Sandoz, Ltd., Basle, for samples of the pure ergot alkaloids.

ELMORE H. TAYLOR
EGIL RAMSTAD

Department of Biopharmacognosy,
School of Pharmacy,
Purdue University.

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PHYSIOLOGY

Rectifying Properties of Heart Muscle

A FACTOR of likely importance in the genesis of the long-lasting action potentials of vertebrate heart muscle is the influence of membrane potential on the potassium permeability of the fibres. That a difference here exists between cardiac muscle and nerve is evident from Weidmann's[1] demonstration of a low slope conductance during the plateau of the action potential. But little information is so far available on the voltage dependence of the membrane conductance under conditions designed to avoid the generation of action potentials.

To study this point, so far as the technical limitations imposed by the structure of cardiac muscle at present allow, excised Purkinje fibres from sheep ventricles were used. While sharing the essential electrical properties of myocardial tissue, these fibres do not produce a mechanical response on depolarization; they are also insensitive to parasympathomimetic substances so that choline chloride or sucrose may be used as a substitute for extracellular sodium chloride to abolish excitability.

Fig. 1, *A* and *B*, shows how the membrane conductance of a Purkinje fibre in sodium-deficient solution depends on the direction and magnitude of the polarizing current. An outward current of 0·31 μamp., for example, produced the same voltage deflexion as is caused by nearly twice as great an inward current. Allowing for the cable properties of the fibre, this means that the conductance to a depolarizing current may be about four times less than to a hyperpolarizing current. Since the contribution of chloride ions to the membrane conductance of cardiac muscle is small[2], a decrease in the potassium conductance presumably occurs on depolarization. The direction of the potassium conductance change in Purkinje fibres is thus opposite to the predominant change in nerve[3], but has a parallel in skeletal muscle[4].

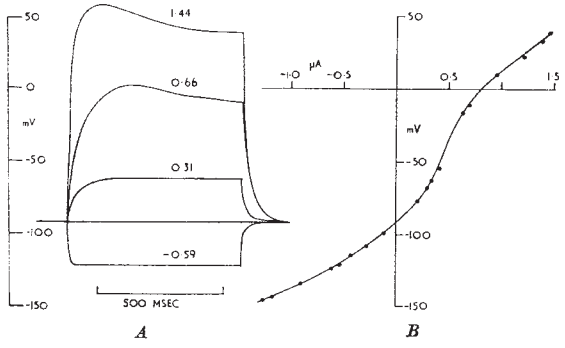

Fig. 1 *A*. Superimposed tracings of electrotonic potentials from a sheep's heart Purkinje fibre. Sodium ions in Ringer's solution replaced by choline. Depolarization is shown in an upward deflexion from a resting potential of −92 mV. Two closely spaced KCl-filled intracellular electrodes were used to record the membrane potential and to pass practically rectangular current pulses of a strength indicated by the figures on each record. *B*, Current-voltage relation for the same preparation. Ordinate, membrane potential at end of current pulse lasting 700 m.sec. Abscissa, total membrane current

With strong depolarizing currents a decline in the electrotonic potentials during the pulse may be observed, suggesting a gradual increase in conductance. This second effect probably accounts for the S-shaped relation between the amplitude of the electrotonic potential at the end of a long pulse and the polarizing current (Fig. 1 *B*), but over the physiological range the conductance remains below its value at the resting potential.

The slope conductance decreases appreciably over the range occupied by the slow diastolic depolarization in spontaneously beating preparations[1]. This alinearity may require consideration in interpreting the resistance changes observed during the pacemaker potential[5].

O. F. HUTTER
D. NOBLE

Department of Physiology,
University College London,
Gower Street, London, W.C.1.

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Cardiac Action and Pacemaker Potentials based on the Hodgkin-Huxley Equations

SINCE the equations describing the nerve action potential were formulated by Hodgkin and Huxley[1], the range of phenomena to which they have been shown to apply has been greatly extended. Huxley[2] has applied them to the influence of temperature on the propagated response and to the repetitive firing observed in low calcium concentrations. More recently, Fitzhugh[3] has shown that the long action potentials induced by tetraethylammonium ions in squid nerve may also be reproduced.

The computations described in this communication were carried out with the aim of reconstructing the long-lasting action potential and pacemaker potential of cardiac muscle. Although this work was done independently, the results agree with those of Fitzhugh in showing that action potentials of long duration may be accounted for by Hodgkin and Huxley's formulation of the membrane properties. The description of the potassium current, however, differs from that used by Fitzhugh and provides a better description of the conductance changes.

The equations I have used to describe the sodium current are very similar to Hodgkin and Huxley's,

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those for h (the variable describing the availability of sodium carriers) being based on Weidmann's[4] experiments on the availability of sodium carriers in Purkinje fibres.

The behaviour of the potassium battery in cardiac muscle differs from that in nerve in that the potassium conductance (G_K) falls when the membrane is depolarized[5]. A small delayed increase in conductance appears to be present when large currents are used, but this is not great enough for the conductance of the depolarized membrane to exceed the resting conductance. For the purpose of setting up equations to describe this behaviour, it is convenient to suppose that potassium ions move through two types of channel in the membrane. In one, G_K is an instantaneous function of the membrane potential and falls when the membrane is depolarized. A simple empirical equation has been used to describe the current in this channel. In the other type of channel the rectification is in the opposite direction and occurs with a delay. The conductance of this channel may be described by Hodgkin and Huxley's equations for delayed rectification with two modifications. First, the magnitude of this conductance must be small; accordingly the maximum conductance ($\overline{G_K}$) has been made less than 1/50 of that in nerve. Secondly, the time constants have been made very much longer to take account of the slow onset of the effect.

The membrane capacity was taken as 12 μF./cm.^{2} (ref. 4), and the absolute values of the various conductances were adjusted to give a resting membrane resistance of about 2,000 ohms/cm.^{2} (ref. 4).

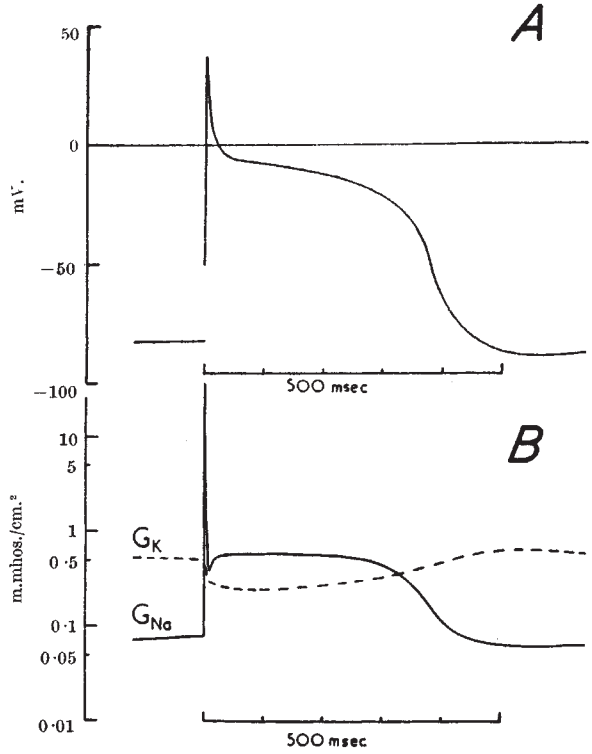

Fig. 1. *A*, Computed action potential. The integration was started by displacing the membrane potential to −50 mV., which is equivalent to a very short cathodal pulse of $3{\cdot}6 \times 10^{-7}$ coulombs/cm.^{2}. *B*, Time course of computed conductance changes on a logarithmic scale. G_K, potassium conductance; G_{Na}, sodium conductance. The potassium and sodium equilibrium potentials were set at −100 mV. and + 40 mV. respectively

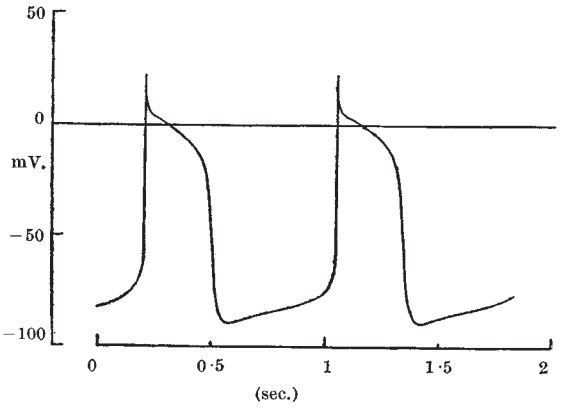

Fig. 2. Solution to equations in which the membrane potential is unstable in diastole so that pacemaker activity occurs. In this case, the potential at which the steady state sodium and potassium currents are equal and opposite is −38 mV. and an unstable state at this potential corresponds to a second solution to the equations

The equations were set up on a digital computer and were integrated by a numerical approximation using a step length of 0·05 m.sec. during the initial spike of the action potential and 0·3 m.sec. during the slower phases. In this connexion I should like to thank Dr. R. A. Buckingham, director of the University of London Computer Unit, for permission to use *Mercury* for this purpose, and Dr. M. J. M. Bernal and other members of the Computer Unit staff for advice on programming.

It was found that computed responses resembling action potentials of different durations could be obtained by inserting appropriate values for the constants in the equations for m (the variable describing the activation of sodium carriers). One of the solutions is shown in Fig. 1. It can be seen that the general shape of the action potential (A) corresponds very closely to that observed experimentally in Purkinje fibres[4]. The time-course of the computed conductance changes is shown in Fig. 1*B*. After an initial 'spike', the sodium conductance (G_{Na}) settles down to a value during the plateau which is about 8 times its resting value. G_K by contrast is below its resting value throughout the duration of the plateau.

The total membrane conductance ($G_K + G_{Na}$) rises during the plateau, but the slope conductance (determined by adding current to the equations at different times during the integration) was found to decrease. This is the result of a decrease in the sodium slope conductance which becomes negative towards the end of the plateau. Although the slope conductance during the plateau falls to a value which is equal to the resting conductance, the decrease was not so great as that observed experimentally[4]. This may be the result of a defect in my equations which over-estimate the potassium current produced by large depolarizations of the membrane.

If a large enough repolarizing current is added to the equations during the plateau, an all-or-nothing repolarization is initiated. The threshold for this phenomenon is about −30 mV. at the middle of the plateau, which is in fair agreement with that found experimentally by Weidmann[4].

In these equations there is always at least one potential at which the steady state sodium and potassium currents are equal and opposite. In the computations just described, this forms the resting potential and the system is stable unless excited. A

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small change in the constants in the equations for m is sufficient to make the system unstable in diastole, and pacemaker activity then occurs. Such a solution is shown in Fig. 2. However, in order to bring about repolarization in this case a larger delayed potassium conductance had to be assumed. It is not yet known whether this is a necessary feature of the equations and further computations are being made to test this point. Nevertheless, the sensitivity of the computed pacemaker potential to changes in ionic conductance has been shown to correspond quite well with the experimental information available[6].

D. Noble

Department of Physiology,
University College,
London.

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Relative Vascularity of Certain Anatomical Areas of the Brain and Other Organs of the Rat

It need scarcely be pointed out that for the meaningful interpretation of the results of work where an exogenous material is administered to an animal and the assay performed on dissected areas of tissues with their content of blood, as is usually the case, it is frequently necessary to know the contribution of blood to the respective areas. This is particularly true if the concentration of a given substance is much greater in the blood than in the tissues.

Several approaches were made by different investigators[1–5] to determine the vascularity of the central nervous system of the cat, but so far as we know, nothing similar has been reported for the rat since the earlier work of Craigie[6]. He used the method of direct capillary counts of specimens from animals injected with carmin gelatin to determine the relative vascularity, but the limitations of this method are obvious. Furthermore, it has not included all the anatomical areas of interest to us. In 1955 Oeff and König[7] reported the blood content of several organs of the rat including whole brain in the non-bled out, bled out, and perfused animals using erythrocytes labelled with phosphorus-32. They did not, however, determine the blood content of the various anatomical areas of brain. In their work on the cat, Barlow *et al.*[1] have approached this problem using human serum albumin labelled with iodine-131 and demonstrated the relative vascularity by means of densitometric measurements of the autoradiograms.

Their technique consisted of killing the animal by intracardiac injection of a saturated solution of potassium chloride, dissecting out the brain, hardening it on a bed of dry ice followed by sectioning. The whole operation was performed at room temperature (29°). They observed that "tissue radioassay data of an anatomical structure within a single animal and of the same structure from animal to animal showed wide variation and were, therefore, useless for quantitative purposes. It is clear that dissection-sampling technique inadvertently included varying amounts of large blood vessels containing highly concentrated radioactivity giving rise to gross inconsistencies". However, by selecting areas on the autoradiogram corresponding to tissue areas containing blood within only small blood vessels and capillaries and avoiding large blood vessels, they were able to obtain values in good agreement with those found by others using capillary counting techniques. The problem referred to by Barlow *et al.* is a general one and arises also in distribution and metabolism work where such techniques are utilized.

It seems that the major factors which contribute to the inconsistencies in similar experiments are: (1) Varying amounts of residual blood that is retained in the brain tissue as a result of the varying loss which is inevitable in the process of dissection. (2) Contamination from blood which is also unavoidable. It is not difficult to see that in this case, where there is a tremendous concentration gradient from blood to the adjoining tissue, how adversely this situation can affect the results. (3) Since the operation is performed at room temperature, there is some condensation of moisture on the cold surface of brain slices which may lead to spreading, leaching, and further contamination of areas. The influence of these factors is greater when one is dealing with the very small areas of brain. In the present work, we have avoided these difficulties by using the following technique.

White male rats, weighing 250–300 gm., were injected by the tail vein with 10 μc./100 gm. of human serum albumin labelled with iodine-131 (*RISA*, Abbott). Pentobarbital sodium (4 mgm./100 gm.) was also administered along with the albumin to facilitate withdrawal of blood by intracardiac puncture, minimizing at the same time influences of stress on the animal. At the end of 15 min., 1 ml. of blood was withdrawn from the heart, and the animal was killed by rapid immersion in acetone–carbon dioxide mixture (−78°). It was then transferred to a refrigerated room (−10°), allowed to remain for 1 hr., and all subsequent operations were carried out there. The brain was chiselled out of the frozen animal with a 6 mm. wide chisel and after removal of the meninges and the larger blood vessels on the surface, 2–3 mm. thick serial coronal sections were obtained by free-hand sectioning with a razor blade. The areas dissected from the central nervous system were: cerebral cortex, cerebral white matter, caudate nucleus, thalamus, hypothalamus, hippocampus, inferior colliculi, cerebellar cortex, medulla and spinal cord. Similarly, sciatic nerve, kidney cortex, kidney medulla, periphery of the left lateral lobe of liver, skeletal muscle, and adrenals were also dissected. Of these, the cerebral white matter and the cerebellar cortex were the most difficult to dissect with any degree of assurance. It is to be noted that during the whole operation, in view of the fact that it is performed in the refrigerated room on the frozen animal, there was neither loss of blood nor contamination by blood; nor was there any spreading of activity from the blood as a result of condensing moisture. The areas dissected were homogenized in 1·5 ml. of water, 1 ml. of the homogenate transferred to weighed glass tubes and the radioactivity determined in a well-type scintillation counter. The tubes containing homogenate were then dried to constant weight in a desiccator. Table 1 shows the results from 15 animals. The results are expressed as a ratio of the specific activity of the area over the activity of a microlitre of hæmolysed blood of the same animal taken at the time of killing, times 10^2. An absolute determination of the vascularity was not attempted in this investigation, but it merely sought to establish a set

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