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INFLUENCE OF (DL)-PROPRANOLOL AND Ca^{2+} ON MEMBRANE POTENTIAL AND AMINO ACID TRANSPORT IN EHRLICH ASCITES TUMOR CELLS

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Summary

(1) (DL)-Propranolol and Ca^{2+} are shown to alter the transmembrane potential difference of Ehrlich ascites tumor cells as measured by means of the cyanine dye, 3,3′-dipropyl-2,2′-thiodicarbocyanine iodide, whose fluorescent intensity changes as a function of membrane potential.

(2) The changes in membrane potential elicited by these agents are dependent on the external K^+ concentration in a manner which suggests that the potential changes result from a specific increase in the permeability of the plasma membrane to K^+.

(3) Na^+-dependent amino acid transport in the presence of propranolol can be modulated by varying the external K^+ concentration (K_o^+). The initial rate of uptake is stimulated by propranolol at low K_o^+ and inhibited at high K_o^+. The change in transport rate is nearly directly proportional to the natural logarithm of $[K^+]_o$ in the presence of propranolol.

(4) ATP depletion of the cells by preincubation with rotenone abolishes the changes in fluorescence and amino acid uptake seen with propranolol as a function of K_o^+. Restoration of cellular ATP with glucose in presence of Ca^{2+} restores both fluorescence and amino acid transport changes which occur in response to propranolol.

(5) The fluorescence changes and amino acid transport changes in response to propranolol are pH dependent, with little effect seen at pH 6.

(6) It is concluded that the rate of Na^+-dependent amino acid uptake is a function of membrane potential and is dependent on the electrochemical potential difference for Na^+.

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Introduction

Propranolol, an adrenergic β-receptor blocking agent, induces human red cells to lose K^+. The outflow of K^+, which is enhanced when external Ca^{2+} is raised, is not accompanied by an equivalent increase in Na^+ uptake. In fact, the permeability change is highly specific for K^+, while Na^+ permeability is little, if at all, affected at low cellular Ca^{2+} levels [1—4]. At elevated Ca^{2+} levels (greater than 20 μM) permeability to other ions such as Na^+ may be increased in the presence of propranolol [2]. The effect of propranolol on red cell K^+ permeability may be related to the drug's antiarrhythmic properties [2,5,6]. Propranolol has been shown in some cases to compete for negatively charged fixed sites on the internal aspect of cell membranes resulting in a release of bound Ca^{2+} [2] which, in turn, plays an important role in the regulation of the membrane permeability to Na^+ and K^+ in red cells [7] and nerve cells [8,9].

The effects of propranolol on red cells bear a close resemblance to those of Ca^{2+} in ATP-depleted cells obtained by incubation with fluoride, iodoacetate and adenosine [10] or by prolonged starvation [11]. This mechanism has been extensively studied and it has been suggested that the increase in K^+ permeability in cells depleted of ATP is a direct result of an increase of internal Ca^{2+} [7, 12—14].

A further ramification of the effects of these agents should be an influence upon the electrical properties of the membrane. Ca^{2+}-induced changes in membrane potential have been observed in HeLa cells [15] and *Amphiuma* red cells [16—18] as well as nerve cells [9], as determined by means of microelectrode impalement.

This communication describes studies on the effects of propranolol and Ca^{2+} on the membrane potential of Ehrlich ascites tumor cells determined by means of the cyanine dye, 3,3′-dipropyl-2,2′-thiodicarbocyanine iodide and the consequent alteration of amino acid transport by propranolol under a variety of ionic and metabolic conditions. The fluorescence of several cyanine dyes has been shown to be sensitive to changes in membrane potential in a number of preparations including the Ehrlich ascites tumor cell [19] and has been recently reviewed in detail [20].

A preliminary account of a portion of this work has been presented elsewhere [21].

Methods

The procedures for maintenance and harvesting [22] as well as for preparation of Ehrlich cells for use in measurements of fluorescence have been previously described in detail [19]. Na^+-Ringer solution contained 154 mM NaCl, 6 mM KCl, 1.5 mM $MgSO_4$ and 10 mM sodium phosphate, pH 7.4. In K^+-free Na^+-Ringer solution, NaCl was substituted for KCl. K^+-Ringer and choline-Ringer were prepared by substituting equivalent concentrations of KCl and choline chloride for NaCl. In experiments with Ca^{2+}, the Ringer solution contained 1.2 mM $CaCl_2$ and was buffered with 3 mM sodium phosphate at pH 7.4. In some experiments the buffer employed was 10 mM morpholinopropane sulfonic acid (MOPS) or 10 mM *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid

(HEPES) and brought to pH 7.4 with equimolar Tris (hydroxymethylamino methane) hydroxide or KOH, respectively.

The fluorescent dye, 3,3'-dipropyl-2,2'-thiodicarbocyanine iodide (referred to as "the dye") employed in these studies was obtained from Dr. S. Parsons (University of California, Santa Barbara, Calif.) who synthesized it according to a method described by Sims, Waggoner, Wang and Hoffman [23]. The procedure for measurement of dye fluorescence in cell suspensions has been previously described in detail [24]. The dye was added from a stock solution in ethanol (0.5 mg/ml) such that its final concentration was always $3.0 \cdot 10^{-6}$ M. In general, results from fluorescence experiments are given in relative terms.

DL-Propranolol hydrochloride, ouabain and rotenone were purchased from Sigma, St. Louis, Mo., and valinomycin from Calbiochem, U.S.A.

The transport experiments were carried out as described in previous publications [25]. The conditions were similar to those for the fluorescence measurements except that the cell density was about 1.6% compared to 0.3% for fluorescence.

Results

The addition of propranolol to cell suspensions containing dye results in rapid changes in fluorescence, the rates, magnitudes and directions of which are similar to those which occur upon the addition of valinomycin under the same conditions (see Fig. 1 here and Fig. 1 in ref. 19). The changes in the steady level of the fluorescence of the dye in the presence of propranolol are dependent on external K^+ concentration (Fig. 2), the cells becoming hyperpolarized (indicated by a decrease in fluorescent intensity) at low external K^+ concentrations and depolarized (increase in fluorescent intensity) at high K_o^+.

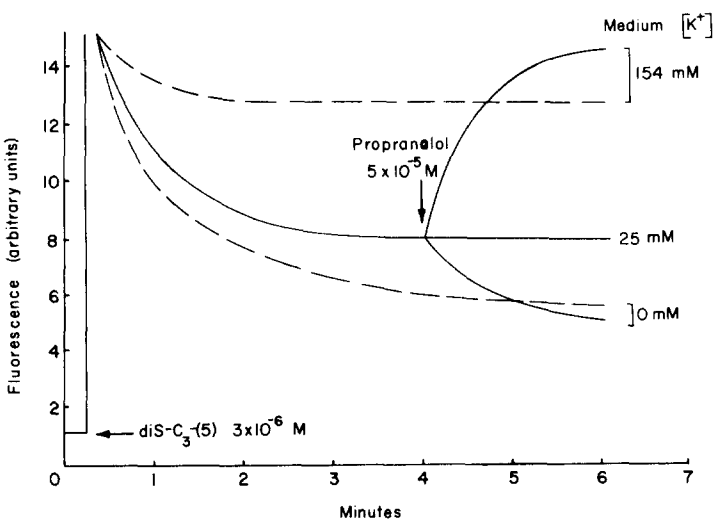

Fig. 1. Fluorescent intensity of the dye ($3.0 \cdot 10^{-6}$ M) with time in a 0.3% suspension of Ehrlich ascites tumor cells. Mixtures of Na^+-Ringer and K^+-Ringer were used to give the external K^+ concentration shown on the figure. Solid lines, propranolol added where indicated; dashed lines, propranolol added immediately before dye. All incubations were done in air at 37°C.

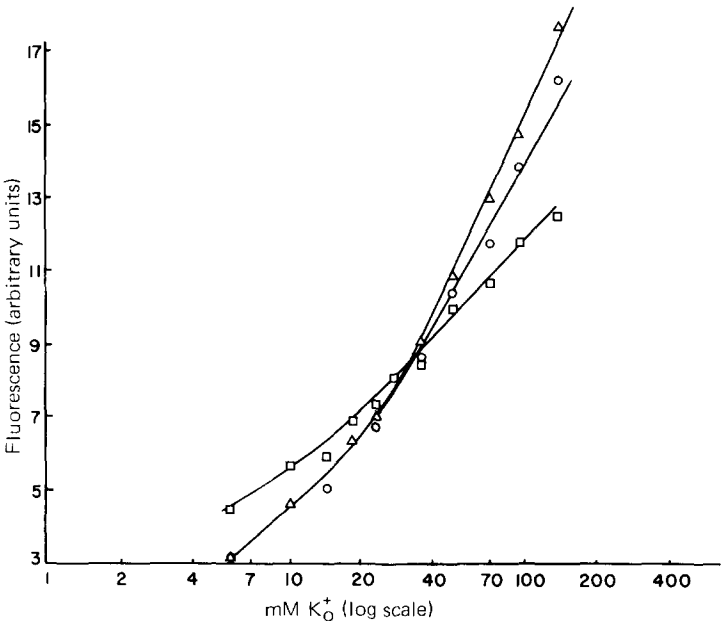

Fig. 2. Constant level of fluorescent intensity of dye ($3.0 \cdot 10^{-6}$ M) in cell suspensions attained in the presence of $1.2 \cdot 10^{-3}$ M Ca^{2+} (□), $5 \cdot 10^{-5}$ M propranolol (○) and $1 \cdot 10^{-6}$ M valinomycin (△) as a function of log K^+ concentration of the medium. Mixtures of Na^+-Ringer and K^+-Ringer each containing 1.2 mM $CaCl_2$ were made to give the external K^+ concentrations shown.

The magnitude of the change in fluorescence of cells in K^+-Ringer is dependent (Fig. 3) on the concentration of propranolol in the range $2—60 \cdot 10^{-6}$ M. Above 10^{-4} M propranolol, interactions are seen (an increase in fluorescent intensity) between propranolol and the dye. Hence the relation between fluorescence and

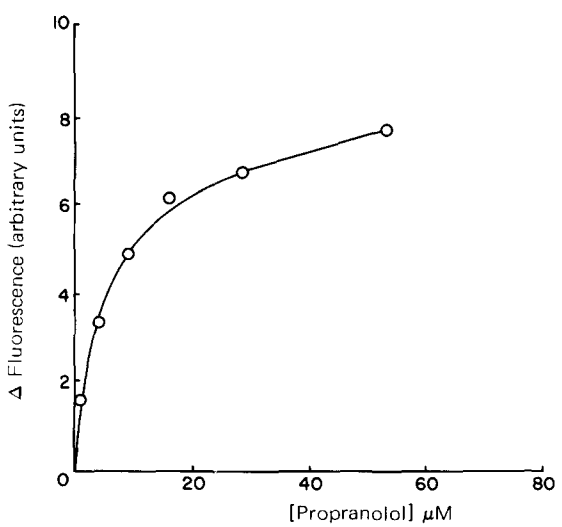

Fig. 3. Constant level of fluorescent intensity of dye attained in the presence of various concentrations of propranolol in K^+-Ringer in air at 37°C.

propranolol concentration could not be studied over a wider range of concentrations. The data in Fig. 3, however, suggest that the phenomenon is a saturable process.

In addition to propranolol, Ca^{2+} induces changes in fluorescence in response to changes in K_o^+. In the absence of Ca^{2+}, alteration of extracellular K^+ does not influence the fluorescence (ref. 19 and this communication). In contrast to propranolol, the Ca^{2+}-induced changes in membrane potential are slow and require incubation with Ca^{2+} for 5—10 min to elicit a full response. The effects of Ca^{2+}, propranolol and valinomycin on fluorescence as a function of K_o^+ are shown in Fig. 2. In these experiments, valinomycin and propranolol were added to cells in Ca^{2+}-containing Ringer solutions. The same results with valinomycin and propranolol are obtained with cells incubated in Ca^{2+}-free Ringer solutions over the same range of K^+ concentrations, thus showing that extracellular Ca^{2+} is not required for the response to propranolol or valinomycin (results not shown). Omission of Mg^{2+} from the medium does not alter the response to propranolol. Lassen et al. [26] have reported that a 15 min incubation of Ehrlich cells in Ca^{2+} containing all K^+-Ringer abolished the transient peak potential (which, according to Lassen et al. [26], represents the true membrane potential) of about 25—35 mV. When transferred back to Ca^{2+}-containing Na^+-Ringer these cells again showed the transient peak potentials upon impalement. These observations are compatible with our fluorescence data and suggest that the membrane potential in Ehrlich cells may or may not be a function of external K^+ depending on the presence or absence of Ca^{2+}. In a recent paper, Burckhardt [27] concluded that Ehrlich cells become depolarized when extracellular K^+ is raised, unlike our original observations [19]. It is interesting to note that Burckhardt's experiments were done in presence of 2.5 mM Ca^{2+}.

The above observations also suggest that propranolol and to a lesser extent, Ca^{2+} produce an increase in the K^+ permeability. A comparison of the per cent change in fluorescence upon addition of propranolol to cells suspended in Ringer solutions where various monovalent cations are substituted for K^+ suggests that the effect of propranolol on membrane potential is highly selective for K^+ and Rb^+. Depolarization in presence of propranolol is seen when K^+ or Rb^+ are substituted for Na^+, whereas with Na^+, Li^+ and Cs^+ hyperpolarization is seen. This observation is consistent with increased K^+ permeability in presence of propranolol. Using $^{86}Rb^+$ as a K^+ substitute [28], it may be seen that there is an increase in ^{86}Rb efflux with propranolol (Fig. 4). (Note the difference in cell densities and higher propranolol concentrations for these efflux measurements than for fluorescence changes.) At 10^{-4} M propranolol, the efflux of ^{86}Rb is double the control rate compared to the 10-fold difference seen with 10^{-3} M propranolol.

Effect of pH

The changes in fluorescent intensity recorded upon addition of either propranolol or Ca^{2+} are influenced by the pH (range 6.0—8.0) of the medium. It is apparent that the effects of propranolol and Ca^{2+} are reduced (or absent) below pH 7.0 (or below pH 8 for Ca^{2+}). With valinomycin, however, neither the leveling-off point following the addition of dye, nor the change in fluorescence intensity varies appreciably over the same pH range (Table I).

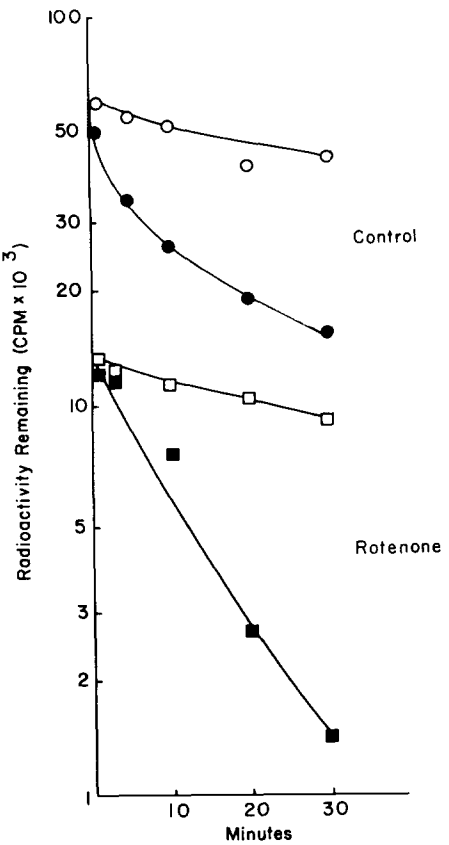

Fig. 4. Action of propranolol on ^{86}Rb efflux. Cells were incubated with ^{86}Rb (1 μCi/ml medium) for 10 min in Na^+-Ringer containing 156 mM NaCl, 4 mM KCl, 1.5 mM $MgSO_4$, 1.5 mM phosphate and 10 mM Na^+-HEPES buffer (pH 7.4) at 37°C. The cytocrit was 8% during the loading period. After 10 min, rotenone (32 μg/ml) was added to one flask and the incubation continued. After 30 min the cells without rotenone (marked control) were centrifuged, washed once in isotonic choline chloride, centrifuged and then divided into two equal portions suspended in 1 ml of isotonic Ringer solution. The cells were introduced into fresh medium containing 148 mM NaCl, 12 mM KCl, 1.5 mM $MgSO_4$, 1.5 mM phosphate and 10 mM HEPES buffer (pH 7.4) at 37°C in the presence (●) and absence (○) of 10^{-3} M propranolol. Rotenone-treated cells were handled in an identical way except that the pre-incubation period was longer (40 min). Rotenone was present at the same concentration during efflux which was measured in the presence (■) and absence (□) of 10^{-3} M propranolol. Cytocrit was 1.0% during efflux. 2-ml samples were taken at intervals and injected into 10 ml ice-cold choline chloride to stop the reaction. The cells were extracted with 5% trichloroacetic acid and the supernatant fluid was counted.

Effect of ATP depletion

Cells depleted of ATP by incubation with rotenone (32 ng/ml) at 37°C do not exhibit changes in fluorescence upon the addition of propranolol. With time of exposure to rotenone, the change in fluorescence at low K_o^+ (i.e., hyperpolarization) upon the addition of propranolol becomes progressively smaller and finally nonexistent after 20—25 min in Ca^{2+}-free Na^+-Ringer, and after 45—50 min in Na^+-Ringer containing 1.2 mM Ca^{2+} (Fig. 5). The diminution in response to propranolol in rotenone-treated cells is not due to depletion of cell K^+ since neither the depolarizing action at high K_o^+ nor the polarizing action at low K_o^+ are seen. The former response would be expected to increase when

TABLE I

INFLUENCE OF pH ON PERCENT CHANGES IN FLUORESCENCE OF A 0.3% SUSPENSION OF CELLS WITH DYE IN THE PRESENCE OF Ca^{2+}, PROPRANOLOL AND VALINOMYCIN

Cells were suspended in Na^+- or K^+-Ringer. Non-equilibrated: cells maintained at 1 : 20 dilution at room temperature (21—22°C) and diluted to 1 : 300 before dye addition. Pre-equilibrated: cells diluted 1 : 300 and incubated at 37°C for 30 min before dye addition. Fluorescence measurements made at 31°C.

Medium	pH	Percent change in fluorescence				
		Non-equilibrated cells		Pre-equilibrated cells		
		Propranolol (10^{-4} M)	Val (10^{-6} M)	Propranolol (10^{-4} M)	Ca^{2+} (2.5 mM)	Val (10^{-6} M)
K^+-Ringer	8.0	+57	+78	+44	+10	+26
	7.0	+47	+76	+35	+4	+26
	6.0	+13	+81	+2	0	+23
Na^+-Ringer	8.0	—39	—31	—28	—31	—32
	7.0	—42	—45	—26	—5	—39
	6.0	—6	—42	0	0	—27

cell K^+ decreased. Control cells incubated under the same conditions, but without rotenone, exhibit a rapid decrease in fluorescence (hyperpolarization) upon addition of propranolol. When ATP levels are restored to about 1/3 the original level by subsequent addition of 10 mM glucose, and if Ca^{2+} is present, the response to propranolol reappears. Ouabain (1 mM) did not have any effect on the Ca^{2+}- or propranolol-induced changes in membrane potential, making it unlikely that the Ca^{2+} or propranolol-induced fluorescence changes are associated with the (Na^+ + K^+)-ATPase.

Action of propranolol on amino acid uptake

Since studies of fluorescence intensity indicate that the membrane potential is a function of medium K^+ concentration in the presence of propranolol

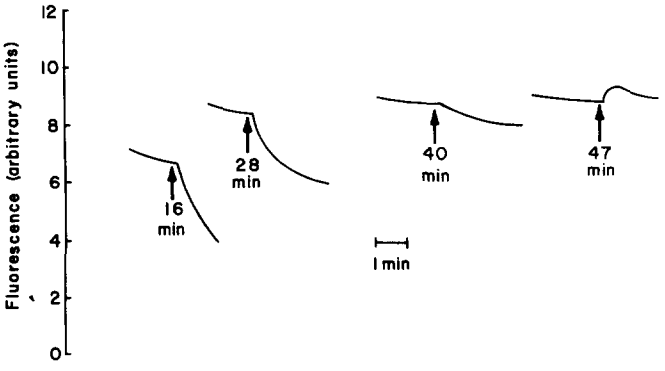

Fig. 5. Influence of rotenone on the response to propranolol. Rotenone (32 ng/ml) was added to a 0.3% suspension of cells in Na^+-Ringer containing 1.2 mM $CaCl_2$ at zero time. Periodically the suspension was sampled; dye was added and the fluorescence was recorded. The times given above indicate the time when propranolol ($5 \cdot 10^{-5}$ M) was added. Temperatures of incubation and fluorescence measurements as in Table I.

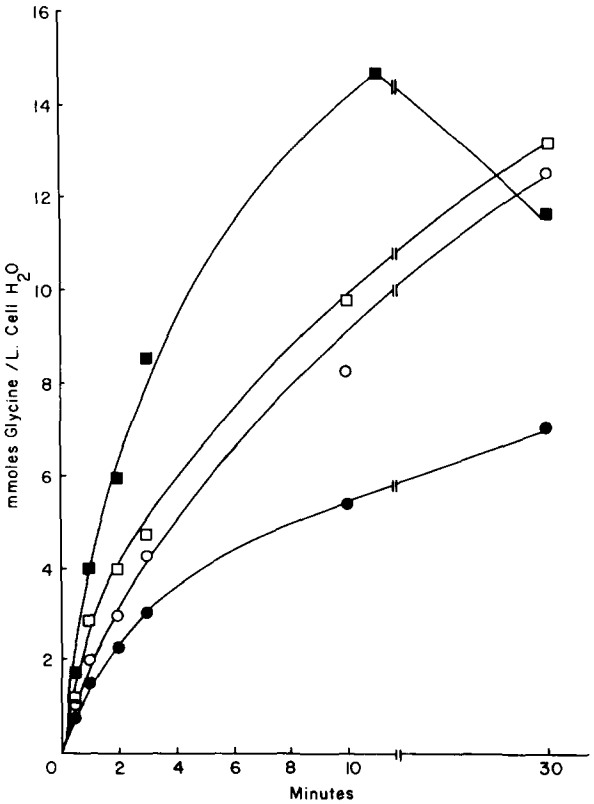

Fig. 6. Action of propranolol on amino acid uptake. Cells were incubated at 37°C in Na^+-Ringer containing 2.5 mM $CaCl_2$ and 10 mM K^+-HEPES buffer (total K_o^+ = 12 mM (■,□) and with cells in which some of the Na^+ was replaced by KCl (K_o^+ = 65 mM; Na_o^+ = 97 mM) (●,○). The cells were allowed to equilibrate for 10 min then propranolol (10^{-3} M) was added, followed, one min later by [1-^{14}C]glycine (1 mM, 300 cpm/nmol). Samples were taken periodically. Open symbols, without propranolol; closed symbols, with propranolol.

(hyperpolarization at low K_o^+ and depolarization at high K_o^+), we examined whether propranolol affected amino acid transport in a manner dependent on external K^+.

Propranolol at 10^{-4} M was without effect on amino acid transport. However, at 10^{-3} M, particularly if 2–3 mM Ca^{2+} is present, propranolol affects glycine uptake in a K_o^+-dependent manner. A representative experiment of the action of propranolol on glycine uptake at low K_o^+ and high K_o^+ levels is shown in Fig. 6. It is clear that at high K_o^+ (depolarization) uptake is reduced, whereas at low K_o^+ (hyperpolarization) uptake of glycine is increased. Although propranolol changes the ATP level, the decrease (about 30%) is the same at either K_o^+ concentration.

In contrast to the data on fluorescence, exogenous Ca^{2+} had little effect on glycine uptake. This observation confirms a much earlier one made by Riggs et al. [29] that Ca^{2+} is not required for amino acid transport. The effect of propranolol on amino acid uptake is enhanced by Ca^{2+}.

Earlier studies [19,30,31] have shown that during sodium-coupled amino

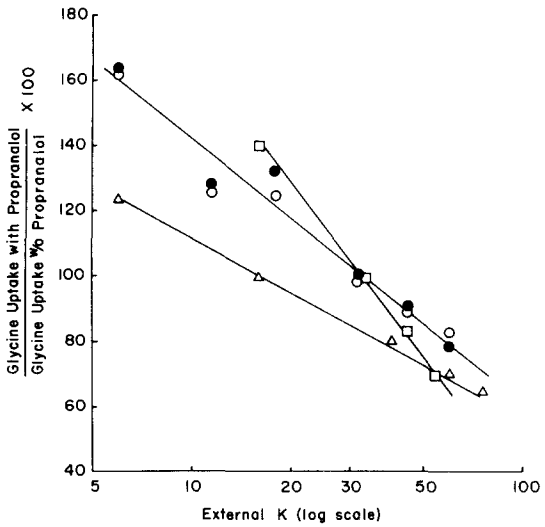

Fig. 7. Glycine uptake as a function of K_o^+ concentration in the presence of propranolol. All incubations were carried out in Ringer solution containing 95 mM NaCl and 2.0 mM $CaCl_2$. Osmolarity was maintained with choline chloride or KCl. The buffer used was 10 mM K^+-HEPES. The uptake with 10^{-3} M propranolol is expressed as a percentage of the corresponding control without propranolol at the equivalent K_o^+ concentration. Uptake of [1-^{14}C]glycine was measured at 37°C. The uptakes recorded during 3 different experiments are shown (○,□,△). Samples were taken at 1, 2, 3, 4 and 5 min. The percentage change caused by propranolol is given after either 2 min (○,□,△) or as an average of the first 4 min (●) of the experiment represented by open circles.

acid transport there is depolarization of the Ehrlich cell. In addition, studies with intact cells [32] and various vesicular preparations of mammalian cells [33—39] have shown that imposition of a membrane potential (inside negative) increases Na^+-coupled organic solute transport. If the rate of Na^+-dependent amino acid transport is a function of the membrane potential, and if with propranolol the membrane potential tends to equal (RT/F) ln $([K]_o/[K]_i)$, changes in K_o^+ should be reflected by changes in transport as long as the membrane remains relatively impermeable to other ions. The relationship between membrane potential and ln K_o^+ (with K_i^+ constant) may, of course, not be a linear one at all levels of K_o^+. If the rate of transport is a function of the electrochemical difference for sodium, however, one would predict some type of relationship between K_o^+ and the rate of amino acid transport. To ascertain whether such a relationship exists, we examined the initial rate of amino acid transport with and without propranolol at a variety of K_o^+ levels (6—50 mM) with Na_o^+ maintained at 95 mM. Choline was used to maintain osmolarity. Since the rate of uptake of amino acid in absence of propranolol is also influenced by the external K^+ concentration [40,41] we plotted the percentage difference of glycine uptake with and without propranolol at a series of K_o^+ concentrations. The data in Fig. 7 show that between 6 and 50 mM K_o^+ there is a linear (or near linear) relationship between in K_o^+ and the percentage change in rate of glycine transport.

Glycine uptake in metabolically altered cells

We find that in cells depleted of ATP by rotenone, amino acid uptake is not

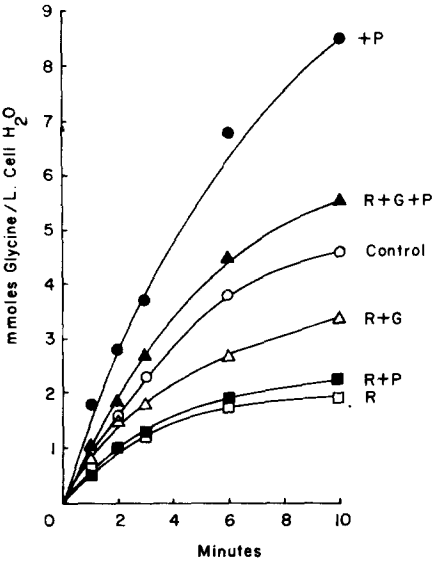

Fig. 8. Action of propranolol on rotenone-treated cells. Cells were pretreated with 32 μg/ml rotenone (+R) for 20 min. Then glucose (to 10 mM) was added where indicated (+G). After 6—7 min propranolol (to 1 mM) was added (+P). 7—8 min later [1-^{14}C]glycine (1 mM, 300 cpm/nmol) was added and samples taken. ○, Control (no addition); ●, + propranolol alone; □, + rotenone alone; ■, + rotenone and propranolol; △, + rotenone and glucose; ▲, + rotenone, glucose and propranolol. The incubation medium was Na^+-Ringer with 2.5 mM $CaCl_2$ buffered with K^+-HEPES at pH 7.4. Temperature was 37°C.

stimulated by propranolol at any K_o^+ concentration (Fig. 8) despite the fact that K^+ permeability is still increased by propranolol. ^{86}Rb efflux (Fig. 4) from Ehrlich cells is increased by propranolol in presence or absence of rotenone. Rotenone is known to reduce or abolish the membrane potential in Ehrlich cells in absence of glucose [19]. Restoration of cellular ATP levels with glucose restores the stimulatory effect of propranolol on glycine uptake at low K_o^+. These data are in line with the observations on fluorescence changes in rotenone-treated and glucose-restored cells.

That the action of propranolol on amino acid uptake or fluorescence measurements is not due to its β-adrenergic blocking action is suggested by the observations that (1) the action of propranolol is not antagonized by isoproterenol, (2) isoproterenol has an effect similar to propranolol and (3) the concentrations of propranolol required are orders of magnitude greater than those required for the adrenergic response (results not shown).

Propranolol action at different pH values on amino acid uptake

The action of propranolol on amino acid uptake appears to be pH dependent. While stimulation of glycine uptake by propranolol may be observed at pH values between 6.5 and 7.5, at pH 6.0 there is no effect (not shown). The sensitivity to pH in this system is reminiscent of the effect shown by Lassen et al. on Ca^{2+}-induced hyperpolarization in *Amphiuma* red cells [18].

One of the problems encountered in this work is that the response to propranolol, particularly on amino acid uptake, is not consistent. That is, in a sizable percentage of experiments, propranolol at low K_o^+ levels (below 20 mM), is

TABLE II

STIMULATION OF GLYCINE UPTAKE BY PROPRANOLOL IN PRESENCE OF OUABAIN

The cells were incubated at 37°C for 3 min with ouabain before addition of propranolol, followed by a preincubation for 2 min with propranolol before adding [1-^{14}C]glycine to start the reaction. Incubation was at 37°C in normal Ringer solution containing 2 mM $CaCl_2$ with K^+-HEPES buffer at pH 7.4. K_0 concentration was 12 mM. [1-^{14}C]Glycine concentration was 1 mM at 300 cpm/mmol. A typical experiment is shown.

	Glycine uptake (mmol/l cell water) at			
	3 min	Δ	14 min	Δ
Control	1.9		4.7	
Ouabain (10^{-3} M)	1.7		2.8	
Propranolol (10^{-3} M)	3.3	1.4	7.0	2.3
Ouabain (10^{-3} M) Propranolol (10^{-3} M)	2.9	1.2	5.9	3.1

either without effect or inhibitory to amino acid influx. In thirty experiments in which the effect of propranolol at low K_o^+ was tested, an inhibition or absence of response was noted in eight experiments. The reason for this variability is not known. It suggests, however, that either propranolol has a variable effect on other cellular activities, possibly on the permeability of the cell to other ions or the cells themselves have variable conductances, which may counteract the hyperpolarizing effect of propranolol.

Effect of ouabain on propranolol stimulation of amino uptake

Ouabain has long been known to inhibit Na^+-dependent amino acid uptake in this system [44] as well as many others. In Ehrlich cells, ouabain has been shown to prevent repolarization of depolarized cells [19,30,31] suggesting that at least under some conditions (for example with elevated cell Na^+) the (Na^+ + K^+)-ATPase is electrogenic. If the action of propranolol were associated with an effect on the (Na^+ + K^+)-ATPase there should be no response or less response to propranolol in presence of ouabain. The data in Table II show that propranolol overcomes the inhibition of amino acid uptake by ouabain indicating that the effect of propranolol does not involve the (Na^+ + K^+)-ATPase.

Discussion

The data presented above demonstrate that propranolol alters the membrane potential in Ehrlich cells in a K^+-dependent manner. In conjunction with effects on the membrane potential, changes are seen on the rate of amino acid uptake which suggest that hyperpolarization increases glycine uptake, whereas depolarization decreases glycine uptake. The data obtained are consistent with the conclusion that with propranolol, changes in K^+ permeability are reflected in changes in membrane potential which affect amino acid uptake. The direction and extent of the propranolol effect on fluorescence and glycine transport are dependent on the K^+ concentration in the medium.

The data obtained argue for a causal relationship between the changes in membrane potential and changes in amino acid transport for the following

reasons. (1) The change in transport rate is nearly directly proportional to ln K_o^+ in the presence of propranolol, as would be predicted if transport responded to a membrane potential largely determined by the K^+ gradient. A linear increase in uptake would be expected under conditions where only the electrical component of the electrochemical potential for Na^+, increased in a linear manner by varying external K^+. The influence of propranolol on fluorescent intensity indicates that this drug changes the membrane potential in a manner that is dependent upon external K^+. Unfortunately, the exact relationship between fluorescent intensity and membrane potential is not yet known. (2) The fluorescence changes and the amino acid transport changes in response to propranolol with variable K_o^+ are pH dependent, with little effect seen at pH 6. (3) Preincubation of the cells in rotenone abolishes the changes in both fluorescence and amino acid transport seen with propranolol at variable K_o^+. This alteration in response to propranolol due to ATP depletion is not secondary to K^+ depletion for the following reasons. (a) Cells preincubated in high K^+ during rotenone treatment maintain nearly normal K^+ levels. These cells do not respond to propranolol. (b) After rotenone treatment, Ca^{2+} is required in addition to glucose to restore the propranolol effect. Cell K^+ is the same with and without Ca^{2+}. (c) After rotenone treatment, incubation in high K_o^+ does not bring about an increase in fluorescence of dye. Had cell K^+ been lowered, an increase in fluorescence (depolarization) greater than with control cells would occur (data not shown). (4) In rotenone-treated cells, restoration of cellular ATP (by addition of glucose) restores the fluorescence changes and the amino acid transport changes in response to propranolol.

There is some difference, however, in the response to propranolol seen in the two systems examined, glycine transport and fluorescence. Ca^{2+} alone elicits a change in dye fluorescence but has no effect on amino acid uptake at low or high K^+ in the medium. Moreover, Ca^{2+} alone does not increase ^{86}Rb efflux (not shown). The lack of consistency may be due to the different conditions required to execute these measurements, for example, the cell densities used to measure fluorescence changes are one fifth to one tenth those used for transport studies. Whether the use of a higher cell density means that medium Ca^{2+} is elevated due to leakage from cells is unknown.

It is also difficult to explain the fact that in rotenone-treated cells ^{86}Rb efflux is still increased by propranolol, whereas the effects of propranolol on fluorescence (membrane potential) changes and amino acid uptake are abolished. A likely explanation for this difference may be that the permeability of the cell to other ions is also increased by depletion of ATP. In the latter circumstances the depolarizing or hyperpolarizing action of propranolol would not be apparent, although increased ^{86}Rb flux would nonetheless remain.

Another explanation is also possible. Simons [42] has shown that in red cell ghosts, Ca^{2+}-induced K^+ flux is reduced by this dye. Therefore, the absence of fluorescence changes in response to propranolol in rotenone-treated cells, although ^{86}Rb flux is still increased by propranolol, could be due to absence of the dye during ^{86}Rb flux measurements. Experiments showed however that the dye did not affect ^{86}Rb efflux in response to propranolol in rotenone-treated cells with or without glucose.

Overall, there is agreement between the effect of propranolol on fluores-

cence changes and on amino acid uptake. Earlier experiments [19,30,31] showed that the membrane potential is changed during amino acid transport. The present experiments permit us to conclude that the rate of Na^+-dependent amino acid uptake is a function of the membrane potential and support the conclusion that amino acid transport is dependent on the electrochemical potential difference for Na^+. This conclusion is consistent with the proposal originally made by Gibb and Eddy [43] for amino acid transport in this type of cell. Since the magnitude of the membrane potential is also a function of the metabolic state of the cells, the requirement for ATP in the transport process may be due to its role in maintaining a potential difference probably via the ion pumping mechanisms and/or control of passive permeability.

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