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ORIGINAL ARTICLE

Differential vasodilation response to olprinone in rabbit renal and common carotid arteries

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Abstract
Purpose Olprinone, one of the most frequently used phosphodiesterase-3 inhibitors, exerts its positive inotropic and vasodilation effects by inhibiting the degradation of intracellular cyclic adenosine monophosphate (cAMP). The vasodilation response to olprinone is not uniform among the different vascular beds. This study was designed to compare the vasorelaxation response to olprinone between renal and common carotid arteries, and investigate its underlying mechanisms.
Methods Isometric force measurement, enzyme immunoassay, and western blotting techniques were used to investigate the vasorelaxation action of olprinone in isolated rabbit renal and common carotid arteries.
Results Olprinone inhibited the contractile response to phenylephrine (PE) both in the renal and carotid arteries in a concentration-dependent manner with IC_{50} values of 40 ± 10 and 103 ± 43 nM, respectively. The IC_{50} value was lower ($P = 0.004$) and the maximal inhibition was greater ($P = 0.002$) in the renal artery compared with the carotid artery. A cell-permeable cAMP analogue, 8-bromo-cAMP, also inhibited the contractile response to PE in the renal and carotid arteries with IC_{50} values of 581 ± 150 and 740 ± 179 μM, respectively; however no differences were observed both in the IC_{50} value and the maximal inhibition between two arteries. Olprinone (0.1 μM) increased the intracellular cAMP level in the renal arterial smooth muscle cells (ASMCs) but not in the carotid ASMCs. The expression of PDE3A was greater ($P = 0.008$) in the carotid ASMCs than the renal ASMCs.
Conclusion The enhanced vasodilator action of olprinone in the renal artery is presumably because of its ability to stimulate the cAMP production, which might be attributable to the heterogeneous expression of PDE3A.

Keywords cAMP · Olprinone · Phosphodiesterase · Vascular smooth muscle

Introduction

Phosphodiesterase-3 (PDE3) inhibitors are frequently used for treatment of heart failure because of their positive inotropic and vasodilation properties. PDE3 inhibitors block the hydrolysis of cyclic adenosine monophosphate (cAMP), thereby resulting in accumulation of intracellular cAMP. In vascular smooth muscle cells, elevation of the cAMP level reduces the intracellular Ca^{2+} concentration and myofilament calcium sensitivity. This subsequently induces vasodilation [1, 2].

Olprinone, a relatively newly commercially available PDE3 inhibitor, is known to increase both cerebral [3] and visceral organ blood flow [4, 5]. The vasodilation response to PDE3 inhibitors including amrinone and milrinone is not uniform among the different vascular beds including the renal and cerebral arteries [6, 7]. Although olprinone was also reported to exert differential vasodilator action on splanchnic and peripheral arteries [8], whether the effect of olprinone on the cerebral vasculature is similar to that on the systemic arteries is poorly understood. The carotid artery supplies cerebral blood flow from systemic circulation, and thus serves to regulate cerebral blood flow. This study was designed to investigate and compare the

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olprinone-induced vasorelaxation response of the renal and common carotid arteries, and to elucidate the underlying mechanism.

Methods

The experimental protocol was approved by the Wakayama Medical University Animal Care and Use Committee.

Isometric force measurement

Male Japanese white rabbits weighing 2.2–3.2 kg were sacrificed by bleeding from the abdominal aorta after laparotomy under halothane anesthesia. Bilateral renal and common carotid arteries of similar external diameter (2.0–3.0 mm) were carefully dissected and immersed in ice-cold Krebs bicarbonate solution (KBS) of composition (mM): NaCl 118.2, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, and dextrose 10. The arteries were cleared of adherent connective tissue and cut into rings 3 mm long. The intimal surface was gently rubbed with a stainless-steel needle to remove the endothelium. Afterwards, the rings were suspended between wire hooks in a 10-ml organ bath. Each organ bath was filled with KBS which was maintained at a temperature of 37°C and continuously aerated with a mixture of 95% O_2 and 5% CO_2. The isometric tension was measured as described previously [9–11]. Before the start of the experiment, the rings were allowed to equilibrate for 60 min, during this time the organ bath was replaced with fresh KBS every 20 min.

Renal and carotid arterial rings were exposed to KCl (30 mM) to assess their overall contractile responsiveness. Rings that did not develop at least 2.0 g contractile force were discarded. Removal of endothelium was confirmed by the lack of relaxation response to acetylcholine (10 μM) in rings precontracted with phenylephrine (PE 0.3 μM).

The arterial rings were contracted with PE (0.3 μM), which had been determined to achieve 80–90% of the maximum PE-induced response in both renal and carotid arteries in the preliminary study. After a plateau had been reached, the concentration–relaxation responses of the renal and common carotid arteries to olprinone (1 nM to 10 μM) were obtained.

The relaxation response of the renal and carotid arteries to 8-bromo-cAMP (10 μM to 3 mM), a membrane-permeable analogue of cAMP, was compared to determine whether these arteries differ in their response to an increased level of in intracellular cAMP. Bilateral renal and common carotid arterial rings from each of seven different rabbits ($n = 7$) were randomly assigned to be exposed to olprinone or 8-bromo-cAMP. Each ring was exposed to only one drug.

Measurement of cyclic nucleotides

The level of c-AMP and cyclic guanosine monophosphate (cGMP) were measured in isolated renal and common carotid arteries from which the endothelium had been mechanically removed. After 60 min equilibration, the arteries were incubated with PE (0.3 μM) for 15 min. They were then exposed to olprinone (0.1 μM) for 10 min. (This exposure time was chosen because the relaxation response to olprinone reaches its maximum level after 10 min.) Afterwards, the preparations were frozen in liquid nitrogen. The tissues were then homogenized in 5% trichloroacetic acid at 4°C with a homogenizer. The homogenized tissue was centrifuged at 1500*g* for 10 min. An ether extraction procedure was carried out on the supernatant. An enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) was used to determine the presence of cAMP and cGMP from an aliquot of the extract. The concentrations of cAMP and cGMP were expressed per milligram of wet tissue. Cyclic nucleotides from renal and common carotid arterial preparations were measured from five different rabbits ($n = 5$).

Measurement of PDEs expression

To examine whether the distribution of PDEs differs in renal and common carotid arteries, western blotting analysis was used to measure expression of PDE3A, PDE4, and PDE 5A in both arteries. PDE3A, a major isoform of the PDE3 family, is identified in cardiac and vascular myocytes [12]. The renal and common carotid arteries were excised from five animals ($n = 5$). The endothelium of the arteries was mechanically removed and rapidly frozen with dry ice. Frozen tissues were cut into small pieces and homogenized in ice-cold lysis buffer with 0.2% Triton X-100. The homogenates were centrifuged at 10000*g* for 30 min at 4°C. The supernatants were assayed for protein concentration using bicinchoninic acid methods [13]. Samples with equal total protein content (25 μg) were separated using sodium dodecylsulfate polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. The membranes were incubated with anti-PDE3A (1:500), anti-PDE4 (1:1000), anti-PDE5A (1:1000), or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:200) for 2 h. This was followed by incubation with horseradish peroxidase-conjugated antibody (1:2000) for 1 h. The densities of immunoreactive bands were detected using chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and were assessed with image analysis software (NIH Image 1.62, Bethesda, MD, USA). The ratio of each PDE to GAPDH was used as an indicator of each PDE expression.

Materials

All drugs were of the highest purity commercially available. Acetylcholine, PE, and 8-bromo-cAMP were obtained from Sigma–Aldrich Fine Chemicals (St Louis, MO, USA). Olprinone HCl was provided by Eisai (Tokyo, Japan). Specific antibodies to the PDEs were obtained from Novus Biologicals (Littleton, CO, USA) and secondary antibody labeled with horseradish peroxidase was obtained from Zymed Laboratories (Carlsbad, CA, USA). Anti-GAPDH antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All drugs except antibodies were dissolved in distilled water and added directly to the bath in volumes of 100 μl or less.

Data analysis

Statistical analysis was performed using the software StatMate (Atoms, Tokyo, Japan). Vasorelaxation responses to olprinone and 8-bromo-cAMP are expressed as the percentage of relaxation relative to the PE precontraction. The drug concentration eliciting 50% of the maximum relaxation response (IC_{50}) was calculated by linear regression analysis, and the maximum relaxation response (R_{max}) was determined. IC_{50} and R_{max} were expressed as mean ± SD, and compared using the unpaired Student's *t* test. For nonparametric comparison, data were expressed as medians with the 25th and 75th percentiles, and were evaluated using Mann–Whitney *U* test for observations between two groups. *P* values <0.05 were considered significant. Sample size (*n* values) represents the number of animals from which renal and common carotid arteries were taken.

Results

Isometric force measurement

Olprinone induced a concentration-dependent relaxation response in both renal and common carotid arteries that had been precontracted with PE. The concentration–relaxation curve of the renal arteries was significantly shifted to the left compared with those of the carotid arteries ($P = 0.004$). R_{max} value of the renal artery was also significantly greater than that of the carotid artery ($P = 0.002$). The IC_{50} values of the renal and carotid arteries in response to olprinone were 40 ± 10 and 103 ± 43 nM, respectively; the R_{max} values of the renal and carotid arteries in response to olprinone were 90 ± 3 and 80 ± 7%, respectively ($n = 7$ each) (Fig. 1). In contrast, there were no differences between the values of IC_{50} ($P = 0.10$) and R_{max} ($P = 0.26$) of the renal (581 ± 150 μM, 92 ± 3%) and carotid (740 ± 179 μM, 85 ± 8%) arteries induced by 8-bromo-cAMP (Fig. 2).

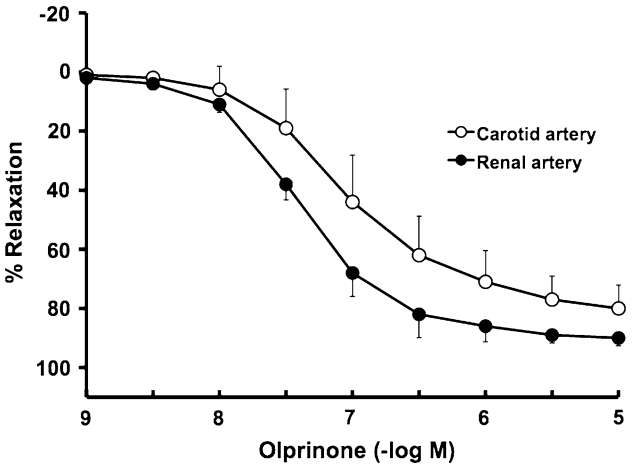

Fig. 1 Concentration-dependent relaxation induced by olprinone (10^{-9} to 10^{-5} M) in rabbit renal and common carotid arteries. Both arterial preparations were endothelium-denuded and precontracted with phenylephrine (0.3 μM). Relaxation responses are expressed as the percentage of relaxation relative to phenylephrine precontraction. The IC_{50} value of the renal artery was significantly smaller than that of the carotid artery ($P = 0.004$). The maximum relaxation response in the renal artery was also greater than that in the carotid artery ($P = 0.002$)

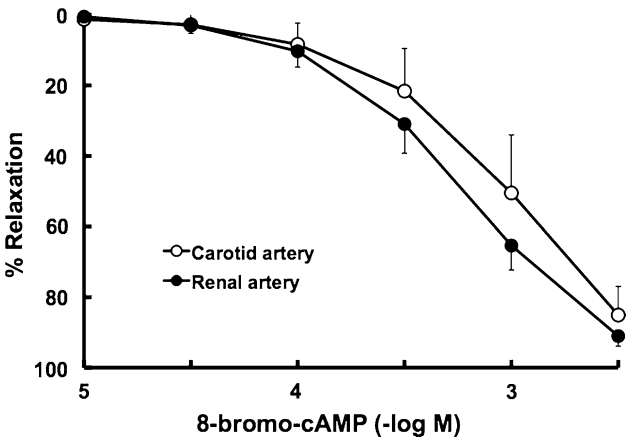

Fig. 2 Concentration-dependent relaxation induced by 8-bromo-cAMP (10^{-5} to 3×10^{-3} M), a membrane permeable analogues of cAMP, in endothelium-denuded rabbit renal and common carotid arteries. Both arterial preparations were precontracted with phenylephrine (0.3 μM). Relaxation responses are expressed as the percentage of relaxation relative to phenylephrine precontraction. There was no significant difference in the relaxation response to 8-bromo-cAMP between the renal and carotid arteries

Measurement of cyclic nucleotides

Ten minutes exposure of renal and carotid arteries to olprinone (0.1 μM) caused a significant increase in intracellular cAMP accumulation in the renal artery ($P = 0.032$,

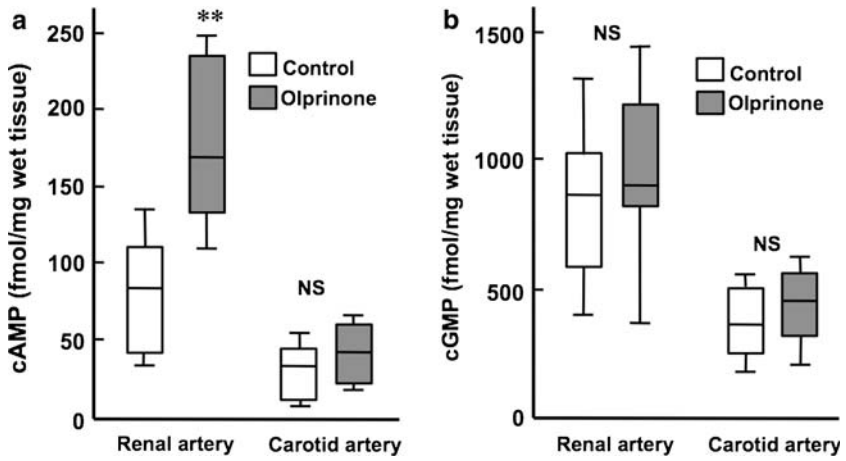

Fig. 3 The effect of olprinone (0.1 μM) on intracellular cAMP (**a**) and cGMP (**b**) levels in rabbit renal and common carotid arterial smooth muscle. Arterial preparations were exposed to phenylephrine (0.3 μM) for 15 min, followed by exposure to olprinone (0.1 μM) for 10 min, and then frozen and assayed for cAMP measurement. Values are expressed as fmol mg^{-1} wet tissue weight. *Horizontal bars* represent medians, *boxes* represent 25th and 75th percentile ranges, and *T-bars* the minimum and maximum values. Compared with the control (no olprinone exposure), exposure to olprinone induced a significant increase in cAMP level in the renal artery (**$P = 0.032$), but not in the carotid artery (**a**). The level of cGMP was not increased by olprinone in either artery (**b**)

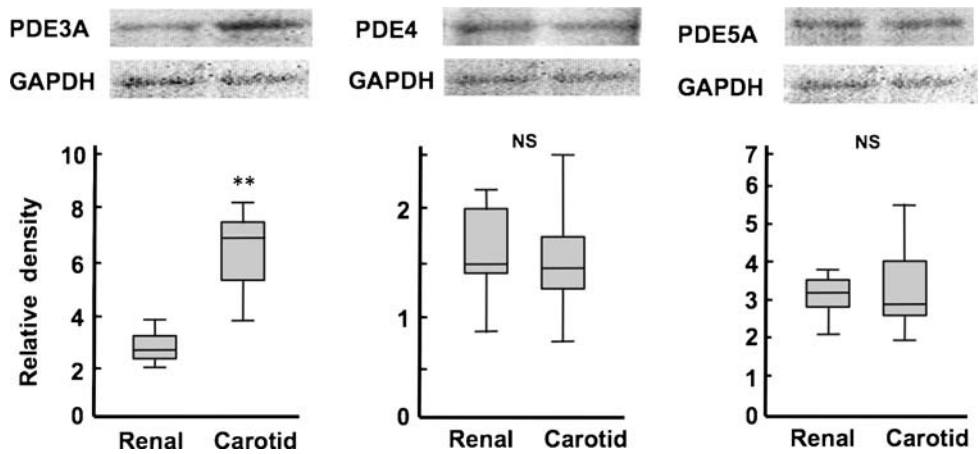

Fig. 4 The expression of PDE3A (*left*), PDE4 (*middle*), and PDE5A (*right*) in rabbit renal and common carotid arterial smooth muscle as detected by western blot analysis. The quantitative result for the amount of each PDE isozyme was normalized for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). *Horizontal bars* represent medians, *boxes* represent 25th and 75th percentile ranges, and *T-bars* the minimum and maximum values. Expression of PDE3A, but not of PDE4 and PDE5A, is significantly greater in the carotid artery (**$P = 0.008$) than in the renal artery

$n = 5$), but not in the common carotid artery ($P = 0.42$, $n = 5$) (Fig 3a). Olprinone did not affect the intracellular cGMP level in either artery (Fig 3b).

Measurement of PDEs expression

PDE3A expression normalized by GAPDH was greater in the common carotid artery than in the renal artery ($P = 0.008$, $n = 5$) (Fig. 4). In contrast, the expression of PDE4 and PDE5A was similar in both arteries.

Discussion

The key findings of this study are as follows. Olprinone induces a concentration-dependent relaxation response in the rabbit renal and common carotid arteries. The extent of the relaxation is greater in the renal artery than in the carotid artery. The relaxation response to 8-bromo-cAMP is similar in both arteries. Olprinone at a concentration of 0.1 μM significantly increases the intracellular c-AMP level in the renal artery, but not in the carotid artery.

Western blotting analysis reveals greater expression of PDE3 in the carotid artery than in the renal artery.

Olprinone is now one of the PDE3 inhibitors most frequently used to treat acute heart failure [14]. It inhibits the degradation of cAMP. The intracellular accumulation of cAMP in the myocardium and in vascular smooth muscle enhances myocardial contraction and induces vascular relaxation, respectively. These actions contribute to improving cardiac output. Various types of arteries in human [8] and animal [1, 6] experiments have differential pharmacological sensitivities to PDE3 inhibitors, although the mechanism remains unknown. It has been shown that olprinone increases cerebral blood flow and that there is no direct correlation between changes in cerebral blood flow and cardiac output [3]. Although this finding suggests that the direct vasodilation may play an important role in the olprinone-induced change in cerebral blood flow, the direct effect of olprinone on the cerebral artery is poorly understood. The carotid artery plays a role in the regulation of cerebral blood flow, and recent studies have also revealed that the distensibility of the wall of the common carotid artery is improved by olprinone [3, 14]. PDE3 inhibitors are known to have a potent vasodilation effect on renal and splanchnic arteries [4–6, 15]. Therefore, we tried to characterize and compare the pharmacological properties of olprinone in the carotid and renal arteries.

As a result, olprinone dilated the renal artery to a greater extent than the carotid artery. However, the vasodilation in response to 8-bromo-cAMP was similar in both arteries. This suggests that the different vasodilation response to olprinone may not be due to the ability of each artery to respond to cAMP. Instead, the different vascular responses may be due to the different intracellular levels of cAMP induced by olprinone in the renal and carotid arteries. A greater increase in the intracellular cAMP concentration in the renal artery was indeed observed in this study. These findings are consistent with those of other investigators [8] who found that olprinone-induced relaxation is more potent in human gastroepiploic and radial arteries than in the internal mammary artery, whereas the relaxation response to folskolin, an activator of adenylate cyclase that generates cAMP, was similar in these arteries. Taken together, the differences in cAMP accumulation induced by olprinone may contribute to the different vasodilation responses in the renal and carotid arteries. No significant increase in the cGMP level in response to olprinone was observed. This indicates that cGMP is not involved in olprinone-induced vasodilation.

Western blot analysis revealed greater expression of PDE3A in the common carotid artery than in the renal artery. This suggests that olprinone-induced inhibition of PDE3 activity may differ in these arteries. It may be speculated that the greater expression of PDE3A results from the carotid artery's being more resistant to the effect of olprinone. This would cause less intracellular accumulation of cAMP in carotid vascular smooth muscle, thereby resulting in a reduced level of vasodilation. Heterogeneous distribution and regulation of PDE3 isozymes has been reported in previous studies and different effects of PDE3 inhibitors on each artery can be explained, at least in part, by the varying distribution of the PDE3 isozyme [14, 16, 17].

When administered at 0.2 μg kg^{-1} min^{-1}, the plasma concentration of olprinone in patients was reported to range from 22 to 59 ng ml^{-1} (approximately 70–200 nM) [18, 19]. Because plasma protein binding of olprinone is 81.3% [20], the plasma-free concentration of olprinone could be estimated at 15–40 nM. This concentration of olprinone is close to the IC_{50} value observed in the isometric force experiment in the renal artery (IC_{50} = 40 nM), but lower than that seen in the carotid artery. Considering that protein binding of a drug is not constant and is affected by many factors, for example plasma albumin concentration and plasma pH, and that the maximum infusion rate of olprinone is up to 0.4 μg kg^{-1} min^{-1}, olprinone could cause a differential vasodilation effect in the renal and carotid arteries when used at a clinically relevant concentration.

The limitation of this study is that the findings were obtained from endothelium-denuded vessels. The endothelium plays an essential role in regulating the underlying smooth muscle tone under in-vivo conditions. However, previous studies have demonstrated that the presence of the endothelium does not affect the vasodilation of isolated arteries induced by PDE3 in humans [8] or in experimental animals [1, 21]. This indicates that the vasodilation effect of olprinone is endothelium-independent. Furthermore, organ blood flow is regulated by cardiac output and peripheral vascular resistance. The latter is mainly determined by the resistance of small arteries rather than by large arteries such as the renal or carotid arteries. Although the findings from this in-vitro study cannot be directly extrapolated to in-vivo situations, the preferential dilation of the renal artery by olprinone may contribute to an increase in renal blood flow.

In conclusion, at a clinically relevant concentration olprinone induces greater vasodilation of the renal artery than of the common carotid artery. The intracellular accumulation of cAMP induced by olprinone is greater in the renal artery than in the carotid artery. The heterogeneous expression of PDE3A in each artery may be involved in the differential vasodilation effect of olprinone.

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