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39. Kakuichi Sakai: Studies on Chemical Transmission in Taste Fibre Endings. II.*1 The Effect of Cholinesterase Inhibitor on the Taste.

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A. F. Baradi, G. H. Bourne, *et al.*[1] found histochemically that various enzymes including cholinesterase and phosphatase were distributed predominantly in the gustatory epithelium and the gustatory nerve endings and the activities of these enzymes were activated or inhibited by taste-bestowing substances. They presumed, therefor, that this may be a part of the gustatory mechanism.

In the present report, electrophysiological and sensory investigation were performed to find out what changes in gustatory effects will be caused by inhibiting the enzymatic action of cholinesterase on the tongue. Experiments were carried out by analysis of the action potential derived from the chorda tympani of rats and by employing 10 human subjects for sensory test.

Experimental

1) Electrophysiological Experiments——As experimental animals, male and female rats were used. The rat was anesthetized with pentobarbital, trachea cannulated, and the lingual nerve dissected free from the point of entry into the tongue down to the branching of the chorda tympani from the lingual nerve. The chorda tympani, completely isolated from the surrounding tissues, was placed in a small vessel filled up with Ringer's solution, and then the connective tissues were thoroughly removed from the nerve under an observation with the binocular microscope and dissected away from the lingual nerve for about 1 cm. The tongue was placed in a flow-chamber[2] and the electrical action of the nerve was led with a non-polarized electrode enclosed in the circuit. To C-R coupled 5 stage amplifier were introduced the impulses produced, of which those above 50 μV were picked up through an integration circuit to an osillograph for visible observation.

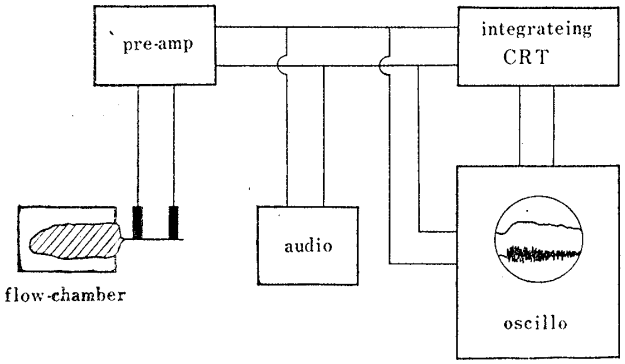

Fig. 1.

The impulses gained at non-polarized electrode (Ag-AgCl type) was amplified by pre-amplifier and then were led into an integration circuit and recorded by oscillograph. On the other hand, the amplified impulses were directly introduced into audio circuit and oscillograph without integration.

At the oscillograph, the upper trace shows how much activity appears when processed through the integrator; the lower trace shows a typical impulses, multifiber discharge from a large number of nerve fibers.

10^{-3} mol. solution of eserine, neostigmine and diisopropylfluorophosphate (DFP) were used as cholinesterase inhibitors. Each solution was poured into the flow-chamber through the input and removed through the output after the inhibition of cholinesterase on the tongue for 60 sec. Thereafter the tongue was simply washed with refined water and a tasting solution (sour, sweet, bitter or salty) was given subsequently. The impulse produced was integrated for 20 sec. and the integration value obtained in this method was compared with that obtained without the pretreatment of the cholinesterase inhibitors. All the solutions were kept at a temperature of $20 \pm 1°$ throughout the experiment.

2) Method of the Sensory Test——The threshold value for each of the tasting substances was determined using 10 human subjects (5 boys and 5 girls, 14.7 years of age on an average). They were let hold 10 ml. of 10^{-3}

*1 Part 1. K. Sakai: This Bulletin, **12**, 1159 (1964).

*2 Kita-4, Sendai, Miyagi-Ken (酒井格一).

1) A. F. Baradi, G. H. Bourne: J. Histochem. Cytochem., **7**, 2 (1959).

2) Lloyd M. Beidler: J. Neurophysiol., **16**, 595 (1953).

mol. solution of each cholinesterase inhibitor ($20 \pm 1°$) in the mouth for 60 sec., then rinsed their mouth with refined water ($20 \pm 1°$). The taste after application of 20 ml. of a tasting substance of the threshold concentration was recorded.

Results

1) Electrophysiological Results

Fig. 2 indicates the integrated value of impulses for 20 second, which were produced by the application of 10^{-3} mole solutions of the cholinesterase inhibitors on the surface of the tongue. The frequency level of the background activity caused by rinsing the tongue with refined water was also shown as control. The integral values of the impulse obtained after the treatment with eserine, neostigmine and diisopropylfluorophosphate were 1.72, 1.76, and 1.40 times as much as those of the control, respectively, showing an effect of the pre-treatment with those substances.

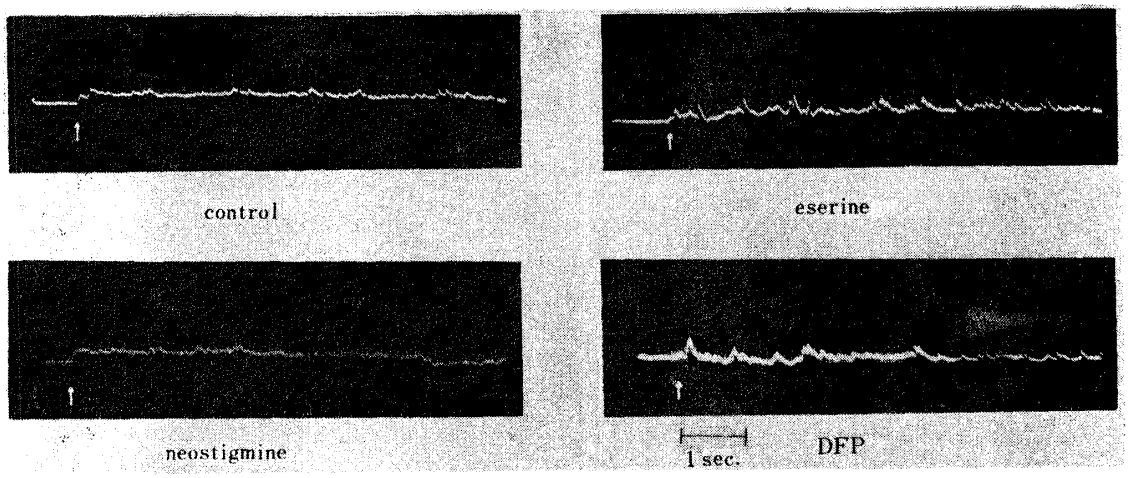

Fig. 2. Integrated Electrical Response of Chorda Tympani to various Cholinesterase Inhibitors ($10^{-3}M$) flowed over Tongue of Rat

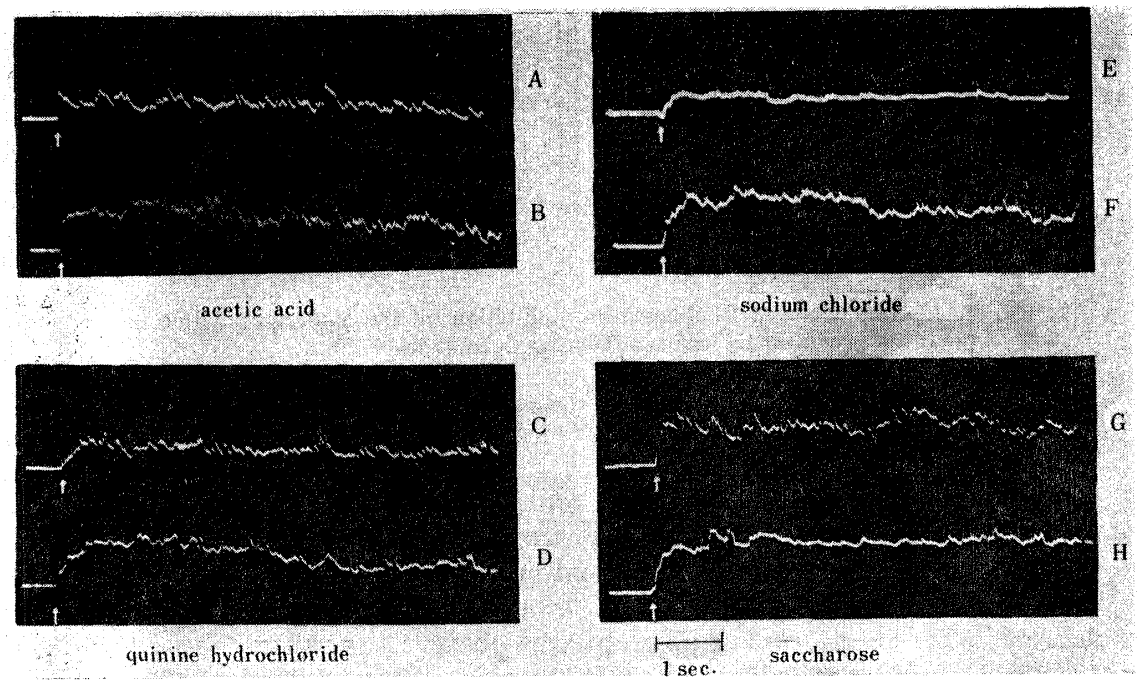

Fig. 3. The Comparison between the Integrated Values obtained after the Application of the Tasting Substances with or without the Pre-treatment of the Cholinesterase Inhibitors

The comparison was made between the values obtained after the application of the tasting substances with or without the pre-treatment of the cholinesterase inhibitors. Fig. 3 shows a change of the value, observed on the oscilloscope, for 4 examples of the tasting substances :

A : the value obtained with acetic acid solution (5×10^{-2} mole.).B : the value obtained with acetic acid solution with the pre-treatment of neostigmine. The ratio (B/A) is 2.14, showing an increase of the value caused by the cholinesterase inhibitor. C : the value obtained with quinine hydrochloride (2.5×10^{-4} mole.). D : that with quinine hydrochloride and eserine. (D/C), being 1.2, shows little increase of the value due to the pre-treatment with eserine. E : the value obtained with the application of sodium chloride solution (3×10^{-1} mole.). F : that with DFP and sodium chloride solution. The ratio (F/E) is 2.29. G : the value obtained with sucrose solution (2×10^{-1} mole.). H : that with eserine and sucrose solution. The ratio (H/G) is 1.17, showing little increase due to the pre-treatment.

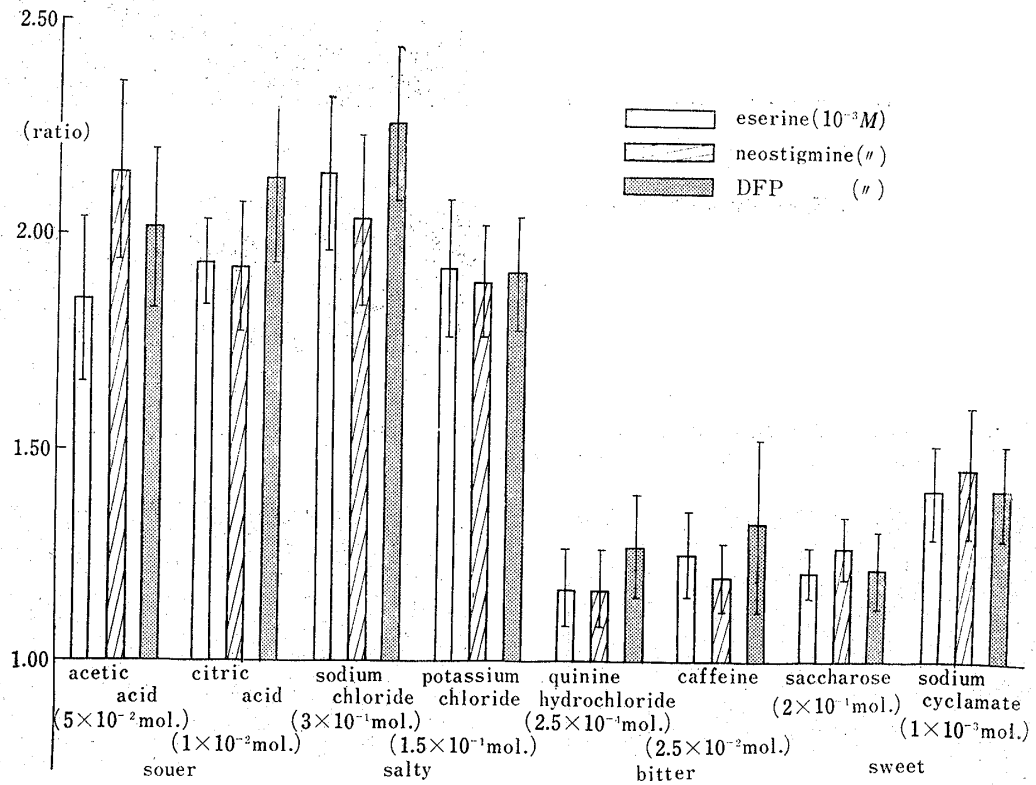

Fig. 4. Influences of Cholinesterase Inhibition on the Integrated Value of Impulses produced by various Tasting Substances

Ratio was calculated as follow :

$$\text{Ratio}=\frac{\text{Integrated value when treated by cholinesterase inhibitor}}{\text{Integrated value without inhibition}}$$

Fig. 4 indicates the increasing ratio of the value with the pre-treatment to that without it. As recognized from the figure, the number of the impulse becomes nearly twice as many as that without the pre-treatment in the case of such substances that taste sour as acetic acid and citric acid, or taste salty as sodium chloride and potassium chloride, whereas no remarkable changes observed in bitter substances such as quinine hydrochloride and caffeine and in sucrose. But in the case of sodium-cyclamate, one of the artificial sweet-flavouring agent, the number of the impulse showed an increase nearly 1.4 times as much.

2) Results of the Sensory Test

Table I shows a lowering of the threshold value obtained in the sensory test on 10 human subjects for the above mentioned 8 kinds of tasting substances.

The results showed, in the same manner as those of the electrophysiological test, a lowering of the threshold value up to 4 times as much for acid and salty taste, but no remarkable changes in it for bitter taste and sweet taste of sucrose.

Table I. Changes of Threshold Concentration in Sensory Test by the Inhibition of Cholinesterase

	Without pre-treatment (M)	Pre-treatement of cholinesterase (M)		
		eserine	neostigmine	DFP
Acetic acid	2.3×10^{-3}	0.45×10^{-3}	0.58×10^{-3}	0.40×10^{-3}
Citric acid	0.4×10^{-3}	0.10×10^{-3}	0.13×10^{-3}	0.09×10^{-3}
Sodium chloride	6.2×10^{-2}	1.50×10^{-2}	1.59×10^{-2}	1.55×10^{-2}
Potassium chloride	3.1×10^{-2}	0.82×10^{-2}	0.80×10^{-2}	0.78×10^{-2}
Quinine hydrochloride	2.1×10^{-6}	2.07×10^{-6}	2.14×10^{-6}	1.98×10^{-6}
Caffeine	1.5×10^{-4}	1.59×10^{-4}	1.52×10^{-4}	1.48×10^{-4}
Saccharose	4.0×10^{-2}	3.22×10^{-2}	3.30×10^{-2}	3.21×10^{-2}
Sodium cyclamate	2.0×10^{-4}	1.47×10^{-4}	1.38×10^{-4}	1.37×10^{-4}

Discussion

Both in the electrophysiological test and in the sensory test it was observed that the taste of substances which taste sour as acetic acid and citric acid or which taste salty as sodium chloride and potassium chloride was increased by the inhibition of cholinesterase on the tongue (an increase of the impulse in the former test and a lowering of the threshold value in the latter).

These facts mean that cholinesterase may play a certain role at the gustatory nerve endings and also lead us to the presumption that cholinesterase acts on a mediater which brings gustatory informations to the centre and inhibits function. It is also presumed that intensity and durability of acid or salty taste will be obliged to the intensity and durability of this inhibiting action.

On the other hand, in the case of a bitter substance such as quinine hydrochloride or caffeine no significant changes are recognized regardless of the inhibition of cholinesterase. It may due to the fact that the bitter substance itself has an inhibiting effect on cholinesterase activity, as stated in our previous report.

However, the question why a remarkable increase of the impulse cannot be recognized in the case of sweet substances as sucrose is not clarified yet and must be solved by further investigation.

The author expresses his deep gratitude to Prof. H. Ozawa and Assist. Prof. M. Uchiyama of this institute for their kind advice.

Summary

Both electrophysiological test and sensory test revealed that the gustatory effect of substance which show taste sour, as acetic acid and citric acid, or taste salty, such as sodium chloride and potassium chloride, was remarkably reinforced by inhibition of cholinesterase on the tongue. Where as almost no changes were observed for bitter taste or sweet taste of sugar. From these results it could be concluded that cholinesterase which is found at the gustatory nerve ending may play some important roles in the gustatory mechanism of sour and salty taste.

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