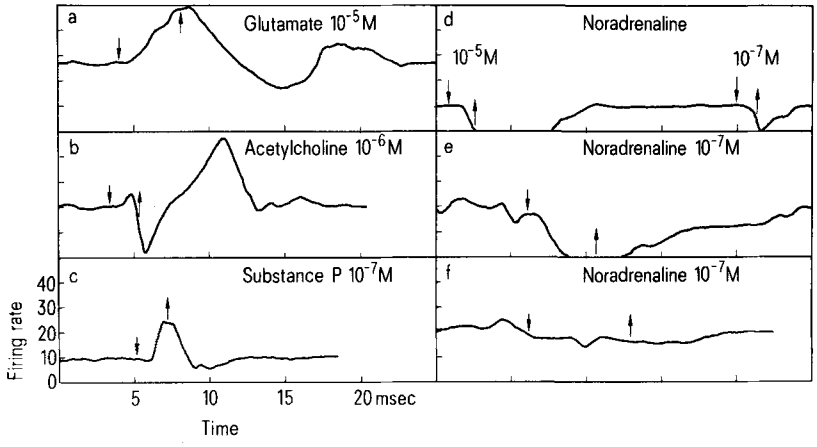

Fig. 2. Effects of various drugs on the spontaneous firing rate of the neurons in the brain stem slice. Extracellular single cell discharges recorded in magnetic tapes were digitized by an A-D converter, and processed by a general-purpose computer Nihon-Kohden ATAC 1200. Firing rate is indicated in the ordinate as the number of spikes per 10 sec. Upward and downward arrows represent the commencement of the drug application and washing by the standard medium, respectively. *A-C* Interpeduncular nucleus; *D* and *E* mamillary body; *F* Hypothalamus. Noradrenaline (10^{-7} M) usually depressed the firing rate of the hypothalamic neurons. However, in some neurons, it exerted no effect as shown in *F*.

neurons in vitro. Therefore, it might be considered alternatively that the in vitro neuron is relevant to the examination of drug effect on the discrete central neuron without interference from the surrounding neuronal activities as compared with the in vivo neuron. In view of the recent findings that acetylcholine[5] and substance P[6] play an important role in the habenulo-interpeduncular system, and that several putative peptide transmitters such as vasoactive intestinal polypeptide (VIP)[7] or endorphins[8] are rich in the hypothalamus, the brain stem slice will be a useful experimental model for pharmacology of the hypothalamic and interpeduncular neurons.

1 Acknowledgment. I thank Prof. H. Kuriyama for discussions and encouragement.
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Receptor potential of rat taste cell to potassium benzoate

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Summary. Rat taste cells responded to relatively low concentrations of K-benzoate with a hyperpolarization and to the high concentrations with a depolarization. During both responses the membrane resistance of a taste cell decreased. Depolarization elicited by application of a combination of 0.25 M NaCl and 0.05 M K-benzoate was smaller than that by the NaCl alone, indicating a depressant action of K-benzoate.

The effect of low concentration of K-benzoate applied to rat taste receptors is quite unusual in that the spontaneous activity in the taste nerve is depressed and a transient excitation occurs when a water rinse is applied. At higher concentrations, the stimulus behaves as most other stimuli in that it excites the taste receptors, although a large response still occurs with a water rinse (figure 1). Miller[2] studied the single taste fibre response to low concentrations of K-benzoate. Several fungiform papillae on rat tongue, each of which has only 1 taste bud, are innervated by peripheral branches of a single chorda tympani nerve fibre[2]. During stimulation of 1 fungiform papilla with NaCl, stimulation of the other neurally connected fungiform papillae with a relatively low concentration of K-benzoate caused a depression of gustatory neural impulses being elicited by the NaCl stimulus[2]. To understand this mechanism we studied the electrical properties of receptor potentials in rat taste cells elicited by K-benzoate applied to the tongue surface.

Materials and methods. Female adult Sprague-Dawley rats, anesthetized with urethane, were used. The intracellular responses of single taste cells in the fungiform papillae were recorded with a 3 M KCl-filled micropipette. The details of method for recordings have been given elsewhere[3]. Distilled water was flowed continuously on the tongue surface and, when a taste stimulus solution was delivered, the water flow was switched to the stimulus flow. The method of stimulus application was the same as that described previously[3]. The experiments were carried out at room temperature of 25–28 °C.

Results and discussion. Figure 2 illustrates an example of the concentration-receptor potential curve for K-benzoate of a taste cell. The receptor potentials in response to 0.003 M, 0.03 M and 0.1 M K-benzoate solutions are shown in the inset. As seen in the 3 records, the receptor potentials were composed of an initial transient hyperpolarization and a subsequent hyperpolarization or depolarization of a relatively steady magnitude. An off-depolarization was initiated after K-benzoate was rinsed with water (arrows in 2 right records). Since the tongue was adapted to distilled water, of course, the application of the water did not produce any change in the membrane potential. In this cell, only hyperpolarizations occurred below 0.05 M of K-benzoate but depolarizations preceded by initial transient hyperpolarizations appeared at concentrations above 0.05 M. The amplitude of initial phasic hyperpolarizations

increased gradually up to 0.03 M and then decreased with increasing concentrations. The steady-state responses measured 20 sec after K-benzoate application are plotted with open circles. In 4 other taste cells examined, 0.1 M K-benzoate caused only hyperpolarizing receptor potentials at both the initial phasic and the steady phase.

Figure 3 illustrates the relationship between concentrations of K-benzoate and membrane resistances in a taste cell. The data were obtained from the same cell as in figure 2. The membrane resistance in the ordinate is expressed as percent of the control input resistance of the taste cell membrane under water adaptation. The filled circles denote the resistance accompanying the initial phasic hyperpolarizing responses and the open circles the resistance accompanying the steady hyperpolarizing or depolarizing responses measured 20 sec after stimulation by K-benzoate. Only the 3 open circles with asterisk show the values of resistances at the time of depolarizing responses.

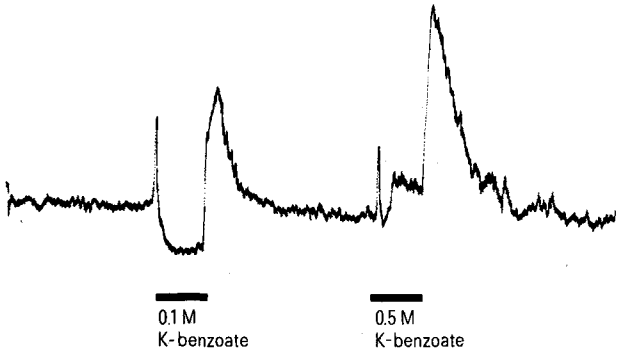

Fig. 1. Integrated responses of rat chorda tympani taste nerve to potassium benzoate flowed over the tongue. Water is applied continuously before and after stimulus application. Note initial transient when K-benzoate is applied and massive discharge when rinsed from the tongue with water. An inhibitory response is observed with first stimulus and an excitatory with second. First stimulus is 0.1 M and second is 0.5 M K-benzoate applied for 20 sec duration.

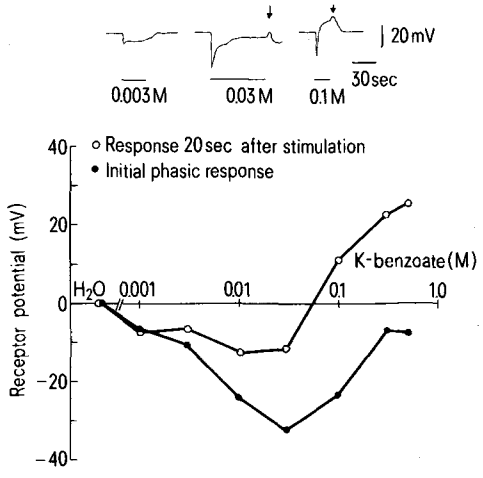

Fig. 2. Relation between molar concentration of K-benzoate and amplitude of receptor potentials in a rat taste cell. The filled circles denote the values of initial phasic hyperpolarizing responses and the open circles the values of steady hyperpolarizing or depolarizing responses measured 20 sec after the onset of K-benzoate stimulation. Positive numerals in ordinate denote depolarizations. The inset shows recordings of intracellular responses to K-benzoate solutions at concentrations shown underneath bars, which mean the period of stimulation. The arrows indicate off-depolarizations.

It is of interest that a decrease in the membrane resistance occurred when both the depolarizing and hyperpolarizing responses were evoked. One possible explanation for this is that the depolarization resulted from an increase in Na permeability of the taste cell membrane and the hyperpolarization an increase in K or Cl permeability. Figure 4 illustrates an example of receptor potentials in a taste cell in response to 0.25 M NaCl (A), 0.05 M K-benzoate (B) and a mixture containing the both (C). It is seen that the mixture which contained 0.05 M K-benzoate inducing a hyperpolarizing receptor potential depressed the amplitude of depolarizing receptor potential elicited by 0.25 M NaCl.

The observations[4,5] that background gustatory neural activities in rat are depressed by application of low concentrations of K-benzoate (figure 1) are accounted for by an increase in the taste cell membrane potential during the K-benzoate stimulation as seen in figure 2. In addition, the transient firing in the taste nerve when K-benzoate is rinsed with water may be due to an off-depolarization in the taste cell as shown in the records of figure 2.

The experiment by Miller[2] using rat tongue that gustatory neural impulses being produced by application of NaCl to 1 fungiform papilla is suppressed by application of K-benzoate at low concentrations to the surround may be explainable as follows: Stimulation of the surrounding papillae with K-benzoate would produce hyperpolarizing responses in the taste cells of the papillae. The hyperpolarizations would cause an increase in the postsynaptic membrane voltage of gustatory afferent fibre terminals, which is likely to be always maintained at a depolarized level as

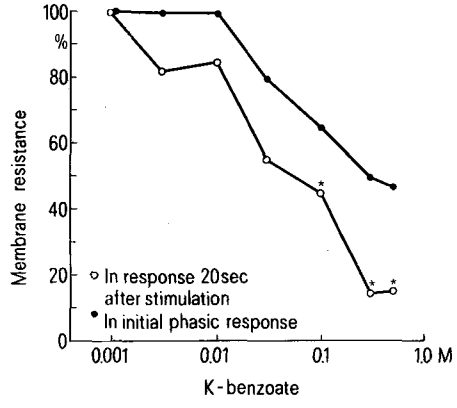

Fig. 3. Relation of molar concentrations of K-benzoate to the membrane resistances of a taste cell. The resistances are represented relative to the input resistance at rest. The filled circles show the resistances at the peaks of initial phasic hyperpolarizations, and the open circles show those in hyperpolarizing (the 1st 4 points) or depolarizing (the remaining 3 points with asterisks) responses measured 20 sec after K-benzoate presentation. All the values of the filled and open circles were obtained from the corresponding response values in figure 2.

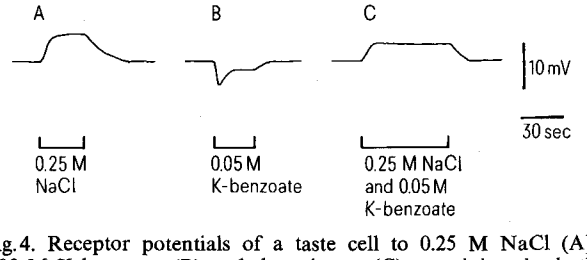

Fig. 4. Receptor potentials of a taste cell to 0.25 M NaCl (A), 0.05 M K-benzoate (B) and the mixture (C) containing the both substances at the same concentrations each.

estimated from the existence of spontaneous impulses discharged without chemical stimulation. That is, stimulation with K-benzoate may shift the depolarized level of the postsynaptic membrane to a hyperpolarizing direction. This corresponds to the phenomenon of the so-called disfacilitatory hyperpolarization found in some cells[3,6,7]. The K-benzoate-induced hyperpolarization at the postsynaptic axon membrane in the surrounding papillae might spread out electrotonically up to branching point of a gustatory nerve fibre, as has been suggested by Miller[2]. The hyperpolarization spread will add, at the branch point, to the depolarization elicited by NaCl stimulation of the other taste bud, so that the resultant initiation of impulses to NaCl may be reduced.

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The effects of dopamine on renin release in the isolated perfused rat kidney

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Summary. In the isolated and perfused kidney of the rat, the stimulant effect of dopamine on renin release is blocked by propanolol and not by haloperidol. This suggests that the release of renin induced by dopamine is due to the activation of β-receptors.

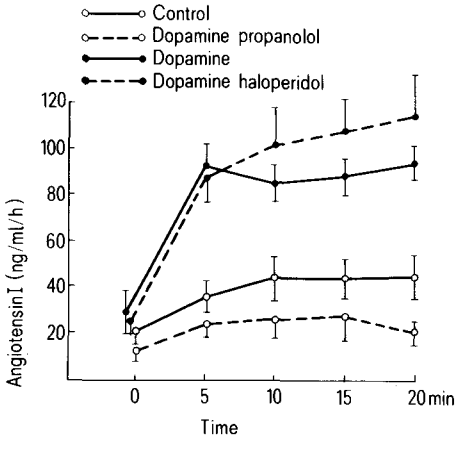
It has been shown that renin secretion is increased by dopamine[1-4]. However, the mode of action of dopamine on renin release is still not clear. It has been suggested that dopamine could act through dopamine[3] or β-adrenergic receptors[4]. Using the isolated perfused kidney of the rat, we have studied the direct effect of dopamine on renin release and the influence on this effect of haloperidol, a dopamine receptor blocker, and propanolol, a β-adrenergic blocking agent.

Materials and method. The method of isolation and perfusion of kidney has previously been described[5] elsewhere. The perfusion fluid was Krebs-Ringer dextran saline equilibrated with 95% O_2 and 5% CO_2 at 37 °C and was delivered as pulsatile flow at a constant rate (8 ml/min).

Perfusion pressure was monitored (Desvices M2). Samples were collected at 0, 5, 10, 15 and 20 min. Drugs were administered from 1 to 20 min. Renin was measured in the perfusion fluid by incubating with rat renin substrate and radioimmunoassay of angiotensin I, and the results are expressed as ng/ml/h of angiotensin I generated. The drugs used were: dopamine (Merck) at doses of $4.7\times$ (10^{-8} 10^{-7} and 10^{-5} M) dissolved in perfusion buffer with 6×10^{-4} M of ascorbic acid, propanolol (ICI) (2×10^{-4} M) and haloperidol (Latino SA) (5×10^{-5} M).

Results. The perfusion pressure was not altered by any of the drugs used in the present study. Neither propanolol (n = 7) nor haloperidol (n = 7) caused significant changes on renin secretion compared with the control kidney (n = 14). Dopamine was administered in 3 doses: 4.7×10^{-8} M and 4.7×10^{-7} M did not alter the renin release, but the 3rd doses 4.7×10^{-5} M produced a significant increase ($p < 0.01$) of renin release. The elevation of the renin secretion obtained with this dose of dopamine was not altered by haloperidol but was inhibited significantly ($p < 0.01$) by propanolol (figure).

Discussion. It is clear from the present results that dopamine (4.7×10^{-5} M) causes a significant increase of renin release in the isolated perfused kidney of the rat. Low doses of dopamine (10^{-8} M and 10^{-7} M) have been shown to have a stimulant effect on renin 'in vitro', but the monoamine oxidase inhibitor, pheniprazine, was used[4]. The possibility of an intrarenal conversion of dopamine into noradrenaline could be discarded, since there were no changes in the perfusion pressure during any experiment. Therefore, the action of dopamine on renin secretion described here could be attributed to its direct effect on the juxtaglomerular cells through an activation of β-receptors. The increase in renin release mediated by dopamine was blocked completely by propanolol and not by haloperidol.



The effect of dopamine (n = 6), dopamine plus propanolol (n = 7) and dopamine plus haloperidol (n = 9) on renin secretion on the isolated perfused kidney. Values given are means±SEM. For significances see text.

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