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Taste Preference and Taste Nerve Responses of Rats under Copper Toxicosis

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YAMAMOTO, T., T. KOSUGI AND Y. KAWAMURA. *Taste preference and taste nerve responses of rats under copper toxicosis.* PHARMAC. BIOCHEM. BEHAV. 9(6)799–807, 1978.—Effects of administration of cupric chloride on the taste preference for the four basic taste solutions were studied by means of two bottle preference tests in rats. The intraperitoneal injection of 450 μg $CuCl_2$/100 g B.W./day for 2 weeks induced no significant changes in the taste preference for both NaCl and sucrose solutions. On the other hand, the taste preference for tartaric acid and quinine-HCl solutions was influenced by $CuCl_2$ injection; 10 out of 27 rats took tartaric acid or quinine solutions as much as water, and 4 out of 27 rats showed a marked increase in fluid intake for tartaric acid or quinine solutions over water. Discontinuation of copper administration restored the altered preference responses to the preference level seen in the control period. Patterns of the summated taste nerve response to various concentrations of the four basic taste stimuli in the copper-injected rats were similar to those in the control rats. The possible mechanisms by which copper injection alters preference behavior are discussed.

Copper   Taste preference   Electrophysiology   Chorda tympani

A SMALL amount of heavy metals is well known to be indispensable to the life processes of most animals. Decrease or increase of these trace metals in the body beyond the normal range will induce various disorders of the body functions. Concerning taste sensation, recent studies by Henkin and his colleagues [2, 6, 7, 8, 9, 10, 15], have indicated that copper, zinc and nickel ions play an important role in maintaining the normal taste sensitivity in man. Among them, several studies have dealt with the role of copper in taste sensation. For example, Henkin *et al.* [9] and Keiser *et al.* [15] have shown that hypogeusia is produced as a side effect of treatment with D-penicillamine in a significant number of patients suffering from scleroderma, systinuria, rheumatoid arthritis and idiopathic pulmonary fibrosis. The impairment of taste sensitivity was generally associated with the lowered concentrations of ceruloplasmin induced by D-penicillamine administration, and administration of copper salts restored the impaired taste sensitivity to the normal level associated with increases of serum-ceruloplasmin to the normal level. From this finding, it is suggested that copper metabolism plays an important role in taste sensitivity in man [9]. Animal experiments by means of preference behavior have confirmed the occurrence of taste impairment after administration of D-penicillamine [7, 12, 13, 24].

On the other hand, it is known that patients suffering from Wilson's disease with excessive body levels of copper do not exhibit abnormalities of taste [9]. Few reports are available concerning changes in taste sensitivity after administration of copper to increase the tissue copper content over the normal range. The purpose of the present study is to examine effects of administration of copper on the preference behavior for four basic taste solutions and on taste nerve responses.

METHOD

Behavioral Experiment

Forty-five Wistar male rats (150–200 g B.W.) were used, 35 for experimental and 10 for control groups. The rats were acclimated to our laboratory for at least 5 days before testing. The animals were individually housed in cages $35\times25\times18$ cm at a temperature of around 25°C and a humidity of about 60%. Each cage was provided with an inverted 100 ml graduated glass cylinder with a curved drinking spout. During the acclimation period, animals were kept on a maintenance diet (Oriental Yeast Co. MF) and tap water ad lib. Preferences for taste solutions were determined with the two bottle choice technique. The test solution and distilled water were presented to the animal for 24 hr and the daily fluid intake was measured. To help eliminate position preferences bottles were removed after 24 hr, washed, filled with fresh solution, and the position switched. The amount of taste solution consumed during a 24-hr period was expressed as a percent of the total fluid intake.

As test solutions, 150 mM NaCl, 30 mM surcrose, 0.25–5 mM tartaric acid and 0.0125–0.05 mM quinine-HCl were used. All the test chemicals were reagent grade and solutions were made up with distilled water. After measuring the fluid intake for 7–10 days, 450 μg $CuCl_2$/100 g B.W./day was injected intraperitoneally for 2–6 weeks. At 900 μg $CuCl_2$/100 g B.W./day, the rats became dehydrated and emaciated, and were not suitable for the behavioral experiment. Although a quantitative analysis of copper in tissues and plasma was not performed, the amount of $CuCl_2$ administrated in the present study is supposed to be sufficient enough to cause copper toxicity in the rat, since the histopathological and histochemical surveys revealed some abnormal structures of the liver tissue and a severe deposition of copper in the liver [20]. Reagent grade of $CuCl_2$ was used and the copper solution made up of 0.9% saline was stocked such that 0.5 ml solution contained 450 μg $CuCl_2$. In some animals, effects of withdrawal of injection and re-injection of $CuCl_2$ on the fluid intake were examined. Physiological saline (0.5 ml/100 g B.W./day) was injected intraperitoneally for 2–6 weeks in the control rats.

Each experimental animal was not presented with each taste quality, i.e., each animal was used for either one of the four taste qualities; 4 were tested for sucrose, 4 for NaCl, 13 for tartaric acid and 14 for quinine-HCl. Each control animal was used for either two of the four taste qualities.

Two animals (one tested for tartaric acid and one for quinine) which did not show any position preferences in the control period developed a marked change in fluid consumption depending on bottle locations after the start of copper injection. The basis for determining position drinking was that the animal always showed a marked preference for the taste solution over water (>75% preference) when the solution was placed at a particular side, while the animal showed a marked avoidance for the taste solution (<25% preference) at the other side. In these rats, the daily fluid intake was evaluated as a mean of the fluid intake of the successive two days.

Electrophysiological Experiment

After preference testing electrical responses of the chorda tympani to various concentrations of the four basic taste stimuli (reagent grade of NaCl, sucrose, tartaric acid and quinine-HCl dissolved in distilled water) were recorded. The animals were anesthetized by intraperitoneal injection of a mixture of Nembutal (40 mg/kg B.W.) and urethane (150 mg/kg B.W.). The trachea was cannulated and bilateral hypoglossal nerves were cut to prevent possible tongue movements. The chorda tympani was exposed by the lateral approach, and was placed on a platinum wire electrode (100 μm diameter). An indifferent electrode was placed on the adjacent tissues. The electrodes were connected to a conventional amplifier and the summated response of the whole chorda tympani was recorded with an electronic summator (time constant: 0.5 sec) on an ink-writing recorder. Each solution of 3 ml was applied to the anterior region of the tongue by means of a pipette. After each stimulation the tongue was washed with distilled water, followed by tap water. The test solutions and rinsing water were kept at 37 ± 1°C. The magnitude of the summated responses of the whole chorda tympani to each of the four taste solutions was measured by a height of the maximum response from the baseline. The relative magnitude of response (r.m.r.) to NaCl (N), sucrose (S), tartaric acid (T) or quinine-HCl (Q) was expressed as a percent of the total magnitudes of responses to all the four taste stimuli:

$$\%\ \text{r.m.r.} = \frac{(N)\ \text{or}\ (S)\ \text{or}\ (T)\ \text{or}\ (Q)}{(N) + (S) + (T) + (Q)} \times 100$$

Histology

For histochemical determination of deposition of copper in tissues, each animal which showed a significant change in the preference behavior was given a lethal dose of Nembutal

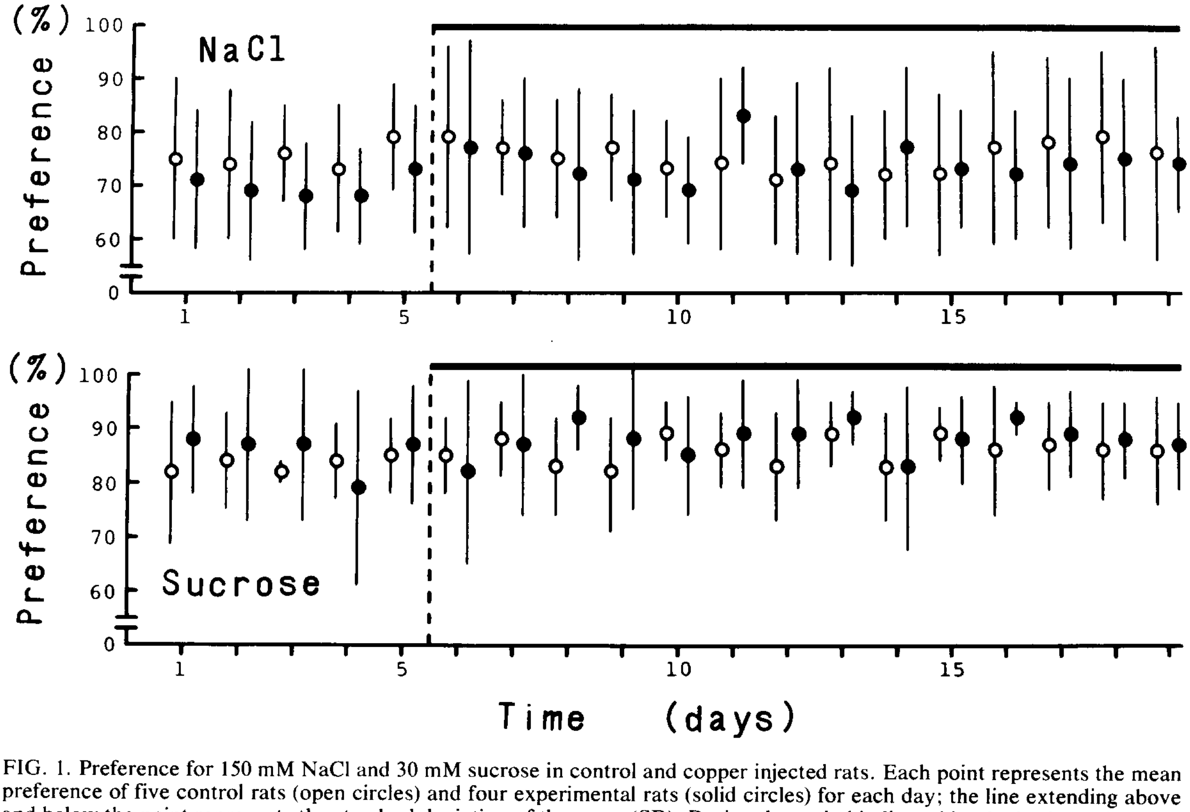

FIG. 1. Preference for 150 mM NaCl and 30 mM sucrose in control and copper injected rats. Each point represents the mean preference of five control rats (open circles) and four experimental rats (solid circles) for each day; the line extending above and below the point represents the standard deviation of the mean (SD). During the period indicated by the horizontal bar 0.5 ml saline/100 g B.W./day was administrated in the control rats and 450 μg $CuCl_2$/100 g B.W./day in the experimental rats. There is no significant difference in the mean daily preference in both groups of rats throughout the experimental period.

at the end of electrophysiological experiment, and perfusion was carried out transcardially with 0.9% saline followed by 10% Formalin. The brains and liver were removed from the animal and fixed in 10% Formalin for a week. The tissues were embedded in paraffin and sectioned at 10 μm. One or several series of sections separated by 0.1 mm was stained following the p-dimethylamino-benzylidene-rhodanine method introduced by Okamoto and Utamura [17] to find the location of copper histochemically. Sections were counterstained with hematoxylin.

RESULTS

Behavior Test

The preference responses of both control rats and experimental rats are shown in Figs. 1 and 2. In Fig. 1 the preference responses for 150 mM NaCl and 30 mM sucrose are shown. During the period indicated by a horizontal line, saline was injected in the control animals (open circles) and copper was injected in the experimental animals (solid circles). The mean daily preference of the copper-injected rats for sucrose or NaCl was essentially the same as that of the control saline-injected rats. That is, the mean daily preference of the control rats for NaCl varied between 71 ± 12% (mean ± SD, n=5) and 79 ± 16%, and that of the experimental rats between 68 ± 10% (n=4) and 83 ± 9%; the mean daily preference of the control rats for sucrose varied between 82 ± 2% (n=5) and 89 ± 6%, and that of the experimental rats between 79 ± 18% (n=4) and 92 ± 6%. In Fig. 2 the preference responses for 0.25 to 5 mM tartaric acid and 0.0125 to 0.05 mM quinine-HCl are shown. Concentration of the solutions was adjusted so that the rats drank them at around a 15% preference level. During the period indicated by a horizontal line, saline was injected in the control animals (open circles) and copper was injected in the experimental animals (solid circles). The mean daily preference of the control rats for tartaric acid or quinine-HCl was essentially the same as that of the experimental rats during the control period (first day to fifth day). On the other hand, after administration of copper the mean daily preference of the experimental rats for tartaric acid or quinine-HCl increased gradually, while that of the control rats remained almost unchanged after injection of saline. That is, the mean daily preference of the control rats for tartaric acid varied between 11 ± 8% (mean ± SD, n=5) and 19 ± 7% during the period in which saline was injected, and that of the experi-

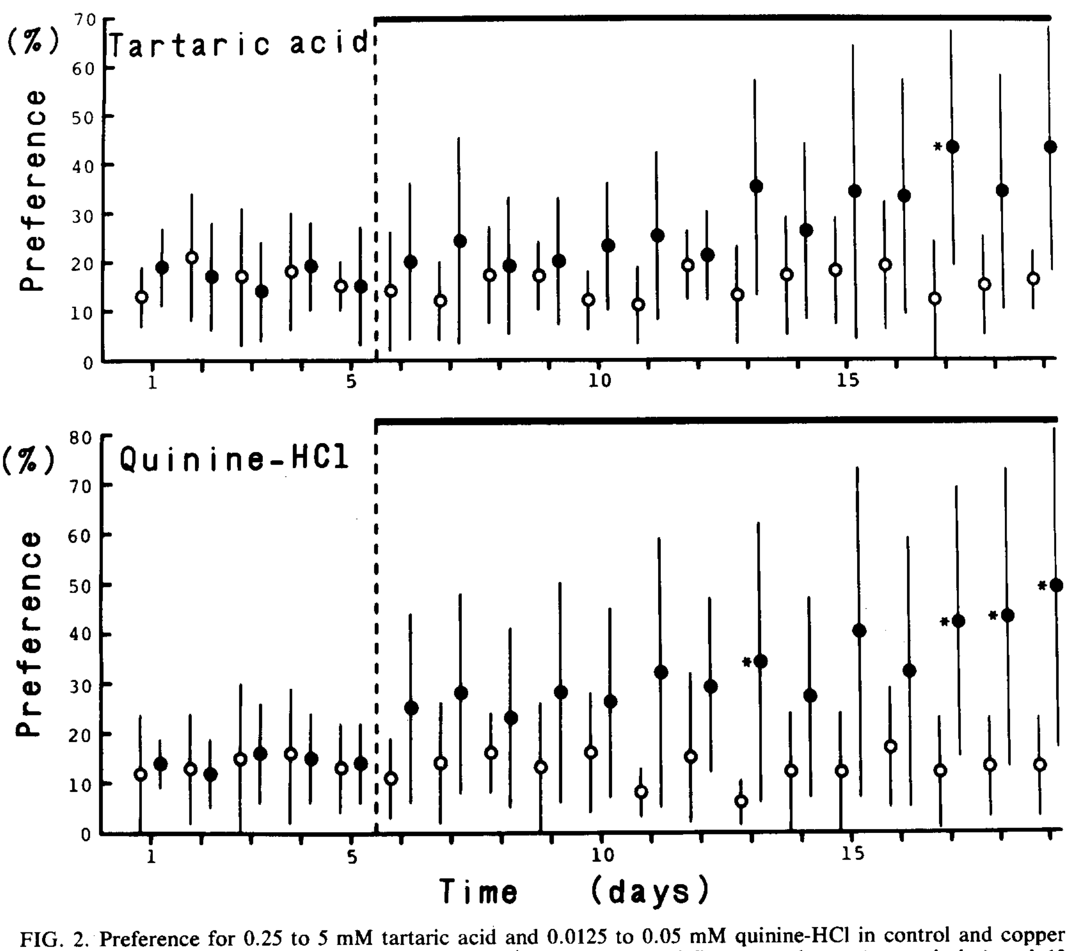

FIG. 2. Preference for 0.25 to 5 mM tartaric acid and 0.0125 to 0.05 mM quinine-HCl in control and copper injected rats. Each point represents the mean ± SD preference of five control rats (open circles) and 13 experimental rats (solid circles) for tartaric acid and 14 experimental rats (solid circles) for quinine-HCl. During the period indicated by the horizontal bar 0.5 ml saline/100 g B.W./day was administrated in the control rats and 450 μg $CuCl_2$/100 g B.W./day in the experimental rats. After administration of copper the mean daily preference of the experimental rats increased gradually to nearly 50% level. The mean daily preferences of the experimental rats indicated by the star mark in the graphs are significantly higher compared to those of the control rats.

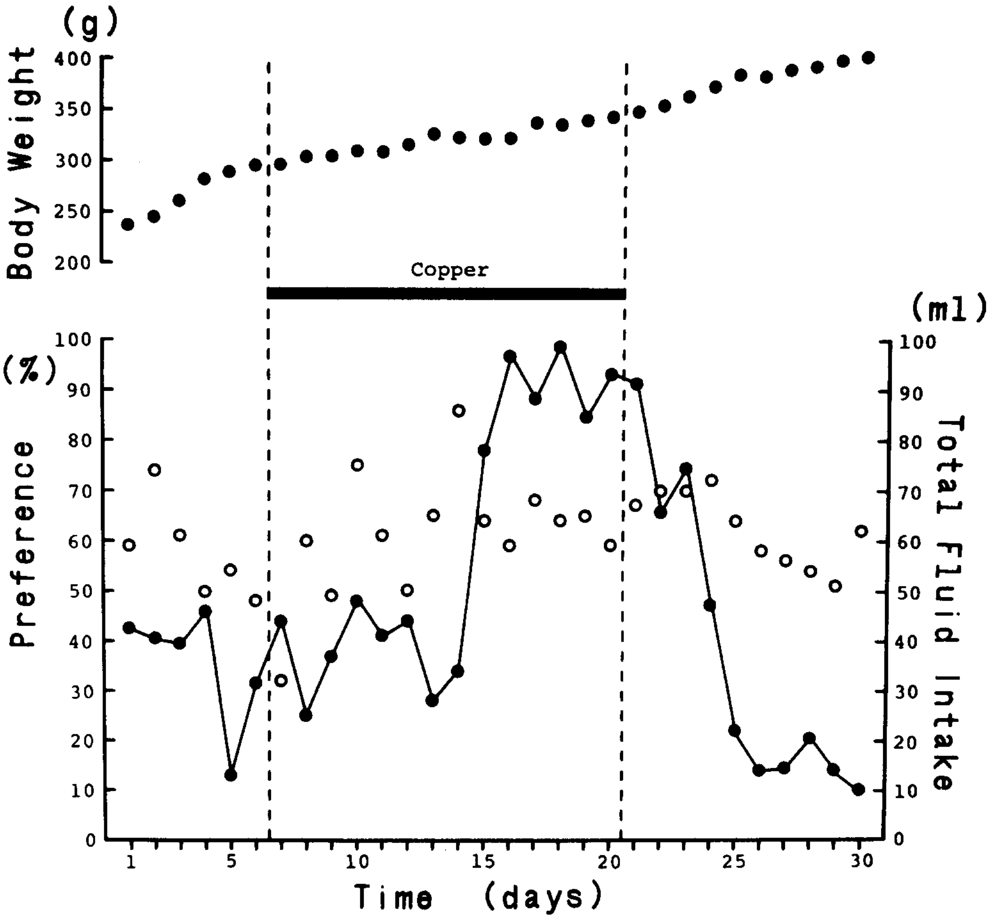

FIG. 3. Daily percent preference for 5 mM tartaric acid, total fluid intake and body weight. Solid line connects points representing daily percent preference, and open circles show daily total fluid intake of tartaric acid and water. During the period indicated by the horizontal line, 450 μg $CuCl_2$/100 g B.W./day was injected intraperitoneally.

mental rats gradually increased from 20 ± 16%(n=13) to finally 43 ± 25% after injection of copper; the mean daily preference of the control rats for quinine-HCl varied between 6 ± 4% (n=5) and 18 ± 12% during the period in which saline was injected, and that of the experimental rats gradually increased from 23 ± 18% (n=14) to finally 49 ± 32% after injection of copper. The standard deviation of the means was very large in the experimental copper-administrated rats because of a great individual variation of the preference responses. The mean daily preference for tartaric acid in the experimental rats was statistically significantly higher ($p<0.05$, t-test) than that in the control rats at the 17th day (or 12th day after the start of copper injection), and the mean daily preference for quinine-HCl in the experimental rats was significantly higher ($p<0.05$) than that in the control rats at the 13th, 17th, 18th and 19th days (or 8th, 12th, 13th and 14th days after the start of copper injection).

As mentioned above, no significant effects of copper administration on the preference behavior for sucrose and NaCl solutions were observed. On the other hand, out of 27 experimental rats (13 tested for tartaric acid and 14 for quinine-HCl), 14 rats (7 for tartaric acid and 7 for quinine-HCl) showed a significant change in their daily preferences after administration of copper, and 4 (2 for tartaric acid and 2 for quinine-HCl) out of these 14 rats took more tartaric acid or quinine solutions than water after daily intraperitoneal injection of copper; they preferred tartaric acid or quinine solutions which were rejected during the control period. Typical examples are shown in Figs. 3 and 4. In Fig. 3 the daily preference for 5 mM tartaric acid and the daily body weight are shown. After 6 days of the control period, 450 μg $CuCl_2$/100 g B.W./day was injected intraperitoneally for 14 days as indicated by a horizontal bar and vertical broken lines. Increase of body weight was slightly suppressed during the period in which copper was injected. At the 9th day after injection, the animal began to prefer tartaric acid solution over water. At the 5th day after cessation of copper injection, preference returned to the control level. There were no apparent differences in the total daily fluid intake of tartaric acid solution and water during the course of the experiment. In Fig. 4 the daily intake of 0.05 mM quinine-HCl is expressed as a percent preference and the daily body weight is also plotted. The period of copper injection (450 μg $CuCl_2$/100 g B.W./day) is indicated by a horizontal bar and vertical broken lines. After injection of copper, the preference for quinine solution increased gradually and at the 9th day the animal preferred quinine markedly over water. After cessation of injection, the animal showed a preference behavior similar to that in the control period within 3 days. The animal again showed a preference for quinine solution soon

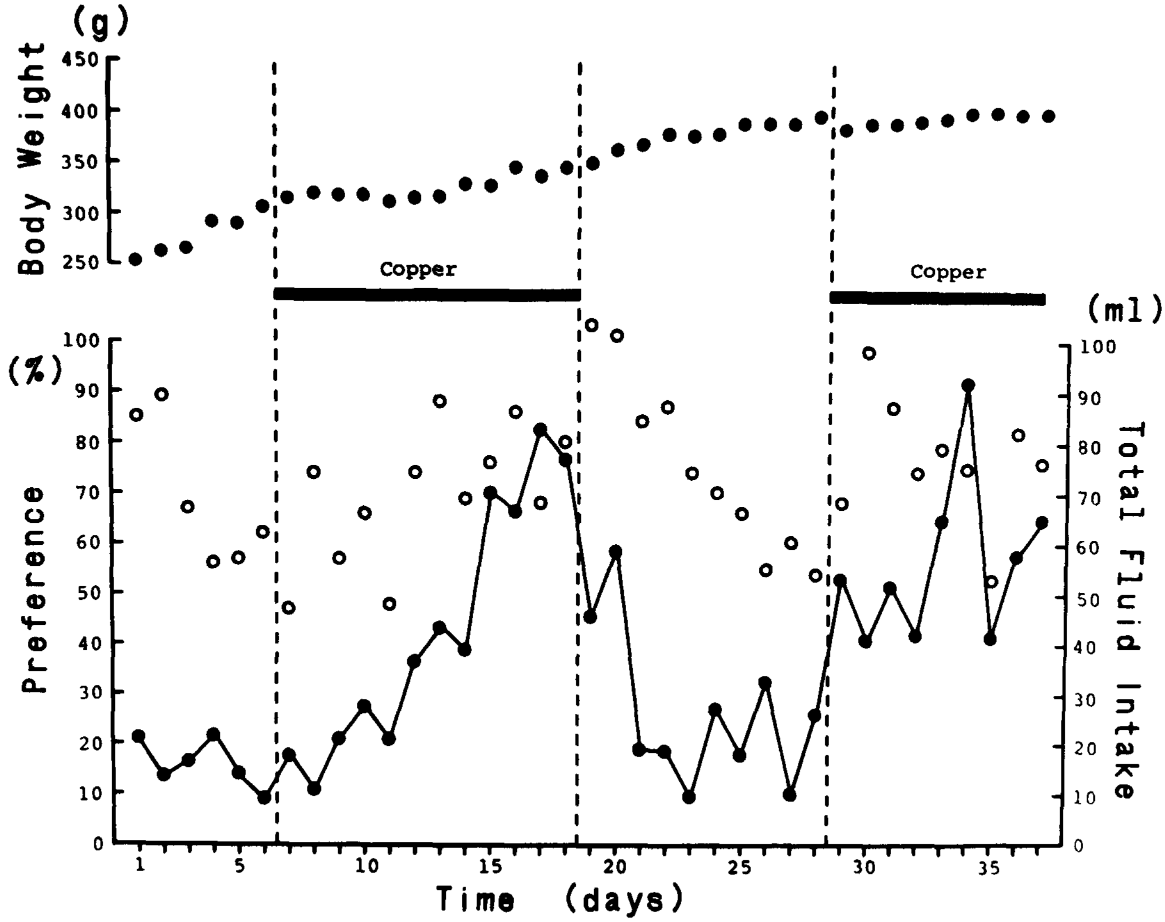

FIG. 4. Daily percent preference for 0.05 mM quinine-HCl, total fluid intake and body weight. Solid line connects points representing daily percent preference, and open circles show daily total fluid intake of quinine-HCl and water. During the period indicated by the horizontal lines, 450 μg $CuCl_2$/100 g B.W./day was injected intraperitoneally.

after reinjection of copper. Although total daily fluid intake of quinine solution and water tended to be greater during the period in which copper was injected than during the period in which copper was not injected, the difference was not significant because of a large variation in the amounts of daily fluid intake.

Ten out of 14 rats also increased the daily preference for tartaric acid or quinine after copper administration. However, in contrast to the above-mentioned highly increased preference for the taste solutions, they took the taste solutions equally with water or slightly over water. A typical example is shown in Fig. 5. In Fig. 5 the daily percent preference for 0.05 mM quinine-HCl and the daily body weight are shown. As the animal showed a marked position preference, the percent preference and the daily fluid intake are expressed as a mean of recordings of the successive 2 days, and the body weight is plotted every other day. Daily 450 μg $CuCl_2$/100 g B.W. was injected intraperitoneally during the period indicated by a horizontal bar and a vertical broken line. Increase of body weight was suppressed slightly by injection of copper. The animal began to prefer quinine solution within a few days after the start of copper injection. The percent preference was nearly 50% or slightly over 50% level, suggesting insensitiveness to the aversive taste of quinine. The total fluid intake of quinine solution and water per day was almost similar during the course of the experiment.

Electrophysiological Test

Chorda tympani responses to the four basic taste stimuli in copper-administrated rats were not significantly different in magnitude and in time course of activities from those in the control rats. Figure 6 shows the relative magnitude of summated responses of the chorda tympani to 100 mM NaCl, 50 mM tartaric acid, 5 mM quinine-HCl and 500 mM sucrose in the copper-injected rats (n=7) and in the control rats (n=10), where the magnitude of responses to each taste stimuli is expressed as a percent ratio to the sum of the magnitude of response to all the four basic taste stimuli. Each value shows mean ± SD. No significant difference was observed in the two sets of taste responses. The copper-injected animals used for the electrophysiological experiment were those which showed a significant change of preference behavior by copper injection. The control animals used were those which had been injected with 0.9% saline intraperitoneally for at least 2 weeks.

There were no apparent differences in the magnitude of responses to each concentration of the four basic taste stimuli between the copper-injected rats (n=6) and the con-

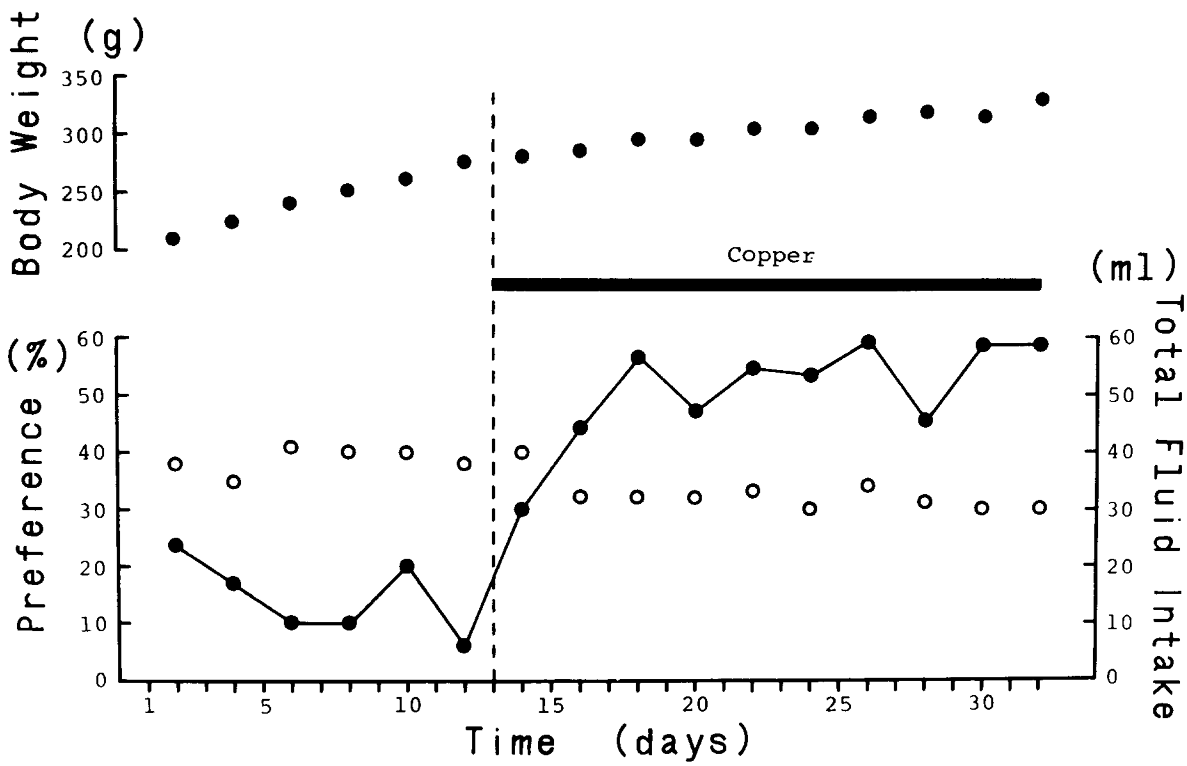

FIG. 5. Daily percent preference for 0.05 mM quinine-HCl, total fluid intake and body weight. Solid line connects points representing daily percent preference, and open circles show daily total fluid intake of quinine-HCl and water. During the period indicated by the horizontal line, 450 μg $CuCl_2$/100 g B.W./day was injected intraperitoneally. As the animal showed a marked position preference, the percent preference and the daily fluid intake are expressed as a mean of recordings of the successive 2 days, and the body weight is plotted every other day.

trol rats (n=10) as shown in Fig. 7. Each plot shows mean ± SD. Also these graphs indicate similar thresholds for each of the basic tastes in both groups of rats.

Histological Examination

The histochemical survey did not reveal any deposition of copper in the brain tissues of the copper-injected rats. However, obvious amounts of copper were detected in the liver. There were also marked pathological changes in the liver such as discoloration, loss of elasticity, fusion of the liver with adjacent organs, fusion of the lobules, proliferation of Kupfer cells, infiltration of small round cells, thickening of the liver capsule and partial destruction of the liver structures. Histochemical study in the saline-injected control rats did not show any copper in the liver with the present technique.

DISCUSSION

In the present study, we did not evaluate the blood or urine copper levels, but the histopathological and histochemical examinations of the liver were performed at the end of the experiments. Although it is limited to evaluation of copper levels in the body by the present histological measurements, a large deposition of copper in the liver in the copper-injected experimental rats may suggest an elevation of copper level in the body together with copper toxicity. Such a concept is derived from a series of studies on the copper metabolism in the rat [18, 19, 20]. Owen [18] and Owen and Hazelrig [19] have shown that the liver rapidly stores excessive copper, synthesizes ceruloplasmin and excretes it at least to furnish copper in a usable form to the tissues. They also suggested the approximate parallelism between the content of hepatic copper and amount of ceruloplasmin.

Copper injected markedly altered the taste preferences for tartaric acid or quinine solutions in 14 out of 27 rats (51.9%) tested for tartaric acid or quinine, while it did not significantly affect the preference behavior for both sucrose and NaCl. Ten out of these 14 rats took acid or quinine equally with water or slightly over water. Patton and Ruch [21] showed that rats accepted quinine solution slightly over water when the concentration of quinine was below the rejection threshold, and Engel [4] showed that very low concentrations of tartaric acid and quinine were pleasant in human subjects. In our pilot study, we have found that some rats showed a preference for tartaric acid over water at very low concentrations. Hence, the present results obtained in the copper-injected rats could be interpreted to mean that copper administration produced a decrease in taste sensitivity to tartaric acid and quinine solutions in the rat. Four out of these 14 rats showed a marked change of preference behavior for acid and quinine solutions; the percent preference reached nearly 100%. This phenomenon may not be interpreted simply by a decrease in taste sensitivity. Other more complex mechanisms may be involved.

In contrast to the present results, clinical studies [9,15] have demonstrated that 20–40% of the patients treated with

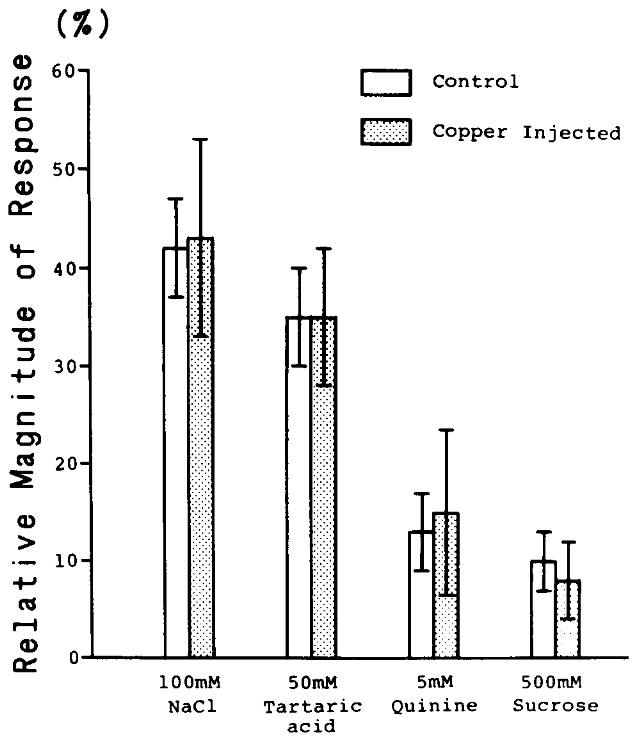

FIG. 6. Comparison of the summated response of the chorda tympani to the four basic taste stimuli in the control saline-injected rats and in the experimental copper-injected rats. The magnitude of response to each taste stimulus is expressed as a percent ratio to the sum of the magnitude of responses to all the four basic taste stimuli. Each value shows mean ± SD of 10 control and 7 experimental rats.

D-penicillamine showed a decreased taste sensitivity to four basic taste qualities, but the most noticeable subjective manifestation was an inability to recognize the taste of salts and sweets. In the rats treated with D-penicillamine, Henkin and Bradley [7] recognized an increased preference for 0.15 M and 0.3 M NaCl associated with the reduced copper concentration in plasma. Kare and Henkin [13] also observed a significant increase in the preference for NaCl solutions. These authors suggested that D-penicillamine administration produced a decrease in taste sensitivity to NaCl and D-penicillamine-treated rats preferred high concentration of NaCl which was rejected in the normal rats. Meanwhile, Zawalich [24] showed that D-penicillamine-treated rats preferred high concentrations of Na-saccharin, NH_4Cl, KCl and NaCl solutions, while tartaric acid, quinine-HCl and $CaCl_2$ solutions were not obviously preferred. More recently, Ito and Sato [12] showed a lowered taste sensitivity to NaCl and sucrose in D-penicillamine-treated rats in a behavioral experiment, while the preference for HCl and quinine were not significantly altered. It is of great interest that there is a contrary change in the preference behavior for tastes in the copper-injected rats and in the copper-deficient rats.

Chorda tympani nerve responses to the taste stimuli in the copper-injected rats were not significantly different in magnitude from those in the control rats. However, one cannot simply exclude the peripheral changes of the gustatory system; there still remain possibilities that the altered preferences for tartaric acid and quinine are due to effects of copper excreted from the salivary glands on taste receptors [23] or due to the direct effect of copper on the function of taste receptors or taste nerves because of the following reasons. First, other taste nerves innervating circumvallate, folliate or palatal taste buds were not examined electrophysiologically in the present study. These nerves may have shown some alterations in electrical activity. Second, the activity of single taste fibers in copper-injected rats may be changed without any manifestation of altered whole nerve activity. This latter possibility has been reported by Contreras [3] in sodium-deprived rats where the activity of single taste fibers revealed differences not evident in previous whole nerve preparations [16,22].

At least two other possible mechanisms exist to explain the effects of copper on the preference behavior. First, copper affects the activity of central neurons directly to induce insufficient processing of taste information. However, the histological attempt to locate the deposition of copper in the brains of the rats which showed the altered taste preferences was not successful. Methods other than histochemistry might reveal a localization of accumulated copper in the brain tissues. Alternatively, copper may not accumulate in the rat brain, since it is known that it is difficult for ceruloplasmin to pass the blood-brain barrier [1]. Second, copper may exert its effect indirectly. The gastrointestinal toxicity such as diarrhea was observed during the course of administration of copper, and histological and histochemical observations revealed severe pathological changes of the liver tis-

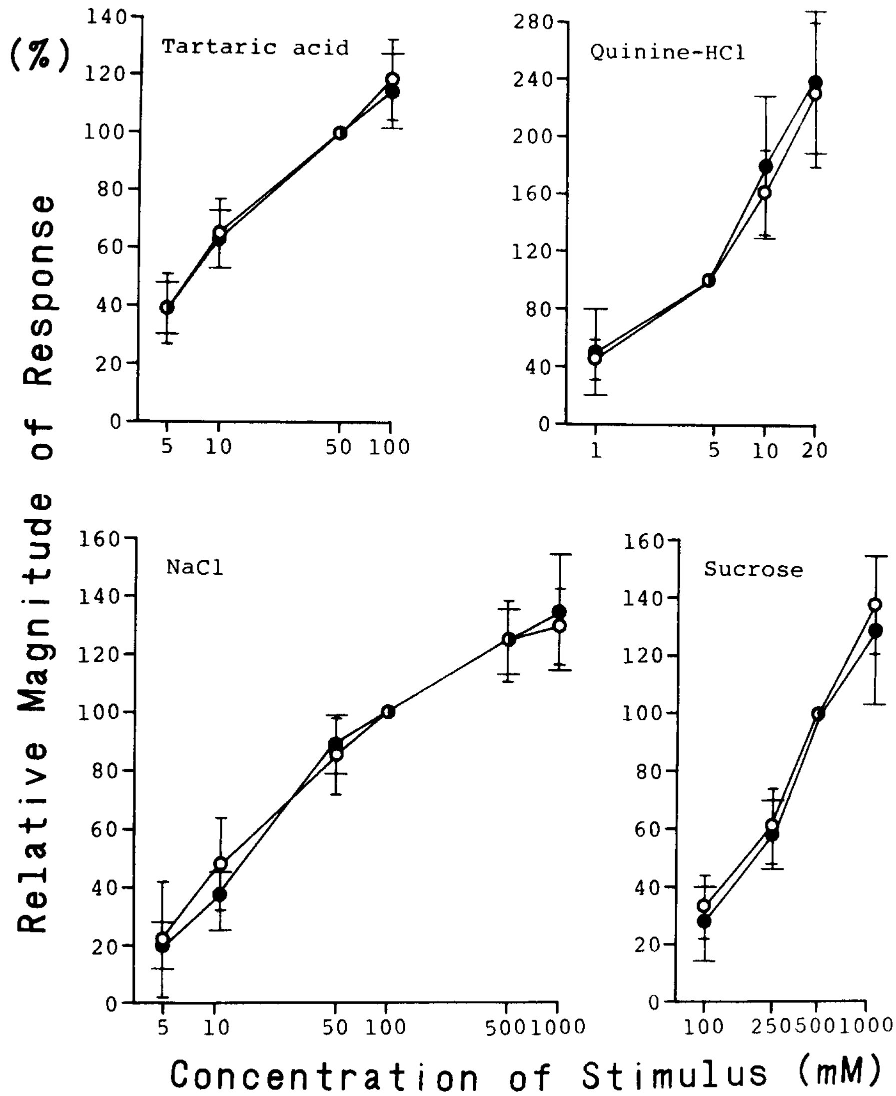

FIG. 7. Comparison of the summated response of the chorda tympani to various concentrations of the four taste stimuli in the control saline-injected rats (open circles) and in the experiental copper-injected rats (solid circles). The ordinate is a percent relative magnitude of response. Responses to 50 mM tartaric acid, 5 mM quinine-HCl, 100 mM NaCl and 500 mM sucrose are taken as the standard for each basic taste stimulus. Each plot represents mean value ± SD of 10 control and 6 experimental rats.

sues and a severe deposition of copper in the liver. Clinical studies [11,14] have shown changes of taste sensitivity in patients suffering from gastrointestinal diseases and/or a disfunction of the liver. Moreover, copper plays an essential role in the biochemistry of the living body, that is, copper is an essential constituent of many enzymes and other biological catalysts [5]. An overdose of copper in the body may affect the function of enzymes. There is a possibility that changes of the preference behavior for taste induced by increase or decrease of copper content in the body may be controlled at the biochemical level.

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