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Central Angiotensin Converting Enzyme Blockade and Thirst

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DI NICOLANTONIO, R., F. A. O. MENDELSOHN AND J. S. HUTCHINSON. *Central angiotensin converting enzyme blockade and thirst.* PHARMACOL BIOCHEM BEHAV **18**(5) 731–735, 1983.—The role of endogenous brain angiotensin II (AII) in various thirst states was examined in the rat using the angiotensin converting enzyme inhibitor, captopril. Intracerebroventricular (ICV) captopril (7 μg) significantly attenuated the dipsogenic response to centrally administered angiotensin I (AI) (200 ng) for up to 2 hours. The same dose of captopril significantly potentiated the dipsogenic response to ICV AII (100 ng) but failed to alter the dipsogenic response to ICV carbachol (200 pmoles). Central pretreatment with captopril (7 μg), for 30 minutes, failed to alter markedly the cumulative water intake of 24 hour water deprived rats. However, a small, significant 8% decrease in water intake was noted in ICV captopril treated rats 60 minutes following the return of water. The same dose of captopril, administered intraperitoneally, significantly potentiated the cumulative water intake of 24 hour water deprived rats. Central pretreatment with captopril (7 μg), for 30 minutes, failed to alter the cumulative water intake of rats treated intraperitoneally with hypertonic saline (0.75 M given at a dose of 1% of the body weight). From these studies it would appear that central angiotensin converting enzyme plays only a minor role in thirst induced by water deprivation.

Angiotensin Water intake Drinking behaviour Captopril Carbachol Renin-angiotensin system Intraperitoneal hypertonic saline

ANGIOTENSIN II (AII) has been shown to be a potent dipsogen in every species tested [12]. As little as 10^{-16} mole of AII, when applied to circumventricular structures such as the subfornical organ of the brain, causes a normal, water replete rat to seek out and ingest water [24]. The finding that all the components of the renin-angiotensin system (RAS) are present within the central nervous system [13, 21, 22] and that centrally administered components of the RAS are equipotent with AII in stimulating thirst [28] suggests a functional role for the RAS in the brain. Consequently it has been suggested that local generation of AII in the brain may play a physiological role in the control of thirst [13,14].

In order to assess the role of brain AII in thirst Summy-Long and Severs [25] examined the effect of intracerebroventricular (ICV) administration of the octa-peptide competitive AII-receptor antagonist, saralasin, or the nona-peptide inhibitor of angiotensin converting enzyme, SQ20881, on thirst produced by intraperitoneal hypertonic saline or hyperoncotic polyethylene glycol injection in the rat. They found in acute studies that ICV pretreatment with either saralasin or SQ20881 failed to modify the water intake following either of these thirst stimuli and the authors concluded that central AII was not important in eliciting thirst [25]. Malvin and co-workers infused saralasin into the cerebral ventricle of rats in order to investigate the role of AII in thirst due to water deprivation [18]. They found that an infusion of saralasin commenced 30 minutes prior to the return of water to 30 hour water deprived rats markedly increased the subsequent water intake. It was suggested that this potentiation may have been due to the partial agonist action of saralasin [18]. In order to avoid this effect, in a second experiment ICV infusion of saralasin was commenced 75 minutes prior to the return of water to 30 hour water-deprived rats [18]. Under these conditions, saralasin was found to markedly attenuate the cumulative water intake of the water-deprived rats upon return to water [18]. Hoffman and co-workers showed that combined central pretreatment with saralasin and atropine, but neither treatment alone, significantly attenuated the water intake of water deprived rats [14]. Recently, however, Lee *et al.* have failed to duplicate Malvin's findings and the role of endogenous AII in thirst is still controversial [16].

Saralasin has agonist activity in a variety of systems [19]. This makes interpretation of drinking experiments using peptide antagonists of the renin-angiotensin system difficult [12]. Furthermore, it has been suggested that saralasin and SQ20881 have diminished tissue access when compared to the newer low molecular weight, angiotensin converting enzyme inhibitor, captopril [2]. Captopril is believed to be a potent and specific inhibitor of angiotensin converting enzyme with no other significant demonstrated pharmacological activity [1] at low doses.

We therefore decided to reassess the role of brain AII in water deprivation induced thirst using the angiotensin converting enzyme (ACE) inhibitor, captopril.

METHOD

Animals

Male Wistar Kyoto (WK) rats (350–400 g) were used in this study. The rats were maintained in individual metabolic cages and offered tap water from bottles fitted with dripless stainless steel spouts. The animals were housed under conditions of 12 hour light:12 hour dark (lights on 0700) and room temperature was maintained at approximately 22°C. The drinking response was estimated by weighing water bottles before and after the various treatments.

Surgery

Animals were lightly anaesthetised with ether and a length of PE50 cannula was implanted into the left lateral cerebral ventricle at co-ordinates AP1.0, L1.5, H4.0 using the bregma as reference. Following surgery, animals were returned to their home cages and a four day recovery period was allowed before experiments.

Effectiveness and Specificity of Central ACE Blockade with ICV Captopril

Eight WK rats were pretreated for 30 minutes, in a randomized fashion, with an ICV injection of either 10 μl of artificial CSF or 7 μg of captopril in 10 μl of artificial CSF [10]. This dose and regime had previously been shown to significantly inhibit central ACE [27]. Following this pretreatment period each animal received an ICV injection of 200 ng of AI in 10 μl of artificial CSF. The drinking response was followed for 30 minutes. Drinking was generally completed after 10–15 minutes. Following a 2 day recovery period, the experiment was repeated with those animals which were pretreated with vehicle receiving captopril and vice versa. Hence each animal served as its own control.

An identical experimental design was used to examine the effect of ICV captopril, at the same dose, on drinking elicited by ICV injection of 100 ng AII. This dose of AII has previously been shown to be an effective dipsogen in the rat [5]. It was found that AI was a less potent dipsogen than AII on an equimolar basis. Hence the dose of AI used above is that giving a similar dipsogenic response to 100 ng AI. Similarly, an identical crossover design was used to examine the effect of ICV captopril, at the same dose, on drinking elicited by ICV injection of 200 pmole of carbachol; an agent believed to cause thirst by mechanisms independent of AII [12]. This dose of carbachol has previously been shown to be an effective dipsogen in the rat [5].

Time-Course of Central ACE Blockade Following ICV Captopril

Eight WK were pretreated in a random fashion with an ICV injection of either 7 μg captopril in 10 μl of CSF or 10 μl of artificial CSF. Those rats which received CSF alone were given an ICV injection of 200 ng of AI 30 minutes and also 5 hours following the CSF-pretreatment. Those rats receiving captopril were given ICV injections of 200 ng of AI 30 minutes, 1, 2 and 5 hours following the captopril pretreatment. The drinking responses following ICV AI were observed for 30 minutes. Following a 2 day recovery period the experiment was repeated using a crossover design, as described above.

The drinking responses of the rats which were captopril-pretreated were expressed as a percentage of the mean water intake of CSF-pretreated rats at 30 min and 5 hours.

Effect of ICV Captopril on Drinking Due to Water Deprivation

Ten WK rats were deprived of water for 24 hours and then, 30 minutes prior to the return of water bottles, rats were treated in a randomized fashion with an ICV injection of either 10 μl of CSF or 7 μg of captopril in 10 μl of CSF. Following the return of water bottles, the cumulative water intake was monitored at 15 minute intervals for one hour. Following a three day recovery period the animals were again deprived of water. The experiment was repeated with those animals which were previously pretreated with vehicle receiving captopril and vice versa. The three day recovery period was chosen on the basis of previous work [5,6] and the normal reactions of the animals to various stimuli at this time.

Effect of Intraperitoneal Captopril on Drinking Due to Water Deprivation

Eight WK rats were deprived of water for 24 hours and then, 30 minutes prior to the return of water bottles, rats were treated in a randomized fashion with an intraperitoneal (IP) injection of 0.9% saline (1 ml/kg) or 7 μg of captopril in 0.9% saline (1 ml/kg). Following the return of water bottles, the cumulative water intake was monitored at 15 minute intervals for one hour. Following a 3 day recovery period the animals were again water deprived and the experiment repeated using the crossover design described above.

Effect of ICV Captopril on Drinking Due to IP Hypertonic Saline

Eight WK rats were pretreated in a random fashion with an ICV injection of either 7 μg of captopril in 10 μl of CSF or 10 μl of CSF alone. Thirty minutes following ICV pretreatment all rats received an IP injection of 0.75 M saline at a dose of 1% of the body weight. This dose of saline has been shown to be an effective dipsogen in the rat [5]. The cumulative water intake after this manouvre was followed at 15 minute intervals for one hour.

Statistics

The significance of changes was assessed by paired t-test and two-way analysis of variance comparing the vehicle and treatment periods for each rat.

RESULTS

Following central injections, no changes in gross behaviour, other than drinking behaviour, were observed and animals appeared normal.

Effectiveness and Specificity of Central ACE Blockade with ICV Captopril

The effect of ICV administration of captopril (7 μg) or vehicle (CSF) on the drinking response to AI (200 ng), AII (100 ng) or carbachol (200 pmole) is shown in Fig. 1. While captopril caused a marked, significant suppression of AI-induced drinking ($t=11.2$, $p<0.01$), it caused a small but sig-

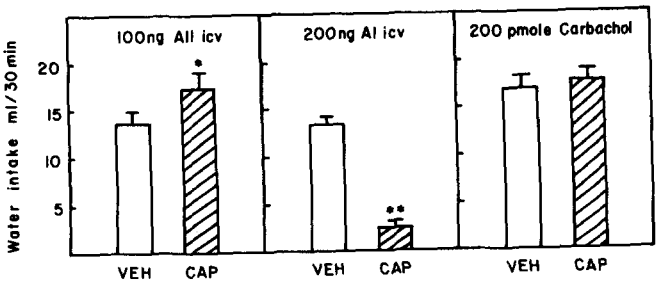

FIG. 1. The effect of ICV pretreatment, for 30 minutes, with either captopril (7 μg, hatched bars) or vehicle (CSF, open bars) on the dipsogenic response to ICV AII, AI or carbachol. Results are the mean±SEM of eight observations. $^{*}p<0.05$, $^{**}p<0.01$.

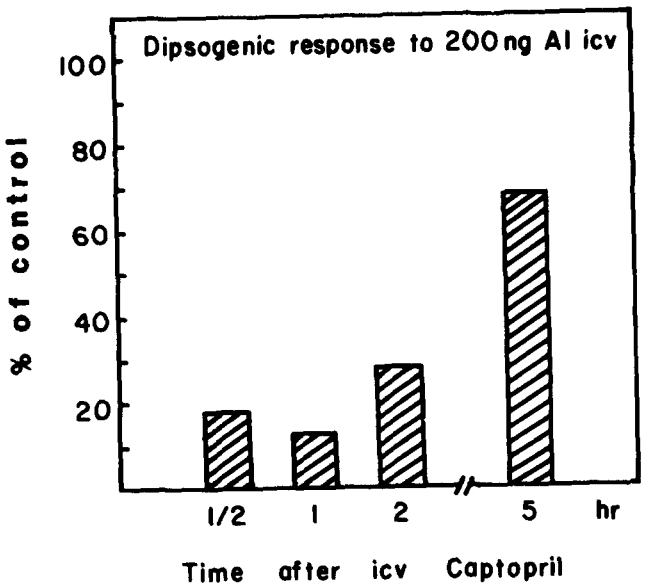

FIG. 2. Time course of central angiotensin converting enzyme blockade following ICV captopril (7 μg). Results represent the response to AI following ICV captopril-pretreatment expressed as a proportion of the AI response in ICV vehicle-pretreated controls at the same time periods.

nificant potentiation of AII-induced drinking ($t=2.3, p<0.03$) and did not significantly alter carbachol induced drinking ($t=0.7$, $p>0.2$) compared to their respective vehicle-treated controls. This indicates that ICV captopril, at the dose we used, effectively inhibited central angiotensin converting enzyme.

Time Course of Central ACE Blockade Following ICV Captopril

The time course of central ACE blockade, as assessed by AI drinking responses, following ICV captopril (7 μg) is shown in Fig. 2. The AI drinking response was suppressed by approximately 70% for at least 2 hours following the ICV treatment with captopril. By 5 hours the AI drinking response had returned to 75% that of vehicle treated rats.

Effect of ICV Captopril on Drinking Due to Water Deprivation

The effect of ICV captopril (7 μg) on drinking following

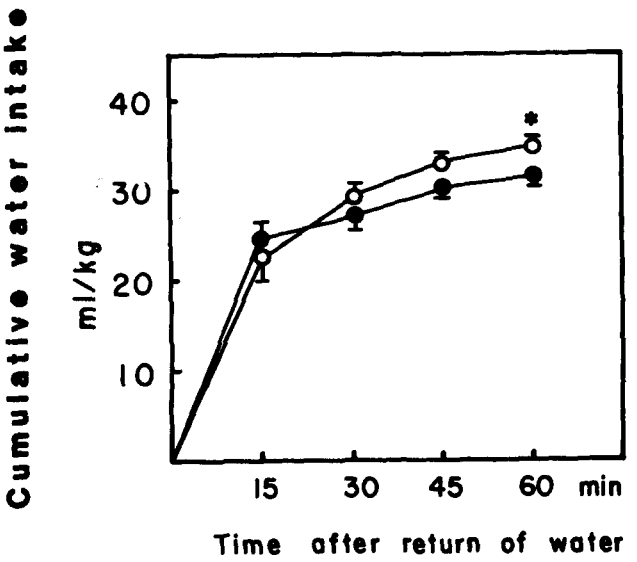

FIG. 3. The effect of ICV pretreatment, for 30 minutes, with either captopril (7 μg, ●) or vehicle (CSF, ○) on the cumulative water intake of 24 hour water deprived rats. Results are the mean±SEM with ten rats in each group; $^{*}p<0.05$, paired t-test.

water deprivation is shown in Fig. 3. Central captopril failed to alter the cumulative intake of 24 hour water-deprived rats at 15, 30 or 45 minutes following the return of water. However, there was a small, significant 8% decrease ($t=1.8$, $p<0.05$) in cumulative water intake of captopril treated rats compared to vehicle treated rats 60 minutes following the return of water. When compared over the entire time period there was no significant difference in water intake between the captopril and vehicle treated groups, $F(1,96)=0.6$, $p>0.4$.

Effect of Intraperitoneal Captopril on Drinking Due to Water Deprivation

The effect of IP captopril on the water intake of 24 hour water-deprived rats is shown in Fig. 4. Peripherally administered captopril (7 μg) significantly potentiated the cumulative water intake of 24 hour water deprived rats at 15 ($t=3.0$, $p<0.01$), 30 ($t=2.9$, $p<0.01$), 45 ($t=3.8$, $p<0.002$) and 60 minutes ($t=3.1$, $p<0.01$) following the return of water. Furthermore when compared over the entire time period captopril treated animals had a significantly greater cumulative water intake than the vehicle treated group, $F(1,80)=11.1$, $p<0.002$.

Effect of ICV Captopril on Drinking Due to IP Hypertonic Saline

The effect of ICV captopril (7 μg) on the cumulative water intake of rats treated with IP hypertonic saline is shown in Fig. 5. Central captopril pretreatment did not significantly alter the cumulative water intake at any time following IP hypertonic saline injection, $F(1,48)=0.1$, $p>0.5$.

DISCUSSION

Intracerebroventricular injection of a low dose of captopril in this study markedly attenuated the dipsogenic response

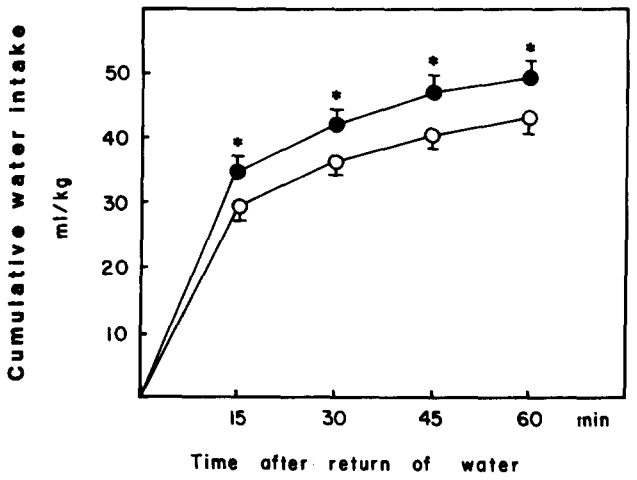

FIG. 4. The effect of IP pretreatment, for 30 minutes, with either captopril (7 μg, ●) or vehicle (0.9% saline, ○) on the cumulative water intake of 24 hour water deprived rats. Results are the mean±SEM with eight rats in each group; *$p<0.05$, paired t-test.

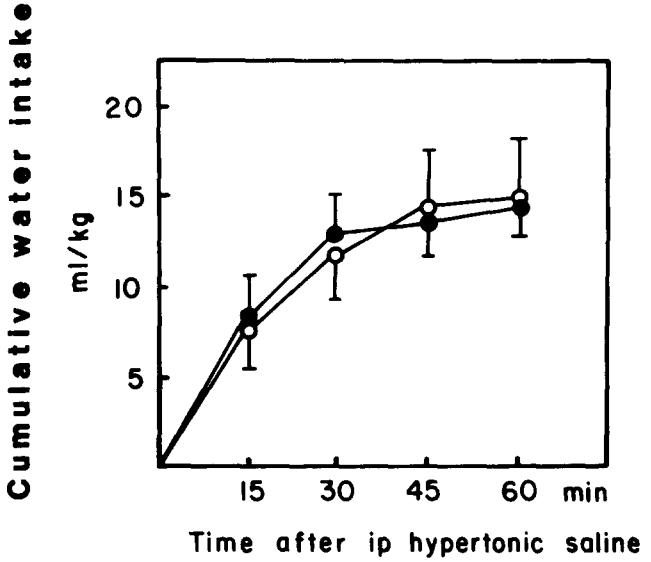

FIG. 5. The effect of ICV pretreatment, for 30 minutes, with either captopril (7 μg, ●) or vehicle (CSF, ○) on the cumulative water intake of rats treated with IP hypertonic saline (0.75 M, in 1% of the body weight). Results are the mean±SEM with eight rats in each group.

to ICV administration of AI. This indicates effective blockade of angiotensin I converting enzyme (ACE) under these conditions. At the same time the drinking response to exogenous ICV AII was potentiated; this is consistent with reduced endogenous brain AII which would be expected following blockade of AI conversion. The same regime did not alter drinking following ICV carbachol illustrating the specificity of the drug since carbachol is a dipsogen believed to act independently of central AII mechanisms [12]. The finding that ICV captopril inhibited central ACE, as measured by the dipsogenic action of AI, for up to 2 hours allowed us to examine the effect of central ACE blockade in short-term drinking studies following various manouvres.

Intracerebroventricular captopril, at the dose used in this study, did not markedly alter the fluid intake of water-deprived rats upon the return of water. However, there was a small, significant 8% decrease in the cumulative water intake of ICV captopril treated rats compared to vehicle treated controls. This small effect of captopril on water-deprivation induced drinking cannot be explained by a non-specific effect of the drug as ICV captopril failed to modify the water intake of rats treated with IP hypertonic saline. The dipsogenic effect of hypertonic saline is believed to act independently of the renin-angiotensin system [12]. Angiotensin converting enzyme has been shown to degrade bradykinin [7] and enkephalin [9] and, therefore, central ACE blockade could raise the central levels of these peptides. However, since the opioids stimulate ingestive behaviour [4,20] and in one study central administration of bradykinin failed to elicit drinking in the rat [11], it seems most likely that the effect of converting enzyme blockade reflects decreased AII formation rather than changes in these other neuropeptides.

Peripheral administration of ACE inhibitors has been shown to cause increased drinking in the undisturbed [23] and water deprived [3, 17, 23] rat. Recent evidence suggests that this effect is due to an increased level of circulating AI, with subsequent conversion to AII, at brain regions inaccessible to peripherally administered captopril, where it stimulates thirst [23]. Similarly in this study a low dose of captopril significantly potentiated the water intake of water deprived rats. Hence the small fall in water intake following ICV captopril treatment cannot be due to a peripheral effect following leakage from central sites.

Intraperitoneal pretreatment with a large dose of captopril (50 mg/kg) for 45–60 minutes has been reported to significantly attenuate drinking in 24 hour water deprived rats [3]. Lower doses of captopril and shorter pretreatment periods were without effect. However, it has been shown that blockade of peripheral angiotensin conversion occurs at 10-fold lower doses of captopril and within minutes of peripheral administration [26]. Hence the effect of these high doses of captopril on drinking seen in this previous study may have been due to either a non-specific effect of the drug or alternatively reflect the dose necessary to gain access to a restricted site in the brain [8, 13, 14, 23]. This possibility is supported by our previous finding that following a single intravenous injection of captopril none is detectable in rat CSF although after chronic treatment significant amounts are found in CSF [15]. Thus central actions of peripherally administered captopril may be dose- and time-dependent. In this study we have avoided the problem of access to central sites by using the ICV route of administration of a low dose of captopril (~0.02 mg/kg).

While the small effect of ICV captopril on the water intake of water-deprived rats is statistically significant, its biological significance is unclear. The current findings do not support a major role for central angiotensin II in stimulating thirst after 24 hours of water deprivation in the rat. However, further studies using longer periods of captopril pretreatment may be warranted.

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