Pharmacology Biochemistry and Behavior, Vol. 46, pp. 751–758, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Bradykinin Suppresses Alcohol Intake and Plays a Role in the Suppression Produced by an ACE Inhibitor

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Received 14 October 1992

ROBERTSON, J. M., S. HARDING AND L. A. GRUPP. *Bradykinin suppresses alcohol intake and plays a role in the suppression produced by an ACE inhibitor.* PHARMACOL BIOCHEM BEHAV 46(4) 751–758, 1993.—The possible role of the endogenous kinins in the control of alcohol intake was assessed in two experiments. In Experiment 1, naive rats, maintained on ad lib food and water, were given daily 40-min access to a 6% (w/v) alcohol solution and water. Daily intraperitoneal (IP) injections of captopril (20 mg/kg) significantly reduced alcohol intake, while pretreatment with subcutaneous (SC) injections of the bradykinin antagonist [D-Phe^7]-bradykinin (100–300 μg/kg) attenuated the suppressive effect of captopril on alcohol intake. The saline vehicle or the bradykinin antagonist alone did not alter alcohol intake. In Experiment 2, bradykinin was administered daily at 100, 200, and 400 μg/kg doses SC either alone or in combination with captopril 10 mg/kg IP. Neither bradykinin nor captopril by themselves changed alcohol or water intake. Bradykinin combined with captopril stimulated water intake and reduced alcohol intake by up to 70%. This effect was not due to drug-induced changes in the pharmacokinetics of alcohol. The angiotensin II receptor antagonist [Sar^1,Thr^8]-angiotensin II at 250 and 500 μg/kg SC attenuated the stimulation of water intake but not the reduction in alcohol intake. It is suggested that by inhibiting kininase II, ACE inhibitors extend the duration of action of bradykinin and thereby unmask a potent inhibition of alcohol intake mediated by kinins—an effect that is dissociable from the accompanying stimulation of water intake. Taken together, these results point to an involvement of the kinin system in the regulation of alcohol intake and in particular to a role of bradykinin in the suppressive effect of ACE inhibitors on alcohol intake.

Alcohol drinking Bradykinin Angiotensin converting enzyme inhibitor Angiotensin II Rat Bradykinin antagonist

ANGIOTENSIN converting enzyme (ACE) inhibitors are drugs that prevent the conversion of angiotensin I to angiotensin II (2,30) and are used clinically in the treatment of hypertension. In addition to this action, preclinical studies have shown that ACE inhibitors reduce voluntary alcohol intake in rats (1,13,15,28). The mechanism of action of these drugs in terms of reducing alcohol intake is not understood. ACE inhibitors stimulate water intake (25,29) and salt intake (5,20), both of which are thought to be mediated through a rise in angiotensin (ANG) II production in the brain (24). Since alcohol intake is suppressed when ANG II activity is enhanced [see (11) for a review], it was hypothesized that a rise in central ANG II might be responsible for the reduction in alcohol intake by ACE inhibitors (11,15). This hypothesis was not supported by recent work showing that the reduction in alcohol intake produced by ACE inhibitors could not be attenuated either by central ACE inhibition or by central angiotensin II receptor blockade (23).

Besides their effect on the synthesis of ANG II, the ACE inhibitors also elevate levels of enkephalins (21), substance P (3), and neurotensin (4) by inhibiting the enzymes that promote their degradation. Angiotensin converting enzyme is identical to kininase II, the enzyme responsible for the degradation of bradykinin (31). Thus, ACE inhibitors can enhance the activity of bradykinin by preventing its degradation. Recent studies with other drugs affecting the kinin system have demonstrated that NH_2 senktide can promote a reduction in alcohol intake (22).

The present experiments explore the possible role of bradykinin in the regulation of alcohol intake. In the first experi-

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ment, we examine the interaction between bradykinin and an ACE inhibitor by assessing the ability of a kinin antagonist to attenuate the effect of captopril on alcohol intake. In the second experiment, we examine the ability of bradykinin itself to directly influence alcohol intake.

GENERAL METHOD

Subjects

Naive male Wistar rats weighing 220–290 g were individually housed on a reversed light/dark cycle with lights on at 7 a.m. and off at 7 p.m. Food and water were available ad lib in home cages.

Experimental Protocol

The effect of the drug treatments on alcohol intake was examined using the limited access procedure (16,17). Each day during the active dark cycle, the animals were removed from their home cages, weighed, and placed for 40 min in individual "drinking cages" that had two graduated drinking tubes at the front, one containing a solution of alcohol and tap water and the other containing tap water. No food was available in the drinking cage. After 40 min the amounts of water and alcohol consumed were recorded and the animals were returned to their home cages. The positions of the two tubes in the drinking cages were alternated daily to control for position preference. For 12–14 days a 3% (w/v) alcohol solution was offered followed by a 6% (w/v) alcohol solution for the remainder of the experiment. After intake stabilized at this concentration and the animals were divided into groups equated for alcohol intake (baseline phase, 12–14 days), the different drug treatments were administered during the treatment phases. Rats were first ranked from highest to lowest consumption according to their mean intake in the baseline phase. Beginning with the highest drinkers and using a random numbers table, the first four rats were assigned each to one of the four groups in the experiment. Successive clusters of four rats were similarly assigned, each rat in the cluster to a different group. In this way the groups could be matched for alcohol intake but randomly assigned to the different groups. Only rats who habitually drank more than 5 ml/kg of the 6% w/v alcohol solution were ranked and used. This level of consumption exceeds the Wistar rat's metabolic rate of 300 mg/kg/h (i.e., 5 ml/kg of the 6% w/v concentration) and therefore indicates that the animals were experiencing a pharmacodynamic effect (16). The limited access procedure produces a "bout" type of alcohol intake and average blood alcohol levels approximating 50 mg% (16).

Data Analysis

Alcohol and water intake for each rat was averaged over the last 6 days of each phase when the drug effects were at asymptote. Group averages were calculated for all phases of the experiment. Alcohol and water intake data were analyzed separately using two-way repeated measures analyses of variance (ANOVA) with phase as the within-group factor and treatment as the between-group factor.

One-way ANOVA followed by post hoc Duncan's tests examined relevant pairwise group comparisons. For all analyses alpha was set at 0.05.

EXPERIMENT 1: ATTENUATION OF THE CAPTOPRIL-INDUCED REDUCTION IN ALCOHOL INTAKE BY THE BRADYKININ RECEPTOR ANTAGONIST [D-Phe^7]-BRADYKININ

METHOD

Subjects

Thirty-two naive male Wistar rats (220–290 mg) were used.

Drugs

The angiotensin converting enzyme inhibitor captopril (Squibb SQ14,225) and the B_1,B_2 bradykinin receptor antagonist [D-Phe^7]-bradykinin (Bachem) were used in this experiment. Both drugs were dissolved in saline and injected in a volume of 1 ml/kg.

Treatment

Rats were divided into four groups of eight animals each, equated for drinking during the baseline phase when no drugs were administered. In the subsequent phases, the VEHVEH group received two saline injections, one by the IP route and one by the SC route. The VEHDPHE group received saline IP and [D-Phe^7]-bradykinin SC. The CAPVEH group received captopril IP and saline vehicle SC, and the CAPDPHE group received captopril IP and [D-Phe^7]-bradykinin SC. Intraperitoneal injections of captopril or saline vehicle were performed 30 min prior to the drinking session; SC injections of [D-Phe^7]-bradykinin or saline vehicle were performed immediately prior to the drinking session.

There were four drug treatment phases that followed the baseline phase, which were distinguished by changes in the doses of the captopril and [D-Phe^7]-bradykinin. Captopril was given in doses of 5, 10, and 20 mg/kg in drug phases 1 (14 days), 2 (14 days), and 3 (12 days), respectively, and at 20 mg/kg in the fourth phase (10 days). The [D-Phe^7]-bradykinin dose was 100 μg/kg in the first three drug phases and 300 μg/kg in the fourth phase.

RESULTS

One rat in the CAPDPHE group died and its data was not included in the analysis.

Alcohol Intake

Figure 1A shows the alcohol intake for each group over the course of the experiment. The two-way ANOVA revealed a significant effect of treatment, indicating significant differences in alcohol intake among the different treatment groups, $F(3, 27) = 4.52$, $p < 0.02$. A significant effect of phase, $F(4, 104) = 8.54$, $p < 0.0001$, and the interaction of treatment and phase, $F(12, 108) = 4.93$, $p < 0.0001$, show that while alcohol intake was reduced, the reduction was brought about by only certain drug treatments. One-way ANOVAs showed that alcohol intake in the control VEHVEH group was unchanged throughout the entire experiment, $F(4, 28) = 2.32$, NS, while the group receiving captopril alone (CAPVEH) showed a significant reduction in alcohol intake, $F(4, 28) = 6.7$, $p < 0.001$, that occurred in the final two phases where the dose was 20 mg/kg. The VEHDPHE group receiving the bradykinin antagonist alone did show a significant increase in

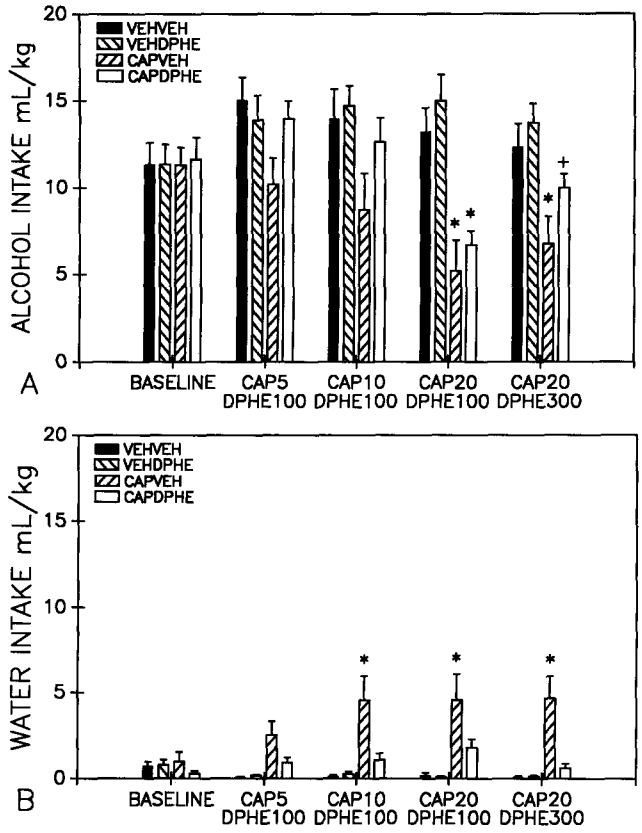

FIG. 1. Alcohol (A) and water (B) intake during baseline and the four subsequent phases of Experiment 1. The drugs and doses administered during each phase are given on the abscissa. VEHVEH group received saline IP and SC. VEHDPHE received saline IP and [D-Phe^7]-bradykinin SC. CAPVEH received captopril IP and saline SC. CAPDPHE received captopril IP and [D-Phe^7]-bradykinin SC. No injections were given in baseline. *Indicates significance ($p < 0.05$) relative to baseline; +indicates significance relative to CAP20 + DPHE100 phase ($p < 0.05$). An intake of 1 ml/kg of the 6% w/v alcohol solution is equivalent to a dose of 60 mg/kg.

intake, $F(4, 28) = 3.33$, $p < 0.03$, relative to baseline; however, Duncan's test indicated that the level of intake in this group during the drug treatment was not greater than the amount of alcohol consumed by the animals in the VEHVEH group. Thus, the equally small but likewise significant increase in alcohol intake in the VEHDPHE group does not signify a significant effect of the antagonist. Alcohol consumption in the CAPVEH group receiving the combination of captopril and bradykinin antagonist was significantly changed by the drug treatments, $F(4, 24) = 13.67$, $p < 0.0001$. Duncan's test revealed that neither the 5 mg/kg nor the 10 mg/kg doses of captopril in combination with the 100 μg/kg of [D-Phe^7]-bradykinin had any effect on alcohol intake. In the third phase, where the captopril dose was raised to 20 mg/kg, a significant reduction in alcohol intake compared to baseline was observed. However, in the fourth phase, when the dose of the antagonist was raised from 100 to 300 μg/kg, alcohol intake was no longer different from baseline levels, and the absolute amount consumed compared to the third phase was significantly elevated. Taken together, these findings suggest that the bradykinin antagonist was able to attenuate the effect of the ACE inhibitor on alcohol intake.

Water Intake

Figure 1B shows the water intake throughout the experiment. A two-way ANOVA revealed significant effects of treatment, $F(3, 27) = 9.28$, $p < 0.0005$, phase, $F(4, 108) = 3.69$, $p < 0.01$, and treatment × phase interaction, $F(12, 108) = 4.85$, $p < 0.0001$. Water intake was stimulated in some of the groups and only at some doses of the drug treatments. One-way ANOVAs showed that water intake in the VEHVEH group, $F(4, 28) = 7.12$, $p < 0.0005$, showed a very small but significant decrease over the course of the experiment compared to baseline. Thus, the equally small but likewise significant decrease in the VEHDPHE, $F(4, 28) = 4.60$, $p < 0.01$, group does not signify an effect of the antagonist on water intake per se. Captopril alone (CAPVEH group) significantly stimulated water intake, $F(4, 28) = 4.99$, $p < 0.005$, compared to baseline, but only at the 10 and 20 mg/kg doses administered over the last three drug treatment phases. The combination of captopril and the bradykinin antagonist (CAPDPHE group) did not produce any significant change in water intake, $F(4, 24) = 2.53$, NS. Thus, the stimulation of water intake produced by captopril was completely suppressed by the kinin antagonist in all of the drug treatment phases.

DISCUSSION

This experiment shows that the bradykinin antagonist [D-Phe^7]-bradykinin can significantly attenuate the reduction in alcohol intake produced by the ACE inhibitor captopril. Furthermore, the attenuation produced was significantly greater as the dose of the antagonist rose from 100 μg/kg to 300 μg/kg. This effect appeared to be a pharmacological antagonism, since the bradykinin antagonist itself did not exert an effect on alcohol intake that was different from control. Along with the diminished effect of captopril on alcohol intake, the bradykinin antagonist also reduced the captopril-induced stimulation in water intake, an effect that occurred at the lowest dose of the antagonist. Thus, the stimulation of water intake by captopril was more sensitive to attenuation by the kinin antagonist than was the reduction in alcohol intake.

The reports showing that ACE inhibitors that prevent the synthesis of ANG II could reduce alcohol intake [(1,13,15,28), but see (7) for a contrary report] stood in contradistinction to an array of findings showing that drugs that raise ANG II activity could reduce alcohol intake (11). The attempt to harmonize these conflicting findings by hypothesizing that the ACE inhibitors reduced alcohol intake by raising *central* levels of ANG II was not confirmed in studies that attempted to alter the ability of ACE inhibitors to reduce alcohol intake by manipulating central ANG II activity [(7), Grupp et al., unpublished observations]. ACE inhibitors such as captopril not only prevent the synthesis of ANG II through an inhibition of the angiotensin converting enzyme, but also raise kinin levels by inhibiting kininase II the enzyme responsible for the breakdown of bradykinin. The present experiment tested the hypothesis that the ACE inhibitor, captopril, reduces alcohol intake, at least in part, by raising bradykinin activity. The finding that the bradykinin antagonist [D-Phe^7]-bradykinin was indeed able to attenuate the reduction in alcohol intake produced by captopril can be taken as evidence supporting the hypothesis that the ability of the ACE inhibitors to reduce alcohol intake is mediated through an elevation of bradykinin.

EXPERIMENT 2: BRADYKININ REDUCES VOLUNTARY ALCOHOL INTAKE IN CAPTOPRIL-TREATED RATS

The results of Experiment 1 suggest that bradykinin may be involved in the reduction in alcohol intake produced by an ACE inhibitor. A more straightforward way of examining the role of bradykinin would be to assess its effect directly on voluntary alcohol intake. Because of rapid degradation, bradykinin has a very short half-life in vivo (9,18), and parenteral injections of bradykinin alone do not usually exert measurable biological activity. For example, Fregly and Rowland (8) found that bradykinin alone had no effect on water intake, but when combined with captopril, an inhibitor of kininase II (the enzyme that degrades bradykinin), a significant stimulation of water intake was produced. In that experiment, the dose of captopril by itself was too low to stimulate water intake, suggesting the contribution of captopril lay solely in extending the duration of action of bradykinin. Thus, captopril unmasked a dipsogenic effect of exogenously administered bradykinin by preventing its degradation in vivo.

The present experiment examined the effect of bradykinin on alcohol intake using the methodological approach of Fregly and Rowland (8). Thus, the effect of bradykinin alone and in combination with captopril on alcohol and water intake was measured. Other groups included a captopril-alone group and a saline control group. In addition, since the kallikrein-kinin and renin-angiotensin systems interact (26), we also examined the effect of an angiotensin II receptor antagonist on changes in alcohol and water drinking produced by the bradykinin/captopril combination.

METHOD

Subjects

The subjects were 36 naive male Wistar rats weighing 240–300 g at the beginning of the experiment. They were individually housed in cages with ad lib access to water and food and were kept on a reverse 12L : 12D cycle with lights off at 7:00 a.m.

Drugs

Bradykinin-(1-9) (Sigma), captopril (Squibb SQ14,225), and the ANG II antagonist [Sar^1,Thr^8]-angiotensin II (Bachem) were used in this experiment. All drugs were dissolved in saline and injected in a volume of 1 ml/kg.

Procedure

The limited access alcohol drinking procedure was used as described in the General Method section. The first phase (14 days) established a baseline level of alcohol intake at the end of which the animals were divided into four groups (n = 9 per group) matched for alcohol intake. In the subsequent three treatment phases, the VEHVEH group received saline vehicle IP and SC. The CAPVEH received captopril (10 mg/kg) IP and vehicle SC. The VEHBDK group received vehicle IP and bradykinin in ascending doses of 100, 200, and 400 μg/kg SC sequentially in the three treatment phases. The CAPBDK group received captopril (10 mg/kg) IP and bradykinin in ascending doses of 100, 200, and 300 μg/kg SC sequentially in the three treatment phases. The first three treatment phases were 16, 14, and 10 days in duration, respectively. Intraperitoneal injections were given 30 min prior to the drinking session and SC injections were given immediately prior to the drinking session.

In the fourth and fifth treatment phases, the CAPBDK group was split into two groups of five and four rats equated for drinking during the third treatment phase. The CAPSARBDK group (n = 5) continued to receive its doses of 10 mg/kg captopril and 400 μg/kg bradykinin at the usual intervals, but in addition received the angiotensin II receptor antagonist [Sar^1,Thr^8]-ANG II SC 10 min prior to drinking. The CAPVEHBDK group also received 10 mg/kg captopril and 400 μg/kg bradykinin at the usual intervals, but in addition received the [Sar^1,Thr^8]-ANG II saline vehicle 10 min prior to the drinking session. The [Sar^1,Thr^8]-ANG II dose was 250 μg/kg in phase 4 (6 days) and 500 μg/kg in phase 5 (6 days).

At the end of the experiment, a blood alcohol elimination curve was established for the VEHVEH, CAPVEHBDK, and CAPSARBDK groups Each group received its respective drug treatments, and at the time the drinking session would have begun the rats were injected IP with 2.5 g/kg alcohol diluted in saline to 17.5% w/v. Blood samples (50 μl) were taken from tails at intervals of 15, 30, 60, 120, 180, 240, 300, and 360 min postalcohol. Blood alcohol concentrations were measured using gas liquid chromatography.

RESULTS

Effect of Captopril, Bradykinin, and Captopril/Bradykinin on Alcohol and Water Intake

Alcohol intake. Alcohol intake for each animal was averaged across the last 6 days of each phase, and the group means in all four groups during baseline and the first three treatment phases of the experiment are depicted in Fig. 2A. A repeated measures two-way ANOVA yielded a nonsignificant effect of phase, $F(3, 96) = 1.29$, NS, but a significant effect of group, $F(3, 32) = 10.20$, $p < 0.0002$, indicating that the drug treatments did alter the amount of alcohol consumed. A significant group × phase interaction, $F(9, 96) = 7.53$, $p < 0.0001$, indicated that differences in alcohol consumption did occur among the groups but only with certain drug treatments.

Post hoc analysis revealed no significant differences in alcohol consumption in the VEHVEH control group, $F(3, 24) = 2.67$, NS, showing that the procedures themselves including the two injections did not have an effect of their own on alcohol consumption. The 10 mg/kg dose of captopril in the CAPVEH group was indeed low and did not in itself produce a significant reduction in alcohol intake, $F(3, 24) = 1.64$, NS. Similarly, none of the three ascending doses of bradykinin (100, 200, or 400 μg/kg) in the VEHBDK group significantly altered alcohol consumption relative to the baseline phase, $F(3, 24) = 2.04$, NS. In contrast, the one-way ANOVA yielded a highly significant effect in the CAPBDK group, $F(3, 24) = 11.09$, $p < 0.0002$, which received both captopril and bradykinin in sequence before the limited access period. Duncan's test showed that alcohol consumption was significantly reduced compared to baseline in all of the three drug treatment phases and that the reduction in alcohol intake reached its maximum with the 10 mg/kg captopril/100 μg/kg bradykinin combination.

Water intake. Water intake for each animal was averaged across the last 6 days of each phase, and the group means in all four groups during baseline and the first three treatment phases of the experiment are depicted in Fig. 2B. A repeated measures two-way ANOVA yielded a significant group effect, $F(3, 32) = 80.63$, $p < 0.0001$, showing group differences in

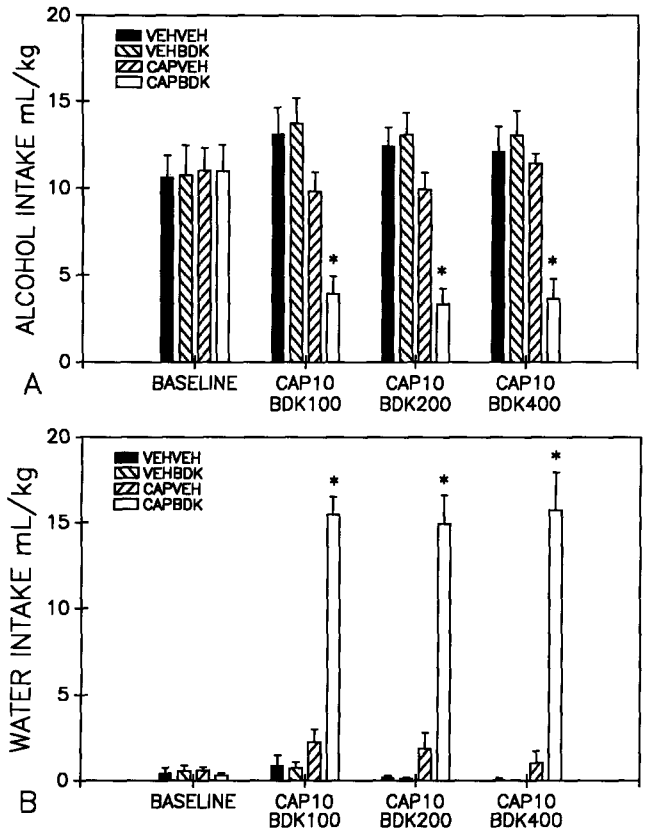

FIG. 2. Alcohol (A) and water (B) intake during baseline and the three subsequent phases of Experiment 2. The drugs and doses administered during each phase are given on the abscissa. VEHVEH group received saline IP and SC. VEHBDK group received saline IP and bradykinin SC. CAPVEH group received captopril SC and saline SC. CAPBDK group received captopril IP and bradykinin SC. No injections were given in baseline. *Indicates significance relative to baseline ($p < 0.05$). An intake of 1 ml/kg of the 6% w/v alcohol solution is equivalent to a dose of 60 mg/kg.

the amount of water consumed. A significant effect of phase, $F(3, 96) = 34.95$, $p < 0.0001$, illustrates the ability of the drug treatments to increase water intake relative to baseline, and a significant group × phase interaction, $F(9, 96) = 30.30$, $p < 0.0001$, shows that only some of the drug treatments were able to produce the rise in water intake. Post hoc analysis did not reveal any significant changes in water intake produced by the administration of the saline vehicles in the VEHVEH group, $F(3, 24) = 1.61$, NS, or by any of the three bradykinin doses in the VEHBDK group, $F(3, 24) = 2.48$, NS. The 10 mg/kg dose of captopril in the CAPVEH group did produce a rise in water intake, but this was too small to be significant, $F(3, 24) = 2.27$, NS. In contrast, water intake was profoundly increased in the CAPBDK group. Duncan's test revealed that water consumption was significantly increased compared to baseline in all of the three drug treatment phases and that this increase was at its maximum with the 100 μg/kg dose of bradykinin.

In summary, the doses of captopril and bradykinin alone had no effects on either alcohol or water intake, yet when combined acted synergistically to produce profound changes in the intake of both alcohol and water.

Effect of an Angiotensin II Antagonist on the Changes in Drinking Produced by the Combination of Captopril/Bradykinin

As noted above, the CAPBDK group was divided into the CAPSARBDK and CAPVEHBDK subgroups for the final two phases. The baseline level of intake for these two subgroups during the third phase and the intake during phases 4 and 5, where the ANG II antagonist or its vehicle was added, is depicted in Fig. 3. A two-way repeated measures ANOVA of the alcohol intake data (Fig. 3A) revealed no significant effect of treatment, $F(1, 7) = 0.00$, NS, phase, $F(2, 14) = 1.52$, NS, or the interaction of treatment and phase, $F(2, 14) = 1.31$, NS, demonstrating that the ANG II antagonist [Sar^1,-Thr^8]-ANG II at the two doses tested did not attenuate the reduction in intake produced by the combination of captopril and bradykinin. These doses of the ANG II antagonist have previously been shown to attenuate the effect of ANG II on alcohol intake (10).

A two-way ANOVA of the water data, (Fig. 3B) did not reveal an effect of treatment, $F(1, 7) = 0.65$, NS, or the interaction of treatment and phase, $F(2, 14) = 2.93$, $p = 0.0866$, NS, but did show a significant effect of phase, $F(2, 14) = 11.79$, $p < 0.002$. A one-way ANOVA revealed no effects on

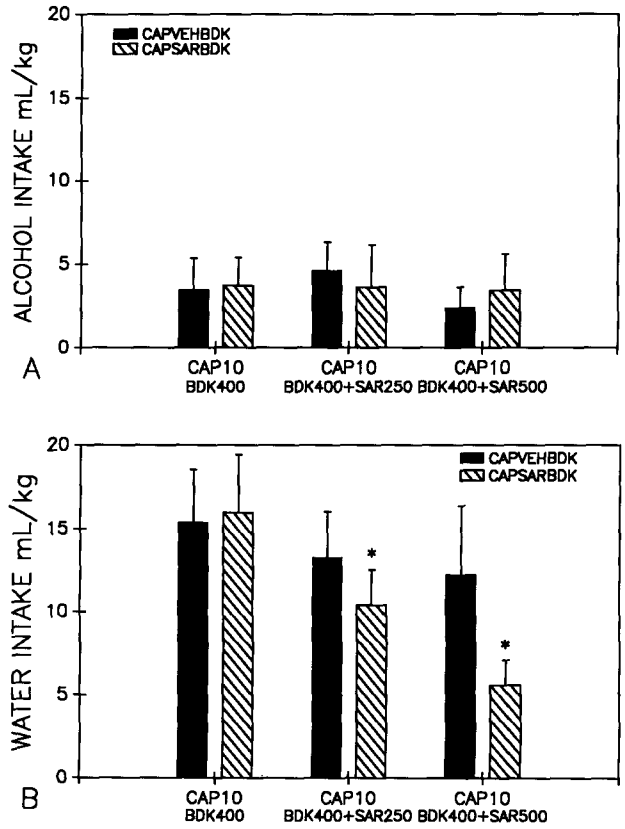

FIG. 3. Alcohol (A) and water (B) intake during angiotensin II receptor blockade in Experiment 2. The drugs and doses administered during each phase are given on the abscissa. CAPSARBDK received captopril IP, [Sar^1,Thr^8]-angiotensin II SC, and bradykinin SC. CAPVEHBDK received captopril IP, saline SC, and bradykinin SC. *Indicates significance relative to CAP10 + BDK400 ($p < 0.05$). An intake of 1 ml/kg of the 6% w/v alcohol solution is equivalent to a dose of 60 mg/kg.

water intake in the CAPVEHBDK group, $F(2, 6) = 0.84$, NS, showing that a third injection per se had no effect on water consumption. There was, however, a significant effect in the CAPSARBDK group, $F(2, 14) = 16.97$, $p < 0.002$, indicating that water intake stimulated by captopril/bradykinin was reduced by [Sar^1,Thr^8]-angiotensin II and in a dose-dependent fashion (Duncan's $p < 0.05$). This finding indicates that while the rise in water intake produced by the captopril/bradykinin combination is, at least in part, produced by ANG II, the reduction in alcohol intake is not an ANG II mediated effect.

Effect of Captopril/Bradykinin Treatment on the Pharmacokinetics of Alcohol

Figure 4 illustrates the blood alcohol concentrations in the VEHVEH, CAPSARBDK, and CAPVEHBDK groups. Two-way repeated measures ANOVA with time after injection as the within-group factor and treatment as the between-group factor showed a significant effect of time, $F(7, 105) = 49.67$, $p < 0.0001$, reflecting the elimination of alcohol over the 6-h period. Neither treatment, $F(2, 15) = 1.04$, NS, nor the interaction of time and treatment, $F(14, 105) = 0.62$, NS, had any significant effect on blood alcohol levels. Thus, bradykinin plus captopril does not appear to mediate the reduction in alcohol intake through a change in the pharmacokinetics of alcohol.

DISCUSSION

This experiment shows that while bradykinin alone and captopril alone in the doses used had no effects of their own on alcohol or water intake, in combination they acted to produce a profound reduction in alcohol intake and rise in water intake. The effect of the bradykinin/captopril combination was maximal at the 100 μg/kg dose of bradykinin, suggesting that still lower doses of bradykinin might also have been effective.

There are at least two possible mechanisms by which the bradykinin/captopril combination could have produced its attenuation in alcohol intake. Since captopril is a kininase II inhibitor, it is possible that it prolonged the action of the injected bradykinin by preventing its degradation. Thus, the reduction in alcohol intake and the enhancement of water intake could be due to the protracted action of bradykinin. Such an interpretation was put forward by Fregly and Rowland (8) to explain the profound thirst they observed with captopril and bradykinin. A second explanation is that while both doses of bradykinin and captopril were too low to exert an observable effect on intake, they each were producing a subthreshold effect that, when added together, was large enough to exert an observable effect. Regardless of the mechanism, it is clear that this combination is an extremely potent way to reduce alcohol consumption.

Finally, the reduction in alcohol intake produced by captopril/bradykinin was not the result of treatment-mediated changes in the pharmacokinetics of alcohol, since the blood alcohol elimination curve comparing the vehicle-treated and bradykinin/captopril groups was similar. In the final phases of the experiment, the addition of an angiotensin receptor antagonist failed to attenuate the reduction in alcohol intake produced by the combination, but did produce a dose-dependent attenuation in water intake. This finding suggests that the mechanisms influencing the stimulation of water intake and the reduction in alcohol intake are independent. The former appear to be angiotensin related while the latter are not. This finding also indicates that the reduction in alcohol intake by the captopril/bradykinin combination is not simply a reflection of an increase in water intake.

GENERAL DISCUSSION

The present experiments examined the role of bradykinin in the control of alcohol intake in two different ways. The first experiment explored the possibility that bradykinin might be involved in the reduction in alcohol intake produced by the ACE inhibitor captopril. The second experiment examined the direct effect of bradykinin on alcohol consumption.

A number of independent reports have demonstrated that

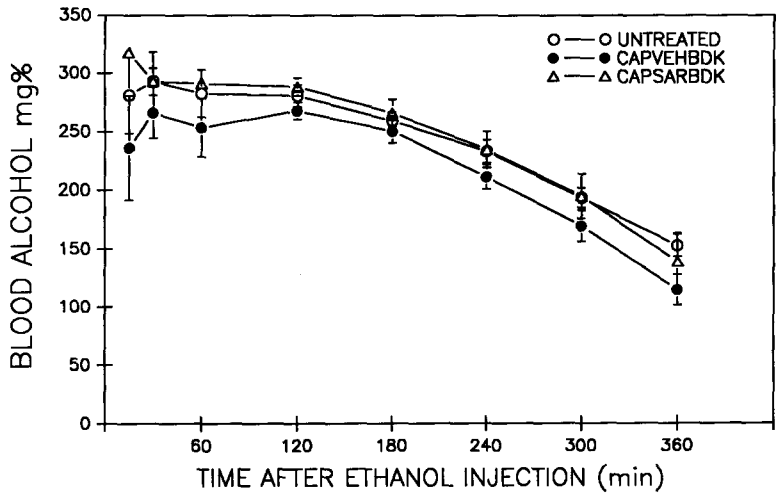

FIG. 4. Serial blood alcohol measurements after acute injection of 2.5 g/kg ethanol IP on the day following the last drinking session. CAPSARBDK received captopril IP, [Sar^1,Thr^8]-angiotensin II SC, and bradykinin SC. CAPVEHBDK received captopril IP, saline SC, and bradykinin SC. VEHVEH was untreated. No difference was found between groups in absolute blood alcohol levels at any of the time points.

ACE inhibitors can reduce alcohol consumption (1,13,15,28), yet the mechanism of this effect has not been identified. Because ACE inhibitors are thought to raise central ANG II levels (8), our laboratory suggested that the ACE inhibitors might reduce alcohol intake through the elevation of central ANG II (11,15). Recent experiments, however, showing that the reduction in alcohol intake produced by the ACE inhibitor, enalapril, could not be attenuated by manipulations that blocked central ANG II activity (i.e., the central administration of either an ANG II antagonist or an ACE inhibitor), failed to confirm this hypothesis (23).

ACE inhibitors are also enkephalinase inhibitors and thereby raise levels of endogenous opiates. However, the endogenous opiate system is not likely to be involved in the effect of ACE inhibitors on alcohol intake, since a rise in opiate activity has repeatedly been shown to increase alcohol intake [see (14) for a review]. Thus, raising the level of endogenous opiates would be expected to have an effect opposite to the one observed.

Other effects of the ACE inhibitors include inhibition of the enzyme that degrades bradykinin, substance P, and neurotensin. Since a number of studies have shown that the antihypertensive effect of some ACE inhibitors, including captopril, is partly due to the vasodilatory effects of bradykinin (6, 12,27), we chose to examine the kinin system and indeed demonstrated in Experiment 1 that a bradykinin antagonist can partially reverse the reduction in alcohol intake and rise in water intake produced by captopril. This effect showed some degree of dose dependence, since the 100 μg/kg dose of the antagonist had a small but not statistically significant effect while the larger 300 μg/kg dose did significantly attenuate the ability of captopril to reduce alcohol drinking. This finding suggests that the ability of the ACE inhibitors to suppress alcohol intake is mediated, at least in part, through bradykinin.

In order to further assess the involvement of bradykinin, Experiment 2 examined the effect of a bradykinin agonist on alcohol intake. A reduction in alcohol intake would provide additional supportive evidence for the role of bradykinin in the control of alcohol intake in particular and implicate the kallikrein-kinin system in general as a mechanism involved in the regulation of alcohol drinking.

The results of Experiment 2 confirmed the involvement of the kallikrein-kinin system in the control of alcohol intake. The agonist, bradykinin-(1-9), produced a potent reduction in alcohol intake and a stimulation of water intake when spared degradation by a low dose of the ACE inhibitor captopril. Neither the bradykinin nor the captopril doses by themselves were able to reduce alcohol intake, so that the effect of this combination was a synergistic one.

This effect on alcohol intake was not secondary to a change in the pharmacokinetics of alcohol and furthermore did not appear to be dependent on activity in the R-A system, since an ANG II receptor antagonist did not counteract the reduction in alcohol consumption observed. On the other hand, the stimulation of water intake did seem to be ANG II related, since the ANG II antagonist [Sar^1,Thr^8]-ANG II did oppose, in a dose-dependent fashion, the increase in water intake. This latter finding dissociates the effect of bradykinin on alcohol and water intake and further demonstrates that the reduction in alcohol intake is not a function of a change in water intake per se.

In summary, these two experiments demonstrate that bradykinin in particular and the kallikrein-kinin system in general is involved in regulating alcohol intake. Furthermore, it appears that the mechanism by which the ACE inhibitors reduce alcohol drinking includes enhanced bradykinin activity. In this regard it is interesting to note that ANG II itself is able to stimulate the release of kallikrein, the enzyme that converts kininogens to active kinins (19). Thus, ANG II and drugs that raise activity in the R-A system may increase the production of bradykinin. The possibility that such manipulations reduce alcohol intake through the release of endogenous kinins merits further investigation.

ACKNOWLEDGEMENTS

This research was supported by the Addiction Research Foundation of Ontario and by a grant from the Bristol/Myers Squibb Company. We also thank Dr. R. Purpura, Bristol/Myers Squibb Company, for the generous supply of captopril.

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