A Study on Taste Effectiveness of Cycloheximide as a Repellent to Rats

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In the present study, the electrical responses of the taste nerve and the lingual nerve of a rat to cycloheximide applied on the tongue were analyzed to determine the taste effectiveness of this chemical. The residue of cycloheximide on the tongue after rinsing with water was also measured to evaluate the possible effective period of this chemical. In addition, the residual effect of previously applied cycloheximide on the drinking behavior of the rat when fed with quinine hydrochloride solution was also determined. Results obtained are as follows: cycloheximide applied to the tongue induced obvious response in the whole chorda tympani, and the bitter-sensitive unit fiber within the whole chorda tympani was specially sensitive to cycloheximide. However, when higher concentrations of this chemical was used, nerve response to the 2nd trial was not elicited, and the response recovery of the nerve to this chemical varied depending on its concentration. Even after rinsing the tongue surface with water, the previously applied cycloheximide still remained, suggesting a firm binding of cycloheximide with the sites of bitter taste receptors in the tongue. Responses of the lingual nerve to mechanical and thermal stimuli applied to the tongue did not change at all even after application of cycloheximide.

The present results indicate that the repellency of cycloheximide to rat is primarily attributed to the sense of taste transmitted through the bitter-sensitive unit fiber in the taste nerve and to its long lasting effect on the bitter taste receptors of the tongue.

INTRODUCTION

Various kinds of acute or subacute toxic rodenticides have been widely accepted as effective means for killing rats and mice. In addition, chemicals effective to prevent or to minimize rodent attacks upon packaged materials and the invasion of rodents into warehouses, dwelling houses, stores and buildings have also been well considered. Since Traub et al. (1950) proposed the strong repellent action of cycloheximide to rodents, cycloheximide has generally been accepted as one of the active repellents to rat. Concerning the repellency of cycloheximide to rats, Okuda (1959) assumed that the repellent action of this chemical may be due to its taste rather than to its olfactory effectiveness. However, there are still not enough neurophysiological evidences

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concerning the taste mechanism of cycloheximide, and behavioral or electrophysiological studies on the mechanism of effectiveness of this chemical are urgently needed.

In the present experiment, the electrical responses of the chorda tympani and the lingual nerves to cycloheximide were recorded in rats, and the residual effectiveness of cycloheximide applied to the tongue surface was also evaluated. On the basis of the results obtained the mechanisms of repellency of cycloheximide have been discussed.

**MATERIALS AND METHODS**

Fifty male adult rats of the Wistar strain, weighing 100-200 g, were used. The following chemicals were used as test solutions: 0.0000018-0.05 M cycloheximide, 0.5 M sodium chloride, 0.2 M tartaric acid, 0.9 M sucrose and 0.03 M quinine hydrochloride. All of these taste solutions were kept at 25-30°C to avoid thermal effects of the solutions on receptors of the tongue, except in experiments which were designed to observe the lingual nerve response to thermal stimulation.

A pure preparation of cycloheximide (Naramycin, Tanabe Pharmaceutical Co.) was used in this experiment, and it was the same compound (actidione) found by Leach et al. (1947).

*Responses of Taste and Lingual Nerves to Cycloheximide Solution*

Rats were anesthetized with a mixture of Nembutal (50 mg/kg) and 15 per cent urethane (intraperitoneal injection), and the trachea was cannulated. The masseter muscle and mandibular ramus were partially removed and the chorda tympani nerve or lingual nerve was exposed. The nerve was separated from the surrounding connective tissues under observation with a stereoscopic microscope. Responses of the whole chorda tympani nerve or those of the functional single fiber to taste solutions and to cycloheximide solution applied to the tongue surface were recorded by a platinum wire electrode (100 µ in diameter). A silver plate was placed on the adjacent tissue as an indifferent electrode. The electrodes were connected to a 5 stage R–C coupled amplifier and the integrated response was recorded by a cathode-ray oscilloscope and a recording camera or with an electronic summator on an ink-writing recorder. After

![Fig. 1. Schematic diagram of experimental procedures. AMP : amplifier, INT : integrator, OS : oscilloscope, IWO : ink-writing oscillograph, ct : chorda tympani.](image-url)
the application of cycloheximide, the responses of the lingual nerve to pressure or cooling stimulation to the tongue were recorded with an electronic summator on an ink-writing recorder (Fig. 1). The tongue surface was rinsed well with plain water after each trial. In these experiments, the test solutions were all prepared with deionized water.

Residue of Cycloheximide in the Tongue

One ml of a 0.036 M cycloheximide solution was dropped on the tongue surface of the rat anesthetized with ether, and within 1.0 min the tongue was cut off at the base. The epithelial tissue of the tongue was separated free from underlying muscle tissues and weighed. The material, in distilled water of ten times the weight as that of the epithelial tissue, was ground with the homogenizer of the Potter-Elvehjem type and the supernatant was obtained by centrifugation at 3000 r.p.m. for 15 min. The content of cycloheximide in the supernatant was determined by bioassay (cup method) with yeast, Saccharomyces cerevisiae AJ-4008.

Effect of Cycloheximide on the Discriminative Ability for Quinine Hydrochloride

One ml of cycloheximide solution (0.006 M and 0.05 M) was dropped on the tongue surface of the rat and after 30 sec the tongue surface was rinsed five times with one ml of distilled water. After 1 hr, the daily intake of distilled water and 0.0003 M quinine solution were determined by a two bottle preference method.

RESULTS

1. Integrated Response of the Whole Chorda Tympani to Cycloheximide

As shown in Figs. 2 and 3, when cycloheximide solution (0.05 M and 0.07 M) was applied to the tongue surface, a definite response was recorded from the chorda tympani. However, even after rinsing the tongue with distilled water, the chorda tympani

Fig. 2. Responses of the whole chorda tympani to sodium chloride and cycloheximide solutions. Upper trace of each record is integrated response, and lower is spike discharge. 1: 0.5 M NaCl (control), 2: the first trial with 0.05 M cycloheximide, 3: the second trial with 0.05 M cycloheximide.
Fig. 3. Integrated response of the whole chorda tympani following repetitive application of 0.07 M cycloheximide solution. 1: the first trial, 2: the second trial after rinsing with running water for 30 sec. Downward arrows indicate onset of cycloheximide application and upwards arrows indicate onset of application of running water. No response at the second trial. Time is in seconds.

Fig. 4. Relationship between magnitude of response of the whole chorda tympani and concentration of cycloheximide solution.

The chorda tympani did not show any response to a second trial of the same concentration of cycloheximide. In some cases, an off type response was observed at the time of rinsing but it disappeared following sufficient rinsing of the tongue.

A relationship between the concentration of cycloheximide and the magnitude of the integrated response of the chorda tympani was determined to exist within the range of 0.0001 M to 0.003 M. The integrated response of the chorda tympani increased in proportion to increase of the cycloheximide concentration, and in higher concentrations of more than 0.003 M, the magnitude of the integrated response of the chorda tympani decreased slightly and showed a steady value in spite of increases in the concentration (Fig. 4).
Fig. 5. Recovery of response of the whole chorda tympani following repeated application of cycloheximide solution. 1: 0.0018 M, 2: second trial immediately after rinsing with running water for 30 sec., 3: rest of two hours between the first and the second trial. Time is in seconds.

Table 1. Integrated Response of the Whole Chorda Tympani to Cycloheximide and Quinine Hydrochloride Solution Following Application of Cycloheximide

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>0.00078</th>
<th>0.006</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%) of responsibility Two hours after the first trial</td>
<td>90</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Decline (%) of responsibility at the second trial</td>
<td>30</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Decline (%) of response to quinine solution (0.03 M) after application of cycloheximide</td>
<td>0</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

Even immediately after the first trial, the chorda tympani responded to the second trial, when concentrations of cycloheximide below 0.003 M were used, although the magnitude of response was slightly lower than that of the first trial. When the interval between successive trials was more than two hours, sensitivity of the receptor recovered regardless of the concentration of cycloheximide and the chorda tympani showed similar magnitude of response as the first trial (Fig. 5, Table 1). However, when concentrations of cycloheximide over 0.003 M were used, the chorda tympani did not respond to repetitions of the trial in spite of the two hour intermission.

The integrated response of the chorda tympani to quinine hydrochloride decreased when there was a previous application of strong cycloheximide (0.05 M), but in lower concentrations (0.00078 M and 0.0009 M), cycloheximide did not induce such depressive effect on the taste effectiveness of quinine hydrochloride (Figs. 6 and 7, Table 1). However, cycloheximide showed no effect on the integrated responses of the chorda tympani to salt, acid and sweet solutions.
Taste Effectiveness of Cycloheximide

Fig. 6. Effects of high concentrations of cycloheximide solution on integrated response of the whole chorda tympani to four standard taste solutions. A—1: 0.5 M NaCl, 2: 0.2 M tartaric acid, 3: 0.9 M sucrose, 4: 0.03 M quinine hydrochloride, B—1: 0.05 M cycloheximide, 2: the second trial immediately after rinsing with running water for 30 sec., C—1: 0.5 M NaCl, 2: 0.2 M tartaric acid, 3: 0.9 M sucrose, 4: 0.03 M quinine hydrochloride. Time is in seconds.

Fig. 7. Effect of low concentrations of cycloheximide solution on integrated response of the whole chorda tympani to four standard taste solutions. A—1: 0.5 M NaCl, 2: 0.2 M tartaric acid, 3: 0.9 M sucrose, 4: 0.03 M quinine hydrochloride, B—1: 0.0009 M cycloheximide, C—1: 0.5 M NaCl, 2: 0.2 M tartaric acid, 3: 0.9 M sucrose, 4: 0.03 M quinine hydrochloride. Time is in seconds.
Fig. 8. Response of a quinine sensitive fiber in the chorda tympani to cycloheximide solution. 1: 0.03 M quinine hydrochloride, 2: 0.025 M cycloheximide, 3: second trial of 0.03 M quinine hydrochloride, 4: second trial of 0.025 M cycloheximide.

Fig. 9. Effect of cycloheximide solution of the lingual nerve. 1—arrow indicates application of 0.05 M cycloheximide, 2—arrow indicates application of tap water, 3—first arrow: one gram pressure application, second arrow: application of cooled tap water (10°C).

2. Single Unit Fiber Analysis of the Effect
A single quinine sensitive fiber in the chorda tympani which responds particularly to quinine hydrochloride applied to the tongue showed a marked response to
cycloheximide, but this response became weaker at the second trial of cycloheximide (Fig. 8).

3. **Response of the Lingual Nerve to Cycloheximide**

Possible effects of cycloheximide on the touch, pressure and temperature receptors of the tongue were examined through analysis of the lingual nerve response. The lingual nerve did not at all respond to cycloheximide and responses of the lingual nerve to touch, pressure and thermal stimuli applied to the tongue was not affected by any previously applied cycloheximide (Fig. 9).

4. **Residue of Cycloheximide on the Tongue**

As shown in Table 2, in the case of non-rinsed epithelium, an amount ranging from 12 \( \gamma \) to 29 \( \gamma \) of cycloheximide remained on the tongue surface after application of 0.036 M cycloheximide, and this residual amount corresponds to 0.42-0.29 per cent of the total applied amount. When the tongue was rinsed twice with distilled water (1 ml each time) after application of 0.036 M of cycloheximide, the residual amount of cycloheximide decreased markedly compared to the case without rinsing.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Residual amount of cycloheximide (( \gamma ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.036 M cycloheximide Non-rinse</td>
<td>11.6～28.5</td>
</tr>
<tr>
<td>0.036 M cycloheximide Two rinsings with 1 ml of water each time</td>
<td>2.7～3.8</td>
</tr>
<tr>
<td>0.036 M cycloheximide Five rinsings with 1 ml of water each time</td>
<td>1.1～1.8</td>
</tr>
</tbody>
</table>

Fig. 10. Antibiotic activity of the supernatant extracted from the tongue epithelial tissue of the rat. 0.036 M cycloheximide solution was applied first and then rinsed twice with one ml of water. 1: supernatant, 2: 0.0000072 M cycloheximide, 3: 0.0000018 M cycloheximide, 4: four-fold diluted supernatant.
and the supernatant extracted from the tongue showed antibiotic activity as shown in Fig. 10. However, a cycloheximide solution of 0.000018 M itself did not show any antibiotic activity. When the tongue was rinsed five times with distilled water (1 ml each time), the residual amount of cycloheximide (applied concentration-0.036 M) on the tongue was from 1.1 to 1.8 μg and this residual amount corresponds to the rate of 1/10—1/20 of the total applied amount of the non-rinsed case.

5. Drinking Behavior of the Rat after Treatment with Cycloheximide to Quinine Solution

Cycloheximide of 0.006 M or 0.05 M was applied to the tongue surface first, and then the rat’s drinking behavior to quinine solution (0.0003 M) and distilled water for 24 hours was observed by a two bottle preference method. Awakening from the ether anesthesia, the rat became restless with profuse salivation and rubbed its mouth against the wire net cage. Such behavior was marked when higher concentrations of cycloheximide (0.05 M) was applied to the tongue.

After the application of 0.006 M cycloheximide to the tongue, rats always hated to drink quinine hydrochloride solution and drank large amounts of distilled water, the mean daily intake of distilled water being 19 to 35 ml. That of quinine solution was a very small amount (less than 0.5 ml).

DISCUSSION

Through behavioral studies, OKUDA (1959) assumed that the specific odour of cycloheximide may act as a conditioning stimulus and a direct stimulating effect of cycloheximide on the oral mucous membrane may act as an unconditioning stimulus in rat, although the odour of cycloheximide is unperceivable to human-being. Once this conditioning reflex is established, rats will show rejective behavior to the smell of cycloheximide.

KIMISHIMA and KADO (1959) reported that, when cycloheximide was injected intravenously or into the cisterna magna of a rabbit, no electrical responses were recorded from the amygdaloid area of the brain where possibly the function of olfactory perception exists.

OMURA et al. (1961) noted that the application of cycloheximide to the tongue surface or into the nostrils of rats did not induce any changes of electrical activities of the chorda tympani and the olfactory bulb.

Furthermore, they revealed that rats lose the ability to discriminate cycloheximide solution from tap water after denervation of the glossopharyngeal nerve or after destruction of the olfactory bulb. From these results, they concluded that the specific smell of cycloheximide may act as a conditioning stimulus to induce an escape reaction in rat. Recently, KUWABARA et al. (1970) also reported similar results. As such, there are still big controversies and discrepancies about the concept of the biological effectiveness of cycloheximide on rat.

The present paper revealed that cycloheximide stimulated the taste receptors innervated by the bitter sensitive fibers in the chorda tympani. This fact suggests that cycloheximide has an unfavorable bitter taste to rat and the rat averses it. OMURA et al. (1961) and KUWABARA et al. (1970) reported that cycloheximide did not induce any responses in the chorda tympani nerve. The reasons of discrepancy between their results and our present results is not uncertain, but their conclusion was derived only from routine record of the whole nerve activity and OMURA et al. (1961)
did not use the electrical summator. Moreover, as we mentioned in this report once strong cycloheximide is applied to the tongue successive application of similar doses of this chemical does not induce responses in the chorda tympani nerve. Therefore, cycloheximide may have no effects on the chorda tympani nerve, if we pick up the results obtained through repetitive application of cycloheximide.

In addition, the present results suggest a close and firm binding of cycloheximide with the bitter receptors of the tongue and also that once cycloheximide comes in contact with the taste receptor, it will be difficult to remove it from the epithelium with mere water rinsing. Generally, taste substances are easily removed from the tongue with water rinsing and if the tongue is rinsed with water after each trial the chorda tympani may readily respond to repetitive application of the solution. In spite of water rinsing, of course, some chemicals may still remain on the tongue and the previous application of such a chemical may suppress the taste nerve response to successively applied routine taste substances. Tannic acid is one such suppressive taste chemical (Kawamura et al., 1968, 1969). Therefore, taste effectiveness of cycloheximide is somewhat similar to tannic acid, although tannic acid, more or less, suppresses the integrated response of the chorda tympani to the four standard taste solutions and cycloheximide suppressed only the response to quinine solution.

Dastoli et al. (1968) indicated that protein receptors having specific affinity to quinine hydrochloride and to sweet substances were located in specific areas of the tongue, and the interaction between receptors and taste substrates might play a prominent part to induce the bitter taste sense or the sweet taste sense. From such concepts, the firm binding of cycloheximide with the bitter receptors of the tongue may induce a continuous effect of unpalatable taste.

Cycloheximide is a kind of taste repellent, and it did not influence the lingual nerve response concerned with the general sense of the tongue.

Taste receptors are located at various parts of the oral cavity (Paffmann, 1960), and cycloheximide may stimulate taste receptors in fairly spread areas of the oral mucous membrane. Since the unpalatable taste of cycloheximide is initially perceived by rats following nibbling or licking, the practical application of cycloheximide by painting or spraying is most meaningful for protection from rat invasion. Okuda et al. (1959) showed that a paper box containing some normal diets and the outer surface of which was treated with 26 \( \gamma/cm^2 \) of cycloheximide was repellent to rats. Particularly, a painted amount of 52 \( \gamma/cm^2 \) showed the most marked repellency. The present report also showed that when one ml of 0.036 M cycloheximide solution was applied to the tongue 12-29 \( \gamma \) of cycloheximide still remained on the tongue surface even after adequate rinsing, and further, the amount of cycloheximide corresponding to such residual amount also elicited a marked integrated response of the chorda tympani. This result will be a good evidence to support the above mentioned experimental results of Okuda et al. (1959).

ACKNOWLEDGEMENTS

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