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Role of Angiotensin in the Dipsogenic Effect of Bradykinin in Rats

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ROWLAND, N. E. AND M. J. FREGLY. *Role of angiotensin in the dipsogenic effect of bradykinin in rats.* PHARMACOL BIOCHEM BEHAV **57**(4) 699–705, 1997.—We have previously shown that peripheral administration of bradykinin (BK) induces water intake in rats acutely pretreated with captopril, a kininase II inhibitor. We now show that BK-induced drinking is also observed in rats treated chronically with dietary captopril, and that this is reversed by Hoe 140, a BK receptor antagonist. Both acute and chronic captopril in combination with BK caused a large increase in plasma renin activity. Fos-like immunoreactivity (Fos-ir: used as a marker of cellular activation) was induced by BK + captopril in regions of the brain previously associated with action of angiotensin (Ang) II, including the circumventricular organs and the magnocellular hypothalamic nuclei. However, while water intake induced by peripheral administration of Ang I was potentiated by acute administration of captopril, it was suppressed by chronic captopril treatment. Fos-IR induced in brain by Ang I was not markedly affected by either acute or chronic treatment with captopril. The simultaneous occurrence of potentiated drinking to BK and inhibited drinking to Ang I following chronic treatment with captopril suggest that different mechanisms of action are involved. In order to further examine this possibility, rats were given lesions of the anterodorsal third ventricle region. Lesions that completely abolished the water intake following administration of Ang II only partly attenuated water intake induced by BK + captopril. Further, Fos-IR induced by BK + captopril was only partly (31%) reduced in the supraoptic and paraventricular nuclei of lesioned rats compared with sham operated controls. We suggest that at least two mechanisms, one Ang-related, underlie drinking after BK + captopril. © 1997 Elsevier Science Inc.

Kininase II Angiotensin I Spillover hypothesis Captopril Subfornical organ Anterodorsal third ventricle lesion

PERIPHERAL administration of bradykinin (BK) to rats is not dipsogenic, possibly because it is rapidly degraded by ubiquitous enzymes such as kininase II (K2). Administration of a K2 inhibitor such as captopril retards the degradation of BK (2,3,24). Several years ago, we showed that peripheral administration of BK to rats acutely pretreated with captopril produced a vigorous drinking response (7). More recently, we reported that plasma renin activity (PRA) of rats was greatly elevated by the combination of captopril and BK (18). Further, the angiotensin (Ang) II type I receptor (AT-1R) antagonist, losartan, partially blocked the drink induced by BK and the K2 inhibitor, enalapril (18).

These results complement the potentiation by K2 inhibitors of the water intake induced in rats by administration of either Ang I or hypotensive agents that increase PRA such as isoproterenol (5,6,17). It is widely believed that this potentiation results from increased plasma concentrations of Ang I which then gain access to brain circumventricular organs (CVOs) that have both a weak blood brain barrier and high concentrations of K2. Low doses of the K2 inhibitors are assumed to have little effect on K2 activity in the CVOs, so conversion of Ang I to Ang II may still proceed despite maximal inhibition of the enzyme in the periphery (4,5,6,10,12,14,15,25). Consistent with this view, we have shown that low doses of either captopril or enalapril potentiate neuronal activation, assessed by induction of Fos, by isoproterenol in the subfornical organ (SFO) and organum vasculosum laminae terminalis (OVLT) (19).

The situation after chronic administration of K2 inhibitors is less clear. Rats given dietary treatment with either captopril or ramipril for 5–6 days no longer show significant potentiations of Ang I-induced water intake (17). Another study using a 3–4 week treatment found either no change or a decrease in Ang I-induced drinking (7). This loss of potentiation of Ang I-induced drinking by K2 inhibitors during chronic administration contrasts with the ability of the same doses of

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these agents to induce sodium appetite after several days in rats (4,15,17,22). The natriorexigenic mechanism of K2 inhibitors is often assumed to be similar to that proposed for water intake (4,15). However, if these agents progressively lose their Ang I-potentiating effect, it is difficult to reconcile this with an increasing sodium appetite. In order to assess the generality of the apparent loss of effect of K2 inhibitors on water intake with chronic treatment, we now examine BK-induced water intake. In order to assess further the involvement of Ang II in BK-related drinking, we examined the effects of either treatment with losartan or lesions aimed at the anterodorsal third ventriclular region (AD3V), on BK drinking with both acute and chronic captopril treatment.

METHOD

Animals and Housing

Eighty adult (360–420 g) male Sprague–Dawley rats (Harlan Industries) were used in this study. They were housed singly in stainless steel cages in a standard vivarium (12L:12D cycle (on 0600–1800 h), temperature 23 ± 2°C) with food and tap water available ad lib except as noted.

Drugs and Reagents

Captopril HCl was a gift from the Squibb Pharmaceutical Company, Princeton NJ. Losartan potassium was a gift from Dr. Ronald Smith of the DuPont-Merck Company, NJ. Ang I and BK were from the Sigma Chemical Company, St Louis, MO (catalog #s A9650 & B3259). Hoe 140 was from Bachem, Torrance, CA (catalog # PBRA 92). The Fos polyclonal primary antibody (SC52) was from Santa Cruz Biotechnology (Santa Cruz, CA), and the secondary biotinylated goat anti-rabbit IgG from Zymed Labs (San Francisco, CA). The avidin-biotin complex (ABC) reagents were the Vectastain Elite kit from Vector Laboratories (Burlingame, CA). The Ang I radioimmunoassay kit was from DuPont-New England Nuclear.

Acute and Chronic Captopril, BK Drinking and Plasma Renin Activity

For six days prior to the drinking test, 24 rats were fed powdered Purina Rodent Chow (#5001) from glass jars attached to the inside of the cage with a spring. The other 24 rats were fed powdered Chow to which captopril had been thoroughly mixed at a concentration of 1 g/kg Chow. This yields a dose of about 75 mg captopril/kg body weight/day that is known to induce sodium appetite (7). On the day of the drinking test, food and water were removed from the cages at 0900 h. Starting at 1030 h, 12 rats each from the captopril-treated and control groups received an IP injection of 0.15M NaCl (vehicle; 1 ml/kg) and the other 12 rats from each dietary group received an injection of captopril (3 mg/kg). Twenty min later, rats from each diet and preinjection condition received an SC injection of either vehicle (n = 6) or BK (250 μg/kg; n = 6). Rats were returned immedately to their home cage on the front of which a graduated tube of tap water, with a sipper spout, was attached. Water intake was recorded 30, 60 and 120 min later. In all cases intake was complete within 60 min so, for brevity, only the 60 min intakes will be presented.

After the drinking test, food and water were returned to each rat. The following day, the rats received the same injections as on the first day. However, neither water nor food was available after injection. Twenty min after the second injection, the rats were sedated briefly with methoxyflurane and a 0.3 ml blood sample taken by cardiac puncture. The blood was saved in ice-cold EDTA-treated tubes, plasma separated, and later assayed for PRA by radioimmunoassay as the Ang I generated from a 10 μl sample in 1 h at 37°C.

Acute and Chronic Captopril and Ang I-induced Drinking

In order to confirm prior results with Ang I, additional rats were given either regular food (n = 12) or captopril-added food for 6 days, as described above. A drinking test, as described above, was performed on the 6th day. Six of the rats fed the regular diet received an acute injection of captopril (3 mg/kg, SC) followed 15 min later by Ang I (250 μg/kg, SC). The remaining 6 rats fed regular diet and the 6 rats fed the captopril diet were injected with saline, followed 15 min later by Ang I. Water intake was measured, in the absence of food, after 1 h.

Effect of Either BK or Ang II Receptor Antagonists

In order to demonstrate that the dipsogenic action of BK after chronic captopril was mediated by BK type 2 receptors, additional rats (n = 6/group) were injected acutely with captopril (3 mg/kg) and either Hoe 140 (50 μg/kg, SC) or saline, followed 15 min later by BK (300 μg/kg). Water intake was measured as above. Immediately after this test, the rats were fed the diet with added captopril for 6 days. On the 7th day, the drinking test with BK was repeated with pretreatment with either Hoe 140 or saline (no extra captopril injection was given, since this had no effect in the first study).

In order to assess whether the involvement of Ang II differed between acute and chronic captopril conditions, additional rats (n = 6/group) were given either Ang I (250 μg/kg SC) or acute captopril (3 mg/kg) followed by BK (250 μg/kg SC) with either losartan (20 mg/kg IP) or saline given 20 min before the dipsogenic peptide. Water intake was measured as before. Following these acute tests, the rats were given dietary captopril for 5–6 days as before, and the effects of either saline or losartan pretreatment on either BK- or Ang I-induced drinking again determined.

Effect of Acute or Chronic Captopril on BK-induced Fos-IR

To assess whether BK activates the same brain regions in rats given chronic captopril, compared with those we have reported after acute captopril (19), six rats were given dietary captopril for 6 days as above. On the test day, all received BK (300 μg/kg SC) but were given no water prior to being anesthetized (pentobarbital Na, 50 mg/kg) 60 min later. (Two of these rats additionally received 3 mg captopril/kg 15 min before the BK but, since this produced no effect above that of the dietary captopril, their data were combined). Other rats received the following treatments: dietary captopril and vehicle injection (n = 3), dietary captopril plus Ang I (250 μg/kg SC; n = 3), acute captopril (3 mg/kg) plus Ang I (n = 2), Ang I only (n = 4), and Ang II (200 μg/kg SC, n = 2) only. All rats were without water and were anesthetized 60 min after the last injection.

They were then perfused intracardially with heparinized saline followed by 4% buffered paraformaldehyde, as described previously (20). The brains were sectioned coronally at 100 μm on a Vibratome, the floating sections treated with sodium borohydride (1%), rinsed well, then incubated with Fos primary antibody (SC52: Santa Cruz; 1 : 20,000) for 24 h

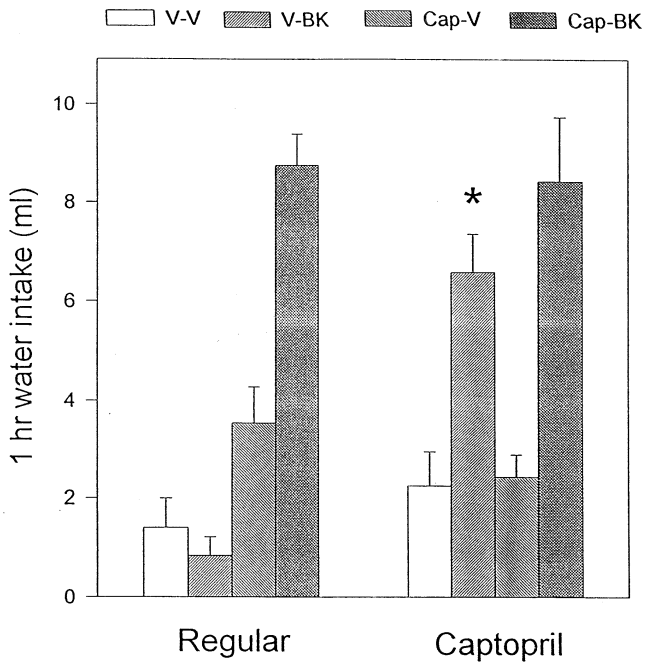

FIG. 1. Mean (± SE; $n = 6$) 1 h water intake of rats fed either regular (left) or captopril-added (right) food for 6 days and then treated acutely, as indicated in the legend above the graph, with either Vehicle or captopril (cap) followed by either vehicle (V) or bradykinin (BK). *$p < 0.01$ difference between dietary groups.

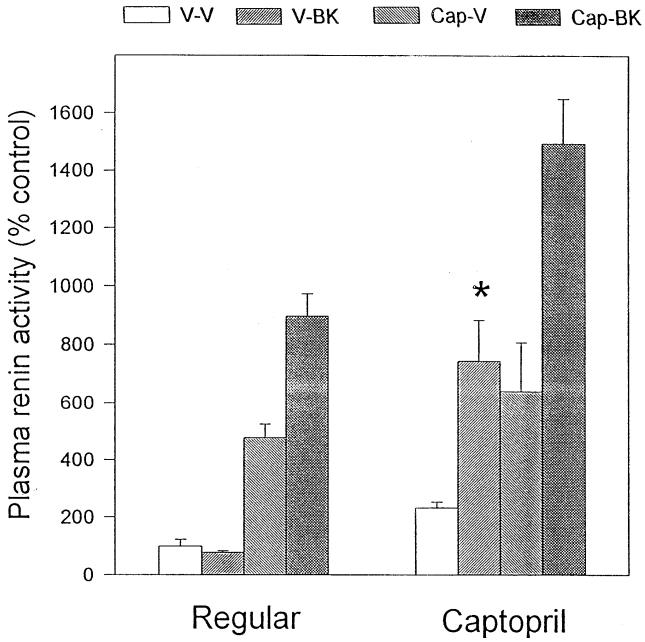

FIG. 2. Mean (± SE; $n = 6$) plasma renin activity of rats fed either regular (left) or captopril-added (right) food for 6 days and then treated acutely as described in Figure 1. Values are expressed as % of the mean (8.8 ± 0.9 ng Ang I/ml/h) of the regular diet V-V group. *$p < 0.01$ difference between dietary groups.

at 4°C, in secondary antibody for 3 h at room temperature, then processed with the ABC reagent (24 h at 4°C). Fos-IR was developed using diaminobenzidine (0.03%) with cobalt/nickel. Sections were then mounted on slides, coverslipped, and Fos-IR was rated semiquantitatively in the OVLT, SFO, SON and PVN. The scale was 0: no Fos-IR, 1: light and scattered Fos-IR, 2: scattered or moderate Fos-IR, 3: many cells, some intensely stained, 4: most cells heavily stained. The results were clear-cut using this scale and cell counts were not performed.

Effect of AD3V Lesions on Drinking to BK and Either Acute or Chronic Captopril

Eight rats received, under sodium pentobarbital (50 mg/kg) anesthesia, electrolytic lesions aimed at the SFO and/or the MnPO immediately rostral, as described before (20). Briefly, the ventricular surface was located electrophysiologically, and the lesion electrode placed at three anterior-posterior sites. Six rats had the electrode lowered to above the SFO but no current passed (sham group). Starting 1 wk after surgery, a series of behavioral tests were performed at 2 day intervals. First, the dipsogenic action of a high dose of Ang II (333 μg/kg) was assessed to determine functional lesions. Second, the acute dipsogenic action of captopril (3 mg/kg) and BK (500 μg/kg) was measured. Third, the dipsogenic effect of BK (500 μg/kg) was assessed after 4 days' chronic dietary captopril. The next day, with the rats still on captopril diet, the dipsogenic effect of Ang I (250 μg/kg SC) was determined. Two days later, Fos-IR was determined as above after injection of BK (500 μg/kg, $n = 5$ lesion, $n = 3$ sham; rats still on captopril diet). The remaining rats (3 lesion, 3 sham) were returned to normal food for 4 days and Fos was determined after injection of Ang II (333 μg/kg SC). In all cases, the rats were sacrificed 1 h after peptide injection, with no water available. The location of the lesions was assessed from the immunocytochemistry sections. In this study, we determined the number of Fos-IR cells in the MnPO (dorsal to the anterior commissure), the mid-regions of the SFO and SON, and the magnocellular PVH. For each rat and area, the cells were counted in the section with the most Fos-IR in that region.

Statistics

Intakes and PRA were analyzed by ANOVA and follow-up t-tests or Duncan post hoc tests, using $p < 0.05$ as the criterion for significance.

RESULTS

BK-Induced Water Intake After Acute and Chronic Captopril

The water intakes are shown in Fig. 1. In rats fed the regular diet, acute administration of captopril produced a small drinking response, and the combination of BK and captopril produced a substantially higher intake. In the rats with 6 days' exposure to dietary captopril, the combination of BK and captopril was comparably dipsogenic. The only difference between the diet conditions was in the groups receiving BK only, after which the captopril diet rats drank almost as much as after BK plus the additional captopril injection.

BK-Induced PRA After Acute and Chronic Captopril

The PRAs are shown in Fig. 2. In the regular diet groups, captopril alone elevated PRA by about 5-fold over basal, and

TABLE 1

REVERSAL OF DRINKING BY A BRADYKININ RECEPTOR ANTAGONIST (Hoe 140)

Captopril Treatment	Other Treatments	1 h Water Intake (ml)
Acute	Vehicle + BK	8.5 ± 2.1
Acute	Hoe 140 + BK	4.9 ± .7*
Acute	Hoe 140 + Vehicle	2.8 ± .9*
Chronic	Vehicle + BK	8.3 ± 1.2
Chronic	Hoe 140 + BK	4.7 ± 1.4*
Chronic	Hoe 140 + Vehicle	3.1 ± 1.0*

Shown are M ± SE for $n = 6$ group. *$p < 0.05$ differs from Vehicle + BK condition.

TABLE 2

MEDIAN FOS-IR RATINGS AFTER VARIOUS TREATMENTS

Treatment	OVLT	SFO_{post}	SON	PVN_{magno}
BK only ($n = 2$)*	0	0	0	1
Acute CP + BK ($n = 3$)*	1	1.5	3	3
Chronic CP + BK ($n = 6$)	2	2	4	4
Chronic CP + sal ($n = 3$)	1	1	0	0
Ang I ($n = 4$)	2	3	3.5	3.5
Acute CP + Ang I ($n = 2$)	1	3	3	3
Chronic CP + Ang I ($n = 6$)	2	3	4	4
Ang II ($n = 2$)	3	3.5	3	3

Note: CP = captopril. Doses and routes are given in text. *Data from [7].

the BK + captopril treatment induced a 9-fold elevation. In the dietary captopril groups, chronic treatment induced modest elevations in PRA in the vehicle-vehicle and vehicle-captopril groups, and also potentiated the effects of BK alone ($p < 0.05$) and BK + acute captopril (the latter was not significant due to higher variability).

Effect of Acute or Chronic Captopril on Ang I-Induced Drinking

Water intake of rats treated with Ang I only was 2.8 ± 0.3 ml/h (M ± SE). Acute pretreatment with captopril enhanced Ang I-induced intake to 5.1 ± 0.6 ml ($p < 0.05$). Conversely, dietary treatment with captopril reduced Ang I-induced intake to 1.2 ± 0.4 ml ($p < 0.05$).

Reversal of Drinking by BK or Ang II Receptor Antagonists

The results are shown in Table 1. Hoe 140 reduced by about 70% the intake induced by acute captopril and BK. Hoe 140 reduced by about 65% the intake induced by chronic captopril and BK. Losartan completely reversed the drinking induced by either Ang I or captopril and BK in either the acute or chronic captopril conditions.

Fos-IR After Chronic Captopril

The OVLT, posterior SFO, SON and PVN showed moderate to heavy Fos-IR following chronic captopril and BK. This pattern was quite similar to our previous observations with acute captopril and BK (Table 2). Ang I alone, or in combination with either acute or chronic captopril induced a similar pattern of Fos-IR to captopril plus BK, although with greater relative intensity in the SFO. The pattern also is similar to that observed after Ang II administered either intravenously (20) or SC ((9) and Table 2).

Effect of AD3V Lesions on Drinking

Sham lesion rats drank 8.9 ± 1.9 ml in 1 h following injection of Ang II. Six of the 8 SFO lesion rats drank less than 1 ml (the maximum intake in a vehicle control test) and were considered to have functionally complete lesions. The other two rats drank 4.7 and 7.7 ml, respectively, and were considered to have ineffective lesions.

Following acute injection of captopril and BK, sham lesion rats drank 8.3 ± 1.2ml in 1 h. The group mean intake of the AD3V lesion rats was 4.4 ± 1.0 ml ($p < 0.05$ vs sham). However, all but one of the lesion rats consumed some fluid. The two rats assigned ineffective lesions on the basis of the Ang II test consumed 1.3 and 7.7 ml (average 4.5 ml), which represent the second lowest and the highest intakes in this group. There was no relationship between Ang II and BK + captopril responsiveness.

After injection of BK following chronic exposure to captopril, sham lesion rats consumed 5.7 ± 0.6 ml, and the lesion group 4.4 ± 0.7 ml (not significantly different). In this case, only one lesion rat (zero intake) consumed less than the lowest control (4.0 ml). Again, there was no difference on the basis of prior Ang II responsiveness. The following day, using Ang I as the dipsogen, sham lesion rats drank 6.6 ± 1.2 ml while lesion rats drank 0.9 ± 0.4 ml ($p < 0.01$). Further, comparing intakes at the last BK and Ang I tests across groups revealed a significant (2 way ANOVA, $p < 0.01$) interaction between dipsogen and lesion: that is, the lesion rats were severely impaired relative to sham controls in their drinking response to Ang I, but not to BK.

Fos-IR and Lesion Evaluation

Representative photomicrographs are shown in Figs. 3 and 4. Of the 3 lesion rats given Ang II for the Fos study, one was a drinker to Ang II and the others were nonresponders. The lesions all extended from dorsal to the main organ to ventral and anterior to the main organ, including the dorsal MnPO, hence the present descriptive term "anterodorsal third ventricle". The lesion in the Ang II-responsive rat was slightly more dorsal and spared most of the SFO. In each of the lesioned rats, there were fewer Fos-positive cells in the SON and PVN than in the sham controls (Fig. 3, Table 3). Part of the SFO was intact in each rat, and again showed reduced Fos-IR cells than controls. Fos-IR in the OVLT and in undamaged parts of the MnPO was similar in sham and SFO lesion rats.

In sham lesion rats, captopril + BK induced Fos-IR in the same regions as Ang II, and to approximately the same degree. Of the five lesion rats given BK for the Fos study, one was a drinker to Ang II and the others were nonresponders. The lesions in all five rats were mainly in the anterior SFO and MnPO, as before. Fos-IR was reduced in SON, PVN and SFO relative to shams, and was also markedly reduced in the OVLT and posterior bed nucleus of the stria terminalis (Table 3).

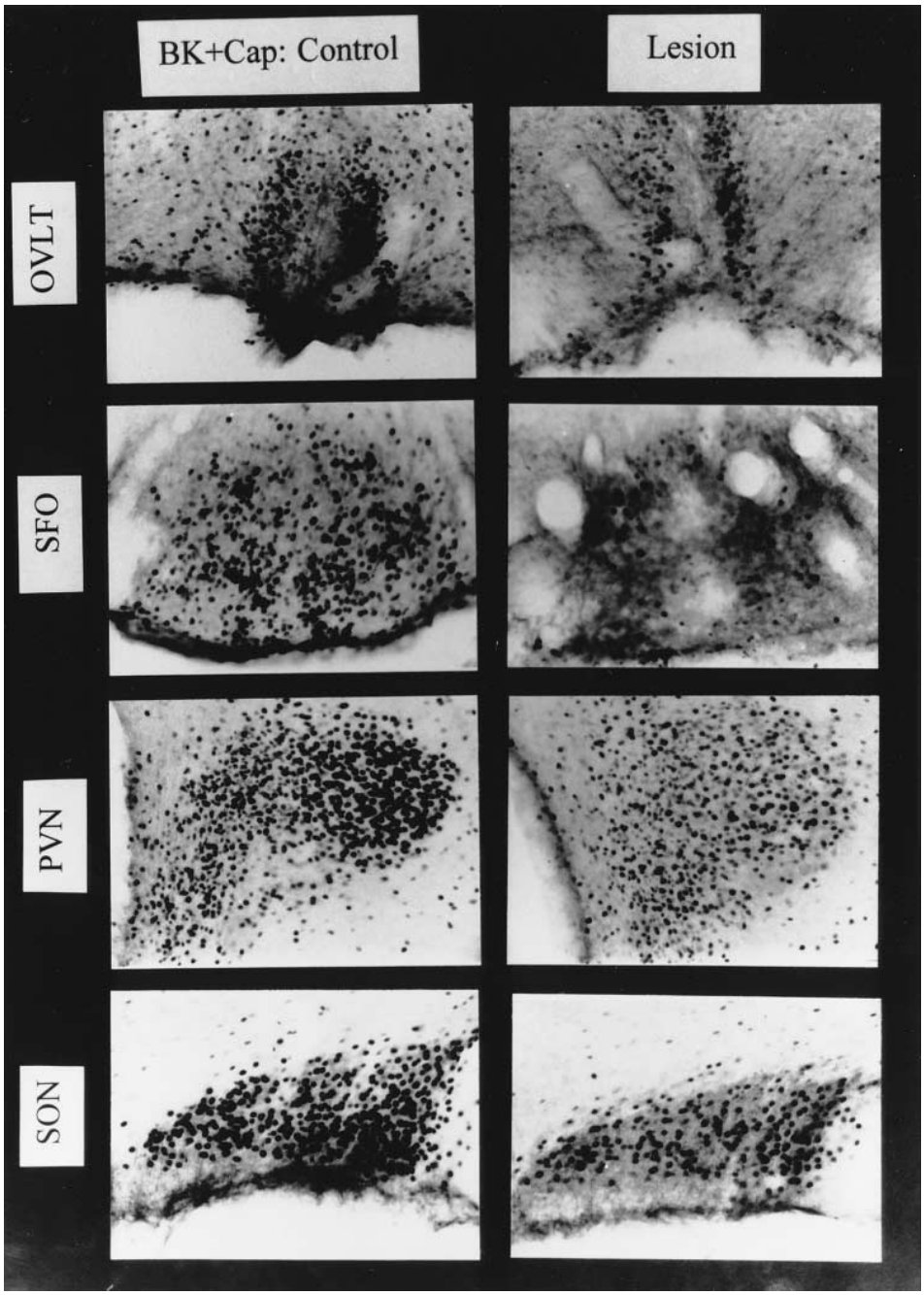

FIG. 3. Photomicrographs of Fos-IR from a representative sham operated (left) and a AD3V-lesioned (right) rat following administration of BK + captopril. Reductions in the number of stained cells are evident in magnocellular PVN and SON, as well as in the remaining posterior SFO and OVLT, of the lesioned rat.

DISCUSSION

These data confirm our previous reports (8,18) that acute treatment with K2 inhibitors reveal a stimulatory effect of BK on both water intake and PRA. Further, we show that chronic dietary administration of captopril induces water intake and elevates PRA after acute BK injection. This is consistent with the view that the dietary captopril, which is ingested primarily at night, is still present during the following day and inhibits BK degradation. Other work from our laboratory on the time of day at which NaCl appetite occurs with K2 inhibitors (22) indicates a fairly long bioavailability. The rats given chronic captopril showed no additional drinking or PRA responses when acute captopril was added, either with or without BK. A strong positive correlation was found ($r = 0.89$, $p < 0.01$) between the group mean PRA (Fig. 2) and mean water intake (Fig. 1). This suggests that PRA, and by inference the associated concentrations of Ang I in plasma and circumventricular organs of the brain, are directly responsible for the BK-

TABLE 3

NUMBERS OF FOS-IR CELLS IN VARIOUS BRAIN REGIONS OF INTACT AND AD3V-LESION RATS AFTER INJECTION OF EITHER BRADYKININ + CAPTOPRIL OR ANGIOTENSIN II

Group	Treatment	MnPO	SFO	SON	PVN
Sham op. (3)	Ang II	145 ± 15	430 ± 26	367 ± 14	280 ± 8
SFO lesion (3)	Ang II	98 ± 12	160 ± 39*	167 ± 22*	197 ± 3*
Sham op. (3)	BK + captopril	85 ± 14	287 ± 46	387 ± 18	317 ± 40
SFO lesion (5)	BK + captopril	12 ± 12*	33 ± 11*	267 ± 27*	220 ± 21

induced drink. Indeed, we have shown previously that the AT-1R antagonist, losartan, attenuates BK-related water intake (18). The generally similar patterns of Fos-IR in brain after Ang II or BK + captopril further suggest that a crucial event in BK-related dipsogenesis is penetration and/or generation of Ang II in brain.

We report, however, some differences between BK- and Ang I-related drinks that seem to require additional explanation. The first difference is that, while BK-induced drinking was similar after either acute or chronic captopril, Ang I-induced drinking was facilitated by acute captopril and inhibited by chronic captopril. The Ang I data, which confirm in a single study what has previously been shown in separate studies, could most readily be interpreted according to an "accumulation hypothesis". In this hypothesis, progressive accumulation of captopril may occur in brain during chronic administration so that after several days the effective concentrations in brain are comparable to those achieved with large acute doses. It is known that acute captopril in low doses (ca 3 mg/kg) potentiates, but in high doses (ca 30 mg/kg) attenuates, Ang I-induced drinking (5,6). In turn, this has been explained by a "spillover hypothesis" in which low doses of captopril do not completely block the high K2 activities in the circumventricular organs, while high doses do produce a block (4,5,6,14,15,25). Both low and high doses in this context completely block peripheral K2, hence cause large increases in plasma Ang I that spill over into the parenchyma of the circumventricular organs, but only with the lower doses of captopril can this be converted into active Ang II by K2. However, while the combined accumulation and spillover hypotheses can explain the Ang I data, they are not consistent with Ang I being the mediator of BK-induced drinking because (i) PRA was comparable after either acute or chronic captopril and BK, and so the spillover of Ang I should have been comparable, and (ii) captopril shows an inverted U-shaped dose-effect curve on both induction of BK (18) and facilitation of Ang I (6) drinks. Both of these observations would lead us to expect parallel changes, if any, in BK and Ang I drinks as a function of acute and chronic captopril administration. Further, we were unable to show major changes in the pattern or intensity of Fos-IR induced by either Ang I or BK as a function of either acute or chronic captopril. We assume that the Fos-IR seen after Ang I reflects conversion to Ang II, so had expected to see increases and decreases in intensity or number of Fos-positive cells as a function of acute or chronic captopril, respectively. Neither we nor others have as yet published formal dose-response data for Ang II administration and Fos-IR, but in preliminary work it is clear to us that such a dose-response relationship holds, although perhaps one that is not linear and may be "saturated" at doses commonly used in drinking studies. Thus, in principle, the spillover and accumulation hypotheses could be testable using Fos-IR. We previously demonstrated this using isoproterenol (which causes renin release from the kidney and drinking by generation of Ang II) and captopril and showed a facilitation of isoproterenol-induced Fos-IR in SFO by 3 mg and a complete inhibition by 30 mg captopril/kg (19). Thus, the failure of chronic captopril to block Ang I-induced Fos-IR could be taken as evidence against an accumulation hypothesis. However, the contexts within which Ang I acts—hypotension after isoproterenol, hypertension after Ang I—may prove to be a crucial difference. Further study will be needed to examine this aspect. A second possible difference between acute and chronic captorpil may relate to changes in Ang II receptor numbers in critical brain regions. However, this will not affect Ang I- and BK-induced intake differentially if they both depend on Ang I.

Because the above differences suggested, but did not definitively prove, that factor(s) other than Ang I may contribute to BK-related drinking, a second line of evidence was sought in the lesion study. The lesions were aimed at the AD3V, including rostral SFO and dorsal MnPO. As is the case for SFO lesions (11,23), AD3V lesions almost completely abolished Ang I- and Ang II-induced drinking but had either no (chronic captopril) or a partial (acute captopril) attenuating effect on BK-related drinking. This constitutes a major difference between Ang and BK-related mechanisms. We have previously shown that lesions of the AD3V completely abolished Fos-IR in SON and PVN induced by either intravenous infusion (20) or SC injection (9) of Ang II. Thus, the presence in SON and PVN of lesion rats of about 69% of the number of Fos-IR cells found in the sham operated controls following BK + captopril, despite much greater reductions in the lesioned area, suggests that non-Ang mechanisms may be driving Fos in these regions. In other studies, we have found an increase in the number of Fos-IR cells in SON during hypovolemia in rats treated with sufficient losartan to block Fos-IR in the SFO (21). One potential candidate stimulus for Fos under these conditions is baroreceptor input. It is thus possible that both the drinking and the Fos-IR (at least in SON and PVN) in these lesioned rats is due to hypotension alone. This may also be the case for hypovolemia induced in SFO lesion rats by PEG, in which the drink is only slightly attenuated ralative to sham controls (11). We reported that blood pressure, measured indirectly at a single time point, was not significantly reduced in BK + captopril-treated rats (18). However, even with K2 inhibition, the hypotensive effect lasts only a few minutes (2), so there is a need for more detailed, direct measurements. Further evidence for a role of endogenous BK comes from a study in sodium-depleted marmosets (16): acute treatment with captopril caused hypotension and this was reversed by treatment with the BK receptor antagonist, Hoe 140. The issue of hypotension and/or peripheral angiotensin as a thirst stimulus in hypotensive rats is not a new issue: analysis of the actions of isoproterenol led to a

concensus that both mechanisms may be operative (1). Our current interpretation of Fos-IR patterns suggests that Ang II (SFO) and baroreceptor (SON, PVN) afferent inputs can be separated with selective lesion or pharmacological manipulations. A cautionary note should be added because, in the few lesioned rats studied, Ang II-induced Fos-IR was not completely abolished. This differs from the complete loss in our previous study using a lower dose (200 μg/kg, SC) of Ang II (9). Either the higher dose or the slightly more rostral (but with great overlap) lesion in this study is less effective. More caudal lesions and/or lower doses of BK + captopril may be needed to interpret fully the BK data.

Lastly, the present data do not address whether the BK actions are exclusively peripheral, or include some central effects. In contrast to its peripheral action, BK injected into the cerebral ventricles causes a substantial rise in blood pressure that most likely depends on the rostral 3rd ventricle (13). In preliminary work, we have been unsuccessful in attempts to elicit drinking with cerebroventricular injection of BK in rats treated with a low peripheral dose of captopril. The fact that the clinical antihypertensive effects of K2 inhibitors last much longer than measured blockade of peripheral K2 blockade (10) suggests that late central actions may occur. This also suggests that behavioral long-term actions of K2 inhibitors, such as induction of sodium appetite in rats (4,7,17), may involve mechanisms other than simply increasing the availability of blood-borne Ang I to brain.

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