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# Effect of des-aspartate-angiotensin I on the actions of angiotensin II in the isolated renal and mesenteric vasculature of hypertensive and STZ-induced diabetic rats

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**Abstract**

The present study investigated the action of des-aspartate-angiotensin I (DAA-I) on the pressor action of angiotensin II in the renal and mesenteric vasculature of WKY, SHR and streptozotocin (STZ)-induced diabetic rats. Angiotensin II-induced a dose-dependent pressor response in the renal vasculature. Compared to the WKY, the pressor response was enhanced in the SHR and reduced in the STZ-induced diabetic rat. DAA-I attenuated the angiotensin II pressor action in renal vasculature of WKY and SHR. The attenuation was observed for DAA-I concentration as low as $10^{-18}$ M and was more prominent in SHR. However, the ability of DAA-I to reduce angiotensin II response was lost in the STZ-induced diabetic kidney. Instead, enhancement of angiotensin II pressor response was seen at the lower doses of the octapeptide. The effect of DAA-I was not inhibited by PD123319, an $AT_2$ receptor antagonist, and indomethacin, a cyclo-oxygenase inhibitor in both WKY and SHR, indicating that its action was not mediated by angiotensin $AT_2$ receptor and prostaglandins. The pressor responses to angiotensin II in mesenteric vascular bed were also dose-dependent but smaller in magnitude compared to the renal vasculature. The responses were significantly smaller in SHR but no significant difference was observed between STZ-induced diabetic and WKY rat. Similarly, PD123319 and indomethacin had no effect on the action of DAA-I. The findings reiterate a regulatory role for DAA-I in vascular bed of the kidney and mesentery. By being active at circulating level, DAA-I subserves a physiological role. This function appears to be present in animals with diseased state of hypertension and diabetes. It is likely that DAA-I functions are modified to accommodate the ongoing vascular remodeling.


*Keywords:* Des-aspartate-angiotensin I; Renal; Mesenteric; Vasculature; Angiotensin II; Streptozotocin

## 1. Introduction

The renin–angiotensin system (RAS) plays a major role in the regulation of blood pressure as well as sodium and water balance. Most of the modulating effects are mediated via angiotensin II, acting at the $AT_1$ subtype of the angiotensin receptor [1]. Changes in the renin–angiotensin system have been implicated in the pathophysiology of cardiovascular diseases [2]. Des-aspartate-angiotensin I (DAA-I), a nine amino acid peptide has been shown to attenuate the action of angiotensin III in the aortic rings of the rabbit [3] and the SHR [4], and in the renal and mesenteric vasculature of SHR and Wistar–Kyoto (WKY) rats [5]. Intracerebroventicular administration of DAA-I attenuated the central pressor actions of angiotensin II and III in the SHR and WKY rats [6,7]. Data obtained from electrically contracted endothelium-denuded rabbit pulmonary arteries showed that DAA-I

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acted as an agonist on the $AT_1$ receptor [8]. In the renal and mesenteric vasculature, DAA-I action was not blocked by PD123319, a specific $AT_2$ receptor agonist, which suggests that DAA-I may act through the $AT_1$ receptor [5]. The present study was designed to investigate the effects of DAA-I on the pressor action of angiotensin II in the renal and mesenteric vasculature of SHR, WKY and STZ-induced diabetic rats. Angiotensin II is implicated in the pathophysiology of these two disease states, and the extent its pressor action could be modulated by DAA-I would determine the likely roles of the nonapeptide.

## 2. Materials and methods

### 2.1. Animals

Male Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) age 10 weeks were obtained from Animal House in the University of Malaya Medical Centre. The animals were fed standard rat chow and tap water ad libitum for 2 weeks and diabetes was induced at age 12 week.

### 2.2. Induction of diabetes

WKY was made diabetic by administration of streptozotocin (STZ) 75 mg/kg intraperitoneally. Age matched controls received equal volume of vehicle. Body weight and blood glucose levels of each rat were taken every 2 weeks until the 8th week. Animals were considered diabetic if their blood glucose level was >17 mM.

### 2.3. Isolated kidney and mesenteric vascular bed

To isolate the right kidney, rats were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.). The right kidney was exposed by midline laparotomy. The renal artery was cannulated with a catheter (PET-50) via the superior mesenteric artery and perfusion was started in situ. The right renal vein and ureter were cut. The right kidney was ligated, excised and placed in a water-jacketed chamber maintained at 37 °C and perfused with an oxygenated (95% $O_2$ and 5% $CO_2$) Kreb's solution by means of a peristaltic pump (Minipuls 3 Model 312, Gilson Villiers Le Bel, France) at a rate of 5 ml/min. In order to isolate the mesenteric arterial bed, the remaining length of the superior mesentery artery was cannulated with a catheter according to the method of McGreggor [9]. The mesentery was carefully excised from the intestine and placed in a water-jacketed chamber maintained at 37 °C and perfused with an oxygenated Kreb's solution by means of a peristaltic pump at a rate of 5 ml/min. The composition of the Kreb's solution was as follows (mmol/l): NaCl, 120; KCl, 4.7; $CaCl_2$, 2.4; $MgCl_2$, 1.2; $NaHCO_3$, 20; EDTA, 0.06; and glucose, 10. Changes in perfusion pressure were measured by means of a pressure transducer (Model P23XL, Ohmeda Medical Devices Division Inc, USA) and recorded via a MacLab data acquisition system (AD Instruments, Australia).

### 2.4. Experimental protocol

After an equilibration period of 20 min, the preparation was preconstricted with phenyleprine (PE, $10^{-5}$ M) and the increase in perfusion pressure was recorded until a 5-min plateau was observed. This contractile response to phenylephrine was taken as a unity and responses to other pressor compounds were normalized against this unit. The preparation was then perfused with a Kreb's solution that contained 30 μM captopril. Following 1 h of perfusion, various concentrations ($10^{-13}$–$10^{-6}$ M) of angiotensin II was used to produce a contractile response. The angiotensin was administered as a single bolus injection of 20 μl (renal) and 50 μl (mesenterial bed) into the perfusion system. The minimum time interval between successive bolus injections was 10 min or the time till the basal pressure was again recorded.

The effects of various concentrations ($10^{-18}$–$10^{-9}$ M) of DAA-I on the response to angiotensin II were studied with the following protocol. The preparation was first perfused with Kreb's solution containing 30 μM captopril and a concentration of DAA-I for 30 min, prior to initiating a concentration response to angiotensin II. Each concentration of DAA-I was studied using a new set of preparation. A similar protocol was used to study the direct effect of indomethacin ($10^{-7}$ M) or PD123319 ($10^{-5}$ M) on the concentration response to angiotensin II. The effect of PD123319 or indomethacin on the actions of DAA-I on the concentration responses to angiotensin II was studied by perfusing the preparation with DAA-I and PD123319 (or indomethacin) for 30 min prior to bolus injection of angiotensin II.

### 2.5. Drugs

Captopril, angiotensin II, streptozotocin and indomethacin were purchased from Sigma. Des-aspartate-angiotensin I was purchased from BACHEM AC, Bubendorf, Switzerland. PD123319 was a generous gift from Parke-Davis Pharmaceutical Research, Michigan, USA.

### 2.6. Statistical analysis

Data are presented as mean ± SEM. Significant difference ($p<0.05$) between means was evaluated using Student's $t$-test when comparing two groups. When more than two groups were compared, and for the comparison of the dose–response curves, data were evaluated by one-way analysis of variance (ANOVA) followed by Bonfferoni post hoc test. Results with $p<0.05$ were considered statistically significant.

## 3. Results

### 3.1. Renal vasculature

A $10^{-5}$ M concentration of PE caused an average increase in perfusion pressure of 250 ± 20 mm Hg in SHR, and 210 ± 25 mm Hg in the WKY and STZ-induced diabetic rats. The lower and higher limits of detection of the system were 5 and 350 mm Hg, respectively. Fig. 1 shows the dose–response of renal perfusion pressure to angiotensin II in WKY, SHR and STZ-induced diabetic rats. The response to the higher five of the eight increasing doses of angiotensin II in the SHR was significantly greater than those in the WKY. Angiotensin II pressor responses in the STZ-induced diabetic rats were significantly lower than those in the WKY.

Pressor responses to angiotensin II in the WKY and SHR were attenuated by DAA-I. The attenuation was seen at the higher angiotensin II concentration ($10^{-9}$–$10^{-6}$ M) in both animal groups (Fig. 2). In STZ-induced diabetic rats, DAA-I significantly potentiated the pressor action of lower concentrations of angiotensin II ($10^{-13}$–$10^{-11}$ M) and had no effect on the high concentrations of the octapeptide. DAA-I, by itself, had no effect on the basal perfusion pressure (data not shown). The action of DAA-I was not affected by PD123319 and indomethacin in both WKY (Fig. 3, upper set of histograms) and SHR (data not shown).

### 3.2. Mesenteric vasculature

A $10^{-5}$ M concentration of PE caused an average increase in perfusion pressure of 100±25 mm Hg. This response was smaller than the increase recorded for the renal vasculature. The responses for angiotensin II recorded for mesenteric bed

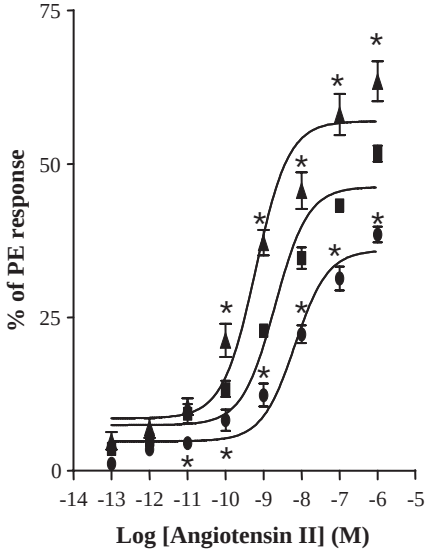


Fig. 1. Angiotensin II-induced pressure response in the kidney of WKY (■), SHR (▲) and STZ-induced diabetic (●) rats. Each point is the mean ± SEM of 5–6 animals. *Indicates significant difference from WKY.

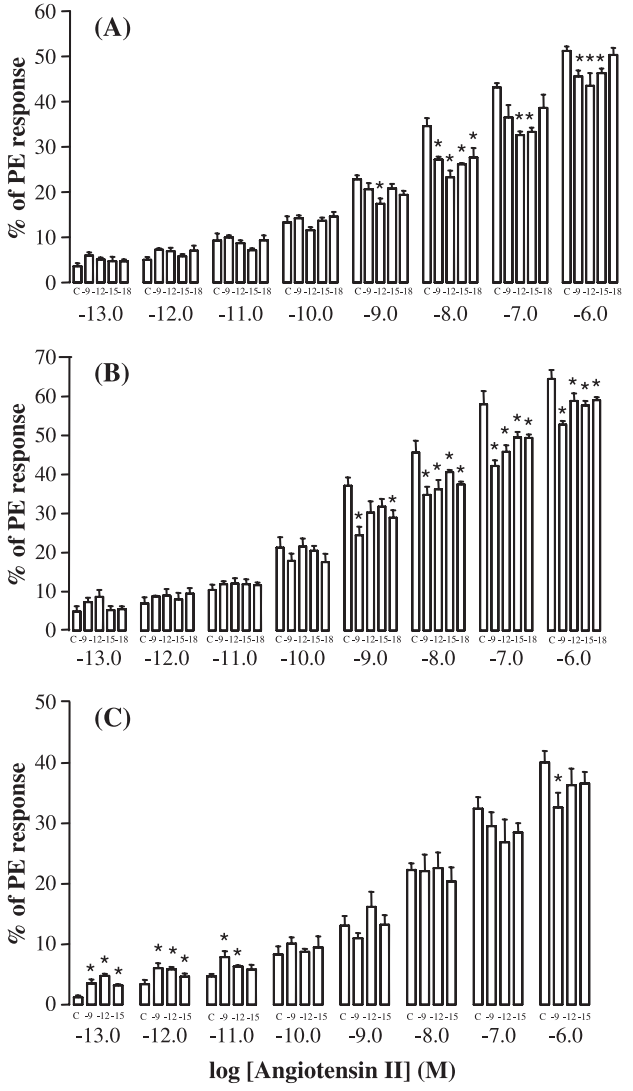


Fig. 2. Effects of DAA-I on the angiotensin II-induced pressure response in the renal vasculature of WKY (A), SHR (B) and STZ-induced diabetic (C) rats. Alphabet and numbers immediately below histogram: C, control (not treated), −9, pretreated with $10^{-9}$ M DAA-I; −12, pretreated with $10^{-12}$ M DAA-I; −15, pretreated with $10^{-15}$ M DAA-I; −18, pretreated with $10^{-18}$ M DAA-I. Each histogram and bar represents the mean ± SEM of 5–7 separate preparations. *Indicates significant difference from the control.

were also smaller, and the threshold dose was $10^{-10}$ M for WKY and STZ-induced diabetic rats, and $10^{-9}$ M for SHR. Fig. 4 shows the dose–response of mesenteric perfusion pressure to angiotensin II in WKY, SHR and STZ-induced diabetic rats. The responses in SHR were significantly smaller than those in WKY. There were no significant differences in angiotensin II contractile responses between WKY and STZ-induced diabetic rats. DAA-I ($10^{-18}$–$10^{-9}$ M) attenuated the angiotensin II response in WKY and SHR (Fig. 5). Similar to the renal vasculature, attenuation by DAA-I was also not seen with the higher concentrations of angiotensin II in the STZ-induced diabetic mesenteric vasculature (Fig. 5). The action of DAA-I on angiotensin

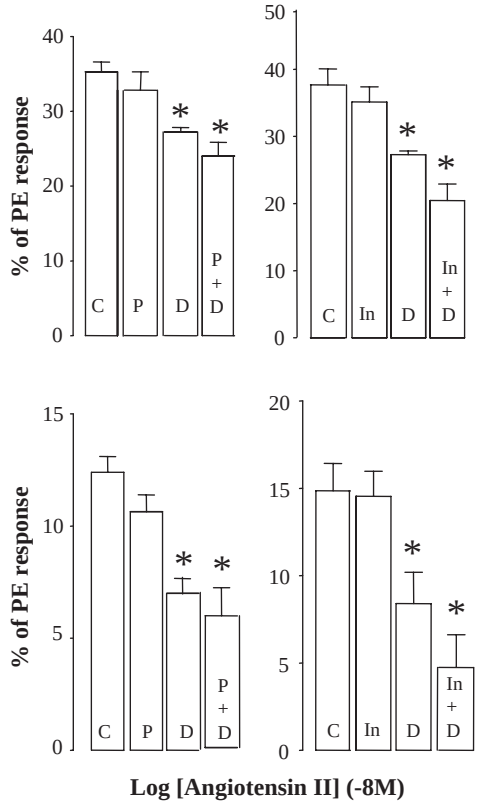


Fig. 3. Effects of PD 123319 (left histogram) and indomethacin (right histogram) on the inhibitory actions of DAA-I on the angiotensin II-induced pressure response in the renal vasculature (upper histograms) and mesenteric vasculature (lower histograms) of the WKY. Alphabets inside histograms: C, control (not treated); P, pretreated with $10^{-5}$ M PD 123319; D, pretreated with $10^{-9}$ M DAA-I; P+D, pretreated with PD 123319 and DAA-I; In, pretreated with $10^{-7}$ M indomethacin; In+D, pretreated with indomethacin and DAA-I. Each histogram and bar represents the mean ± SEM of 5–7 separate preparations. *Indicates significant difference from the control.

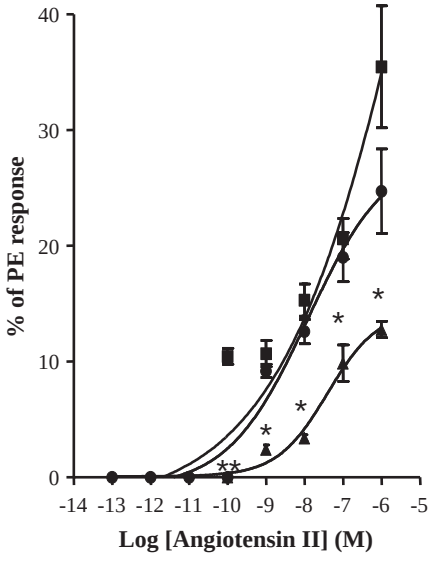


Fig. 4. Angiotensin II-induced pressure response in the mesenteric arterial bed of WKY (■), SHR (▲) and STZ-induced diabetic (●) rats. Each point is the mean ± SEM of 5–6 animals. *Indicates significant difference from WKY.

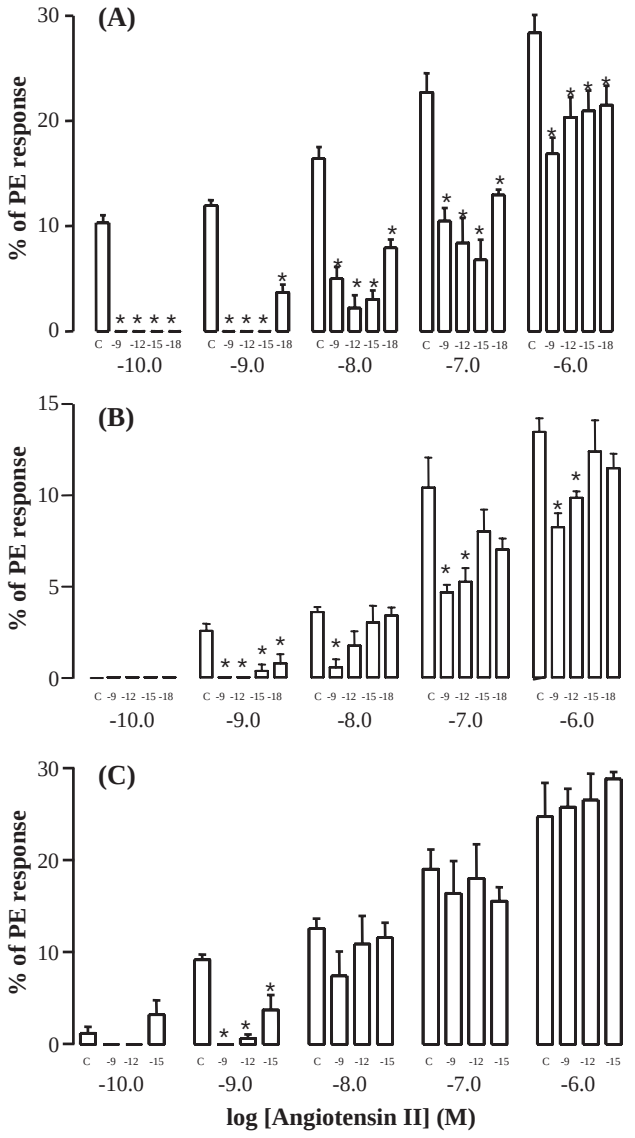


Fig. 5. Effects of DAA-I on the angiotensin II-induced pressure response in the mesenteric vascular bed of WKY (A), SHR (B) and STZ-induced diabetic (C) rats. Alphabet and numbers immediately below histogram: C, control (not treated), −9, pretreated with $10^{-9}$ M DAA-I; −12, pretreated with $10^{-12}$ M DAA-I; −15, pretreated with $10^{-15}$ M DAA-I; −18, pretreated with $10^{-18}$ M DAA-I. Each histogram and bar represents the mean ± SEM of 5–7 separate preparations. *Indicates significant difference from the control.

II-induced vasoconstriction was not affected by PD123319 and indomethacin in WKY (Fig. 3, lower set of histograms) and SHR (data not shown).

## 4. Discussion

### 4.1. Renal vasculature

Angiotensin II produced a greater pressor response in the SHR than WKY. A similar observation has also been reported by earlier investigators [10–12]. The mechanisms

responsible for increased vascular resistance have been the subject of intense investigation. Studies by Makarios et al. [13] and Haddad and Garcia [14] have demonstrated that the hyperresponsiveness observed in SHR correlates with increased angiotensin II receptor density. Various other mechanisms such as reduced offsetting activity of vasodilator prostaglandins [15], greater negative influence on phosphodiesterase induced increase in cAMP [16], defective interaction between receptor and G-protein activation [17] by angiotensin II have been alluded to cause hyperresponsiveness to angiotensin II in the SHR.

In contrast to SHR, angiotensin II pressor action was reduced in STZ-induced diabetic rats. This is in line with earlier work done by Sarubbi et al. [18]. They have demonstrated that angiotensin II-induced vasoconstriction was significantly impaired at 2 and 8–12 weeks diabetes. In addition to animal studies, attenuated renal and systemic responsiveness to angiotensin II has been shown in type 1 diabetic patient [19]. The reduced angiotensin II reactivity in diabetes has been associated with a downregulation of glomerular angiotensin II receptors [20,21]. Study by Sharma et al. [22], however, did not find a downregulation in angiotensin II receptor level but demonstrated that impaired angiotensin II response in diabetes is caused by decreased expression of the type 1 inositol 1,4,5-triphosphate ($InsP_3$) isoform receptor. Increased basal nitric oxide (NO) production in diabetes has also been reported [23] and has been suggested as a cause of reduced angiotensin II pressor response in diabetes.

DAA-I attenuated angiotensin II pressor action in both WKY and SHR. The attenuation was observed at the higher concentration range of angiotensin II ($10^{-9}$–$10^{-6}$ M). Similar attenuation has also been reported for the pressor action of angiotensin III [5]. However in the present study, the effective dose of DAA-I was a thousand-fold lower ($10^{-18}$ M) and the effect was seen in both the WKY and SHR. At this atto concentration, the concentration of DAA-I is below the reported circulation (pico molar) level [24]. The renal vasculature is an ex vivo preparation devoid of circulating DAA-I and re-introduction of DAA-I provides a means for measuring the effective physiological concentration of the peptide and possible roles it exerts in normal and pathological conditions. The present findings may indicate that, at circulating level, DAA-I attenuates pressor action to angiotensin II only when the local concentration of the octapeptide rises above a certain level i.e. nano molar concentration. In this way, DAA-I regulates the action of angiotensin II and prevent the latter from exerting excessive and damaging pressor effect. However, the attenuation in the SHR though equally significant was not sufficient to match the absolute value seen in the WKY indicating that the circulating level of DAA-I was not able to ameliorate the hypertensive malady. Effective at atto concentration, DAA-I is indeed the most specific angiotensin peptide that is known to attenuate the pressor action of angiotensin II.

A different profile of DAA-I action was seen in the renal vasculature of STZ-induced diabetic rats. Unlike in the WKY and SHR, DAA-I had no effect on the higher pressor concentrations of angiotensin II. Instead, DAA-I potentiated the pressor action of the lower concentrations ($10^{-13}$–$10^{-11}$ M) of the octapeptide. Although the exact mechanism for this reversal is not known, changes in levels of cellular diacylglycerol (DAG) and isoforms of protein kinase C (PKC) following induction of diabetes [25–27] could be contributory factors, as the contractile actions of different agonists are mediated by different isoforms of PKC [28,29], which are in turn regulated by different PKC-interacting proteins [30]. Whatever the cellular mechanisms responsible, it is tempting to speculate that DAA-I normalizes the STZ-induced lower pressor response to angiotensin II to near normal and, in this way, maintains the physiological vascular tone in the diabetic renal vasculature. The need to exert negative control on the higher concentrations of angiotensin II may not be critical in the diabetic renal vasculature as the response to the octapeptide is below normal.

PD 123319 and indomethacin were without effect on the action of DAA-I in WKY and SHR, indicating that the $AT_2$ angiotensin receptors and prostaglandins were not involved in its action. This is in agreement with our earlier finding with angiotensin III [5]. These findings suggest that DAA-I probably acts via the angiotensin $AT_1$ receptor as has been shown in other preparations.

### 4.2. Mesenteric vasculature

Several studies have documented enhanced contractile response as well as no change in SHR compared to WKY [31–34]. This equivocal finding could be due to the use of the different sub-branches and sizes of the mesenteric vessel. In the present study, pressor response of the mesenteric vasculature to angiotensin II has been found to be significantly smaller in SHR than WKY. Noting the in situ preparation includes the venules, the decrease in response of the venular circulation could have contributed to the observation in the SHR as angiotensin II has been shown to be a stronger venoconstrictor than arterioconstrictor [35].

The absence of differences in response to angiotensin II between the WKY and diabetic mesenteric vasculature is a contrast to that observed for the SHR. Although the vascular renin–angiotensin system (RAS) has been known to be activated in diabetes and is a cause of vascular remodeling, sensitivity of mesenteric vessels to vasoconstrictors such as noradrenaline and serotonin remains controversial [36]. With 4 weeks of STZ treatment, the vasoconstrictor responses induced by noradrenaline, endothelin-I and angiotensin II were significantly increased [37], but longer treatment with STZ has been shown to increase blood flow in the superior mesenteric artery [38]. It is likely that the observation of no significant difference

from WKY for the STZ-treated rats could be temporal dependent.

There are subtle differences between the effects of DAA-I on the renal and mesenteric vascular bed. In the latter, DAA-I was more effective in attenuating the pressor action of angiotensin II in the WKY and less so in the diabetic animals. It is difficult to speculate on the underling mechanisms for the differences. One possibility could be the ongoing remodeling caused by an activated RAS and the different roles the blood vessels play in the kidneys and intestines. In terms of the effects of DAA-I, PD123319 and indomethacin on angiotensin II contraction, similar patterns to those of the renal vasculature were observed. This suggests that DAA-I action is via the angiotensin $AT_1$ receptors and is unlikely to be mediate by prostaglandins.

The present findings reiterate a regulatory role for DAA-I in vascular bed of the kidney and mesentery. By being active at circulating level, DAA-I subserves a physiological role. This function appears to be present in animals with diseased state of hypertension and diabetes. The vascular RAS is activated and angiotensin II contributes to remodeling in vascular tissues of hypertensive and diabetic rats. It is likely that DAA-I functions are modified to accommodate the ongoing vascular remodeling.

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