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Nature of the calcium dependent potassium leak induced by (+)-propranolol, and its possible relevance to the drug's antiarrhythmic effect

I. M. GLYNN AND ANNE E. WARNER

Physiological Laboratory, University of Cambridge, and Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, London

Summary

1. Manninen (1970) recently reported that in the presence of calcium ions, (+)-propranolol causes a massive loss of potassium ions from human red cells, together with a marked inward 'counter-transport' of potassium ions from the medium.

2. This paper shows, theoretically, that counter-transport of the kind he observed may be accounted for by the setting up of a diffusion potential across the cell membrane and does not imply that, under the influence of propranolol, potassium ions cross the membrane by some process other than free diffusion.

3. The physiological and pharmacological significance of this interpretation of Manninen's findings is briefly discussed.

Introduction

When propranolol is added to warmed suspensions of human red cells in media with an electrolyte composition similar to that of plasma, the cells become highly permeable to potassium ions though the permeability to sodium ions is increased very little (Manninen, 1970). The effect is more marked with (+)-propranolol than with the (−)-form or the racemic mixture; it does not occur unless calcium ions are present in the medium, and it is antagonized by extracellular magnesium ions in high concentration (Manninen, 1970). In the selectivity for potassium and the need for calcium this effect of propranolol on red cells is strikingly reminiscent of the effects of fluoride (Wilbrandt, 1940; Gárdos, 1959; Lepke & Passow, 1960) of iodoacetate plus adenosine (Gárdos, 1956, 1958a, b, 1959), of iodoacetate after prolonged starvation (Blum & Hoffman, 1970), of prolonged starvation alone (Hoffman, 1966) and of triose reductone (Passow, 1964).

A further similarity seems to be the marked 'counter-transport' (Wilbrandt & Rosenberg, 1961) that is associated with the leakage of potassium from the cells. In Manninen's experiments, when (+)-propranolol was added to cells incubated in an equal volume of medium containing 6 mM potassium labelled with ^{42}K, the bulk outflow of K was accompanied by an entry of ^{42}K so rapid that 5 min after the addition of the drug the ratio (quantity of ^{42}K in cells)/(quantity of ^{42}K in an equal volume of medium) was about 3:1 and already falling rapidly. Counter-transport of ^{42}K during the leakage of potassium from stored cells treated with iodoacetate has recently been reported by Blum & Hoffman (1970). Both Manninen and Blum & Hoffman regard the existence of counter-transport as evidence that the leakage of

potassium is not brought about by free diffusion of potassium ions through the cell membrane. Their argument would be valid if one could be sure that the inward movement of ^{42}K ions, which was certainly up a concentration gradient, was also up an electrochemical potential gradient. An alternative interpretation of the experimental results, however, is that the net outward movement of potassium ions so hyperpolarized the membrane that the inward movement of ^{42}K was thermodynamically downhill. Manninen considered this possibility but rejected it on the grounds (i) that the very high permeability of the red cell membrane to small anions would prevent a potassium diffusion potential from being set up, and (ii) that ' no abrupt change in the distribution of bromide ions could be demonstrated '. The primary purpose of this note is to show that rejection on these grounds is not justified, and that the setting up of a diffusion potential is a likely explanation of the counter-transport observed by Manninen—and perhaps also of the counter-transport observed by Blum & Hoffman, though details of their experiments are not available.

Theory

Since the observations of Chappell & Crofts (1966) on the effects of valinomycin on red cells, it has been clear that the rate at which small anions can diffuse through the red cell membrane is much less than the rate at which internal and external anions may be exchanged for each other. Very recently, Hunter (1971) has made a careful study of the effects of external potassium concentration on the potassium loss induced by valinomycin, and, by assuming a constant electric field through the thickness of the red cell membrane, he has been able to estimate the non-exchange-restricted chloride permeability. He calculates that the rate constant for chloride loss by free diffusion is about 0·04 min^{-1}, equivalent to a chloride permeability of $3{\cdot}8 \times 10^{-8}$ cm/s, that is about four orders of magnitude less than the Cl^-–Cl^- exchange permeability.

The question to be considered is: if the true chloride permeability is as low as Hunter's calculations suggest, can the counter-transport observed by Manninen be explained electrically without assuming impossibly high values for the potassium permeability?

For a suspension of red cells at any time, the rate of change in the concentration relative to cell water of any internal ion X is given by

$$\frac{dX_i}{dt} = -\varphi_x \frac{A}{V} - \frac{X}{V}\frac{dV}{dt} \tag{1}$$

where φ_x is the net outward flux of the ion per unit area of cell membrane, A is the total area of cell membrane and V is the volume of cell water at that time. The rate of change in the concentration of ions of the same species in the external solution is given by

$$\frac{dX_o}{dt} = -\frac{dX_i}{dt}\frac{V}{V_s - V} \tag{2}$$

where V_s is the total volume of water in the system. Because the cells are freely permeable to water, a net transfer of ions from one side of the membrane to the other will be accompanied by the movement of sufficient water to keep both solutions isotonic. Ignoring the effects of changes in pH on the ionization of haemoglobin,

the total concentration of osmotically active particles in the red cell should, ideally, remain constant at approximately 300 mosmol/litre. We may therefore write

$$\frac{dV}{dt} = -\frac{A}{300}\Sigma\varphi_x \tag{3}$$

To solve these equations we need expressions for φ_x. For K, ^{42}K, and Cl, we assume that under the conditions of Manninen's experiments the net fluxes were virtually entirely the result of free diffusion, the active influx of potassium being negligible compared with the passive fluxes. Assuming, arbitrarily, a constant electric field through the thickness of the membrane (cf. Goldman, 1943) we may write

$$\varphi_K = -P_K \frac{EF}{RT} \frac{K_i \exp(EF/RT) - K_o}{1 - \exp(EF/RT)} \tag{4}$$

$$\varphi_{42K} = -P_K \frac{EF}{RT} \frac{{}^{42}K_i \exp(EF/RT) - {}^{42}K_o}{1 - \exp(EF/RT)} \tag{5}$$

$$\varphi_{Cl} = -P_{Cl} \frac{EF}{RT} \frac{Cl_o - Cl_i \exp(-EF/RT)}{1 - \exp(-EF/RT)} \tag{6}$$

$$\text{where } E = -\frac{RT}{F} \ln \frac{P_K(K_i + {}^{42}K_i) + P_{Na} Na_i + P_{Cl} Cl_o}{P_K(K_o + {}^{42}K_o) + P_{Na} Na_o + P_{Cl} Cl_i} \tag{7}$$

For sodium the contribution of the pump flux will not be negligible, but it can be approximated by an expression of the form $\alpha\ Na_i/(\beta + Na_i)$ where α is the rate of pumping in the presence of saturating concentrations of internal sodium and β is the intracellular Na concentration at which pumping is at half the maximal rate. Therefore

$$\varphi_{Na} = -P_{Na} \frac{EF}{RT} \frac{Na_i \exp(EF/RT) - Na_o}{1 - \exp(EF/RT)} + \frac{\alpha\ Na_i}{\beta + Na_i} \tag{8}$$

Solutions to the set of 8 equations can be computed by setting the boundary conditions and choosing values for P_K, P_{Na}, P_{Cl}, α and β.

Results

Figure 1 shows the results of some computations of this kind. For the purpose of these computations it was assumed that at zero time:

^{42}K was present only in the external solution

$V/V_g = 1/2{\cdot}43$ (equivalent to the haematocrit of 50% used in Manninen's experiments)

$K_i = 136$ mmol/l. cell water

$K_o = 4{\cdot}8$ mM

$Cl_i = 107$ mmol/l. cell water

$Cl_o = 150$ mM

$Na_i = 10$ mmol/l. cell water

$Na_o = 146$ mM.

The values of the various constants were set as follows:

$A = 1{\cdot}63 \times 10^4$ cm^2 per cm^3 of packed cells. This area was assumed to remain constant despite changes in cell volume.

$\alpha = 1{\cdot}7 \times 10^{-7}$ (μmol/cm^2)/s

$\beta = 25$ mmol/l. cell water

$P_{Na} = 6{\cdot}6 \times 10^{-10}$ cm/s

$P_{Cl} = 3{\cdot}8 \times 10^{-8}$ cm/s

$P_K = 1$, 3, 10, 30 or $100 \times P_{Cl}$.

The curves in Fig. 1 show that if, in the presence of propranolol, the permeability to potassium ions increases to 10 or more times the permeability to chloride ions, the ratio (quantity of ^{42}K in cells)/(quantity of ^{42}K in an equal volume of medium) peaks sharply within the first few minutes of the experiment. The greater the ratio of permeabilities, the higher the peak and the earlier it occurs. This peaking is, of course, the result of the inward driving force on potassium ions provided by the transient hyperpolarization of the cell membrane. In normal human red cells the membrane potential is generally assumed to equal the chloride equilibrium potential, which, for cells incubated in a medium containing 146 mM NaCl and 4·8 mM KCl, is about −9 mV (inside negative). If the permeability to potassium ions is instantaneously increased to a high value, the membrane potential will move towards

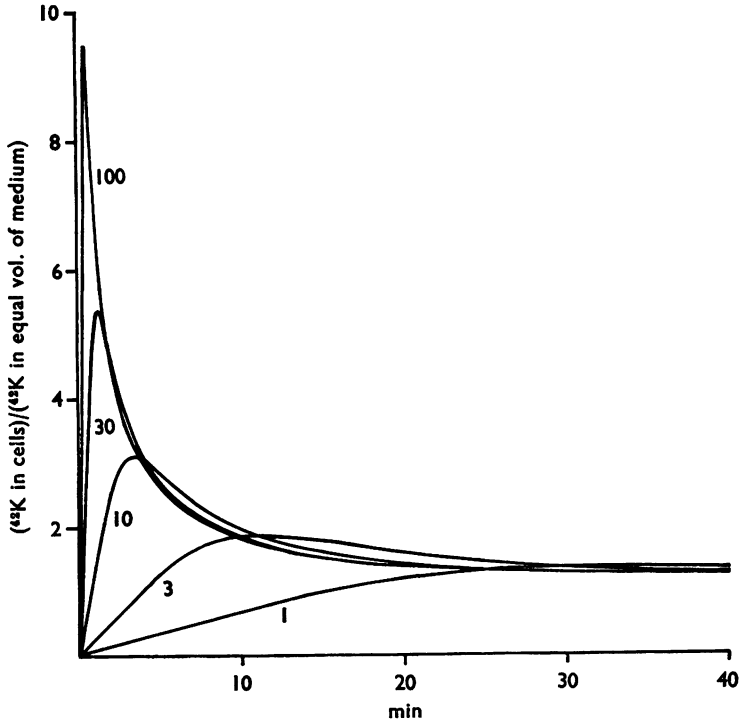

FIG. 1. Computed values of the ratio (quantity of ^{42}K in cells)/(quantity of ^{42}K in an equal volume of medium) after a sudden increase in K permeability from its normal very low value to 1, 3, 10, 30 or 100 times the chloride permeability ($3{\cdot}8 \times 10^{-8}$ cm/s). The increase in K permeability is supposed to occur at zero time. By 40 min the cells have lost just over half their water, and the value of 1·25 for the ratio (quantity of ^{42}K in cells)/(quantity of ^{42}K in an equal volume of medium) then corresponds to a value of 2·4 for the ratio (concentration of ^{42}K in cell water)/(concentration of ^{42}K in medium). For further details see text.

the potassium equilibrium potential, which is about −90 mV. If the ratio P_K/P_{Cl} is set equal to 100, 30, 10, 3 and 1, the calculated membrane potentials are respectively −84 mV, −75 mV, −61 mV, −41 mV and −25 mV. As potassium and chloride ions leave the cell, the membrane potential will fall until the distribution of potassium and chloride ions reaches a Donnan equilibrium. For the starting conditions considered in the computation, this equilibrium is reached when $[K]_i/[K]_o = [Cl]_o/[Cl]_i = 2{\cdot}4$, and the membrane potential is about −23 mV. (The terms $[K]_i$ and $[Cl]_i$ refer to concentrations in cell water.) By the time equilibrium is reached, just over half of the original cell water will have been lost. The computation shows that if P_K is 10^{-7} cm/s, or more, the equilibrium is approached closely within 40 minutes. With lower values of P_K the same equilibrium is approached more slowly, unless P_K is so low that equations (4) and (5) are invalid because the active inward transport of potassium cannot be neglected in comparison with the passive movements. In this situation—which is, of course, the situation in normal red cells—a steady state is reached with $[K]_i/[K]_o > [Cl]_o/[Cl]_i$.

The starting conditions assumed in the computation are approximately the same as in Manninen's experiments except that both bicarbonate and chloride are treated as chloride. No attempt has been made to find the best fit to Manninen's results, chiefly because the theoretical curves differ most in the period before Manninen took his first sample (5 min). In comparing theoretical and experimental curves it is important to note, however, that there is no reason to assume that the ratio observed experimentally at 5 min was the maximum ratio, as the continuous lines in Manninen's figures suggest. It might be worth attempting to obtain measurements of the effects of propranolol over much shorter periods, but such measurements would be technically difficult and a strict comparison with the theoretical curves would, anyway, be valid only if the change in permeability produced by propranolol was known to be complete within a period much shorter than the period preceding the first time point. In comparing theoretical and experimental curves, it is also worth noting that when red cells lose potassium and chloride, the observed changes in volume depart greatly from the changes predicted assuming ideal osmotic behaviour. In an experiment of Manninen's in which the cells lost 50 mmol of potassium and gained 5 mmol of sodium per litre of cells, the decrease in volume was only 22% instead of an expected 30%. A further difficulty in making detailed comparisons between the theoretical and the published experimental curves is that there are minor differences between Manninen's individual experiments. For example, with 0·5 mM (+)-propranolol the rapid loss of potassium ions, measured by flame photometry, appeared to be complete when the ratio (mmol K/l. cells)/(mmol K/l. medium) reached 1·4 ; in a different experiment, in which radioactive potassium was used, the final ratio (mmol ^{42}K/l. cells)/(mmol ^{42}K/l. medium) was near 1.

Without making detailed comparisons, however, it is clear that the sort of countertransport Manninen observed could be the result of the setting up of a diffusion potential. The fact that he was unable to detect abrupt changes in bromide distribution is not incompatible with this hypothesis, since the mechanism responsible for the very rapid exchange of small anions would have ensured that bromide was distributed across the membrane in the same ratio as chloride whatever the membrane potential may have been.

If this interpretation of Manninen's results is correct, his experiments show that, in the presence of Ca, 0·5 mM (+)-propranolol causes red cell membranes to become

selectively leaky to potassium ions, with a permeability of at least 4×10^{-7} cm/s and possibly very much more.

Discussion

The close resemblance between this effect of propranolol and the effects of fluoride, iodoacetate plus adenosine, and so on mentioned earlier, points to a common mechanism. Whittam (1968) suggested that the increase in potassium permeability in cells treated with fluoride or iodoacetate was secondary to a net entry of calcium ions resulting from the reduced activity of the outwardly directed calcium pump. This view is supported by the experiments of Lew (1970, 1971) and of Romero & Whittam (1971), and Lew has wondered whether the 'ATP-dependent mechanism that keeps the red cells virtually Ca^{2+}-free exerts a physiological control on the potassium permeability'. In this connexion it is interesting that van Rossum (1970) has evidence suggesting that the entry of calcium ions into liver cells makes the cells leaky to potassium, and Meech & Strumwasser (1970) have shown that calcium ions injected into *Aplysia* neurones increase the potassium permeability of the neuronal membrane. An apparent difference between the potassium leak induced by propranolol in fresh red cells and the potassium leak induced by iodoacetate in energy-depleted cells is that the latter may be strongly inhibited by ouabain (Blum & Hoffman, 1970), though no such inhibition was found by Romero & Whittam (1971). Both the inhibition and the disagreement may perhaps be explained by a suggestion of Lew (personal communication). He points out that by inhibiting the sodium pump ouabain would conserve adenosine triphosphate and, if conditions were critical, might therefore prevent the uptake of calcium that follows complete ATP depletion.

Relevance to pharmacological actions

It is worth considering whether the selective increase in potassium permeability produced by (+)-propranolol in red cells could, if produced in other tissues, contribute to the drug's antiarrhythmic or local anaesthetic effects.

Noble (1962) has shown that the pacemaker potential of Purkinje fibres is likely to be very sensitive to changes in potassium permeability, a 10% increase in resting potassium permeability completely abolishing pacemaker activity in his computed action potentials. The membrane resistance of Purkinje fibres during diastole is about 2,000 Ωcm^2, which is equivalent to a P_K of about 8×10^{-6} cm/s if potassium ions carry nearly all the current. A 10% increase therefore corresponds to a ΔP_K of about 8×10^{-7} cm/s which is roughly $20\times$ Hunter's estimate of the red cell chloride permeability and therefore of the same order of magnitude as the minimum estimate of potassium permeability necessary to account for the counter-transport observed by Manninen. Manninen used concentrations of propranolol about two orders of magnitude greater than those known to have antiarrhythmic effects in mammalian hearts (Howe & Shanks, 1966; Lucchesi, Whitsitt & Brown, 1966; Lucchesi, Whitsitt & Stickney, 1967; Parmley & Braunwald, 1967; Barrett & Cullum, 1968), and it will obviously be necessary to make careful measurements of the effects of low concentrations of propranolol on potassium permeability in mammalian cardiac tissue. In preliminary experiments, on frog atria, using intracellular microelectrodes, we have found that (±)-propranolol at a concentration of 2×10^{-5} M causes a rapid and reversible drop in input resistance of about 12%. The geometry of the preparation makes it difficult to correct for the effects of

current spread, which will cause the magnitude of any conductance change to be underestimated. It is not certain that the fall in input resistance is caused by an increase in potassium conductance, but that is the most likely explanation. An increase in potassium permeability is also suggested by the accelerated repolarization of dog Purkinje fibres observed by Davis & Temte (1968) with concentrations of (±)-propranolol as low as 3×10^{-6} M; that is, concentrations sufficient to have an antiarrhythmic effect but insufficient to diminish the rate of rise or the magnitude of the overshoot of the action potential. Davis & Temte did not test the action of propranolol on Purkinje fibres under conditions in which they would be expected to show pacemaker activity. The fact that propranolol does not hyperpolarize unstimulated Purkinje fibres is not evidence against an increase in potassium permeability, since, under the conditions of Davis & Temte's experiments, the hyperpolarization to be expected as a result of a 10% increase in potassium conductance is less than 1 mV. (*Cf.* the accelerated repolarization, without change in resting potential, brought about by vagal stimulation in frog auricles—see Fig. 9 of Hutter & Trautwein, 1956.) Unfortunately, though Davis & Temte's results point to an increase in potassium permeability, they do not enable one to decide whether that increase represents a change in background potassium permeability or, conceivably, an effect of propranolol on Noble & Tsien's (1968) 'i_{K_2} system'. Hauswirth, Noble & Tsien (1968) found that pronethalol, which has similar antiarrhythmic properties to propranolol (Sekiya & Vaughan Williams, 1963), reversed the effect of adrenaline on the i_{K_2} system in sheep Purkinje fibres, but they give no information about the effects of pronethalol alone, and its action might merely have been the result of its β-adrenoceptor blocking activity.

If propranolol increases potassium permeability by increasing calcium entry, it may be asked why it decreases the force of contraction of heart muscle (Parmley & Braunwald, 1967) when studies of the staircase phenomenon (Niedergerke, Page & Talbot, 1969) and the therapeutic action of cardiac glycosides (see Glynn, 1969) suggest that an increase in calcium content is associated with an increased force of contraction. We know too little about the distribution of calcium within cardiac muscle to answer this question satisfactorily. Possibly the potassium permeability depends on the calcium-loading of particular sites within the membrane, whereas the force of contraction depends on the amount of calcium released from some other store.

The local anaesthetic effect of propranolol is unlikely to be the result of an increase in potassium permeability, since, although a sufficient increase in the potassium permeability of the axon membrane would block conduction by holding the membrane potential near the potassium equilibrium potential, the very high membrane conductances found in myelinated fibres—see, for example, the data of Frankenhaeuser & Huxley (1964) on *Xenopus*—make the magnitude of the requisite potassium permeability improbably large.

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