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Involvement of AT_1 angiotensin receptors in the vasomodulatory effect of des-aspartate-angiotensin I in the rat renal vasculature

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ABSTRACT

Angiotensin II is known to act primarily on the angiotensin AT_1 receptors to mediate its physiological and pathological actions. Des-aspartate-angiotensin I (DAA-I) is a bioactive angiotensin peptide and have been shown to have contrasting vascular actions to angiotensin II. Previous work in this laboratory has demonstrated an overwhelming vasodepressor modulation on angiotensin II-induced vasoconstriction by DAA-I. The present study investigated the involvement of the AT_1 receptor in the actions of DAA-I on angiotensin II-induced vascular actions in the renal vasculature of normotensive Wistar–Kyoto rats (WKY), spontaneously hypertensive rats (SHR) and streptozotocin (STZ)-induced diabetic rats. The findings revealed that the angiotensin receptor in rat kidney homogenate was mainly of the AT_1 subtype. The AT_1 receptor density was significantly higher in the kidney of the SHR. The increase in AT_1 receptor density was also confirmed by RT-PCR and Western blot analysis. In contrast, AT_1 receptor density was significantly reduced in the kidney of the streptozotocin-induced diabetic rat. Perfusion with 10^{-9} M DAA-I reduced the AT_1 receptor density in the kidneys of WKY and SHR rats suggesting that the previously observed vasodepressor modulation of the nonapeptide could be due to down-regulation or internalization of AT_1 receptors. RT-PCR and Western blot analysis showed no significant changes in the content of AT_1 receptor mRNA and protein. This supports the suggestion that DAA-I causes internalization of AT_1 receptors. In the streptozotocin-induced diabetic rat, no significant changes in renal AT_1 receptor density and expression were seen when its kidneys were similarly perfused with DAA-I.

1. Introduction

The RAS plays an important role in the regulation of blood pressure and fluid homeostatis and exerts this effect through the production of angiotensin II. Angiotensin II increases the renal vascular resistance by constricting afferent and efferent arterioles including interlobular arteries [6]. The effects of angiotensin II are mediated through high-affinity membrane bound receptors, classified as AT_1 and AT_2 receptor subtypes. Most of the known effects of angiotensin II have been attributed to the AT_1 receptor. In the kidney, AT_1 receptors are present in the preglomerular arteries and arterioles,

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glomeruli, vasa rectae and proximal convoluted tubules [8]. In addition, angiotensin II plays a key role in the initiation and amplification of pathobiological events that underlie several vascular diseases. The findings of other bioactive angiotensin peptides, i.e., DAA-I and angiotensin-(1–7) with contrasting vascular actions to angiotensin II has generated interest in the pleiotropism of the AT_1 receptor and other receptor subtypes that mediate their actions.

Angiotensin peptides may bind to multiple angiotensin receptor subtypes that often exhibit different and sometimes opposing actions. For example, angiotensin II has been shown to bind to both AT_1 and AT_2 receptor subtype which are coupled to specific effector systems that elicit different biological responses. DAA-I, has been shown to have cardioprotective action by modulating the expression the AT_1 and AT_2 receptor differentially to attenuate cardiac hypertrophy in rats with abdominal aortic coarctation [9]. This nonapeptide has also been demonstrated to prevent myocardial infact expansion through suppression of inflammatory cytokines and immune cell infiltration [1]. Studies with rabbit [31] and rat aortic rings [19], and rabbit pulmonary artery [30] demonstrated that DAA-I acts as an agonist on losartan-sensitive angiotensin receptor.

In a recent study, we observed increased contractile responses to angiotensin II in the kidney of SHR rats and decreased responses in the kidney of STZ-induced diabetic rat, and suggested that the differences could be due to changes in the angiotensin receptor density [13]. We also demonstrated that DAA-I had a vasodepressing action on the contractile action of angiotensin II in the WKY and SHR renal vasculature and ruled out the involvement of AT_2 receptor in mediating the observed responses [13]. As an extension of the earlier investigation, the present study investigated the effect of DAA-I on the profile of renal AT_1 receptors in the WKY, SHR and STZ-induced diabetic rats.

2. Methodology

2.1. Animals

Male Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) weighing 250–300 g (11–12 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.

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. Prior to injection, animal weights and blood glucose levels were recorded. Body weights and blood glucose were taken every 2 weeks until the 8th week. Animals were considered diabetic if their blood glucose concentration was >17 mM.

In our earlier part of the work [13], DAA-I was perfused through the kidney and the possibility was considered that this peptide acted on the angiotensin receptors present on the membrane of any part of the whole kidney. Thus, we used the whole kidney in the current study.

2.3. Receptor binding assay

The right kidney from WKY, SHR and STZ-induced diabetic rats were isolated and perfused with Krebs solution containing only captopril (30 μM) (control) or captopril and DAA-I (10^{-9} M to 10^{-15} M) for 90 min prior to its use for the preparation of kidney membranes. At these concentrations, DAA-I was effective in attenuating the contraction of kidney vasculature induced by angiotensin II [13]. Kidney membrane was prepared as described by Brown et al. [5] with slight modification. Briefly, each kidney was minced into small pieces and suspended 1:10 (w/v) in Tris–HCl buffer (50 mM, pH 7.4), homogenized and the homogenate was centrifuged at 30,000 × *g* at 4 °C for 40 min. The resultant pellet was resuspended and recentrifuged twice under the same conditions. The final pellet was resuspended in Tris–HCl (20 mM, pH 7.4), containing NaCl (135 mM), KCl (10 mM), glucose (5 mM) and $MgCl_2$ (10 mM). Protein concentration was determined using the Biuret assay. The prepared membranes were kept as aliquots at −80 °C for not longer than 3 months.

The displacement radioligand binding experiment was carried out using the homologous competitive binding method described by Brown et al. [5] with slight modification. Each aliquot of membrane suspension equivalent to 40 μg of protein was added to a solution of ^{125}I-Sar^1–Ile^8-Ang II (2.3–2.5× 10^4 c.p.m.) together with a different (increasing) concentration of non-radioactive Sar^1–Ile^8-Ang II (0.0001–300 nM) in 300 μl of 50 mM Tris–HCl buffer (pH 7.4) containing 0.3 mM phenylmethylsulphonyl fluoride (PMSF), 0.1 mM bacitracin and 0.2% bovine serum albumin (BSA). The assay mixture was incubated at 22 °C for 2 h with continuous shaking. The bound and unbound ligand was separated by addition of cold bovine gamma globulin and polyethylene glycol and centrifugation at 5000 × *g* and 4 °C for 40 min. The radioactivity trapped in the pellets was counted using a gamma counter (Wizard Gamma Counter, PerkinElmer). Efficiency of the counter was 95%. Receptor density was calculated using Graph Pad Prism software. The assay was conducted in duplicate and repeated three to four times per group.

2.4. Semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR)

The right kidneys from WKY, SHR and STZ-induced diabetic rats were isolated and perfused with Krebs and captopril (30 μM) (control) or with DAA-I (10^{-9} M to 10^{-15} M) for 90 min. The kidneys were frozen in liquid nitrogen and kept at −80 °C for further RNA isolation. Total RNA was isolated from the kidneys using Reagent® system according to the manufacturer's instructions. The tissue (100 mg) was homogenized in the provided denaturing solution. Two molar sodium acetate (pH 4) was then added, followed by phenol:chloroform:isamyl alcohol. This mixture was then kept in ice for 15 min before centrifuging at 10,000 × *g* for 20 min at 4 °C. After centrifugation, the top aqueous phase containing the RNA was carefully removed and equal volume of isopropanol was added to the aqueous phase and incubated at −20 °C to allow precipitation of RNA. The RNA was pelleted by ultracentrifugation and was washed in ice-cold 75% ethanol, re-pelleted and dissolved in nuclease-free water. The amount of RNA was quantified by

absorbance at 260 nm and stored at −20 °C for not more than 6 months.

cDNA was synthesized using Revertaid™ H Minus First Strand cDNA Synthesis Kit, according to the manufacturer's protocols. One microlitre of 0.5 μg/μl oligo $(dT)_{18}$ primer was added to 1 μg of total RNA. The final total volume was 12 μl. The mixture was mixed gently, spun down and incubated at 70 °C for 5 min. Then, 4 μl of 5× reaction buffer, 1 μl of RiboLock™ Ribonuclease inhibitor (20 U/μl), and 2 μl of 10 mM dNTP mix were added to the mixture and spun down. The mixture was incubated at 37 °C for 5 min. Lastly, 1 μl of RevertAidTM H Minus M-MuLV reverse trancriptase was added and the mixture incubated at 42 °C for 60 min. The reaction was stopped by heating at 70 °C for 10 min. The synthesized cDNA was kept at −20 °C.

One microlitre of synthesized cDNA was added to the reaction mixture containing reaction buffer, 0.2 μM of each of the two primers, 2 mM $MgCl_2$, 0.2 mM of each dNTP and 1.25 U of Taq polymerase. The final volume was 25 μl. The mixture was spun down and placed in the thermal cycler (PTC-200, MJ Research). After denaturation at 95 °C for 15 min, amplification cycles of 94 °C, 60 °C and 72 °C for 30 s were performed. Final elongation was carried out at 72 °C for 2 min. GADPH was chosen as the internal standard. The PCR products were electrophoresed using 1.5% agarose gel containing ethidium bromide 0.5 μg/ml. The gel was subjected to ultraviolet light, photographed (Kodak Digital Science) and analyzed.

	Primer (5′–3′)	PCR cycle	Size (bp)
AT_1	Fwd: ATCTCGGCTTGGCTGACTTA; Rvs: GACTTCATTGGGTGGACGA	35	200
GADPH	Fwd: GGTGCTGAGTATGTCGTG; Rvs: TTCAGCTCTGGGATGACC	35	400

List of primers used in semi-quantitative RT-PCR.

2.5. Western blotting

The right kidneys from WKY, SHR and STZ-induced diabetic rats were isolated and perfused with Krebs and captopril (30 μM) (control) or with DAA-I (10^{-9} M to 10^{-15} M) for 90 min prior to the homogenate preparation. Tissue homogenate was prepared according to the method described by Zelezna et al. [36] with slight modification. Kidneys were homogenized in phosphate-buffered saline (PBS) containing protease inhibitors (30 μg PMSF, 300 μg EDTA, and 0.5 μg bacitracin/ml). Homogenates were centrifuged at 20,000 × *g* for 10 min, and pellets were re-suspended in PBS with protease inhibitors and rinsed twice by centrifugation. Final pellet were re-suspended and protein concentration were determined using Biuret assay. The supernatant was heated to 95 °C for 5 min in the presence of 2× treatment buffer (0.125 M Tris–HCl, 4% SDS, 20% glycerol, 0.2 M DTT, 0.02% Bromophenol blue, pH 6.8).

The protein (80 μg) was resolved on a 12.5% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane. The blotted PVDF membrane was saturated with 3% gelatin in PBS, pH 7.4 containing 0.1% Tween-20 for 1 h at room temperature to block non-specific binding. The membrane was then sequentially incubated with AT_1 receptor antibody (Santa Cruz Biotechnology, 1:1100) overnight at 4 °C, washed with Tris-buffered saline containing 0.1% Tween-20 and followed by incubation with goat anti-rabbit IgG–HRP conjugated (1:1300) for 2 h at room temperature. After thorough washing, the blot was developed by incubating with HRP-complex until the bands have adequate intensity. The blot was dried, scanned and analyzed.

2.6. Statistical analysis

Data were expressed as mean ± S.E.M. One-way ANOVA and Dunnett post hoc test were employed to determine the significance differences, with an accepted level of significance of $p < 0.05$. B_{max} and K_d value for receptor binding were determined using the homologous competitive equation (GraphPad Prism). The band intensities for RT-PCR and Western blot were measured using a software package (Scion image). The signals for RT-PCR were expressed relative to the intensity of the GADPH amplicon for each co-amplified sample.

3. Results

3.1. Receptor binding assay

The binding of ^{125}I-Sar^1–Ile^8-Ang II to the rat kidney membranes was displaced by losartan and Sar^1–Ile^8-Ang II in a concentration-dependent manner with an almost 100% displacement at 10^{-7} M concentration (Fig. 1). On the other hand, PD 123319, an AT_2 receptor antagonist, displaced less than 30% of the labelled ligand at 10^{-6} M. This shows that the kidney membranes contain mainly AT_1 receptors and a very small proportion of AT_2 receptors. Renal AT_1 receptor density was significantly higher in SHR (739.6 ± 44.0 fmol/mg) and lower in STZ-induced diabetic (271.5 ± 34.94 fmol/mg) compared to WKY (430.0 ± 8.12 fmol/mg) (Fig. 2). AT_1 binding affinity (K_d) between WKY, SHR and STZ-induced diabetic rats were not significantly different (Table 1).

In functional studies reported earlier [13], the involvement of AT_2 receptor in the vasomodulatory role of DAA-I was excluded. Thus, subsequent experiments were focused on

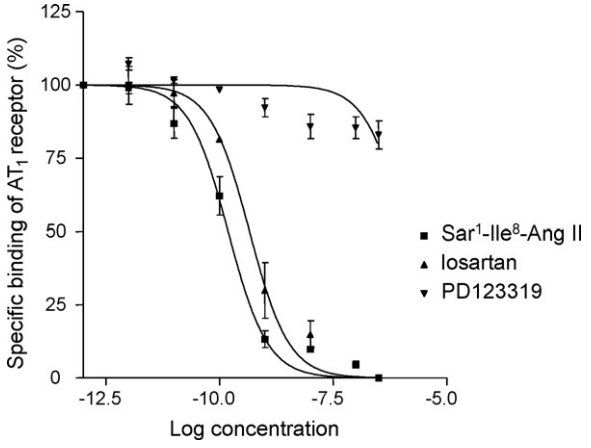

Fig. 1 – Displacement of ^{125}I-Sar^1–Ile^8-Ang II by losartan (■), Sar–Ile-Ang II (▲) and PD123319 (▼) in kidney membrane preparations of untreated rats (control).

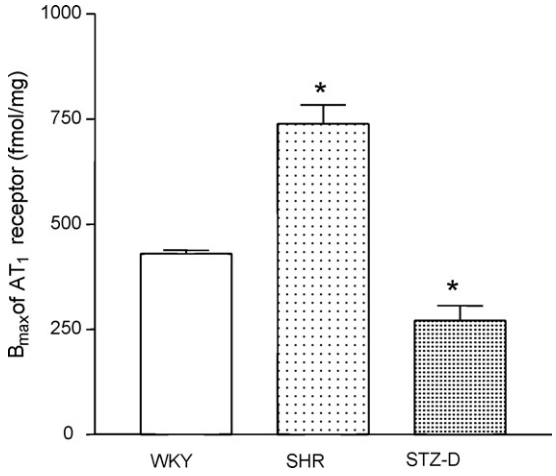

Fig. 2 – Maximal binding capacity of [^{125}I]-Sar^1–Ile^8-Ang II (B_{max}) in kidney membranes of WKY-, SHR- and STZ-treated rats. Bars represent the mean value ± S.E.M. of at least four experiments performed in duplicates. *Indicates significant difference from WKY ($p < 0.05$).

evaluating the involvement of the AT_1 receptors. Pretreatment with 10^{-9} M DAA-I caused a significant reduction in renal AT_1 receptor density in WKY and SHR rats (Fig. 3). DAA-I does not affect the receptor density of AT_1 receptor in the STZ-induced diabetic rat kidney (Fig. 3). DAA-I did not affect the K_d of AT_1 receptor for the iodinated ligand in renal membranes of the three different groups of rats (Table 1).

3.2. RT-PCR

The mRNA expression of AT_1 receptors in the kidneys of WKY, SHR and STZ-induced diabetic rats is shown in Fig. 4. GADPH was used as an internal standard. The densitometic values of the AT_1 receptor were normalized against the corresponding GADPH values. Compared to the WKY, there was an increase in renal AT_1 receptor mRNA in the SHR (1.051 ± 0.066 vs. 0.794 ± 0.067, SHR and WKY, respectively) and a decrease in the STZ-induced diabetic rats (0.592 ± 0.052 vs. 0.794 ± 0.067,

Table 1 – Homologous competitive binding parameters

Groups	K_d value (nM)
WKY	1.187 ± 0.068
DAA-I (10^{-9} M)	0.889 ± 0.092
DAA-I (10^{-12} M)	1.012 ± 0.152
DAA-I (10^{-15} M)	1.465 ± 0.182
SHR	1.440 ± 0.125
DAA-I (10^{-9} M)	1.284 ± 0.140
DAA-I (10^{-12} M)	1.680 ± 0.136
DAA-I (10^{-15} M)	1.638 ± 0.143
STZ-induced diabetic	0.784 ± 0.229
DAA-I (10^{-9} M)	0.931 ± 0.194
DAA-I (10^{-12} M)	0.864 ± 0.196
DAA-I (10^{-15} M)	1.315 ± 0.277

Values are expressed as mean ± S.E.M. of 3–4 binding experiments. B_{max} values are in fmol/mg protein; K_d values are in nM.

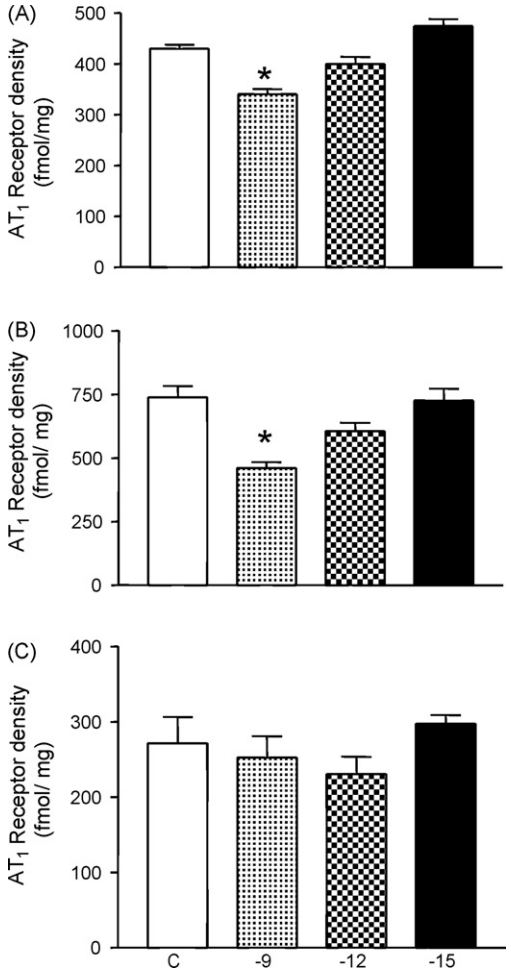

Fig. 3 – AT_1 receptor density in kidney of WKY (A), SHR (B) and STZ-induced diabetic rats (C) pre-treated with DAA-I, respectively. The bars represent the mean value ± S.E.M. of four to five experiments. Empty bar represent control groups and the shaded bars represents groups treated with graded concentrations (10^{-9} M, 10^{-12} M and 10^{-15} M) of DAA-I. *Indicates significant difference from respective control group ($p < 0.05$).

STZ-induced diabetic and WKY, respectively). The effects of DAA-I (10^{-9} M to 10^{-15} M) on the renal AT_1 mRNA expression are shown in Fig. 5. DAA-I did not significantly alter the level of AT_1 mRNA in the kidney of WKY, SHR and STZ-induced diabetic rats.

3.3. Western blotting

The AT_1 receptor antibody has a molecular weight of 43 kDa. The expression of AT_1 receptor protein in the kidney of WKY, SHR and STZ-induced diabetic rats is shown in Fig. 6. Compared to the WKY, there was an increase in AT_1 receptor protein in the SHR and a decrease in the STZ-induced diabetic rats. The effects of DAA-I (10^{-9} M to 10^{-15} M) on the expression of AT_1 receptor protein in the kidney of WKY, SHR and STZ-induced diabetic rats are shown in Fig. 7. Ninety minutes of perfusion with DAA-I perfusion did not affect the AT_1 receptor

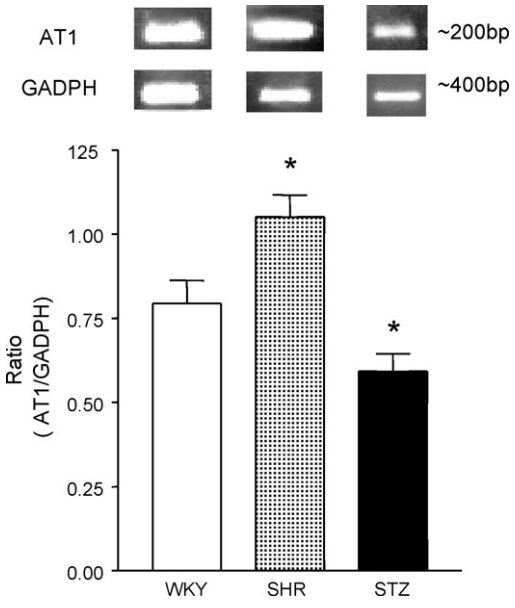

Fig. 4 – AT_1 mRNA expression level in the kidney of WKY, SHR and STZ-induced diabetic rats: (A) agarose gel electropherogram of AT_1 and GAPDH cDNA; (B) quantification of AT_1 mRNA (normalized against GADPH mRNA). The bars represent the mean ± S.E.M. of three to four rats. *Indicates significant difference from the WKY value ($p < 0.05$).

expression in the kidney for WKY, SHR and STZ-induced diabetic rats.

4. Discussion

4.1. *Profile of renal angiotensin receptor*

In the present study, homologous competitive binding assay performed with specific AT_1 receptor antagonist, losartan and the AT_2 receptor antagonist, PD123319 revealed that the AT_1 receptor was the main angiotensin receptor subtype present in the kidney membrane. Several binding studies have also shown exclusive presence of AT_1 receptors in the renal glomeruli, tubular and outer medullary membranes of Sprague–Dawley rats [14,15]. Autoradiographic techniques have also localized a predominant expression of AT_1 receptor in the adult rat kidneys [11]. RT-PCR and Western blot analysis have additionally showed a predominance of AT_1 receptor localization in the rat kidneys [21,24,36].

The binding study also shows that AT_1 receptor density was significantly higher in the SHR compared to the WKY rats. The finding supports those of earlier investigators [5,16,17]. These authors have correlated the increase in AT_1 receptor to the enhancement of angiotensin II-induced renal contractility. The receptor affinity (K_d) for the AT_1 receptor remained unchanged in the WKY and SHR animals. The lack of alteration in AT_1 receptor affinity in hypertensive animals have also been reported by other authors [7,17]. RT-PCR and Western blot analysis are in agreement with the result from the receptor binding assay. Increases in AT_1 receptor mRNA have been observed in the brain [25,26], aorta [23] and heart of the SHR [20], and glomeruli of stroke-prone SHR [22].

In contrast, the AT_1 receptor density in the kidney of STZ-treated rats was significantly lower when compared to the density present in the WKY. This reduction mirrors the reduced responsiveness to angiotensin II observed in the perfused kidney in our previous work [13]. Reduction in AT_1 receptor density was similarly demonstrated in whole kidney homogenates [5] and glomerulus [2,3,35] of STZ-induced diabetic rats. No significant differences in AT_1 receptor affinity was observed between WKY and STZ-treated rats. Similar observations were also documented by other researchers [2,3]. RT-PCR results and Western blot analysis are in agreement the binding data and with our earlier work where a lower AT_1 mRNA level was observed in the kidney of STZ-induced diabetic rats. Reduced renal expression of the AT_1 receptor has been reported in renal proximal tubules [10] of diabetic SHR animals [4] and diabetic patients [33]. However, a study by Sharma et al. [29] found that kidneys from diabetic rats exhibit decreased expression of the type I inositol triphosphatase receptor ($InsP_3R$) isoform and speculated that the impaired angiotensin II-mediated vasoconstriction in diabetic animals might be caused by down-regulation of $InsP_3Rs$. In a later study, this author showed that AT_1 receptor mRNA remains unchanged in aorta from STZ-induced diabetic rats [28]. In contrast, Wehbi et al. [34] showed no significant change of the AT_1 receptor but reduced AT_2 receptor expression in the kidney of diabetic rats. The different profiles of the angiotensin AT_1 receptors seen in diabetic animals reflect the complex metabolic disorder of the disease. In addition, such differences could also be due to the different animal strains and the period used to induce diabetes.

4.2. *Effect of DAA-I on renal AT_1 receptor*

Perfusion of the kidney with 10^{-9} M DAA-I significantly reduces AT_1 receptor density in both the WKY and SHR kidney membranes. This suggests that the vasodepressor actions of DAA-I may in part be due to a down-regulation of the AT_1 receptor in the kidney of WKY and SHR rats. Changes in receptor protein synthesis have been identified as late signalling events, and take hours to occur [32]. As DAA-I was perfused into the kidneys for more than 1 h; it is possible that an adequate concentration of the nonapeptide could down-regulate the AT_1 receptors. However, the RT-PCR and Western blot analysis did not show down-regulation of AT_1 receptors as there were no changes in gene and protein expression with pre-treatment of 10^{-9} M DAA-I concentration. It is possible that the reduced AT_1 receptor density observed at 10^{-9} M DAA-I may be due to receptor internalization.

Receptor internalization leads to a reduction in the number of receptors present on the membrane surface [27]. This may explain the findings from our binding assay as DAA-I may have repositioned the AT_1 receptor into the membrane and lysis of the membrane for Western blot may have exposed the internalized receptors. Agonist-induced receptor internalization especially of AT_1 receptor has been reported [12,18]. Studies on endogenous AT_2 receptors have demonstrated that it is an internalization-deficient receptor [18]. Internalization

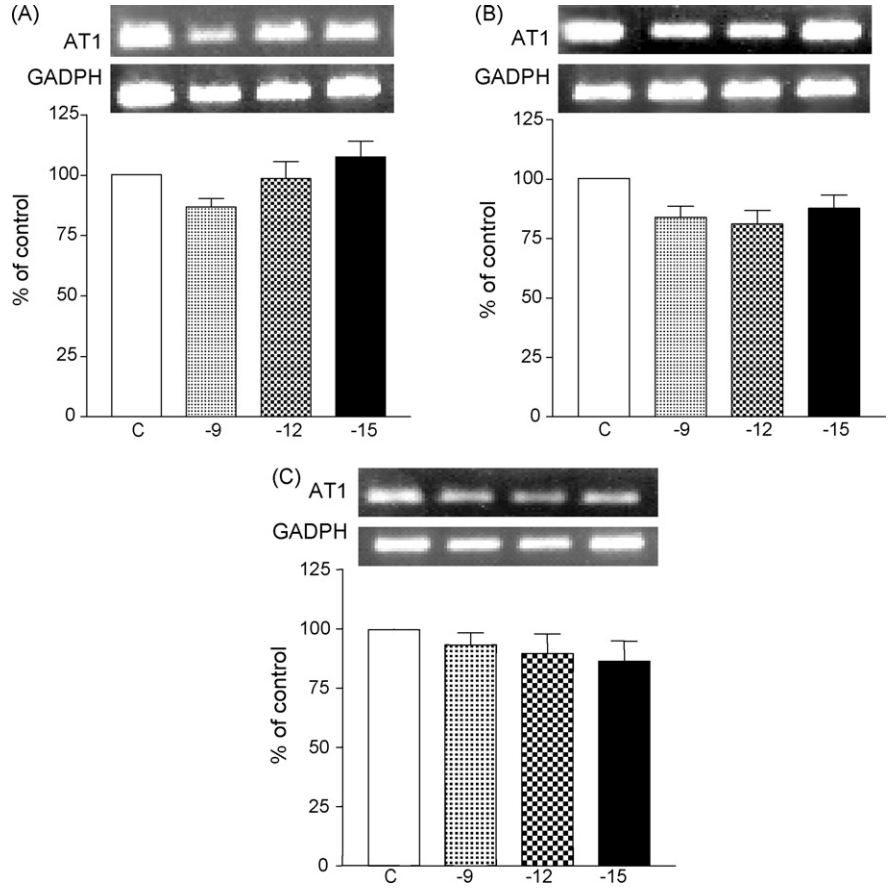

Fig. 5 – Effect of DAA-I on the expression of AT_1 mRNA in perfused kidney of WKY (A), SHR (B) and STZ-induced diabetic rats (C). Above each bar chart is the respective agarose gel electropherogram of AT_1 and GADPH cDNA. The bars represent the mean value ± S.E.M. of four to five experiments. Empty bars represent control groups and the shaded bars represent groups treated with graded concentrations (10^{-9} M, 10^{-12} M and 10^{-15} M) of DAA-I. Each bar is expressed as a percentage of the control. *Indicates significant difference from respective control group ($p < 0.05$).

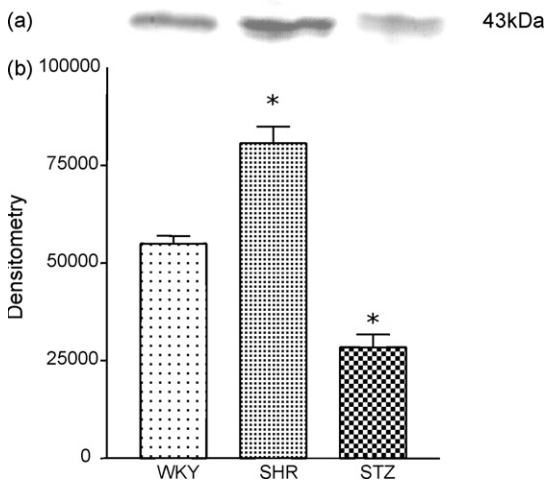

Fig. 6 – AT_1 receptor expression levels in the kidney of WKY, SHR and STZ-induced diabetic rats: (A) Western blot of AT_1 receptor; (B) densitometric quantification of AT_1 receptor level. The bars represent the mean ± S.E.M. of three rats. *Indicates significant difference from the WKY value ($p < 0.05$).

of the AT_1 receptor may explain the vasodepressor actions observed earlier in the isolated perfused kidneys of WKY and SHR animals.

Pre-treatment with 10^{-12} M and 10^{-15} M DAA-I did not affect the AT_1 receptor density although reduction in contractility was observed in the ex vivo renal study [13]. A slight reduction in receptor density was seen in 10^{-12} M pre-treated kidneys especially in the SHR. A similar pattern was also observed with RT-PCR and Western blot analysis. At these subphysiological concentrations, a small difference in receptor availability could still contribute to the observed attenuation of angiotensin II contraction. It is also possible that DAA-I binds to the AT_1 receptor and activate different second messenger pathways, which contributed to its vasodepressor actions. In such a scenario, internalization of the AT_1 receptors is a normal negative feedback mechanism occurring at concentrations above paraphysiological level and absent at lower concentrations.

Pre-treatment with DAA-I did not affect the renal AT_1 receptor density in STZ-treated kidney. This perhaps explains our earlier result, where DAA-I had no effect on angiotensin II-induced vasoconstriction in STZ-induced diabetic rat kidney [13]. The actual underlying mechanisms for this absence of

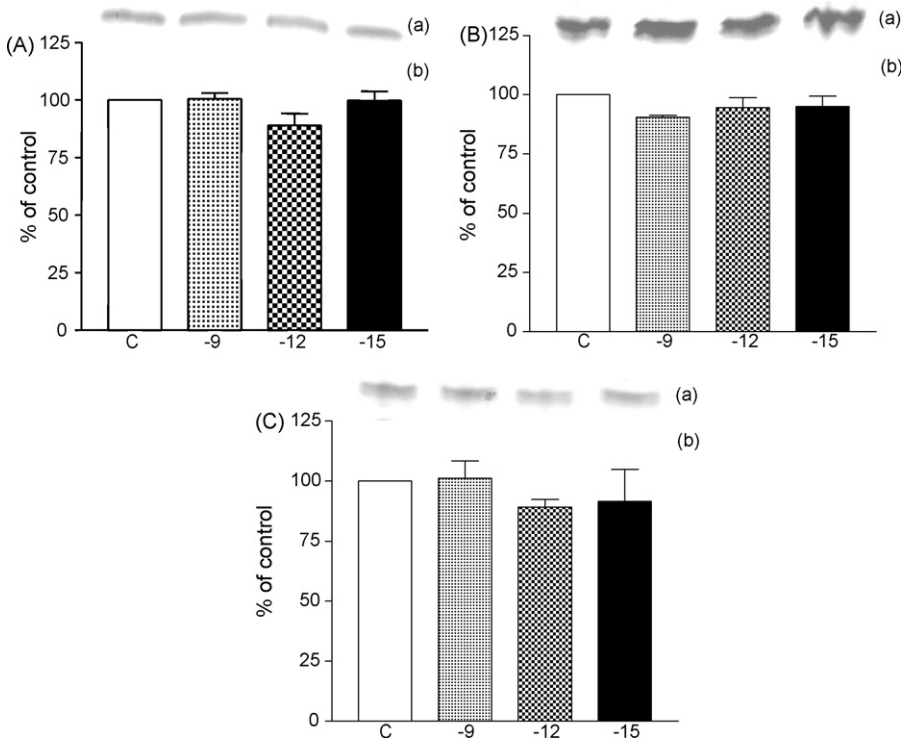

Fig. 7 – Effect of DAA-I on the expression of AT_1 receptor levels in perfused kidney of WKY (A), SHR (B) and STZ-induced diabetic rats (C). Above each bar is the respective blot for AT_1 receptor. Empty bars represent control groups and the shaded bars represent groups treated with graded concentrations (10^{-9} M, 10^{-12} M and 10^{-15} M) of DAA-I. Each bar represents the mean ± S.E.M. of three rats and expressed over its control. *Indicates significantly different from its control value ($p < 0.05$).

effects are not known. In agreement with the binding assay, no changes in AT_1 receptor gene and protein expression was seen in the STZ-induced diabetic rat kidney. As structural changes occur during the diabetes, it is possible that the binding site for DAA-I at AT_1 receptor may be altered, thus reducing the ability of DAA-I to bind to AT_1 receptor. A slightly lower AT_1 receptor affinity was detected in the STZ-induced diabetic untreated and DAA-I treated animals compared to the WKY. No significant difference in receptor affinity was observed between the DAA-I treated group with its own control further suggests that the DAA-I binds to other binding site than Ang II to modulate the AT_1 receptors.

In summary, the present finding demonstrated the accompanying changes in AT_1 receptor profile following effective vasomodulatory concentrations of DAA-I in the kidneys of WKY and SHR. The data support the contention that DAA-I acts via the AT_1 receptor in that it internalizes the receptors in these two animal strains and not in the STZ-induced diabetic rats where DAA-I has no vasodepressor action.

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