Reduced Urination Rate while Drinking Beer with an Unpleasant Taste and Off-flavor

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A lowered subjective evaluation of the taste and flavor of beer due to staleness or to the addition of an unpleasant taste and flavor was found to be closely correlated with the urination rate. Beer in the same lot was compared immediately after shipment from the brewery and after leaving at room temperature for 1 month or 5 months. Each beer sample was given to volunteers at the rate of 3 ml/kg/15 min for 2 hours, and the urine volume was measured every 30 minutes. The urination rate was highest from the volunteers who drank fresh beer and lowest from those who drank 5-month-old beer. The subjective evaluation of both the taste and drinkability of 5-month-old beer was significantly lower than that of fresh beer. Beer samples with various unpleasant taste and flavor substances added lowered the urination rate. The results suggest that the perception of an unpleasant taste and off-flavor would lower the urination rate.

Key words: beer; taste; off-flavor; urination rate; drinkability

The drinkability of beer refers to the characteristic of allowing one to keep on drinking beer without tiring of its tastiness to the last drop. Drinkability is an important factor in the evaluation of beer, but its physiological meaning has not yet been clarified. Beer contains a large amount of potassium, but only a small amount of sodium. This is in contrast to the composition of electrolytes in blood. Drinkable beer is known to cause rapid urination. The blood flow of the body fluid may be maintained by the rapid transfer of unnecessary electrolytes and water into urine. On the contrary, beer which does not cause rapid urination for some reason or other cannot be drunk in large quantities.

The effect of taste and flavor on the drinkability of beer has long been known. Fresh beer is said to be highly drinkable, and beer with an unpleasant flavor such as staleness cannot be drunk in large quantities. However, the mechanism for the drinkability of beer has not yet been scientifically clarified. Drinkable beer seems to be closely correlated with the urination rate during the drinking period. We thus assumed that an unpleasant taste and off-flavor would lower the drinkability of beer. These effects could be reflected in a decrease in the urination rate. We considered that unfavorable components in beer could delay gastric emptying and urination through the perception of an unpleasant taste and off-flavor.

The diuretic effect of beer is thought to be due to its alcohol content or to be characteristic of beer itself. According to Sogawa, beer has some facilitating substances for urination, and the derivatives of nucleic acid in beer may be such substances. On the other hand, since our previous study revealed a significant correlation between the subjective evaluation of beer tastiness and the rate of gastric emptying, it is likely that the urination rate during the drinking period would be controlled not only by some facilitating substance for urination but also by the subjective evaluation of beer tastiness. Some investigators have reported that the afferent signals arising from the oropharynx and larynx by drinking beverages may play an important role in body water regulation. However, a relationship between sensorial evaluation of beer and urination rate has not previously been reported.

In the present study, we clarify the correlation between the subjective evaluation of taste and flavor and urine volume, using fresh beer and stale beer which had been kept at room temperature for a long period. We also examined the urination rate after drinking beer with various unpleasant taste and flavor substances added, and clarify that the perception of an unpleasant taste and off-flavor reduces the urination volume.

Materials and Methods

Materials.

Experiment 1. Bottom-fermented Pilsner beer (Kirin Brewery Co. Ltd., Tokyo, Japan) in the same lot was transported from the brewery and divided into three batches. For one batch, the beer was immediately placed

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in a refrigerator at 4°C. For the remaining two batches, the beer was placed in a refrigerator after being kept at room temperature (25°C) for 1 or 5 months. They were then stored at 4°C until needed.

**Experiment 2.** Bottom-fermented Pilsner beer (Kirin Brewery Co. Ltd., Tokyo, Japan) was used.

[A] The beer was poured into a transparent container (polyethylene bottle) and placed in the shade outdoors for 2 hours in the morning on a clear day. After the existence of a sunstruck flavor was confirmed after the 2nd hour, the beer, referred to as sunstruck-flavor beer, was stored at 4°C in a refrigerator.

[B] Twenty grams of hop cones were added to 750 ml of a 0.025 N NaOH solution, which was then boiled for 30 minutes. After being cooled, the hop cones were removed from the solution. The solution was neutralized by 0.01 N HCl and topped up to 750 ml, this being referred to as the hop extract. The hop extract was added to fresh beer transported from the brewery at a concentration of 2 µl/ml, and this is referred to as the hop-extract beer. Beer in the same lot, but without any treatment and without any additives, was used as the control.

**Experiment 3.** Bottom-fermented Pilsner beer (Plzensky Prazdroj, Plzen, Czech Republic) was used.

[A] The beer was passed through a Sep-Pak C18 column (Millipore Co., Bedford, MA, U.S.A.) to exclude hydrophobic components, and stored at 4°C. Table 1 shows the difference in the composition of the beer before and after the treatment.

[B] The beer was boiled to 1/10 of its original volume, removing CO2, alcohol and other volatile components, and then topped up to its original volume by adding distilled water. It was then stored at 4°C. Beer in the same lot, but without treatment, was used as the control.

In experiments 1 and 2, the beer was directly transported from the brewery, because we needed to use fresh beer without any unpleasant taste or off-flavor as the control. Its quantity was limited, so it could not be used in experiment 3. In experiment 3, the beer which was rich in isohumulon, a bitter component, was used, as we considered it important that the exclusion of hydrophobic components such as isohumulon would induce a change in the beer taste.

**Subjects.** The subjects were 11 healthy volunteers (10 males, 1 female, 26.3±2.0 years old (mean ± SE) with a range of 22–44 years, 61.3±3.6 kg in body weight with a range of 38–77 kg) in experiment 1, 7 healthy volunteers (6 males, 1 female, 26.9±2.5 years old with a range of 22–41 years, 60.9±3.8 kg with a range of 43–73 kg) in experiment 2, and 8 healthy volunteers (7 males, 1 female, 25.6±2.1 years old with a range of 22–40 years, 63.9±2.1 kg with a range of 55–73 kg) in experiment 3. In our previous study,2,3 gender difference didn’t cause any variation of the results, so we didn’t take into account the gender difference of the subjects. According to the Helsinki Declaration (1966, revised in 1989), we obtained written informed consent after explaining the details of the experiments, and any subject could drop out upon request. The subjects were ascertained not to be alcohol dependent or alcohol dehydrogenase deficient.

**Experimental procedure.** The subjects were fasted after finishing lunch at 1:00 p.m., and were not allowed to drink water after 4:00 p.m. The experiment started at 6:00 p.m., and was conducted with each subject sitting in a chair in a quiet room, where the temperature was kept at 25°C. Conversation among the subjects was allowed, but any exchange of opinions on evaluation of the test beer was prohibited. The experiment was conducted two or three times for each beer sample; in experiment 1, for example, the number of experiments performed for each subject was from six to nine in total. The subjects were not informed about the storage period or treatment of the test beer. Each subject drank beer cooled to 10°C at a rate of 3 ml per 15 minutes per kg of body weight for 2 hours. The beer was poured into a glass every 15 minutes, and the subjects were asked to drink at a constant rate. Each subject ate 25 g of pretzel (Ezaki Glico Co. Ltd., Osaka, Japan), a crisp salty biscuit made in the shape of a stick, during the 2 hours. They were instructed to urinate every 30 minutes, and the urine volume was measured. To determine the urine volume, the volunteers were weighed precisely on a digital scale (100 kg full scale, 10 g unit; FW-100K, AND Co. Tokyo, Japan) before and after urination, the difference being regarded as the urine volume discharged. The urine volume is shown as a cumulative amount during the experiment. The measurement of urine volume was continued for 180 minutes in experiment 1, and for 150 minutes in experiments 2 and 3.

In experiment 1, a subjective evaluation of the taste of the beer samples was made together with the measurement of urine volume. The subjects recorded the follow-

<table>
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<th>Component</th>
<th>Before</th>
<th>After</th>
<th>Difference</th>
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<tr>
<td>Alcohol (v/v%)</td>
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<td>4.12</td>
<td>0.65</td>
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<td>pH</td>
<td>4.63</td>
<td>4.76</td>
<td>-0.07</td>
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<tr>
<td>Nitrite (g/100 g)</td>
<td>66.8</td>
<td>39.5</td>
<td>27.3</td>
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<td>Polyphenol (mg/l)</td>
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<td>97</td>
</tr>
<tr>
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<td>0</td>
<td>31.3</td>
</tr>
<tr>
<td>Anion (mg/l)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cl−</td>
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<td>134</td>
<td>12</td>
</tr>
<tr>
<td>SO42−</td>
<td>84</td>
<td>73</td>
<td>11</td>
</tr>
<tr>
<td>PO43−</td>
<td>894</td>
<td>803</td>
<td>91</td>
</tr>
<tr>
<td>Sugar (mg/l)</td>
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</tr>
<tr>
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<td>2600</td>
<td>300</td>
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<td>300</td>
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<tr>
<td>Maltopentose</td>
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<td>100</td>
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<tr>
<td>Amino acid (mg/l)</td>
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<td>951</td>
<td>163</td>
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<tr>
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ing items every 30 minutes after starting drinking, 5 times in total, on a subjective scale of 5: tastiness, desire to drink and bitterness. The maximum tastiness, strong desire to drink, and strong bitterness were each ranked as 5, and the lowest as 1.

The sequence of beer tested was fresh, 1-month-old and 5-month-old in this order in experiment 1, and was randomized in experiments 2 and 3. The minimum interval between experiments for each subject was one week.

Measurement of E-2-nonenal. E-2-nonenal was measured by the HPLC method reported by Verhagen et al.\(^9\)

**Statistics.** In each experiment, measurements were made two or three times for each subject, and the mean values for each subject were used for a statistical analysis. There was a large individual variation in the urination rate. Therefore, cross experiments, in which all kinds of beer were given to the same individual, were conducted, and the results from the same individual (subject) were analyzed in pairs. For comparisons among the cumulative urine volume and the subjectively evaluated scores for the beer at certain times, we used one-way repeated-measure ANOVA and a contrast test in experiment 1 and 3, and a paired Student's t-test in experiment 2. We consider differences significant when \(P<0.05\). Statistics were calculated with the Stat View software package (Macintosh version 4.5, Abacus Concepts, Berkeley, CA, U.S.A.) and the Super ANOVA software package (Macintosh version 1.11, Abacus Concepts, Berkeley, CA, U.S.A.).

**Results.**

Beer immediately after transport from the brewery (referred to as fresh beer hereafter), and that kept at room temperature for 1 month and 5 months (referred to as 1- and 5-month-old beer, respectively) were used. The 5-month-old beer was clearly inferior in taste and flavor to the 1-month-old or fresh beer. The concentration of E-2-nonenal, an indicator of the flavor of beer,\(^11-13\) did not change during the first month; that is, it was 0.09 \(\mu g/l\) in fresh beer and 0.08 \(\mu g/l\) in the 1-month-old beer. However, it was as high as 0.15 \(\mu g/l\) in the 5-month-old beer, which is above the threshold value detectable by humans.\(^10\) Figure 1 shows the urination rate. The cumulative volume of urine was significantly larger from the subjects who drank the fresh beer than from the subjects who drank the 5-month-old beer, the volume from those who drank the 1-month-old beer being intermediate.

The subjects evaluated tastiness, desire to drink, and bitterness on a subjective scale of 5. As shown in Fig. 2(A), the subjects evaluated the 5-month-old beer as not tasty, the score for this beer being significantly lower than that for the fresh beer throughout the experimental

*Fig. 1. Effect on the Cumulative Urination Volume during the Period of Drinking Stale Beer. The subjects drank each beer cooled to 10°C at different sittings. They were instructed to urinate every 30 minutes after starting drinking, and the urination volume was measured. Each value shows the mean ± SEM (n=11). A significant difference between each beer was observed in cumulative urination volume (*\(P<0.05\), **\(P<0.01\), ***\(P<0.001\) by one-way, repeated-measure ANOVA and a contrast test).*

*Fig. 2. Effect on the Subjective Evaluation of Taste during the Period of Drinking Stale Beer. The subjects drank each beer cooled to 10°C at different sittings. At the beginning and every 30 minutes thereafter, they evaluated (A) tastiness, (B) desire to drink and (C) bitterness. Each value shows the mean (n=11). A significant difference was apparent between each beer (\(P<0.05\); **fresh vs 1-m, fresh vs 5-m, 1-m vs 5-m; ***fresh vs 5-m; *1-m vs 5-m by one-way, repeated-measure ANOVA and a contrast test).*
Reduced Urination Rate from Unpleasant Taste and Flavor of Beer

Fig. 3. Effect on the Cumulative Urination Volume during the Period of Drinking Beer with an Added Unpleasant Taste and Flavor.

The subjects drank the beer to which (A) sunstruck flavor or (B) hop extract had been added. They were instructed to urinate every 30 minutes from the start of experiment and the urination volume was measured. As a control, fresh beer transported from the brewery and without any treatment or any additives was used. Each value is the mean ± SEM (n=7). A difference in the cumulative urination volume was observed for each beer (*P<0.05, **p<0.1 by paired Student's t-test).

Fig. 4. Effect on the Cumulative Urination Volume during the Period of Drinking the Beer after Removing Hydrophobic Components or Volatile Components.

The subjects drank each beer cooled to 10°C at different sittings. They were instructed to urinate every 30 minutes from the start of experiment, and the urination volume was measured. Beer in the same lot but without any treatment was used as the control. Each value is the mean ± SEM (n=8). A significant difference in the cumulative urination volume was observed between each beer (*P<0.05, **p<0.01 by one-way, repeated-measure ANOVA and a contrast test).

Discussion

The present study has revealed that gustation and olfaction controlled by the central nervous system, in par-
ticular the evaluation of an unpleasant taste and off-flavor, markedly reduced the urination rate. There was a large individual variation in the peak time for urination; therefore, the urination volume is shown as a cumulative figure. Each subject ate 25 g of pretzel during the drinking period. Although eating food during the test may have created an effect that we must consider, drinking beer without food in a hungry state could be very stressful on the body. In our previous experiment about beer drinkability and gastric emptying, the subjects ate the same amount of pretzel, but it didn’t affect the rate of gastric emptying. Therefore, it may have similarly had no effect in the present experiment. Storage at room temperature for 5 months is a mild treatment, but the production of E-2-nonenal was 0.15 μg/l, which is nearly equal to the threshold amount to give an unpleasant smell to humans. The formation E-2-nonenal is one of the main causes of beer staling. Evaluation scores for the tastiness and desire to drink were markedly low for the 5-month-old beer. The urination rate after drinking this beer was also significantly lower than that after drinking the fresh beer. Figure 1 shows the cumulative urination volume; the difference in urination volume was already evident within 60 minutes and reached the maximum at 120 minutes.

The sunstruck flavor is the very unpleasant smell of 3-methyl-2-butene-1-thiol, which is produced from humulon, a bitter component of hop, by combination with hydrogen sulfate after breakdown of the side chains by ultraviolet rays. This component is a characteristic of the stale flavor of beer. Another name for this smell is that of a racoon dog’s cage, or the smell of fox urine. This smell often results when commercial beer is stored under unfavorable conditions. Beer with a sunstruck flavor resulted in a lower urination rate during the drinking period than that from fresh beer.

There are two kinds of bitterness, one being pleasant that disappears rapidly, and the other being unpleasant that hangs about the tongue for a long period. Isohumulon, a bitter component, has numerous derivatives, and the quality of bitterness has not yet been thoroughly analyzed. However, beer with rapidly disappearing bitterness is considered preferable. Hops boiled for a long period have strong, unpleasant bitterness. The beer to which this bitterness had been added tended to result in a lower urination rate than that from the control beer, but the reduction in urination rate was less than that caused by the unpleasant smell of 3-methyl-2-butene-1-thiol. However, the degree of lowering of the urination rate may depend on both the quality and quantity of the bitter solution added. In the present study, the amount of bitter component added was equivalent to 4 ppm of isohumulon. If greater unpleasant bitterness had been added, the effect on lowering the urination rate might have been more marked.

Both the beer passed through the Sep-Pak column and that after boiling had a strange taste and flavor quite different from the original beer, and all subjects were surprised by the taste. From the beginning, all subjects evaluated it as unpleasant, and none rated it preferable. Both of these beers resulted in a decreased volume of urination.

Neither storage at room temperature for a long time, nor the addition of sunstruck flavor or uncomfortable bitterness changes the major components of beer. On the other hand, exclusion of the hydrophobic fraction by passing through a Sep-Pak column removes many hydrophobic components of beer almost completely, but not the highly polar components such as electrolytes, sugar and alcohol. Boiling down removes many volatile components such as alcohol. The beer resulting from such treatments has a disagreeable taste due to different causes, but all such samples resulted in a lower urination rate. Common to these beers is the judgement via the central nervous system that they are not tasty, and the signal concerning taste from the central nervous system is considered to have affected the urination rate, although the authors cannot completely exclude post-ingestive influences since the beer samples consumed in each case were chemically different.

The urination rate after drinking beer is controlled by two main steps. One is gastric emptying, and the other is the transfer of water from blood to urine by the action of kidneys. From the present series of experiments, we suggest that a signal from the central nervous system reduced the urination rate mainly by acting at the step of gastric emptying. This is because many subjects recorded the complaint of stomach fullness after drinking the beer to which sunstruck flavor or bitterness had been added. In previous experiments with 5 kinds of beer brewed differently, we observed a close correlation between the evaluation of an unpleasant taste and the rate of gastric emptying. These results support the idea that the judgement by the central nervous system influences gastric emptying.

On the other hand, whether the signal from the central nervous system affected the water absorption from the small intestines and colon following gastric emptying is not clear due to experimental difficulty. Furthermore, the degree of the contribution of effert signal transmission from the central nervous system to the kidneys is also unclear. Acceleration of the action of the sympathetic nerve has been found to promote the production of adrenaline, angiotensin II and prostaglandin. It is possible for these substances to affect the renal function, but this remains to be examined further.

In conclusion, the unpleasant taste and off-flavor of beer were found to result in a lower urination rate while drinking beer. The mechanism for this decrease in urination rate has not yet been clarified, but it is considered to be due to delayed gastric emptying which is caused by an unpleasant perception at the central nerve that is transmitted via the nervous system.

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