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The receptor subtype mediating the action of angiotensin II on intracellular sodium in rat proximal tubules

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1 An investigation was undertaken to explore the subtype of receptor involved in mediating the actions of angiotensin II on intracellular sodium content in suspensions of isolated proximal tubules of the rat.

2 Intracellular sodium content of the proximal tubules was measured with ^{23}Na n.m.r. spectroscopy and under these conditions basal sodium content of the tubular cells was 69.04 ± 1.73 nmol mg^{-1} dry weight and the ATP levels, at 8.3 ± 0.9 nmol ATP mg^{-1} protein, were consistent with active respiration by the tissue.

3 In the presence of 10^{-4} M PD123319, a selective non-peptide AT_2 receptor antagonist, intracellular sodium levels rose from steady state by 30% ($P<0.01$; $n=7$) within 10 min of exposure to angiotensin II 10^{-11} M. Over the subsequent 30 min steady state levels were re-established. Administration of angiotensin II 10^{-11} M, in the presence of the selective AT_1 receptor antagonist, losartan at either 10^{-6} M ($n=5$) or 10^{-4} M ($n=6$), was without effect on intracellular sodium levels, which were significantly different ($P<0.001$) from those observed when PD 123319 was present.

4 Angiotensin II 10^{-5} M, administered to the tubular suspension in the presence of 10^{-4} M PD123319, decreased ($P<0.01$, $n=6$) intracellular sodium content by 16% in the first 5 min, but in the following 25 min returned to steady state levels. However, in the presence of losartan 10^{-4} M, angiotensin II 10^{-5} M had no effect on intracellular sodium content which was markedly different ($P<0.001$) from that obtained in the presence of PD123319.

5 These findings show that at both the high and low concentrations of angiotensin II, its modulation of intracellular sodium levels within the proximal tubule cells is mediated via the activation of AT_1 receptors. The intracellular mechanism underlying this effect remain to be investigated.

Keywords: ^{23}Na n.m.r. spectroscopy; proximal tubules; angiotensin II receptor subtypes; losartan; PD123319; sodium transport

Introduction

Angiotensin II (AII) has been shown to have a powerful influence on sodium transport within the kidney through both direct and indirect mechanisms. Indirectly the peptide can decrease renal haemodynamics and consequently the sodium load delivered to the nephron, and hence excreted, while at the level of the nephron itself, AII can directly regulate epithelial cell sodium reabsorption. More specifically, AII has been demonstrated to have a potent biphasic effect on fluid and sodium transport at the proximal tubule (Harris & Young, 1977; Cogan, 1990). AII receptors have been isolated extensively along the brush border and basolateral membranes of the proximal tubule and the consensus is that AII utilizes multiple intracellular signalling pathways in the course of its action to control sodium transport (Liu & Cogan, 1989; Gesek & Schoolwerth, 1990; Chatsudthipong & Chan, 1991; Romero *et al*., 1991).

The presence of the different signalling mechanisms and second messengers associated with AII receptor activation suggests the possibility of receptor heterogeneity and with the advent of the selective non-peptide AII receptor antagonists, losartan (Chiu *et al*., 1989) and PD123177 and its analogues (Dudley *et al*., 1991), it has been possible to characterize and identify AII receptor subtypes. The AII receptor subtypes have now been classified by their affinity for their selective antagonists. The AT_1 receptors is sensitive to losartan and the receptor antagonized by PD123177 has been classed as an AT_2 type receptor.

The presence of AII receptor subtypes in the kidney has now been firmly established (Edwards *et al*., 1992; Sechi *et al*., 1992), but the functional roles of the AT_1 and AT_2 receptors still require clarification. Within the kidney and specifically the proximal tubule, where AII exerts a powerful influence on sodium handling, the functional characteristics of the AII receptor subtypes have attracted great interest. There have been consistent findings which indicate that the AT_1 receptor mediates the majority of haemodynamic responses to AII, although there is evidence of a functional role for the AT_2 receptor at the level of the kidney in respect of water handling (Keiser *et al*., 1992).

Studies have also been conducted on the role that the receptor subtypes play in mediating the direct action of AII on electrolyte and fluid transport at the proximal tubule. Cogan *et al*. (1991) demonstrated that PD123177 inhibited electrolyte transport and inhibited fluid and sodium reabsorption in the S1 segment of the proximal tubule of the rat kidney, and the magnitude of response was comparable to that seen with losartan in a previous study (Xie *et al*., 1990).

At present the available data suggest that most functional responses to angiotensin II at the level of the kidney appear to be mediated via AT_1 receptors, whereas the significance of the AT_2 receptors remains enigmatic with a suggestion that they may be clearance receptors. We have previously shown that AII has a biphasic action on levels of intracellular sodium in rat proximal tubular cells. Thus, AII at 10^{-11} M caused a transient increase in intracellular sodium content, whilst AII 10^{-5} M produced a transient decrease in intracellular sodium content which has been taken as reflecting stimulation and inhibition of sodium transport across the cells (Wong & Johns, 1996). The aim of this study was to attempt to establish

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whether this biphasic action of AII on proximal tubular intracellular sodium could be attributed to the different AII receptor subtypes. To achieve this, the effect of high and low concentrations of AII on rat proximal tubules was determined in the presence of the selective non-peptide AT_1 and AT_2 receptor antagonists losartan and PD123319, respectively.

Methods

General dissection

The techniques used to produce suspensions of rat proximal tubules have been described in detail by Wong *et al.* (1994) and were adapted from techniques utilized by Balaban *et al.* (1980), Vinay *et al.* (1981) and Gesek *et al.* (1987). In brief, male Wistar rats weighing between 350–400 g were anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1}, i.p.) and the kidneys were exposed via a midline incision. The blood vessels rostral to the kidneys were tied off and heparin, 500 u, was administered intravenously. Both kidneys were then perfused, via a cannula in the aorta below the level of the kidneys, with a physiological buffer solution containing (in mM): NaCl 115, $NaHCO_3$ 25, KCl 5, glucose 5, $MgSO_4$ 1, L-alanine 1, sodium lactate 4, mannitol 25, $CaCl_2$ 2, dextran 0.6%, NaH_2PO_4 2 which had been adjusted to pH 7.4, aerated with 95% O_2/5% CO_2 and kept at 37°C.

When the kidneys were cleared of blood the perfusate was changed to the physiological buffer minus the mannitol but containing collagenase 1000 u ml^{-1}. Following this *in situ* digestion, the kidneys were immediately removed, decapsulated and sliced into transverse sections approximately 2 mm thick. The medulla was excised and the cortical slices were minced with a razor blade and incubated for 35 min at 37°C in 15 ml of the buffer/collagenase solution. The suspension of dissociated tubules was then washed three times by centrifugation on a MSE Mistral 3000 for 1 min at 500 r.p.m. and 4°C.

The proximal tubules were then purified by differential centrifugation in a 45% Percoll solution as described previously by Wong *et al.* (1994).

Physiological viability

Tubular viability was estimated by means of the trypan blue exclusion test and was further supported by adenosine 5′-triphosphate (ATP) measurements of the tissues at the end of the experimental procedure. An ATP analysis kit (Sigma, Poole, Dorset, U.K., procedure no 366-UV) was utilized to determine ATP concentrations. Simultaneously, samples were also taken for protein determination, which was performed with a Sigma kit (procedure no. P5656).

Nuclear magnetic resonance methods

The nuclear magnetic resonance (n.m.r.) estimations were performed in the presence of the shift reagent dysprosium tripolyphosphate, ($Dy(PPP_i)_2^{7-}$), which was prepared immediately before use from 100 mM $DyCl_3$ in a 50 mM HEPES-buffered solution containing 200 mM sodium tripolyphosphate. The working solution of 4 mM of $Dy(PPP_i)_2^{7-}$ was obtained by diluting the $Dy(PPP_i)_2^{7-}$ in a solution containing (in mM): NaCl 85, $NaHCO_3$ 25, KCl 5, glucose 5, $MgSO_4$ 1, L-alanine 1, sodium lactate 4, $CaCl_2$ 2, dextran 0.6%, NaH_2PO_4 2. The low sodium buffer was used for the dilution in order to accommodate the sodium in the $Dy(PPP_i)_2^{7-}$ and the final sodium concentration in the shift reagent was around 146 mM. The proximal tubules were then suspended in the buffer/shift reagent to give a protein concentration of approximately 40 mg protein ml^{-1}.

The n.m.r. spectra were collected with either a Bruker 250 spectrometer operating at 66 MHz or a Jeol JNM-GX270 spectrometer operating at 71 MHz. The Bruker accumulated 5000 scans for each spectrum by use of an acquisition time of 70 ms which gave a total accumulation time of approximately 5.8 min. Application of 90°C pulses every 70 ms ensured that the spectra were fully relaxed. The Joel accumulated 1024 scans per spectrum with an acquisition time of 102 ms, plus a pulse delay of 150 ms, giving an accumulation time of approximately 4.5 min.

Between 1.5–2.5 ml of the suspension was placed into a silicone lined 10 mm diameter n.m.r. tube. The shift reagent contained 10% deuterium oxide, which acted as a frequency lock, and one drop of antifoam reagent B (Sigma, Poole, Dorset, U.K.) was added to the suspension before it was placed in the n.m.r. probe. The suspension was continuously aerated with 95% O_2/5% CO_2.

The temperature of the probe was kept at 37°C and the sample reached steady state levels in approximately 10 min after the n.m.r. tube had been placed into the probe.

Drug treatments

Addition of the vehicle alone to the tubular suspension (0.5 ml) acted as the control group. AII was added to the suspension 25–30 min after the tubule suspension had been placed into the probe, at which time the tubule suspension had attained steady state levels at 37°C. The AII was dissolved in the physiological buffer and added to the suspension via an infusion line in a volume of 0.5 ml to give the final concentrations of either 10^{-5} M or 10^{-11} M. After the AII had been delivered to the suspension, data were continuously acquired over the subsequent 25–30 min. Changes were recorded as deviations from the steady state level, which was given a value of 100%, just before the addition of the drug. To determine the receptor subtype(s) utilized by exogenously applied AII, either the non peptide AII receptor antagonist losartan (AT_1 selective) at 10^{-4} M or 10^{-6} M or PD123319 (AT_2 selective) 10^{-4} M was introduced to the proximal tubule suspension before the n.m.r. procedure.

Materials

Glassware was silicone lined where appropriate with Repelcote (Hopkin and Williams, Essex, U.K.). Losartan was kindly donated by Dupont Merck (Wilmington, DE, U.S.A.) and PD123319 ((S)-1-[[(4-di-methylamino)-3-methylphenyl]-methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo [4,5,-c]pyridine-6-carboxylic acid, ditrifluoroacetate, dihydrate) was generously provided by Parke-Davis (Ann Arbor, MI, U.S.A.).

Calculations

The spectral peaks were analysed by integration, by use of either Bruker or Jeol software, which gave an area under the curve for each peak. It was assumed that the intracellular sodium was 100% visible (Gullans *et al.*, 1985), therefore the sodium content within the cells of the rat proximal tubules was calculated as described previously (Wong & Johns, 1996). This

gave the sodium content of the sample in a specific n.m.r. spectra in nmol mg^{-1} protein. Assuming that there was 0.75 mg protein mg^{-1} dry wt (Balaban *et al.*, 1980), the sodium content could be converted to units of nmol mg^{-1} dry wt.

Statistical analysis

The n.m.r. data were tested statistically by use of paired Student's *t* test, comparing the steady state intracellular sodium content with changes due to the effect of the drug over the time period of observation. Comparisons between treatment groups were tested by one way analysis of variance. All statistical calculations were undertaken with statistical software, Superanova and Statview (Abacus Concepts, Berkeley, CA, U.S.A.). All values are quoted as means ± s.e.mean. Significant differences were taken when P was less than 5%.

Results

Tubular viability

The tubular suspension retained after the Percoll separation procedure yielded tubules which, when viewed under the microscope, were found to be greater than 95% proximal tubules with less than 5% glomerular contamination. Further microscopic examination revealed that the proximal tubules exhibited a characteristic granular yellow cytoplasm and brush border and had open lumens which indicated that they were capable of transepithelial transport. The trypan blue exclusion test further supported the view that the tubules were still viable and ATP values of 8.3 ± 0.9 nmol ATP mg^{-1} protein ($n=6$) indicated that the cells were still undergoing active respiration.

N.m.r. results

The shift difference between the intra- and extracellular sodium peaks seen on the n.m.r. spectra was between 8–10 p.p.m which was comparable to that obtained previously (Wong & Johns, 1996). This was sufficient to provide a good resolution between the two sodium peaks and permitted qualitative and quantitative analysis. Data are presented in real terms as nmol mg^{-1} dry wt and as % change from the steady state value which was arbitrarily set at 100%. In the control experiments ($n=7$), as the tubular suspension warmed up to the ambient temperature of the n.m.r. probe, set at 37°C, the intracellular sodium content decreased significantly ($P<0.001$) and reached a steady state level within 20 min. The rate constant for the rate of sodium efflux in the control experiments was $k=0.17\pm0.08$ min^{-1}. The rate constant in the test studies were not significantly different from that of the vehicle controls. In the vehicle control studies the steady state value, for intracellular sodium content was 69.04 ± 1.73 nmol mg^{-1} dry wt. This value was not significantly different from the values observed in our previous studies (Wong *et al.*, 1994; Wong & Johns, 1996). By converting this figure by means of the calculation derived by Gullans *et al.* (1985), the intracellular sodium concentration could be estimated as 21.92 ± 0.55 mM. The steady state intracellular sodium content values observed in the experimental groups were comparable to those seen in the vehicle control group and there were no significant differences between these groups.

The effect of losartan and PD123319 on the action of angiotensin II 10^{-11} M on $[Na]_i$

In the vehicle control experiments, the introduction of physiological buffer, as a vehicle to the tubular suspension, after steady state had been achieved did not significantly change intracellular sodium content from that of the steady state level during the course of the subsequent 30 min period of measurement (Figure 1).

AII 10^{-11} M was previously shown to increase intracellular sodium content (Wong & Johns, 1996) and the effect of the antagonist was studied on the action of the peptide at this concentration. The tubular suspensions were pretreated with losartan to achieve a final concentration of 10^{-4} M before the initiation of the n.m.r. procedure. Following the attainment of steady state, addition of AII 10^{-11} M ($n=6$) had no effect on the steady state value of 66.85 ± 1.58 nmol mg^{-1} dry wt (Figure 1) and the sodium levels remained stable over the course of 30 min, which was comparable to that seen when vehicle alone was added.

The effect of losartan, at a lower dose of 10^{-6} M, was also studied to establish the sensitivity of the AT_1 receptor to the antagonist. In the presence of losartan 10^{-6} M, the administration of AII to a final concentration of 10^{-11} M did not significantly change the steady state level of 64.20 ± 9.56 nmol mg^{-1} dry wt ($n=5$) over 30 min (Figure 1) and the profile of the steady state level was similar to that seen with the addition of the vehicle alone and in the presence of AII plus losartan 10^{-4} M.

A further set of experiments was performed to establish the functional characteristics of the AII receptor subtype AT_2 by use of the AT_2 selective non-peptide AII receptor antagonist PD123319. Following the addition of AII 10^{-11} M ($n=7$), to a suspension of proximal tubules which had previously been treated with PD123319 at 10^{-4} M, the steady state intracellular sodium content of the proximal tubule suspension, at 68.34 ± 1.87 nmol mg^{-1} dry wt, increased significantly ($P<0.01$) by approximately 18% (Figure 1) within 5 min to a new value of 83.68 ± 3.76 nmol mg^{-1} dry wt. The intracellular sodium content reached a peak of approximately 32% of the steady state level at 10 min after exposure to the

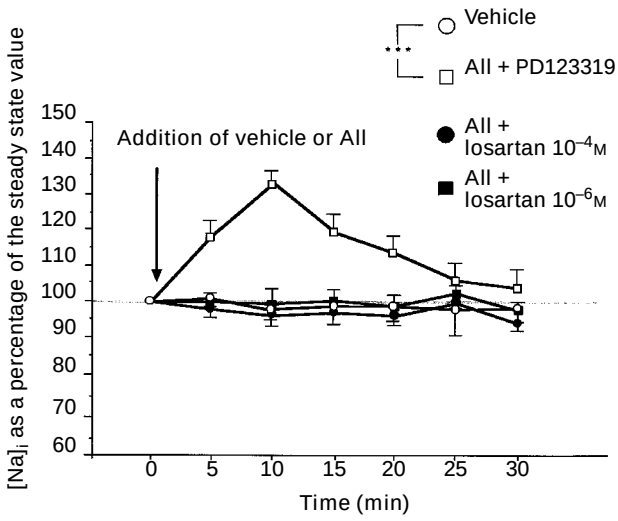

Figure 1 This shows the percentage values of intracellular sodium content ($[Na]_i$) compared with steady state (100%) obtained following stimulation with AII at 10^{-11} M, in the presence of PD123319 10^{-4} M ($n=7$), losartan, 10^{-4} ($n=6$) and 10^{-6} M ($n=5$) or in a set of tubules ($n=7$) incubated with vehicle alone. ***$P<0.001$ compared with vehicle response (ANOVA).

peptide and then began to decline and returned to baseline levels over the next 20 min. This response was significantly different from that seen in the vehicle controls ($P<0.001$) and from the effect seen in the presence of low or high concentration of losartan (both $P<0.001$), whereas it was not different from our previous observations when AII was added alone (Wong & Johns, 1996).

The effect of losartan and PD123319 on the action of angiotensin II 10^{-5} M on $[Na]_i$

In six experiments the addition of angiotensin II at a final concentration of 10^{-5} M to a suspension of proximal tubules incubated in the presence of losartan 10^{-4} M, did not significantly change the intracellular sodium steady state level of 67.4 ± 1.11 nmol mg^{-1} dry wt (Figure 2) and remained at a stable level for the duration of the observation period.

In a series of six further experiments, in the presence of PD123319 10^{-4} M, the addition of AII, at 10^{-5} M, to a suspension of proximal tubules after steady state had been achieved, caused an immediate decrease in the intracellular sodium content (Figure 2). Within the first 5 min of adding AII, the intracellular sodium content decreased significantly ($P<0.01$), by approximately 16% from the steady state sodium level of 61.20 ± 6.12 nmol mg^{-1} dry wt to a new value of 48.79 ± 4.13 nmol mg^{-1} wt, and over the subsequent 25 min returned to the starting steady state level. This response seen after the addition of AII 10^{-5} M in the presence of PD123319 was significantly different from that seen in the control group given vehicle and the group incubated with losartan (both $P<0.001$).

Discussion

The objective of this study was to assess the AII receptor subtype(s) which might be involved in mediating the action of AII on sodium handling at the proximal tubular epithelial cells. The effect of AII on proximal tubular sodium transport has been recognized to be biphasic (Harris & Young, 1977; Wong & Johns, 1996) and the primary aim was to determine whether the same or different receptors might be involved. In earlier studies from this laboratory, ^{23}Na n.m.r. spectroscopy had been used to follow dynamic changes in the intracellular levels of sodium in rat proximal tubule cells (Wong *et al*., 1994)

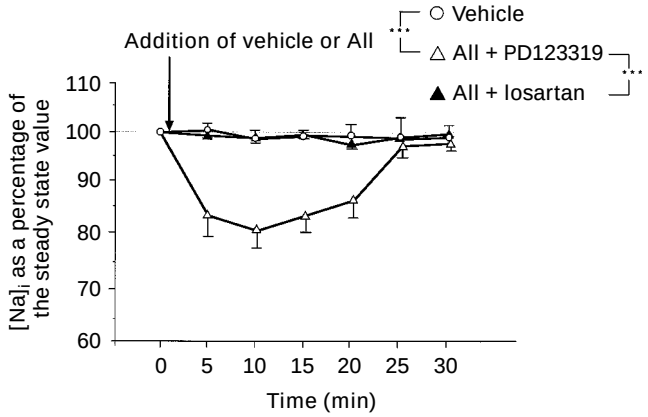

Figure 2 This illustrates the changes in intracellular sodium content ($[Na]_i$), from steady state, in response to AII at 10^{-5} M, in the presence of PD123319 10^{-4} M ($n=6$) or losartan, 10^{-4} M ($n=6$). Vehicle represents the levels in tubules given neither agonist nor antagonist. ***$P<0.001$ compared with vehicle response (ANOVA).

in response to a number of agents. It had been found to be an effective methodology in that the estimated levels of intracellular sodium measured in the present and previous studies were comparable to those utilizing different methods, for example fluorescence microscopy and electron microprobe x-ray analysis (Beck *et al*., 1980; Reilly *et al*., 1995). Under similar experimental conditions we had shown that ouabain, an inhibitor of the Na^+/K^+/ATPase, caused the sodium content to increase, similarly, the diuretic acetalzolamide, which blocks carbonic anhydrase activity and hence sodium transport, was found to elevate intracellular sodium levels. Moreover, in an initial study in which the action of AII was investigated (Wong & Johns, 1996), the precaution was taken of silicone lining all glassware, including the n.m.r. tubes, to prevent surface absorption of the peptide. A further concern was the possible degradation of AII by peptidase enzymes, which are in high concentrations at the proximal tubule brush border membranes, during the incubation, but we had shown previously (Wong & Johns, 1996) that preincubation with the endopeptidase inhibitor, phosphoramidon (10^{-6} M), did not change either the magnitude or the duration of the intracellular sodium responses induced by AII.

AII has been known for some time to have a biphasic action on sodium transporting epithelial cells of the proximal tubules. This was illustrated by the early work of Harris & Young (1977) who used micropuncture studies in the rat, which showed that peritubular capillary perfusion of low (10^{-12}–10^{-10} M) and high (10^{-7}–10^{-6} M) concentrations of AII increased and decreased, respectively, fluid transport across the proximal tubular epithelial cells. Such a pattern was also observed by Schuster *et al*. (1984) in the rabbit isolated perfused tubule, where fluid reabsorption was increased at low and decreased at high concentrations of AII in the perfusion medium. Indeed, studies in rat isolated proximal tubules (Cogan, 1990) have confirmed these findings. The underlying mechanisms which might explain this biphasic effect are unclear, but may be a consequence of the intracellular signalling mechanisms which come into play. The current view is that at low concentrations, 10^{-11} M, AII activates a receptor coupled to an inhibitory G-protein which reduces adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels. This reduction in intracellular cyclic AMP removes the inhibitory effect of cyclic AMP on the Na^+-H^+ exchanger and thus increases the activity of the transporter. Conversely, activation of a receptor coupled to a G-protein by AII at 10^{-5} M would stimulate phosphoinositol metabolism, which would increase the intracellular calcium concentration leading to an inhibition of the Na^+-H^+ exchanger (Harris, 1992). An alternative suggestion involving phospholipase A_2 as the mediator of the AII inhibitory effect has also been proposed (Douglas & Hopfer, 1994).

In a previous study, we had shown that AII at 10^{-11} M caused an increase in intracellular sodium content in suspensions of rat proximal tubules which was transient in nature, falling back to steady state levels by 20–30 min (Wong & Johns, 1996). This was explained as being due to AII initially stimulating the Na^+-H^+ exchanger allowing increased entry into the cells, but thereafter, there would be an increase in the Na^+/K^+/ATPase activity, such that intracellular sodium levels were returned to pre-existing values, with the consequence that actual sodium transport across the cells would be at a higher rate, consistent with an increased sodium reabsorption. In the current study, PD123319, a recognized selective antagonist of the AT_2 receptor was used and the dose chosen, 10^{-4} M, was a maximally effective concentration without any crossover effect on the AT_1 receptor (Dudley *et al*., 1990). It was clear that even

at this concentration of PD123319 (10^{-4} M), the antagonist did not prevent AII at 10^{-11} M from causing a transient increase in intracellular sodium levels. Indeed, it is important to emphasize that the size of this response was essentially of the same magnitude as that obtained previously with AII 10^{-11} M alone (Wong & Johns, 1996). The conclusion drawn from this finding was that the AT_2 receptor subtype was not involved in this response. The converse study was then undertaken, which was to utilize a selective ATP_1 receptor antagonist, and in this case losartan was used. The concentrations of losartan chosen have previously been demonstrated to block selectively the AT_1 receptor (Wong *et al*., 1990). It is evident from the data that at both the high (10^{-4} M) and moderate (10^{-6} M) concentrations of losartan, the transient rise in intracellular sodium content was completely inhibited. Thus, this result supported the view that it was the AT_1 receptor which mediated the action of the low concentration of AII to cause the initial increase in intracellular sodium.

Exposure of the proximal tubular suspension to high concentrations of AII (10^{-5} M) had been shown in our earlier study to cause a transient decrease in intracellular sodium content of some 25% within the first 5 min, but then returned over the next 30 min to the original steady state values (Wong & Johns, 1996). This was interpreted as being due to an initial inhibition of the luminal Na^+-H^+ exchanger whilst the Na^+/K^+/ATPase continued to function at its normal rate, resulting in a fall in the intracellular sodium levels. However, in the later phase, the level of activity of the sodium pump would fall because of the reduced availability of sodium, with the result that intracellular sodium would return to its original levels, but with a reduced rate of transport of sodium across the cells. The current results showed that in the presence of the AT_2 receptor antagonist, PD123319 at 10^{-4} M, the magnitude and pattern of response of intracellular sodium levels to AII was virtually identical to that observed previously (Wong & Johns, 1996). Thus, this makes it very unlikely that the AT_2 receptor subtype was involved in the proximal tubular response to AII. By contrast, in the presence of 10^{-4} M losartan, the AT_1 selective antagonist, the transient reduction in intracellular sodium content in response to 10^{-5} M AII was abolished. Together, these observations are compatible with the conclusion that the action of the high concentration of AII on sodium levels in this tissue was mediated by the activation of the AT_1 receptor subtype. However, some researchers have observed an effect on renal function by selective AT_2 receptor antagonists (Cogan *et al*., 1991; Macari *et al*., 1993), but the high doses involved may have some crossover effect which could explain these observations.

Interestingly, Hiranyachattada & Harris (1996) and Leyssac *et al*. (1997), have provided micropuncture evidence *in vivo* that proximal tubular luminal concentrations are likely to be very high and can cause raised fluid reabsorption under physiological circumstances and that this effect was mediated by AT_1 receptors. In the present study performed *in vitro*, it is unlikely that the high levels of endogenously produced AII would be able to build up and therefore it was possible to examine the influence of the action of both the low and high concentrations of the peptide at both extremes of the biphasic dose-response curve. At both concentrations of AII, AT_1 receptors were involved and these findings are consistent with those of Hiranyachattada & Harris (1996) and Leyssac *et al*. (1997). The observations of the present study add further information in demonstrating that the initial dynamic intracellular sodium responses of the proximal tubules to AII also required the activation of AT_1 receptors. What remains to be elucidated are the exact intracellular mechanisms and pathways which are activated by AII in order to modulate sodium handling by the epithelial cells in this biphasic fashion.

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