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185. Kakuichi Sakai and Hikaru Ozawa: Studies on Chemical Transmission in Taste Fibre Endings. III.*1 The Action of Acetylcholine and 2-Dimethylaminoethanol on the Taste Fibre Endings.

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It is generally believed that the sense of taste corresponds with some changes which are produced in the taste buds in response to gustatory stimuli provided by taste substances. The taste substances are thought to emanate action potential in gustatory nerve and transmit the information of taste. But, the mechanism by which taste substances are able to stimulate the specific end-organs of the taste buds is still obscure.

However, as described in the previous report,[1] it is considered likely that an acetylcholine-like substance plays some important role in the initiation of impulses in chemoreceptors. In facts, there are some reports by Zotterman,[2] and Skouby, *et al.*[3] that the number of impulses produced by taste substances was increased after the application of a small amount of acetylcholine (ACh) to the tongue but was reduced after the application of a large amount of ACh. It seems, however, that absorption of ACh in aquous solution from the surface of tongue leaves something doubtful since ACh is quaternary ammonium compound and it is unlikely to be absorbed easily through the normal surface of tongue.

Therefore, based on the findings mentioned above, attempts have been made to clarify the physiological functions of ACh and related compound involved in the sense of taste. In the experiments described here, ACh in 10% propylene glycol solution and 2-dimethylaminoethanol (DMAE) were administered to frog's tongue to see the changes of the response against the taste substances. The former, ACh in propylene glycol, is considered to be absorbed easily[4] the latter is reported to be absorbed more freely than ACh and to be the precursor of ACh in animal body.[5] The physiological functions of ACh and DMAE in the production of sense of taste were explained partly by the results obtained by the investigations of the electrical action of the gustatory nerve which was induced by taste substances.

Experimental

A series of *Rana nigromaculata* (summer frog) weighing 25 to 30 g. irrespective of the sex were employed as an experimental animal. The tongue sectioned and excised at the root with glossopharyngeal nerve left intact was used in these experiments. Glossopharyngeal nerve, 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. The tongue was placed in lucits flow-chamber in a similar way as described in the previous report,[6] the flow velocity of the test solution being adjusted to 0.2 ml./sec.

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*1 Part 2. K. Sakai: This Bulletin, **13**, 304 (1965).
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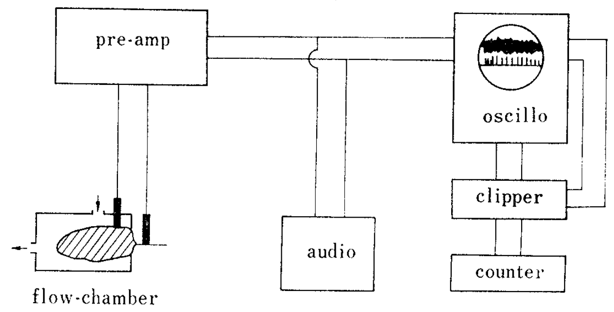

Fig. 1. A Block Diagram of the Recording System

The impulses gained at electrode (Ag-AgCl type) was amplified by pre-amplifier and then led into oscilloscope and counted by electronic-counter by mean of clipper.

The action potential of glossopharyngeal nerve was induced to an oscilloscope through C-R coupled five stage amplifier using Ag-AgCl electrode, and the number of impulses above 25 μV was counted with a electronic-counter by means of a clipper.

As for experimental methods, taste substances were given first and then the number of impulses on glossopharyngeal nerve obtained therefrom was counted each second for 20 sec. Then the tongue was treated with ACh in 10% propylene glycol solution ($10^{-3}M$) for 2 min., rinsed with Ringer's solution, and 20 min. later when the level of impulses reached sufficiently that of background activity, the above substances were applied to count the number of impulses produced for 20 sec. in a similar way.

The same procedure was tried on $2\times10^{-2}M$ of DMAE as well as on mixed solution of ACh and DMAE. The taste substances used included quinine hydrochloride and caffeine as bitter substance, acetic acid and citric acid as sour substance, saccharose and sodium cyclamate as sweet substance and sodium chloride and potassium chloride as salty substance.

The temperature of the test solution was maintained at $20\pm2°$ throughout the experiments to keep out the reaction of warm or cold fibre.

Results

Curve A in Fig. 2 shows the change of impulses with time obtained from glossopharyngeal nerve after acetic acid was administered to the surface of tongue as a taste substance. Curve B indicates results obtained when the tongue was treated with ACh in 10% propylene glycol solution ($10^{-3}M$) for 2 minutes before administration of acetic acid solution. Curves C and D represent those obtained following application of acetic acid solution after treatment of the tongue for 2 minutes with DMAE ($2\times10^{-2}M$) and mixed solution of ACh and DMAE respectively. In Fig. 3 these results are expressed in terms of ratios of impulses produced by administration of acetic acid solution to the tongue treated with ACh, DMAE and mixed solution of ACh and DMAE respectively to those obtained from the untreated tongue.

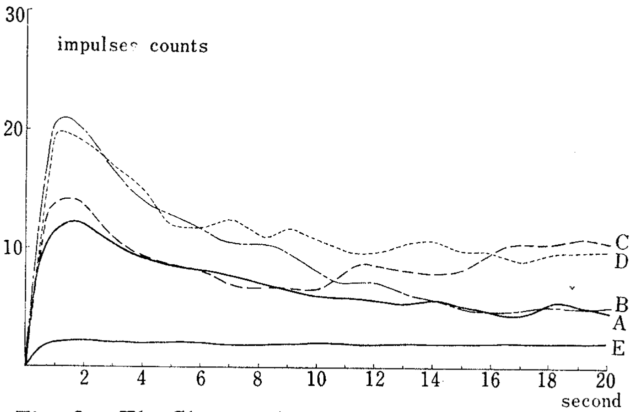

Fig. 2. The Change of Impulses with Time obtained from Glossopharyngeal Nerve

A : after acetic acid ($2\times10^{-2}M$) was administered to the surface of frog's tongue as a taste substance.
B : treated with ACh in 10% propylene glycol solution for 2 minutes and then administered acetic acid.
C and D : represent obtained following application of acetic acid after treatment of the tongue for 2 minutes with DMAE and mixed solution of ACh and DMAE respectively.
E : is level of background activity.

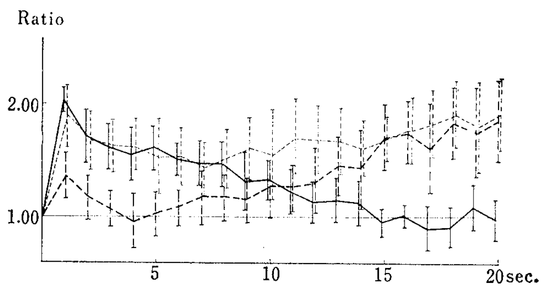

Fig. 3.

This figure shows time versus ratio (impulses counts treated/impulses counts nontreated) at $2\times10^{-2}M$ acetic acid. In this figure, it shows ——: ACh, - - -: DMAE, ········ ACh+DMAE and confidence limits (5%).

As shown in Fig. 3, impulse sproduced by ACh treatment increased rapidly and almost doubled in the first 1~5 seconds. However, their ratios gradually decreased with significant difference be observed between them and the control ratio. With DMAE, on the contrary, an increase was noticed in the impulses produced during the initial 1~2 seconds: then the ratio remained nearly the same as the control ratio until about 10 seconds after application and thereafter showed a gradual increase.

For the tongue treated with mixed solution of ACh and DMAE, the ratio was found to increase until about 10 seconds after application following a similar curve as treated with ACh and to follow a similar trend as treated with DMAE thereafter.

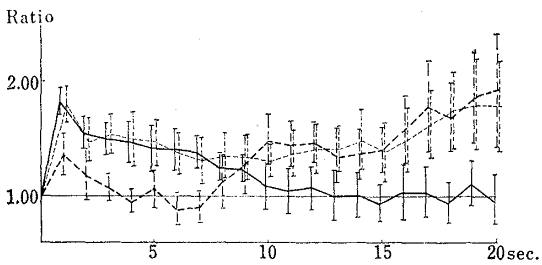

Fig. 4. Quinine Hydrochloride ($4\times10^{-4}M$)

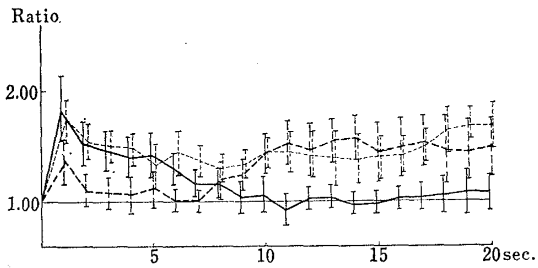

Fig. 5. Caffeine ($2.5\times10^{-2}M$)

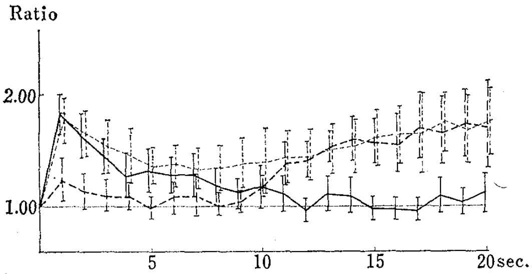

Fig. 6. Citric Acid ($10^{-2}M$)

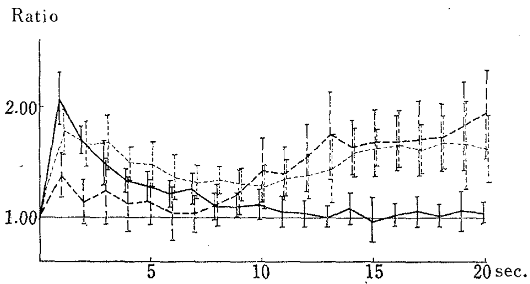

Fig. 7. Saccharose ($2\times10^{-1}M$)

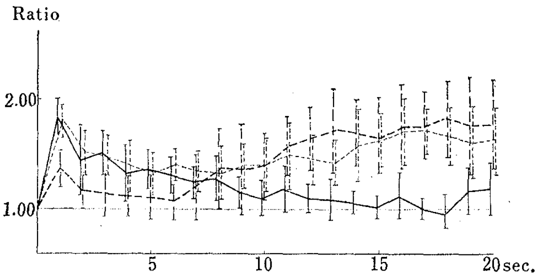

Fig. 8. Sodium Cyclamate ($10^{-2}M$)

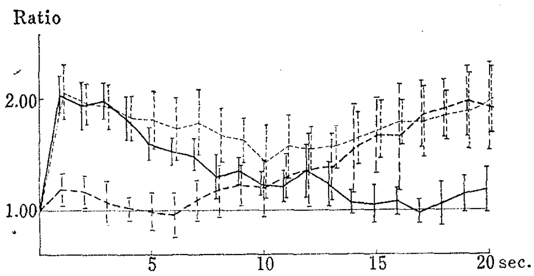

Fig. 9. Sodium Chloride ($2.5\times10^{-1}M$)

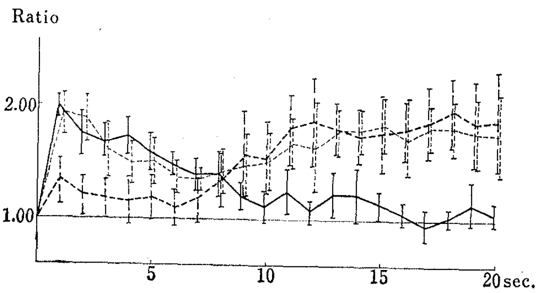

Fig. 10. Pottasium Chloride ($2.5\times10^{-1}M$)

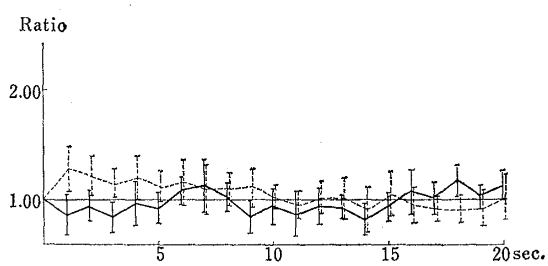

Fig. 11. Blank Test

············ ACh in aqueous solution

———— treated after 10% propylene glycol solution

Similarly, ratios before and after treatment with ACh, DMAE and mixed solution of ACh and DMAE were determined and plotted on graphs on bitter substances, *i.e.* quinine hydrochloride (Fig. 4) and caffeine (Fig. 5), sour substance, *i.e.* citric acid (Fig. 6), sweet substances, *i.e.* saccharose (Fig. 7) and sodium cyclamate (Fig. 8) and salty substances, *i.e.* sodium chloride (Fig. 9) and potassium chloride (Fig. 10). As a result, essentially, although not perfectly, the same was found to pertain to any of these taste substances as described previously on acetic acid solution.

Curve A in Fig. 11 represents the average values obtained by administration of the eight kinds of taste substances to the surface of tongue treated, as in the experiment methods used by Zotterman and Skouby, *et al.*, with ACh in aqueous solution for 2 minutes. With any of these eight substances, gustatory action was found somewhat enhanced in 1~3 seconds as compared with control figure. In curve B the influence of the 10% propylene glycol solution used in these experiments to dissolve ACh on gustatory action was examined, where no significant difference, consequently no influence could be observed.

Discussion

The use of ACh in 10% propylene glycol solution in consideration of absorption of ACh from the mucous membrane of tongue in these experiments has led to very satisfactory results as compared with those from the experiments with ACh in aqueous solution. This might be attributable to the poor permeability of the mucous membrane of tongue since ACh in aquous solution is in the form of quaternary ammonium compound. This is illustrated by the fact that impulses produced by ACh in aqueous solution was about 4 spikes/sec. while that in propylene glycol solution was approximately 7 spikes/sec. (after subtracting the number of impulses due to propylene glycol). The application of $2\times10^{-2}M$ of DMAE, which is almost tasteless, produced impulses of 7 to 13 spikes/sec. during the initial 10 seconds or so and thereafter those of 30 spikes/sec, showing rapid increase in the number of impulses.

Consequently, marked increase in the number of impulses following the application of taste substances to the tongue treated with ACh after impulses produced reached sufficiently the level of background activity, as compared with that obtained from the untreated tongue, may probably be due to enhanced excitability of gustatory receptors which ACh entered. With DMAE, on the other hand, an increase in the number of impulses was noticed during the initial 1~2 seconds after application and the excitability of gustatory receptors persisted even when it would begin to fall down with ACh, a fact which suggests that DMAE may have entered into the meta-

Table I. The Influence of Gustatory Action of Adrenergic Druds

Taste substance	Adrenaline ($10^{-3}M$)	Noradrenaline ($10^{-3}M$)	Serotonin ($5\times10^{-4}M$)	Histamine ($10^{-3}M$)
Acetic acid	±	±	±	±
Citric acid	±	±	±	±
Saccharose	+	+	±	±
Sodium cyclamate	±	±	±	±
Quinine hydrochloride	+	+	±	±
Caffeine	±	+	±	±
Sodium chloride	±	±	±	±
Potassium chloride	±	±	±	±

In this table, ± : not significance increased, + : slightly increased, ++ : increased.

bolic system of ACh to accelerate its synthesis. Therefore, an ACh-like substance can probably be supposed to play some important role in the transmission of sense of taste.

Furthermore, the influence on gustatory action was examined on catecholamine, serotonin and histamine as well. The result showed no such influence for sour and salty substances except for slight increase in gustatory action noticed for a sweet substance, saccharose, and bitter substances. The difference, however, was not so much as could be regarded as significant (Table I).

Above findings can imply that gustatory nerve may be cholinergic rather than adrenergic as shown by the previous experiment results. It is interesting that Barady[7] and Igata,[8] *et al.* have observed a large amount of ChE present in taste buds, the gustatory receptor, by histochemical methods, which seems to allow us to assume the role of ACh in gustatory receptors.

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Summary

Taste substances were administered to the frogs tongue treated with acetylcholine (ACh) in 10% propylene glycol solution and 2-dimethylaminoethanol (DMAE) which is considered to be precursor of ACh in animal body, and the changes in the sense of taste were studied by electrophysiological method. As a result, it was found that sense of taste was enhanced after treatment of the tongue with ACh as compared with before treatment. In addition, a phenomenon of sustained action of sense of taste was observed with DMAE.

This suggests that as ACh-like substance may play an important role in the transmission of sense of taste.

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