation of Kv7 channels may be a novel strategy to treat hypotension induced by the kynurenine.

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1. Introduction

An amino acid kynurenine is reportedly a potential novel contributor to hypotension in animal and human sepsis (1–3). Indoleamine 2,3-dioxygenase, which is a key enzyme in the kynurenine pathway, biologically transforms an essential amino acid tryptophan to kynurenine in many tissues including blood vessels (4–6). Cytokines and endotoxin induce indoleamine 2,3-dioxygenase protein expression in the vascular endothelial cells, leading to increased production of kynurenine (7, 8). Indeed, the activation of indoleamine 2,3-dioxygenase is associated with increased plasma levels of kynurenine in patients with sepsis (2, 3). These results suggest that inflammation in the endothelial cells contributes to augmented plasma levels of kynurenine. However, vasodilator mechanisms of kynurenine, especially toward smooth muscle cells, have been still unclear although a previous study indicated a partial involvement of guanylyl and adenylyl cyclase in the relaxation (1).

K⁺ channels have multiple functions in the vascular smooth muscle cell, and among them, conventional voltage-dependent K⁺ channels are postulated to be a major determinant of vascular tone (9, 10). The voltage-dependent K⁺ channel encoded by the KCNQ gene family (Kv7 channel) comprises five members (Kv7.1-5) (11). Kv7.1 is mainly expressed in the heart whereas Kv7.2, Kv7.3, Kv7.4 and Kv7.5 are distributed in various organs and tissues (11, 12). Gene and protein expression of all Kv7 members has been shown in rodent and human vascular smooth muscle cells (13–17). These results suggest that Kv7 channels may play a major role in the vasomotor function related to endogenous substances. However, no experiments have been performed to study directly the actions of kynurenine on Kv7 channels.

The present study was designed to examine whether Kv7 channels in the smooth muscle cells mediate vasodilator effects of...
kynurenine and whether modulation of these channels ameliorates hypotension caused by this amino acid.

2. Materials and methods

The Animal Care and Use Committee (#2013-3, dated June 5, 2012), and the responsible Human Ethics Board (#12-010, dated June 22, 2012) at Aichi Medical University School of Medicine (Aichi, Japan) approved this study. This investigation conforms to the principles outlined in the Declaration of Helsinki. The written informed consent was obtained from each patient in this study.

2.1. Tissue preparation for ex vivo studies

Thirty-eight male Wistar rats (12–16 weeks of age) were anesthetized with inhalation of 5% sevoflurane. Thoracic aortas and the first order (main) mesenteric arteries were simultaneously harvested after the rats were sacrificed by exsanguination. The aortal arteries (0.5–1.0 mm in diameter) in the greater omentum, which was obtained from patients scheduled for the elective gastric surgery, were also harvested. These patients (five patients [male four, female one], 39–70 yr) were without heart disease as well as coronary risk factors including diabetes mellitus, hypertension, hypercholesterolemia and smoking habit. All in vitro experiments were performed using arteries without endothelium in modified Krebs–Ringer bicarbonate solution (control solution) of the following composition (mM): NaCl 119, KCl 4.7, CaCl2 2.5, MgSO4 1.17, KH2PO4 1.18, NaHCO3 25, and glucose 5.5. Endothelial cells of these arterial rings were removed mechanically by gentle rubbing of the lumen with a cotton swab or needle insertion to avoid the modification mediated by endothelium-derived vasodilator substances. The reason of endothelial cell removal in this study is to avoid endogenous kynurenine production in the endothelial cells (7, 8).

2.2. Vasomotor reactivity studies

Each ring was connected to an isometric force transducer and suspended in an organ chamber filled with 10 ml of control solution (37 °C, pH 7.4) bubbled with 95% O2–5% CO2 gas mixture. The ring was gradually stretched to the optimal point of its length-tension curve as determined by the contraction to an α-adrenoceptor agonist phenylephrine (0.3 or 1 μM for rat aortic and mesenteric arterial rings, respectively) or a prostaglandin H2/thromboxane receptor agonist 9,11-dideoxy-11z,9z-epoxymethanoprostaglandin F2α, (U46619, 0.03 μM for human omental arterial rings) (18, 19). The optimal resting force was achieved at approximately 1.5 g for rat aortic rings and 1.0 g for rat mesenteric and human omental arterial rings (18, 19). The removal of endothelial cells was verified by the absence of relaxation in response to acetylcholine (1 μM) for rat aortic and mesenteric arterial rings and bradykinin (1 μM) for human omental arterial rings (18, 19). Thereafter, rings were incubated with a depolarizing agent, potassium chloride (KCl, 30 mM), a conventional voltage-dependent K+ channel inhibitor 4-aminopyridine (1 mM) or a selective Kv7 inhibitor linopirdine (10 μM) for 15 min. The concentration of 4-aminopyridine is relatively selective to block conventional voltage-dependent K+ channel current (9, 20) and that of linopirdine has been proved to selectively and completely inhibit Kv7 channel activity in arterial smooth muscle cells (21). Some rat mesenteric arteries were simultaneously incubated with a soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, 10 μM) (22), an adenylyl cyclase inhibitor, 9-(Tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22536, 100 μM) (23), an activator of protein kinase C, phorbol-12-myristate-13-acetate (PMA, 0.1 μM) or [arg8]-vasopressin acetate salt (AVP, 0.1 μM). Five min after completion of the incubation, phenylephrine (0.1–1 μM for rat aortic and mesenteric arterial rings) or U46619 (0.01–0.03 μM for human omental arterial rings) was added to the chambers to obtain submaximal contraction of arterial rings. These concentrations of agonists were selected to achieve the same submaximal contraction in arterial rings studied in parallel. During submaximal contraction, which reached plateau 10–15 min after the addition of contractile agents, concentration-response curves to l-kynurenine (10 μM–1 mM) or a selective Kv7 channel opener flupirtine maleate salt (flupirtine, 0.1–10 μM) were obtained in a cumulative fashion with 4–6 min interval. The relaxation was expressed as a percentage of the maximal relaxation in response to papaverine (300 μM), which was added at the end of experiments to produce the maximal relaxation (100%) of arterial rings (18, 19).

2.3. Measurement of membrane potential

Rat mesenteric arterial rings without endothelium were fixed on the bottom of an experimental chamber. The arteries were continuously perfused with control solution (37 °C) bubbled with 95% O2–5% CO2 gas mixture. A glass microelectrode (tip resistance 40–80 MΩ) filled with KCl (3 M) and held by a micromanipulator (Narishige, Tokyo, Japan), was inserted into a smooth muscle cell from the adventitial side of the vessel (18, 25). A recording amplifier (Electro 705, World Precision Instruments Inc., Sarasota, FL, U.S.A.) amplified the electrical signal, and a data-acquisition program (PowerLab™, ADInstruments Pty Ltd., Bella Vista, Australia) displayed and recorded changes in membrane potential on a computer. The validity of a successful impalement was assessed by a sudden negative shift followed by a stable negative voltage for more than 2 min (18, 25). Resting membrane potentials and changes in membrane potentials in response to l-kynurenine (1 mM) and the subsequent linopirdine (10 μM) were evaluated.

2.4. Measurement of hemodynamics

Six male Wistar rats inhaled sevoflurane (5%) in oxygen (50%) and catheters were indwelled in the femoral artery and vein. After the preperation, l-kynurenine (1 mM) was intravenously injected under sevoflurane (3%) inhalation. Linopirdine (10 μM) was subsequently administrated 5 min after the injection. These intravenous doses were determined by referring the maximum vasodilator effect of l-kynurenine in the current study as well as a previous study (1) and the previously reported maximum inhibitory effect of linopirdine on Kv7 channel activity in arterial smooth muscle cells, respectively (21). The rat blood volume of 6 ml per 100 g body weight was employed to the calculation (24). Changes in blood pressure and heart rate were simultaneously monitored by the BSM-2400 monitoring system (Nihon Kohden Co, Tokyo, Japan).

2.5. Drugs

The following pharmacological agents were used: 4-aminopyridine, AVP, dimethyl sulfoxide, flupirtine, KCl, l-kynurenine, phenylephrine, ODQ, PMA, SQ22536 and U46619 (Sigma Aldrich Inc., St. Louis, MO, U.S.A.), and linopirdine (Tocris Cookson Ltd., Bristol, UK). This study used l-kynurenine, but not D-kynurenine since L-kynurenine is a key intermediate in the breakdown pathway of tryptophan (1). Drugs were dissolved in distilled water such that volumes of <60 μl are added to the perfusion system. The stock solution of l-kynurenine or those of flupirtine, ODQ, PMA, and Tiron were prepared in hydrochloric acid and dimethyl sulfoxide, respectively. The highest concentration of hydrochloric acid or dimethyl sulfoxide was 290 and 1.74 μM, respectively. Our
preliminary, as well as previous studies, confirmed that these vehicles do not affect vasomotor function in our experimental condition (19). The concentrations of drugs are expressed as the final molar concentration.

2.6. Statistical analysis

Statistical analysis was performed using PASW Statistics 18™ (IBM Japan Inc., Tokyo, Japan). The distributions of the continuous variables were expressed as the means ± SE. Data were analyzed by one-way ANOVA or two-way ANOVA with Scheffe's test for statistical significance. Differences were considered to be statistically significant when P is < 0.05.

3. Results

3.1. Vasomotor reactivity studies

l-kynurenine induced concentration-dependent relaxation in rat mesenteric arteries (0.3 μM–1 mM) and aortas (1 μM–1 mM), whereas the selective Kv7 inhibitor linopirdine, as well as a depolarizing agent KCl, but not the conventional voltage-dependent K⁺ channel inhibitor 4-aminopyridine abolished the relaxation (Fig. 1). Consistently with these results, l-kynurenine (0.3 μM–1 mM) caused relaxation in the human omental arteries, which was abolished by linopirdine (Fig. 1). Linopirdine (10 μM) induced significant contraction in the rat aortas (62.0 ± 4.4% compared with phenylephrine [0.1 μM]-induced contraction), whereas it did not produce the contractile response in rat mesenteric arteries (Table 1). By adjustment of the doses of phenylephrine (0.1–1 μM for rat aortic and mesenteric arterial rings) or U46619 (0.01–0.03 μM for human omental arterial rings), submaximal contraction to contractile agents did not differ among studied groups in parallel (0.75–0.85 g for rat mesenteric arterial rings, 1.20–1.32 g for rat aortic rings, and 1.70–1.98 g for human omental arterial rings, respectively, NS, Fig. 1).

In rat mesenteric arteries, the selective Kv7 channel opener flupirtine (0.1–10 μM) induced concentration-dependent relaxation, which was completely inhibited by linopirdine (10 μM), but not by 4-aminopyridine (1 mM) (Fig. 2). In Fig. 2, submaximal contraction to phenylephrine did not differ among the studied groups in parallel (1.06–1.15 g, NS). The soluble guanylyl cyclase inhibitor ODQ (10 μM), the adenylyl cyclase inhibitor SQ22536 (100 μM), the protein kinase C activator PMA (0.1 μM) or AVP (0.1 μM) did not alter l-kynurenine-induced relaxation of rat mesenteric arteries (Figs. 3 and 4). In Figs. 3 and 4, submaximal contraction to phenylephrine did not differ among studied groups in parallel (0.74–0.85 g for Fig. 3 and 0.77–0.99 g for Fig. 4, NS).

3.2. Measurement of membrane potential

Consistently with results in the hemodynamic measurements, l-kynurenine (1 mM) hyperpolarized rat mesenteric arteries and subsequent linopirdine (10 μM) reversed the hyperpolarization (Fig. 5).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Phenylephrine (0.3 μM, g)</th>
<th>Linopirdine (10 μM, g)</th>
<th>% Contraction</th>
</tr>
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<tbody>
<tr>
<td>Mesenteric artery</td>
<td>0.75 ± 0.06</td>
<td>0.06 ± 0.02</td>
<td>8.85 ± 2.86</td>
</tr>
<tr>
<td>Aorta</td>
<td>1.02 ± 0.13</td>
<td>0.63 ± 0.10*</td>
<td>62.0 ± 4.35*</td>
</tr>
</tbody>
</table>

Mean ± SE; *: P < 0.05 vs. Mesenteric artery.
3.3. Measurement of hemodynamics

L-kynurenine (1 mM) iv caused hypotension, which was reached a plateau at five min after the injection (Fig. 6). Administration of linopirdine (10 μM) partially reversed the hypotension (Fig. 6). Neither L-kynurenine nor linopirdine iv affected heart rate throughout the experiment (Fig. 6).

4. Discussion

L-kynurenine induced relaxation or hyperpolarization of human omental arteries as well as rat mesenteric arteries and aortas, which was abolished by a selective Kv7 inhibitor linopirdine, but not by a conventional voltage-dependent K⁺ channel inhibitor 4-aminopyridine, indicating that this l-tryptophan metabolite causes vasodilation largely via Kv7 activation (14, 17). This conclusion is supported by the results that linopirdine completely inhibited a selective Kv7 channel opener flupirtine-induced vasorelaxation, verifying the selectivity of linopirdine on Kv7 channels (26). Indeed, linopirdine-sensitive currents are activated at more negative voltages than conventional voltage-dependent K⁺ channels, and, therefore, they are insensitive to 4-aminopyridine in the rat mesenteric arterial smooth muscle cells (21). It is also crucial to note that KCl (30 mM) did not completely remove a component of the kynurenine-induced relaxation. Factors other than K⁺ channels...
should mediate it since the part was seen in the condition with the relatively higher concentrations of a depolarizing agent (9).

The Kv7 channel family comprises five members (Kv7.1-5) (11). Kv7.1 is mainly expressed in the heart whereas Kv7.2, Kv7.3, Kv7.4 and Kv7.5 are widely distributed in various organs and tissues (11, 12). In the blood vessels, Kv7.4 is the most abundant mRNA in the mouse aorta, carotid artery femoral artery and mesenteric artery (14), whereas Kv7.1 is the most dominant in the mouse portal vein (13). In rats, expression of Kv7.1 or Kv7.4 was proved in the third-order mesenteric arteries, thoracic aorta, intrapulmonary arteries and the Gracilis muscle artery (15, 16). In human mesenteric arteries, Kv7.4 mRNA is consistently expressed, whereas expression of Kv7.1, Kv7.3, and Kv7.5 mRNAs is more variable, and Kv7.2 mRNA is undetectable (17). Therefore, Kv7.4 is probably the most likely candidate to mediate kynurenine-induced arterial dilation including that in humans. It should also be emphasized a crucial role of Kv7 expressed on vascular smooth muscle cells in the kynurenine’s vasodilator effect since Kv7 subunits are located in the arterial smooth muscle layer (17). In the current study, linopirdine induced contraction of the rat aortas but not of the rat main mesenteric arteries. These results indicate the roles of Kv7 in basal vascular tone are variable dependently on regions where the artery exists.

Accumulating evidence suggests that protein kinases A, G, and C regulate conventional voltage-dependent K⁺ channels in vascular smooth muscle cells (10). Vasodilators stimulate adenylyl cyclase, which leads to increased cyclic AMP production and protein kinase A activity, resulting in activation of these channels (10). Activation of guanylyl cyclase by vasodilators is also capable of causing an increase in cyclic GMP production and protein kinase G activity, which enhances conventional voltage-dependent K⁺ channel activity (10). In the current study, neither a soluble guanylyl cyclase inhibitor ODQ nor an adenylyl cyclase inhibitor SQ22536 altered l-kynurenine-induced vasorelaxation, indicating that the kynurenine causes Kv7 activation independently of these kinases. These results are in contrast with a previous study that the kynurenine at least partly induces vasodilation via activation of guanylyl and adenylyl...
cyclase pathways (1). Experimental conditions and species differences (cultured rat aortic smooth muscle cells and porcine coronary arteries vs. isolated rat aortas and mesenteric arteries, and human omental arteries in the current study) may play roles in such a discrepancy between previous and current studies. Vasoconstrictors induce diacylglycerol and consequent protein kinase C activation, resulting in inhibition of conventional voltage-dependent K+ channels in vascular smooth muscle cells (10). Indeed, the physiological concentrations of AVP (0.01–0.1 nM) via activation of protein kinase C reportedly inhibit the Kv7 current caused by synthetic Kv7 openers including flupirtine (21, 27, 28). Kynurenine probably acts on Kv7 channels via different mechanisms from synthetic Kv7 openers since the higher concentration of a protein kinase C activator PMA (0.1 μM) or AVP (0.1 μM) failed to alter vasorelaxation in response to i-kynurenine in the current study. More importantly, a Kv7 inhibitor linopirdine completely inhibited i-kynurenine-induced vasorelaxation, and it partially reverses hypotension and hyperpolarization induced by i-kynurenine. These results suggest that a potentially therapeutic role of selective Kv7 inhibitors, but not AVP, in the hypotension mediated by increased production of kynurenine in variable pathological conditions as follows.

Indoleamine 2,3-dioxygenase, which is a key enzyme in the kynurenine pathway, biologically transforms an essential amino acid tryptophan to kynurenine in many tissues including blood vessels, resulting in normal plasma kynurenine concentration of about 1 μM (4–6). Plasma levels of kynurenine are expected to reach the pathological range since many pathological changes are known to cause substantial effects on the kynurenine generation and excretion (4, 5). The activation of indoleamine 2,3-dioxygenase is associated with increased plasma levels of kynurenine up to 20 μM in patients with sepsis (2, 3). Interferon-γ augments the indoleamine 2,3-dioxygenase protein expression in the human aortic endothelial cells, leading to increased levels of kynurenine to 10 μM in a culture medium (8). These results suggest that inflammation plays a major role in augmented plasma levels of kynurenine. Renal dysfunction including uremia in humans is capable of enhancing plasma kynurenine levels up to 5 μM, being supported by the physiological evidence that the glomeruli filter kynurenine (29, 30). Augmented metabolism of tryptophan by indoleamine 2,3-dioxygenase contributes to a three-times increase in plasma kynurenine levels in patients after major trauma (31). Obesity is accompanied by the activation of indoleamine 2,3-dioxygenase in the adipose tissues, resulting in doubled plasma levels of kynurenine (6). These studies draw a conclusion that co-existence and the combination of any above disease state may further increase plasma levels of kynurenine in humans to the concentration range that was evaluated in the current study. It is unclear, however, whether co-existence of different disorders can further increase plasma levels of kynurenine in humans up to the concentration range used in this study.

This is the first study to demonstrate that pathological concentrations of an amino acid kynurenine dilute arteries from rats as well as humans via activation of Kv7 channels in the vascular smooth muscle cells. Consistently with these results, this tryptophan metabolite caused hypotension, which was partially reversed by the Kv7 channel inhibition. These results suggest that Kv7 channels play a crucial role in the regulation of vasomotor function in the pathological conditions. Modulation of these channels and the blockade of kynurenine formation may be novel strategies to treat hypotension induced by kynurenine.

Conflict of interest

The authors declare no conflict of interest.

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References


(26) Morecroft I, Murray A, Nilsen M, Gurney AM, MacLean MR. Treatment with the Kv7 potassium channel activator flupirtine is beneficial in two independent mouse models of pulmonary hypertension. Br J Pharmacol. 2009;157:1241–1249.


