muscle cells were increased[5]. Hence, the effect of dibutyryl cyclic AMP, permeable to the muscle cell membrane, on the responses to CA was examined, and it was observed that dibutyryl cyclic AMP (10^{-7} to 10^{-8} g/ml) augmented the responses to both NA and MX (Figure 2).

Propranolol (10^{-5} g/ml) inhibited the response to NA and, in contrast, it was potentiated with tetracaine (10^{-6} g/ml) (Figure 3). Therefore, the inhibitory action of propranolol may be caused not by a local anesthetic action, but by a β-blocking one. And so there is a possibility that the inhibitory effect of propranolol is mediated by its suppressive action on CA to increase cyclic AMP concentration in the smooth muscle cells[5,6]. When 0.1 μg and 1.0 μg of NA were applied repeatedly and alternately every 15 min, the responses were augmented gradually. In the presence of imidazole (2.4×10^{-4} g/ml), a phosphodiesterase activator, the response to NA was enhanced initially, then its gradual increase ceased and finally reached an almost constant level. An application of propranolol (10^{-5} g/ml), in addition to imidazole, strongly inhibited the response (Figure 4). On the other hand, caffeine, a phosphodiesterase inhibitor, potentiated the response to NA in lower concentration (2×10^{-7} g/ml), while its higher concentration (9.7×10^{-4} g/ml) suppressed them.

The results described above show that adrenergic β-stimulant potentiates the action of adrenergic α-stimulant, since the response to NA is increased in the presence of IP or dibutyryl cyclic AMP. The stimulating effect of adrenergic α-receptor, therefore, appears to be more marked, when the stimulation of β-receptor does coexist. Since, in the cutaneous resistance vessels, adrenergic receptors mainly consist of α-receptor, not of β-receptor, a relaxation of the vessels by stimulation of β-receptor hardly occurs. As mentioned above, dibutyryl cyclic AMP augmented the responses to NA and MX. Therefore, it is suggested that the inhibitory action of propranolol may be due to the suppression of the increase in cyclic AMP levels. This insight may be supported by the observation that an increase in tissue content of cyclic AMP potentiates the contraction of the rat's aortic strip produced by NA[7]. The aortas of spontaneously hypertensive rats (SHR) contained significantly lower intracellular concentrations of cyclic AMP then their controls, and the response of adenyl cyclase activity to IP was reduced, while phosphodiesterase activity was significantly elevated[8]. Therefore, regarding adrenergic receptors and adenyl cyclase system, it is speculated that a similar relationship exists between the aorta of SHR and the cutaneous resistance vessels of rabbit ear, and such characteristics may be associated with an increase in the vasoconstrictor response to NA in the artery of SHR[9].

Zusammenfassung. Die Wirkung von Noradrenalin auf den peripheren vaskulären Widerstand wurde am isolierten Kaninchenohr untersucht und festgestellt, dass die Wirkung einer α-adrenergen Stimulation durch β-adrenerge Stimulation verstärkt wird.

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[5] L. TRINER, G. G. NAHAS, Y. VULLIEMOZ, N. I. A. OVERWEG, M. VEROSKY, D. V. HABIF and S. H. NGAI, Ann. N.Y. Acad. Sci. *185*, 458 (1971).
[6] R. ANDERSSON, Acta physiol. scand. *87*, 84 (1973).
[7] H. J. BARTELSTONE, P. A. NASMYTH and J. M. TELFORD, J. Physiol., Lond. *188*, 159 (1967).
[8] M. S. AMER, Science *179*, 807 (1973).
[9] B. FOLKOW, M. HALLBÄCK, Y. LUNDGREN and L. WEISS, Acta physiol. scand. *80*, 93 (1970).

Resting Membrane Potential of the Stria Cells of the Guinea-Pig

The object of this paper is to examine the effect of potassium ions on the resting membrane potential of the stria cells. A higher concentration of potassium ions in the external environment of muscle cells or nerve cells has been known to depolarize the resting membrane potential[1-3]. The present study was undertaken because the stria cells face the endolymph, and the endolymph contains a high concentration of potassium ions (150 mEq/l)[4], and the resting membrane potentials have not been previously measured, although BÉKÉSY[5-7] did report that inside the stria cells the potential was negative, but gave no measurements.

Method. Electrode. Fine microglass electrode with tip diameter between 0.5 μm to 0.8 μm were used to record the endocochlear DC potential from the scala media and the resting membrane potential of the stria cells. The electrodes were filled with 3 *M* KCl. The resistances were checked before using, only those in the range between 15 $M\Omega$ to 30 $M\Omega$ and with low tip potential (between 5–8 mV) were selected for measurements. A high input impedance differential electrometer amplifier Keithley 604 or Nihon Koden microglasselectrode amplifier was used for recording.

Measurements of resting membrane potentials in vivo. Coloured and white guinea-pigs were used throughout this study. The animal was deeply anaesthetized under Nembutal, and the head was firmly fixed on a headholder. The bulla was opened as previously described[8].

A small hole (diameter about 50–80 μm) just above the middle region of the stria-ligamentum was made on the bony cochlear wall by means of a fine steel-needle which bare 3 sharpened edges. Care was taken to avoid bleeding.

The Ag-AgCl-microglass electrode was adjusted to a 90° direction so that it could pass into the hole and through the cells of ligamentum spirale and the 3 layered cells of stria vascularis. The insertion of the electrode with the aid of a Leitz manipulator was advanced gently in order to show the clear potential drop negative potential just before the registration of the endocochlear DC potential. This negative potential probably represented the resting membrane potential of the stria cells in vivo.

[1] A. L. HODGKIN and B. KATZ, J. Physiol., Lond. *108*, 37 (1949).
[2] A. F. HUXLEY and R. STAMPFLI, J. Physiol., Lond. *112*, 496 (1951).
[3] G. LING and R. W. GERARD, J. comp. Cell Physiol. *34*, 383 (1949).
[4] C. A. SMITH, O. H. LOWERY and M.-L. WU, Laryngoscope, St. Louis *64*, 141 (1954).
[5] G. v. BÉKÉSY, Nature, Lond. *169*, 241 (1952).
[6] G. v. BÉKÉSY, J. acoustic Soc. Am. *24*, 72 (1952).
[7] G. v. BÉKÉSY, *Experiments in Hearing* (McGraw and Hill, New York 1962).
[8] J. T.-Y. CHOU, Arch. Ohr.-Nas.-u. KehlkHeilk. *195*, 246 (1970).

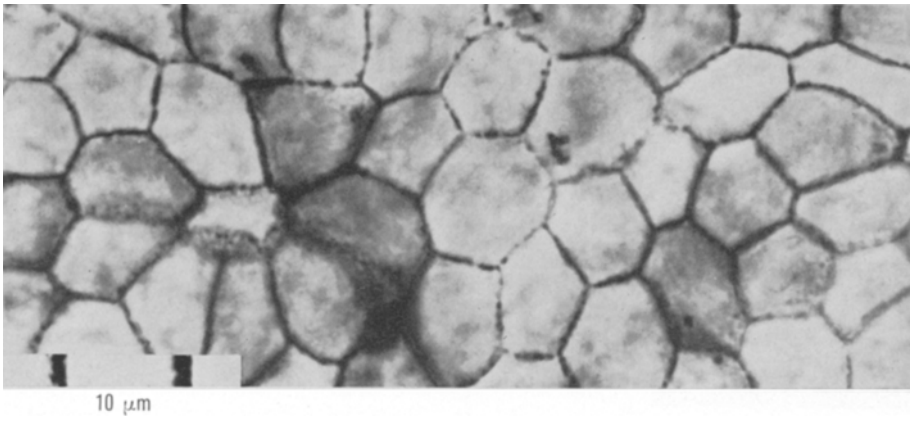

Fig. 1. Surface view of the stria vascularis cells of the guinea-pig. Silver-nitrate preparation. The scale represents 10 µm.

Measurements of resting membrane potentials of the stria cells in vitro. After recording the endocochlear DC potential, the cochlea was cut in half, and the part without the modiolus but with the intact stria vascularis was used for measurements of the resting membrane potentials. The opened cochlea was washed thoroughly either in Ringer's solution or in artificial endolymph. Substrates were added sometimes in both solutions and phosphate buffer at pH 7.2 to 7.6. Simple media as 150 m*M* KCl or 150 m*M* NaCl were also used. All these media were saturated with oxygen before using.

The surface of the stria vascularis was placed facing upwards in a small glass chamber and fixed with Histoacryl blue. In this manner the electrode was brought into contact with the surface of a stria cell. Each surface cell had an area about 100 µm² (Figure 1). The medium was replaced continuously, except when the recording was in progress.

Two methods were used for inserting the electrode in order to obtain the resting membrane potential: 1. with a Leitz manipulator and 2. combination with a Leitz manipulator and a 'Jolter' (manufactured by Nihon-Koden Electronic Co. Tokyo, Japan)[9]. This instrument the 'Jolter' can vibrate through its driver and is triggered with a stimulator, in an upwards direction with an accuracy of e.g. 1 µ, 2 µ, or 3 µ. After first observing on the oscilloscope that the tip of the electrode was very close or in contact with the surface of the stria cell, current was then applied from the stimulator; a sudden and definite vibration was produced by 'Jolter', then the tip of the electrode was inserted into the cell. All the experiments were measured at room temperature and some were measured at 22 °C; a few at 36 °C.

Results. Resting membrane potential of stria cells in vivo. Figure 2 shows the endocochlear DC potential recording from the scala media. As the fine electrode passed through the ligamentum spirale and the 3 layers of the stria cells, the voltage dropped to negative. The value was between negative 12 mV to negative 20 mV in 30 different insertions. The negative potential immediately before the registration of positive endocochlear potential must be from the resting membrane potential of stria cells, since the stria cells are the only cells in direct contact with the endolymph in this region. Nevertheless, as the electrode passed through the cell, either totally or partially damaged it, the exact value of the resting membrane potentials could not be assessed by this method. Many attempts were made to record the resting membrane potentials of the stria cells from an opening made at the apex of the cochlea, the electrode being arranged in a way that it could pass through the Reissner's membrane into the scala media and onto the stria cells. No stable recordings were obtained. It was necessary to record the resting potential in vitro.

Resting membrane potential in vitro. When the electrode was inserted into a surface cell of the stria vascularis with the aid of the Leitz manipulator, there was a sharp negative potential often followed by rapid depolarization until the potential reached a somewhat stable level. This rapid depolarization was probably due to an injury of the

[9] Information from Nihon-Kohden Electronic Company, Tokyo, Jap.

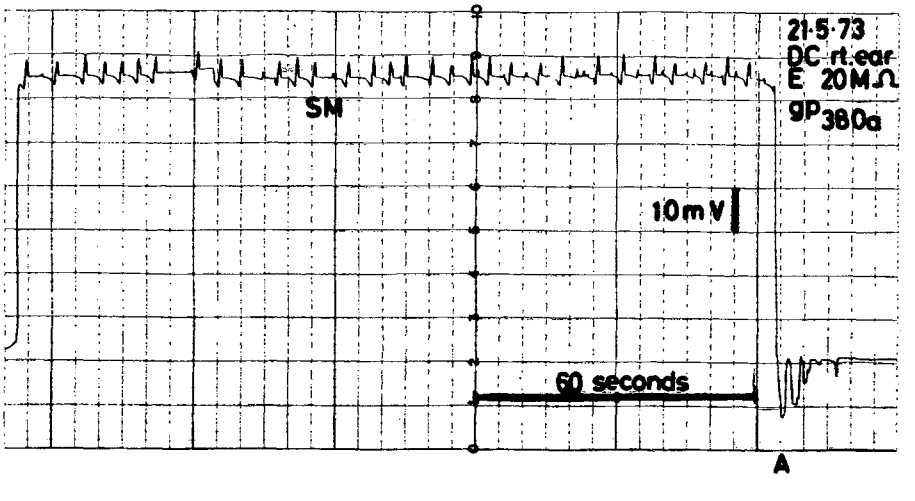

Fig. 2. Endocochlear potential recording (DC potential) from the scala media (in SM) of the guinea-pig. The negative potential shown on the recording just before the positive endocochlear DC potential represents the resting membrane potential (A) of the stria cells in vivo. The series of regular small responses shown during the DC recording corresponds to the movements of the animal's breathing. These responses can only be observed when the tympanic membrane and the 3 auditory ossicles are in perfectly intact condition.

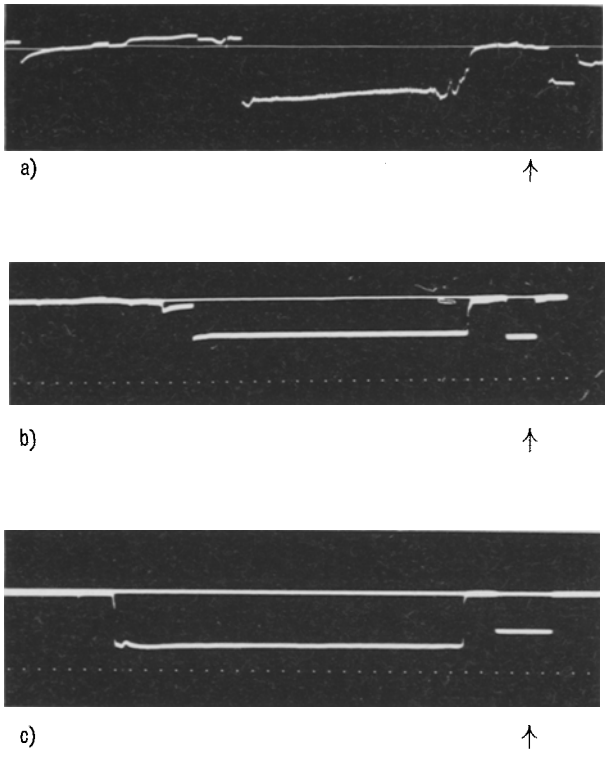

Fig. 3. Resting membrane potential of stria cells of the guinea-pig in vitro. A standard scale 20 mV is shown at the end (left) of every recording (↑). a) Insertion with the aid of Leitz manipulator. A rapid depolarization shown after the insertion was probably due to an injury of cell membrane. Medium: Ringer's solution. b) Insertion with the combined Leitz-Jolter method. Medium: Ringer's solution. c) Insertion with the combined Leitz and Jolter method. Medium: artificial endolymph (K: 150 m*M*; Na: 3 m*M*).

Resting membrane potential of the stria vascularis cells in Ringer's solution and in artificial endolymph

Media	No. of insertions	Resting membrane potential (mV)		Average (mV)
		Lowest value	Highest value	
Ringer's solution	86	−18	−58	−29.69
Artificial endolymph	51	−19	−56	−27.38

cell membrane by the penetration of the electrode (Figure 3a). Although the tip of the electrode was very fine, the shaft of the electrode immediately behind the tip was big enough to cause such change; furthermore, the electrode could have advanced too deep into the cell.

With the combined Leitz-Jolter insertion method, very stable and consistent resting membrane potentials have been recorded (Figures 3b and c). All the recordings, thereafter, were carried out with Leitz-Jolter method. The results of the resting membrane potentials of the stria cells recorded in Ringer's solution and in artificial endolymph are summarized in the Table.

In control experiments with an exactly similar electrical setup, the resting membrane potential of the sartorius of frogs and that of the sterno-hyoid muscle of guinea-pigs were measured in Ringer's solution. The measurements were the expected negative 95 mV and negative 78 mV respectively. A 10-fold change in potassium concentration of external medium caused a voltage shift of about 50 mV in the observed resting membrane potential of both muscles. This may be contrasted with the fact that the high potassium concentration in the surrounding medium did not alter the resting potential of the stria cells.

A few experiments to record the resting membrane potential were also measured at 36 °C. An average value was between negative 26 mV to negative 29 mV in Ringer's solution and in artificial endolymph.

Discussion. The unusual result in this study is the negative resting membrane potential when the stria cells were exposed to the endolymph medium (composition: 150 m*M* KCl and 3 m*M* NaCl). Resting membrane potential could rise in medium containing low concentration of calcium ions; this was due to the increase in permeability potassium of the cell membrane[10]. The concentration of calcium ions in the media showed little influence on the potential of the stria cells, since in normal Ringer's solution and in artificial endolymph containing very little calcium ion, or no calcium ions, an average negative 30 mV could still be recorded.

Normally, in muscle cells or in nerve cells, the resting membrane potential is regarded as a potassium concentration potential if an effective impermeability of the cell to sodium ions is taken into consideration. The possible explanation of the result might be attributed to the fact that the permeability of the cell membrane of the stria vascularis to potassium ions as well as to sodium ions was so low that the resting membrane was determined by chloride ions. It is known that intracellular chloride concentration can affect certain potential in motoneurons of cat[11]. When preliminary experiments were carried out in medium without chloride ions, the potential did decrease by about 75% to 80%. More work is definitely needed to define the nature of the resting membrane potential of the stria cells[12].

Zusammenfassung. Das Ruhemembranpotential der Stria-vascularis-Zellen wurde innen mit einer Mikroglaselektrode gemessen. Bei 22 °C betrug das durchschnittliche Ruhemembranpotential negativ 29 mV in normaler Säuger-Ringerlösung und auch in künstlicher Endolymphe. Hohe K-Ionenkonzentration im Medium beeinflusste das Ruhemembranpotential nicht.

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[10] W. I. V. OSTERHOUT and S. E. HILL, J. Physiol., Lond. *22*, 139 (1939).

[11] R. LLINAS, R. BAKER and W. PRECHT, J. Neurophysiol. *37*, 522 (1974).

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