hybrids, have obtained data that could be explained by rapid phenotypic variation. Without an appropriate clonal analysis of the parental lines, one cannot evaluate this possibility.

DVORA AVIV
E. BRAD THOMPSON
Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20014

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2 June 1972

A New, Long-Lasting Competitive Inhibitor of Angiotensin

Abstract. *An analog of angiotensin II, [Sar¹,Ile⁸]-angiotensin II, has a potent and long-lasting competitive antagonistic effect against angiotensin II when tested for its myotropic action on the isolated rabbit aorta and for its effect on blood pressure in anesthetized cats and dogs. Compared to [Ile⁸]-angiotensin II, the new analog has equal antagonistic potency on the isolated system but a much greater potency in vivo. It is assumed that sarcosine in position 1 protects the peptide against enzymatic degradation and enhances its half-life. This study demonstrates that the modification in both positions 1 and 8 are important for the in vivo antagonistic potencies of angiotensin analogs.*

Several analogs of angiotensin II (A II) with substitutions in position 8, which were synthesized in this laboratory (*1, 2*), competitively inhibit some of the pharmacological effects of the parent peptide. One of these analogs, [Ala⁸]-A II (Ala, alanine) competitively blocks the myotropic effect of both angiotensin I (A I) and A II, but in doses from 1 to 10 μg/kg it does not antagonize the pressor response to A II in anesthetized cats (*3*). This analog, however, has an inhibitory effect against A II in anesthetized rats (*4*). The other analog, [Ile⁸]-A II (Ile, isoleucine) is a more potent competitive inhibitor of the parent peptide both in vivo and in vitro (*5*). We also reported that A II with aminophenylisobutyric acid (Apib) substituted in position 8 ([Apib⁸]-A II) is a short-acting competitive inhibitor of A II both in vivo and in vitro in several pharmacological preparations (*6*).

Since all these peptides are 8-substituted analogs of the parent peptide, it is possible that they are inactivated by the same enzymes that metabolize A II. This may be an explanation of the failure of [Ala⁸]-A II to block the pressor effect of the parent peptide in anesthetized cats (*3*). One of the enzymes that degrade A II is an aminopeptidase that acts on the NH_2-terminal amino acid (*7*). Replacement of this group by sarcosine (Sar) produced a longer-acting blocking peptide; [Sar¹,-Ala⁸]-A II is an effective competitive inhibitor of A II in rat and dog (*8*). Sarcosine, in the NH_2-terminal position, possibly protects the peptide from aminopeptidase degradation and allows it to reach the receptor site and block the effect of A II. The analog [Sar¹,-Ile⁸]-A II (molecular weight, 1003.63) has been synthesized in this laboratory (*2*). This report contains initial results of the pharmacological studies of this compound.

In vitro experiments were performed on isolated, spirally cut rabbit aortic strips in Krebs solution (*9*). The log dose-response curves of A II with and without [Sar¹,Ile⁸]-A II were determined. At the same time, the dose ratios of agonist were estimated when different concentrations of [Sar¹,Ile⁸]-A II were present. From these ratios, log K_2 values were calculated for each experiment from the equation (*10*):

$$\log K_2 = \log \frac{\text{dose ratio} - 1}{[\text{Sar}^1, \text{Ile}^8]\text{-A II}} \quad (1)$$

where the denominator is the molar concentration of the analog. Although the log K_2 value is equal to the pA_2 value when two drugs antagonize each other in a competitive manner (*11*), the pA_2 value of [Sar¹,Ile⁸]-A II was also calculated separately for each experiment. The pA_2 value of [Sar¹,Ile⁸]-A II was found to be 9.33 ± 0.2 (standard error of mean) in eight experiments. The calculated ratios in the presence of different molar concentrations of [Sar¹,Ile⁸]-A II and log K_2 values are summarized in Table 1. Log K_2 values were equal at different concentrations of [Sar¹,Ile⁸]-A II; also, the pA_2 value was not significantly different from the log K_2 values. These criteria are satisfactory for competitive antagonism (*10*). Also, the log dose-response curve of A II was a straight line, shifted to the right and parallel to the control, when [Sar¹,Ile⁸]-A II was added to the muscle bath (Fig. 1).

The antagonistic effect of continuous infusion of [Sar¹,Ile⁸]-A II was also tested on the blood pressure of cats

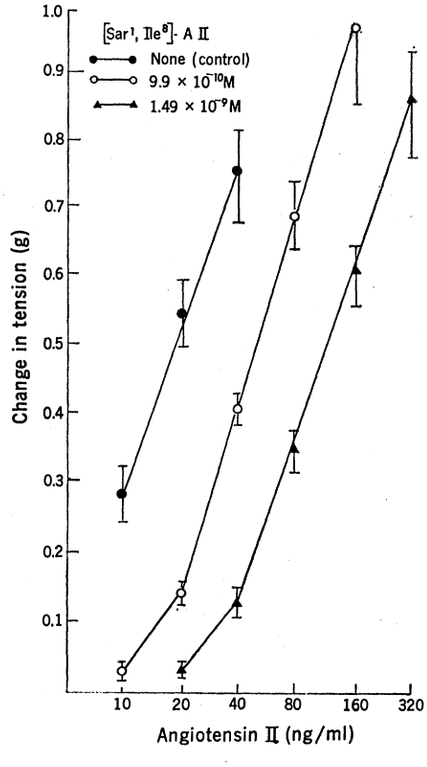

Fig. 1. Log dose-response curve of A II before and after addition of [Sar¹,Ile⁸]-A II. The ordinate shows changes in tension induced by A II on the rabbit aorta. The line to the left was derived from the mean values for three different doses of A II (32 experiments for each dose). When [Sar¹,Ile⁸]-A II ($9.9 \times 10^{-10}M$) was added to the muscle bath the dose-response curve for A II was shifted to the right and was parallel to the control (mean, eight experiments). When the concentration of [Sar¹,Ile⁸]-A II was increased (five experiments) to $1.49 \times 10^{-9}M$ the A II dose-response curve was shifted further to the right. The vertical bars indicate standard error of mean.

and dogs anesthetized with chloralose (80 mg per kilogram of body weight, given intravenously). Figure 2A shows the log dose-response curve of A II. To obtain the pressor response observed with a low dose of A II alone, twice as much A II was required when [Sar¹,Ile⁸]-A II (30.8 ng/kg per minute) was given, and about eight times as much A II was needed when the dose of [Sar¹,Ile⁸]-A II was increased to 121.2 ng/kg per minute. In contrast, the dose of antagonist was almost ten times lower than that of [Ile⁸]-A II for a similar antagonistic response (*5*). Partial blockade of the pressor response of A II was still present 100 minutes after the infusion of [Sar¹,Ile⁸]-A II was stopped (Fig. 2B). This recovery time has been found to be 30 to 45 minutes for [Ile⁸]-A II and 5 to 10 minutes for [Apib⁸]-A II (*6*), a result suggesting that [Sar¹,Ile⁸]-A II is a longer-lasting competitive inhibitor than the others. The analog [Sar¹,Ile⁸]-A II did not inhibit the effect of norepinephrine, serotonin, and prostaglandin F_{2a} on the isolated rabbit aorta or on cat blood pressure; this indicates the specificity of this analog for A II.

In dogs, [Sar¹,Ile⁸]-A II selectively inhibited the pressor effect of A II in a competitive manner. The mean pressor responses in five dogs given single intravenous injections of A II were 38 ± 6 mm-Hg for a dose of 50 ng/kg, 50 ± 7 mm-Hg for a dose of 100 ng/kg, and 67 ± 7 mm-Hg for a dose of 200 ng/kg. During intravenous infusion of [Sar¹,Ile⁸]-A II (200 ng/kg per minute), these responses were significantly decreased for each dose of A II ($P < .001$). The response was 5 ± 2 mm-Hg for an A II dose of 50 ng/kg, and 9 ± 4 mm-Hg for a dose of 100 ng/kg.

Table 1. Antagonism by [Sar¹,Ile⁸]-A II of A II responses on rabbit isolated aortic strips. Log K_2 values were estimated by equation 1; M, mean; S.E.M., standard error of mean; *n*, number of experiments.

Analog (10^{-9} *M*)	Dose ratio (M ± S.E.M.)	Log K_2 (M ± S.E.M.)	*n*
0.495	1.65 ± 0.12	9.037 ± 0.17	7
0.99	3.60 ± 0.6	9.38 ± 0.2	7
1.49	5.88 ± 0.94	9.46 ± 0.08	6

The complete recovery of the pressor response of A II occurred 45 to 50 minutes after the infusion of [Sar¹,Ile⁸]-A II was stopped. Because of the limited number of experiments with [Ile⁸]-A II we cannot compare the antagonistic potency and the duration of the antagonistic effect of [Sar¹,Ile⁸]-A II in the dog with those of other inhibitors. However, initial in vivo experiments in this species suggest that [Sar¹,Ile⁸]-A II is about ten times more active than [Ile⁸]-A II. Responses to norepinephrine during the infusion of the analog were not significantly altered from those obtained before the infusion. The analog [Sar¹,Ile⁸]-A II had no agonistic activity in the isolated rabbit aorta, even when 1 to 10 μg/ml was added to the muscle bath. However, low agonistic activity for blood pressure in the cat and dog was obtained for the analog at doses as high as 1 to 10 μg/kg, almost 100 to 500 times higher than antagonistic doses.

In the aortic strips the pA_2 value of this compound was almost equal to that of [Ile⁸]-A II, which indicates that the antagonistic potencies of these two analogs are equal. However, the antagonistic activity of [Sar¹,Ile⁸]-A II lasted longer than those of [Ile⁸]-A II and the other analogs. The only structural difference between these two analogs is the replacement of the NH_2-terminal group with sarcosine in the former. Changes made only in position 1 of A II have not produced competitive antagonists. However, these changes have increased the duration of the agonist response, as demonstrated by [βAsp¹]-A II (*7*). It is assumed that sarcosine in the NH_2-terminal position protects the analog from degradation. The analog may occupy the specific receptor site for longer periods of time, or may possibly have a longer in vivo half-life and a greater chance of reaching or remaining at the active receptor site. On the other hand, substitution of succinic acid at position 1 of [Phe⁴,Tyr⁸]-A II decreased its potency as an inhibitor (*2*). However, [Phe⁴,Tyr⁸]-A II itself was not a potent competitive antagonist (*12*). Furthermore, the introduction of succinic acid into A II decreased the agonist properties somewhat (*13*). The exact mechanism by which sarcosine exerts its influence in the parent peptide remains to be determined. This study further suggests that positions 8 and 1 of the synthetic analogs are important for the potency and duration of antagonistic activities.

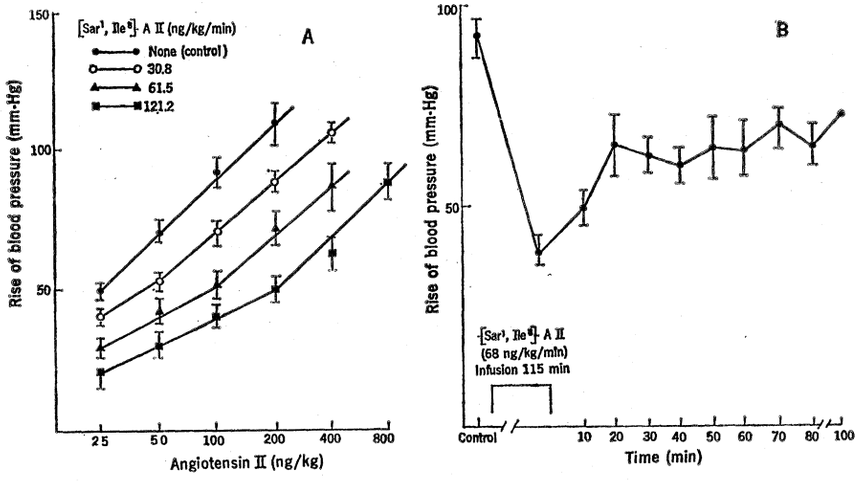

Fig. 2. Effect of [Sar¹,Ile⁸]-A II on pressor response to A II in cats anesthetized with chloralose. (A) Dose-response curves of A II were determined before and after intravenous infusion of [Sar¹,Ile⁸]-A II at three different doses. When the dose of the analog was increased, the curves obtained were parallel to the control and to the right. Each point is the mean for seven experiments, and vertical bars show the standard error of mean. (B) Pressor responses to injections of A II (100 ng/kg) given before, during, and after infusion of [Sar¹,Ile⁸]-A II were determined. Only 25 percent recovery was observed 20 minutes after the [Sar¹,Ile⁸]-A II infusion was stopped. This response did not change significantly 100 minutes after the analog infusion was stopped. Complete recovery occurred after 3 to 3.5 hours.

R. K. TÜRKER
M. M. HALL, M. YAMAMOTO
C. S. SWEET, F. M. BUMPUS
Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106

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Human Prolactin: 24-Hour Pattern with Increased Release during Sleep

Abstract. *Human prolactin was measured in plasma by radioimmunoassay at 20-minute intervals for a 24-hour period in each of six normal adults, whose sleep-wake cycles were monitored polygraphically. A marked diurnal variation in plasma concentrations was demonstrated, with highest values during sleep; periods of episodic release occurred throughout the 24 hours.*

Many anterior pituitary hormones in humans are released episodically over the 24-hour day, and the frequency and magnitude of the episodes are controlled in part by central nervous system rhythms related to time of day and the sleep-wake cycle. These include the sleep-onset release of human growth hormone (*1*), the circadian rhythm of episodic secretion of adrenocorticotrophic hormone (ACTH) and cortisol (*2*), and the episodic release of gonadotrophins (*3*). The demonstration that human prolactin is distinct from growth hormone and circulates in plasma (*4*) and the development of a radioimmunoassay technique for its measurement (*5*) made possible this study of 24-hour release patterns of prolactin. We show that plasma prolactin concentrations in normal young men and women undergo episodic changes and that there is a diurnal variation in release, with highest concentrations during the nocturnal sleep period and the lowest during waking hours.

Three men and three nulliparous women, ages 21 to 30 years, all within 10 percent of ideal body weight, were each acclimated to the sleep laboratory for a period of 24 hours. None had ever received hormone therapy or was taking drugs. During the succeeding 24 hours, between 10 a.m. and 2 p.m., a catheter was inserted into an antecubital vein and blood was sampled every 20 minutes from an adjoining room (*6*). Plasma was immediately separated and frozen until radioimmunoassays of prolactin and human growth hormone were performed.

Surface electrodes for electroencephalography, chin muscle electromyography, and electrooculography were applied by standard techniques at approximately 10 p.m. to monitor sleep polygraphically (*7*). Subjects remained in a sound-attenuated room and ate meals at 8 a.m., 12 noon, and 6 p.m. Lights were turned off and subjects allowed to go to sleep at 11 p.m. Five of the six fell asleep within 40 minutes of the time when lights were turned out and were awakened at 7 a.m.; the sixth did not fall asleep until 1:30 a.m. and was allowed to sleep until 9 a.m.

Polygraphic sleep data were scored according to standardized criteria (*7*). Sleep stage percentages were within expected normal limits on the night when the catheter was in place. Total sleep time ranged from 6 hours, 20 minutes up to 7 hours, 40 minutes,

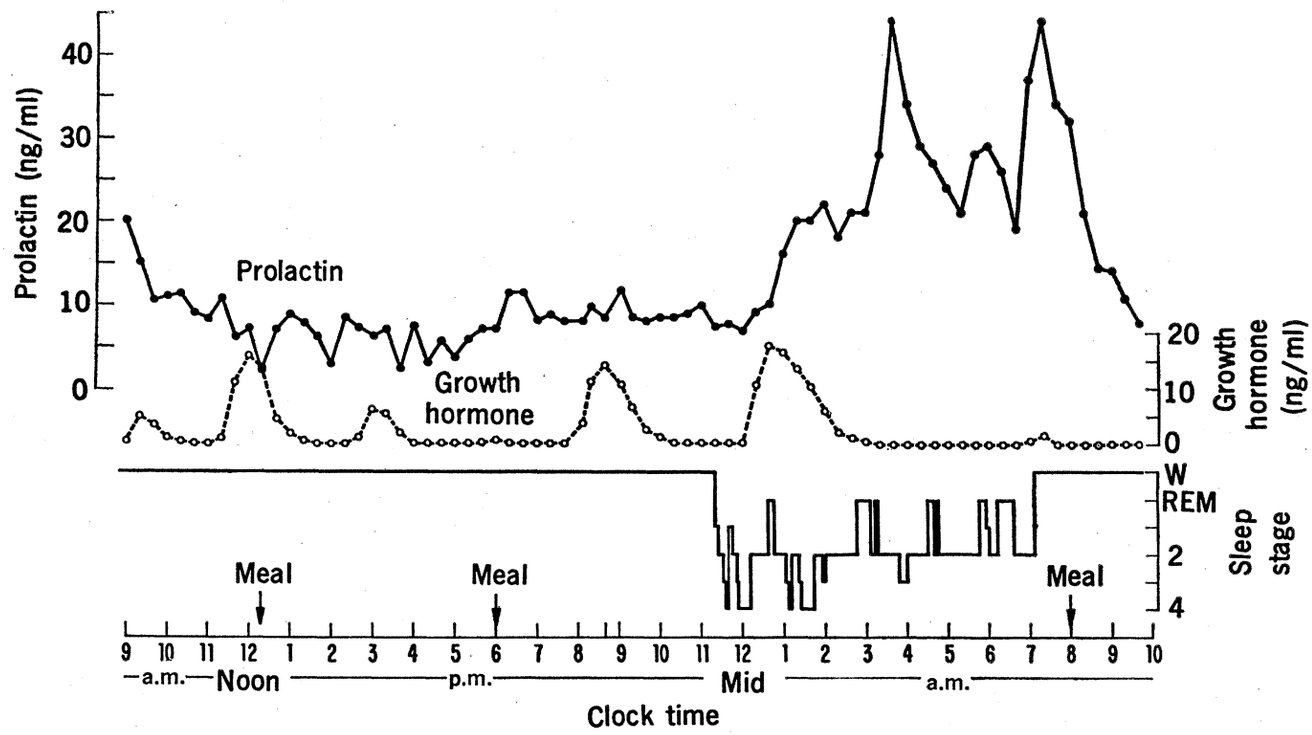

Fig. 1. Prolactin, growth hormone, and sleep stage in a male subject, age 23. The results of this 24-hour catheter study are representative of 24-hour pattern seen in all six subjects; *W*, wake.