Ionic Events During the Volume Response of Human Peripheral Blood Lymphocytes to Hypotonic Media

II. Volume- and Time-dependent Activation and Inactivation of Ion Transport Pathways

B. SARKADI, ESTHER MACK, and A. ROTHSTEIN

From the Research Institute, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada

ABSTRACT Hypotonic dilution of human peripheral blood lymphocytes (PBL) induces large conductive permeabilities for K^+ and Cl^-, associated with the capacity of the cells to regulate their volumes. When rapid cation leakage is assured by the addition of the ionophore gramicidin, the behavior of the anion conductance pathway can be independently examined. Using this technique it is demonstrated that the volume-induced activation of Cl^- transport is triggered at a threshold of ~1.15× isotonic cell volume. If the volume of a cell is increased to this level or above, the Cl^- transport system is activated, whereas if the volume of a swollen cell is decreased below the threshold value, the Cl^- transport is inactivated. Activation and inactivation are independent of the relative volume changes and of the actual cellular Na^+, K^+, or Cl^- concentrations, as well as of the changes in membrane potential in PBL. When net salt movement and thus volume change are inhibited by specific blockers of K^+ transport (e.g., quinine, or Ca^{2+} depletion), volume-induced Cl^- conductance shows a time-dependent inactivation, with a half-time of 5–8 min. The Cl^- conductance, when activated, appears to involve an all-or-none response. In contrast, volume-induced K^+ conductance is a graded response, with the increase in K^+ flux being roughly proportional to the hypotonicity-induced increase in cell volume. The data indicate that during lymphocyte volume response in hypotonic media, anion conductance increases by orders of magnitude, exceeding the K^+ conductance, so that the rate of the volume decrease (KCl efflux) is determined by a graded alteration in K^+ conductance. When the cell volume approaches the isotonic value, it is stabilized by the inactivation of the anion conductance pathway.

Address reprint requests to A. Rothstein, Research Institute, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario M5G 1X8, Canada. Dr. Sarkadi's permanent address is National Institute of Haematology and Blood Transfusion, Budapest, Hungary.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/84/04/0513/15$1.00

Volume 83 April 1984 513–527

INTRODUCTION

During the volume-regulatory response of lymphocytes to hypotonic media, independent K^+ and Cl^- transport pathways are activated, allowing the release of KCl and osmotically obliged water (Grinstein et al., 1982*a*, *b*; Sarkadi et al., 1984). In the present paper the kinetics of opening and closing of these ionic pathways are examined. As shown previously, the ionophore gramicidin greatly increases the permeability of the lymphocyte membrane to small monovalent cations and the volume changes then directly reflect the anion conductance of the cell membrane. Simultaneous measurements of tracer K^+ (Rb^+) and Cl^- fluxes can provide a complete picture of the ionic events during volume response.

MATERIALS AND METHODS

For the detailed description of the materials and methods used in these experiments, see the preceding paper (Sarkadi et al., 1984).

Membrane potential in peripheral blood lymphocytes (PBL) was determined essentially as described by Grinstein et al. (1982*b*). The fluorescent dye 3,3′-dipropylthiadicarbocyanine, referred to hereafter as diS-C_3-(5), or the carbocyanine dye, was added in a final concentration of 0.5 μM and the fluorescence was recorded in an Aminco-Bowman Ratio Spectrofluorometer (Silver Spring, MD). The excitation and emission wavelengths were 620 and 670 nm, respectively. Calibration of the resting membrane potential of PBL by valinomycin, as described by Grinstein et al. (1982*b*), gave values between −55 and −60 mV.

RESULTS

As previously reported, hypoosmotic shock of lymphocytes suspended in high-K^+ media induces an initial osmotic swelling caused by water equilibration, followed by a secondary swelling caused by a rapid KCl influx governed primarily by the inward Cl^- gradient. Addition of gramicidin facilitates this response (Grinstein et al., 1982*a*, *b*), while oligomycin blocks the secondary volume changes by inhibiting the volume-induced K^+ and Cl^- permeability pathways (Sarkadi et al., 1984).

Fig. 1 shows the effect of decreasing osmolarity of high-K^+ media on the volume of PBL, measured 10 min after changing the osmolarity. In the presence of oligomycin the increase in cell volume is proportional to the decrease in the tonicity of the media, which is consistent with simple osmometric behavior (water equilibration with no net salt movement). In the control or gramicidin-treated cells, tonicity values lower than 0.9× isotonic induce an additional component of swelling because of KCl uptake. In the control cells this response increases gradually at tonicity values below 0.85× isotonic, while in the presence of gramicidin it rises more rapidly, especially at ~0.8× isotonic. Since gramicidin in the concentration applied (1 μM) produces an extremely rapid K^+ flux (see Fig. 4, Sarkadi et al., 1984), the more rapid volume changes in gramicidin-treated cells indicate that the limiting factor in the rate of swelling in the control cells is the K^+ permeability and that this limitation is largely removed by addition of gramicidin. Consequently, the rate of swelling becomes limited by Cl^- permeability and gramicidin-treated cells can be used to assess the properties of the Cl^- transport pathway.

The relative Cl^- conductance is low in resting cells (less than one-sixth that of K^+) but is greatly increased by as much as two orders of magnitude following a substantial hypotonic shock (Grinstein et al., 1982*b*). The data of Fig. 1, however, with a sharp break in the swelling curve for the control and gramicidin-treated cells at tonicities between 0.9 and 0.85× isotonic, suggest that a threshold phenomenon in the Cl^- response may be involved.

This supposition was tested by experiments presented in Fig. 2. As shown by the cell volume distribution graphs produced with the Coulter Channelyzer

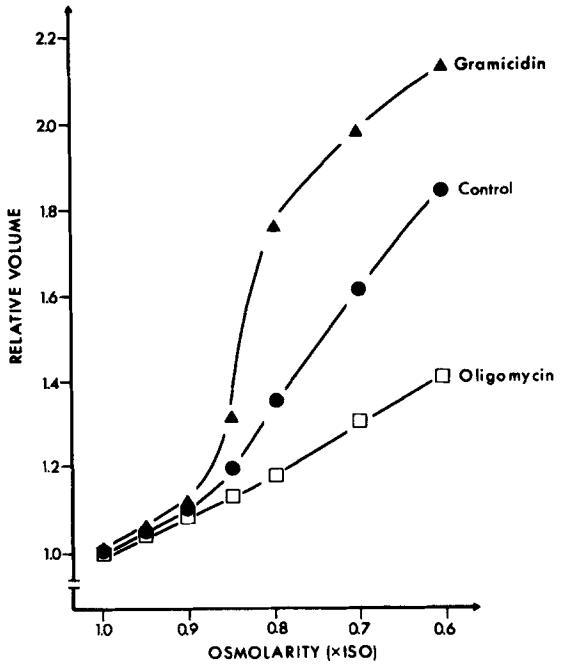

FIGURE 1. Volume changes of human PBL in hypotonic K^+-rich media. Cell volume was measured 10 min after placing the cells in media containing KCl as a predominant salt with the tonicities indicated. Control (●); 0.5 μM gramicidin (▲); 5 μg/ml oligomycin (□).

(Coulter Electronics, Inc., Hialeah, FL), in isotonic cells the addition of gramicidin did not produce any change in the mean cell volume, while in 0.8× isotonic media a single population of swollen cells could be seen. In contrast, exposure of cells to 0.85× isotonic high-K^+ media resulted after 10 min in the appearance of two distinct cell populations. One population had essentially the same volume distribution as all the cells immediately after the change of osmolarity, while a second population had a distinctly higher mean volume, shifting to the right (larger volumes) after longer periods of time. Slight modifications of the tonicity of the media around 0.85× isotonic introduced large variations in the ratio of the two populations as seen in the Channelyzer.

If gramicidin-treated cells suspended in choline-Cl (rather than KCl) are

exposed to hypotonic shock, they initially undergo osmotic swelling, followed by a shrinking phase that results in a return to about isotonic volume (see Grinstein et al., 1982*c*; Sarkadi et al., 1984, Fig. 1). The shrinking phase is associated with a loss of KCl driven largely by the outward K^+ gradient. The threshold for this

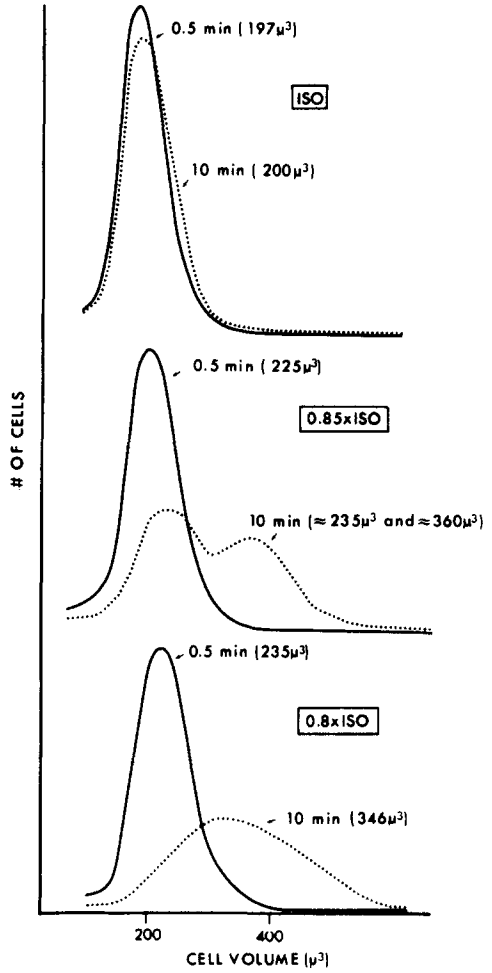

FIGURE 2. Changes in cellular volume distribution of human PBL, as depicted by the Coulter Channelyzer system. The cells were incubated in media of the tonicities indicated, which contained KCl as a predominant salt plus 0.5 μM gramicidin. Measurements were carried out 0.5 (——) or 10 min (· · · ·) after placing the cells into the media.

rapid shrinkage was also ~0.85× isotonic, and the development of two populations with different mean volumes could also be observed (data not shown). It can be noted, however, that because the Coulter Counter is more sensitive in the higher cell volume range, the two populations are more readily discriminated

in the swelling type of experiment illustrated in Fig. 2. These observations on cell swelling or shrinking suggest the presence of an all-or-none type of response in the increased Cl^- conductance of hypotonically shocked lymphocytes with a threshold value of ~0.85× isotonic media, that is, a cell volume of ~1.15× isotonic value.

The effects of various experimental parameters on the activation and inactivation of the volume-induced Cl^- conductance were examined. In the experi-

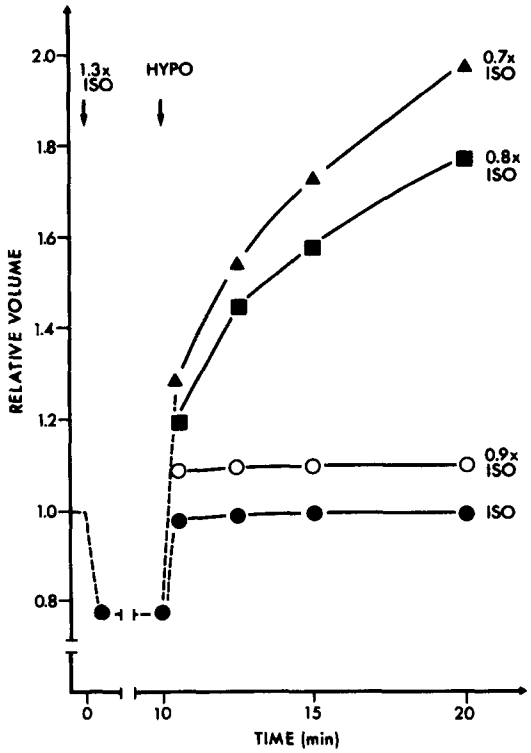

FIGURE 3. Effects of the relative changes in the tonicities of the media on the threshold for secondary swelling in hypotonically shocked human PBL. The media contained KCl as a predominant salt plus 0.5 μM gramicidin. The tonicity was first increased to 1.3× isotonic by the addition of adequate volumes of 1 M KCl, and after 10 min the tonicity was decreased as indicated.

ments presented in Fig. 3, gramicidin-treated cells were first exposed to hypertonic high-K^+ media (1.3× isotonic) before application of a hypotonic shock. As seen, the preincubation had no effect on the threshold for secondary swelling; that is, the rapid increase in cell volume (reflecting high Cl^- conductance) was still initiated at 0.8–0.9× isotonic media. In similar experiments cells were preexposed to media of 1.2–1.5× isotonic. In each case the Cl^- conductance was triggered at a tonicity of ~0.85× isotonic (a cell volume ~1.15× isotonic). These experiments show that preshrinking the cells to various degrees does not alter

the threshold for triggering of the Cl^- conductance. Thus, the degree of dilution or the amount of cell swelling per se is not a controlling factor but the effect appears to be triggered at a particular osmolarity or cell size.

Osmolarity per se was eliminated as a controlling factor by the following experiments (Fig. 4). Cells were diluted in 0.7× isotonic medium. After their return to their isotonic volume by a typical swelling and shrinking cycle, they were diluted a second time by 30% (medium now 0.49× isotonic). They underwent a second swelling and shrinking cycle that was almost indistinguishable from the first (a third cycle usually could not be produced, presumably because of the loss of most of the permeant anions from the cells). If the once-cycled cells

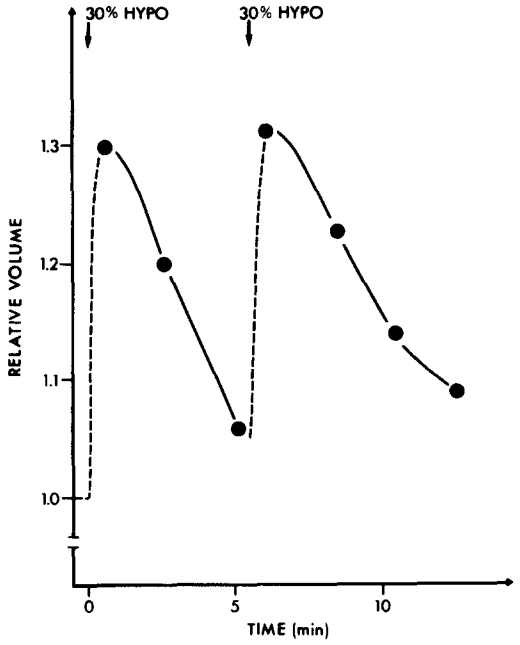

FIGURE 4. Effects of repeated hypotonic dilutions on the volume changes in human PBL. The media contained NaCl as a predominant salt. At the times indicated by the arrows, the media were diluted by 30% with distilled water.

were placed in a K^+-rich medium with gramicidin, the threshold for the secondary swelling was similar to that shown in Fig. 2 for uncycled cells, at a cell volume of 1.15× isotonic value. This experiment suggests that osmolarity is not the controlling factor. It also suggests that internal and external concentrations of anions are not the triggering factors either, for they are reduced by at least 30% before the start of the second cycle (see Grinstein et al., 1982*b*, *c*). Furthermore, it has been demonstrated that virtually complete replacement of internal K^+ by Na^+ by preincubation with gramicidin in a K^+-free medium does not substantially alter the secondary swelling following hypotonic shock (Grinstein et al., 1982*b*, and confirmed in this study). Nor does replacement of Na^+ by $choline^+$ or Cl^-

by SO_4^- or gluconate$^-$ in the medium alter the kinetics of the volume response (Grinstein et al., 1982*b*, *c*; Sarkadi et al., 1984).

The observations noted above appear to eliminate osmolarity, ionic strength, external or internal ion compositions, and the initial cell volume as controlling factors in activating the Cl^- conductance in hypotonically shocked lymphocytes. Rather, the event seems to occur whenever the cells swell to a volume of >1.15× the isotonic value. Furthermore, the Cl^- conductance activated by cell swelling appears to be inactivated when swollen cells shrink toward isotonic volume. As already noted, when gramicidin-treated cells are suspended in choline-Cl and exposed to hypotonic shock, the initial osmotic swelling is followed by a rapid shrinkage, driven by the outward K^+ gradient and terminating when the cells

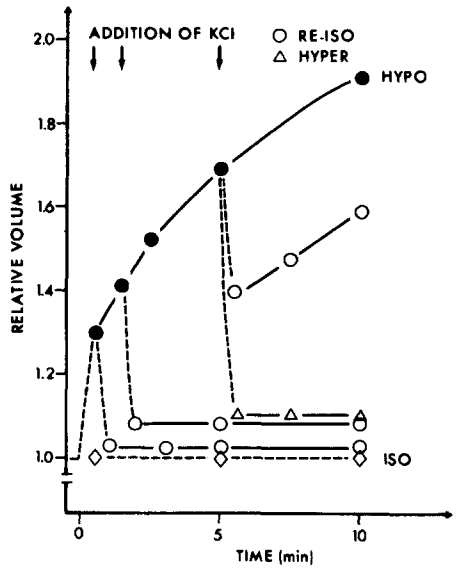

FIGURE 5. Effect of the restoration of the cell volume on the secondary swelling of hypotonically shocked human PBL. The media contained KCl as a predominant salt and 0.5 μM gramicidin. Isotonic medium (◇); 0.7× isotonic medium (●). At the times indicated by the arrows, adequate amounts of 1 M KCl were added to restore isotonicity (○) or adjust the media to 1.3× isotonic (△).

are of approximately isotonic size (Sarkadi et al., 1984). Under these conditions the K^+ permeability is high because of the presence of gramicidin. Consequently, the termination of the shrinking process must indicate an inactivation of the Cl^- conductance. The inactivation is experimentally demonstrated in Fig. 5. Lymphocytes were placed in hypotonic (0.7× isotonic) high-K^+ media containing 1 μM gramicidin; thus, a secondary swelling of the cells occurred. After different time intervals, the tonicity was rapidly increased by the addition of concentrated KCl solutions and the changes in cell volume were followed. Because of the rapid osmotic water equilibration, the cell volume could be rapidly adjusted. In this and other similar experiments, if the cell volume was reduced below ~1.1–1.15× isotonic value, there was no rapid secondary volume increase, despite the contin-

uous presence of gramicidin. If, on the other hand, the increase in tonicity of the medium was insufficient to bring the cell volume below the threshold level, rapid swelling persisted. These experiments suggest that increased Cl^- conductance is terminated by a reduction of cell volume to a threshold value similar to that required to trigger rapid Cl^- transport.

In addition to the volume-dependent termination of Cl^- conductance, a time-dependent inactivation also occurs. This phenomenon can be observed if the lymphocyte volume is kept constantly high (above threshold) by specifically blocking volume-induced K^+ transport after a hypotonic shock. In the experi-

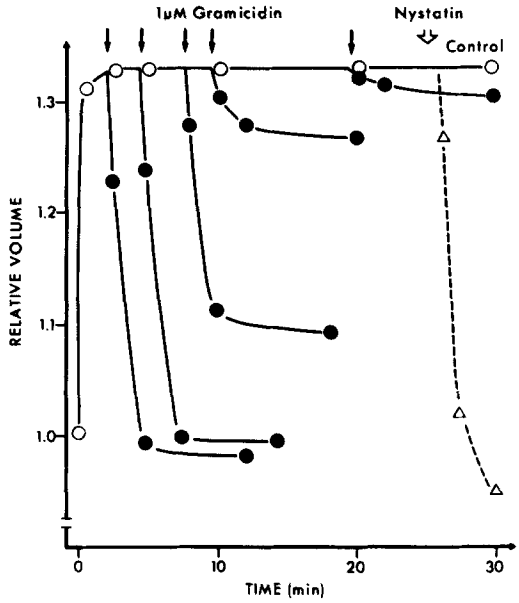

FIGURE 6. Effect of gramicidin on the shrinkage of hypotonically shocked human PBL. The 0.7× isotonic media contained choline-Cl as a predominant salt plus 100 μM quinine. At the times indicated by the arrow, 1 μM gramicidin was added to the media. The open arrow indicates the addition of 50 μg/ml nystatin to the media.

ments represented in Fig. 6, PBL were placed into hypotonic choline-Cl media containing 100 μM quinine. In this case the initial osmotic swelling occurs, but because of quinine inhibition of K^+ transport (Armando-Hardy et al., 1975), no regulatory volume response is observed. The addition of gramicidin, nevertheless, produces a rapid shrinkage of the cells, which indicates the presence of a high Cl^- conductance (see Fig. 1, Sarkadi et al., 1984). If gramicidin is added at various times after the hypotonic shock, the conductance is maximal for 5–6 min but diminishes rapidly between 8 and 10 min and practically disappears by 10–15 min (Fig. 7), even though the cell volume greatly exceeds the threshold value noted above. The lack of response after 10–15 min appears to be due to low Cl^- permeability because addition of nystatin, a nonselective ionophore (which increases membrane permeability to Na^+, K^+, and Cl^- but much less for choline$^+$),

to the lymphocytes after 25 min results in a rapid shrinkage of the cells, even though they are unresponsive to gramicidin (which increases the permeability to Na^+ and K^+ but not Cl^-). A similar, time-dependent inactivation of the Cl^- conductance was observed if the gramicidin effect was examined in Ca^{2+}-depleted PBL in which volume-induced K^+ transport is practically absent (Grinstein et al., 1982*b*; Sarkadi et al., 1984). Such depleted cells remain swollen after exposure to hypotonic media, but reshrink after addition of gramicidin. Delayed addition of the antibiotic produced progressively less shrinkage in a hypotonic choline-Cl medium, similar to the quinine experiment of Fig. 6, which indicates the time inactivation of hypotonicity-induced Cl^- conductance (data not shown). This

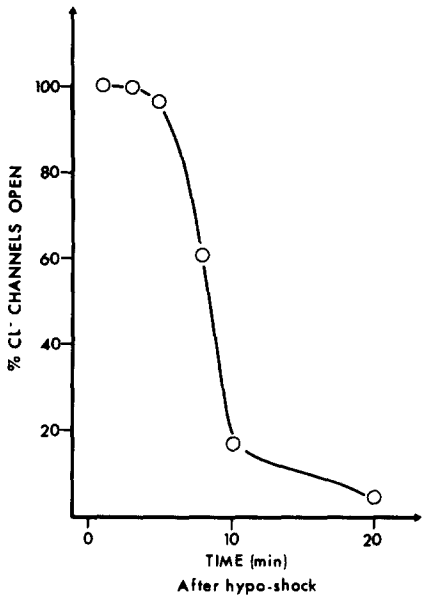

FIGURE 7. Percent of Cl^- channels open after hypotonic shock of human PBL. The calculations were based on experimental data such as shown in Fig. 6.

experiment indicates that the inactivation of the Cl^- system illustrated in Figs. 6 and 7 is not due to any delayed effect of quinine.

The osmolarity- and time-dependent activation and inactivation of Cl^- conductance could also be followed by membrane potential measurements. As shown in Fig. 8, the changes in the fluorescence of the carbocyanine dye diS-C_3-(5) indicate a significant depolarization of the PBL membrane after cell swelling following hypotonic dilution in a NaCl medium. As analyzed by Grinstein et al. (1982*b*), the osmotic swelling results in an increase in conductive permeabilities to K^+ and Cl^-, but the conductance to Cl^- considerably exceeds that to K^+. Consequently, because E_{Cl} is more positive than E_m in the resting cells, the cells become depolarized. As demonstrated in Fig. 8, restoration of isotonicity, and therefore of cell volume, results in an immediate repolarization (trace 2). A slower spontaneous repolarization of the cells remaining in hypotonic media can

also be observed (trace 3). After this spontaneous repolarization has occurred, restoration of isotonicity has only a negligible effect on membrane potential. Gramicidin, by increasing both Na^+ and K^+ permeabilities, induces a rapid depolarization of the cell membrane. Addition of oligomycin C (1 μM), an inhibitor of the Cl^- pathway (Sarkadi et al., 1984), prevents hypotonicity-induced depolarization but does not affect the gramicidin-induced potential changes (data not shown).

In contrast to the threshold type of response found for Cl^-, the volume-induced increase in K^+ (Rb^+) permeability appears to involve a graded response:

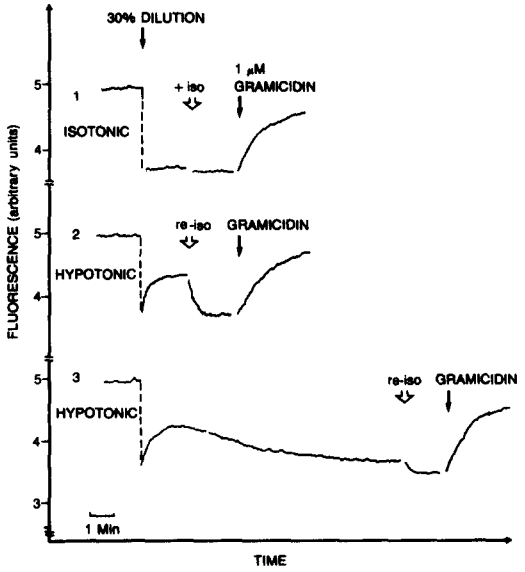

FIGURE 8. Changes in the membrane potential of PBL as measured by the carbocyanine dye diS-C_3-(5). The media contained NaCl as a predominant salt, 2×10^6 PBL/ml, and 0.5 μM dye. After fluorescence reached a steady state value, the media were diluted 30% with isotonic solution or with distilled water. The symbols "+iso" and "re-iso" represent the addition of 5 volume percent of isotonic buffer or concentrated NaCl to restore isotonicity, respectively. The final concentration of gramicidin was 1 μM. The traces are representative of three similar experiments.

the conductance, as assessed by measuring $^{86}Rb^+$ efflux from hypotonically shocked lymphocytes, increases with the degree of swelling (Fig. 9), which confirms the volume-response data reported by Cheung et al. (1982). The restoration of the cell volume by the addition of concentrated salts (which terminates the increased Cl^- conductance; Fig. 5) only partially reduces the activated Rb^+ flux.

DISCUSSION

In this paper we demonstrate that the activation of the Cl^- conductance by hypotonic shock in PBL involves an all-or-none type of switching phenomenon.

Above a distinct threshold cell volume (1.15× isotonic value), the Cl^- conductance is very high, while below this volume Cl^- conductance is very low. Factors such as ionic strength, osmolarity, external or cytoplasmic ion concentrations, the amount of volume change, or the membrane potential (which is substantially different in gramicidin-treated cells suspended in Na^+- or K^+-rich media), do not control the activation of the Cl^- conductance. General changes in cytoplasmic pH can also be excluded because none were observed during hypotonic volume regulation (Rink et al., 1983). The activation of Cl^- conductance occurs when the cells swell to a finite volume. Furthermore, inactivation occurs when swollen

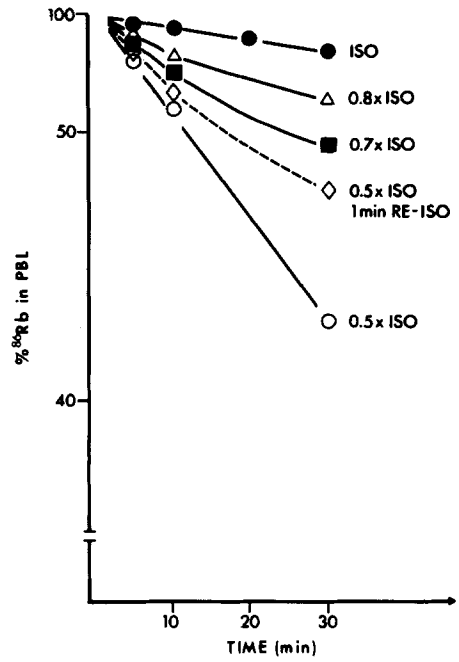

FIGURE 9. Effect of various hypotonic dilutions of human PBL on the rate of $^{86}Rb^+$ efflux. The media contained KCl as a predominant salt plus 0.5 mM ouabain. Isotonic medium (●); 0.8× isotonic (△); 0.7× isotonic (□); 0.5× isotonic (○); 0.5× isotonic (◇), restored to isotonic by the addition of concentrated KCl after 1 min incubation.

cells shrink to a similar or slightly lower volume (Fig. 5). Thus, the mechanism acts like a reversible on-off switch that operates at a given cell volume. At around this threshold volume, a population response can be observed (Fig. 2) with certain cells that show no secondary swelling response, whereas others swell substantially. Thus, particular cells appear to be switched on or off independently. Such a behavior can be interpreted to indicate the presence of a small number of highly conductive "channels" in the cell membrane (see Latorre and Miller, 1983). The difference in the activated or nonactivated cells may be based on the statistical distribution of channels with various sensitivities to the given trigger and the

response is either maximum opening or no opening at all. The actual number of transporters necessary to accomplish the anion flow may be very small. If, for example, channels are involved, which have the same conductance for Cl^- as gramicidin does for Na^+ or K^+, only a few (five to six) channels per cell would be required to account for the 2-min half-time of Cl^- efflux.[1]

The Cl^- conductance of isotonic cells is very low, so that gramicidin treatment results in no measurable volume changes (there is, however, a substantial nonconductive anion exchange flux, with a half-time of ~15 min; Grinstein et al., 1982*b*). It can be estimated that the hypotonic shock increases the Cl^- conductance by at least 100-fold, and that above the threshold size, this conductance appears to be fully turned on, regardless of the degree of hypotonicity (or cell swelling). The half-time for shrinking of hypotonically shocked, gramicidin-treated cells (suspended in 0.7× isosmotic choline-Cl) is ~2 min or less (Sarkadi et al., 1984). The same half-time is found if Cl^- efflux is directly measured (Grinstein et al., 1983*b*; Sarkadi et al., 1984), with no apparent difference at tonicities between 0.7 and 0.5× isotonic (data not shown). It must be noted, however, that the present techniques for volume and flux measurements cannot accurately resolve rates with half-times of <2 min, so the question of the Cl^- conductance being fully turned on at any volume above the threshold cannot be unequivocally answered based on these data.

As shown by the membrane potential measurements of Grinstein et al. (1982*b*) and by the data presented in Fig. 8 of this paper, hypotonic shock induces a rapid depolarization of the PBL membrane. Since the resting potential in these cells is about −50 to −55 mV and reflects mostly a K^+ distribution potential (see Grinstein et al., 1982*b*), depolarization must indicate an increased Cl^- conductance significantly overriding K^+ conductance (E_{Cl} is more positive than E_m, and Na^+ conductance is practically unchanged). The membrane potential changes demonstrated in Fig. 8 further indicate an osmolarity- and time-dependent variation in Cl^- conductance of the PBL membrane.

In contrast to the volume-induced Cl^- conductance change, hypotonicity-induced K^+ conductance is definitely a graded type of response: decreasing osmolarities in the media continuously increase the rate of K^+ movement (Fig. 9, and previous observations by Cheung et al., 1982, and Deutsch et al., 1982). Even with large dilutions, however, the K^+ fluxes are considerably lower than the Cl^- fluxes. For example, at 0.5× isotonic media the half-time for K^+ efflux is ~8–10 min, compared with <2 min for Cl^- efflux. The shutting off of the K^+ pathway during cell shrinkage does not follow the same pattern as the Cl^- pathway. For example, if swollen cells are returned to isotonic size after 1 min of hypotonic shock by the addition of salt (Fig. 9), the Rb^+ flux is reduced by ~40% but is still about four times the normal flux, whereas the Cl^- flux is

[1] The magnitude of K^+ flux through gramicidin channel is $\sim 10^7$ ions/s at 0.1 M salt concentration, 200 mV potential, and 25°C, as measured in artificial black lipid membranes (Finkelstein and Anderson, 1981; Pressman, 1976). Thus, a 60 mmol K^+ loss/liter of PBL (5×10^{12} cells) in 2 min (during RVD, a similar loss of Cl^- occurs) would require the functioning of six gramicidin channels per cell.

reduced to very low values (Fig. 5). Thus, the reduction of K fluxes appears to be largely time dependent and only partially volume dependent.

Given the described characteristics of the K^+ and Cl^- conductive pathways, their role in the volume-regulatory phenomenon can be described. In the isotonic cell the anion conductance is very low and acts to limit the salt and volume changes that could be driven by the outward K^+ or by the inward Na^+ gradients. After hypotonic swelling (of >15%), two apparently independent systems are triggered that are relatively quiescent in the isotonic cell (Grinstein et al., 1982*a–c*; Sarkadi et al., 1984), namely conductive K^+ and Cl^- transport pathways. The former involves a graded response, roughly proportional to the degree of swelling, and the latter is apparently an all-or-none response triggered at a particular cell volume (1.15× isotonic). Once triggered, the Cl^- conductance considerably exceeds the K^+ conductance, so the latter process becomes the rate-limiting step in the loss of KCl and osmotically obliged water. For this reason, the rate of cell shrinking is also a graded response, almost proportional to the amount of dilution and cell swelling (Cheung et al., 1982; Deutsch et al., 1982). As the cells approach isotonic size, the K^+ fluxes decrease and the rate of shrinking also decreases. When the cells reach near the isotonic size, the Cl^- pathway shuts off and the rate of shrinking falls to a low level, so the final volume is usually a little larger than the isotonic size. At this point the Cl^- conductance is again the limiting factor for volume change. Another possible controlling factor is the timed shut-off of the Cl^- conductance that occurs between 6 and 10 min after osmotic swelling at room temperature (Figs. 7 and 8). Its importance in volume regulation is not clear, for under the conditions of the experiments described in this paper, most of the volume-regulating phase is complete by 10 min.

The triggering event is not known for either the K^+ or Cl^- conductance. The former may be mediated by a localized release of intracellular Ca^{2+} (Grinstein et al., 1982*a*; Sarkadi et al., 1984) and may involve mechanisms similar to Ca^{2+}-mediated K^+ transport, the "Gárdos phenomenon," described in several cell membranes. If so, the connection between hypotonic swelling and Ca^{2+} release is not known.

As to the trigger for Cl^- conductance, it has already been noted that no factors have been identified except cell size (volume). Factors that appear to be eliminated include ion composition, ionic strength (or osmolarity), the amount of swelling, cytoplasmic pH, and membrane potential. We have also examined the effects of noradrenalin, carbamylcholine, dibutyril-cAMP, and ionophore A23187 as possible inducers of Cl^- conductance, but none of these compounds produced any appreciable change in the Cl^- conductance of isosmotic lymphocytes. Modulators of the cytoskeleton such as colchicine and cytochalasin B were also without effect (data not shown). When isolated, sealed vesicles were prepared from thymocyte plasma membranes, the volume-induced increase in ion permeabilities was lost and could not be reactivated (Grinstein et al., 1983). Thus, at present we have no serious lead in this respect.

For the possible in vivo physiological significance of the volume-induced ion transport pathways, we can offer only speculative theories at present. The system

is most probably inherited from the early stages of evolution when cells had to be protected against large changes in the osmotic concentrations in their surroundings. In humans, relatively modest changes in tonicity of body fluids may also occur, so that some capacity for rapid cell volume regulation may be important. In general, however, in the well-regulated milieu of human blood, a volume-stabilizing mechanism of such large capacity certainly has no major role. Selective changes in K^+ or Cl^- conductance of the lymphocyte membrane may be important in producing sudden alterations in the membrane potential. This value in the resting lymphocytes is about −30 to −50 mV (Deutsch et al., 1979; Grinstein et al., 1982*b*). A selective increase in the K^+ conductance would result in a K^+ potential of about −70 mV, while a selective increase in Cl^- conductance would result in a membrane potential of about −30 mV (see Grinstein et al., 1982*b*). Such potential changes may be involved in the initiation of cell multiplication by mutagenic agents, associated with an increased calcium entry and stimulation of a rapid K^+ transport (Freedman, 1979; Segel et al., 1979; Lichtman et al., 1980; Szász et al., 1981; Deutsch and Price, 1982; Kaplan, 1979; Tsien et al., 1982).

We thank Drs. S. Grinstein and E. Gelfand for the stimulating discussions and Joan Green, Canadian Red Cross, Blood Transfusion Service, Toronto, for providing buffy coat-rich blood preparations.

This research was supported by the Medical Research Council and the National Cancer Institute, Canada.

Received for publication 26 May 1983 and in revised form 28 September 1983.

REFERENCES

Armando-Hardy, M. C., C. J. Ellory, H. G. Ferreira, S. Flemminger, and V. L. Lew. 1975. Inhibition of the calcium-induced increase in the potassium permeability of human red blood cells by quinine. *J. Physiol. (Lond.).* 250:32p–33p.

Cheung, R. K., S. Grinstein, H. M. Dosch, and E. W. Gelfand. 1982. Volume regulation by human lymphocytes: characterization of the ionic basis for regulatory volume decrease. *J. Cell. Physiol.* 112:189–196.

Deutsch, C. J., A. Holian, S. K. Holian, R. P. Daniele, and D. F. Wilson. 1979. Transmembrane electrical and pH gradients across human erythrocytes and human peripheral lymphocytes. *J. Cell. Physiol.* 99:79–94.

Deutsch, C., and M. A. Price. 1982. Cell calcium in human peripheral blood lymphocytes and the effect of mitogen. *Biochim. Biophys. Acta.* 687:211–218.

Deutsch, C., L. Slater, and P. Goldstein. 1982. Volume regulation of human peripheral blood lymphocytes and stimulated proliferation of volume-adapted cells. *Biochim. Biophys. Acta.* 721:262–267.

Finkelstein, A., and O. S. Anderson. 1981. The gramicidin A channel: a review of its permeability characteristics with special reference to single-file aspect of transport. *J. Membr. Biol.* 95:155–171.

Freedman, M. H. 1979. Early biochemical events in lymphocyte activation. I. Investigation of the nature and significance of early calcium fluxes observed in mitogen-induced T and B lymphocytes. *Cell. Immunol.* 44:290–313.

Grinstein, S., C. A. Clarke, and A. Rothstein. 1982*a*. Increased anion permeability during

volume regulation in human lymphocytes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 299:509–518.

Grinstein, S., C. A. Clarke, and A. Rothstein. 1982*b*. Volume-induced increase in anion permeability of human lymphocytes. *J. Gen. Physiol.* 80:801–803.

Grinstein, S., A. DuPre, and A. Rothstein. 1982*c*. Volume regulation in human lymphocytes. Role of calcium. *J. Gen. Physiol.* 79:849–868.

Grinstein, S., S. Cohen, B. Sarkadi, and A. Rothstein. 1983. Induction of $^{86}Rb^+$ fluxes by Ca^{2+} and volume changes in thymocytes and their isolated membranes. *J. Cell. Physiol.* 116:352–362.

Kaplan, J. G. 1979. Activation of cation transport during lymphocyte stimulation: the molecular theory of spinning metabolic wheels. *Trends Biochem. Sci.* 4:N147–N149.

Latorre, R., and C. Miller. 1983. Conduction and selectivity in potassium channels. *J. Membr. Biol.* 71:11–30.

Lichtman, A. H., G. B. Segel, and M. A. Lichtman. 1980. Total and exchangeable calcium in lymphocytes: effects of PHA and A23187. *J. Supramol. Struct.* 14:65–75.

Pressman, B. C. 1976. Biological applications of ionophores. *Annu. Rev. Biochem.* 46:501–530.

Rink, T. J., A. Sanchez, S. Grinstein, and A. Rothstein. 1983. Volume restoration in osmotically swollen lymphocytes does not involve changes in cytoplasmic pH or free Ca^{2+} concentration. *Biochim. Biophys. Acta.* 762:593–596.

Sarkadi, B., E. Mack, and A. Rothstein. 1984. Ionic events during the volume response of human peripheral blood lymphocytes to hypotonic media. I. Distinctions between volume-activated Cl^- and K^+ conductance pathways. *J. Gen. Physiol.* 83:497–512.

Segel, G. B., W. Simon, and M. A. Lichtman. 1979. Regulation of sodium and potassium transport in phytohemagglutinin-stimulated human blood lymphocytes. *J. Clin. Invest.* 64:834–841.

Szász, I., B. Sarkadi, and G. Gárdos. 1981. Calcium sensitivity of calcium-dependent functions in human red blood cells. *Adv. Physiol. Sci.* 6:211–221.

Tsien, R. Y., T. Pozzan, and T. J. Rink. 1982. T-cell mitogens cause early changes in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. *Nature (Lond.).* 295:68–71.