phocytes and fibroblasts of normal persons and then on lymphocytes of alcohol addicts under chronic disulfiram treatment who, 15–20 min before blood sampling, had been allowed to drink about 0.7 ml/kg of alcohol in the form of wine. Disulfiram is well-known to raise the blood acetaldehyde level and to cause thereby a characteristic vasodilatatory episode.

3. Acetaldehyde (analytical grade distilled twice immediately before use) added to the culture in an amount of 800 μM was toxic, killing the great majority of cells. At 400 μM it inhibited cell multiplication; after 72 h the majority of cells was M_1 and of these 12% showed labile chromosomal aberration. In the M_2 cells, SCE was increased 4–6fold over the background. An acetaldehyde level of 40 μM still affected cell progression and SCE was twice the control, while lower amounts had no adverse effect.

4. From patients under the influence of disulfiram and a minimum amount of alcohol, lymphocytes were obtained in the hypotensive period and cultured in a 1:4 mixture of their own serum and TC medium. After 72 h most cells were in the M_1 phase and SCE was elevated to about 3 times the control frequency. In normal humans, the elimination rate of alcohol is about 100 mg/kg/h irrespective of the amount consumed, and the blood acetaldehyde level does not exceed 25 to 30 μM except under the influence of disulfiram when it may attain levels well above 400 μM. Our results might therefore mean that alcohol causes no disturbance under normal conditions but may have grave consequences whenever the acetaldehyde level rises above 40 μM. This must be due to some low specific activity of aldehyde dehydrogenase. Such a defect was reported in animals during pregnancy[5], but may also be inherited as in some rat[6] and mouse[7] strains, or acquired, as it has been shown in alcoholics[8], and such a defect would presumably be responsible for the fetal alcohol syndrome.

The assumption is supported by the fact that practically all of the mothers of such babies had chronic liver disease[9], and especially by our observation of a family where the heavily drinking mother after having had 6 miscarriages gave birth to an afflicted child; during the early weeks of this pregnancy she was on disulfiram treatment but confessed to having occasionally consumed a little alcohol. Another female alcohol addict had a child with fetal alcohol syndrome; on consuming 0.4 g/kg of alcohol, her blood acetaldehyde level rose to 140 μM. In our experiments, fetal development was affected neither by considerable doses of alcohol nor by disulfiram, while their combination induced 60% fetal mortality.

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Depression of frog gustatory neural responses to quinine-HCl after adaptation of the tongue to various taste stimuli

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Summary. After the frog tongue was adapted to salt, sugar and bitter solutions, the amplitudes of initial phasic gustatory neural responses to quinine-HCl (Q-HCl) were depressed. However, adaptation to acid solutions did not affect the responses to Q-HCl.

It is well-known psychophysically and neurophysiologically that the gustatory response to a taste solution changes after adaptation to other taste solutions[2,3]. In the previous papers[4], we revealed that adapting the frog tongue to Q-HCl of a typical bitter compound enhances the amplitudes of gustatory neural responses to salts, acids and sugars, but generally depresses the neural responses to other bitter solutions. This study aims to investigate how the gustatory neural response to Q-HCl changes after adaptation to various solutions including those representing 4 taste qualities.

Materials and methods. Bullfrogs (*Rana catesbeiana*) anesthetized with urethane were used in the experiments. Whole glossopharyngeal nerve impulses recorded in situ were integrated with an electronic integrator having a time constant of 0.4 sec. Membrane potential changes of single taste cells elicited by chemical stimuli were recorded with 3 M KCl-filled glass microelectrodes having a resistance of 20–50 MΩ by inserting them into the taste disc of the fungiform papillae.

Taste solutions made up in deionized water were applied to the tongue surface using a semiautomatically controlled gustatory stimulator described previously[5]. The tongue was adapted to various taste stimuli for 10 sec, after which test Q-HCl was delivered successively. The flow rate of solutions was 0.78 ml/sec for nerve recording and 0.13 ml/sec for intracellular recording. The experiments were carried out at room temperature of 20–26 °C.

Results and discussion. An example of integrated gustatory neural responses is shown in figure 1. The record A represents the control response elicited by application of 1 mM Q-HCl immediately after adaptation of the

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tongue to water. The record B represents the response to 1 mM Q-HCl after adaptation to 10 mM caffeine. It is seen that the amplitude of initial phasic response to Q-HCl is greatly reduced after caffeine. Figure 2 shows the diminution of initial phasic neural responses to 1 mM Q-HCl following adaptation to 4 other bitter solutions such as 1 mM Q-H_2SO_4, brucine, picric acid and 10 mM caffeine. The amplitudes of responses are expressed as percent of the control responses to Q-HCl after water. The depressing effects of the adapting bitter solutions upon Q-HCl responses became greater in the order of Q-H_2SO_4 > brucine = picric acid > caffeine. These bitter compounds are diverse in chemical structure. Remarkable reduction of Q-HCl response after another bitter substance suggests that both substances possess a similar structural feature.

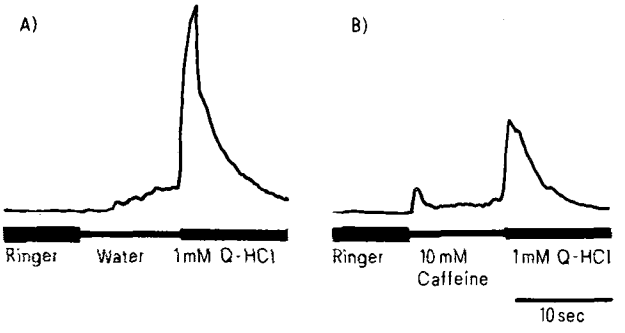

Fig. 1. Integrated whole glossopharyngeal nerve responses to 1 mM Q-HCl. *A* After 10 sec adaptation to water. *B* After 10 sec adaptation to 10 mM caffeine. The tongue was pre-adapted to Ringer before application of adapting solution and was rinsed with it after the end of test stimulation.

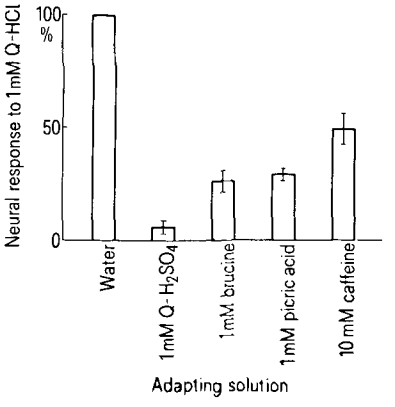

Fig. 2. Histograms showing changes of gustatory neural responses to 1 mM Q-HCl after 10 sec adaptation to 4 bitter solutions. In this and the next figure, responses are expressed as percent of the control responses to Q-HCl after water adaptation. Each value represents the mean ± SE of 4 to 5 preparations.

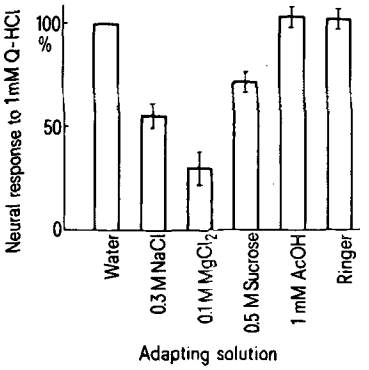

Fig. 3. Histograms showing changes of gustatory neural responses to 1 mM Q-HCl after 10 sec adaptation to various taste solutions. Each value represents the mean ± SE of 4 to 15 preparations.

With taste cells, correlation coefficients between the magnitudes of depolarizing responses elicited by a pair of 1 mM Q-HCl and 1 mM picric acid and by a pair of 1 mM Q-HCl and 1 mM Q-H_2SO_4 were calculated. No significant correlation ($r = 0.23$, 21 cells) was found between the former pair, whereas a significant correlation ($r = 0.89$, 17 cells) was found between the latter pair. These results may suggest that Q-HCl and Q-H_2SO_4 molecules react with the same receptor site on taste receptor membranes, while Q-HCl and picric acid molecules react with different receptor sites. Despite the absence of significant correlation between taste cell responses to Q-HCl and picric acid, the neural response to 1 mM Q-HCl after 1 mM picric acid was reduced to 29%, as shown in figure 2. In contrast, adaptation to 1 mM Q-HCl did not depress the neural response to 1 mM picric acid. It is considered that application of picric acid induces inactivation of taste receptor membranes following the stimulating action, resulting in depression of Q-HCl response after picric acid.

Figure 3 illustrates changes of initial phasic neural responses to 1 mM Q-HCl when adapted to 0.3 M NaCl, 0.1 M $MgCl_2$, 0.5 M sucrose, 1 mM acetic acid and Ringer solution. The responses are represented as percent of the control responses to Q-HCl after water. Adapting the tongue to the first 3 solutions depressed the responses to Q-HCl in various degrees, but adapting to the last 2 solutions did not affect the responses. The Q-HCl response was usually more depressed with increasing concentration of the adapting solutions, while the Q-HCl response after acetic acid was independent of the acid concentration. Although neural responses to 0.5 M sucrose were very poor in the present experiments, a mean of neural responses to 1 mM Q-HCl decreased to 72% following adaptation to the sucrose. This reduction may be attributable to chemical modification of Q-HCl binding site by the sucrose stimulation.

The amplitudes of depolarizing receptor potentials in taste cells in response to 1 mM Q-HCl decreased, like the reduction of neural responses in figure 3, by adapting the taste cells to salt, sugar and bitter solutions.

Microelectrode studies[6,7] on taste cells have suggested that receptor sites to 4 basic taste stimuli are independent of one another, but various combinations of receptor sites exist in single taste cells. It is known that a great number of taste cells converge a single gustatory nerve fibre[6] and that the excitability of the fibre terminals decreases following generation of impulses by taste stimuli[8]. From these lines of evidence, the depression of gustatory neural responses to Q-HCl after adaptation to various taste solutions may be explainable by the following 3 factors: a) similarity between receptor sites to an adapting stimulus and a test stimulus; b) chemical modification of the site to Q-HCl by adapting solutions; c) inactivation of the postsynaptic membrane or impulse firing zone in gustatory nerve fibres during application of adapting solutions.

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