Renin-Angiotensin Role in Thirst: Paradoxical Enhancement of Drinking by Angiotensin Converting Enzyme Inhibitor

Abstract. *A competitive angiotensin converting enzyme antagonist SQ 20, 881 (SQ), was used to examine the role of the renin-angiotensin system in putative renin-dependent thirst in the albino rat. Significant enhancement of "renin-dependent" as well as renin-independent drinking was observed in the presence of peripheral SQ. Intraventricular SQ obviated this enhancement of drinking but did not affect the water intake caused by the original stimulus itself, whereas it sharply reduced drinking evoked by peripheral renin in nephrectomized rats. Prior renin depletion likewise had no influence on so-called renin-dependent thirst.*

The peripheral renin-angiotensin system has been ascribed an important role in the mechanism of a number of thirst-evoking stimuli that are accompanied by substantial elevations of plasma renin activity, such as ligation of the inferior vena cava and peripheral injection of isoproterenol or of other "hypotensive" drugs that induce preferential beta-adrenergic activation.

The concept is based on the following persuasive, albeit circumstantial, evidence. (i) Bilateral nephrectomy, which obviates the rise in plasma renin activity, also inhibits or abolishes the drinking response (*1*, *2*). (ii) The intraperitoneal injection of renin or intravenous infusion of angiotensin II elicits drinking which is enhanced in the absence of the kidneys. (iii) When applied directly to the preoptic area of the hypothalamus, angiotensin II evokes copious drinking in nanogram amounts (*3*).

Taken together, these findings appear to provide an adequate framework for the hypothesis that thirst stimuli dependent upon the presence of the kidneys elicit their action, at least in part, by triggering excess release of renin, which, in turn, produces angiotensin I in the plasma. Angiotensin I, after enzymatic conversion to angiotensin II, enters the brain in sufficient quantity to induce a dipsogenic response.

Fitzsimons (*4*), on the basis of his studies on the relation of the renin-angiotensin system to thirst, concluded that the extent of the intervention of this system in normal thirst mechanisms is "very difficult to judge." He warns against reliance on the effect of nephrectomy on a drinking response as an index of the degree of renin dependency, because the resulting anuria and other thirst mechanisms may compensate for the absence of renin secretion.

The availability of SQ 20, 881 (SQ), an angiotensin converting enzyme inhibitor (*5*), appeared to offer the opportunity to test the role of the renin-angiotensin system in certain thirst mechanisms in a more direct and physiologic manner, that is, without the radical and inexorably progressive deterioration of homeostasis and vital body functions which follow bilateral nephrectomy. Several investigators have demonstrated in both rat and dog (*6*) that SQ inhibits the pressor response to angiotensin I but not to angiotensin II. It was reasoned, therefore, that abolition or significant reduction of thirst in animals prevented from forming peripheral angiotensin II would support the idea that a drinking response is dependent on this octapeptide, whereas undiminished thirst would seriously challenge such contention.

We have unexpectedly found that prior treatment with SQ caused a significant enhancement of the dipsogenic effect of both "renin-dependent" and renin-independent thirst stimuli, but not of exogenous renin itself. Moreover, prior renin depletion did not affect so-called renin-dependent thirst. Our findings appeared to militate against the hypothesis that peripheral formation of angiotensin II is essential in putative renin-dependent drinking. Specifically, the copious water intake following beta-adrenergic activation, which was claimed to be mediated entirely by the renin-angiotensin system (*7*), as well as that resulting from inferior vena cava ligation appeared to be independent of this system.

We used groups of intact and bilaterally nephrectomized male albino rats, and we selected peripheral injection of isoproterenol and ligation of the inferior vena cava as putative renin-dependent drinking stimuli. These stimuli were compared with the dipsogenic potency of exogenous renin, and with the effect of subcutaneous polyethylene glycol (PEG) injection and of water deprivation as prototypes of renin-independent drinking stimuli. The technical aspects of our methods have been described (*8*). Only modifications of previous procedures are mentioned here. SQ (3.0 mg/kg, injected subcutaneously) was administered 10 minutes prior to the actual drinking period. The animals were given immediate access to the tap water source after the parenteral administration of isoproterenol or renin, whereas such access was delayed for 4 hours after injection of PEG (5 ml of Carbowax 20, subcutaneously) or after caval ligation, and for 18 hours in tests of water deprivation thirst. Water intake was measured at half-hour intervals for the succeeding 3 hours in all experiments, and food was withdrawn during this period. Data were analyzed by Student's *t*-test for the statistical significance between groups.

The effect of inhibition of the peripheral angiotensin converting enzyme on the various drinking mechanisms investigated is illustrated for intact and bilaterally nephrectomized rats in Fig. 1. The subcutaneous injection of SQ in the dosages employed did not noticeably alter the general behavior or motor activity.

When intact (unoperated) rats were given SQ (Fig. 1a), putative renin-dependent and renin-independent drinking responses (isoproterenol and caval ligation as compared to PEG and water deprivation) were enhanced, whereas

Table 1. The effect of renin-depletion on thirst induced by either beta-adrenergic stimulation or inferior vena cava ligation. Results are expressed as means ± standard errors of the means. Statistical comparisons were made between experimental and control groups for intact and DCA-saline pretreated animals, respectively. SQ was given subcutaneously; *N*, number of animals.

Treatment	Three-hour cumulative water intake (ml)					
	Intact animals (ml)	*N*	*P*	DCA-saline treatment (ml)	*N*	*P*
Control	1.22 ± 0.36	23		2.81 ± 0.48	16	
β-Adrenergic stimulation	7.50 ± 0.90	12	< .001	5.53 ± 0.52	15	< .001
Caval ligation	7.59 ± 0.92	17	< .001	6.42 ± 1.25	12	< .05
Caval ligation + SQ	18.92 ± 1.19	24	< .001	7.90 ± 1.79	10	< .001

drinking induced by renin given intraperitoneally was not significantly affected. Since the pressor effect of renin was obviated by the dose of SQ given, it would seem that the dipsogenic effect of renin in the intact rat is not dependent upon angiotensin formation, but may be predicated upon an alternate mechanism in the peripheral circulation (*9*). Figure 1b compares the effect on thirst evoked by four of the same stimuli in the presence and absence of SQ in bilaterally nephrectomized rats.

It is apparent that isoproterenol thirst is abolished in the absence of the kidneys, as has been reported (*2, 7, 10*), and that SQ is now without effect. Drinking induced by caval ligation was likewise considered to be kidney dependent, since nephrectomy significantly reduces this thirst stimulus (*4*). Although the enormous thirst seen in cavally ligated rats in the presence of SQ is obviated by prior removal of the kidneys, bilaterally nephrectomized animals still exhibit a significantly larger water intake with than without SQ.

Drinking after the peripheral injection of hog renin is greatly enhanced by prior removal of the kidneys. This is in line with the increased blood pressure–raising potential of renin in bilaterally nephrectomized animals, which has been interpreted as being due to enhanced availability of renin substrate and elimination of an important control mechanism (renal renin inhibitor). Prior treatment of these nephrectomized rats with SQ reduced the renin drinking response by approximately 60 percent, suggesting that, in the absence of the kidneys, peripheral conversion of angiotensin I to angiotensin II is an important factor in the elicitation of renin drinking. This finding contrasts sharply with the lack of interference by SQ with the more modest drinking response elicited by double the dose of renin in the intact rat (Fig. 1a).

The minor increase in water intake produced by peripheral SQ alone in unoperated rats did not occur in the bilaterally nephrectomized animals. The comparative values for the mean (± S.E.M.) intake of water during a 3-hour period for all control experiments were as follows: intact control, 1.22 ± 0.30 ml ($N = 23$); intact + SQ, 2.79 ± 0.38 ml ($N = 24$), $P < .0025$; bilaterally nephrectomized control, 1.00 ± 0.54 ml ($N = 10$); bilaterally nephrectomized + SQ, 0.83 ± 0.21 ml ($N = 12$), $P > .05$.

On the basis of the experiments in Fig. 1, it seemed that, in the intact rat, the formation of peripheral angiotensin II was not important in the mechanism of putative renin-dependent drinking responses. There remained the possibility, however, that angiotensin I might be accumulating in the bloodstream under the influence of peripheral SQ and then might cross the blood-brain barrier in increased concentration. In the brain, it could then be converted to the known dipsogen angiotensin II.

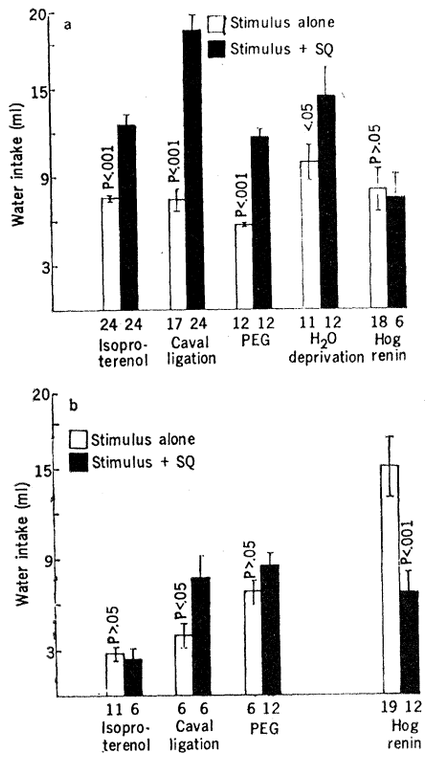

Fig. 1. (a) The effects of the angiotensin converting enzyme inhibitor SQ on putative renin-dependent and renin-independent thirst stimuli in the intact rat. Isoproterenol was administered subcutaneously (0.1 mg per kilogram of body weight), PEG was injected subcutaneously (5 ml of a 20 percent solution per rat), and renin was injected intraperitoneally (6.0 units per 100 g of body weight). The clear bars indicate the effect of the drinking stimulus alone, and the black bars indicate the effect in the presence of SQ (given subcutaneously). The S.E.M.'s are indicated for each group, and the number of animals per group are listed at the bottom of the bars. The water volumes represent the cumulative intake over a 3-hour period per rat. (b) The effects of SQ on putative renin-dependent and renin-independent thirst in the acutely nephrectomized rat. Identical procedures were followed, except that the dose of renin was 3.0 units per 100 g of body weight and that all animals underwent bilateral nephrectomy under ether anesthesia approximately 60 to 120 minutes prior to being used in the drinking experiments.

The possibility of entry of angiotensin I into the brain was studied in a group of rats implanted stereotaxically with cannulas directed at the right lateral ventricle (*11*). On the basis of the work of Severs *et al.* (*12*), it was expected that angiotensin converting enzyme would be selectively inhibited in the brain by the intraventricular injection of SQ, since Severs *et al.* had demonstrated that this procedure inhibited the dipsogenic effect of intraventricular angiotensin I but not that of angiotensin II. They showed, moreover, that peripherally administered SQ did not block central angiotensin I thirst and concluded that SQ does not gain access to cerebral structures when given peripherally and that the central renin-angiotensin system demonstrated by Ganten *et al.* (*13*) was likewise sensitive to the enzyme inhibitor. Swanson *et al.* (*14*) have reported that centrally applied SQ failed to antagonize the dipsogenic effect of angiotensin I in the rat. The disparity between the work of Severs *et al.* and Swanson and co-workers may be due to the difference in both route of administration (intraventricular as compared to intracerebral) and to the time allowed for SQ to act (15 minutes as compared to 1 minute).

In our experiments intraventricular injection of SQ did not modify either isoproterenol or caval ligation thirst (Fig. 2, stippled bars 1 and 3), whereas the intense thirst following intraperitoneal renin administered to the bilaterally nephrectomized rat (blank bar 2) was inhibited by central SQ (stippled bar 2). The enhancement of isoproterenol drinking produced by peripheral SQ (black bar) was likewise obviated by concommitant intraventricular application of the enzyme inhibitor (cross-hatched bar).

These data suggest that the potentiation of drinking in the presence of peripheral SQ may be due to enhanced production of peripheral angiotensin I and its subsequent increased entry into the central nervous system, where, shielded from the action of the enzyme inhibitor, it could be converted to angiotensin II. It would seem, then, that penetration of the decapeptide across the blood-brain barrier in amounts adequate to evoke significant drinking responses occurs only under conditions which favor the formation of high concentrations of angiotensin I in the bloodstream, as, for instance, when under continued substantial renin

release from the kidneys, conversion of the decapeptide to angiotensin II is blocked by SQ.

This view is in line with recent findings in the dog that blocking of the peripheral formation of angiotensin II in hypovolemic or hypotensive states may greatly intensify the release of renin into the bloodstream (*15*). If the presence of a similar mechanism can be demonstrated in the rat, it may account for potent dipsogenic activity of the type originally proposed by Fitzsimons, Epstein, and others, except that under such circumstances it could clearly not be angiotensin II, but its precursor angiotensin I, which passes across the blood-brain barrier. The selective prevention of the *additional* water intake, otherwise evoked by peripheral SQ in isoproterenol and caval ligation drinking, by the intraventricular injection of SQ, without interference with the basic drinking response of these two thirst stimuli, is taken as strong evidence for the renin independency of isoproterenol and caval ligation thirst. By the same token the *additional* drinking is clearly renin dependent and probably the result of angiotensin I entry into the brain. This interpretation is also in line with Epstein's observation that injection of angiotensin I into the superior vena cava of the rat proved a more potent thirst stimulus than angiotensin II, although we believe that the greater potency may likewise be predicated upon direct entry of angiotensin I into the brain (*16*).

Our studies lead us to conclude that, contrary to prevailing opinion (*1–3*), the *primary* mechanism responsible for both isoproterenol and caval ligation drinking does not encompass a significant contribution from the renin-angiotensin system. This view is supported by additional experiments in which participation of this system was suppressed by an entirely different approach.

Adult rats were implanted subcutaneously with 25 mg of deoxycorticosterone acetate (DCA) pellets and offered 1 percent sodium chloride as the exclusive drinking fluid for 21 days. Under this regimen, the kidneys become depleted of renin by more than 95 percent (*17*). Such renin-depleted rats showed an almost undiminished drinking response to beta-adrenergic stimulation and ligation of the inferior vena cava (Table 1).

The minor diminution of total water intake after both beta-adrenergic stimulation and caval ligation in the rats that had been treated with DCA and saline, as compared to untreated animals, is interpreted as due to the difference in baseline values, since the treatment alone causes a significant increase in water intake (from 40 ml to 250 ml in 24 hours), thus slightly blunting the response to the additional dipsogenic stimulus. In contrast, the dramatic enhancement of caval ligation drinking in the presence of peripheral SQ was completely obviated by the depletion of renin stores.

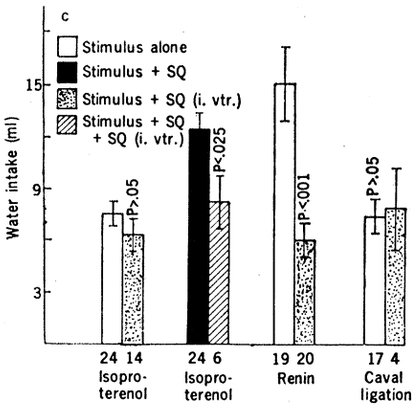

Fig. 2. The effect of angiotensin converting enzyme inhibition in the brain on isoproterenol, caval ligation, and renin drinking. Intraventricular SQ was administered in a dose of 30 μg in 6 μl of isotonic saline, 5 to 10 minutes prior to the start of a drinking study. All other dosages were identical to those utilized in the experiments in Fig. 1, a and b. Nephrectomized rats were used in the renin study. The S.E.M.'s are indicated for each group, and the number of animals per group are listed at the bottom of the bars. The water volumes represent intake over a 3-hour period per rat; *i. vtr.*, intraventricularly; *s.c.*, subcutaneously.

Undiminished thirst that follows caval ligation or peripheral beta-adrenergic stimulation, despite major interference with the renin-angiotensin system either by depletion of renin stores or peripheral and central blockade of angiotensin II formation, puts into question a cause and effect relation between the humoral system and these dipsogenic stimuli. The effectiveness of bilateral nephrectomy, however, in inhibiting or obviating the drinking resulting from these procedures points to the existence of some other renal factor or mechanism that is essential in so-called renin-dependent drinking responses.

Our findings indicate that the mere demonstration of elevated plasma renin levels or abolition of drinking by bilateral nephrectomy should not be taken as adequate evidence for primary involvement of the renin-angiotensin system in dipsogenic activity. The inference derived from our data supporting the proposal that angiotensin I does indeed penetrate the blood-brain barrier may have additional implications with regard to the cardiovascular effects of angiotensin II mediated by the central nervous system, especially when the decapeptide is excessively elevated in the peripheral circulation (*18*).

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References and Notes

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11. Cannula placement was confirmed at the termination of the experiment by the intraventricular injection of 6 μl of India ink. Proper placement was verified by the uniform distribution of ink throughout the ventricular system while intracerebral injection was discernible by local accumulation in the tissue. Intraventricular coordinates were AP = −0.7 (from bregma), L = +1.5, V = −4.0 (from skull surface).
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18. Recent work appears to offer a solution to the perplexing problem created by the persistent failure to actually demonstrate significant penetration of angiotensin (A) I or II across the blood-brain barrier. For one, it

was shown that the subfornical organ, situated in the roof of the third ventricle and *outside* the blood-brain barrier, is extremely sensitive to the dipsogenic effect of A II [J. B. Simpson and A. Routtenberg, *Science* **181**, 1172 (1973)]. Second, it was found that A II, applied intracerebrally at the predilection site in the preoptic area, elicits drinking only when the brain cannula passes through the lateral ventricle and thus permits the entry of injected A II through the punctured blood-brain barrier into the ventricular system (A. K. Johnson, paper presented at the Eastern Psychological Association meeting in Washington, D.C., 1973), providing direct access of A II to the subfornical organ. There is thus no longer any need to postulate passage of peripherally injected A I or A II across the blood-brain barrier in the explanation of their dipsogenic activity.

19. We thank Dr. Giancarlo Guideri for suggestions during the course of this study.

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Color Changes, Unusual Melanosomes, and a New Pigment from Leaf Frogs

Abstract. *Melanosomes of phyllomedusid frogs are unusually large and are composed of an amorphous matrix of thick fibers. Their hitherto undescribed dark red pigment is neither phaeomelanin nor eumelanin, but seems to be related to melanins. Melanophores of at least one of these species,* Agalychnis dacnicolor, *exhibit color change in direct response to illumination, and it is suggested that these chromatophores are innervated.*

Melanophores of all vertebrate classes are remarkably consistent in structure and composition. They contain melanin pigments which show little chemical variation between taxonomic groups and which are found in melanosomes that, in organisms as unrelated as fishes and man, are much alike in origin, development, shape, and size (*1*). The first deviation from this consistent pattern was observed in the dermal melanophores of the phyllomedusid leaf frog, *Agalychnis dacnicolor*, which contains melanosomes that, at more than 1.00 nm in diameter, are twice as large as the usual organelle (*2*). Moreover, these melanosomes are of a dark red color and consist of an electron-dense core that is surrounded by a large concentric matrix of dense fibers.

In order to ascertain whether the red pigment might be the first example of the presence of phaeomelanin in a poikilotherm, skins were removed from the dorsal surface of adult *A. dacnicolor* collected in Sinaloa, Mexico. The skins were preserved in 80 percent ethanol and were subsequently extracted. The most satisfactory results were obtained with 80 to 85 percent dimethyl sulfoxide (DMSO) which produced an extract of an intense red color which when diluted with water formed a red precipitate. The pigment material so precipitated could be purified further by redissolving it in DMSO and by subsequent reprecipitation. The ultraviolet absorption spectrum of the pigment (in DMSO) revealed absorption maximums at 558, 520, 492, 358, 350, 336, and 263 nm, which were little affected by addition of acid or alkali. However, in basic media, for example, 0.1*N* NaOH, the dark red pigment proved to be unstable and was rapidly converted to a yellow product that has an absorption maximum at 400 nm (Fig. 1). On the basis of these preliminary observations, it is evident that the properties of the dark red pigment differ markedly from those of either eumelanin or phaeomelanin; thus, we are dealing with a new type of chromatophore pigment.

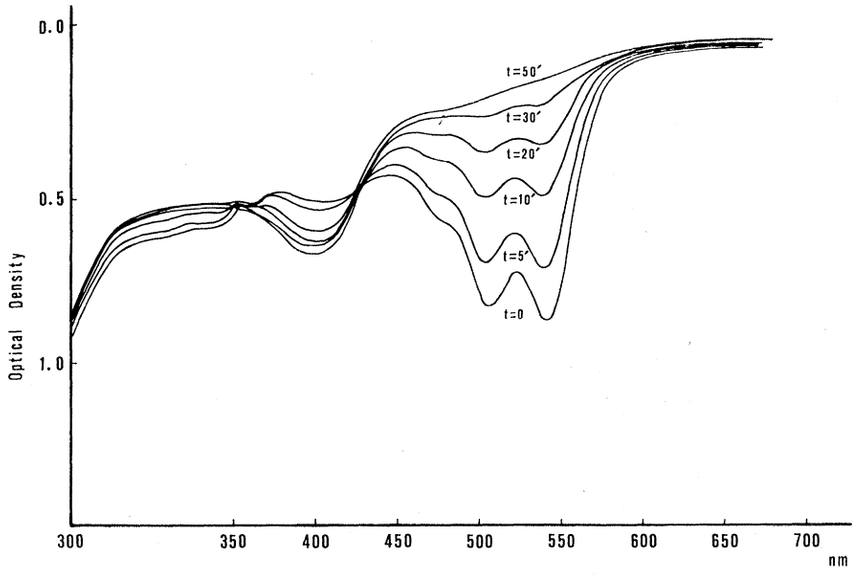

Fig. 1. Time-dependent changes (0 to 50 minutes) in the ultraviolet spectrum in 0.1*N* NaOH of the red pigment from *A. dacnicolor* as it is converted to a yellow product having an absorption maximum at 400 nm.

It is most likely that this new pigment is in some way related to melanins because it is localized in cells that, in terms of morphology, function, and position in the dermal chromatophore unit, are undeniably melanophores (*3*). Accordingly, skins removed from the dorsal surface of *A. dacnicolor* were incubated in Ringer solution containing [^{3}H]dopa and [^{3}H]tyrosine, both labeled in the 2, 3 positions of the side chain, in order to learn whether these melanin precursors were selectively taken up by the skin. In a series of experiments, pieces of dorsal skin from adults of *A. dacnicolor* and from *Rana pipiens*, which served as a control, were washed in amphibian Ringer solution for 3 hours. The skins were then cut into pieces of identical size and incubated for 20, 30, 60, or 90 minutes in fresh Ringer solution which contained either [^{3}H]dopa, [^{3}H]tyrosine, or [^{3}H]-alanine (DL-[3-^{3}H]alanine), the latter serving as a nonspecific control. The radioactivity of the incubation medium for each of the compounds tested was 40 to 50 μc/ml. At the end of the incubation periods the skins were extracted with 10 percent ice-cold trichloroacetic acid for 30 minutes, dehydrated, and analyzed in an LS-200 liquid scintillation spectrometer (Beckman) for radioactivity incorporated into the acid-insoluble skin residue. It was revealed that dopa and tyrosine were quickly incorporated by both *A. dacnicolor* and *R. pipiens* and that uptake increased linearly with time. Skins of *A. dacnicolor* selectively incorporated dopa and tyrosine as indicated by the results of