Original Articles

Gustatory Response and Acceptability of Thallium Salts in Rats

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The acceptability of thallium salts based on the behavioral response of rats was good in low concentrations, but decreased at concentrations over 0.125%, and the discrimination between the poison solutions and the plain water was clear, although a high degree of mortality was achieved at the concentrations of wide range and total ingested amount of fluid was normal value or near by it. The integrated response of the chorda tympani nerve against the application of thallium sulphate was clear in higher concentrations over 0.13% and the magnitude of the taste nerve response in five thallium salts at the concentration of the same molecule (0.48–1%, 0.02 M) was almost the same. Accordingly, a cause for the low acceptability of thallium sulphate at higher concentrations is possibly due to unpleasant taste, although a toxic action of thallium salts also involves in the process of discrimination between water and the poison solution.

The gustatory effectiveness of thallium salts, both inorganic and organic salts, coumarin and its derivatives when measured through the chorda tympani nerve of rats, suggested that the gustatory effectiveness of thallium salts might be mainly caused by thallium ions.

INTRODUCTION

Despite of its high toxicity, thallium sulphate has been still used as a rodenticide because of a high effectiveness without appreciable bait-shyness and development of resistance. The usefulness of thallium sulphate for control of voles in fields was evaluated in a previous paper.¹⁾

Evaluating a new compound as a rodenticide, the acceptability of the poison is one of the important properties to be tested. Kusano²⁾ reported already on some findings involving the acceptability of thallium sulphate as measured with the Osaka University type of Skinner's box. The present paper describes acceptabilities of thallium salts measured by the choice responses and the electrical response of the gustatory nerve to these salts in rats.

MATERIALS AND METHODS

Male adult rats of Wistar strain, weighing 150–250 g were used. The rats were housed in individual rearing cage (38×26×21 cm). After these rats were purchased from The Animal Breeding Laboratory (Ōmiya City), they were reared in our laboratory for one week and employed as test animals. The room temperature during the present experiment was from 17°C to 22°C.

1. Acceptability Thresholds

Thresholds-taste, discrimination, and absolute rejection of various thallium salts and sodium salts were determined by the gradual two-bottle preference method used in a previous paper.³⁾ The starting concentration of these salts was 0.000002% as salt. The data on the sodium chloride used in this paper are those published previously.⁴⁾ The concentra-

tion at which the rats began to take less of both the poison solution and the plain water was referred to as the "toxic symptom" threshold by Richter.⁵⁾

2. Acceptability and Toxicity of Thallium Sulphate

In a direct two-bottle preference test, the preference of albino rats for the plain water (distilled water) and the poison solution of various concentrations was observed by the same method as described in a previous paper.³⁾ Thallium sulphate was used at concentrations near or above those expected to produce toxic symptoms. After 24 hours, the amount of intake of both the poison solution and plain water was determined. Afterwards the test rats were given plain water and diet *ad libitum* (MF, the product of Oriental Yeast Industry Company, Ltd.), and observed for seven days for mortality.

3. Gustatory Nerve Response

Integrated responses of the chorda tympani nerve to various thallium salts, sodium sulphate, sodium acetate, G-100, G-105, G-1, G-107, and coumarin applied to the tongue surface of the rat were recorded using a platinum wire electrode (100 μ in diameter), a 5 stages R-C coupled amplifier (0.003 sec in time constant), an integrator (1.0 sec in time constant), and an ink-writing recorder. The exposure of the chorda tympani nerve and the recording method of nerve response were similar to those described in a previous report.³⁾

The candidate toxic compounds were thallium sulphate, thallium nitrate, thallium

acetate, thallium formate, thallium chloride, G-100 (Tl coumarin-3-carboxylate), G-105 (Na coumarin-3-carboxylate), G-1 (coumarin-3-carboxylic acid), G-107 (K coumarin-3-carboxylate), and coumarin. Also sodium chloride, sodium acetate, sodium sulphate, sodium nitrate, and sodium formate were used as the standard gustation substances.

RESULTS

1. Acceptability Thresholds

As shown in Table 1, the thresholds for discrimination of thallium salts were obtained in one to six rats out of ten rats. The threshold for discrimination of thallium sulphate was about two times larger than that of thallium formate, thallium chloride, thallium nitrate and thallium acetate. Many rats died without discriminating between the plain water and the poison solution. In rats showing the discrimination between two test solution, intake amount of the poison solutions and the plain water declined together within one or two days after the discrimination and they died. Accordingly, very few rats with thallium acetate and thallium nitrate showed the absolute rejection threshold. Averaged daily intake curves for ten rats on five thallium salts were considerably similar to Richter's result⁵⁾ with thallium sulphate in domesticated brown rats. The following results on the toxic symptom threshold in this experiment were obtained: thallium sulphate, 0.0182% for 6 rats; thallium chloride, 0.018% for 7 rats; thallium formate, 0.018% for 7 rats; thallium acetate, 0.029% for 7 rats; thallium nitrate, 0.023% for 4 rats.

Table 1 Discriminating ability of albino rats for thallium salts in the case of the gradual two-bottle preference test.

Test compound	No. of rats tested	Threshold for discrimination		Absolute rejection threshold		Mortality (%)
		(%)	rats*	(%)	rats*	
TlCl	10	0.016 $\pm$ 0.005	3		0	100
CH ₃ COOTl	10	0.016 $\pm$ 0.010	3	0.125	1	100
HCOOTl	10	0.012	1		0	100
Tl ₂ SO ₄	10	0.023	1		0	100
TlNO ₄	10	0.011 $\pm$ 0.002	6	0.125 $\pm$ 0.088	3	90

* Number of the rats of which the threshold for discrimination and the absolute rejection threshold were able to determine.

Table 2 Acceptability thresholds of various sodium salts in albino rats.

Test compound	No. of rats	Threshold for discrimination (%)	Maximum acceptance concentration (%)	Absolute rejection concentration (%)
NaCl	10	0.23±0.22	0.94±0.32	2.25±1.56
Na ₂ SO ₄	10	0.31±0.20	1.15±0.63	3.00±0.00
NaNO ₃	10	0.20±0.13	0.93±0.24	1.95±0.73
CH ₃ COONa	10	0.13±0.09	0.69±0.57	2.70±0.63
HCOONa	10	0.17±0.12	0.73±0.30	1.65±0.47

Body weight of albino rats : 200–250 g.

Table 3 Direct two-bottle preference test with thallium sulphate in albino rats.

Concentration (%)	Averaged amount of intake (ml)		Ingested dose (mg/kg)	Ratio of ingested amount* (%)	Preference response			Mortality	Survival (Days)
	Plain water	Poisoned water			P** > W***	P=W	P<W		
1	34.7±14.1†	1.5±1.3†	76±72†	95±35†	0	0	5	3/5	2
0.5	29.3±8.4	3.5±1.6	74±30	87±24	0	0	5	4/5	2–4
0.25	22.9±10.0	10.5±6.4	111±77	86±11	2	0	3	4/5	2–5
0.125	10.4±6.9	12.0±7.7	81±49	75±22	3	0	2	4/5	2–3
0.0625	21.5±4.7	22.1±3.7	52±10	114±8	3	0	2	4/5	4–6
0.0313	15.0±4.7	24.8±12.6	39±19	100±17	3	0	2	2/5	2–5

* Ingested amount of poisoned water and plain water

Intested amount of plain water per day during preliminary preference test (3 days) × 100

** Poisoned water, *** Plain water, † Mean±S.D., Body weight of albino rats: 200–300 g.

The acceptability of sodium chloride, sodium acetate, sodium sulphate, sodium formate and sodium nitrate was determined by the same method as described in the above experiment. All of these salts were well accepted with the increase of the concentration showing the threshold for discrimination of the concentration from 0.13% to 0.31% and the maximum acceptance concentrations were from 0.7% to 1.2%, while above these concentrations these salts were markedly rejected (Table 2).

2. Acceptability and Toxicity of Thallium Sulphate

The results are given in Table 3. The poison solution of 0.0313% to 0.125% was more acceptable than the plain water, and above the concentration 0.125%, the plain water was more acceptable than the poison solution. The rat mortality was 80% for the concentrations ranging from 0.0625% to 0.5%, and at the concentrations of 1% and 0.0313%, lower mortalities were achieved. The total amount

of both the plain water and the poison solution ingested was normal at 0.0313% and 0.0625%, and above the concentration of 0.0625%, it decreased slightly.

3. Response of Gustatory Nerve

The magnitudes of the response in the chorda tympani nerve against the application of five thallium salts (0.02 M) were almost the same. In the case of sodium sulphate and sodium acetate at 0.02 M, the magnitude of the response of the chorda tympani nerve was as much as that of thallium salts (Fig. 1). As shown in Fig. 2, the magnitude of the response in the chorda tympani nerve against the application of sodium sulphate, sodium nitrate, sodium acetate, sodium formate and sodium chloride at the concentration of 0.02 M also was almost the same. At the concentration of 0.03% (0.0013 M) to 0.5% (0.02 M), the magnitude of the integrated response of the chorda tympani nerve to the application of thallium sulphate increased with the increase of the concentra-

Fig. 1 Integrated responses of the chorda tympani nerve to thallium salts, sodium sulphate, sodium acetate, G-105 and G-100.

A-1. 0.5 M (2.9%) NaCl, 2. 0.02 M (1.0%) thallium sulphate, 3. 0.02 M (0.53%) thallium nitrate, 4. 0.02 M (0.53%) thallium acetate, 5. 0.02 M (0.53%) thallium formate, 6. 0.02 M (0.48%) thallium chloride, 7. 0.02 M (0.28%) sodium sulphate, 8. 0.02 M (0.16%) sodium acetate, B-1. 0.5 M (2.9%) NaCl, 2. 0.02 M (1.0%) thallium sulphate, 3. 0.02 M (0.4%) G-105, 4. 0.02 M (0.8%) G-100. Arrow indicates onset of application of test solution. (Time in second)

tion of the poison (Fig. 3). When one ml of G-100 (0.8%) and G-105 (0.4%) at the concentration of 0.02 M was applied to the tongue surface of the rat, a definite integrated response was obtained and the gustatory effect of G-105 was slightly larger than that of G-100. No integrated response was obtained with coumarin (supersaturated solution), G-1, and G-107 at the concentration of 0.02 M.

DISCUSSION

The toxic symptom thresholds on thallium

Fig. 2 Integrated responses of the chorda tympani nerve to five sodium salts.

1. 0.02 M (0.28%) sodium sulphate, 2. 0.02 M (0.17%) sodium nitrate, 3. 0.02 M (0.16%) sodium acetate, 4. 0.02 M (0.14%) sodium formate, 5. 0.02 M (0.12%) sodium chloride. Arrow indicates onset of application of test solution. (Time in second)

Fig. 3 Integrated responses of the chorda tympani nerve to thallium sulphate (TS).

1. 0.5 M (2.9%) NaCl, 2. 0.02 M (1.0%) TS, 3. 0.01 M (0.5%) TS, 4. 0.005 M (0.25%), 5. 0.0025 M (0.13%) TS, 6. 0.0013 M (0.06%) TS, 7. 0.0006 M (0.03%) TS. Arrow indicates onset of TS application. (Time in second)

salts in the present experiment were slightly larger than the threshold value (averaged value—0.065% for 6 rats) of thallium sulphate obtained by Richter,⁵⁾ although the starting concentration of thallium salts in both experiments was very close. As the test animals in this experiment were albino rats and those in Richter's experiment were domesticated brown rats, the difference of the toxic symptom threshold in both experiments might reflect that of test animals. As the total fluid consumption of tested rats decreased without discrimination between thallium sulphate and plain water, the threshold for discrimination of thallium sulphate was not obtained from the averaged daily intake curve and no all the individuals were able to perceive a taste of thallium sulphate on the taste tests on human subjects. Richter⁵⁾ concluded that thallium sulphate was tasteless and the decrease of the total fluid consumption was due to the toxic effect of thallium compound. In the present experiment, many rats died before the threshold for discrimination and the absolute rejection threshold were obtained. Although few rats were able to discriminate between plain water and solution of thallium salts, these concentrations were the toxic symptom threshold or near by it and lower than the concentration necessary to exert gustatory effect of thallium sulphate. As thallium salts have strong subacute toxicity, the discrimination between plain water and solutions of thallium salts in few rats using the gradual two-bottle preference method might be indicative a response of rats to the cumulative toxic effect of thallium salts. In the direct two-bottle preference test, the solution of thallium sulphate in higher concentrations than the toxic symptom threshold was slightly more acceptable than the plain water, and in concentrations above 0.25%, the poison solution was markedly less acceptable than the plain water, although high mortality was obtained in the concentrations over 0.0625%. Also, total ingested amount of poison solution and plain water was about equal to total amount of plain water during the preliminary preference test or slightly lower. These results suggest that the solution of thallium sulphate in higher concentrations could be discriminated from the plain

water by albino rats. In the preference test between the plain pellet and the poison pellet with the modified Skinner-box, thallium sulphate pellet in the concentration of 1% showed fair acceptability by albino rats and the ratio of eaten amount of poison pellet to the plain pellet (weight unit) was the mean value of 63%.²⁾ Thallium sulphate gave clearly gustatory effect to albino rats in the concentrations over 0.0025 M (0.125%) and its effect was marked in 0.5% and 1%. Although the toxicity of thallium sulphate to rodents is strong, the speed of toxic effect is very slow.^{6,7)} Nachman and Hartley⁷⁾ showed that taste aversion against a 15% sucrose solution in rats did not obtain through an ill effect after the injection of thallium sulphate and suggested that one cause against it might be deduced from the delayed onset of toxic symptoms. From these informations and the present findings, a cause for the low acceptability of thallium salts at higher concentrations in the direct preference test is possibly a toxic effect. Richer⁵⁾ showed that thallium sulphate in the concentration of 0.1% had no taste to human subjects. This result is possibly due to the low concentration of thallium sulphate and possibly species difference. Also, it is clear from the present experiments that the concentration of thallium sulphate from 1% to 0.5% in commercially available solid bait is a little too high and not suitable to get superior control efficiency.

Generally, cations play an important role in the taste quality of inorganic salt solution, anions exert only supplementary role, and this view is due to the results of electrophysiological responses of both the chorda tympani nerve of rats⁸⁾ and the glossopharyngeal nerve of frogs.⁹⁾ In the present experiment, five kinds of thallium salts, and five kinds of both inorganic and organic salt in equal molecular concentrations showed about the same order of gustatory effectiveness to the chorda tympani nerve of rats. Furthermore, thallium salts were clearly rejected and sodium salts were greatly preferred in the range of similar concentrations. Therefore, the difference between gustatory effect of two kinds of salts is mainly due to sodium ions and thallium ions.

G-100 and G-105 exerted the gustatory

Fig. 4 Chemical structure of coumarin derivatives.

1. coumarin, 2. G-100, 3. G-105, 4. G-1, 5. G-107.

effectiveness to the chorda tympani nerve of rats but in coumarin, G-1, and G-107 the gustatory effectiveness was very slightly detected. The difference in the chemical structure of these chemicals is side chain of $-\text{COOTl}$, $-\text{COONa}$, $-\text{H}$, $-\text{COOH}$ and $-\text{COOK}$ attached to the 3-position of coumarin nucleus (Fig. 4). Consequently, the primary factor in the gustatory effectiveness of G-100 and G-105 is believed to be thallium ions and sodium ions, respectively. A deficiency of gustatory effectiveness in coumarin is probably due to insolubility in water and that in G-107 is probably due to very low stimulating activity, because the responsibility of the chorda tympani nerve against kalium chloride in rat is very low, compared with the stimulating force of sodium chloride.⁸⁾

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REFERENCES

- 1) T. Kusano: *J. Fac. Agric. Tottori Univ.* **12**, 8 (1977)
- 2) T. Kusano: *ibid.* **10**, 15 (1975)
- 3) T. Kusano, Y. Kasahara & Y. Kawamura:

Appl. Entomol. Zool. **6**, 40 (1971)

- 4) T. Kusano: *Botyu-Kagaku* **41**, 107 (1976)
- 5) C. P. Richter: *J. Comp. Physiol. Psychol.* **43**, 358 (1950)
- 6) T. Kusano: *J. Fac. Agric. Tottori Univ.* **5**, 15 (1969)
- 7) M. Nachman & P. L. Hartley: *J. Comp. Physiol. Psychol.* **89**, 1010 (1975)
- 8) L. M. Beidler: *J. Neurophysiol.* **16**, 595 (1953)
- 9) M. Sato: *Science* **29**, 124 (1959)

要 約

タリウム塩のネズミに対する味覚効果ならびに摂取性

草野忠治

シロネズミで、間接二瓶法により5種類のタリウム塩の識別閾値、絶対拒否閾値を測定すると、亜急性中毒のために実験途中で死亡するものが多く、この方法でタリウム塩の味覚効果を明らかにすることはできなかった。直接二瓶法で硫酸タリウムの摂取性を調べると、それは低濃度で良好であったが、濃度が高くなると(>0.125%), 硫酸タリウムよりも蒸留水のほうがより多く選好され、総摂取量は正常値に近い値を示した。しかし、広い濃度範囲で80%の高い死亡率が得られた。

硫酸タリウム0.13%以上でシロネズミの鼓索神経を介して明白な味覚効果が認められ、5種類のタリウム塩は等モル濃度でほぼ等しい味覚効果を与えることが明らかとなった。高濃度のタリウム塩がネズミに識別される過程における、その味覚効果の役割について論議された。クマリン核を共通にもつ5種類のタリウム塩に対するシロネズミ鼓索神経の電気生理学的反応からタリウム塩の味覚効果は主としてタリウムイオンによるものである。