CDP Increases Ingestion and Alters Taste Palatability across Specific Tastants in Rats

Phillip Neill

Isaac Rankin

Michael Schecter

A research thesis submitted in partial completion of PSY451 senior research thesis, at

Wofford College
Abstract

Anti-anxiety drugs containing benzodiazepines are commonly given to people suffering from Generalized Anxiety Disorder (GAD) and other forms of anxiety. Benzodiazepines are depressants that act on the inhibitory neurotransmitter gamma-amino butyric acid (GABA). GABA is associated with neurotransmitter inhibition in the parabrachial nucleus (PBN), specifically in the gustatory region. Benzodiazepines have been shown to result in hyperphagia and hyperdipsia as a result of their effects on taste palatability. Research has linked the hyperphagia evident after benzodiazepine consumption with obesity and weight gain. The changes in taste palatability observed after benzodiazepine consumption is a likely explanation of hyperphagia and subsequent weight gain. Previous research has focused on sweet and salty tastants such as saccharin and monosodium glutamate (MSG). This study expands that research to include capsaicin and ethanol, two tastants with inconclusive effects on taste palatability, in addition to saccharin and MSG. We predicted that CDP would have less of an effect on the rate of ingestion for the capsaicin and ethanol solutions than for sweet and salty tastants based on evidence from previous studies. Fourteen male Sprague-Dawley rats were tested under water-deplete and water-replete conditions in a Davis Rig to measure rate of ingestion of different concentrations of the four stimuli used. Results showed that CDP decreased aversiveness of the high concentrations of saccharin, MSG, and ethanol, but not of capsaicin. These results support previous research as well as provide new direction for research pertaining to the interaction of taste-mediated stimulants and those with alternative orosensory detection.

Introduction

Anti-anxiety drugs containing benzodiazepines are commonly given to people suffering from Generalized Anxiety Disorder (GAD) and other forms of anxiety. Chlordiazepoxide (CDP)
is a kind of benzodiazepine, a common anti-anxiety drug (Berridge and Treit, 1986).

Benzodiazepines are depressants that act on the inhibitory neurotransmitter gamma-amino butyric acid (GABA) (Berridge and Pecina, 1995). GABA is associated with neurotransmitter inhibition in the parabrachial nucleus (PBN), specifically in the gustatory region (Higgs and Cooper, 1996). Benzodiazepines increase the affinity of GABA receptors to GABA in areas such as the parabrachial nucleus (PBN) of the pons.

Benzodiazepine consumption has been connected to causing noticeable increases in food and water consumption known as hyperphagia and hyperdipsia, respectively. Findings demonstrate that hyperphagia and hyperdipsia are connected to benzodiazepines altering taste palatability, or the perceived pleasantness of food (Treit, Berridge, and Schultz, 1987; O’Hare, Kim, and Tierney, 2006). Research has linked the hyperphagia evident after benzodiazepine consumption with obesity and weight gain (Treit et al., 1987). Berridge and Treit (1986) and O’Hare et al. (2006) propose that CDP and other benzodiazepines heighten the hedonic value of tastants, while reducing the aversion to less palatable tastants. The changes in taste palatability observed after benzodiazepine consumption are the likely explanation of hyperphagia and subsequent weight gain (Berridge and Treit, 1986). Both studies suggest a direct change in taste palatability as a result of the consumption of benzodiazepines such as CDP. The palatability of tastants after injection of CDP is determined by the eating habits and level of consumption in rats. Treit et al. (1987) suggests that CDP might not play as significant a role in the reduction of aversion to negative tastants like capsaicin. Their findings suggest that CDP positive palatability reactions were facilitated by CDP but those neutral or aversive reactions to tastes were not as affected by CDP when comparing findings to control groups. This study replicated findings from Berridge and Treit (1986). In addition, benzodiazepine antagonists Ro 15-1788 and CGS 8216
were found to negate the enhancement of positive reactions induced after CDP consumption, further suggesting that benzodiazepines, and specifically CDP, alter taste palatability.

If benzodiazepines such as CDP alter responses of aversion in rats as a result of a direct change in taste palatability, then understanding the anatomy of the brain is essential in determining the chemical interaction that leads to these results. The PBN is a likely area of the brain and a part of the taste system where benzodiazepines may have taste-mediated effects (Higgs and Cooper, 1996). Upon injection of the benzodiazepine receptor agonist Midazolam into the PBN, food consumption was significantly increased in rats (Higgs and Cooper, 1996). The benzodiazepine receptor antagonist Flumenazil blocked the same hyperphagic response in rats injected with CDP, leading to reduced food consumption. Considering the effects of both the benzodiazepine agonist and antagonist, the benzodiazepine receptors of the PBN are likely the site of GABA receptors that are affected by the injection of CDP and mediate palatability and food intake (Higgs and Cooper, 1996).

This study sought to measure the effects of CDP on eating habits and palatability and consumption of tastants such as saccharin, monosodium glutamate (MSG), ethanol, and capsaicin. Using the Davis Rig to present different concentrations of the aforementioned tastants, responses to tastants were measured through the latency before licking and the number of total licks for brief 15-second presentations of different concentrations. Previous studies such as Berridge and Treit (1986) assessed the effects of CDP on primarily sweet tastants, while Miller, McGinnis, and Richardson (2008) presented findings concerning sweet, salty, bitter, and sour tastants. Taste palatability was inferred from the level of consumption and ingestive behavior in rats when consuming these tastants after CDP injection.

Sweet stimuli like saccharin generally elicit positive appetitive responses, and saccharin
can be perceived as bitter at higher concentrations as demonstrated by decreased ingestion in several studies (Miller, McGinnis, and Richardson, 2008; Parker, 1991). Concentrations greater than 10 mM of saccharin will likely be aversive (Berridge and Treit, 1986).

Previous studies demonstrate that ingestion of MSG salt at concentrations greater than 1.0 M are aversive to rats, suggesting that the palatability of MSG is more contingent on the concentration of the substance. Findings from Miller, McGinnis, and Richardson (2008) suggest that lower concentrations of MSG ranging from 0.1 M to 0.5 M are palatable to rats, and CDP enhances the palatability of these concentrations as compared to saline controls.

Capsaicin is a highly aversive compound that is an ingredient in chili peppers and stimulates free nerve ending to elicit pain. As a tastant, it is predicted that responses to capsaicin will be highly aversive, but that CDP will reduce the negative response observed in control groups. Capsaicin is also likely to elicit a response mediated through the trigeminal nerve ganglions in response to pain, and so the reaction could be less influenced by CDP as the response will not be entirely taste mediated.

Ethanol is also considered an aversive stimulus that will elicit similar reactions to capsaicin; however, ethanol is grain alcohol, and the effects of alcohol on neurochemical processes could lead to cognitive and physical impairment that overshadows the taste-mediated effects of CDP. A study by Soderpalm and Hansen (1998) showed that benzodiazepines significantly increased the hedonic value and consumption of ethanol (8%, 10%) in comparison to saline control. This suggests that CDP might have a more pronounced effect on consumption of ethanol than capsaicin.

The purpose of this experiment is to measure the effects of benzodiazepines, specifically CDP, on the patterns of ingestive behavior in rats consuming MSG, saccharin, capsaicin, and
ethanol, in order to draw conclusions on the effects of CDP on taste palatability in rats.

Based on the results of Miller, McGinnis, and Richardson (2008) and Parker (1991) it is predicted that CDP will significantly increase the palatability of saccharin at the concentration levels of 2.5 mM, 5 mM, and possibly at 10 mM, while decreasing the negative taste quality of 50 mM. It is also predicted that CDP will increase the palatability of MSG at concentrations 0.1 M and 0.3 M, while reducing the negative taste quality of 0.5 M and 1.0 M concentrations of MSG.

Considering that capsaicin is a trigeminal nerve irritant that will likely have more pain-mediated effects than taste-mediated, it is predicted that CDP will not significantly decrease the aversiveness at any concentration. The findings of Soderpalm and Hansen suggest that CDP could enhance the positive taste qualities of ethanol at higher concentrations, and it is predicted that the CDP will significantly increase the amount of licking at 10% and 12% ethanol.

Overall it is predicted that CDP will have less of an effect on the rate of ingestion for the capsaicin and ethanol solutions than for sweet and salty tastants.

During the second and third phases of testing, the water replete schedule will likely alter the overall amount of licking and consumption in rats, but it is predicted that the consumption of MSG and saccharin will be similar to the pattern of consumption in the first phase of water deplete testing.

Methods

Animal Subjects

Fourteen male Sprague-Dawley rats were used in this experiment. Each rat was housed under a 7:00 a.m. to 7:00 p.m. light/dark cycle, where lights were off from 7:00 p.m. until 7:00
a.m., and on from 7:00 a.m. until 7:00 p.m. during the first phase of testing in individual plastic cages. Rats were housed on a 2:00 a.m. to 2:00 p.m. light/dark cycle during the second and third phases of testing. Rats were restricted on a water deplete schedule for 23 hours prior to training and during the first phase of the experiment. Water was available ad libitum before being removed for four hours prior to testing during the second phase, a water replete schedule, and 30 minutes prior to testing during the third phase, also a water replete schedule.

**Chemical Stimuli**

Five different taste stimuli were used to test the effects of benzodiazepines on food consumption and palatability of tastants. Four concentrations of each tastant were tested as follows: saccharin (2.5, 5, 10, 50 mM), MSG (0.1, 0.3, 0.5, 1.0M), capsaicin (5, 10, 15, 30 µM), and ethanol (2%, 4%, 8%, 12% with water). Water was utilized as the control solution. The same tubes were used for each concentration of tastant so as not to dilute solutions. The second and third phases of testing used only the tastants MSG (25, 50, 100, 300 mM) and saccharin (1.25, 2.5, 5, and 10 mM) at the same concentrations used in phase one.

**Behavioral Procedures**

Rats were run in a Davis Rig, a caged environment where automated sequences of tastant presentation make one tastant available at a time for a set time interval. The Davis Rig contained 16 alternating solution tubes that were presented to rats individually and available for the same interval. Rats underwent pilot design testing to determine baseline responses to tastants, and then subsequently underwent one day of water testing to acclimate the subjects to the testing environment. Two training sessions were conducted to measure the effects of the environment on water consumption, and injections were given during both training days utilizing a counterbalanced schedule alternating the rats between CDP and saline injections on successive
days. Injections were given 20 minutes prior to each individual rat’s testing session to maximize the effects of the CDP injection during the test. Rats then underwent 6 days of testing with presentation of MSG, saccharin, and capsaicin, each solution presented in random order for 2 consecutive days. On days 7 and 8 of testing, ethanol was presented to all rats. During each test session 3 tubes were filled with filtered water. Due to days with insufficient data, six rats were tested with capsaicin, MSG, and saccharin on make-up day 9. Tastants were presented at random during 30-second windows for consumption. Ten seconds were given between tastants, and if rats did not initiate licking during the first 30-second window, two additional 30-second intervals were presented to rats before moving on to the next tastant. Once a rat initiated licking, a 15-s trial began. During the water replete testing of the second and third phases, only saccharin and MSG were tested. Rats were alternated daily between CDP and saline on a counterbalanced schedule. CDP and saline injections were administered 20 minutes prior to each individual rat’s testing session to maximize the effects of the CDP injection during the test.

Statistical Analysis

Average licks and latencies for each concentration of tastant were compared between CDP and Saline conditions using a within- subjects Analysis of Variance (ANOVA). Significant differences were then assessed further using post-hoc Pairwise Comparisons T-tests with a statistical criterion of p<0.05.

Results

Saccharin

Saccharin was tested at 4 different concentrations along with water: 2.5, 5, 10, and 50 mM. The rats had been water deprived and were therefore motivated to lick. There was an overall main effect of CDP on the number of licks [$F_{(1, 13)} = 7.23, p < 0.05$] as
shown in Figure 1. There was also an overall main effect of concentration on the number of licks \[ F(4, 52) = 37.87, p < 0.01 \] as shown in Figure 1. CDP increased the number of licks for the 5 mM \( t_{13} = 2.53, p < 0.05 \), 10 mM \( t_{13} = 2.88, p < 0.05 \), and 50 mM \( t_{13} = 3.08, p < 0.01 \) concentrations of saccharin. Water as well as the lowest concentration of saccharin did not show any effect of CDP on licks. A significant interaction between CDP and concentration \[ F(4, 52) = 7.14, p < 0.01 \] can be seen in Figure 1. Even though the rats are water deprived and therefore highly motivated to lick the tastants, they still seem to avoid the aversive concentration of 50 mM when under both the control condition and the experimental condition. They avoid it less, however, in under the influence of CDP.

**MSG**

MSG was tested at 4 different concentrations along with water: 0.1, 0.3, 0.5, and 1 M. The rats had been water deprived and were therefore motivated to lick. There was no overall main effect of CDP on the number of licks as shown in Figure 2. There was a significant main effect of concentration on the number of licks \[ F(4, 52) = 57.25, p < 0.01 \] as shown in Figure 2. A significant interaction between CDP and concentration \[ F(4, 52) = 5.56, p < 0.01 \] can be seen in Figure 2. CDP increased the meal licks for the highest concentration (1 M) only \( t_{13} = 2.85, p < 0.05 \). CDP only affected certain concentrations of MSG. Like the results with saccharin, again it can be seen in Figure 2 that the rats avoided the highest concentration of MSG when under both the control and the experimental condition. They avoid it less, however, when given CDP.

**Ethanol**

Ethanol was tested at 4 different concentrations along with water: 2, 4, 8, and 12 %
with water. The rats had been water deprived and were therefore motivated to lick. There was no overall main effect of CDP on the number of licks as shown in Figure 3. There was a significant main effect of concentration on the number of licks \([F (4, 52) = 49.49, p < 0.01]\) as shown in Figure 3. A significant interaction between CDP and concentration \([F (4, 52) = 5.38, p < 0.01]\) can be seen in Figure 3. CDP selectively affected the highest concentration of ethanol. CDP increased the meal licks for the highest concentration (12%) only \((t_{13} = 4.22, p < 0.01)\). Again, the highest concentration was clearly avoided by rats regardless of whether they were given a saline injection or CDP. The rats avoid the highest concentration, however, significantly less when given CDP.

**Capsaicin**

Capsaicin was tested at 4 different concentrations along with water: 5, 10, 15, and 30 µM. Capsaicin is typically an aversive stimulus and is thought to elicit the pain pathways more than the taste system. The rats had been water deprived and were therefore motivated to lick. There was no overall main effect of CDP on the number of licks as shown in Figure 4. There was a significant main effect of concentration \([F (4, 52) = 27.69, p < 0.01]\) as shown in Figure 3. There was not a significant interaction between CDP and the concentration, as can be seen in Figure 4. T-tests do not show any significance of CDP at any of the concentrations. As Figure 4 demonstrates, the rats were avoiding the two highest concentrations of capsaicin (15.00 µM and 30.00 µM) regardless of drug and therefore clearly considered such stimuli aversive.

**Water Replete – MSG**

MSG was also tested at 4 different concentrations along with water in a water-replete scenario: 0.1, 0.3, 0.5 and 1 M. There was an overall main effect of CDP on the
number of licks \[F_{(1, 13)} = 19.56, p < 0.01\], as Figure 5 demonstrates. There was an overall main effect of concentration on the number of licks \[F_{(1, 13)} = 5.26, p < 0.01\], as Figure 5 demonstrates. There was no significant interaction between CDP and the concentration, as Figure 5 demonstrates.

*Water Replete – Saccharin*

Saccharin was also tested at 4 different concentrations in a water-replete scenario along with water: 2.5, 5, 10 and 50 mM. There was an overall main effect of CDP on the number of licks \[F_{(1, 13)} = 15.41, p < 0.01\] as shown in Figure 6. There was a significant main effect of concentration \[F_{(4, 52)} = 20.84, p < 0.01\] as Figure 6 demonstrates. There was not a significant interaction between CDP and the concentration, as Figure 6 demonstrates.

The latency differences for each of the taste stimuli were not significantly different between the experimental and control groups with the exception of ethanol. However, it should be noted that latency differences with ethanol were only statistically significant at the highest concentration \[t_{13} = 4.22, p < 0.01\] but the difference in latency was so minimal that it did not affect the difference in the number of licks.

**Discussion**

The results indicate that CDP caused rats to exhibit decreased aversion to MSG and saccharin in concentrations of 1.0 M and 10 mM, respectively. In addition, CDP did not significantly affect the average number of licks to capsaicin at any concentration, refuting the prediction that the aversiveness of capsaicin in solution would be reduced by CDP. CDP's effect on ethanol showed a significant difference in average number of licks.
to the highest concentration of the solution, indicating that the concentration at which ethanol becomes an aversive stimulus is affected by CDP. This is consistent with the findings of Soderpalm & Hansen (1998) that the effects of CDP produced increased licking behavior despite the rats' otherwise lethargic behavior; it is interesting to note that this effect was observed using a dose of 5-10mg/kg which is greater than the 1mg/kg dose used in our study. This is also supportive of our prediction that CDP would have a more pronounced effect on ethanol consumption than on consumption of the capsaicin solution.

This evidence points to the likelihood that CDP's action on GABA receptors in the PBN to exert an effect on taste palatability, potentially the result of decreased aversion to typically unpalatable or aversive stimuli. This means that the primary way in which CDP affects hyperphagia is by increasing palatability to all taste stimuli. It is important to note that CDP did not affect the aversiveness of capsaicin in solution (see Figure 3), which indicates that its effects on the transduction process of pain signals in the trigeminal nerve ganglions is not as marked as the effect of the drug on incoming taste signals. In addition, CDP did not affect the mean number of licks to water at any point, indicating that the test subjects were not simply licking because they were thirsty, but rather there was an effect of CDP on taste palatability, resulting in differences in the number of licks observed to appetitive as opposed to aversive concentrations of the particular stimuli.
One potential problem with this experiment was that 6 of the test subjects had to be re-tested as a result of an extremely poor performance on certain test days. This could have been a result of lethargy as a side-effect of the effects of CDP or it could have been a response to the aversiveness of the test environment itself. It is possible that the rats began to associate the Davis Rig with highly aversive stimuli such as the 30µM capsaicin solution. While it is unlikely given that the different concentrations of the stimuli were presented in random order, this association could be related to the poor performance demonstrated on six different occasions.

Water replete testing was included as part of this experiment to examine whether or not the rats’ thirst was a powerful enough motivator to cause them to ingest aversive concentrations of the various solutions during the water deplete phase. Between the second and third phases of the experiment, the length of water deprivation was decreased from four hours to thirty minutes. This resulted in more consistent licking behavior, although a ceiling effect was observed during both phases. Worth noting is that the observed results in the second phase of the water-replete testing followed an interesting pattern in that the mean number of licks increased as the concentrations of the tastants increased; that is, the mean number of licks was fewest for water and highest for the highest concentrations of both saccharin and MSG. This could possibly be explained by the fact that the test subjects had free access to water up to thirty minutes before testing began and therefore might have been more motivated to ingest the solutions containing tastants than they were to ingest water.
The main problem with the first phase of water-replete testing was that the rats showed decreased or no motivation to lick more frequently than during the water-restricted trials. This could potentially be a result of the lack of a designated training day, as occurred before the first water-restricted tests to acclimate the animals to the testing environment. Additionally, the four-hour water deprivation may not have been enough to significantly motivate the rats to drink while in the test chamber. In the second phase of water-replete testing, a shorter period of water deprivation (30 min prior to testing) was used and more consistent results were observed.

Further research to determine more distinctly the differences between gustatory stimuli and irritants such as capsaicin in regards to the way the different signals are processed in the brain. In addition, researchers might consider testing rats on varying doses of benzodiazepines to determine if there is a certain dose at which the aversiveness of capsaicin will be decreased as seen in taste-mediated stimuli. Further research could also examine differences between oral administration of benzodiazepines and direct injection to the PBN or other areas of the brain. Similarly, observing the results of administering a GABA antagonist in conjunction to benzodiazepines might help strengthen the assertion that the taste-mediated effect of the drug is in fact the primary factor in the observed difference in the licking behavior in the test subjects. Another important topic to cover would be the practical implications of these and other findings as they relate to human use of benzodiazepines. Research to examine options to help
mediate hyperphagia associated with benzodiazepine use could potentially lead to new ways of dealing with the growing obesity epidemic.
References


Figure 1. Displays number of licks/15s for increasing concentrations of saccharin in Phase 1 water-deplete trials. Significant drug effects for specific concentrations are indicated by cross (p<0.05) or star (p<0.01).
Figure 2. Displays number of licks/15s for increasing concentrations of MSG in Phase 1 water-deplete trials. Significant drug effects for specific concentrations are indicated by cross (p<0.05) or star (p<0.01).
Figure 3. Displays number of licks/15s for increasing concentrations of ethanol in Phase 1 water-deplete trials. Significant drug effects for specific concentrations are indicated by a star (p<0.01).
Figure 4. Displays number of licks/15s for increasing concentrations of capsaicin in Phase 1 water-deplete trials.
Figure 5. Displays number of licks/15s for increasing concentrations of MSG in Phase 3 water-replete trials. Significant drug effects for specific concentrations are indicated by a cross (p<0.05) or a star (p<0.01).
Figure 6. Displays number of licks/15s for increasing concentrations of saccharin in Phase 3 water-replete trials. Significant drug effects for specific concentrations are indicated by a cross (p<0.05) or a star (p<0.01).