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158. Kakuichi Sakai : Studies on Chemical Transmission in Taste Fibre Endings. I. The Action of Acetylcholinesterase on Bitter Taste.

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Recently the physiological characteristic of taste has begun to be clarified gradually. Baradi and Bourne[1~3] discovered that certain enzyme such as acetylcholinesterase (AChE), phosphatase, lipase, dehydrogenase, etc. are localized closed to the taste buds and these enzymes were found to be activated or inhibited *in vitro* by applying the taste-bestowing substance.

On the other hand, Landgren, Liljestrand, and Zotterman[4] reported from the electrophysiological point of view that the formation of impulse in glossopharyngeal nerve was not observed only ACh was applied on the surface of the tongue but a clear increase in impulse was observed when taste-bestowing substance was applied after ACh had been applied and that ACh was playing some role in the cause of initiation of impulse. It was assumed, therefore, that the bitter substance stimulates the taste nerves in the taste buds and simultaneously converts the inactive ACh to active form. The resulted active ACh transmits the information of bitterness to the nerve center.

The fact that AChE activity is inhibited by bitter tasting substances[5] reveals that AChE is acting, at least partially, the important role on the control of bitter taste information.

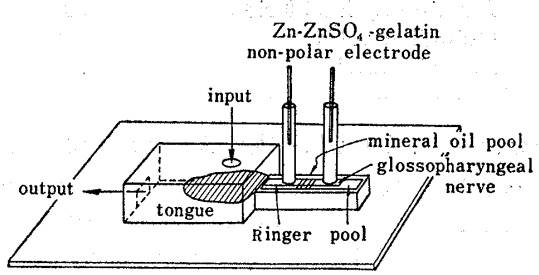
These facts are very interesting for explaining the mechanism of the sense of taste when a clear relationship between the sense of taste and chemical structure of the taste-bestowing substance has not been so far presented. It is considered that the electrophysiological investigation of the influence of AChE on impulse formation and the comparative observation of the strength and the durability of impulse formation will offer the valuable approach for resolving the mechanism of taste-production.

The present experiment was carried out to clarify these points.

Materials and Methods

As experimental animals, Rana nigromaculata (summer frog) of both sex weighing 30~35 g. were used. Frog is reported to be suitable as experimental animal, since frogs respond similar to human beings electrophysiologically against the four basic taste[6] (bitter, souer, sweet, salty), when analyzed electrophysiological procedure.

The tongue of frog was cut off from the base with the attaching glossopharyngeal nerve. As shown in Fig. 1 the tongue was placed in a lucite flow-chamber and glossopharyngeal nerve was made straddled on two Ringer pools which are separated by a mineral oil pool of 1.5 mm. width. The application of taste-bestowing substances on tongue produces the corresponding impulses. The impulse was visualized on synchroscope by employing the Zn-$ZnSO_4$-gelatin non-polarized electrode and a five-stage amplifier. At the same time the impluse was counted with a electronic-counter by means of a clipper.



(Lucite Flow-chamber)

Fig. 1.

*1 Kita-4, Sendai, Miyagi-ken (酒井格一).

1) A. F. Baradi, G. H. Bourne : J. Histochem. Cytochem., **7**, 2 (1959).
2) G. H. Bourne : Science, **113**, 660 (1951).
3) *Idem* : Nature, **161**, 445 (1948).
4) S. Landgren, G. Liljestrand, Y. Zotterman : Acta Physiol. Scand., **30**, 105 (1954).
5) K. Okazaki, S. Tutiya : Unpublished data.
6) K. Kusano, M. Sato : Jap. J. physiol., **7**, 324 (1957).

The AChE used was 5 U, 15 U, and 25 U (One unit liberates 1 cu. mm. of CO_2 per minute from a Ringer-bicarbonate buffer equilibrate against 5% CO_2 in N_2 at pH 7.4 when the initial acetylcholine concentration is 0.0092*M*). This enzyme solution was introduced *via* the input and the tongue was completely dipped for 60 sec. Thereafter the enzyme solution was discharged from outlet. Thirty seconds after the remove of enzyme solution the solution of bitter tasting substance (adjusted to pH 7.4) was introduced from the input and the impluse produced at this time was counted with a electronic-counter for 20 sec. The solution was maintained at $17 \pm 1°$ during experiment for preventing the admixture of the influence of thermal receptors. At the same time, the precaution was taken that the effect of pressure produced by the introduction of solution and the sense of contact were kept as small as possible. Also, the surface of the tongue was washed thoroughly with Ringer solution after the test with each sample solution and the next experiment was started after the discharge frequency has returned to the level of self-generation discharge.

Results

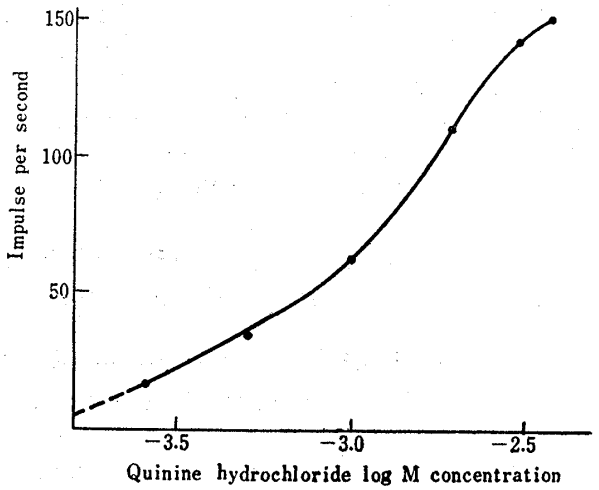


Fig. 2. Relationship between Impulse and Concentration of Quinine Hydrochloride

The Relationship between the Concentration of the Bitter Substance and Impulse Produced

Fig. 2 show the relationship between concentration of quinine hydrochloride, a representative bitter substance, and impulse count. Impulse of over 25 μV was counted with electronic-counter for one second. No marked change can be seen by the application of 2.5×10^{-4} mol. of quinine hydrochloride compared with Ringer solution. However, a linear relationship was observed between impulse count and a concentrations in the vicinity of 10^{-3} mol. The concentrations in experiments of other kinds of substance were estimated by the same method.

Table I.

	(mol.)		(mol.)
Quinine hydrochloride	0.001	Berberine hydrochloride	0.001
Methylephedrine	0.04	Caffeine	0.1
Emetine hydrochloride	0.01	Mephenesin	0.05
Quinidine sulfate	0.001	$MgCl_2$	1.0
Phenobarbital	0.02	$MgSO_4$	0.1

Changes in Impulse Count produced when the Tongue is treated with AChE

In Fig. 3, one of the patterns observed on synchroscope was presented.

Fig. 4 shows one of the resulted impulse caused by quinine hydrochloride when treated with 5 U, 15 U, and 25 U of AChE. Each curve represents the response when bitter substance was applied alone or after the treatment with 25 U, 15 U, and 5 U of AChE. When the tongue was pretreated with 25 U AChE, a marked reduction in impulse count for all bitter substance was observed. Particularly in the case of emetine hydrochloride, methylephedrine, etc. impulse count was reduced to almost the same number of self-generation impulse (Figs. 5, 6). The impulse reducing activity of AChE was found to be the function of amount of AChE pretreated, namely 5 U of AChE could not result a great change in impulse formation and 15 U of AChE showed the medium efficiency. The maximum impulse count was obtained at the first 2 sec. Thereafter the counts decreased with time as show in Figs. 4~6.

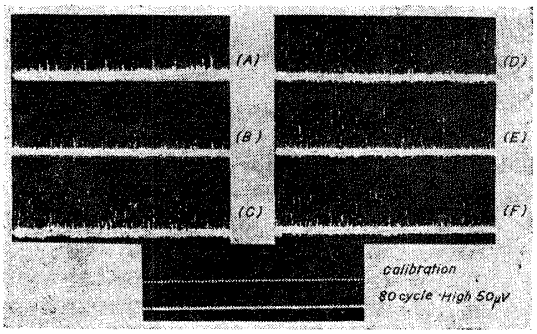
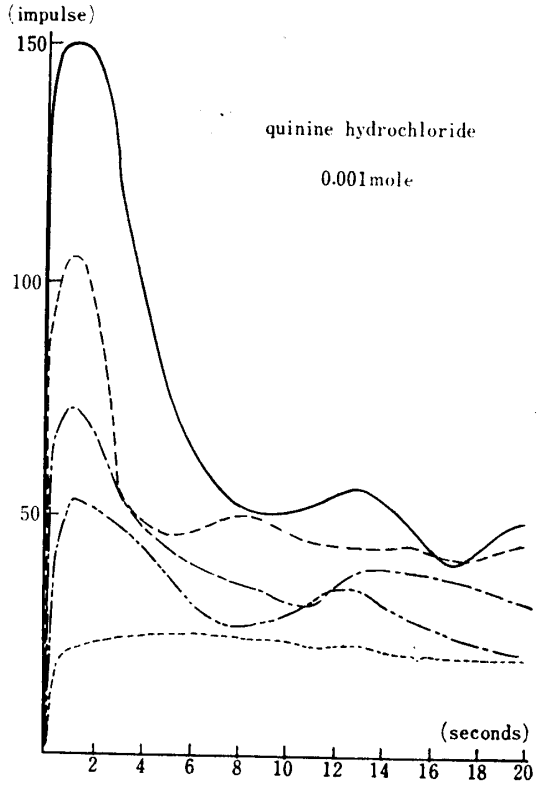


Fig. 3. Impulse from Frogs Glossopharyngeal Nerve produced by Bitter Substance

A : the level of self-generation discharge
B : treated with 25 U of AChE
C : application of 10^{-3} mol. quinine hydrochloride
D~F : 10^{-3} mol. quinine hydrochloride was applied after the treatment of 5 U (D), 15 U (E) and 25 U (F) of AChE



Flg. 4.

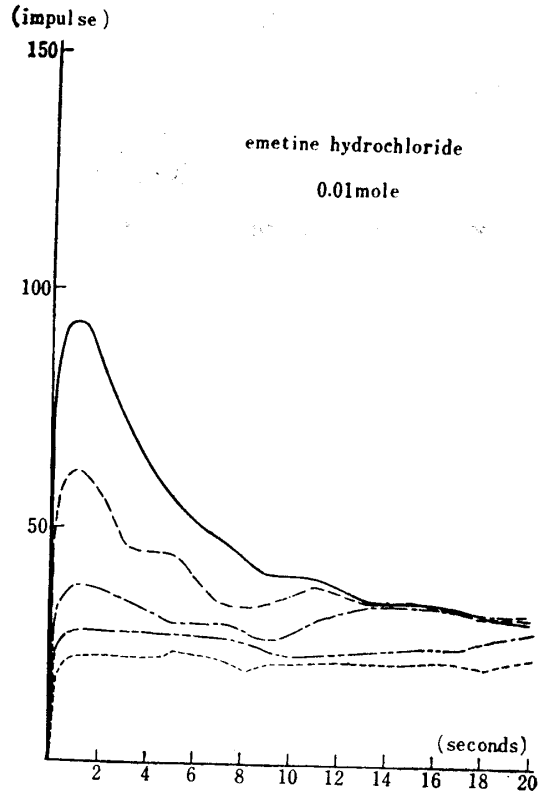


Fig. 5.

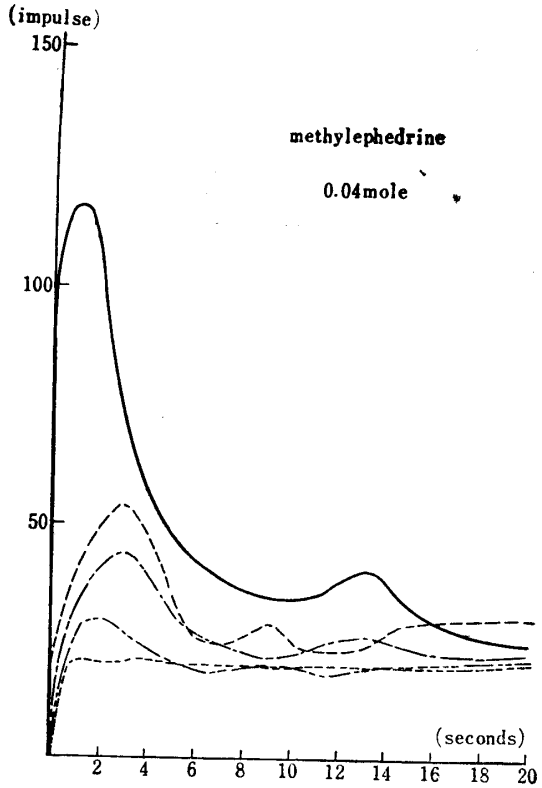


Fig. 6.

Strength and Duration of Impulse

Ten bitter substances listed in Table I were used in this experiment. The impulse count of first 2 sec. 8~10 sec. and 18~20 sec. were listed in Table II. The number of impulse can be considered to represent the strength of bitter taste. In all cases the impulse were suppressed by the pretreatment of AChE.

Table II.

Bitter substance / Time (sec.) / AChE unit	Quinine hydrochloride			Methylephedrine			Emetine hydrochloride			Quinidine sulfate		
	0~2	8~10	18~20	0~2	8~10	18~20	0~2	8~10	18~20	0~2	8~10	18~20
0	149	51	50	116	35	25	93	37	32	148	59	40
5	104	52	44	36	31	30	67	35	35	126	30	37
15	73	35	32	30	23	22	38	25	35	75	31	24
25	53	27	22	27	23	26	28	23	27	60	28	24
Self-generation	23	27	22	22	22	21	20	18	21	24	23	20

Bitter substance / Time (sec.) / AChE unit	Phenobarbital			Berberine hydrochloride			Caffeine		
	0~2	8~10	18~10	0~2	8~10	18~20	0~2	8~10	18~20
0	151	45	29	157	52	24	82	34	34
5	129	50	23	139	26	27	67	24	22
15	110	31	21	111	56	26	45	26	24
25	68	25	23	84	20	22	44	20	16
Self-generation	23	20	22	23	21	21	21	21	19

Bitter substance / Time (sec.) / AChE unit	Mephenesin			$MgCl_2$			$MgSO_4$		
	0~2	8~10	18~20	0~2	8~10	18~20	0~8	4~10	18~20
0	154	69	30	62	40	34	67	33	29
5	101	29	20	43	32	39	49	28	29
15	56	34	25	40	26	24	47	25	28
25	38	29	26	36	22	21	39	25	25
Self-generation	25	25	24	21	21	21	25	25	24

The spike potential is, of course, to be another factor which express the strength of bitter taste (in a sense of fatigue of recepter), but in this experiment all pulses above 25 μV were counted evenly. The potential of spike which can be observed in synchroscope is enough high in earlier stage but in later stage the potential markedly drops particularly when the transient acting bitter substance was employed. The so-called prolong acting bitter substance did not produce so many impulse in the earlier stage, but the potential of spike in later stage was higher than that of transient acting bitter substance. Such a difference in potential of spike at the later stage will be obligatory to the duration of bitter taste.

Discussion

The experimental results thus obtained reveals that AChE, which is known to be located on tongue and inhibited by bitter substances, plays some competitive suppression on the production of impulse with bitter bestowing substances. The application of ChE

inhibiting materials such as eserine (10^{-3} mol.), prostigmin (10^{-3} mol.), DFP (10^{-3} mol.) must cause the complete inhibition of AChE on the tongue, however no increase in impulse was observed. On the contrary bitter substances were applied after the application of such ChE inhibiting materials yields the somewhat increment in the number of impulse than the single application of bitter substances.[7] These results are compatible with the report of Landgren, Liljestrand and Zotterman.[4]

Based on these results it is assumed that the bitter substance stimulates the taste buds and liberate the active ACh from receptor. Resulted active ACh produce the impulse in the nerve and transport the taste. On the other hand the bitter substance inhibits AChE in taste bud simultaneously and this caused the reduction of the capacity decomposing ACh.

It would be premature to conclude the definite mechanism of the bitter transmission, so the further electrophysiological experiment is now under investigation.

The author is grateful to Dr. K. Okazaki for his valuable advices and encouragements throughout this work. Thanks are also due to Dr. H. Ozawa and Dr. M. Uchiyama of this institute for his kind advices.

Summary

A marked decrease in impulse count which is produced by bitter substances when tongue (frog) was pretreated with AChE. It was assumed that there is a close relationship between AChE and the sensation of bitter taste. Factors affecting the strength and durabillity of bitter taste were discussed.

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7) K. Sakai: Unpublished data.

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159. Hiroshi Kugita and Mikio Takeda: Syntheses of Morphine-like Structures. II.*1 2′-Methoxy-9-hydroxymethyl-2,5-dimethyl-6,7-benzomorphan.

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In a previous paper*1 it was described that hydroboration of 9-methylene-2,5-dimethyl-6,7-benzomorphan with diborane followed by hydrogen peroxide oxidation produces stereospecifically the 9β-hydroxymethyl derivative.*3 With such a reaction the corresponding 2′-methoxy compound (I) was expected to provide a new benzomorphan derivative as analgesics.

*1 Part I: This Bulletin, **11**, 986 (1963).

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*3 The hydroxymethyl group is oriented toward nitrogen, *trans* to the 5-methyl group.