Roles of Peripheral and Central Angiotensin-Converting Enzyme (ACE) in Hypovolemic Thirst Induced by Compound 48/80 in Rats

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Abstract—Subcutaneous (s.c.) injection of Hoe 498, an angiotensin converting enzyme (ACE) inhibitor, at the doses of 0.1, 0.5, 1.0 and 4.0 mg/kg produced a dose-related inhibition of compound 48/80-induced hypovolemic thirst in rats. A significant time-response relationship was observed between the pretreatment time of Hoe 498 at a dose of 4.0 mg/kg and the inhibition of compound 48/80-induced water intake. Nearly 90% of plasma ACE activity was inhibited by Hoe 498 at all doses used, and this inhibition at the dose of 4.0 mg/kg of Hoe 498 continued for more than 4 hr. Intracerebroventricular (i.c.v.) or s.c. injection of Hoe 498 in doses ranging from 0.5 to 20 μg comparably inhibited plasma ACE activity in a dose-dependent manner. The compound 48/80-induced water intake was significantly reduced by i.c.v. injection of Hoe 498 (20 μg) 30 min after compound 48/80 administration, but not reduced when the drug was given 15 min prior to injection of dipsogen. The inhibition of water intake by Hoe 498 seems to be dependent on the dose and time between administration of Hoe 498 and compound 48/80. The present data suggest that brain ACE is more involved in compound 48/80-induced water intake than peripheral systemic ACE.

Although the exact role of the brain renin-angiotensin system is still controversial, available data suggest that the brain renin-angiotensin system plays an important physiological role in the control of thirst, blood pressure and the release of pituitary hormones such as ADH (antidiuretic hormone) and ACTH (1, 2). Ganten and Speck (3) have previously suggested that different angiotensin II receptors are involved in cardiovascular responses when angiotensin II is injected centrally or peripherally. This is supported by the following experiment showing that blood-borne angiotensin II could not cross the blood-brain barrier at least in physiological concentrations and reach angiotensin-sensitive sites in the brain (4, 5).

Robinson and Evered (6) have recently reported that SQ 14,225 given i.c.v. leaks out of the brain and inhibits angiotensin-converting enzyme (ACE) peripherally as well as centrally, suggesting that the abolition of the dipsogenic effect following i.c.v. injection of ACE inhibitor is not merely due to the inhibition of brain ACE. While studying the mechanism by which hypovolemia elicited by s.c. injection of compound 48/80 produced water intake in rats (7–9), we found that the inhibition of plasma ACE estimated by measurement of plasma ACE activity did not correlate with the reduction of water intake by inhibitors of ACE. This lack of correlation suggested that the primary site of action for such agents may not be the plasma. The present experiments were designed to investigate the involvement of the peripheral and central ACE in compound 48/80-induced water intake with the aid of an ACE inhibitor, Hoe 498 (10).
Materials and Methods

Animals and housing: Male Wistar rats (200–220 g), purchased from Funabashi Farming, Funabashi, Japan, were used in the experiments. They were housed individually in diuresis cages on a 12:12 light-dark cycle initiated at 07:00 hr. Food (Funabashi Farming Rodent Laboratory Chow) and tap water available ad libitum. An ambient temperature of 20–22°C was maintained throughout the study.

Brain dissection and preparation: The animals were sacrificed by decapitation 15 min after s.c. administration of Hoe 498 or saline between 10:00 and 11:00 hr. The brains were quickly removed and dissected into six regions in ice-cold 50 mM Tris-HCl (pH 7.4) as outlined by Glowinski and Iversen (11). Individual brain tissue was disrupted in approximately 10 volumes (W/V) of the above buffer. The homogenates were centrifuged at 1,000 × g for 20 min at 4°C, and the supernatant was stored at −20°C until assay.

Blood collection: Blood samples were collected from the abdominal aorta into heparinized tubes under light anesthesia with ether and centrifuged at 1,000 × g for 15 min at 4°C. Plasma was stored at −20°C until the assay for ACE activity.

Drinking response: To measure water intake, the animals were placed in test cages with access to water in graduated drinking burettes equipped with glass drinking spouts. Before the drinking tests, which were started between 10:00 and 11:00 hr, each animal was removed from its cage, weighed and injected s.c. with compound 48/80. The volume of water intake was recorded directly from the drinking burettes for 3 hr after compound 48/80 injection and one hr after angiotensin II injection. The animals were placed in the test cages one or two days before the experiment to allow them to adapt to the test cages and the glass drinking spouts.

ACE activity assay: ACE activity was assayed in a 125-μl incubation mixture containing 150 mM Tris-HCl (pH 7.4), 450 mM NaCl and 1.0 mM hippuryl-histidyl-leucine (HHL, synthetic tripeptide substrate for ACE) as described previously (12). The reactions were initiated by the addition of plasma (10 μl) or brain tissue homogenates (25 μl), and the reaction mixture was incubated at 37°C for 30 min (plasma) or 90 min (brain tissue homogenates). Plasma ACE activity is expressed as nmoles hippuric acid formed by one ml plasma per min. In the preliminary experiments, the formation of hippuric acid was linear with the time of incubation (15–120 min) and concentration of homogenate added to the assay under the above conditions.

I.c.v. injection: To prepare rats for the i.c.v. injections, the rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and a permanent stainless steel cannula (18-gauge needle) was implanted stereotaxically in the left lateral cerebral ventricle. The references for localizing the lateral ventricle were based on a stereotaxic atlas for the rat brain (13): 1.7 mm lateral to the bregma and 4.0 mm ventral from the dura. I.c.v. injections were made by inserting an injector cannula into the implanted cannula so that it projected 1.0 mm into the ventricle. The injector cannula was connected through polyethylene tubing (PE 10) to a 10-μl syringe containing Hoe 498, SQ 14,225 or 0.9% saline 15 min before and 30 min after s.c. administration of compound 48/80.

Drugs: Compound 48/80 (N-methylmonanisylamine-formaldehyde condensation product) was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Angiotensin I, angiotensin II, saralasin (P-113) and HHL were obtained from Peptide Institute, Inc., Osaka, Japan. Hoe 498, a novel non-peptide and non-sulfur inhibitor of ACE, was synthesized at the Pharma Synthese of Hoechst Aktiengesellschaft, Frankfurt/Main (Federal Republic of Germany). SQ 14,225 was generously donated by Sankyo Corp. (Tokyo, Japan).

Drug administration: Compound 48/80 solutions, prepared in 0.9% saline daily while avoiding light exposure, were injected s.c. at the dose of 3.0 mg/kg. Hoe 498 or SQ 14,225 was dissolved in 0.9% saline and was injected s.c. or i.c.v. 15 min prior to administration of compound 48/80, except where otherwise noted.

Protein determination: Protein content was determined by the Coomassie Brilliant Blue G-250 binding method with bovine plasma
albumin as the standard (14).

**Statistical analysis:** All numerical data are given as the means±S.E. of the means. The significance of the differences was evaluated using the paired or unpaired Student’s t-test and the Welch analysis. The absence of an asterisk indicates that the apparent difference was not significant at the P=0.05 level.

**Results**

**Effects of Hoe 498 on compound 48/80-induced drinking and plasma ACE:** Figure 1 shows the influence of Hoe 498 pretreatment on water intake caused by s.c. administration of compound 48/80 (3.0 mg/kg). Rats were pretreated with Hoe 498 at the dose of 0.1, 0.5, 1.0 or 4.0 mg/kg 15 min prior to compound 48/80 administration. Hoe 498 produced a dose-related inhibition of compound 48/80-induced water intake. On the other hand, the inhibitory pattern of plasma ACE was different from that described for water intake; nearly 90% of the plasma ACE activity was inhibited by s.c. administration of Hoe 498 at all doses tested, and the inhibition at a dose of 4.0 mg/kg of Hoe 498 continued for

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**Fig. 1.** Cumulative water intake after s.c. administration of compound 48/80 in the absence (control) and in the presence of various doses of Hoe 498 in the rats. Hoe 498 at the dose of 0.1, 0.5, 1.0 or 4.0 mg/kg was injected s.c. 15 min prior to the application of compound 48/80 (3.0 mg/kg). Results are expressed as means±S.E.M. (vertical bars) of 6 rats. Asterisks indicate a statistical difference from the control (compound 48/80 alone) at 180 min after compound 48/80 injection, *P<0.01, **P<0.001. (●) compound 48/80 (3.0 mg/kg), (/) Hoe 498 (0.1 mg/kg)+compound 48/80, (A) Hoe 498 (0.5 mg/kg)+compound 48/80, (E) Hoe 498 (1.0 mg/kg)+compound 48/80, (O) Hoe 498 (4.0 mg/kg)+compound 48/80.

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**Fig. 2.** Effects of Hoe 498 on plasma ACE activity. Rats were injected with Hoe 498 at the dose of 0.1, 0.5, 1.0 or 4.0 mg/kg, s.c. Plasma ACE activity was determined 15 min after injection of 0.9% saline (open column) or Hoe 498 (filled columns). Plasma ACE activities in obliquely hatched or cross-hatched columns were measured 1, 2, 3 and 4 hr after 4.0 mg/kg Hoe 498 injection or 15 min after compound 48/80 injection (3.0 mg/kg), respectively. Plasma ACE activity is expressed as nmols hippuric acid formed by one ml plasma per min. Columns are means±S.E.M. (vertical bars) of 6 rats. Asterisks indicate a statistical difference from the control (0.9% saline alone): *P<0.001.
more than 4 hr (Fig. 2). No relationship was observed between the reduction of compound 48/80-induced water intake and the inhibition of plasma ACE activity following s.c. administration of various doses of Hoe 498. Compound 48/80 per se had no effect on plasma ACE activity.

Effect of pretreatment time of Hoe 498 on compound 48/80-induced water intake: Figure 3 shows the correlation between the inhibition of water intake and pretreatment time of Hoe 498. The rats pretreated with Hoe 498 at a constant dose of 4.0 mg/kg at 0, 30, 60, 90 or 120 min prior to compound 48/80 administration drank 1.35±0.45, 2.33±0.40, 2.82±0.49, 3.10±0.42 or 5.70±0.96 ml, respectively, in 3 hr. The highest significant time-response relationship was observed between the pretreatment time and the inhibition of water intake for all samples taken (r=+0.925; P<0.05). Administration of compound 48/80 by the s.c. route at a dose of 3.0 mg/kg of body weight, when given alone, caused rats to ingest 9.12±0.58 ml water in 3 hr (n=9). This drinking response was significantly greater than that of the control rats injected with 0.9% saline (1.08±0.05 ml/3 hr, n=6, P<0.001).

Effects of s.c. or i.c.v. administration of Hoe 498 on plasma ACE activity: To test if Hoe 498
Table 1. Effects of i.c.v. injection of saralasin, SQ 14,225 or Hoe 498 on compound 48/80- or angiotensin II-induced water intake

| Pretreatment (i.c.v.) | Dipsogens                        | Water intake (ml/rat/3 hr) | (N) |
|----------------------|---------------------------------|---------------------------|-----|
| 0.9% saline          | +Compound 48/80 (3 mg/kg, s.c.) | 9.34±0.63                 | (9) |
| Saralasin (10 μg)    | +                               | 8.53±0.65                 | (6) |
| SQ 14,225 (20 μg)    | +                               | 8.40±0.87                 | (9) |
| Hoe 498 (20 μg)      | +                               | 7.60±0.50                 | (6) |
| Hoe 498t (20 μg)     | +                               | 5.05±1.36*                | (6) |
| Saline               | +Angiotensin II (50 ng, i.c.v.) | 8.44±0.87                 | (7) |
| Saralasin (10 μg)    | +                               | 4.08±1.10**               | (8) |

0.9% saline, saralasin, SQ 14,225 or Hoe 498 was given i.c.v. 15 min before the administration of compound 48/80 or angiotensin II unless otherwise stated. *Hoe 498 was injected i.c.v. 30 min after the s.c. administration of compound 48/80. Water intake was measured over 3 hr or one hr after injection of compound 48/80 or angiotensin II, respectively. The number of rats used is shown in parentheses (N). Results are expressed as means±S.E.M. Asterisks indicate a statistical difference from the corresponding control given physiological saline: *P<0.05, **P<0.01.

injected into the lateral ventricle leaks into the periphery and then inhibits plasma ACE activity, plasma ACE activities were compared after the same dose of Hoe 498 was injected s.c. or i.c.v. As shown in Fig. 4, dose-dependent inhibition of plasma ACE activity was observed when Hoe 498 was injected i.c.v. in doses ranging from 0.5 μg to 20 μg. A similar tendency was observed following s.c. administration of Hoe 498. There were no significant differences in plasma ACE activity between the s.c.- and the i.c.v.-injected groups given the same dose.

Effect of i.c.v. injection of Hoe 498, SQ 14,225 or saralasin on compound 48/80- or angiotensin II-induced water intake: Since Hoe 498 given i.c.v. leaks out of the brain and inhibits plasma ACE (Fig. 4), it is necessary to dissociate the cerebral and peripheral effects of Hoe 498 to isolate the cerebral ACE. For this purpose, Hoe 498 was injected at a dose of 20 μg per rat, since this dose given peripherally (0.1 mg/kg, s.c.) did not produce an inhibitory effect on compound 48/80-induced water intake (Fig. 1). As shown in Table 1, Hoe 498 given i.c.v. 30 min after compound 48/80 administration was effective in reducing compound 48/80-induced water intake. On the contrary, Hoe 498 (20 μg), SQ 14,225 (ACE inhibitor, 20 μg) or saralasin (angiotensin II-receptor blocking agent, 10 μg) given 15 min prior to compound 48/80 administration had no effect at any time measured. Saralasin injected in a similar manner significantly reduced i.c.v. angiotensin II-induced water intake.

Effects of s.c. administration of Hoe 498 on brain ACE activity: Figure 5 represents the effects of s.c. administration of Hoe 498 on the ACE activity in various brain regions of 4 rats. The rats were decapitated 15 min after s.c. administration of Hoe 498 in doses of 0.1, 0.5, 1.0 or 4.0 mg/kg, and the seven regions of the brain were dissected and were assayed for ACE activity as described in Materials and Methods. The control ACE activities in the cerebral cortex, cerebellum, medulla oblongata, hypothalamus, striatum and mid brain+hippocampus were 454.01±80.35, 2526.28±381.90, 590.34±92.32, 541.13±32.05, 4853.53±801.26 and 3205.73±148.13 pmol hippuric acid/mg protein/min, respectively. These values of the brain ACE activity measured by HPLC were approximately one-fifth those determined in the fluorometric assay by Mendelsohn et al. (15). The data presented in Fig. 5 are expressed as % ACE activity of the control given saline s.c. Hoe 498 at a dose of 4.0 mg/kg significantly attenuated the ACE activity of all seven regions. However, we could not detect a dose-dependent inhibition of ACE activity in any brain region measured following s.c. administration of Hoe 498.

Discussion

In previous papers, we suggested that the s.c. administration of compound 48/80, a
well-known histamine liberator (16–19), caused an increase in vascular permeability to protein (increase of plasma extravasation), presumably through histamine H1-receptors, which produced a fall in plasma volume (hypovolemia). Hypovolemia in turn stimulated the juxtaglomerular cells of the kidney to release renin and induced drinking and analgesia in the rats (7–9, 12, 20).

As shown in Figs. 1 and 2, despite a dose-related reduction of compound 48/80-induced water intake by s.c. administration of Hoe 498, this inhibition did not correlate with inhibition of plasma ACE activity; nearly 90% of the plasma ACE was inhibited by Hoe 498 at all doses used, and this inhibition at a dose of 4.0 mg/kg of Hoe 498 continued for more than 4 hr. On the other hand, Fig. 3 shows the correlation between the inhibition of compound 48/80-induced water intake and pretreatment time of Hoe 498. These results indicate that inhibition of plasma ACE and hence conversion of angiotensin I to angiotensin II in the systemic circulation is not so deeply involved in compound 48/80-induced water intake and suggest that brain ACE is more involved in compound 48/80-induced hypovolemic thirst.

The effect of s.c. or i.c.v. administration of ACE inhibitors on thirst are controversial and difficult to interpret. Lehr et al. (21) reported that s.c. injection of SQ 20,881 enhanced drinking in response to a number of stimuli. On the other hand, i.c.v. or s.c. injection of SQ 14,225 or SQ 20,881 was reported to inhibit the dipsogenic effect of s.c. injection of isoproterenol, a beta-adrenergic drug, or i.c.v. injection of angiotensin I or renin (22–27). Our data showing similar plasma ACE activity between the i.c.v.- and s.c.-injected groups at the same dose of Hoe 498 (Fig. 4) suggest that Hoe 498 injected i.c.v. easily leaks into the circulation. These results are in agreement with those of Robinson and Evered (6) who reported that i.c.v. injection of 20 μg SQ 14,225 given 20 min before the determination significantly reduced both the maximal rise in arterial pressure and the duration of the pressor response to i.v. injection of renin.

As shown in Table 1, i.c.v injection of Hoe 498, SQ 14,225 or saralasin 15 min prior to compound 48/80 administration did not
show any inhibitory effect on compound 48/80-induced water intake, despite the significant reduction of i.c.v injected angiotensin II-induced water intake by the similar injection of saralasin. This inhibitory effect of saralasin on angiotensin II-induced thirst was consistent with the results of Fitzsimons et al. (25) and Tonnaer et al. (27). On the other hand, the i.c.v. injection of Hoe 498 30 min after compound 48/80 administration caused a significant reduction of water intake. These data suggest that routes of administration and pretreatment time of ACE inhibitor seem important in evaluating the effect of ACE inhibitor on a thirst stimulus.

The present level of understanding indicates that the inhibition of plasma ACE does not necessarily relate to the reduction of compound 48/80-induced hypovolemic thirst, and suggest the involvement of brain ACE in this thirst. However, a correlation between the inhibition of compound 48/80-induced water intake (Fig. 1) and the inhibition of brain ACE activity in any region (Fig. 5) following s.c. administration of various doses of Hoe 498 was not observed in the present study. Further investigation is necessary to establish more precisely the role of brain ACE in compound 48/80-induced hypovolemic thirst.

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