Recent Advances in Physiology

Edited by R. J. Linden

NUMBER NINE

Recent Advances in Physiology

Edited by R. J. Linden

NUMBER NINE

Churchill Livingstone Edinburgh and London 1974

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by Longman Inc., New York, and by associated companies, branches and representatives throughout the world.

First edition 1925
Second edition 1926
Third edition 1928
Fourth edition 1930
Fifth edition 1936
Sixth edition 1939
Seventh edition 1949
Eighth edition 1963
Ninth edition 1974

ISBN 0 443 01006 4

Library of Congress Catalog Card Number 73–88901

Printed in Great Britain by
Cox & Wyman Ltd., London, Fakenham and Reading

Preface

Physiology, being defined as the science of life, involves a field of study which extends from the interstices of the cell to the function of the whole animal and its reactions to the environment. To cover the whole field of recent advances in such a large subject is not possible; it has therefore been necessary to select some topics which illustrate the advances in certain aspects of physiology. Such a selection of topics has necessitated the retention of the format of the previous volume of *Recent Advances in Physiology* in which a number of authors each subscribed a chapter, an arrangement which allows a more detailed and exciting view of the growing points in physiology.

Each author is an authority on his chosen topic, has an enthusiasm for his subject made obvious by his researches and is experienced in the teaching of undergraduate science honours and medical students. The aim of each author was to write a personal account of his topic, not necessarily a comprehensive account, expressing a point of view based on evidence, and of interest and use not only to undergraduate science honours and interested medical students but to post-graduate students of physiology. Clinicians and teachers of physiology not working primarily in the fields of these topics should find these accounts a suitable starting point for a fuller understanding of the topic. Each author was asked *not* to write a review for the specialist research worker in his field and not to worry about the expert looking over his shoulder. It was hoped that each chapter would help 'students' of physiology to make the transition from a general knowledge of the topic to an understanding necessary for the appreciation of evidence contained in monographs and original papers.

In these respects I can claim, having acted mainly as a mild administrator, that the authors have indeed succeeded in their task. I would like to thank the authors for contributing these chapters even in the midst of their other tasks of research and teaching.

Leeds, 1973. R.J.L.

Contributors

P. F. Baker M.A., Ph.D.
Physiological Laboratory, Cambridge.

E. L. Blair M.D., F.R.C.P.E.
Professor of Physiology, University of Newcastle upon Tyne.

R. S. Comline M.A., Ph.D., B.Sc., M.R.C.V.S.
Physiological Laboratory, Cambridge.

R. H. Fox M.B., B.S., M.R.C.S., M.R.C.P., Ph.D.
Division of Human Physiology, National Institute for Medical Research London.

B. R. Jewell B.Sc., Ph.D., M.B., B.S.
Department of Physiology, University College, London.

R. J. Linden M.B,. B.Ch., M.R.C.P., Ph.D., D.Sc.
Cardiovascular Unit, Department of Physiology, University of Leeds.

F. S. Nashat M.D., Ph.D.
Reader in Physiology, Middlesex Hospital Medical School, London.

D. Noble Ph.D.
University Laboratory of Physiology, Oxford.

Marian Silver M.A., Ph.D.
Physiological Laboratory, Cambridge.

R. M. Simmons B.Sc., M.Sc., Ph.D.
Department of Physiology, University College, London.

H. M. Snow B.Sc., Ph.D.
Cardiovascular Unit, Department of Physiology, University of Leeds.

J. G. Widdicombe M.A., D.M., D.Phil.
Professor of Physiology, St George's Hospital Medical School, London.

Contents

1 D. Noble

Cardiac Action Potentials and Pacemaker Activity

INTRODUCTION

The electrical activity of cardiac muscle largely controls its mechanical activity. The rate of beating is controlled by the action potential frequency, in turn determined by the pacemaker potential. Although the strength of the beat is not solely controlled by the action potential, changes in the duration and magnitude of depolarization nevertheless result in corresponding changes in the strength and duration of contraction (see e.g. Morad and Trautwein, 1968). Thus the relation between the intrinsic electrical and mechanical mechanisms is more elaborate in cardiac muscle than it is in skeletal muscle, where the initiation of electrical activity lies entirely with the nervous system and in which the action potential acts solely as a trigger for the mechanical event.

It is not surprising, therefore, that electrical activity in heart muscle is subject to a wider variety of chemical controls and that the electrical mechanisms should be more complex than in nerve or skeletal muscle.

The responsiveness of cardiac muscle to chemical agents has been known for a long time and it is partly for this reason that cardiac muscle has been popular in physiological and pharmacological studies of drug actions. However, the relative complexity of the underlying electrical mechanisms has been established only in the last eight years, following the application of voltage clamp techniques to cardiac muscle. This statement may appear surprising in view of the fact that apparently large differences between electrical activity in, say, nerve and cardiac muscle have been known for a long time. As shown in Figure 1.1 the action potentials in cardiac muscle last hundreds of times longer than those in nerve fibres and are often followed by the spontaneous depolarization known as the pacemaker potential. They are also quite varied in shape and duration in different parts of the heart. Nevertheless, theoretical studies made

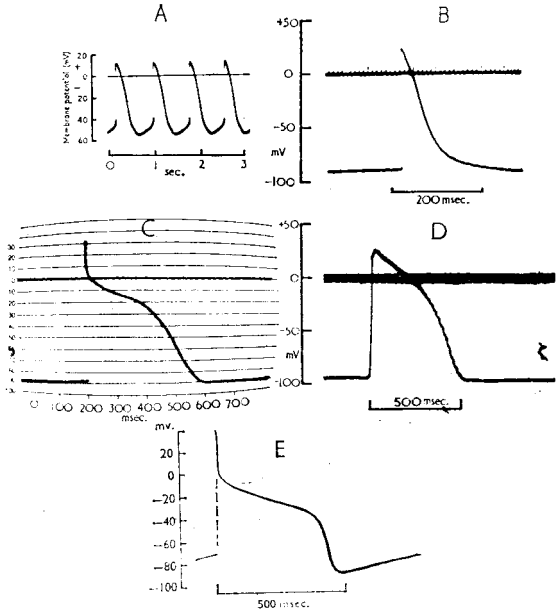

Fig. 1.1. Action potentials and pacemaker activity recorded in different parts of the heart. The natural pacemaker, the SA node (A), is spontaneously active and the membrane potential never becomes more negative than about −60 mV. Each action potential is followed by a slow spontaneous depolarization known as the pacemaker potential. The atrium (B) has a higher resting potential (which may be up to −80 mV), and a triangular-shaped action potential. It is usually quiescent, although pacemaker activity can be induced by steady depolarizing currents (see Fig. 1.23). Purkinje fibres are sometimes quiescent (C) and sometimes show pacemaker activity (E). This pacemaker activity differs from that in sino-atrial tissue in that the pacemaker potential occurs at very negative potentials (−90 to −70 mV). The action potential characteristically shows two phases of fast repolarization separated by a very slow phase known as the plateau. The ventricular fibres (D) have a much higher plateau and show no pacemaker activity.

A. Recorded from frog sinus venosus (Hutter and Trautwein, 1956).
B. Recorded from dog atrium by Hoffman and Suckling (from Weidmann, 1956).
C. Recorded from dog Purkinje fibre by Draper and Weidmann (1951). (photograph from Folkow and Neil, 1971).
D. Recorded from frog ventricle by Hoffman (from Weidmann, 1956).
E. Recorded from sheep Purkinje fibre (Weidmann, 1957).

about twelve years ago (FitzHugh, 1960; Brady and Woodbury, 1960; Noble, 1960, 1962a) revealed that these effects could be produced by making relatively simple modifications of the nerve impulse theory given by Hodgkin and Huxley (1952). This theory showed that the depolarization phase of the action potential in nerve is generated by a rapidly activated sodium conductance and that repolarization occurs as the sodium conductance is inactivated and a slower potassium conductance appears.

Figure 1.2 shows one of the modifications of Hodgkin and Huxley's theory designed to reproduce electrical activity in the heart. As in the Hodgkin–Huxley theory, it is assumed that depolarization activates, and then largely inactivates, a sodium conductance, which is followed (albeit much more slowly than in nerve) by activation of a potassium conductance. The only additional component of current is a background (or non-time-dependent) current (see Background Currents below) which reproduces an important property of cardiac muscle: the *immediate* effect of membrane depolarization is to *reduce* the potassium permeability (Hutter and Noble, 1960; Carmeliet, 1961; Hall, Hutter and Noble, 1963; Deck and Trautwein, 1964; Noble, 1965). The potassium conductance showing this property (sometimes known as inward-going rectification) was called g_{K_1}, while the normal delayed potassium conductance modified from the Hodgkin–Huxley theory was called g_{K_2}.

The calculation shown in Figure 1.2 successfully reproduces the action potential and pacemaker potential of cardiac Purkinje fibres. The conductance curves below the computed potentials show how this is achieved. The sodium conductance is largely, but not quite completely, inactivated following the large initial conductance that generates the fast depolarization phase of the action potential. A residual sodium conductance (in this case only about 2 per cent of the peak conductance), assisted by the decrease in K conductance attributable to inward-going rectification, then maintains the depolarization for a long period of time. This phase is often called the plateau phase and it is eventually terminated by the slow increase in conductance, g_{K_2}. The decay of this conductance following repolarization is then responsible for initiating the pacemaker potential by allowing the resting sodium conductance to depolarize the membrane towards threshold.

Although we now know that the mechanism of electrical activity in the heart is more complex than this, it will be convenient in the remainder of this chapter to fit the various components of current identified in voltage clamp experiments into a general scheme by explaining how they serve one or other of the functions served by the conductance changes shown in Figure 1.2. It will therefore be worth while summarizing the essential features of these conductance changes and how they might be modified.

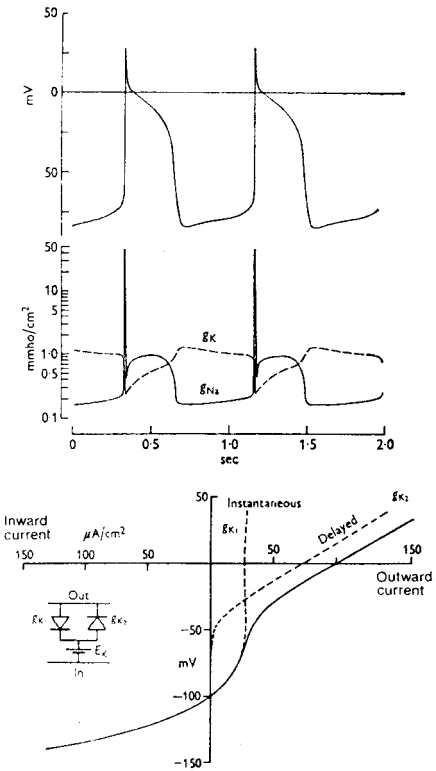

Fig. 1.2. A model of cardiac action potentials and pacemaker activity based on a simple modification of the Hodgkin–Huxley (1952) nerve equations (Noble, 1962a). The sodium current was reproduced using the nerve g_{Na} equations which were slightly modified by allowing sodium activation (m) to be less steeply voltage dependent (see Noble, 1962a, Fig. 4). The potassium conductance was divided into two components: g_{K_1} is dependent on voltage only and becomes small when the membrane is depolarized; g_{K_2} is described by the Hodgkin–Huxley potassium equations using greatly reduced rate constants for activation and decay. These properties were based on measurements of the potassium conductance by Hall, Hutter and Noble (1963).

The top record shows the computed potential changes which closely resemble experimental records (see Fig. 1.1E). Changes in g_{Na} and g_K are shown below. The way in which the total K current was divided into two components is shown in bottom diagram. This model is now known to be too simple to describe all the observed conductance changes. In the rest of this chapter it will be referred to as the 1962 model.

1. The initial transient conductance increase must be large enough to account for the maximum rate of depolarization observed. Despite the long-lasting nature of the cardiac action potential, the initial rate of depolarization is frequently as large as in nerve or skeletal muscle (of the order of 500 mV/msec). The magnitude of the conductance required to produce this rate of depolarization depends also on the amount of membrane capacitance to be discharged. As we shall see later, the magnitude of the initial sodium conductance and the amount of capacitance discharged are still controversial matters (see Sodium Current and the Problem of Capacitance).

2. The residual inward conductance must be large enough to maintain the depolarization. However, it is not essential that this conductance should be controlled by the same mechanism as that responsible for the initial depolarization. In fact, it is now known that a substantial, if not the major, part of the inward conductance during the plateau is attributable to a second inward current mechanism and that calcium ions carry at least part of this inward current (see Calcium Current and Excitation-contraction Coupling).

3. The initial fall in K conductance might be restricted to g_{K_1}, as assumed in Figure 1.2, but this is not an essential feature. Time-dependent conductance mechanisms of the Hodgkin–Huxley type might also respond to *sudden* depolarization with a *reduction* in permeability despite the fact that they subsequently show a slow increase in permeability. We shall see later that the potassium conductance, g_{K_2}, involved in pacemaker activity in Purkinje fibres shows this rather complex behaviour (see Potassium Current and Pacemaker Activity in Purkinje Fibres).

4. Termination of the action potential may be attributed to a slow increase in K conductance as in Figure 1.2 but could equally well be attributed to a slow *decrease* in the inward current responsible for maintaining the plateau phase. Both processes seem, in fact, to contribute in most cardiac preparations studied so far (see Calcium Current and Outward Plateau Currents).

5. The decay of K conductance responsible for pacemaker activity need not involve the same mechanism as that responsible for terminating the action potential. We shall see that, in Purkinje fibres, different K conductance mechanisms are involved.

6. Finally, it is worth emphasizing that there are large variations in the electrical behaviour of different parts of the heart and these may be reflected in the corresponding conductance mechanisms. Thus the mechanism of pacemaker activity in sinus and atrial muscle may not be identical with that in Purkinje fibres (see Pacemaker Activity in Atrial Muscle).

VOLTAGE-CLAMP TECHNIQUES

Before describing the current components identified in recent work, it may be helpful to discuss how voltage-clamp techniques have been applied to cardiac muscle. The object of a voltage-clamp experiment is to control the membrane potential by keeping it uniform over an area of membrane through which the recorded current flows. Hodgkin, Huxley and Katz (1952), following Cole (1949) and Marmont (1949), achieved this in squid nerve by inserting a wire electrode along the axoplasm. The axoplasm resistivity was thus shorted and the membrane of the nerve could be polarized uniformly. This technique is not applicable to cardiac muscle fibres, which are far too small to allow wire electrodes to be inserted. Uniformity of current flow must therefore be achieved in other ways. Two basic techniques have been developed: microelectrode techniques and sucrose gap techniques. A third technique is a hybrid one using a combination of microelectrode recording and sucrose gaps.

Two Microelectrode Techniques

Deck, Kern and Trautwein (1964) were the first to make use of the fact that cut or otherwise damaged cardiac muscle 'heals over', the result being a high resistance seal in place of the damage or cut (see review by Weidmann, 1967; Délèze, 1970). By using this property, it is possible to isolate a small segment (e.g. 1 mm) of a Purkinje fibre and to insert two glass microelectrodes. One is used for recording the membrane potential, the other for passing current through the membrane. The resting space constant λ in Purkinje fibres is about 2 mm (Weidmann, 1952) so that, by placing the electrodes in the centre of the segment, the end of the fibre may be only 0·25 λ away from the electrode. Even in a segment as long as 2 mm, the end is only 0·5 mm away. Under these conditions, the non-uniformity of voltage produced by passing current at the centre of the fibre may be restricted to within 5 to 10 per cent (see Jack, Noble and Tsien, 1973, chapter 4, for a theoretical treatment of this kind of problem). Of course this estimate applies strictly only when the space constant remains as long as 2 mm. Since the space constant is given by $\sqrt{(r_m/r_a)}$, where r_m is the membrane resistance per unit length of fibre and r_a is the axial resistance per unit length, the essential requirement is that r_m should remain large. In studies of the potassium conductance, this is usually the case, since, as already noted in the Introduction, the initial effect of depolarization is to greatly reduce the potassium conductance so that the effective space constant becomes much larger than 2 mm. Provided that the subsequent recovery of conductance is not too great, one may expect the uniformity of polarization to be effectively maintained. Deck, Kern and

Trautwein (1964) checked this point experimentally by inserting a third microelectrode so that they could measure the membrane potential at two different points. Very little difference in potential was observed (see Deck *et al.*, 1964, Fig. 6).

It should be emphasized, however, that the situation is not as encouraging as this when the sodium currents are investigated. As already noted in the Introduction, the sodium conductance increase must be very large and the system may then be expected to be non-uniform (see Sodium Current and the Problem of Capacitance below).

In addition to ensuring that the membrane potential is uniform in the region being studied, the voltage-clamp technique also requires that the potential should be controlled, usually by being made to follow a step-waveform, although other waveforms, such as ramps, have also been used. The introduction of circuitry to achieve this is, in its details, complex and the details vary greatly between one laboratory and another. However, the principle involved is very simple and is summarized in Figure 1.3.

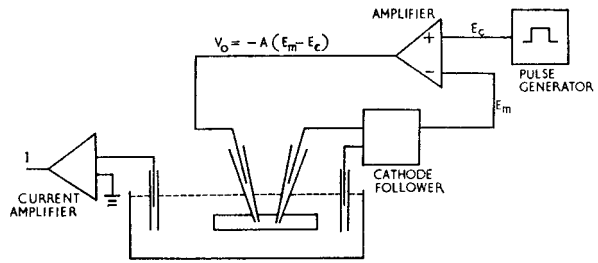

Fig. 1.3. General arrangement of circuit for voltage-clamp work. The membrane potential, E_m, of a short Purkinje fibre is recorded with a microelectrode and, after passing through a cathode follower to prevent significant current being drawn from the fibre, is fed into the negative input of an amplifier, that amplifies the signal by a factor $-A$. The voltage clamp signal, E_c, is fed into the positive input. The output, $-A(E_m - E_c)$, is applied to interior of fibre via a second microelectrode. Current flowing through membrane into bath is collected by an electrode connected to a current amplifier.

The membrane potential, E_m, is used as one input to the summing junction of an amplifier, the other input being the voltage, E_c, produced by a pulse generator. Currents proportional to E_m and $-E_c$ are summed algebraically by the amplifier and then amplified by a factor $-A$ to produce an output proportional to $-A(E_m - E_c)$. The circuit ensures that current flows through the preparation whenever E_m differs from E_c and that the direction of current flow is such as to reduce the difference between E_m and E_c. If the amplification

factor A is sufficiently large, the effect is that E_m is forced to closely follow E_c. In theory, when A is infinitely large E_m equals E_c. In practice, however, there are technical limits on the magnitude of A. When microelectrodes are used these arise from limitations on the amount of current that may flow through the large resistance provided by the current electrode and from the fact that capacitive coupling between the electrodes can lead to unstable oscillatory behaviour at large values of A. Nevertheless, it is usually possible to keep E_m constant to within 2 to 3 mV.

Double Sucrose Gap

Rougier, Vassort and Stämpfli (1968) introduced a technique based on the high resistance gap methods originally used for nerve by Huxley and Stämpfli (1951), Frankenhaeuser (1957) and Julian, Moore and Goldman (1962). The general principle is that the external resistance between two or more regions of the preparation is greatly increased by replacing the normal bathing solution by air, oil, or a sucrose solution. The high resistance 'gap' so created may then be used either to record the potential difference between the areas of preparation on either side of the gap or to force current to flow along the much lower resistance of the interior of the preparation and so apply current to the intracellular fluid.

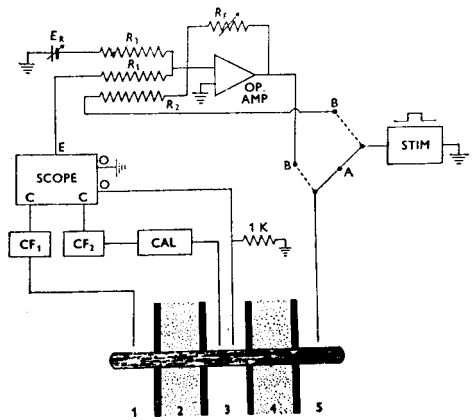

Fig. 1.4. Schematic diagram of bath and circuit used in voltage-clamp experiments on atrial muscle. Chambers 2 and 4 are perfused with sucrose. Chamber 1 is used to record the potential changes with respect to the test chamber 3. Chamber 5 is used to apply currents. When chamber 1 is exposed to isotonic K^+ solution, a potential equal to the resting potential is recorded (see Fig. 1.5). The electronic circuitry is similar to that shown in Figure 3 except that the membrane potential is first amplified using an oscilloscope (SCOPE) before being fed into the voltage clamp amplifier (OP.AMP). (Brown and Noble, 1969a.)

As applied to heart muscle (see Rougier *et al.*, 1968; Brown and Noble, 1969a) the method uses ion-deficient sucrose solutions to create high external resistances. A fine strip of tissue, typically 100 μ or less in diameter and a few millimetres in length, is dissected from the atrium and placed so that it runs across five chambers (see Fig. 1.4). The length of the preparation in the middle chamber is kept very small (e.g. 200 μ) and is exposed to physiological solutions. The chambers on either side are filled with non-conducting sucrose solution. The end chambers are filled with a physiological solution which may be K-rich solution to depolarize the ends of the preparation. The potential difference between one of the end chambers and the middle chamber then gives a measure of the membrane potential in the centre chamber. Current may be made to flow through the membrane of the centre region by applying voltages to the other end chamber. The success of the voltage recording and the current application both depend on the resistance of the interior of the preparation being negligible compared to the external resistance of the sucrose

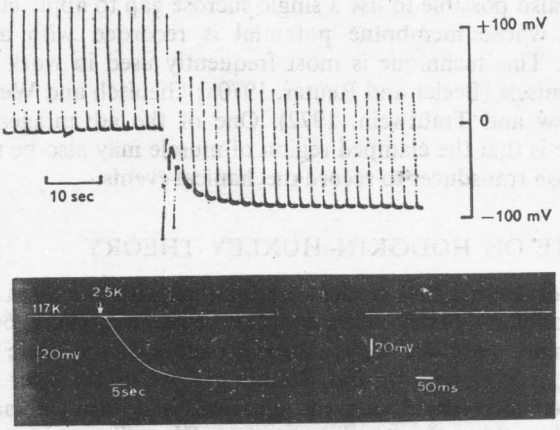

Fig. 1.5. Measurement of resting and action potentials of atrial muscle using sucrose gap technique. Top record shows development of 'resting potential' during application of isotonic K solution to chamber 1 while chamber 3 is perfused with Na Ringer. Tracing shows pen recordings of action potentials in response to repetitive stimuli applied via chamber 5. Note presence of two inverted action potentials arising in chamber 1 during the application of K solution. The resting potential in this case is −80 mV and the action potential overshoot is about +30 mV (Brown and Noble, 1969a). Bottom record shows an alternative method. The control chamber (3) is first depolarized in isotonic K solution and the recovery of potential is then observed following return to normal Ringer (*left*). The action potential (*right*) is similar to that recorded using intracellular electrodes (see Fig. 1.1B) (Ojeda, 1971).

gap. This may be tested by recording the action potentials and resting potentials using the sucrose gap technique and comparing them with those recorded using intracellular microelectrodes. Figure 1.5 shows such a test. The resting potential (— 80 mV) and action potential (120 mV) are as large as those recorded with intracellular microelectrodes. Virtually all the voltage drop must therefore occur in the external sucrose gap across which the voltage is recorded, while negligibly small voltage drops occur within the preparation. Since an identical gap is also used for applying current, this also ensures that very little of the current applied at one of the ends flows through the external resistance of the gap. Most of it must flow through the interior of the preparation and then across the membrane in the central 'test' gap.

Voltage clamp conditions are achieved using circuits of the kind already described in Figure 1.3. The two microelectrodes are simply replaced by metal electrodes lying in the end chambers.

Hybrid Techniques

It is also possible to use a single sucrose gap to apply current to a region whose membrane potential is recorded with a microelectrode. This technique is most frequently used in work on ventricular muscle (Beeler and Reuter, 1970a; Giebisch and Weidmann, 1971; New and Trautwein, 1972). One of the advantages of this technique is that the clamped region of muscle may also be attached to a tension transducer to record mechanical events.

NOTE ON HODGKIN–HUXLEY THEORY

I shall assume that readers are familiar with the main features of the Hodgkin–Huxley nerve equations (see Hodgkin, 1964; Katz, 1966; Noble, 1966, for explanatory accounts of the basic theory). However, it may help in understanding some of the later parts of this chapter to briefly discuss the equations in a form similar to that used in describing the cardiac currents. We will consider a single current component. Each channel conducting the current is assumed to be in one of two states: (*a*) conducting, or (*b*) non-conducting. It is assumed that these states are connected via a first order reaction with an activation rate coefficient α and decay rate coefficient β. These coefficients describe the probabilities that a charged structure (sometimes called the gate) may move into or from a blocking position or configuration. As the electric field is raised in one direction (depolarization) the α rate coefficient is increased while the β rate coefficient is decreased, i.e. in this direction the field favours movement away from the blocking configuration so that the channel becomes conducting. An opposite voltage change (hyperpolarization) increases β and decreases α. The simplest possibility is that the rates

should be exponential functions of voltage. This arises when the reaction has a single activation energy (see Tsien and Noble, 1969), but this result is rarely obtained experimentally. The rate coefficients are usually more complex functions of voltage, e.g. they become linear functions or even show saturation (i.e. reach a limit) at extremes of potential. For simplicity, Figure 1.6 assumes that the rates are exponential functions of voltage.

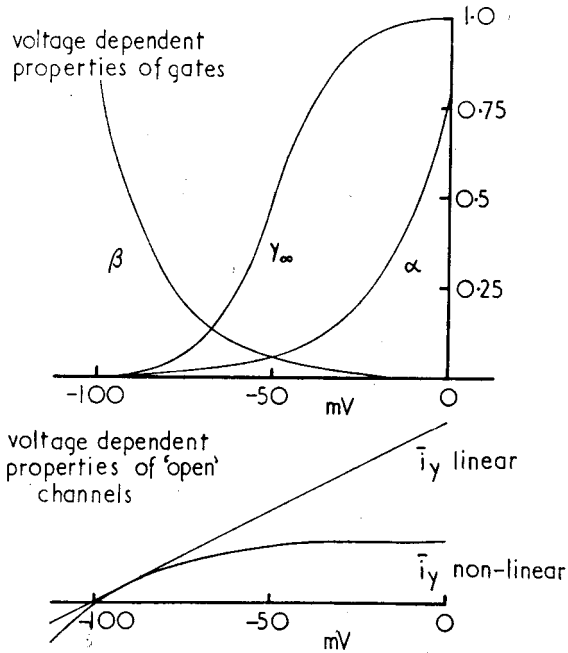

Fig. 1.6. Parameters used in Hodgkin–Huxley theory to describe the time and voltage dependence of an ionic current.
Top: The steady state fraction, y_∞, of open channels is a sigmoid function of membrane potential. The speed of activation is described by a rate coefficient, α, and decay is described by a rate coefficient β.
Bottom: To calculate the current carried we must also know the instantaneous current-voltage relation, $\bar{i}_y$. This may be linear, as in squid nerve, but is more usually non-linear (cf. Fig. 1.18).

If the fraction of channels in the conducting state is y, then that in the non-conducting state will be $1 - y$. The rate of change of y will be given by the difference between the activation and decay rates:

$$\frac{dy}{dt} = \alpha(1 - y) - \beta y \qquad (1)$$