SODIUM TRANSPORT IN HUMAN ERYTHROCYTES-- ABSENCE OF AN EFFECT OF PROSTAGLANDIN E_1

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SUMMARY In contrast to a previous report (1) in which prostaglandin E_1 was reported to produce a significant decrease in the extracellular sodium concentration when human erythrocytes were incubated in plasma, we have demonstrated that prostaglandin E_1 has no significant effect on either sodium outflux or sodium influx in human erythrocytes in vitro.

INTRODUCTION

Prostaglandin E_1 (PGE_1) has been demonstrated to stimulate sodium transport (2-5) and to inhibit vasopressin-induced stimulation of water transport (6, 7) in epithelial tissues. These effects have been attributed to PGE_1-induced alterations of intracellular levels of cyclic 3',5'-AMP (1-7). Human erythrocytes have been demonstrated not to possess adenyl cyclase activity (8). In a recent review, Ramwell and Shaw (1) postulated that PGE_1 can exert a primary effect on ion transport without involvement of the adenyl cyclase system, since they found that addition of PGE_1 (2.8×10^{-8} M) to human erythrocytes suspended in plasma produced a significant decrease in the plasma sodium concentration. Assuming an hematocrit of 50% and no outflux of sodium from the cells, these authors' data require a rather unlikely value for sodium influx of at least 60 mmoles/L cells per hour--a value which is 40 times greater than that normally observed in human erythrocytes. Furthermore, if the hematocrit of the incubation mixture was less than 50% or if sodium outflux was not zero, an even higher value for sodium influx would be required to account for the data of Ramwell and Shaw.

To further explore this reported effect of PGE_1 on sodium transport in human erythrocytes, we have repeated the experiment of Ramwell and Shaw and

have measured the effect of PGE_1 on sodium outflux and sodium influx in human erythrocytes *in vitro*.

METHODS

Heparinized blood was obtained from normal male human volunteers. The cells were separated by centrifugation and the plasma and buffy coat removed by aspiration. Two portions of the cells were resuspended in the original plasma (37°C) at an hematocrit of 35%. At 10-minute intervals aliquots of the incubation mixtures were centrifuged and the sodium concentration in the supernatant was measured. PGE_1 (8×10^{-6} M) was added to one of the incubation vessels after 30 minutes of incubation.

A third portion of cells was washed 3 times with isosmotic choline chloride and placed in two identical preincubation solutions at a 10% hematocrit. ^{24}Na was added to the preincubation mixture containing erythrocytes which were subsequently used for sodium outflux determination. At the end of two hours, samples were taken for determination of hematocrit and hemoglobin concentration. The cells were then separated by centrifugation and washed three times with iced isosmotic choline chloride. A portion of the washed cells was hemolyzed and diluted for determination of sodium and hemoglobin concentrations. Hemoglobin was measured using the cyanmethemoglobin method (9). Sodium concentration was measured with an Instrumentation Laboratories Model 143 flame photometer. Erythrocyte sodium concentration was calculated after the method of Sachs and Welt (10).

The cells which were preincubated in the medium containing ^{24}Na were added to the appropriate incubation solution (prewarmed to 37°C) at an hematocrit of 6%. After mixing, samples were taken at 0 and 30 minutes, the cells separated and an aliquot of supernatant taken for measurement of radioactivity. At some time during the incubation radioactivity was measured in an aliquot of the incubation mixture (i.e. cells plus medium). Fractional sodium outflux (k) was calculated from the following equation:

$$k = \frac{-\ln(A_{30}/A_0)}{t}$$

A_o and A_{30} represent the amount of radioactivity in an aliquot of cells at 0 and 30 minutes. t = time (hrs.)

Sodium outflux was calculated as the product of the erythrocyte sodium concentration and fractional sodium outflux.

The cells which were preincubated in the non-radioactive medium were added to incubation solutions which contained ^{24}Na but were otherwise identical in composition to those used for outflux determination. Aliquots were taken at 0 and 30 minutes, chilled, washed 4 times with isosmotic choline chloride and their radioactivity was measured. The volume of cells counted was calculated from the hemoglobin concentration of the incubation mixture and the previously measured hemoglobin content per volume of cells. Sodium influx (J_{in}) was calculated from the following equation:

$$J_{in} = \left[\frac{kU}{1-e^{-kt}}\right] / SA$$

U is the uptake of radioactivity per liter of cells per 30 minutes.
SA is the specific activity of the incubation medium.

The standard preincubation and incubation solutions had the following composition (mM): NaCl, 146; KCl, 4.0; K_2HPO_4, 1.0; Tris buffer (pH = 7.4), 18; glucose, 11.1. Radioactivity was measured with a Packard Model 3320 liquid scintillation spectrometer and all counts were corrected for decay. Liquid scintillation was performed using 20 ml of a solution composed of 15 parts toluene (J. T. Baker Chemical Co.), 5 parts Triton X-100 ® (New England Nuclear Corp.) and 1 part Liquifluor ® (New England Nuclear Corp.).

RESULTS

The data in Figure 1 illustrate that PGE_1 had no significant effect on the sodium concentration in the incubation medium. The data in Table 1 show that PGE_1 had no significant effect on either sodium outflux or sodium influx in human erythrocytes in vitro. Lower concentrations of PGE_1 (5×10^{-7} and 2×10^{-8} M) likewise had no significant effect on either sodium outflux or sodium influx. We have excluded the possibility that our PGE_1 was not biologically active since it inhibited thrombin-induced platelet aggregation

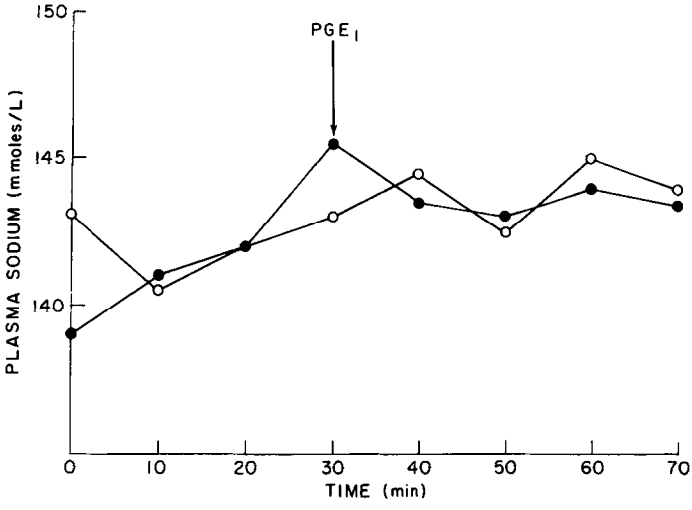

Figure 1. Absence of an effect of PGE_1 (8×10^{-6} M) on plasma sodium concentration in vitro. Open circles represent control values. Closed circles represent values from the incubation mixture to which PGE_1 was added and the time of addition of PGE_1 is indicated by the vertical arrow.

Table 1

ABSENCE OF AN EFFECT OF PROSTAGLANDIN E_1 ON SODIUM OUTFLUX AND SODIUM INFLUX IN HUMAN ERYTHROCYTES IN VITRO

	Control	+PGE_1
Sodium outflux	2.27 ± .31	2.30 ± .41
Sodium influx	1.52 ± .20	1.61 ± .32

Each value represents the mean of 10 experiments ± 1 S.D. Sodium outflux and influx are expressed as mmoles/L cells per hour. PGE_1 was present at concentration of 8×10^{-6} M.

and nucleotide release as has been reported previously (11).

DISCUSSION

In contrast to Ramwell and Shaw (1), we have detected no effect of PGE_1 on sodium transport in human erythrocytes. We are unable to reconcile this discrepancy other than to point out that even the data of Ramwell and Shaw suggest that a significant difference was observed only at 10 minutes but not at 5 and 15 minutes after addition of PGE_1.

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