cAMP and cGMP in the same tissue sample, according to the method of Cailla et al.[12,13]. Protein was assayed by the method of Lowry et al.[14].

Results. The mean level of cAMP measured in SCG of sham-operated animals by radioimmunoassay was 1.11 pmoles ± 0.17 SEM/mg wet weight, in accordance with our earlier experiments using Gilman's protein binding assay[4,5,8]. The mean cGMP level was 0.062 pmoles ± 0.003/mg wet weight, thus being about 18fold lower than cAMP levels. 3 days after decentralization, a rapid decrease in cAMP and cGMP was observed. cAMP decreased to 52% and cGMP to 67% of the contralateral ganglion. After 21 days cAMP returned to 73.9% and cGMP to 78.2% of control (figures 1 and 2). There was a slight but significant effect on the ganglion wet weight 3 days after denervation with an increase to 106.5% followed by a decrease to 96.9% after 21 days. The protein content showed similar changes with a decrease to 80% of control 14 and 21 days after denervation. Thus, when expressed as pmole/mg protein, cAMP and cGMP concentrations reapproached control values after 21 and 14 days respectively.

Discussion. Decentralization of the superior cervical ganglion of the rat has been shown to induce a rapid loss of 90% of the total number of synapses within 24 h of sectioning the preganglionic nerve trunk[11]. A similar effect was found at the synapses of the SIF-cells (small intensely fluorescent cells), which are presumed to be the adrenergic interneurons[15]. The time course of the decrease in cAMP and cGMP in our experiments appears to correlate with the ultrastructural synaptic changes after decentralization.

The permanent decrease of cAMP may be correlated with the decreased turnover of noradrenaline observed 2 weeks after denervation of rat SCG together with an increased total content of noradrenaline[16]. However, cyclic nucleotide levels in tissues are considered to reflect steady state levels of synthesis and degradation, and further interpretations would require measurements of activity of the respective enzymes. The partial restoration of cAMP levels after 7 and 21 days may well correlate with alterations of adrenoceptor sensitivity known to occur after denervation[17].

The less marked decrease after decentralization and faster recovery of cGMP as compared to cAMP suggests that the metabolism of the 2 nucleotides is differently affected by the procedure and hence under different preganglionic control. It remains unclear in which cells the observed changes of cyclic nucleotide levels take place. We have suggested earlier that catecholamine-linked adenylate cyclase may possibly be located in ganglionic satellite cells[5]. Profound structural alterations of these cells, in addition to the changes at neuronal synapses, have been shown to occur early after preganglionic sympathectomy[18].

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An initial phasic depolarization exists in the receptor potential of taste cells[1]

T. Sato[2]

Department of Physiology, School of Dentistry, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo 113 (Japan), 14 March 1977

Summary. When frog taste cells were stimulated by varying salts after adaptation to water, quinine or acetic acid, a phasic depolarization appeared initially in the receptor potential of taste cells. The initial transient depolarization may be related to the enhancement of an initial phasic response in the taste nerve.

When various salt solutions are applied to the frog tongue, an initial rapidly rising discharge (the phasic response) followed by a slower steady discharge (the tonic response) usually appears in the gustatory nerve[3]. On the other hand, microelectrode studies of taste cells[4–7] have demonstrated that depolarizing receptor potentials evoked by salt stimuli consist of a sustained response having no initial overshoot. Therefore, it has been proposed that the initial phasic response in the gustatory nerve may be associated with the rate of rise of depolarization in the taste cells, while the tonic response in the gustatory nerve may be related to the magnitude of steady depolarization[5,6].

Recently it has been found that the magnitude of the initial phasic component in the frog gustatory neural responses elicited by salt stimuli is markedly potentiated by adapting the tongue to water, quinine-HCl (Q-HCl) or acid in comparision with it during Ringer adaptation[3,8]. Therefore, there is a possibility that the enhancement of initial phasic neural response might be correlated with the appearance of an initial phasic depolarization in the taste receptor potential that has not been found under the Ringer adaptation. The purpose of the present study is to examine this possibility.

Materials and methods. Bullfrogs, Rana catesbeiana, were used throughout the present experiments. The animal was anesthetized with urethane. Electrical activities of whole glossopharyngeal nerves and single taste cells were

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recorded with conventional electrophysiological equipments. Amplified gustatory neural impulses were integrated with an electronic integrator of 0.4 sec time constant. Membrane potential changes in individual taste cells elicited by chemical stimuli were measured with a 3 M KCl-filled glass microelectrode of 15–40 MΩ by inserting it into a fungiform papilla of the tongue. Various taste solutions, made up in deionized water, were applied to the tongue surface by means of a semiautomatically controlled gustatory stimulator, described previously[3]. The flow rate used was 0.78 ml/sec for the whole nerve recording and 0.13 ml/sec for the intracellular recording. During the experiments on taste cells, a frog Ringer's solution was continuously flowed over the tongue as a pre-adapting solution and the Ringer flow was stopped when presenting adapting and test solutions. All experiments were carried out at room temperature of 23–26 °C.

Results and discussion. Figure 1 shows integrated glossopharyngeal nerve responses to 0.5 M NaCl when the tongue was adapted for 10 sec to deionized water (A), 0.01 M NaCl (B), Ringer (C) and 0.001 M Q-HCl (D). The magnitude of initial phasic responses became larger in the order of adapting solutions of 0.001 M Q-HCl > water > 0.01 M NaCl > Ringer. However, the magnitude of tonic responses following the phasic ones was almost the same even under different adapting solutions. Thus, as suggested previously[3,5], the 2 components of the gustatory neural response may originate from different mechanisms.

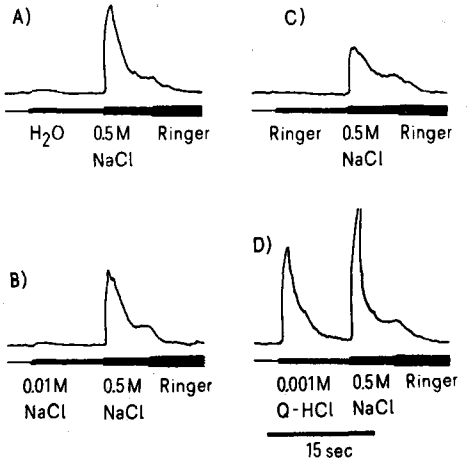

Fig. 1. Integrated glossopharyngeal nerve responses to 0.5 M NaCl following 10 sec adaptation to deionized water (*A*), 0.01 M NaCl (*B*), Ringer (*C*) and 0.001 M Q-HCl (*D*). During about 1 min before application of each adapting solution, the pre-adapting Ringer flow over the tongue was stopped but the tongue was covered with a thin Ringer layer. In this and subsequent figures, lower traces under responses denote applications of adapting and test solutions.

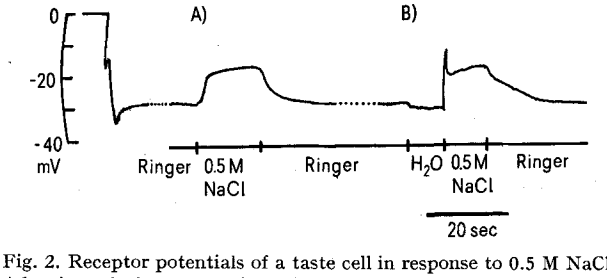

Fig. 2. Receptor potentials of a taste cell in response to 0.5 M NaCl. Adapting solutions were Ringer (*A*) and water (*B*). Resting potential measured just before application of chemicals was –29 mV.

Figure 2 illustrates membrane potential changes in a taste cell caused by 0.5 M NaCl after the adaptation to Ringer (A) and deionized water (B). A microelectrode was inserted into the taste cell inside the fungiform papilla to which nozzles of the gustatory stimulator were positioned at the distance of 1–2 mm. Under Ringer adaptation, no initial phasic depolarization appeared. The slowly rising sustained depolarization alone was recorded in accordance with previous experiments[4–7]. The rate of rise of depolarization occurring upon salt stimulation was more rapid than that of the subsequent gradually increasing depolarization. As previously suggested[6], it is likely that the rate of the initial steeper depolarization is related to the initial phasic gustatory neural response. The reasons for this are: 1. The initial phasic component of neural impulses in response to NaCl after Ringer appeared even when any phasic depolarization was not generated[6]. 2. The amplitude and peak time of the phasic neural response were dependent on the rate of rise of the sustained depolarization having no initial overshoot[6].

In the record B of figure 2, it is obviously seen that an initial phasic depolarization, followed by a steady depolarization, was elicited by 0.5 M NaCl after the tongue had been adapted to deionized water, which frequently hyperpolarized the taste cell membrane. The phasic depolarization might be associated with the enhancement of initial phasic neural responses as in figure 1A, B and D compared with the response in C. As the rate of rise of the NaCl stimulus on the papilla surface, where the microelectrode was inserted became slower, the initial phasic depolarization decreased in amplitude and finally disappeared. Similar overshoot in taste cell depolariza-

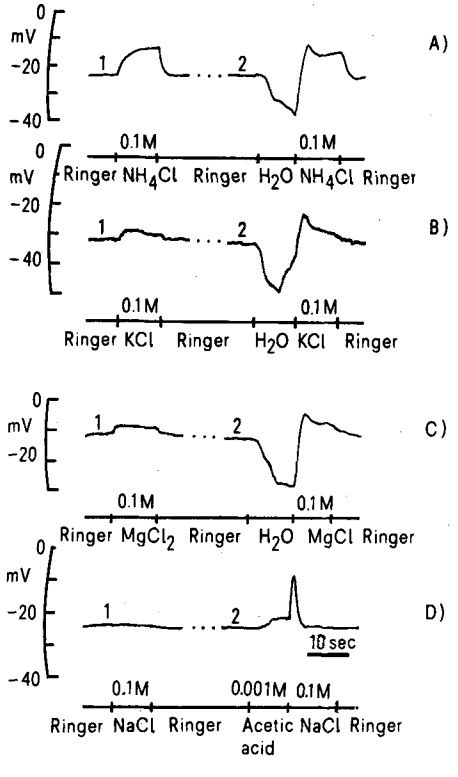

Fig. 3. Receptor potentials recorded intracellularly from 4 different taste cells. Adapting solutions were Ringer in records A1–D1, water in records A2–C2 and 0.001 M acetic acid in D2. Test solutions were 0.1 M NH_4Cl (*A*), 0.1 M KCl (*B*), 0.1 M $MgCl_2$ (*C*) and 0.1 M NaCl (*D*). Resting potentials were –24 mV in *A*, –32 mV in *B*, –12 mV in *C* and –25 mV in *D*.

tions was observed by various other salt stimuli after the tongue was adapted to water, Q-HCl or acetic acid. Some examples are illustrated in figure 3. Intracellular responses to 0.1 M NH_4Cl (A2), 0.1 M KCl (B2) and 0.1 M $MgCl_2$ (C2) following water and to 0.1 M NaCl (D2) following 0.001 M acetic acid are shown. In these and other taste cells studied, the initial phasic depolarization was scarcely observed by application of various salt stimuli under the Ringer adaptation (A1-D1).

2 theories have been presented as to taste receptor stimulation: One, proposed by Beidler[9], suggests that taste response is related to the number of occupancies of receptor sites by a stimulus. The other suggests that taste response is related to the rate of occupancy of receptor sites[10]. Since the initial phasic depolarization of taste cells was sensitive to the rate of stimulus onset, this would appear to support the rate theory concerning taste stimulation. However, the generation of phasic depolarization can probably be explained even by the Beidler's 'occupation' theory, if the amount or rate of conformational change of receptor sites occupied by a taste stimulus is presumed to be larger at the rising phase of the stimulus onset than at the static phase of the stimulus.

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Carbon dioxide sensitivity of pulmonary receptors in the frog[1]

W. K. Milsom and D. R. Jones

Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1W5 (Canada), 8 March 1977

Summary. Pulmonary mechano-receptors have been found in the frog lung that are sensitive to CO_2 concentrations in the lungs and airways comparable to the physiological levels recorded in frogs. These results support the suggestion that a pulmonary receptor with distinct mechano- and chemosensitive properties may represent the functional precursor of the more specialized pulmonary receptor types which have evolved in birds and mammals.

The rate and degree of inflation of the lungs during breathing are monitored by receptors within the lungs and airways in all animals which have been studied[2]. There are at least 2 sets of stimuli which vary with the rate and extent of lung inflation; mechanical factors such as lung volume or transpulmonary pressure and chemical factors such as the concentration of gases in the airways. The pulmonary receptors of mammals respond primarily to the transpulmonary pressure developed during each breathing cycle but their discharge is partially modified by high levels of alveolar CO_2[3-10]. Avian pulmonary receptors have little or no mechanosensitivity responding primarily to changes in airway CO_2 concentration throughout the breathing cycle[11-16]. Reptilian pulmonary receptors are typically mechanosensitive but exhibit a range of variation in their sensitivity to CO_2 which encompasses the different sensitivities to CO_2 found in the avian and mammalian receptor types[17]. Consequently it appears that the divergent receptor types found in birds and mammals may have arisen from a less specialized receptor type such as that found in the more phylogenetically ancient reptiles. Amphibia, however, have evolved from the evolutionary stem line at an even earlier date, possess structurally simple lungs and represent some of the earliest forms of semi-terrestrial lunged vertebrates. This study was undertaken to determine whether there are receptors present in the lungs of these early forms which are sensitive to CO_2.

Frogs (Rana pipiens), weighing between 120 and 160 g, were used in these experiments. The frogs were double pithed and unidirectionally ventilated with a continuous gas flow under slight positive pressure, air entering the lung through a tracheal cannula and leaving the lung by a cannula sewn into the caudal tip of the lung. The lung could be inflated during ventilation at any desired volume by altering the resistance of the outflow cannula from the lung. Single and multi fibre nerve activity were recorded from pulmonary afferent fibres in vagal slips using bipolar silver electrodes. The intratracheal pressure was recorded with a pressure transducer and with neural activity were amplified, visually displayed on an oscilloscope and stored on magnetic tape for later analysis on a PDP Lab 8e mini-computer using conventional software.

On the basis of changes in discharge frequency following lung inflation, frog pulmonary receptors have been classified into 3 groups; rate receptors, proportional receptors and rate plus proportional receptors[18]. The discharge frequency of rate receptors is modulated solely by the rate of increase in lung volume. 6 of 25 fibres we recorded from were of this type. Although these fibres were continuously active, their static rate of discharge was unaffected by the volume of the lung; discharge increased only during the period of lung inflation and then returned

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