Studies on the Peptides Encoded By Rat and Human Angiotensin II Complementary RNA

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Some evidence suggests that RNA complementary to the messenger RNA encoding a peptide hormone encodes a complementary peptide that binds the original peptide hormone. The objective of this investigation was to assess in vivo the ability of complementary angiotensin II (II Ang) peptides to block the biological effects of angiotensin II (Ang II). Increasing concentrations of rat or human II Ang were preincubated with Ang II for 2 hours, and this solution was then infused intra-arterially into the superior mesenteric artery. Human, but not rat, II Ang dose-dependently inhibited Ang II–induced mesenteric vasoconstriction. The in vivo inhibitory potencies of human II Ang and [Sar',Ile']Ang II, with respect to inhibition of the pressor response to Ang II, were compared by infusing intravenously increasing doses of each blocker and determining their effects on a fixed intravenous dose of Ang II. Although human II Ang could abolish the pressor response to Ang II, [Sar',Ile']Ang II was approximately 100 times more potent in this regard. A fixed dose of human II Ang (150 μg/min i.v.) inhibited the effects of increasing doses of Ang II on mesenteric vascular resistance, arterial blood pressure, and aldosterone secretion. The 1H nuclear magnetic resonance spectra of human Ang II and Ang II were determined both separately and when combined in the same cuvette. The spectrum obtained by overlaying the separate spectra for these two peptides was the same as the spectrum obtained from the mixture of these two peptides in the same cuvette. These studies indicate that human, but not rat, II Ang blocks the in vivo effects of Ang II; however, this blockade is mediated by receptor antagonism and not via direct binding to Ang II. (Hypertension 1993;21:42–49)

KEY WORDS • angiotensin II • peptides • RNA

A complementary peptide is defined as a peptide encoded by RNA sequences complementary to that portion of messenger RNA (mRNA) that codes for a particular peptide hormone. Blalock and his group (Blalock and Smith1 and Bost et al2) hypothesized that complementary peptides bind the original peptide hormone by mimicking the receptor binding pocket for that peptide hormone. This hypothesis has predicted correctly the interaction between several hormones and their complementary peptides,3–6 and antibodies to complementary peptides have been shown to recognize a variety of cell surface receptors.3,5–9

In 1988, Elton et al10 applied this hypothesis to angiotensin II (Ang II). These investigators studied a synthetic peptide encoded by an RNA complementary to that portion of rat angiotensinogen mRNA that codes for Ang II. This complementary angiotensin II (II Ang) inhibited 125I-Ang II binding to rat adrenal membranes. Also, antibodies to rat II Ang inhibited binding of 125I-Ang II to rat adrenal membranes and inhibited Ang II–induced aldosterone secretion from rat adrenal cells. Further, anti-rat II Ang was used successfully to purify by immunoaffinity a protein that bound Ang II.

This initial and exciting report by Elton et al10 was quickly followed by three reports that were unable to demonstrate any effects of rat II Ang on Ang II–induced aldosterone secretion from bovine adrenal glomerulosa cells,11 Ang II–induced contraction of the rabbit aorta,11,12 and 125I-Ang II binding to bovine11 and rat12,13 adrenal membranes, rat aortic smooth muscle cell membranes,13 or human uterine membranes.13 In addition, anti-rat II Ang did not inhibit 125I-Ang II binding to rat smooth muscle cell membranes.13 Finally, in an elegant study, Eaton et al14 failed to detect any interaction between Ang II and rat II Ang by NMR spectroscopy. Therefore, it remains controversial whether rat II Ang can inhibit interactions of Ang II with its receptor.

Due to wobble in the genetic code, that portion of human angiotensinogen mRNA that codes for Ang II is different compared with rat angiotensinogen.15,16 As a result, human II Ang does not have the same sequence as rat II Ang. In one report, human II Ang was shown to inhibit Ang II–induced contraction of the rat uterus,17 and in another study, human II Ang blocked Ang II–induced contraction of the rabbit aorta and inhibited 125I-Ang II binding to rat adrenal membranes. This latter report concluded that the interaction of human II Ang with Ang II was at the receptor level rather than the peptide level since the Kd for inhibition of contrac-
tion of the rabbit aorta was similar for three different Ang II receptor agonists. However, very recently Budisavljevic et al. demonstrated that both $^{125}$I- and $^{3}$H-human II Ang failed to bind to hepatic or mesangial cell receptors and failed to bind to seven monoclonal antibodies defining four distinct epitopes on the Ang II molecule. In this same study it was shown that human II Ang inhibited $^{125}$I-Ang II binding to rat hepatocyte membranes and to the seven monoclonal antibodies against Ang II. Also, human II Ang blocked the effect of Ang II on mesangial cells and inhibited the pressor effects of Ang II infused into rats. These investigators concluded that human II Ang does indeed interact with Ang II at the peptide level and that II Ang peptides may serve as a model for a new class of antihypertensive drugs. The above discussion indicates that there is still much confusion and inconsistency regarding the effects and mechanism of action of II Ang peptides. While many of the studies mentioned above were being conducted, we too were investigating the ability of II Ang to block the effects of Ang II. In this regard, we asked two questions: 1) Does either rat or human II Ang block the biological effects of Ang II in vivo? 2) If so, how does II Ang inhibit the actions of Ang II? To date only one study has examined the in vivo effects of II Ang. That study focused only on blood pressure responses to Ang II and only examined the in vivo effects of human II Ang. In our present study, we compared the in vivo efficacy of both rat and human II Ang, and in the case of human II Ang, examined its ability to inhibit several actions of Ang II. Although the recent study by Budisavljevic et al. suggests that human II Ang blocks the effects of Ang II by directly binding to Ang II, peptide-peptide interactions were not assessed in that study. Therefore, we investigated with $^{1}$H NMR spectroscopy whether human II Ang directly binds to Ang II.

**Methods**

**Animals**

Male Sprague-Dawley rats weighing 353±6 g (mean±SEM; n=44) were obtained from Sasco, Omaha, Neb., and were allowed at least 1 week for acclimation to the animal care facility before commencing the study. Food (Purina rodent chow, St. Louis, Mo. ; 174 meq sodium per kilogram and 281 meq potassium per kilogram) and water were allowed ad libitum, and animals were maintained on a constant 12-hour light/dark cycle, relative humidity of 50% and ambient temperature of 22°C. The night before each experiment food was withheld. All studies were approved by the institutional Animal Care and Use Committee and were performed according to institutional guidelines.

**Study A**

Rats were anesthetized with pentobarbital (50 mg/kg i.p.), and a PE-240 cannula (Clay Adams, Parsippany, N.J.) was placed in the trachea to facilitate respiration. Also, two PE-50 catheters were placed in the jugular vein, one for infusion of 0.9% saline (50 µl/min) and one for administration of supplementary anesthetic. Animals were then prepared for in situ mesenteric perfusion. Briefly, the mesenteric vascular bed was blood perfused via an extracorporeal shunt established between the abdominal aorta and the superior mesenteric artery. Perfusion rate was maintained at 3 ml/min with a peristaltic pump (model 1210, Harvard Apparatus, South Natick, Mass.), and mesenteric perfusion pressure and arterial blood pressure were monitored continuously with pressure transducers (model P23 ID, Statham Division, Gould Inc., Oxnard, Calif.) that were connected to the extracorporeal shunt. Pressures were recorded on a polygraph (model 791D, Grass Instruments, Quincy, Mass.). Since perfusion rate was constant, changes in mesenteric perfusion pressure were a linear function of mesenteric vascular resistance. Each rat received 1,500 units heparin (intravenously) before the extracorporeal shunt was established to prevent blood clotting. Also, a heat lamp was placed above the animal to maintain body temperature. The extracorporeal shunt was constructed from Silastic tubing and contained several access ports that permitted convenient infusions of agents directly into the mesenteric vascular bed. After the extracorporeal shunt was established, the rat was allowed 1 hour to stabilize before proceeding with the experiment. Details for the in situ perfused mesentery procedure as well as details of the design of the extracorporeal shunt have been published previously.19,20

The protocol is shown in Figure 1. Ang II was premixed and incubated for 2 hours at room temperature with rat or human II Ang in a buffer (3 g tris(hydroxymethyl)aminomethane [Trizma Base, Sigma Chemical Co., St. Louis, Mo.], 3.5 g sodium chloride, 500 ml water, 1 g bovine serum albumin; pH adjusted to 7.5 with glacial acetic acid; filtered to 0.22 µm). Premixing and incubation of the peptides was performed before infusing the peptides to allow more than sufficient time for any binding of Ang II with Ang II to occur. After incubating for 2 hours, the peptide mixtures were placed on ice until needed. The concentration of Ang II in the mixture was fixed such that when the mixture was infused into the mesentery at 50 µl/min, Ang II was delivered at a rate of 10 ng/min. The concentration of Ang II was varied so that either 0, 1, 10, 100, or 1,000 µg/min of Ang II was infused with the 10 ng/min of Ang II. Every 30 minutes, one of the five mixtures was infused for 5 minutes, and mesenteric perfusion pressure was noted every minute of the
5-minute infusion. The order of administration of the various mixtures was randomized.

**Study B**

In this study, each rat was anesthetized with pentobarbital (50 mg/kg i.p.) and was placed on a prewarmed Deltapath Isothermal Pad (Braintree Scientific, Inc., Braintree, Mass.). A heat lamp also was positioned above the animal, and rectal temperature probe was inserted, and body temperature was displayed continuously on a digital monitor (Physiotemp Instruments, Inc., Clifton, N.J.). Body temperature was maintained at 37±0.5°C by adjusting the position of the heat lamp above the animal. The trachea was cannulated (PE-240) to facilitate respiration, and three PE-50 catheters were inserted into the jugular vein (one for supplementary anesthetic and the other two for intravenous infusions). Also, a PE-50 cannula was placed in the carotid artery and was connected to a digital blood pressure analyzer (Micro-Med, Inc., Louisville, Ky.) that was set to time-average mean arterial blood pressure every 1 minute (sampling rate=1,100/sec with an 82% duty cycle). After the surgery was complete, each rat received a subcutaneous injection of the ganglionic blocker chlorisondamine (10 mg/kg, Ciba-Geigy Corporation, Summit, N.J.). Thirty minutes later an intravenous bolus of captopril (Squibb Company, Princeton, N.J.) was administered (30 mg/kg). Thirty minutes after the captopril was administered, the protocol was begun.

The experiment consisted of six 3-minute intravenous infusions of Ang II (0.1 nmol/min) separated by 20 minutes. Mean arterial blood pressure was time-averaged 1 minute before and during the last minute of each 3-minute infusion of Ang II. The purpose of the first infusion of Ang II was to determine the baseline pressor response to Ang II, so the conditions of this infusion were the same in all rats. After obtaining a baseline response to Ang II, rats were randomly assigned to receive during the subsequent 23-minute periods either increasing infusion rates of human II Ang (1, 3, 10, 30, or 100 nmol/min), increasing infusion rates of [Sar1,Ile8]Ang II (0.03, 0.1, 0.3, 1, and 3 nmol/min) or vehicle (buffer). Therefore, accumulative dose-response relations (with respect to inhibition of the pressor response to Ang II) for human II Ang and [Sar1,Ile8]Ang II were obtained. The rats receiving only buffer served as a time-vehicle control group to assess whether the pressor response to Ang II was stable over the course of the experiment.

**Study C**

In Study C, rats were also prepared for in situ perfusion of their mesenteric vascular beds as described above, except that three PE-50 catheters were placed in the jugular vein: one for administration of supplementary anesthetic, one for infusion of buffer (and later human II Ang dissolved in buffer) at 20 µl/min, and one for infusion of 0.9% saline (and later Ang II dissolved in saline) at 50 µl/min. In addition, rats received an intravenous bolus of captopril (30 mg/kg) to reduce endogenous synthesis of Ang II. Captopril was administered immediately after surgery was completed, and a 1-hour rest period was allowed before the experiment was continued.

The protocol for Study C is outlined in Figure 2. One hour after administration of captopril, mean arterial blood pressure and mesenteric perfusion pressure were recorded, and a 0.5 ml blood sample was removed from the extracorporeal shunt for later measurement of plasma aldosterone levels. Blood samples were collected into 10 µl of 10% EDTA and centrifuged at 4°C; the plasma was stored at −20°C until assayed for aldosterone with a Coat-A-Count aldosterone radioimmunoassay kit (Diagnostic Products Corp., Los Angeles). To replace blood volume, each time a blood sample was removed, a constant volume of blood obtained from a donor rat was injected intravenously. The donor rat was prepared by placing cannulas in the trachea, carotid artery, and jugular vein and by administering 1,500 units of heparin.

After these baseline measurements were taken, the infusion containing buffer was either maintained throughout the experiment or was switched for the remainder of the experiment to human II Ang (150 µg/min) dissolved in buffer. Twenty minutes later mean arterial blood pressure and mesenteric perfusion pressure were noted, and another 0.5 ml blood sample was obtained for plasma aldosterone determination. Next, the infusion of 0.9% saline was replaced with an infusion of Ang II (10 ng/min per 50 µl, dissolved in 0.9% saline). Twenty minutes into this infusion of Ang II, mean arterial blood pressure and mesenteric perfusion pressure were recorded, and another blood sample for plasma aldosterone was taken. This procedure was repeated every 20 minutes as the infusion rate of Ang II was increased to 33, 100, 333, and 1,000 ng/min.

**Study D**

The purpose of the fourth study was to determine whether human II Ang directly interacts with Ang II. If a direct interaction occurs, then it is highly likely that the magnetic environment of one or more protons on Ang II, human II Ang, or both would change when the two peptides are dissolved in the same solution. Consequently, the ¹H NMR spectrum obtained by overlaying the separate spectra for human II Ang and Ang II would not be the same as the spectrum obtained by a mixture of these two peptides in the same cuvette. On the other hand, if no interaction between human II Ang and Ang II occurs, then the magnetic environment of protons on both Ang II and human II Ang would not be...
affected when the peptides are mixed. Therefore, in this case, the \( ^1H \) NMR spectrum obtained by overlaying the separate spectra for human II Ang and Ang II would be the same as the spectrum obtained by a mixture of these two peptides in the same cuvette.

This approach was recently used by Eaton et al\(^4\) to study potential interactions between rat II Ang and Ang II, and we have followed their method closely. Briefly, anhydrous sodium phosphate monobasic and dibasic (Aldrich Chemical Company, Milwaukee, Wis.) were dissolved in D\(_2\)O, and this solution was freeze-dried. This procedure was repeated four times. Solutions containing human II Ang only, Ang II only, and human II Ang (0.5 mM) plus Ang II (1 mM) were prepared in the sodium phosphate buffer (pH=7.5). The \( ^1H \) NMR spectra for each peptide or mixture of peptides were obtained on a Bruker AM 400 spectrometer at 30°C with a sweep width of 4201.681 Hz and a digital resolution of 0.513 Hz per point. Chemical shifts were referenced to HOD, which was assigned a chemical shift of 4.61 ppm. Spectra for the mixture of human II Ang and Ang II were obtained 1 and 4 hours after codissolving these two peptides.

**Statistical Analysis**

Statistical analyses were conducted using one-factor and two-factor analysis of variance with repeated measures, Fisher's least significant difference test, and paired and unpaired Student's \( t \) test as appropriate. Analyses were performed on the Number Crunchers Statistical System (Kaysville, Utah). All results are shown as mean±SEM.

**Peptides**

[Ile\(^3\)]angiotensin II was used in all protocols and was obtained from Sigma, and [Sar\(^1\),Ile\(^3\)]Ang II was purchased from Peninsula Laboratories (Belmont, Calif.). Rat II Ang (H\(_2\)N-Lys-Gly-Val-Asp-Val-Tyr-Ala-Val-COOH) and human II Ang (H\(_2\)N-Glu-Gly-Val-Tyr-Val-His-Pro-Val-COOH) were custom synthesized, purified, and checked for purity by Peninsula Laboratories. For both peptides, amino acid analysis corresponded closely with theory. Peptide purity was assessed by thin-layer chromatography (ninhydrin), electrophoresis (ninhydrin), and reverse-phase, high-performance liquid chromatography (ultraviolet absorbance) and was approximately 98%.

**Results**

**Study A**

Figures 3 and 4 summarize the changes in mesenteric perfusion pressure (mm Hg) induced by direct intra-arterial infusions of Ang II (0.01 \( \mu \)g/min) when coinfused with 0, 1, 10, 100, or 1,000 \( \mu \)g/min rat (Figure 3) or human (Figure 4) II Ang. In each case, the coinfusion was achieved by mixing Ang II and II Ang in the same test tube for 2 hours at room temperature before infusing the mixtures. Ang II without II Ang increased mesenteric perfusion pressure approximately 20 mm Hg. Rat II Ang did not significantly alter the mesenteric vascular response to Ang II even when the Ang II/II Ang ratio was 1/100,000 (Figure 3). In contrast, human II Ang dose-dependently inhibited the mesenteric vascular response to Ang II (Figure 4) with an \( IC_{50} \) of approximately 10 \( \mu \)g/min (Ang II/II Ang ratio of 1/1,000). Complete inhibition of the Ang II response was achieved with 1,000 \( \mu \)g/min infusion.

**Study B**

Figure 5 illustrates the effects of increasing intravenous infusion rates of [Sar\(^1\),Ile\(^3\)]Ang II (0, 0.03, 0.1, 0.3, 1, and 3 nmol/min) and human II Ang (1, 3, 10, 30, and 100 nmol/min) on pressor responses induced by a fixed intravenous infusion rate of Ang II (0.1 \( \mu \)mol/min). In this experiment, Ang II and II Ang were not preincubated before the infusions and were infused via separate intravenous catheters. Ang II increased mean arterial blood pressure approximately 100 mm Hg. Both [Sar\(^1\),Ile\(^3\)]Ang II and human II Ang significantly and dose-dependently inhibited the pressor response to Ang II; however, [Sar\(^1\),Ile\(^3\)]Ang II was approximately 100 times more potent on a molar basis in this regard. At high infusion rates, both blockers were able to abolish the effects of Ang II on arterial blood pressure. The pressor response to Ang II was stable in the time-vehicle control group (data not shown).

**Study C**

Figure 6 demonstrates the effects of human II Ang (150 \( \mu \)g/min i.v.) on baseline mean arterial blood pressure, mesenteric perfusion pressure, and plasma levels of aldosterone. Human II Ang did not produce any detectable partial agonist activity.

Figures 7, 8, and 9 illustrate the effects of human II Ang (150 \( \mu \)g/min i.v.) on Ang II-induced changes in mean arterial blood pressure, mesenteric perfusion pressure, and plasma aldosterone levels, respectively. Ang II significantly and dose-dependently increased
FIGURE 4. Line graph shows response (change in mesenteric perfusion pressure) induced by intra-arterial infusions of angiotensin II (Ang II) when Ang II was infused alone and when Ang II was coin infused with various amounts of human complementary angiotensin II (II Ang). Ang II and human II Ang were preincubated in the same test tube for 2 hours at room temperature before the infusions. Repeated-measures two-factor analysis of variance indicated a significant effect (p=0.005) of human II Ang on the response induced by Ang II. Fisher's least significant difference test indicated that the response to Ang II was not inhibited by 1 µg/min human II Ang, but was significantly inhibited by 10, 100, and 1,000 µg/min human II Ang. Also, the inhibition by 100 and 1,000 µg/min human II Ang was significantly greater than the inhibition by 10 µg/min human II Ang. Baseline mesenteric perfusion pressures were 80±3, 80±3, 79±3, 84±5, and 83±4 mm Hg before infusion of the solutions containing Ang II plus human II Ang (0, 1, 10, 100, and 1,000 µg/min, respectively).

Each of the measured parameters, and these effects of Ang II were abolished by human II Ang. In control rats, Ang II (10 ng/min) significantly increased mean arterial blood pressure and mesenteric perfusion pressure, yet in human II Ang–treated rats not even 1,000 ng/min Ang II significantly increased these parameters (Figures 7 and 8). To determine whether the inhibition of vascular responses to Ang II by human II Ang was specific, six pilot experiments were conducted in which the effects of human II Ang (150 µg/min i.v.) on mesenteric vascular responses to periarterial nerve stimulation (5 Hz, 1-millisecond biphasic pulses for 20 seconds) were examined. Periarterial nerve stimulation was performed as previously described.
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human II Ang (compared at corresponding periods). Baseline MABP was 88±5 and 88±4 mm Hg in the control group and human II Ang group, respectively.

hours, and these peptide solutions were then infused into the in situ blood-perfused mesentery. Rat II Ang did not attenuate Ang II–induced mesenteric vasoconstriction even when the amount of rat II Ang was 100,000 times greater than the amount of Ang II. These results indicate that in vivo rat II Ang does not directly bind to Ang II and does not antagonize the Ang II receptor. This conclusion is not consistent with the in vitro findings of Elton et al, but does corroborate the in vitro results of other investigators who were unable to detect any biological effects of rat II Ang.

In contrast to rat II Ang, human II Ang dose dependently blocked Ang II–induced mesenteric vasoconstriction when human II Ang was preincubated with Ang II. To determine whether human II Ang could prevent the effects of Ang II even without preincubation, human II Ang was infused intravenously and continuously at increasing rates while pressor responses to brief intravenous infusions (using a separate catheter) of Ang II were tested repeatedly. This protocol also included a group of animals that received [Sar1, Ile8]Ang II (a well-known peptide Ang II receptor blocker) to provide some frame of reference for assessing the potency of human II Ang. Even without preincubation, human II Ang effectively blocked the pressor response to Ang II, although the potency of human II Ang in this regard was 100 times less compared with [Sar1, Ile8]Ang II.

Our third study with human II Ang was designed to examine several questions regarding the in vivo effects of human II Ang: 1) Does human II Ang have partial agonist activity? 2) Does human II Ang block equally the effects of Ang II on several parameters, including arterial blood pressure, mesenteric vascular resistance, and aldosterone secretion? 3) Can the effects of human II Ang be overcome with increasing doses of Ang II? Regarding the partial agonist activity of human II Ang, even in captopril-pretreated animals with low endogenous levels of Ang II, human II Ang did not increase noticeably baseline levels of arterial blood pressure, mesenteric perfusion pressure, or plasma aldosterone. These data suggest that human II Ang is either devoid or nearly devoid of partial agonist activity.
Ang II caused dose-related increases in arterial blood pressure, mesenteric vascular resistance, and plasma aldosterone levels in control rats but not in rats receiving an intravenous infusion of human II Ang. Thus, human II Ang blocked the effects of Ang II in vivo not only on the vasculature, but also on the adrenal cortex. However, despite increasing the infusion rate of Ang II to 1,000 ng/min (10 times maximal), the blockade induced by human II Ang was not reversed (see Figures 7 and 8). This suggests that the effects of human II Ang may not be strictly competitive in nature. However, the fact that human II Ang did not alter mesenteric vascular responses to sympathetic nerve stimulation suggests that the inhibition of Ang II-induced responses by human II Ang was specific. Our in vivo findings with human II Ang are consistent with other published in vitro studies and are in agreement with the limited in vivo studies reported by Budisavljevic et al.

Because human II Ang blocks both the in vitro and in vivo effects of Ang II, it becomes an important issue as to how human II Ang inhibits the biological effects of Ang II. It is possible that human II Ang directly binds to Ang II as predicted by the complementary peptide hypothesis of Blalock and his group and as suggested by the recent studies by Budisavljevic et al. It is also possible that human II Ang just happens to be an antagonist of the Ang II receptor. Of course, these two possibilities are not mutually exclusive.

We suggest that human II Ang inhibits the biological effects of Ang II by antagonizing the Ang II receptor, not by directly binding to Ang II. This conclusion is based on the following considerations: 1) If human II Ang binds directly to Ang II, the 1H NMR spectrum of the peptide-peptide complex most likely would be different compared with the spectrum formed by overlaying the spectra for the individual peptides. Our experiments indicate that this is not the case and, therefore, argue strongly against a direct peptide-peptide interaction. 2) The only a priori rationale for a direct peptide-peptide interaction is that the complementary peptide hypothesis of Blalock and his group predicts that such an interaction should exist. However, both the results of the present study and several previous studies indicate that this hypothesis fails with respect to rat II Ang. This weakens the authority of the complementary peptide hypothesis, particularly regarding its application to complementary angiotensin peptides, and undermines the a priori argument for a direct interaction. This hypothesis is further weakened by recent evidence that neither rat, human, nor bovine angiotensin II subtype 1 receptors contain the amino acid sequences represented in either rat or human II Ang. This weakens the authority of the complementary peptide hypothesis, particularly regarding its application to complementary angiotensin peptides, and undermines the a priori argument for a direct interaction. 3) A previous in vitro study by Wiest et al. demonstrated that human II Ang antagonized with a similar potency the ability of three separate agonists of the Ang II receptor to contract the rabbit aorta. If human II Ang interacts directly with Ang II agonists, it is unlikely that it would possess a similar affinity for all three agonists. However, since potency of an antagonist is not influenced by the affinity of the agonist, the results by Wiest et al. are entirely consistent with the conclusion that human II Ang blocks the Ang II receptor. 4) It is unlikely that any of the four amino acid substitutions in human II Ang would prevent human II Ang from binding to the Ang II receptor. Phe8 to Val8 substitution is well known to confer antagonist activity to Ang II peptides. The Ile8 to Val8 substitution would not influence receptor affinity since this substitution occurs naturally in some species. Substitution of Glu1 for Asp1 would not likely influence
receptor affinity since these amino acids differ only by one methylene group. Finally, a Gly for Arg substitution in Ang II antagonists causes only a 10-fold reduction in antagonist activity.27

The best argument in favor of a direct interaction of human II Ang with Ang II is provided by the recent observations by Budisavljevic et al18 that radiolabeled human II Ang does not bind to the Ang II receptor nor does it bind to seven monoclonal antibodies defining four distinct epitopes on the Ang II molecule. However, the affinity of human II Ang for the Ang II receptor (and antibodies against Ang II) is most likely quite low. For instance, Wiest et al12 estimated the $K_d$ of human II Ang for the adrenal Ang II receptor to be only 0.6 μM. With such a low affinity, any method of separating receptor-bound (or antibody-bound) from free radiolabeled human II Ang would have to be accomplished in approximately 0.01 seconds28 to avoid disruption of binding. It is unlikely that this could have been accomplished with the vacuum filtration or polyethylene glycol precipitation methods used by Budisavljevic et al.18

Since it is unlikely that human II Ang interacts directly with Ang II, it is improbable that human II Ang represents a model for a new class of antihypertensive drugs. From a teleological point of view it is interesting to speculate about the potential physiological relevance of the fact that in humans, one strand of DNA codes for Ang II and the complementary strand codes for an Ang II receptor antagonist. Whether this is by chance or design cannot be determined from the present study. However, we remain skeptical as to the physiological importance of human II Ang because of its low potency and because of its lack of conservation among species.

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