

Capsaicin Reduces Ethanol Consumption in C57BL/6 but not DBA/2 Mice

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Objective: Capsaicin, the pungent analgesic substance of hot peppers which produces a burning sensation and pain is known to affect Substance P and central opioid activities. This experiment was designed to test the effect of capsaicin on alcohol consumption in C57BL/6 and DBA/2 mice. These two strains are known to differ in both their alcohol consumption and their endogenous opioid distribution and response to alcohol. It is hypothesized that this effect may be mediated by both increases Substance P and decreases beta-endorphin.

Methods: After i.p. administration of 0.01 and 0.001 mg/kg of capsaicin with a vehicle or the vehicle alone as the control for eight days in C57BL/6 and DBA/2 mice on limited access alcohol model, Capsaicin's effects on 2-hour alcohol, 22-hours water, 24-hours food intake and body weight were studied.

Results: In this study, as expected, C57BL/6 mice drank significantly more alcohol than DBA/2 mice under baseline conditions. Capsaicin at both doses tested significantly reduced baseline alcohol consumption in C57BL/6 but not DBA/2 mice. These effects were selective for alcohol as capsaicin did not disrupt food or water consumption.

Conclusion: These results demonstrate that capsaicin differentially affects those mechanisms underlying alcohol consumption in two strains of mice known to differ in their preference for and consumption of alcohol. This effect is hypothesized to be related to differences in the response of the endogenous opioid system.

KEY WORDS: Alcohols; Capsaicin; Mice, inbred C57BL; Mice, inbred DBA; Opioid analgesics.

INTRODUCTION

Rodent strains showing high or low preference for alcohol have been especially useful in determining critical differences in those neurotransmitter systems underlying the biological basis of alcoholism. One system that has been suggested to have an important influence on both the acquisition and maintenance of alcoholism is the endogenous opioid system [1-3]. Preclinical studies have shown that low doses of the opioid agonist, morphine, increase ethanol consumption [4,5], while the opioid antagonist, naltrexone, reduces alcohol consumption [6].

Two strains of mice showing differential alcohol consumption are the C57BL/6 and the DBA/2. Given a free choice of alcohol C57BL/6 mice drink significantly more than DBA/2 mice [7]. At basal levels C57BL/6 mice also have lower levels of beta-endorphins when compared to DBA/2 mice, whereas exposure to alcohol produces a significant increase of beta-endorphin response in C57BL/6, but not DBA/2 mice [8]. It is well known that alcohol consumption increases the release of beta-endorphin, which is an endogenous opioid peptide, and beta-endorphin interacts with brain structures involved in the positive reinforcement system [9,10].

Following this logic we hypothesized that pharmacological agents that have an effect on the response of endogenous beta-endorphins should produce differences in behavioral outcome in alcohol consuming C57BL/6 and DBA/2 mice. One such agent is capsaicin, a noxious stimulant known to cause a burning sensation in the hu-

Received: February 9, 2021 / **Revised:** April 5, 2021

Accepted: April 26, 2021

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man body and the primary ingredient that produces a hot or burning taste sensation in red peppers [11]. Capsaicin, which acts on vanilloid receptors (VR1), has a bidirectional effect on pain pathways. Acute administration initially activates presynaptic Substance P receptors leading to the perception of pain [12]. This initial response depletes Substance P, which then blocks the pain signal [11]. There have been studies that capsaicin influences on the degree of activity of the central opioid system. When capsaicin was injected in rats subcutaneously, the β -endorphin level of the cerebrospinal fluid of the cisterna magna increased 45 minutes after the injection [13]. Capsaicin has also been shown to decrease beta-endorphin levels in the central nervous system following intraventricular administration [14,15]. These different results seem to be due to the difference in the injection sites.

This experiment was designed to evaluate the effect of capsaicin on the self-administration of alcohol of C57BL/6 and the DBA/2 mice. These strains are known to differ in their ethanol consumption, their basal beta-endorphin levels, and beta-endorphin response to alcohol. We hypothesize that capsaicin will differentially affect alcohol consumption in these two strains consistent with the differences in beta-endorphin response to alcohol.

METHODS

Animals

Three-week-old C57BL/6 and DBA/2 male mice at the start of the experiment (Hyochang Science, Daegu, Korea) were used. Mice were housed 5 to a cage on a 12/12 hour light/dark cycle and allowed 5 days to acclimate after delivery. During this time they were maintained on ad lib food and water. This research protocol was reviewed and approved by Pusan National University Institutional Animal Care and Use Committee (PNU-2010-000115).

Drugs

Alcohol (Sigma Aldrich, St. Louis, MO, USA) was mixed with tap water to produce a 10% (v/v) solution. Capsaicin (Sigma Aldrich) was dissolved in propylene glycol and mixed with saline to yielded doses of 0.01 and 0.001 mg/kg in a volume of 0.2 ml. Vehicle consisted of a comparable amount of propylene glycol mixed with saline. All mice were injected i.p. with 0.2 ml of vehicle or drug 30 minutes prior to alcohol access. The concen-

tration of alcohol and capsaicin was determined by referring previous studies [16-19].

Experimental Procedure

The mice were given access to food (Purina extrusion, EEGJ30060; Cargill Agri Purina, Seongnam, Korea) and 10% (v/v) alcohol (Sigma Aldrich) with no access to water for 7 days. Mice were then singly housed and given daily 2-hour access to the 10% (v/v) alcohol solution with water available during the other 22-hours. Food (Purina extrusion, EEGJ30060; Cargill Agri Purina) was continuously available. This phase continued for 21 days. Weight of alcohol solution was measured to the nearest 0.001 g at the beginning and end of the 2-hour limited access period. Weight of food and water was taken once each day. Mice were weighed every 2 days. Based on their consumption, mice from each strain were randomly assigned to a Vehicle group (n = 5), a Capsaicin 0.01 group (n = 6 C57BL/6; n = 5 DBA/2), and a Capsaicin 0.001 group (n = 5). For the next 2 days baseline measures of consumption were obtained and for the following 8 days mice were injected with vehicle or capsaicin 30 minutes before the limited access period. Figure 1 shows flow chart of the experiment.

Statistical Analysis

Means for consumption of alcohol, water and food were compared in 2-day blocks across baseline and the 8 injection days using a repeated measures one-way analysis of variance (ANOVA) with 2 levels for strain, C57BL/6 and DBA/2, and 3 levels of treatment, vehicle, capsaicin 0.01 and capsaicin 0.001 mg/kg, repeated across five 2-day blocks.

To minimize the effects of variability seen in daily intake, the mean of 2 day blocks was used. The baseline mean consisted of the 2 days preceding saline and capsaicin administration and means consisted of the four 2 day blocks established across the period when saline and capsaicin were injected. A repeated measures ANOVA (capsaicin treatment group repeated across the four 2 day blocks to each strain) was used for group comparison of daily alcohol, water, and food intake. Statistical significance was set at $p < 0.05$. SPSS version 26 (IBM Co., Armonk, NY, USA) was used for all analyses.

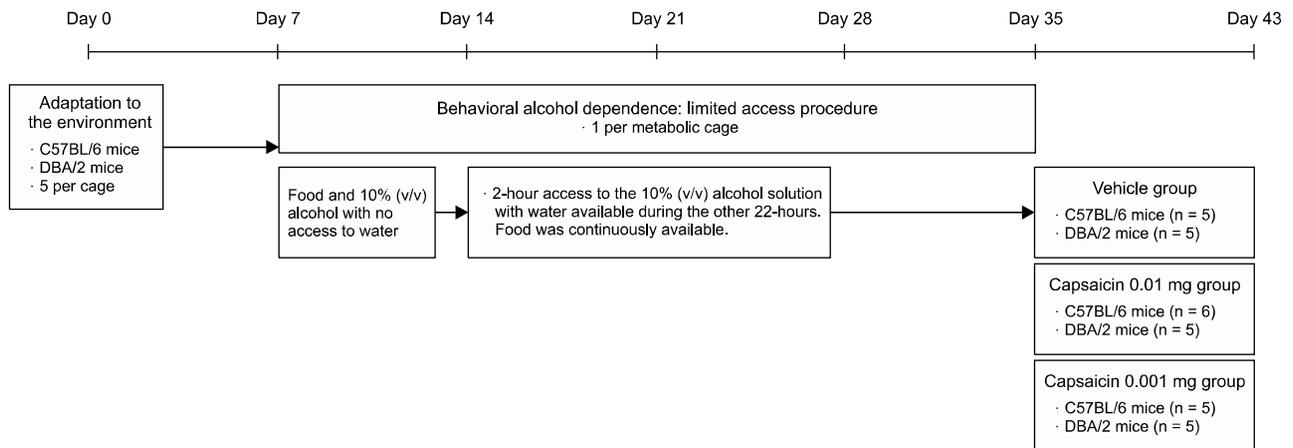


Fig. 1. Flow chart of experiment.

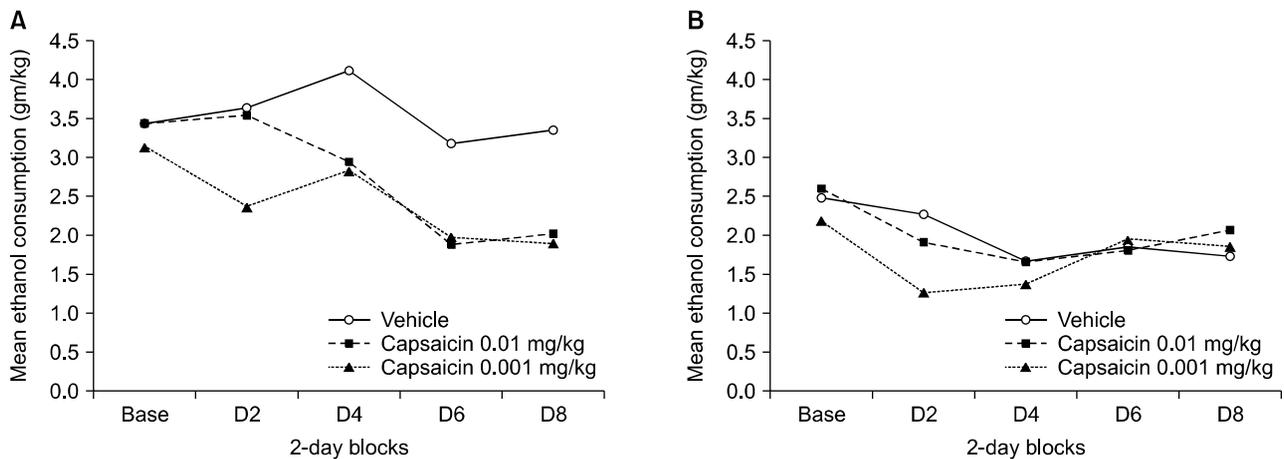


Fig. 2. Mean alcohol consumption from baseline to drug treatment days 10 in C57BL/6 and DBA/2 mice. Panel (A) shows mean alcohol consumption across 2-day blocks for baseline, days 1 and 2, and drug treatment days, days 2 through 10, for C57BL/6 mice across the 3 treatment conditions, saline and 2 doses of capsaicin. Panel (B) shows mean alcohol consumption across 2-day blocks for baseline, days 1 and 2, and drug treatment days, days 2 through 10, for DBA/2 mice across the 3 treatment conditions, saline and 2 doses of capsaicin.

RESULTS

Consistent with the literature C57BL/6 mice consumed significantly more alcohol than the DBA/2 mice. Capsaicin significantly reduced alcohol consumption compared to vehicle only in the C57BL/6 mice. Capsaicin had no effect on the consumption of food or water in either strain. Figure 2 shows alcohol consumption by each strain in each treatment condition across the 2-day blocks of baseline or experimental condition. A two way repeated measures ANOVA with two levels for strain and 3 levels of treatment repeated across the five two-day blocks yielded a significant effect for dose ($F = 3.81$, $p = 0.036$), a significant effect for strain ($F = 24.36$, $p < 0.0001$), a sig-

nificant effect for time ($F = 9.77$, $p < 0.0001$), and a significant time \times strain interaction ($F = 7.42$, $p < 0.0001$).

A subsequent Newman-Keuls *post hoc* test revealed that C57BL/6 mice drank significantly more alcohol compared to DBA/2 mice.

A repeated measures ANOVA of alcohol consumption by C57BL/6 mice in the three treatment conditions repeated across the five 2-day blocks was significant for dose ($F = 29.57$, $p < 0.0001$), a significant effect for time ($F = 10.60$, $p < 0.0001$), and a significant time \times dose interaction ($F = 7.69$, $p < 0.0001$).

A Newman-Keuls *post hoc* test revealed that both capsaicin groups differed from the vehicle group.

A repeated measures ANOVA of alcohol consumption

by DBA/2 mice in the three treatment conditions repeated across the five 2-day blocks was significant for time only ($F = 3.49, p = 0.008$).

Unlike alcohol, DBA/2 mice consumed significantly more water than C57BL/6 mice. Figure 3 shows water consumption by each strain in each treatment condition across the 2-day blocks of baseline or experimental condition.

A two way repeated measures ANOVA with two levels for strain and 3 levels of treatment repeated across the five

two-day blocks yielded a significant effect for strain ($F = 62.46, p < 0.0001$) and a significant effect for time ($F = 10.53, p < 0.0001$).

The effect for time described a decrease in water consumption across 2-days block that was displayed by both the vehicle control animals and capsaicin animals.

Figure 4 shows food consumption by each strain in each treatment condition across the 2-day blocks of baseline or experimental condition.

A two way repeated measures ANOVA with two levels

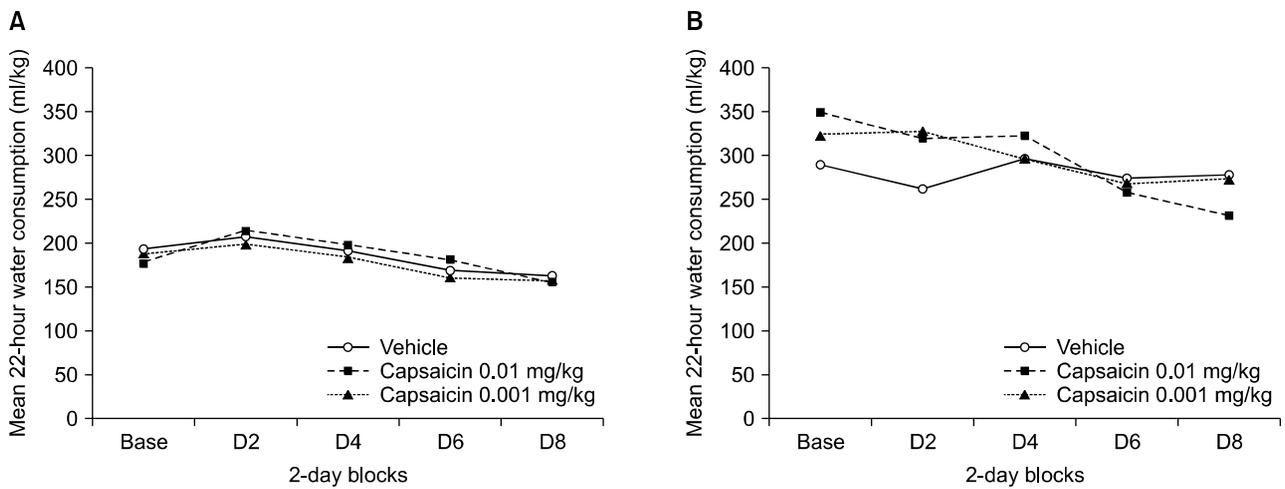


Fig. 3. Mean water consumption from baseline to drug treatment days 10 in C57BL/6 and DBA/2 mice. Panel (A) shows mean water consumption across 2-day blocks for baseline, days 1 and 2, and drug treatment days, days 2 through 10, for C57BL/6 mice across the 3 treatment conditions, saline and 2 doses of capsaicin. Panel (B) shows mean water consumption across 2-day blocks for baseline, days 1 and 2, and drug treatment days, days 2 through 10, for DBA/2 mice across the 3 treatment conditions, saline and 2 doses of capsaicin.

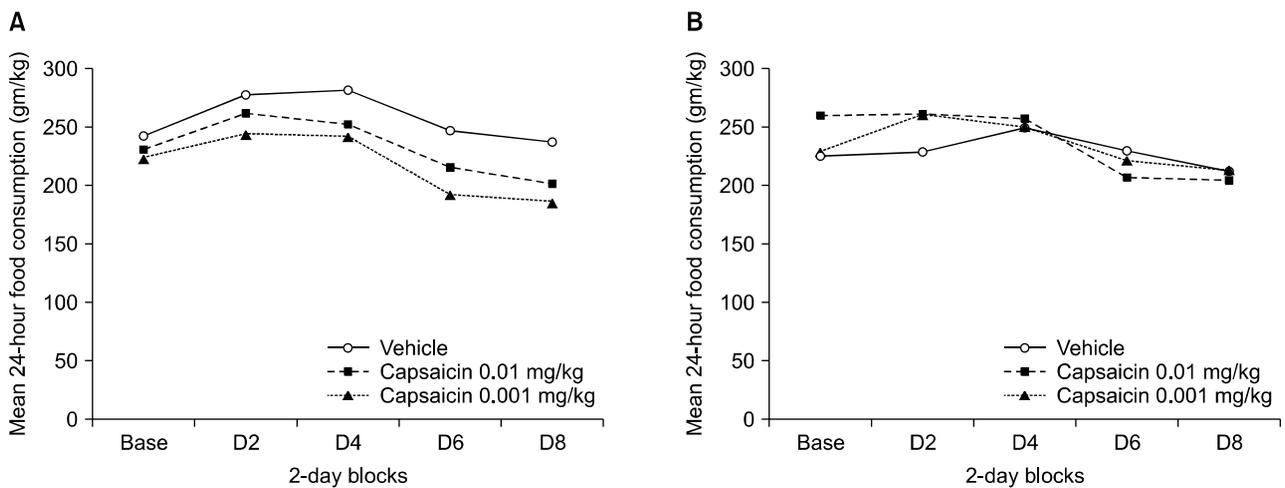


Fig. 4. Mean food consumption from baseline to drug treatment days 10 in C57BL/6 and DBA/2 mice. Panel (A) shows mean food consumption across 2-day blocks for baseline, days 1 and 2, and drug treatment days, days 2 through 10, for C57BL/6 mice across the 3 treatment conditions, saline and 2 doses of capsaicin. Panel (B) shows mean food consumption across 2-day blocks for baseline, days 1 and 2, and drug treatment days, days 2 through 10, for DBA/2 mice across the 3 treatment conditions, saline and 2 doses of capsaicin.

for strain and 3 levels of treatment repeated across the five two-day blocks was significant for time only ($F = 15.78$, $p < 0.0001$).

Similar to water consumption, food consumption showed a decrease in both treatment and drug groups across the 2-day blocks of the experiment.

DISCUSSION

The principle finding of this experiment is that systemic administration of capsaicin significantly reduced alcohol consumption in C57/BL6 but not in DBA/2 mice. This suggests that capsaicin differentially interacted with those neural systems that underlie the difference in alcohol preference in these two strains. This pattern of the results suggests two explanatory frameworks.

Firstly, capsaicin is an agonist of vanilloid (VR1) receptors. Capsaicin's primary use is as a topical analgesic producing its effect indirectly by depleting Substance P. A link between Substance P and alcohol consumption has recently been established in Sardinian alcohol-preferring rats [20]. However, manipulation of Substance P in Wistar rats failed to reduce ethanol consumption [21]. It is unclear if this difference between the Sardinian alcohol preferring and the unselected Wistar rats is directly related to differences in the expression or activity of Substance P. While there is evidence that some of those neural systems underlying the consumption of alcohol differ between C57BL/6 and DBA mice, we are unaware of any reports on differences in Substance P between these two strains.

A second explanation is suggested by evidence that capsaicin has also been shown to deplete hypothalamic beta-endorphin in adult rats 3, 5, and 7 days after intraventricular administration [14]. These changes in beta-endorphin occurred in the absence of any change in Substance P. The doses of capsaicin used in these experiments were high and may have been neurotoxic to beta-endorphin sites. However, beta-endorphin levels returned to normal 15 days after capsaicin injection suggesting a transient effect. The doses chosen for use in the experiments reported here are well below the 1 mg/kg level that has been identified to be toxic [22]. The results of this experiment show that repeated measures ANOVA of alcohol consumption by C57BL/6 mice in the three treatment conditions repeated across the five 2-day blocks was

significant for dose ($F[2,79] = 5.21$, $p < 0.03$), a significant effect for time ($F[4,79] = 12.02$, $p < 0.00001$). A Newman-Keuls *post hoc* test revealed that both capsaicin groups differed from the vehicle group. While the precise mechanism of capsaicin action is not understood, the pattern of the results suggest that the decrease in alcohol consumption seen in C57BL/6 mice in this experiment may have been due to capsaicin's action at central opioid receptors. If alcohol's reinforcing effects are dependent, in part, on its ability to release beta-endorphin, a reduction in tone in this system would have an effect similar to the action of an opioid antagonist like naltrexone.

The involvement of the endogenous opioid system is hypothesized to be responsible, in part, for the reinforcing properties of alcohol. Various theoretical models have been proposed to describe this relationship. The Opioid Surfeit Hypothesis [23] suggests that alcohol is positively reinforcing in some individuals because of inherent excessive opioid activity. The Opioid Deficit Hypothesis [24] suggests that alcohol is negatively reinforcing in some individuals because they experience diminished opioid activity. Data has been provided to support both of these views suggesting that an opioid response may underlie excessive alcohol consumption under a number of biological and environmental conditions. This suggested a third view, which recognizes a relationship between excessive alcohol consumption and both basal differences in the endogenous opioid system and in differences in response of the endogenous opioid system to alcohol [2].

The animal model used in this experiment employed two strains known differ in both basal opioid systems [25], response of the opioid system to alcohol [8], and behavioral consumption of alcohol [7]. Consistent with other reports in the literature the C57BL/6 mice used in this study drank significantly more alcohol than the DBA/2 mice. Those neural systems underlying this behavioral difference have been examined and differences in both basal configuration and response to alcohol have been noted in many of these systems including the endogenous opioid system. Although the evidence in this experiment does not use biomarkers and not directly assess the effect of capsaicin on the central endogenous opioid system, results of this experiment suggest that differences between these strains in basal opioid tone and response to alcohol may be critical factors in determining the differences in behavioral response. While capsaicin reduced alcohol

consumption in C57BL/6 mice, it had no effect on the consumption of food or water in either strain throughout the experiment. Although further investigations are needed, this suggests that capsaicin's effect may be selective for alcohol.

There are some limitations in present study. Firstly, the number of subjects are relatively small. However, the strength of present study is that the experiment was conducted under well controlled condition using two different specific strains. Secondly, present study is an animal experiment, and the results of present study cannot be generalized to humans. However previous studies [26,27] that capsaicin injection can change the activities of the dopamine nervous system and the central opioid system of the nucleus accumbens and the findings [28,29] that the changes in the activities of these nervous systems are also important as the mechanism of addiction. Based on these findings, it could be possible to plan future research on the relationship between capsaicin and alcohol consumption according to races.

This study demonstrates that capsaicin significantly reduced alcohol consumption in C57BL/6 but not DBA/2 mice. These effects were selective for alcohol as capsaicin did not disrupt food or water consumption. These results suggest that capsaicin affects the endogenous opioid system and ultimately raise a possibility that diets can be related to drinking behavior.

■ Funding

This study was supported by Research institute for Convergence of biomedical science and technology (30-2020-007), Pusan National University Yangsan Hospital.

■ Acknowledgments

All authors were involved in the design of the clinical study, acquisition of data, and analysis and interpretation of data. All authors were involved in the drafting and revising of the manuscript and have approved the final version. All authors declare no conflict of interest.

■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Conceptualization: Sung Young Huh, Sung-Gon Kim,

Hyeon-Kyeong Kim. Data acquisition: Hyeon-Kyeong Kim. Formal analysis: Sung Young Huh. Funding: Sung-Gon Kim. Supervision: Sung-Gon Kim. Writing—original draft: Sung Young Huh. Writing—review & editing: Sung Young Huh, Sung-Gon Kim.

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