stain[8]; Gallyas's silver stain[9]. However, these 4 procedures stained every one of the isolated neurons as well as the neuroglial clumps (figures 1 and 2), and, therefore, they are not specific for isolated neuroglia. In the present experiments the neurons and neuroglia have not been sectioned but have been subjected otherwise to all the other techniques of the procedures cited[6-9]. However, in a previous paper neuroglial clumps *in sections* were also stained with these reagents[5]. In view of the present results, we are investigating whether identified neuronal cell bodies in cerebral sections also stain with neuroglial stains.

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Activation of the electrogenic Na-pump of cardiac muscle fibres by ACh in K-free solutions[1]

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Summary. The ionic mechanism of the membrane outward current (ACh-current) of bullfrog atrium muscle, induced by acetylcholine in K-free solution, was analyzed by a voltage-clamp experiment. The results suggested that the ACh-current was induced not only by an increase in K-conductance but also by an activation of the electrogenic Na-pump.

It has been known for long that the membrane of cardiac muscle fibres is hyperpolarized by the action of acetylcholine (ACh)[3-6]. This hyperpolarization has been suggested to be caused by an increase in K-permeability of the membrane on the basis of experimental evidence that the membrane conductance[7,8] and K^+ outflux[9] increase during the hyperpolarization. The present communication reports a new experimental finding that the membrane hyperpolarization induced by ACh in the K-free solution seems to be caused not only by an increase in K-permeability but also by an activation of the electrogenic Na-pump.

Material and methods. Muscle strips (300–500 μm in diameter) isolated from atrium of bullfrog *(Rana catesbeiana)* were used. Recordings of membrane potentials and currents were made by use of an experimental arrangement designed for a voltage clamp experiment[10]. Ionic compositions (mM) of the Ringer solution were 112 NaCl, 2.0 KCl, 0.1 $CaCl_2$, 6 $MgCl_2$, 2.4 $NaHCO_3$ and 2.5 glucose; a lower calcium level was used to reduce contraction and facilitate the prolonged maintenance of the microelectrode in individual cells. The K-free solution was prepared by omitting KCl from the Ringer solution. The sucrose solution used for sucrose-gap contained 230 mM sucrose. The muscle fibres were first equilibrated for 1–2 h in the K-free solution and the experiment started thereafter in this solution at room temperature. The resting membrane potential at this stage was -44 ± 2 mV (SE), n=22.

Results and discussion. The muscle fibres were markedly hyperpolarized by ACh (figure 1, a) in K-free solution; the mean amplitude of the hyperpolarization was 28 ± 4 mV, n=7, at a concentration of 10^{-5} M. The minimal effective concentration to produce the hyperpolarization was 10^{-7} M. During an application of ACh, a large membrane outward current (ACh-current) was produced when the membrane was held at the resting potential level (figure 1, b); the mean amplitude of the outward current induced by 10^{-5} M ACh was 1.6 ± 0.1 μA, n=20.

An interesting finding was that the ACh-current recorded in the K-free solution was sensitive to ouabain. It reduced to $14\pm4\%$, n=7, of its control value within 10 min of an application of 10^{-6} M ouabain (figure 1, c). A small part of ACh-current was, however, resistant to the action of ouabain; it was observed for more than 1 h in the presence of 10^{-5} M ouabain. These results suggest that ACh-current in K-free solution consist of 2 different current components, namely ouabain-sensitive and ouabain-insensitive ones. Both current components were completely blocked by an application of 10^{-5} M atropine to the perfusate.

The membrane conductance was increased by ACh to 1.8 ± 0.1, n=14, times larger than its control value. This

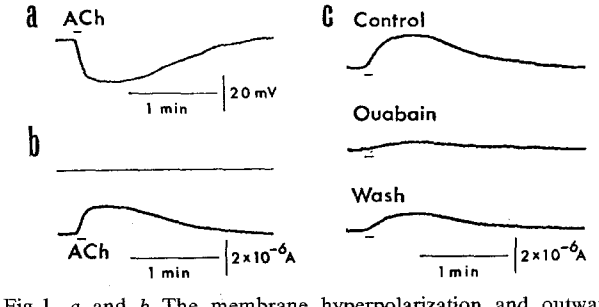

Fig. 1. *a* and *b* The membrane hyperpolarization and outward current by 10^{-5} M ACh, respectively; upper trace in *b* is for voltage recording. *c* The effect of 10^{-6} M ouabain on the ACh-current; the upper and middle records are before and 5 min after application of ouabain, respectively, and the bottom record is 10 min after removal of ouabain. Short horizontal bars in *a*, *b* and *c* indicate periods of 10^{-5} M ACh application.

increase in membrane conductance was unaltered by ouabain. When the membrane was hyperpolarized stepwise, both ouabain-insensitive and ouabain-sensitive ACh-currents were reduced accordingly, and finally reversed their polarities at a potential level between -90 and -120 mV, n=7. These results suggest that an increase in potassium permeability is responsible for those ACh-currents.

Since the ACh-current was sensitive to ouabain, the contribution of an electrogenic Na-pump to the ACh-current was examined by studying the changes of the K-activated current[10] in the presence or absence of ACh. The K-activated current is a membrane outward current produced by an activation of the electrogenic Na-pump when the perfusate is changed from K-free to Ringer (2 mM K) solution[10-12]. In 14 experiments, the K-activated current was markedly depressed (more than 90%, 8 cells) or eliminated (3 cells) and even reversed its direction (3 cells) in the presence of 10^{-5} M ACh (figure 2). Since the K-activated current consists of the net outward current (the electrogenic Na-pump current) and the passive inward K current[10], a reversal of its direction indicates an almost complete loss of the net outward current. These results suggest that the electrogenic Na-pump is already activated in the K-free solution by ACh. A possible reason could be that the increase in K-permeability by ACh increases the net outflux of K^+ (ouabain-insensitive), and consequently the K^+ concentration immediately outside of the membrane; similar extracellular K^+ accumulation was suggested during activity of heart muscles[13]. Such an increase in extracellular potassium ions in the K-free solution could activate the Na-Pump (ouabain-sensitive). According to this concept, the mechanism of ouabain-sensitive ACh-current is similar to that of the K-activated current, and the K-activated current would therefore be depressed during the generation of the ACh-current. To other results presented in this paper could also be reasonably explained by this concept.

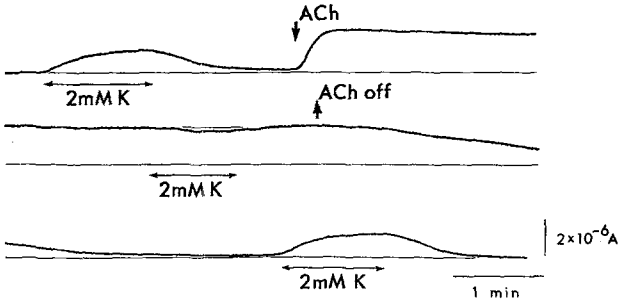

Fig. 2. The effect of 10^{-5} M ACh on the K-activated current; periods of application of 2 mM K and 10^{-5} M ACh are marked by horizontal and vertical arrows, respectively.

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Frog's tongue receptive areas: Neural organization and gustatory function

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Summary. Lateral and medial branch of the frog's IXth nerve innervates rostral third and caudal two-thirds of the tongue surface, respectively. The amounts of gustatory signals in these branches differ in proportion to the area they supply.

Taste organs or the fungiform papillae in the frog tongue receive gustatory as well as somatic fibres only from the glossopharyngeal (IXth) nerve. Neural organization of the tongue receptive area in the frog has thus been considered simple compared with that in the mammalia, whose taste organs in the anterior two-thirds and the posterior third of the tongue are supplied by the chorda tympani and the IXth nerve, respectively. However, because the frog's IXth nerve divides into 2 branches before entering the tongue, it is possible that each of the branches innervates a defined tongue area, as in mammals. We examined this possibility in relation to functional significance on taste reception.

American bullfrogs *(Rana catesbeiana)*, weighing between 270 and 450 g, were mainly used besides the common frogs *(Rana nigromaculata)*. They were anesthetized with 20% urethane solution (15 ml/kg, i.p.). The IXth nerve and its distal ramifications, the medial (m.br.) and lateral (l.br.) branches, were dissected; the latter 2 being cut at the proximal junction, if required. The nerve was mounted on a pair of silver wire electrodes. For stimulation of the nerve, brief electric pulses with 1/sec in repetition rate were employed. To record electrical signals from a single fungiform papilla, it was sucked into a glass capillarly electrode filled with Ringer's solution. The signals were amplified by c.r. coupled amplifiers, summated by an averaging computer and displayed on a X-Y recorder. When recording taste responses from the nerve, the amplified signals were led to an integrator of a time constant of 0.2 sec, and recorded on a pen-recorder. In some instances, the recording was made from bilateral nerves, simultaneously. Taste stimuli employed were 0.5 M sodium chloride, 0.05 mM quinine hydro-chloride, 0.125 mM hydrochloric acid and 0.1 M sucrose solutions, the latter 3 dissolved in 0.01 M saline. They were applied to, and kept for about 5 sec in, a lucite chamber in which the tongue had been fixed. During this period, one or both sides of the lingual artery was perfused with Ringer's solution, the method being similar to that of other authors[1,2]. The fungiform papillae were counted in number after injection of 0.05% methylenblue solution into 1 of the lingual arteries. In this study, the tongue attached