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ION MOVEMENTS IN JUNCTIONAL TRANSMISSION

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TABLE OF CONTENTS

I. INTRODUCTION

The action of many excitatory and inhibitory substances may be explained by supposing that they open additional channels or pathways through the membrane for one or several of the environmental ions. For excitatory substances, these channels are available to Na and at least one other ion species; for inhibitory substances, they are available to K or Cl ions, or both.

In a few situations it has been possible to demonstrate that such channels are opened by transmitter substances by following the movements of radioactive ions. For the most part, however, the evidence comes from studies of the way in which prejunctional nerve stimulation affects the electrical properties of cells; it has therefore been found convenient to account for the ion movements in electrical terms. To those unaccustomed to thinking in such terms, equivalent circuits may at first seem somewhat forbidding; but all that is required is a knowledge of Ohm's law.

A valuable account of many aspects of junctional transmission has recently been given by Katz (83a).

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II. THE ACTION OF TRANSMITTERS THAT INITIATE EXCITATION

A. Equivalent electrical circuit

The use of an electrical model was first described by Fatt and Katz (50) in 1951 to explain the action of acetylcholine, released by nerve stimulation, on the end-plate membrane. A similar model has since been used to account for the action of excitatory transmitters on a variety of chemosensitive membranes (table 1, section II C). With an appropriate modification, it applies also to inhibition (section III), and the same methods have been used to investigate its validity in both cases. It is convenient, however, to restrict the discussion at this stage to excitation.

The essential features are illustrated in figure 1b. The right hand element (E, R) represents the ordinary ion pathways through the membrane of a single cell, in the absence of transmitter action; the left hand element (ϵ, r) represents the pathways that are opened by the action of the transmitter or similar substance. Its effect is thus mimicked by closing the switch, S. If ϵ is less than E, a current will then flow in the direction shown, reducing the potential difference between the terminals.

The value of the current, I is clearly given by $I = (E - \epsilon)/(R + r)$. The reduction in potential difference, e, is therefore given by $e = R(E - \epsilon)/(R + r)$.

The current I corresponds to the flow of positive ions outwards through the ordinary channels (and negative ions inwards, or both) and the equal net flow of positive ions inwards through the channels opened by the transmitter. The reduction in potential difference between the terminals, e, corresponds to depolarization of the cell. This depolarization if sufficient, will cause an action potential to be generated.

Thus a typical frog muscle fibre at rest might be simply represented (*cf.* 51) by a battery corresponding to the resting potential, of 90 mV, in series with a

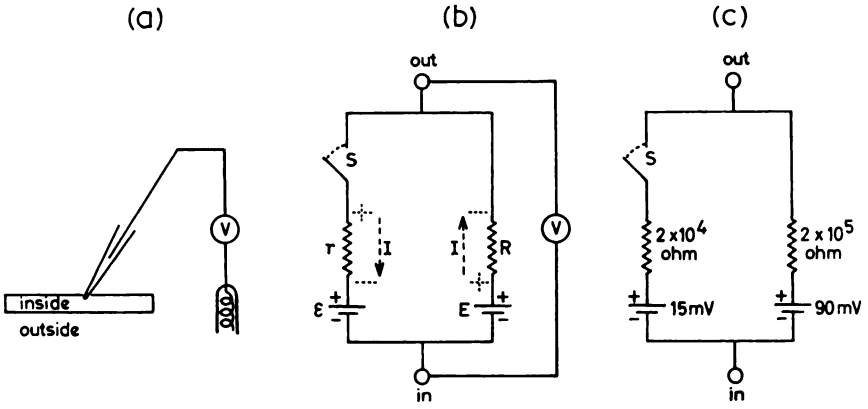

FIG. 1. (a) Intracellular electrode inserted at junctional region of cell: V, potential difference measured between inside and outside. (b) Simplified equivalent circuit: current, I, flows only when S is shut. (c) Equivalent circuit for transmitter action at the end-plate (*cf.* 29, 51).

resistance of 2×10^5 ohm (fig. 1c): the channels through the end-plate membrane may be represented by a battery of 15 mV in series with a resistance of 2×10^4 ohm. When, therefore, the end-plate is activated, a current of

$$\frac{(90 - 15)\text{ mV}}{(2 \times 10^5 + 2 \times 10^4)\text{ ohm}} = 3.4 \times 10^{-7}\text{ amp}$$

flows inwards through the transmitter-induced channels and outwards through the rest of the membrane. Its effect would be to depolarize the muscle fibre by (3.4×10^{-7}) amp $\times$ (2×10^5) ohm = 68 mV at the end-plate, if an action potential were not generated before this depolarization was attained.

According to the model of figure 1, the voltage, of course, changes to its new value immediately the switch is closed and reverts to its initial value immediately the switch is opened. This simplified model will suffice for the present discussion, which is not concerned with the time course of transmitter action. However, in practice the capacity of the membrane slows the rise and fall of the changes in membrane potential. With their more realistic model, which included the capacity and allowed for the cable properties of the muscle cell, Fatt and Katz (50) were able to account for the amplitude and time course of the end-plate potential at different points along the fibre [*cf.* Falk and Fatt, (48, P. 104): for similar models appropriate to other situations see (21, 52, 104)].

In general the ion current that flows inwards through the activated regions of the membrane and outwards through the ordinary channels will, of course, give rise to an exchange of one intracellular ion species for another. This exchange is generally too small to have any significant effect on the intracellular concentrations, unless a cell is exposed to a transmitter substance for a prolonged period.

The equilibrium potential for the action of the transmitter. The values that must be ascribed to the components of the equivalent circuit of figure 1 may be looked on in two somewhat different ways. Clearly, at the more fundamental level, they reflect the permeabilities of the membrane to the various ions and their environmental concentrations (section II C); but they may also be thought of in terms of the way they are measured. Thus E evidently corresponds to the resting potential, or in other words to the potential difference across the membrane in the absence of any net current, and R the resistance between the inside and outside of the cell, is given by the ratio of the displacement of the membrane potential caused by a current to the value of the current. If, during transmitter action, the membrane could be sealed, except for the channels opened by the transmitter, ϵ would then be observed as the potential difference across these channels in the absence of net current through them. This direct form of experiment is clearly impossible; but if the potential difference between the "out" and "in" terminals in figure 1b were set to ϵ with the switch open, then when it was shut, no current would flow through r, and there would be no further change in potential difference. Therefore, ϵ may be defined as the potential difference across the cell membrane that is unaffected by the action of the transmitter. For this reason ϵ is usually referred to as the "equilibrium potential" for the action of the transmitter.

If the argument is carried a stage further, it may be seen that the model of

figure 1 predicts that by displacing the membrane potential, it should be possible not only to abolish the potential change caused by the transmitter, but also to reverse it. That this can be done in practice provides the evidence for the correspondence between the model and transmitter action, and, by implication, for the idea that the transmitter acts by opening additional channels through the membrane, or in other words by increasing its permeability.

It will be recalled that the usual convention is that membrane potentials are referred to in terms of the inside with respect to the outside potential. Batteries with the orientation shown in figure 1, therefore have *negative* numerical values. Since this convention is not always applied consistently, a depolarization from −90 mV to −70 mV, say, is often described as a reduction in membrane potential.

B. Evidence for the model: methods for the determination of transmitter equilibrium potentials

1. INTERACTION METHOD. An ingenious method was described by Castillo and Katz (28) in 1954 for investigating the effect of the transmitter on the end-plate membrane of frog muscle fibres. It has since been applied to frog sympathetic ganglion cells (14) and in a study of the action of procaine on the end-plate (96).

A microelectrode was inserted into a muscle fibre at the end-plate and an action potential generated in the fibre by direct stimulation (*i.e.*, not *via* the nerve). By stimulation of the motor nerve at varying times in relation to the direct stimuli, the transmitter was applied to the end-plate at different stages of the action potential (see fig. 2). It was found that the effect of the transmitter was abolished when the membrane potential was at about −15 mV.

The approximate equivalent circuit is shown in figure 2. When S is shut at such a time that the membrane potential is between −90 mV and −15 mV, current flows inwards through the left hand element (corresponding to the channels opened in the chemosensitive membrane) and outwards through the ordinary channels, increasing the depolarization. When the membrane potential is less negative than −15 mV, current flows in the opposite direction, causing a relative increase in internal negativity.

2. CONSTANT CURRENT METHOD. The most widely used method for investigating the validity of the model and determining the transmitter equilibrium potential was introduced by Fatt and Katz (50) in 1951. The membrane potential was recorded with one intracellular electrode and a second was used for passing different steady currents to displace the membrane potential. Both electrodes were inserted at the end-plate. It was found that the amplitude of the end-plate potential, e say, was increased when the fibre was hyperpolarized; moreover, the relationship between e and the membrane potential, V say, was a straight line which could be extrapolated to predict that e would have been zero at a value of V between 0 and about −14 mV.

The equivalent circuit is illustrated in figure 3. Suppose that a current I is made to flow between the terminals; then with S open,

$$V = E - RI \qquad (1)$$

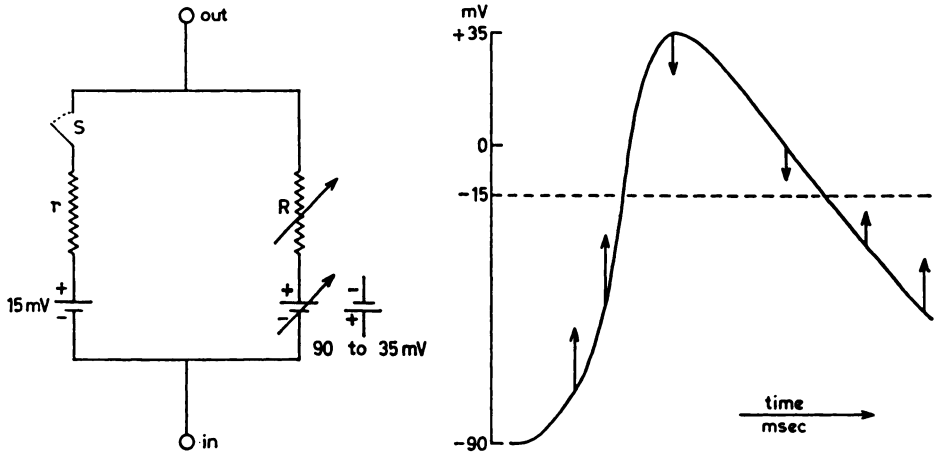

FIG. 2. Simplified equivalent circuit for the interaction method, and the nature of the results obtained [after (28); *cf.* (14, 96)].

When S is shut, provided the total current remains *constant* (*e.g.*, if it were supplied by a battery in series with a very large resistance), part of it, i_r say, flows through the left, and part, i_R say, through the right hand branch; thus

$$I = i_r + i_R \tag{2}$$

If the potential difference between the terminals changes to V^1, then

$$V^1 = E - Ri_R \tag{3}$$

and

$$V^1 = \epsilon - ri_r \tag{4}$$

Equations 2, 3 and 4 are easily solved for V^1 in terms of I. From eqn. 1 it is then found that

$$V^1 - V = [R/(r + R)](V - \epsilon)$$

With the usual sign convention, the depolarization, e say, is given by

$$e = [R/(r + R)](\epsilon - V) \tag{5}$$

Accordingly, under constant current conditions, the depolarization caused by the transmitter should vary linearly with the displacement of the membrane potential from the level of the transmitter equilibrium potential, ϵ. It should evidently be abolished when the membrane potential is equal to ϵ; and if the membrane potential is made less negative than ϵ, the effect of the transmitter on the membrane potential should be reversed in direction.

It was not feasible to abolish or reverse the direction of the end-plate potential by this method (the steady depolarization required would have caused contractions of the muscle fibre, which would have dislodged the electrodes), but

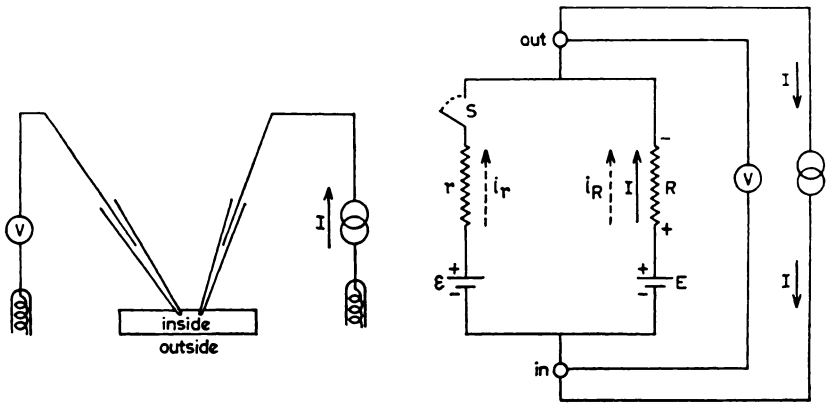

FIG. 3. Experimental arrangement and simplified equivalent circuit for the constant current and voltage clamp methods.

the junctional potentials in other cells have been reversed. They include cat motoneurones (33) and cerebellar cells (46); electroplaques of electric fish (13); and frog sympathetic ganglion cells (103) and slow skeletal muscle fibres (21). Moreover, the "artificial" junctional potentials caused by the ionophoretic application of drugs have been reversed: in the case of acetylcholine, on the end-plates of muscle fibres bathed in solutions in which action potentials do not occur (30, 115) and on chronically denervated muscle (6); and in the case of glutamate, on motoneurones (34). The results from a number of investigations are listed in table 1 (section II C).

The effects of inhibitory transmitters on the membrane potential, which are in principle similar to those of excitatory transmitters, have also been extensively investigated by the constant current method (table 2, section III A).

Effect of non-linear voltage-current relationship. A linear relationship between the amplitude of the junctional potential and the steady membrane potential as predicted by eqn. 5 has not always been found (see *e.g.*, 15, 21, 30). A particularly striking example of a departure from this prediction of the model has recently been described by Kandel and Tauc (82). They have found that for a certain variety of cell in the sea-slug, *Aplysia*, the depolarization caused both by the transmitter and by ionophoretically applied acetycholine was *diminished* when the cell was hyperpolarized by applied currents. However, they have shown that this result does not compromise the essential validity of the model, and that it is explained by the fact that the membrane resistance (R in fig. 3) decreased with hyperpolarization.

When R is not constant, *i.e.*, for a membrane with "rectifier" properties, it is simplest to use a graphical approach (30) based on eqns. 2, 3 and 4. This is illustrated in fig. 4 (c) and (d) for a cell with a voltage-current relationship similar to that described by Kandel and Tauc (82). For comparison, the same method is applied to a non-rectifying case in (a) and (b). Curve A in (a) and (c) is the voltage-current curve in the absence of transmitter action (*i.e.*, for the element

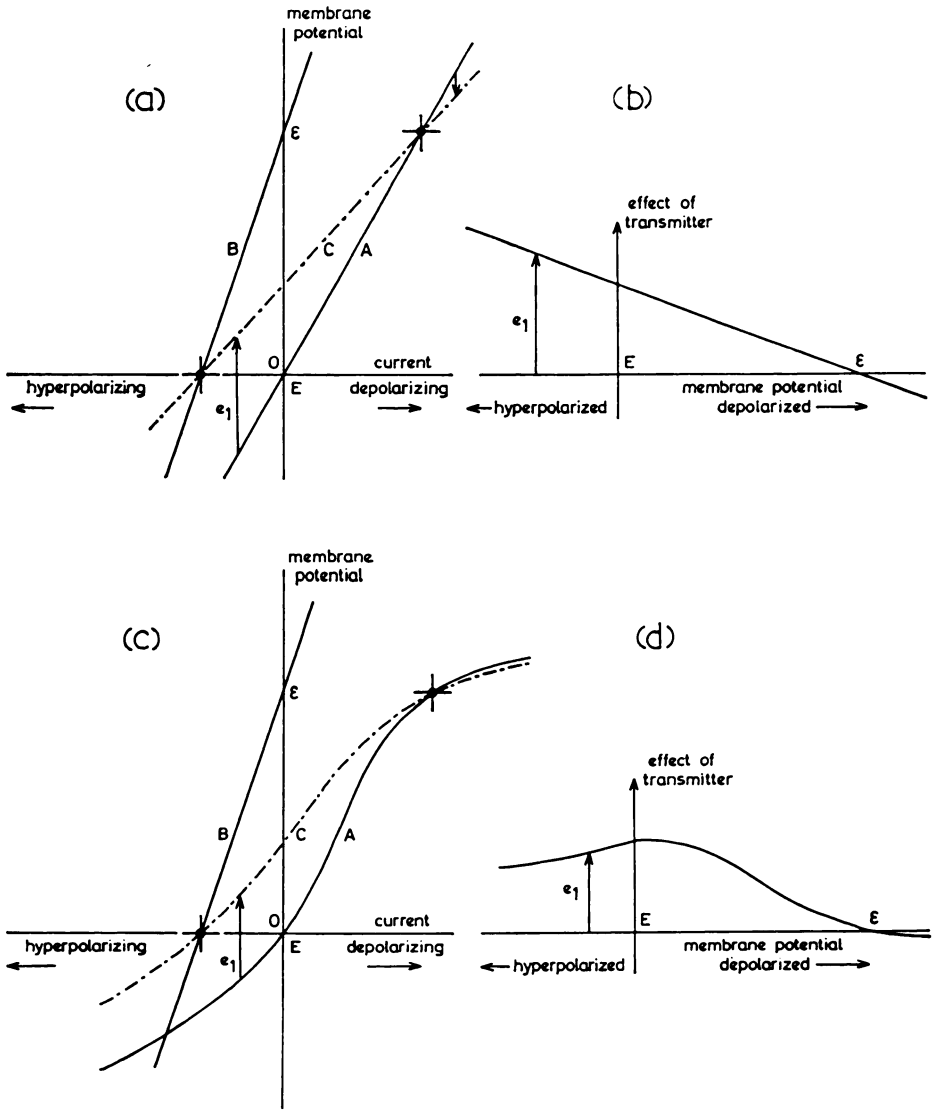

FIG. 4. Effect of rectification on the relationship between the amplitude of the junctional potentials and the membrane potential, in the constant current method (see text).

E, R in fig. 3) and represents eqn. 3, R being variable; and curve B is the relationship for the activated chemosensitive membrane (eqn. 4) (*i.e.*, for the element ϵ, r), r being assumed constant. With regard to the relationship between voltage and current during the action of the transmitter, it is clear that for any particular voltage, the total current is the algebraic sum of the separate currents in A and B (eqn. 2); this addition gives curve C. The change in potential caused by the action of the transmitter, under the constant current condition, is then given

by subtracting the voltage in A from that in C, for the same current. The values so obtained are plotted in (b) and (d) for the linear and rectifying cases, against the initial membrane potentials in A. The relationship between the amplitude of the synaptic potential and the membrane potential observed by Kandel and Tauc (82, fig. 2), is similar to that shown in the left hand section of (d), *i.e.*, for hyperpolarization.

In several other situations, different forms of variation of the membrane resistance, R, with membrane potential have also been shown to account for the observed nonlinearity in the relationship between the amplitude of the junctional potential and the membrane potential (*e.g.*, 21, 30).

There are, however, two curious anomalous results, which cannot be immediately explained in this way. In the motoneurone, it has been found that the excitatory postsynaptic potential (EPSP) increases only over a limited range of steady hyperpolarization, although the membrane resistance appears to be constant (33). The same applies to cells in the vas deferens (11), in which the junctional potentials caused hypogastric nerve stimulation are not decreased by steady depolarization, unless this exceeds 20 mV. These results might be explained if the resistance of only a small part of the membrane, in the neighbourhood of the chemosensitive region, was voltage dependent. Under the conditions in which the equivalent circuit of the following section applies, the nonlinearity would not be observed in the voltage-current relationship in the absence of transmitter action, but would play a significant part during it.

Effect of junctions distant from the site of recording and current injection. In the slow muscle fibres of the frog, where nerve stimulation simultaneously activates junctions distributed along the whole of the fibre, it has been shown that to reverse the direction of the junctional potential at any one point, it is necessary to depolarize the fibre at that point beyond the level of the transmitter equilibrium potential (21). The same is likely to be true wherever there are activated junctions distant from the site of impalement; for instance, in neurones that are impaled in the cell body but have junctions on dendrites (see, *e.g.*, 42) or in smooth muscle cells that form part of a "functional syncytium" (*e.g.*, 123, 124). In these cases there is an obvious departure from the equivalent circuit of figure 3, which corresponds to the situation in which there is a single postjunctional chemosensitive region, close to which the electrodes are inserted. Although it is not generally possible to make a detailed analysis, a simple equivalent circuit shows how the discrepancy between the "reversal" and true equilibrium potential comes about in these cases. Suppose, by way of example, that the cell concerned has a single dendrite on which the junction is situated. The internal resistance of the dendrite is represented by the additional element D, in the equivalent circuit of figure 5. If the transmitter equilibrium potential is -10 mV, the membrane in the region of A must be depolarized to this level, to abolish the effect of the transmitter. A current of 8×10^{-9} amp must therefore be made to flow through the region of the membrane represented by the branch BB′. However, this current will also cause a rise in potential across D, of (8×10^{-9}) amp $\times 10^{6}$ ohm, *i.e.*, of 8 mV. Thus the membrane potential recorded by the electrode will be

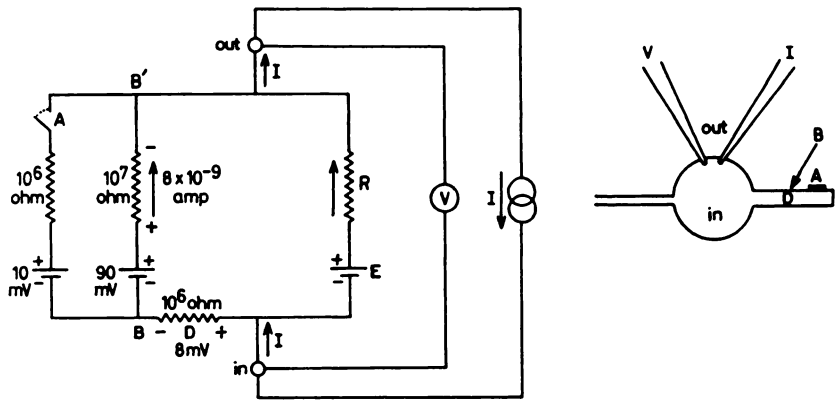

FIG. 5. Effect of a junction at a distance from the site of impalement of the cell in the determination of the transmitter equilibrium potential. The branch R, E corresponds to the remainder of the cell, including the cell body (see text .

−2 mV. It is evident that qualitatively, the nature of the result does not depend on the values for the separate resistances, which in practice could not be determined.

This analysis is of interest for two rather different reasons. First, it suggests how the previously mentioned nonlinear relationships between junction potentials and the membrane potential might arise, in spite of an apparently constant membrane resistance (11, 33). For suppose that the region of the membrane represented by BB′ (fig. 5) had rectifier properties, but its resistance was always large with respect to R; evidently, if R were constant, a nonlinear voltage-current relation would obtain only during the action of the transmitter.

The analysis is also of interest in connection with the identification of unknown transmitter substances. For example, Curtis (34) has applied glutamate and several other excitatory amino acids (see Curtis and Watkins, 35) to motoneurones through the extracellular tip of a triple-barrel microelectrode; one of the intracellular tips was used for recording, and the other for displacing the membrane potential by passage of currents. The upshot of these experiments was that the direction of the artificial synaptic potential was reversed at a greater internal negativity than was the EPSP. This result has been regarded as evidence against the idea that the natural transmitter is an amino acid on the ground that the equilibrium potential for a transmitter should not depend on whether it is applied artificially or by nerve stiumulation. However, the reversal potential of the EPSP may correspond to a smaller internal negativity than the true equilibrium potential, if the excitatory synapses are located on dendrites (*cf.* 12). On the other hand the cell body may also be sensitive to glutamate and the reversal potential for its effect when applied in the way described would then be expected to be closer to the true equilibrium potential. It is of course known that chemosensitivity to a transmitter substance may extend to regions outside those activated by nerve stimulation (*e.g.*, 82, 85, 118); and a discrepancy between the

reversal potentials for the inhibitory effects of artificially applied acetylcholine, and of the transmitter, on *Helix* H-cells has already been explained on the basis of the present discussion (85).

3. VOLTAGE CLAMP METHOD. This method has been applied to the study of the action of acetylcholine at the end-plate (110, 116) and of the (unknown) transmitter on the large cardiac ganglion cells of the lobster (70). In each case both the constant current and voltage clamp methods were applied in the same experiment, and the two methods gave the same result for the equilibrium potential.

The method again requires the impalement of a single cell with both a recording and a current-passing electrode. The current is not, however, kept constant during the action of the transmitter, but is supplied by a feedback amplifier to keep the membrane potential, V say, constant, at a pre-set level. Referring again to figure 3, suppose that with S open, a current I flows in the direction shown, reducing the potential difference between "out" and "in" to $V = E - RI$. Under voltage clamp conditions where V does not change, when S is shut an additional current i_r (which may be in either direction), flows through the left hand element. Since $V = \epsilon - r \cdot i_r$, this current is given by $i_r = (\epsilon - V)/r$. The value of the equilibrium potential is evidently equal to the membrane potential at which $i_r = 0$, *i.e.*, at which no additional current is drawn from the feedback amplifier.

In principle, this is a most powerful method. The amplitude and time course of the current i_r give an exact measure of the change caused by the transmitter, since the fact that the membrane potential is held constant implies that the membrane capacity may be ignored in principle, and not merely by way of simplification It is also clear that the properties of the nonjunctional membrane do not affect i_r. However, if there are junctions distant from the site of impalement of the cell the accuracy will be affected in the same way as with the constant current method.

C. Interpretation of transmitter equilibrium potentials in terms of ion movements

The results obtained by the methods described in the previous section are summarized in table 1, from which it can be seen that the equilibrium potentials for the action of excitatory transmitters lie between 0 and −20 mV. It will be appreciated that if the ion pathways opened by the transmitter allowed the passage of only a single ion species, then with the exisiting intracellular concentrations, $[K]_i$, $[Na]_i$, $[Cl]_i$, and extracellular concentrations $[K]_o$, $[Na]_o$ and $[Cl]_o$, the transmitter equilibrium potential, ϵ, would be given by one of the values of the Nernst potentials:

$$E_K = \frac{RT}{F} \log_e \frac{[K]_o}{[K]_i}; \quad E_{Na} = \frac{RT}{F} \log_e \frac{[Na]_o}{[Na]_i}; \quad E_{Cl} = \frac{RT}{F} \log_e \frac{[Cl]_i}{[Cl]_o}$$

(where R, T and F have their usual meanings, so that $RT/F = 25$ mV at 20°C). Since in the normal environment E_K and E_{Cl} are fairly close to the resting potential, and E_{Na} is positive, the values for ϵ in table 1 indicate that in all these cases,

TABLE 1

Evidence for validity of model, and level of transmitter equilibrium potentials for various cells

Site	Method	Equilibrium Potential	Reference
Striated muscle			
Frog fast fibres			
Nerve stimulation*	Constant current	0 to −14 mV extrapolation	Fatt and Katz (50) figs. 28, 29
Nerve stimulation	Interaction	−10 to −20 mV, reversal	Castillo and Katz (28) fig. 3
Nerve stimulation	Constant current and voltage clamp	−18 mV, extrapolation	Takeuchi and Takeuchi (110) fig. 9.
Ionophoretic Ach	Constant current	0, reversal (high $[K]_o$) −60 mV, reversal (low $[Na]_o$)	Castillo and Katz (30) figs. 4, 7.
Frog slow fibres	Constant current	−10 to −20 mV, reversal	Burke and Ginsborg (21) figs. 1, 2.
Cat denervated tenuissimus ionophoretic Ach	Constant current	−10 mV, reversal	Axelsson and Thesleff (6) fig. 4
Lobster	Constant current	−20 mV, extrapolation†	Grundfest and Reuben [(65 fig. 11) cf. (64)]
Locust	Constant current	0, extrapolation†	Castillo *et al.* (24) fig. 4
Smooth muscle			
Guinea pig vas deferens	Constant current	0, extrapolation†	Bennett and Merrillees (11) fig. 5
Vertebrate neurones			
Cat motoneurone			
Nerve stimulation	Constant current	0, reversal†	Coombs *et al.* (33) figs. 1, 2
Ionophoretic glutamate	Constant current	below zero, extrapolation	Curtis (34)
Toad motoneurone	Constant current	0, extrapolation†	Araki (2) fig. 6
Cat cerebellar cells	Constant current	?, reversal	Eccles *et al.* (46) figs. 13, 14
Frog sympathetic ganglion cells	Constant current	−10 to −20 mV, reversal	Nishi and Koketsu (103) figs. 12, 13
	Interaction	−10 to −20 mV reversal	Blackman *et al.* (14) fig. 8
Invertebrate neurones			
Squid giant synapse	Constant current	0, extrapolation	Hagiwara and Tasaki (69) figs. 10, 11
Lobster ganglion cell	Constant current and voltage clamp	−10 mV, extrapolation	Hagiwara *et al.* (70) figs. 5, 6
Onchidium ganglion cell	Constant current	0, extrapolation	Kusano and Hagiwara (94) fig. 4D

* Unless otherwise stated, responses were obtained by nerve stimulation.

† Values may be overestimates because of distant junctions (see Section II).

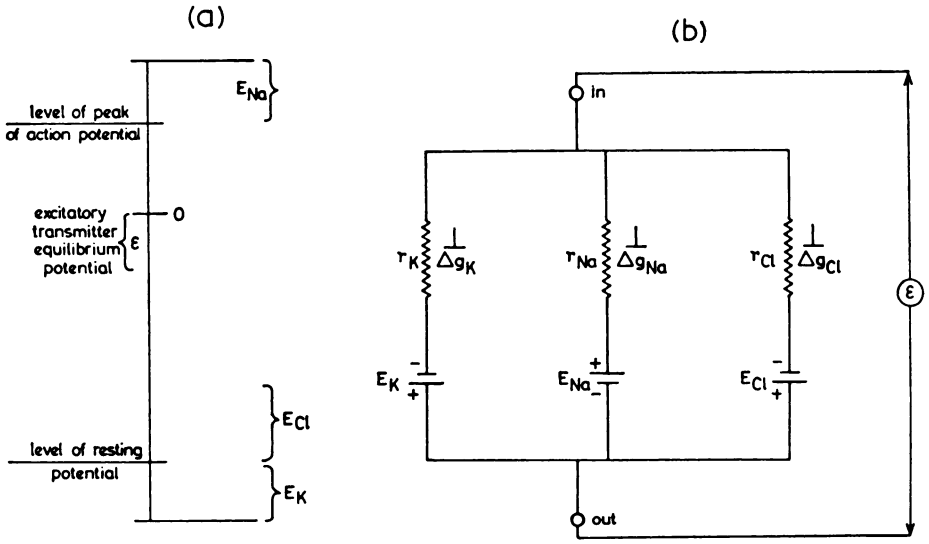

FIG. 6. (a) Typical relationship for different varieties of cell between values of Nernst potentials and membrane potential corresponding to resting, transmitter equilibrium and peak of action potential levels. (b) Equivalent circuit for transmitter equilibrium potential, ϵ, in terms of Nernst potentials and "additional conductances" Δ g produced by transmitter action.

the channels opened by the transmitter must allow the passage of Na and at least one other ion species (see fig. 6a). More detailed information is available for muscle, and the action of inhibitory substances (see section III), from experiments in which changes in ion concentrations have been made, and their effect on the value of ϵ determined. It would therefore be useful to have some model whereby values for ϵ could be interpreted in terms of the properties of the ion pathways. The "constant field" model (63, 73; *cf.* 47) does not appear to be applicable (44, 111), and in the absence of any other theory that allows explicit discussion in terms of permeabilities, a somewhat formal approach has been adopted [(*e.g.*, 96, 111); for some implications see (53)]. It is based on the model used by Hodgkin and Huxley in their analysis of the ion movements underlying the action potential (see *e.g.* 72a). It is supposed that the channels opened by the transmitter may be represented by the circuit shown in figure 6b. Then with the usual sign convention, it is easily seen that

$$\epsilon = \frac{E_K/r_K + E_{Na}/r_{Na} + E_{Cl}/r_{Cl}}{1/r_K + 1/r_{Na} + 1/r_{Cl}}$$

It is more convenient to use conductances, in which case

$$\epsilon = \frac{\Delta g_K E_K + \Delta g_{Na} E_{Na} + \Delta g_{Cl} E_{Cl}}{\Delta g_K + \Delta g_{Na} + \Delta g_{Cl}}$$

where $\Delta g_K = 1/r_K$, *etc.* (This notation is used to avoid confusion between the

conductances attributable to the activated postjunctional membrane and those of the nonjunctional membrane usually denoted by g_K, *etc.*) Δg_K, *etc.* refer to the values at the transmitter equilibrium potential, for the existing ion concentrations since no assumption can be made about the properties of the resistances in figure 6b. These might be functions of both voltage and ion concentrations, and indeed the problem of analysing transmitter action more closely can be regarded as determining these functions. An important step in this direction has been made for the action of acetylcholine on the end-plate (section D); elsewhere the evidence is scanty. However, it is worth noting that since, in general, the channels opened by the transmitter can be represented by a battery and resistance which are at least approximately constant over a wide range of membrane potential, it is unlikely that Δg_K *etc.* are particularly voltage dependent.

It would be of interest to relate the ion conductances to the ion fluxes across the activated postjunctional membrane. If the assumption is made that the ion movements are independent of one another [see Hodgkin and Huxley (72a, p. 467)] then it may be shown that for univalent ions,

$$M_1 = \Delta g \frac{E - E_N}{F}\left[1 - \exp\frac{E_N - E}{RT/F}\right]^{-1}$$

$$M_2 = M_1 \exp\frac{E_N - E}{RT/F}$$

where M_1 and M_2 are respectively outward and inward fluxes for a cation and *vice versa* for an anion, E_N is the Nernst potential for the particular ion and Δg its conductance, and E is the potential difference across the membrane. By way of example, if $E_K = -100$ mV, the outward flux of K ions at a membrane potential of -15 mV would be equal to

$$\Delta g_K(\text{ohm}^{-1}) \times \frac{(-15 + 100)10^{-3}\ (\text{volt})}{96{,}500\ (\text{coulomb. mole}^{-1})} \times \left[1 - \exp\frac{-100 + 15}{25}\right]^{-1}$$

$$= 9.2 \times 10^{-7}\ \Delta g_K\ \text{mole/sec.}\ (\Delta g_K \text{ in ohm}^{-1})\ \text{at } 20°\text{C.}$$

and the inward flux to

$$9.2 \times 10^{-7} \Delta g_K \times \exp\frac{-85}{25} = 3.1 \times 10^{-8}\ \Delta g_K\ \text{mole/sec.}$$

When the membrane potential is equal to the Nernst potential, *i.e.*, $E = E_N$, then $M_1 = M_2 = (RT/F^2)\Delta g = 2.6 \times 10^{-7}\ \Delta g$ mole/sec.

It must, however, be noted that the results described in the following section show that the expressions are unlikely to hold for the end-plate. Thus if Δg_{Na} and Δg_K are not greatly affected by small changes in membrane potential it would be predicted, for example, that a reduction in the external concentration of Na ions from 113 mM to 33 mM would lead to a reduction in the value of $\Delta g_{Na}/\Delta g_K$ by a factor of about $\frac{1}{3}$, whereas in practice no change was observed. This suggests that the ion movements across the activated end-plate do not occur

through simple aqueous channels [see section IV; *cf.* (43)]. No comparable data are available for other kinds of junctional membrane.

It need hardly be mentioned that attention has been focused on K, Na and Cl ions, because these have the highest concentrations and would therefore be expected to carry most of the current. In exceptional circumstances, the movement of Ca ions, for example, may make a significant contribution to the current. It may also be important in other ways (section II F).

D. Ion movements in skeletal muscle

Effect of changes in ion concentration on the transmitter and acetylcholine equilibrium potentials. Both the voltage clamp and constant current methods have been used by A. and N. Takeuchi to investigate the effects of changes in ion concentration on ϵ, the transmitter equilibrium potential (111, 115, 116). The ionophoretic application of acetylcholine allowed experiments to be made under conditions in which nerve conduction is abolished (115, 116).

Changes in the concentrations of Cl ions, with corresponding changes in the Nernst Cl potential, E_{Cl}, of as much as 80 mV, had no effect on ϵ. Evidently, acetylcholine does not increase the Cl-conductance of the membrane (*i.e.*, $\Delta g_{Cl} = 0$, in eqn. 6). On the other hand, as would then be expected, ϵ was sensitive to changes in the concentration of K as well as Na ions. In the case of Na ions, the results could be accounted for quantitatively by the change in E_{Na}, with the assumption that $\Delta g_{Na}/\Delta g_K$ remained constant (111, 115). In contrast, it was necessary to assume that the external concentration of K ions did affect the ratio of the conductances (115). The results could be fitted empirically by the relation $\Delta g_{Na}/\Delta g_K = 129/(2[K]_o + 95)$. Thus under physiological conditions, where $[K]_o = 2.5$ mM, $\Delta g_{Na}/\Delta g_K = 1.29$; and with $E_K = -99$ mV and $E_{Na} = +50$ mV, ϵ is equal to -15 mV.

The effect of changes in Ca ion concentration was also investigated (116). It was found that (a) an increase in $[Ca]_o$ from 2 to 30 mM reduced Δg_{Na} by about 30% and (b) when the preparation was bathed in a Na-free solution containing isotonic $CaCl_2$, the value of ϵ was less negative than the resting potential. This indicates that acetylcholine also causes an increase in Ca-conductance, since in this situation, neither E_K nor E_{Na} can be less negative than the resting potential.

Pharmacological considerations. The results just described have been concerned with the effects on the ion pathways. There are, of course effects at the recepter level both of the environmental ions (*e.g.*, 77, 97, 100, 101) and of the traditional neuromuscular blocking agents. In this connection, it is of interest that the acetylcholine equilibrium potential, and hence $\Delta g_{Na}/\Delta g_K$ is unaffected by tubocurarine, although the individual values Δg_{Na} and Δg_K as would be expected may be greatly reduced (111). Evidently, this is in line with the idea that tubocurarine does not affect the properties of the channels opened by acetylcholine, but reduces their number by combining with acetylcholine receptors (*e.g.*, 77). In contrast, Maeno (96) has recently found that in the presence of procaine, the transmitter equilibrium potential and hence $\Delta g_{Na}/\Delta g_K$, is altered. One explanation is that Na and K ions pass through separate channels, each kind being

under the ultimate control of a different species of receptor, each with a different affinity for procaine. However, this seems unlikely; and it would be necessary to assume that by chance, the two kinds of receptor had the same affinity for tubocurarine. The more likely alternative (*cf.* Shanes, 107) is that procaine modifies the properties of the ion pathways, perhaps in addition to combining with acetylcholine receptors of a single species. It has been suggested (*e.g.*, 107, 120) that other substances, for instance the "depolarizing" neuromuscular blocking drugs such as decamethonium, also have a direct effect on the ion pathways. There is however no compelling evidence against the idea that these drugs are partial agonists. Thus the interaction between decamethonium and acetylcholine at the end-plate (*e.g.*, 31) may be explained by supposing that a large proportion of the receptors is occupied by decamethonium, but that it is a drug with a low efficacy (109). On this basis, the effects of the depolarizing drugs are confined to the receptor level; this conclusion would be strengthened if it were shown that the equilibrium potential for their action was identical with that for acetylcholine.

Specificity of ion pathways. It has been shown by a number of investigators that the channels made available to Na and K ions at the end-plate, by the action of acetylcholine, also allow the inward transfer of certain "foreign" cations. This result has been inferred from the fact that acetylcholine still causes depolarization, even in the total absence of extracellular Na ions, provided that the bathing solution contains cations such as ammonium, tetramethylammonium (99), methylammonium (60), and hydrazinium (90).

Denervated skeletal muscle: tracer experiments. The equilibrium potential for the action of acetylcholine appears to be the same for chronically denervated muscle as for the end-plate of normal muscle (see table 1); the same ion movements are thus likely to underlie the depolarization caused by acetylcholine in the two situations. It is therefore of interest to consider the results of tracer experiments on denervated muscle (81) since the tracer method is capable of demonstrating changes in permeability, rather than of conductance. For such experiments denervated muscle has, of course, the advantage that the chemosensitive region occupies a much greater fraction of the surface of the fibre than in normal muscle (for review, see 121); any change in ion fluxes caused by acetylcholine is not therefore swamped in the ion movements that are always occurring, in parallel, through the nonchemosensitive membrane.

It will be recalled that an increase in the rate of influx or efflux of a labelled ion does not always imply an increase in permeability to that ion. For example if a cell is loaded with ^{42}K, and a drug which depolarizes the membrane is applied, the rate of efflux of ^{42}K may increase as a result of the depolarization rather than because of an increase in permeability to K ions. (Considerations of this kind are especially important in studies of the effect of drugs on spontaneously active tissues, since large "nonspecific" changes in ion fluxes may then occur because of changes in the frequency of action potentials (see, *e.g.*, 79 and section III D). This complication has been avoided by Jenkinson and Nicholls (81), who have studied the ion movements in the chronically denervated rat diaphragm, already completely depolarized in K-enriched solutions. It was shown that no further

change in membrane potential occurred on the application of acetylcholine but that the rates of influx of ^{42}K, ^{24}Na and ^{45}Ca and the rates of efflux of ^{42}K and ^{24}Na were all increased by acetylcholine (10^{-7} to 2×10^{-5} g/ml). In contrast the movements of ^{36}Cl were virtually unchanged. The results are therefore in complete accord with those obtained from the electrical experiments on the normal end-plate.

Nothing is known of the ion movements underlying the stimulant effects of tubocurarine and adrenaline on chronically denervated muscle (see Thesleff and Quastel, 122, for references).

Uptake of depolarizing drugs. The tracer method has also been applied to studies of the uptake of various quaternary nitrogen compounds by muscle (see 120). Among those recently investigated (119) are tritium labelled (N-methyl ^{3}H) decamethonium and carbamylcholine (carbachol). In the normal rat diaphragm, the uptake of decamethonium was significantly greater in the end-plate region, except in the presence of tubocurarine. As might then be expected, a greater uptake was found in chronically denervated muscle, uniformly distributed over its whole length. On the other hand, the uptake of carbachol by normal muscle was not greater at the end-plate, nor was the uptake greatly affected by tubocurarine. The interpretation of these results is somewhat uncertain (see 120; *cf.* 101, 130).

E. Ion movements in intestinal smooth muscle

Electrical experiments. There is no direct evidence that the standard model applies to the action of the transmitter or of depolarizing drugs on intestinal smooth muscle, since the existence of an equilibrium potential for their action has not been established. However, Bülbring and Kuriyama (18) have studied the effect of acetylcholine on single cells in the taenia of the guinea pig; and they have shown that the depolarization is altered by changes in $[K]_o$ and $[Na]_o$ in a way which suggests that acetylcholine increases the conductance to Na and at least K ions (see also Bennett, 7).

Tracer experiments. Durbin and Jenkinson (40) have applied the tracer method to a study of the action of carbachol on the taenia of the guinea pig. The experiments were made on preparations that were depolarized in K-rich solutions, for the reasons given above. The rates of influx and efflux of ^{42}K, ^{36}Cl (and ^{82}Br), and the rate of influx of ^{24}Na and ^{45}Ca were all increased by carbachol (3×10^{-7} g/ml). The results are therefore consistent with the idea that carbachol's primary action on the membrane of cells in the taenia is to produce a non-selective increase in ion permeability.

F. Effects at other sites

Little definite can be said about the ion movements caused by excitatory substances at sites other than those listed in table 1 or discussed in section E.

A number of substances cause complicated potential changes in sympathetic ganglia (see Volle, 128, for review), but external records only are available and it is not possible to interpret these in terms of ion movements.

Acetylcholine has recently been shown to depolarize single chromaffin cells of the adrenal medulla (Douglas *et al.* 37) and it is therefore attractive to suppose that it causes an increase in ion conductance at this site. It is already known that the secretion of catecholamines is in some way related to the uptake of Ca ions (see Douglas, 36), which might now be supposed to result in part from a direct effect of acetylcholine on the permeability to Ca ions. The uptake of Ca ions has been shown to be concerned in the development of tension by depolarized smooth muscle, under the action of acetylcholine and its analogues (see Durbin and Jenkinson, 41; Schild, 106) and by depolarized denervated skeletal muscle (81). It is, of course, also well known that Ca ion movements are intimately related to contraction, under conditions in which transmitters are not involved (see, *e.g.*, Niedergerke, 102).

In at least two situations, it appears that the standard model cannot account for the effects of transmitter substances. Thus it is inappropriate for the action of sympathomimetics on the heart (see Trautwein, 125, for review), and for the initiation of salivary secretion (see Burgen and Emmelin, 19, and Lundberg 95, for reviews). Although nerve stimulation causes changes in potential, they cannot readily be explained by increases in membrane conductance.

Finally, it may be recalled that there are situations in which junctional transmission occurs without the aid of transmitter substances (*e.g.*, Furshpan and Potter, 57; Martin and Pilar, 98). Ion movements must, of course, be concerned, since ions are the only current carriers available, but their identity is determined by the passive properties of the postsynaptic membrane and not by the provision of additional ion pathways.

III. THE ACTION OF INHIBITORY TRANSMITTERS

A. Transmitter equilibrium potentials

The first account of the action of an inhibitory transmitter in terms of a model similar to that of figure 1 was given by Fatt and Katz (52) in 1953, to explain the results of experiments on crustacean muscle fibres. At the resting level, no effect on the membrane potential was produced by inhibitory nerve stimulation; when, however, the membrane potential was displaced, in either direction, by a constant current, inhibitory nerve stimulation then caused a change towards the original resting level. Evidently, there was a transmitter equilibrium potential, in this case at the level of the resting potential.

The existence of equilibrium potentials ϵ' both for the action of transmitters and inhibitory substances applied artificially, has now been established for a variety of cells (table 2). The fact that ϵ' has the same value for the effects of nerve stimulation and of applied γ-amino-n-butyric acid (GABA) supports the idea that GABA may be an inhibitory transmitter in crustacea (*e.g.*, 68, 112) and insects (127) (for review of evidence of other kinds see 35, 54, 85, 88) and possibly in the cat cerebral cortex (91). The same is true in connection with acetylcholine and the mollusc H-cells (87, 118). [A small discrepancy in the case of the *Helix* H-cells has been explained (87) on the basis of the discussion in section II B].

TABLE 2

Levels of inhibitory equilibrium potentials for various cells. In all cases, the responses could be reversed by passage of current across the cell membrane

Site	Method	Difference between Equilibrium and Resting Potential	Reference
Vertebrate			
Heart: dog atrium, Ach*	Constant current	25 mV hyp.†	Trautwein and Dudel (126)
Motoneurone, cat	Constant current	10 mV hyp.	Coombs *et al.* (32) figs. 1, 2
Motoneurone, cat	Voltage clamp	15 mV hyp.	Araki and Terzuolo (4) fig. 9
Motoneurone, toad	Constant current	15 mV hyp.	Fukami (56) fig. 4
Cortical cells, cat			
Nerve stim. / GABA	Constant current	small hyp.	Krnjevic and Schwartz (91)
Mauthner cell, goldfish	Constant current	small hyp.	Furukawa and Furshpan (59) fig. 18
Invertebrate			
Muscle			
Crab	Constant current	0	Fatt and Katz (52) fig. 7
Crayfish			
Astacus			
Nerve stim. / GABA	Constant current	0	Boistel and Fatt (15)
Orconectes	Constant current	small dep.	Dudel and Kuffler (39 fig. 2
Cambarus			
Nerve stim. / GABA	Constant current	small dep.	Takeuchi and Takeuchi (112) fig. 9
Lobster	Constant current	0	Grundfest *et al.* (65) fig. 1
Insect			
Nerve stim. / GABA	Constant current	up to 20 mV hyp.	Usherwood and Grundfest (127) fig. 4
Crayfish giant motor fibre			
Nerve stim. / GABA	Constant current	small dep.	Furshpan and Potter (58) figs. 3, 4
Crustacean stretch receptor			
Nerve stim.	Stretch	small dep.	Kuffler and Eyzaguirre (92) figs. 4, 5
Nerve stim. / GABA	Constant current	small dep. or hyp.	Hagiwara *et al.* (68)
Molluscan neurones			
Aplysia and Helix	Constant current		Tauc (117) figs. 11, 13
Aplysia (H-cells)			
Nerve released Ach	Constant current		Tauc and Gerschenfeld (118) fig. 7
Helix (H-cells)		up to 20 mV hyp.	Kerkut and Thomas (87) fig. 2
Nerve released Ach	Constant current		Gerschenfeld (61)
Cryptomphallus (D-cells)	Constant current		
Onchidium	Voltage clamp / Constant current	small dep. or hyp.	Hagiwara and Kusano (67) fig. 1

* Unless otherwise stated, responses were obtained by nerve stimulation.

† hyp. = the level of the transmitter equilibrium potential is more negative than the resting potential.

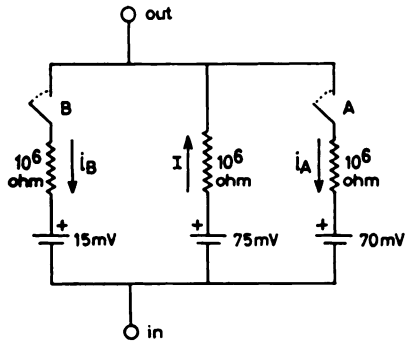

FIG. 7. Simplified equivalent circuit for interaction between inhibitory (A) and excitatory (B) transmitter effects on postjunctional membrane.

Interaction between inhibitory and excitatory effects. Where ϵ' is more negative than the resting potential, the inhibitory transmitter acting alone will cause hyperpolarization. Although the hyperpolarization may contribute, it is not the main cause of the inhibitory effect (52); and the change in membrane potential during the simultaneous actions of inhibitory and excitatory substances is not the algebraic sum of the displacements that each would separately cause. This is illustrated by the equivalent circuit of figure 7, which also indicates how a transmitter may be inhibitory, although when acting alone it causes depolarization (see table 2). It is assumed that the channels opened by the excitatory and inhibitory transmitters are entirely distinct (see 113). The action of the inhibitory transmitter is represented by closing switch A, which produces a "depolarization" of 2.5 mV. The action of the excitatory transmitter, represented by closing switch B, causes a "depolarization" of 30 mV. When A and B are both shut, the "depolarization" e, in mV is evidently given by

$$e = (10^6 \text{ ohm}) \times (I \text{ mA})$$

where,

$$I = i_A + i_B$$

$$75 - 10^6 I = 70 + 10^6 i_A$$

and

$$75 - 10^6 I = 15 + 10^6 i_B$$

whence $e \doteq 22$ mV. The action of A is thus to "inhibit" that of B. It is evident that a transmitter substance will be inhibitory if the level of its equilibrium potential is more negative than the threshold for excitation.

It need hardly be emphasized that this inhibitory action applies also to situations in which excitation is brought about by processes other than excitatory transmitter action, as for example in the heart (see Trautwein, 125), the crayfish motor axon (Furshpan and Potter, 57, 58), the crustacean stretch receptor

(Kuffler and Eyzaguirre, 92) and spontaneously active smooth muscle (see Burnstock and Holman, 22).

Presynaptic inhibition. It was recognized by Fatt and Katz (52), that the change in conductance caused by the inhibitory transmitter was not large enough to account alone for the inhibition of crustacean muscle. It has now been shown conclusively by Dudel and Kuffler (39) that in this situation, the inhibitory transmitter (probably GABA) has the additional (and usually more important) effect (but see 84) of reducing the amount of transmitter liberated by the excitatory nerve. This action, known as presynaptic inhibition, occurs also in the vertebrate central nervous system (see, *e.g.*, Eccles, 42). The action of the transmitter on the presynaptic excitatory nerve terminals may have the same underlying ionic mechanism as that involved in postsynaptic inhibition (38, 114), but its discussion is outside the scope of this article.

B. Ion movements involved in inhibition at particular sites

It is evident from the fact that the levels of ϵ' are close to, or more negative than the resting potential (Table 2) that the additional conductance produced by inhibitory substances must be greater for K or Cl, or both, than for Na ions. Further information has been obtained from observations on the way in which ϵ' changes when the ion concentrations, and hence their Nernst potentials, are altered (section II C). The results are summarized in table 3.

TABLE 3

Effects of changes in Nernst potentials E_K and E_{Cl} on inhibitory equilibrium potential, ϵ'

	Stimulus	E	Effect on ϵ'	Permeating ions	Reference
Vertebrate					
Heart	Ach	E_K	$\epsilon' = E_K$	K only	Trautwein and Dudel (126)
Neurones in cat CNS	Nerve	E_{Cl}	Changed	Cl*	See Eccles (42, 43)
Mauthner cell	Nerve	E_{Cl}	Changed	Cl	Furukawa and Furshpan (59)
Invertebrate					
Astacus muscle	Nerve	E_K	V. small	K?	Boistel and Fatt (15)
	GABA	E_{Cl}	Large	Cl	
Insect muscle	Nerve	E_K	None		Usherwood and Grundfest (127)
	GABA	E_{Cl}	Large	Cl only	
Crayfish giant motor axon	Nerve, GABA	E_{Cl}	Changed	Cl	Furshpan and Potter (58)
Crayfish stretch receptor	Nerve, GABA	E_{Cl}	Changed	Cl*	Hagiwara *et al.* (68)
Helix H-cells	Nerve	E_K	Small	K?	Kerkut and Thomas (87) See also (86)
	Ach	E_{Cl}	Large	Cl	
Cryptomphallus D-Inhi cells	Nerve	E_K	Large	K only	Gerschenfeld and Chiarandini (62)
		E_{Cl}	None		

* Less direct evidence shows that K ions also permeate the activated postsynaptic membrane in the motoneurone (35) and Crayfish stretch receptor [for refs. see (68)].

1. THE HEART. It had already been shown in 1953 by Burgen and Terroux (20) that the action of acetylcholine (and carbachol) could be explained by supposing that it caused an increase in K conductance. This was confirmed by Trautwein and Dudel (126), who showed that the equilibrium potential for the action of acetylcholine on the atrium of the dog heart was, in fact, identical with the Nernst K potential, E_K. Evidently, the channels opened by acetylcholine are available only to K ions. This conclusion had also been reached by Harris and Hutter (71; see also 74, 75) on the basis of tracer studies. In the sinus venosus of frog and tortoise hearts, both the influx and efflux of ^{42}K were increased by the application of acetylcholine, whereas those of ^{36}Cl (and ^{82}Br) were unaffected.

2. VERTEBRATE CENTRAL NEURONES. Controlled changes in the extracellular ion concentrations of vertebrate central neurons which must be studied *in situ* cannot be made; the intracellular concentrations can however be altered by the injection of ions from an intracellular electrode. The most extensive investigations have been made on motoneurones of the cat (32, 45; for a recent summary see Eccles, 43). The changes in ϵ' with changes in $[Cl]_i$ have shown that the transmitter causes an increase in conductance to Cl ions. However, since E_{Cl} is at the level of the resting potential, or perhaps even somewhat less negative, but inhibitory nerve stimulation causes hyperpolarization, it has been assumed that there is also an increase in K conductance (45). This has not been tested directly because of the difficulty in making significant changes to $[K]_i$ and hence to E_K. An increase in $[Na]_i$ had no effect on ϵ', provided that it was not accompanied by an increase in $[Cl]_i$. This condition was achieved by coupled injections of Na and SO_4 ions into the cell, from double-barrelled electrodes (45).

It has been inferred that the Cl conductance in other central neurones is also increased by inhibitory nerve stimulation since the responses are reversed from the hyperpolarizing to the depolarizing direction, when $[Cl]_i$ rises, as a result of diffusion of Cl ions from a KCl-filled intracellular recording electrode.

3. INVERTEBRATE CELLS. The wide variety of invertebrate cells which has been studied is indicated in table 3; in addition, it has been found that GABA and piperazine may cause inhibition of *Ascaris* muscle by increasing the conductance of specific regions of the membrane to Cl ions (26, 27). In this, as in insect muscle (127) [and possibly *Helix* H-cells (86)] inhibition is associated not only with an increase in conductance that is exclusive to Cl ions, but also with hyperpolarization. The Nernst Cl potential, E_{Cl}, for these cells must therefore be more negative that the resting potential. This in turn implies that there must be an outward "Cl-pump". Although for several kinds of cell the existence of an inward "Cl-pump" must be assumed, to account for the excess in $[Cl]_i$ over the value that would be in equilibrium at the resting potential (*e.g.*, 23, 89, 129), there is as yet no independent evidence for a "pump" in the opposite direction (but see 25).

C. Specificity of ion pathways

Attempts have been made to specify the properties of the ion pathways more closely by examining the effect of the injection of various anions into cells, on the change in membrane potential caused by the inhibitory transmitter. The

cells that have been studied are cat motoneurones (3, 32, 76), goldfish Mauthner cells (5) and *Helix* H-cells (87). It was found for all three types, that anions that have a "limiting equivalent conductance" in water, λ^0 (see, *e.g.*, Robinson and Stokes, 105, chapter 6) greater than 64 units (cm^2 ohm^{-1} $equiv^{-1}$) were indistinguishable from Cl ions (λ^0 = 76 units), insofar as the activated postjunctional membrane was concerned; in contrast, anions for which λ^0 was less than 56 units, were totally impermeant. Since λ^0 is a function of the mobility, which in turn depends on the size of the hydrated ion, it has been suggested that the ion pathways are aqueous pores of uniform, fixed diameter (*ca.* 3 Å). Little is known in either theory or practice about ion movements through such small pores; the idea is at present therefore rather speculative.

D. Action of inhibitory substances on intestinal smooth muscle

It is by no means generally agreed that inhibition in smooth muscle is brought about by a process of the kind so far discussed (see Bennett, 7; Bueding and Bülbring, 16; Burnstock and Holman, 22). Nevertheless, although there are almost certainly additional factors (see below), the idea that there is an increase in K conductance, which plays a major role in inhibition, now has considerable supporting evidence.

Alternative explanations which have been suggested (see, *e.g.*, 16, 22), more particularly for the action of adrenaline than of the transmitter, include (1) the activation of an electrogenic "Na-pump," (2) a decrease in Na-permeability, and (3) an increase in the fixation of Ca ions by the membrane, involving a metabolic process and leading to (2). While none of these possibilities can at present be rejected, none is supported by any compelling evidence.

No information is available about the ion movements underlying the inhibitory action of acetylcholine on smooth muscle.

Electrical experiments. Studies of the membrane potential of single cells in the taenia of the guinea pig have been made by Kuriyama (93) and Bülbring and Kuriyama (18). The relationship between the membrane potential and $[K]_o$ was investigated in the absence and presence of adrenaline (93, fig. 6). The effect of adrenaline was similar to that found by Burgen and Terroux (20, fig. 2) for carbachol in connection with its action on the heart. Thus in the presence of adrenaline, the membrane potential in the taenia was closer to the Nernst K-potential, E_K. Furthermore, in Na-free solutions containing K ions, in which it would be expected that the membrane potential would already be closer to E_K than normally, adrenaline had little effect (18). Both these findings are consistent with the idea that adrenaline causes an increase in K conductance. Although they might also be explained by a decrease in Na conductance, such electrical evidence as there is [on frog stomach (108)] suggests that the membrane resistance of smooth muscle is decreased, rather than increased, by adrenaline (see also tracer studies, below).

It has also been shown by Bülbring and Kuriyama (18) that on the application of adrenaline to the bath, the spontaneous action potentials in the taenia are abolished before the cells are hyperpolarized. This may not be, as is sometimes

thought, a serious objection to the "K-hypothesis," since the hyperpolarization is not, in any case, the cause of the inhibition (section III A). The phenomenon might simply reflect the time course of the rise in concentration of the adrenaline at the cell surface during diffusion. Initially the low concentration might increase the K conductance sufficiently to inhibit the process responsible for excitation, but not to cause hyperpolarization. It is of interest, that with sympathetic nerve stimulation, there appears to be no delay between inhibition and hyperpolarization.

Bennett, *et al.* (8) have briefly reported the effect of ion changes on the inhibitory responses of the taenia to intramural nerve stimulation [the transmitter for these nerves is unknown, but it is not noradrenaline (see 10)]. The experiments were made with the "sucrose-gap" technique, and results were obtained that suggested that the transmitter caused an increase in K conductance.

Tracer studies. In a number of studies, although adrenaline was found to increase the influx of ^{42}K into the taenia, it did not increase the efflux. This might be regarded as evidence against the idea that adrenaline causes an increase in K permeability; on the other hand, since the cells are hyperpolarized by adrenaline and the spontaneous action potentials are abolished (18), the rate of loss of K ions from the taenia will be diminished. Thus any increase in ^{42}K efflux that resulted from an increase in permeability might be masked. Using the depolarized taenia bathed in K-rich solution (*cf.* section II D), which eliminates this complication, Jenkinson and Morton (78, 79) have tested the effect of noradrenaline (3×10^{-7} g/ml) on the exchange of ^{42}K and ^{36}Cl and on the influx of ^{24}Na. Their conclusions are that there is no effect on the permeability to Cl or Na ions, but that there is an increase in permeability to K ions. This effect was not shared by isopropylnoradrenaline (isoprenaline) in equimolar concentration, and was blocked by phentolamine, but not by pronethalol. It was therefore concluded (80) that it was mediated *via* "α-adrenergic" receptors (see Ahlquist, 1). It has recently been shown that adrenaline also increases the rates of influx and efflux of ^{42}K in the depolarized taenia (Bülbring, *et al.*, 17).

Inhibition via β-receptors. Jenkinson and Morton (80) have also extended the studies made by Schild and his colleagues (see, *e.g.*, 106) on the relaxation of depolarized smooth muscle induced by catecholamines. The kind of inhibition involved in this situation is not accompanied by changes in membrane potential and would not be explained by an increase in K permeability. It has been found, in fact, that such an increase does not occur during the relaxation caused by isoprenaline, and evidence has been produced to show that the catecholamine relaxation of the depolarized taenia is mediated *via* β-receptors. The extent to which "β-relaxation" is involved under normal conditions and its possible mechanism are discussed by the authors in their recent paper (80).

IV. DISCUSSION

It has been evident for some years that the same substance may produce a variety of permeability changes. Acetylcholine, for example, opens channels in skeletal muscle only to cations, in heart muscle only to K ions and in intestinal

smooth muscle, most probably to both cations and anions. At the end-plate (see Katz, 83) and probably elsewhere, drastic changes in membrane potential are unable to mimic the action of the transmitter; that is to say, the channels cannot be opened by voltage or current (but for possible feeble effects see 49 and 55). They cannot even be opened by the action of the transmitter substance applied from within the cell (29) (see also 91).

For these reasons it is an attractive idea (49) to suppose that the ion pathways are, literally, preformed pores which are "unplugged" as a result of the reaction between the transmitter substance and receptors located on the outer surface of the cell membrane. The exclusion of cations or anions could then be explained by supposing that the pores were negatively or positively charged (15, 49) and discrimination against Na ions where it occurs could be attributed to restricted pore diameter. It must however be admitted that the physical nature of the ion pathways is no better understood now than some 15 years ago, when they were first revealed. It cannot be supposed that the pores, if they exist, are simple aqueous channels. This idea does not account for the ion conductances in the situation which has been most closely studied, namely the end-plate (sections II C and D); that it still seems to fit the action of inhibitory substances may be due only to the relative absence of detailed information.

REFERENCES

1. Ahlquist, R. P.: The adrenotropic receptor-detector. Arch. Int. Pharmacodyn. & Terap. **139**: 38–41, 1962.
2. Araki, T.: Effects of electrotonus on the electrical activities of spinal motoneurons of the toad. Jap. J. Physiol. **10**: 518–532, 1960.
3. Araki, T., Ito, M. and Oscarsson, O.: Anion permeability of the synaptic and non-synaptic motoneurone membrane. J. Physiol. (London) **259**: 410–435, 1961.
4. Araki, T. and Terzuolo, C. A.: Membrane currents in spinal motoneurons associated with the action potential and synaptic activity. J. Neurophysiol. **25**: 772–789, 1962.
5. Asada, Y.: Effects of intracellularly injected anions on the Mauthner cells of goldfish. Jap. J. Physiol. **13**: 583–598, 1963.
6. Axelsson, J. and Thesleff, S.: A study of supersensitivity in denervated mammalian skeletal muscle. J. Physiol. (London) **147**: 178–193, 1959.
7. Bennett, M. R.: Model of the membrane of smooth muscle cells of the guinea pig taenia coli muscle during transmission from inhibitory and excitatory nerves. Nature (London) **211**: 1149–1152, 1966.
8. Bennett, M. R., Burnstock, G. and Holman, M. E.: The effect of potassium and chloride ions on the inhibitory potential recorded in the guinea-pig taenia coli. J. Physiol. (London) **169**:33P–34P, 1963.
9. Bennett, M. R., Burnstock, G. and Holman, M. E.: Transmission from perivascular inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. J. Physiol. (London) **182**: 527–540, 1966.
10. Bennett, M. R., Burnstock, G. and Holman, M. E. Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. J. Physiol. (London) **182**: 541–558, 1966.
11. Bennett, M. R. and Merrillees, N. C. R.: An analysis of the transmission of excitation from autonomic nerves to smooth muscle. J. Physiol. (London) **185**: 520–535, 1966.
12. Bennett, M. V. L.: Nervous function at the cellular level. Annu. Rev. Physiol. **26**: 289–340, 1964.
13. Bennett, M. V. L., Wurzel, M. and Grundfest, H.: The electrophysiology of electric organs of marine electric fishes. I. Properties of electroplaques of Torpedo nobiliana. J. Gen. Physiol. **44**: 757–804, 1961.
14. Blackman, J. G., Ginsborg, B. L. and Ray, C.: Synaptic transmission in the sympathetic ganglion of the frog. J. Physiol. (London) **167**: 355–373, 1963.
15. Boistel, J. and Fatt, P.: Membrane permeability changes during inhibitory transmitter action in crustacean muscle. J. Physiol. (London) **144**: 176–191, 1958.
16. Bueding, E. and Bülbring, E.: The inhibitory action of adrenaline. Biochemical and biophysical observations. In: Proc. 2nd Int. Pharmac. Meeting, ed. by E. Bülbring, vol. 6, pp. 37–54, Pergamon Press, Oxford, 1964.
17. Bülbring, E., Goodford, P. J. and Setekleiv, J.: The action of adrenaline on the ionic content and on the sodium and potassium movements in the smooth muscle of the guinea pig taenia coli. Brit. J. Pharmacol. **28**: 296–307, 1966.
18. Bülbring, E. and Kuriyama, H.: Effects of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of the guinea-pig taenia coli. J. Physiol. (London) **166**: 59–74, 1963.
19. Burgen, A. S. V. and Emmelin, N. G.: Physiology of the salivary glands. Monographs of the Physiological Society, No. 8, Edward Arnold, London, 1961.

20. BURGEN, A. S. V. AND TERROUX, K. G.: On the negative inotropic effect io the cat's auricle. J. Physiol. (London) 120: 449–464, 1953.
21. BURKE, W. AND GINSBORG, B. L.: The action of the neuromuscular transmitter on the slow fibre membrane. J. Physiol. (London) 132: 599–610, 1956.
22. BURNSTOCK, G. AND HOLMAN, M. E.: Effect of drugs on smooth muscle. Annu. Rev. Pharmacol. 6: 129–156, 1966.
23. CASTEELS, R. AND KURIYAMA, H.: Membrane potential and ion content in the smooth muscle of the guinea-pig's taenia coli at different external potassium concentrations. J. Physiol. (London) 184: 120–129, 1966.
24. DEL CASTILLO, J., HOYLE, G. AND MACHINE, X.: Neuromuscular transmission in a locust. J. Physiol. (London) 121: 539–547, 1953.
25. DEL CASTILLO, J., DE MELLO, W. C. AND MORALES, T. Influence of some ions on the membrane potential of *Ascaris* muscle. J. Gen. Physiol. 48: 129–140, 1964.
26. DEL CASTILLO, J., DE MELLO, W. C. AND MORALES, T.: Mechanism of the paralysing action of piperazine on *Ascaris* muscle. Brit J. Pharmacol. 22: 463–477, 1964.
27. DEL CASTILLO, J., DE MELLO, W. C. AND MORALES, T.: Inhibitory action of γ-aminobutyric acid (GABA) on *Ascaris* muscle. Experientia 20: 141–143, 1964.
28. DEL CASTILLO, J. AND KATZ, B.: The membrane change produced by the neuromuscular transmitter. J. Physiol. (London) 125: 546–565, 1954.
29. DEL CASTILLO, J. AND KATZ, B.: On the localization of acetylcholine receptors. J. Physiol. (London) 128: 157–181, 1955.
30. DEL CASTILLO, J. AND KATZ, B.: Local activity at a depolarized nerve-muscle junction. J. Physiol. (London) 128: 396–411, 1955.
31. DEL CASTILLO, J. AND KATZ, B.: Interaction at end-plate receptors between different choline derivatives. Proc. Roy. Soc. B. 146: 369–381, 1957.
32. COOMBS, J. S., ECCLES, J. C. AND FATT, P.: The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. J. Physiol. (London) 130: 326–373, 1955.
33. COOMBS, J. S., ECCLES, J. C. AND FATT, P.: Excitatory synaptic action in motoneurones. J. Physiol. (London) 130: 374–395, 1955.
34. CURTIS, D. R.: Direct extracellular application of drugs. Biochem. Pharmacol. 9: 205–212, 1962.
35. CURTIS, D. R. AND WATKINS, J. C.: The pharmacology of amino acids related to Gamma-aminobutyric acid. Pharmacol. Rev. 17: 347–391, 1965.
36. DOUGLAS, W. W.: The mechanism of release of catecholamines from the adrenal medulla. Pharmacol. Rev. 18: 471–480, 1966.
37. DOUGLAS, W. W., KANNO, T. AND SAMPSON, S. R.: Effects of acetylcholine and other medullary secretagogues and antagonists on the membrane potential of adrenal chromaffin cells: an analysis employing techniques of tissue culture. J. Physiol. (London) 188: 107–120, 1967.
38. DUDEL, J.: The action of inhibitory drugs on nerve terminals in crayfish muscle. Pflügers Arch. Gesamte Physiol. 283: 104–118, 1965.
39. DUDEL, J. C. AND KUFFLER, S. W.: Presynaptic inhibition at the crayfish neuromuscular junction. J. Physiol. (London) 155: 543–562, 1961.
40. DURBIN, R. P. AND JENKINSON, D. H.: The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions. J. Physiol. (London) 157: 74–89, 1961.
41. DURBIN, R. P. AND JENKINSON, D. H.: The calcium dependence of tension development in depolarized smooth muscle. J. Physiol. (London) 157: 90–96, 1961.
42. ECCLES, J. C.: The Physiology of Synapses, 316 pp., Springer Verlag, Berlin, 1964.
43. ECCLES, J. C.: The ionic mechanisms of excitatory and inhibitory synaptic action. Ann. N.Y. Acad. Sci. 137: 473–494, 1966.
44. ECCLES, J. C., ECCLES, R. M. AND ITO, M.: Effects of intracellular potassium and sodium injections on the inhibitory postsynaptic potential. Proc. Roy. Soc. B 160: 181–196, 1964.
45. ECCLES, J. C., ECCLES, R. M. AND ITO, M.: Effects produced on inhibitory postsynaptic potentials by the coupled injections of cations and anions into motoneurons. Proc. Roy. Soc. B 160: 197–210, 1964.
46. ECCLES, J. C., LLINÀS, R. AND SASAKI, K.: The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. J. Physiol. (London) 182: 268–296, 1966.
47. EISENMAN, G.: Some elementary factors involved in specific ion permeation. In: Proc. 23rd Int. Congr. Physiol. Sci. Tokyo, vol. 4, pp. 489–506, 1965.
48. FALK, G. AND FATT, P.: Linear electrical properties of striated muscle fibres observed with intracellular electrodes. Proc. Roy. Soc. B 160: 69–123, 1964.
49. FATT, P.: The changes in membrane permeability during the inhibitory process. In: Nervous Inhibition, ed. by E. Florey, pp. 87–91, Pergamon Press, Oxford, 1961.
50. FATT, P. AND KATZ, B.: An analysis of the end-plate potential recorded with an intracellular electrode. J. Physiol. 115: 320–369, 1951.
51. FATT, P. AND KATZ, B.: The electric activity of the motor end-plate. Proc. Roy. Soc. B 140: 183–186, 1952.
52. FATT, P. AND KATZ, B.: The effect of inhibitory nerve impulses on a crustacean muscle fibre. J. Physiol. (London) 121: 374–389, 1953.
53. FINKELSTEIN, A. and MAURO, A.: Equivalent circuits as related to ionic systems. Biophys. J. 3: 215–237, 1963.
54. FLOREY, E.: Comparative pharmacology: neurotropic and myotropic compounds. Annu. Rev. Pharmacol. 5: 357–382, 1965.
55. FRANK, K. AND TAUC, L.: Voltage-clamp studies of molluscan neuron membrane properties. In: The Cellular

Functions of Membrane Transport, ed. by J. F. Hoffman, pp. 113–135, Prentice Hall, Englewood Cliffs N.J., 1964.

56. FUKAMI, Y.: Postsynaptic potentials in toads spinal motoneurones due to muscle afferent volleys. Jap. J. Physiol. **11**: 596–604, 1961.
57. FURSHPAN, E. J. AND POTTER, D. D:. Transmission at the giant motor synapses of the crayfish. J. Physiol. (London **145**: 289–325, 1959.
58. FURSHPAN, E. J. AND POTTER, D. D.: Slow postsynaptic potentials recorded from the giant motor fibre of the crayfish. J. Physiol. (London) **145**: 326–335, 1959.
59. FURUKAWA, T. AND FURSHPAN, E. J.: Two inhibitory mechanisms in the Mauthner neurons of goldfish. J. Neurophysiol. **26**: 140–176, 1963.
60. FURUKAWA, T. AND FURUKAWA, A.: Effects of methyl- and ethyl-derivatives of NH_4^+ on the neuromuscular junction. Jap. J. Physiol. **9**: 130–142, 1959.
61. GERSCHENFELD, H. M.: A non-cholinergic synaptic inhibition in the central nervous system of a mollusc. Nature (London) **203**: 415–416, 1964.
62. GERSCHENFELD, H. M. AND CHIARANDINI, D. J.: Ionic mechanism associated with non-cholinergic synaptic inhibition in molluscan neurons. J. Neurophysiol. **28**: 710–723, 1964.
63. GOLDMAN, D. E.: Potential, impedence and rectification in membranes. J. Gen. Physiol. **27**: 37–60, 1943.
64. GRUNDFEST, H.: Heterogeneity of excitable membrane: electrophysiological and pharmacological evidence and some consequences. Ann. N.Y. Acad. Sci. **137**: 901–949, 1966.
65. GRUNDFEST, H. AND REUBEN, J. P.: Neuromuscular synaptic activity in lobster. In: Nervous Inhibition, ed. by E. Florey, Pergamon Press, Oxford, 1961.
66. GRUNDFEST, H., REUBEN, J. P. AND RICKLES, W. H., JR.: The electrophysiology and pharmacology of lobster neuromuscular synapses. J. Gen. Physiol. **42**: 1301–1323, 1959.
67. HAGIWARA, S. AND KUSANO, K.: Synaptic inhibition in giant nerve cell of *Onchidium verraculatum*. J. Neurophysiol. **24**: 167–175, 1961.
68. HAGIWARA, S., KUSANO, K. AND SAITO, S.: Membrane changes in crayfish stretch receptor neuron during synaptic inhibition and under action of gamma-amino butyric acid. J. Neurophysiol. **23**: 505–515, 1960.
69. HAGIWARA, S. AND TASAKI, I.: A study of the mechanism of impulse transmission across the giant synapse of the squid. J. Physiol. (London) **143**: 114–137, 1958.
70. HAGIWARA, S., WATANABE, A. AND SAITO, N.: Potential changes in syncitial neurons of lobster cardiac ganglion. J. Neurophysiol. **22**: 554–572, 1959.
71. HARRIS, E. J. AND HUTTER, O. F.: The action of acetylcholine on the movements of potassium ions in the sinus venosus of the heart. J. Physiol. (London) **133**: 58P–59P, 1956.
72. HODGKIN, A. L.: Ionic movements and electrical activity in giant nerve fibres. Proc. Roy. Soc. B **148**: 1–37, 1958.
72a. HODGKIN, A. L. AND HUXLEY, A. F.: Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. J. Physiol. (London) **116**: 449–472, 1952.
73. HODGKIN, A. L. AND KATZ, B.: The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. (London) **108**: 37–77, 1949.
74. HUTTER, O. F.: Mode of action of autonomic transmitters on the heart. Brit. Med. Bull. **13**: 176–180, 1957.
75. HUTTER, O. F.: Ion movements during vagus inhibition of the heart. In: Nervous Inhibition, ed. by E. Florey, pp. 114–123, Pergamon Press, Oxford, 1961.
76. ITO, M., KOSTYUK, P. G. AND OSHIMA, T.: Further study on anion permeability of inhibitory post-synaptic membrane of cat motoneurones. J. Physiol. (London) **164**: 150–156, 1962.
77. JENKINSON, D. H.: The antagonism between tubocurarine and substances which depolarize the motor end-plate. J. Physiol. (London) **152**: 309–324, 1960.
78. JENKINSON, D. H. AND MORTON, I. K. M.: Effects of noradrenaline and isoprenaline on the permeability of depolarized intestinal smooth muscle to inorganic ions. Nature (London) **205**: 505–506, 1965.
79. JENKINSON, D. H. AND MORTON, I. K. M.: The effect of noradrenaline on the permeability of depolarized intestinal smooth muscle to inorganic ions. J. Physiol. (London) **188**: 373–386, 1967.
80. JENKINSON, D. H. AND MORTON, I. K. M.: The role of α- and β-adrenergic receptors in some actions of catecholamines on intestinal smooth muscle. J. Physiol. (London) **188**:387–402, 1967.
81. JENKINSON, D. H. AND NICHOLLS, J. G.: Contractures and permeability changes produced by acetylcholine in depolarized denervated muscle. J. Physiol. (London) **159**: 111–127, 1961.
82. KANDEL, E. R. AND TAUC, L.: Anomalous rectification in the metacerebral giant cells and its consequences for synaptic transmission. J. Physiol. (London) **183**: 287–304, 1966.
83. KATZ, B.: The transmission of impulses from nerve to muscle, and the subcellular unit of synaptic action. Proc. Roy. Soc. B **155**: 455–479, 1962.
83a. KATZ, B.: Nerve, Muscle, and Synapse, McGraw Hill, London, 1966.
84. KENNEDY, D. AND EVOY, W. H.: The distribsion of pre- and post-synaptic inhibition at crustacean neuromuscular junctions. J. Gen. Physiol. **49**: 457–468, 1966.
85. KERKUT, G. A.: Biochemical aspects of invertebrate nerve cells. In: Invertebrate Nervous Systems, ed. by C. A. G. Wiersma, University Press, Chicago, 1967.
86. KERKUT, G. A. AND MEECH, R. W.: The internal chloride concentration of H and D cells in the snail brain. Comp. Biochem. Physiol. **19**: 819–832, 1966.
87. KERKUT, G. A. AND THOMAS, R. C.: The effect of anion injection and changes in the external potassium and chloride concentration on the reversal potentials of the IPSP and acetylcholine. Comp. Biochem. Physiol. **11**: 199–213, 1964.

88. KERKUT, G. A. AND WALKER, R. J.: The effects of L-glutamate, acetylcholine and GABA on the miniature end-plate potentials and contractures of the coxal muscles of the cockroach *Periplaneta americana*. Comp. Biochem. Physiol. **17**: 435–454, 1966.
89. KEYNES, R. D.: Chloride in the squid giant axon. J. Physiol. (London) **169**: 690–705, 1963.
90. KOKETSU, K. AND NISHI, S.: Restoration of neuromuscular transmission in sodium-free hydrazinium solution. J. Physiol. (London) **147**: 239–252, 1959.
91. KRNJEVIC, K. AND SCHWARTZ, S.: Is γ-aminobutyric acid an inhibitory transmitter? Nature (London) **211**: 1372–1374, 1966.
92. KUFFLER, S. W. AND EYZAGUIRRE, C.: Synaptic inhibition in an isolated nerve cell. J. Gen. Physiol. **39**: 155–184, 1955.
93. KURIYAMA, H.: The influence of potassium sodium and chloride on the membrane potential of the smooth muscle of taenia coli. J. Physiol. (London) **166**: 15–28, 1963.
94. KUSANO, K. AND HAGIWARA, S.: On the integrative synaptic potentials of *Onchidium* nerve cell. Jap. J. Physiol. **11**: 96–101, 1961.
95. LUNDBERG, A.: Electrophysiology of salivary glands. Physiol. Rev. **38**: 21–40, 1958.
96. MAENO, T.: Analysis of sodium and potassium conductances in the procaine end-plate potential. J. Physiol. (London) **183**:592–605, 1966.
97. MANTHEY, A. A.: The effect of calcium on the desensitization of membrane receptors at the neuromuscular junction. J. Gen. Physiol. **49**: 963–976, 1966.
98. MARTIN, A. R. AND PILAR, G.: Dual mode of synaptic transmission in the avian ciliary ganglion. J. Physiol. (London) **168**: 443–463, 1963.
99. NASTUK, W. L.: Some ionic factors that influence the action of acetylcholine at the muscle end-plate membrane. Ann. N.Y. Acad. Sci. **81**: 317–327, 1959.
100. NASTUK, W. L. AND LIU, J. H.: Muscle postjunctional membrane: change in chemosensitivity produced by calcium. Science **154**: 266–267, 1966.
101. NASTUK, W. L., MANTHEY, A. A. AND GIESSEN, A. J.: Activation and inactivation of postjunctional membrane receptors. Ann. N.Y. Acad. Sci. **137**: 999–1014, 1966.
102. NIEDERGERKE, R.: Movements of Ca in beating ventricles of the frog heart. J. Physiol. (London) **167**: 551–580, 1963.
103. NISHI, S. AND KOKETSU, K.: Electrical properties and activities of single sympathetic neurons in frogs. J. Cell. Comp. Physiol. **55**: 15–30, 1960.
104. RALL, W.: Theory of physiological properties of dendrites. Ann. N.Y. Acad. Sci. **96**: 1071–1092, 1962.
105. ROBINSON, R. A. AND STOKES, R. H.: Electrolyte solutions. 2nd ed. (revised). Butterworths, London, 1965.
106. SCHILD, H. O.: Calcium and the relaxant effect of isoprotorenol in the depolarized rat uterus. Pharmacol. Rev. **18**: 495–501, 1966.
107. SHANES, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. Pharmacol. Rev. **10**: 59–273, 1958.
108. SHUBA, M. F.: The influence of adrenalin on the electronus of smooth muscle. Sechenov J. Physiol., USSR **47**: 109–113, 1961.
109. STEPHENSON, R. P.: A modification of receptor theory. Brit. J. Pharmacol. **11**: 379–393, 1956.
110. TAKEUCHI, A. AND TAKEUCHI, N.: Active phase of frog's end-plate potential. J. Neurophysiol. **22**: 395–411, 1959.
111. TAKEUCHI, A. AND TAKEUCHI, N.: On the permeability of the end-plate membrane during the action of the transmitter. J. Physiol. **154**: 52–67, 1960.
112. TAKEUCHI, A. AND TAKEUCHI, N.: Localized action of gamma-aminobutyric acid on the crayfish muscle. J. Physiol. (London) **177**: 225–238, 1965.
113. TAKEUCHI, A. AND TAKEUCHI, N.: A study of the inhibitory action of γ-aminobutyric acid on neuromuscular transmission in the crayfish. J. Physiol. (London) **183**: 418–432, 1966.
114. TAKEUCHI, A. AND TAKEUCHI, N.: On the permeability of the presynaptic terminal of the crayfish neuromuscular junction during synaptic inhibition and the action of γ-aminobutyric acid. J. Physiol. (London) **183**: 433–449, 1966.
115. TAKEUCHI, N.: Some properties of conductance changes at the end-plate membrane during the action of acetylcholine. J. Physiol. (London) **167**: 128–140, 1963.
116. TAKEUCHI, N.: Effects of calcium on the conductance change of the end-plate during the action of the transmitter. J. Physiol. (London) **167**: 141–155, 1963.
117. TAUC, L.: Processus post-synaptiques d'excitation et d'inhibition dans le soma neuronique de l'aplysie et de l'escargot. Arch. Ital. Biol. **96**: 78–110, 1958.
118. TAUC, L. AND GERSCHENFELD, H. M.: A cholinergic mechanism of inhibitory synaptic transmission in a molluscan nervous system. J. Neurophysiol. **25**: 236–262, 1962.
119. TAYLOR, D. B., CREESE, R., NEDERGAARD, O. A. AND CASE, R.: Labelled depolarizing drugs in normal and denervated muscle. Nature (London) **208**: 901–902, 1965.
120. TAYLOR, D. B. AND NEDERGAARD, O. A.: Relation between structure and action of quaternary ammonium neuromuscular blocking agents. Physiol. Rev. **45**: 523–554, 1965.
121. THESLEFF, S.: Effects of motor innervation on the chemical sensitivity of skeletal muscle. Physiol. Rev. **40**: 734–752, 1960.
122. THESLEFF, S. AND QUASTEL, D. M. J.: Neuromuscular pharmacology. Annu. Rev. Pharmacol. **5**: 263–284, 1965.
123. TOMITA, T.: Electrical responses of smooth muscle to external stimulation in hypertonic solution. J. Physiol. **183**: (London) 450–468, 1966.
124. TOMITA, T.: Electrical properties of the smooth muscle of the guinea-pig vas deferens. J. Physiol. (London) **186**: 9P–10P, 1966.

125. Trautwein, W.: Generation and conduction of impulses in the heart as affected by drugs. Pharmacol. Rev. 15: 277–332, 1963.
126. Trautwein, W. and Dudel, J.: Zum Mechanismus der Membranwirkung des Acetylcholin an der Hersmuskelfasser. Pflügers Arch. Gesamte Physiol. 266: 324–334, 1958.
127. Usherwood, P. N. R. and Grundfest, H.: Peripheral inhibition in skeletal muscle of insects. J. Neurophysiol. 28: 497–518, 1965.
128. Volle, R. L.: Modification by drugs of synaptic mechanisms in autonomic ganglia. Pharmacol. Rev. 18: 839–869, 1966.
129. Wallin, B. G.: Simultaneous determination of membrane potential and intracellular ion concentrations in single nerve axons. Nature (London) 212: 521–522, 1966.
130. Waser, P. G.: Die cholinergischen Receptoren der Muskelendplatten. Pflügers Arch. Gesamte Physiol. 274: 431–446, 1962.