Journal of Cellular Biochemistry 45:207–212 (1991)

Taurine Release Associated to Volume Regulation in Rabbit Lymphocytes

J. Jesús García, R. Sánchez Olea, and H. Pasantes-Morales

Institute of Cell Physiology, National A. University of Mexico, 04510 México D.F., Mexico

Abstract Rabbit lymphocytes exposed to hyposmotic media first swell and then recover their initial volume within 6 min. During volume recovery, free amino acids (FAA) decrease from 451.1 to 208 nmoles/mg protein. Taurine was the dominating FAA, accounting for 70% of the FAA pool. The time course of ^{3}H-taurine release induced by hyposmolarity followed that of volume recovery. Efflux of ^{3}H-taurine in an 8 min period was 17.8% (of total labeled taurine accumulated during loading) in an isosmotic medium. Reducing osmolarity to 0.87, 0.75, 0.62, and 0.5 increased this release to 24.8%, 38.1%, 56.4% and 70.9%, respectively. The volume-sensitive release of ^{3}H-taurine was unaffected by omission of external Na^+ or Ca^{++} and was reduced by 23% in the absence of Cl^-. It was unaffected by agents disrupting the cytoskeleton or by tetraethylammonium, barium, quinidine, and gadolinium, but was 26% reduced by DIDS. Taurine release was inhibited at 4°C, but was unchanged at 15°C or 25°C. An involvement of FAA, particularly taurine, in lymphocyte volume regulation is suggested.

Key words: hyposmolarity, swelling, free amino acids, DIDS

Cell volume regulation is a process described in several animal cell types exposed to anisosmotic media [1,2]. In hyposmotic conditions, cells rapidly swell because of their high permeability to water. Swelling is followed by a slower recovery phase in which cells return to near-normal volume. This process is known as regulatory volume decrease (RVD) and is mediated by a reduction in the internal solute content. Volume regulation has been observed in epithelial cells [3,6], red blood cells [7,8], Ehrlich ascites tumor cells, astrocytes [10,11], and human lymphocytes [12]. In vertebrate cells, the RVD is mainly supported by a net loss of intracellular K^+ and Cl^- [3,12,13]. In blood cells of euryhaline invertebrates and fishes, free amino acids (FAA), particularly taurine, also contribute to the regulatory process [14–16]. This occurs through the activation of amino acid efflux, thus reducing the osmotic gradient. In mammalian cells, the involvement of taurine and free amino acids in volume adjustment has been described in bird erythrocytes [17], Ehrlich acites cells [9], and cultured MDCK cells [18] and astrocytes [19]. Mammalian lymphocytes contain large amounts of taurine, in the millimolar range [20,21], and also possess the ability to regulate cell volume [22,23]. In order to investigate whether taurine and other FAA participate in the process of volume regulation in lymphocytes, in the present work, the release of taurine and FAA in response to decreases in external osmolarity was examined in rabbit lymphocytes.

MATERIALS AND METHODS

Chemicals and Solutions

A23187, DIDS, quinidine, tetraethylammonium, furosemide, colchicine, and cytochalasin B were purchased from Sigma Chemical Company (St. Louis, MO). Bumetanide was a gift from Leo Laboratories, Denmark.

Krebs-bicarbonate medium contained (in mM) NaCl 118, KCl 4.7, KH_2PO_4 1.17, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 10, pH 7.4, adjusted by bubbling with O_2/CO_2 (95%:5%). Solutions with decreased osmolarity were prepared by reducing the concentration of NaCl without modifying the concentration of other solutes, as follows: 100% osmolarity, NaCl 118 mM; 75% osmolarity, NaCl 79 mM; 62.5% osmolarity, NaCl 57.5 mM; 50% osmolarity, NaCl, 38.5 mM. Drugs were dissolved in water (boiling water for quinidine), except bumetanide and

Received February 26, 1990 accepted September 1, 1990.

Address reprint requests to H. Pasantes-Morales, Instituto de Fisiología Celular, UNAM, Apartado Postal 70-600, 04510 México D.F. Mexico.

A23187, which were dissolved in ethanol, and cytochalasin B, in dimethylsulfoxide.

Lymphocyte Isolation

Lymphocytes were obtained from adult albino rabbits (2–3 kg weight). A constant population of rabbits was used through all the experiments. The procedure of Boyum [24] was used for isolation of lymphocytes. Periferic blood was obtained by cardiac punction with sterile syringes containing 10% EDTA. The blood sample (20 ml) was diluted 1:1 with Krebs-bicarbonate medium, and 4 ml of this dilution was carefully layered on 3 ml of Hystopaque (Sigma) in conical centrifugue tubes of 15 ml. After centrifugation at 400*g* for 40 min, the interphase containing lymphocytes was separated, washed once, and resuspended in Krebs-bicarbonate medium. This preparation contained negligible contamination of platelets and polimorphonuclear cells and was free of erythrocytes. Cell viability, estimated by trypan blue exclusion, was about 98%.

Cell Volume Measurements

Cell volume changes were followed by changes in the optical properties of the lymphocyte suspension incubated in media of different osmolarity. Absorbance at 700 nm was measured in a double-beam spectrometer equipped with a magnetic stirrer and temperature controller. A cell suspension in Krebs-bicarbonate medium was diluted with water to obtain the desired osmolarity in 1 ml final volume. Changes in absorbance were followed during 10 min at 37°C. Except as otherwise indicated, volume measurements were carried out in a calcium-free medium to avoid cell aggregation. Volume regulation is unaffected under these conditions [12].

Release of ^{3}H-Taurine

For release experiments, cells were preincubated in a Krebs-bicarbonate medium containing ^{3}H-taurine (5 μM, 2 μCi/650 μl) at 37°C. After this loading period, cells were filtered in Millipore filters (0.65 μm pore). Filters were then transferred to glass superfusion chambers of 0.25 ml and superfused at a flow rate of 0.8 ml/min with Krebs-bicarbonate medium at 37°C. Fractions of the perfusate were collected at 1 min intervals directly into scintillation vials. After a washing period of 18 min, the baseline efflux was attained and cells were then stimulated during 8 min with media of reduced osmolarity. At the end of the superfusion, radioactivity remaining in cells and that of collected samples was measured by scintillation spectrometry. Results were expressed as fractional release, i.e., the radioactivity in fractions as percent of total radioactivity in the cells at the start of the superfusion, excluding the washing period. In some experiments, filters were transferred to vials containing 1 ml of the different experimental media and incubated during 8 min at 37°C. No difference was found between results of these two experimental procedures. Except when otherwise indicated, the tested drugs were added at the end of the loading period (15 min) and were present during the whole superfusion period. When drugs were dissolved in a solvent other than water, controls were exposed to the same concentration of the solvent used. Cell viability was examined in all experiments with drugs.

Determination of Endogenous Amino Acids

The FAA content of lymphocytes exposed to media of different osmolarity and FAA released by hyposmotic conditions were determined by reversed-phase HPLC in a Beckman chromatograph equipped with an Ultrasphere column. FAA from cells were extracted with 70% ethanol and derivatized with *O*-phthaldialdehyde. FAA in the superfusate were measured directly after derivatization.

RESULTS

Lymphocytes exposed to a medium with reduced osmolarity (159 m Osmoles) rapidly swell, reaching the highest volume within 30 s. Then the volume decreased and almost recovered the initial value after 6 min (Fig. 1).

Decreasing osmolarity of the superfusion medium resulted in a massive release of ^{3}H-taurine accumulated by cells during the loading period. The fractional release of ^{3}H-taurine that in isosmotic conditions corresponded to 17.8% in 8 min increased to 70.9% in the same period upon exposure to a medium with 50% reduction in osmolarity. The time course of the release process is shown in Figure 2. The peak release was attained within the first minute, after substracting the dead space of the superfusion system, and then the efflux declined to reach prestimulation values, despite the persistence of the hyposmotic conditions. A medium containing the reduced amount of NaCl was required to decrease osmolarity, but when made isosmotic with su-

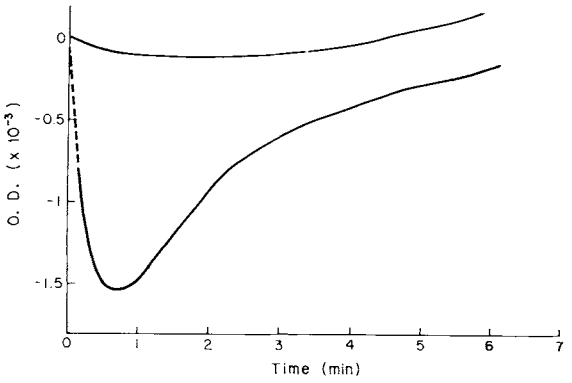

Fig. 1. Regulatory volume decrease in rabbit lymphocytes. Cells were suspended in isosmotic Krebs medium (upper line) or in hyposmotic (0.5) solution (lower line). Changes in absorbance were followed as described in Materials and Methods. Results correspond to a representative experiment from four separate experiments.

crose, was unable to stimulate ^{3}H-taurine release (Fig. 2).

The efflux of ^{3}H-taurine from lymphocytes was clearly associated to reductions in osmolarity. The fractional release of ^{3}H-taurine in media of 318, 270, 238, 197, and 159 Osmoles corresponded to 17.8%, 24.8%, 38.1%, 56.4%, and

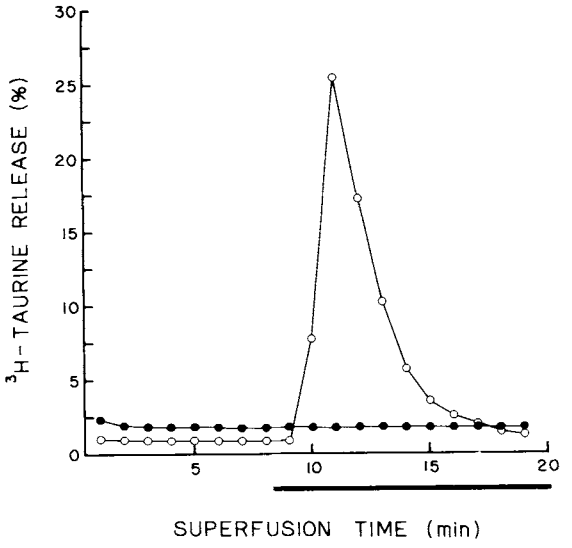

Fig. 2. The time course of ^{3}H-taurine release stimulated by decreased osmolarity. Loading and release conditions are as described in Materials and Methods. During the time indicated by the bar, the superfusion medium (isosmotic) was replaced by a hyposmotic medium (○), (159 mosmoles, 38.5 mM NaCl) or by a medium with reduced NaCl but made isosmotic with sucrose (●). Results are expressed as fractional release as described in Materials and Methods. Results are means of 4–6 separate experiments. S.E.M. range 2%–13%.

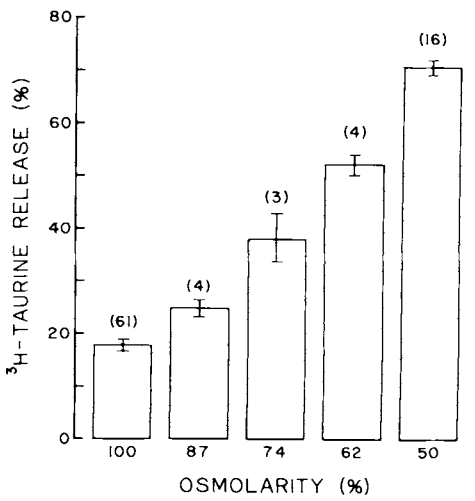

Fig. 3. The effect of decreasing osmolarity on ^{3}H-taurine release from rabbit lymphocytes. Cells were loaded with ^{3}H-taurine and superfused with solutions of decreasing osmolarities as indicated. Bars correspond to the release (%) during 8 min of superfusion. Results are means ± S.E.M. of the number of experiments indicated in parentheses.

70.9% of total accumulated ^{3}H-taurine, respectively (Fig. 3).

The efflux of ^{3}H-taurine stimulated by reduction in osmolarity was unaffected when NaCl was omitted from the superfusion medium and replaced by choline chloride. The osmotically induced ^{3}H-taurine release was significantly reduced (30%) by replacing chloride with the impermeant anion gluconate. Removal of external calcium had no effect on the osmolarity-sensitive release of taurine (Table I).

The release of ^{3}H-taurine evoked by hyposmolarity was independent of pH in the range of

TABLE I. Effect of Replacing External Ions on the Release of ^{3}H-Taurine Evoked by Hyposmolarity*

Conditions	^{3}H-taurine release (%)	
	Isosmotic	Hyposmotic
Control	9.08 ± 0.82 (38)	68.0 ± 7.17 (38)
Na^+-free	6.02 ± 1.3 (4)	72.5 ± 2.5 (4)
Cl^--free	5.70 ± 1.31 (10)	52.5 ± 4.58 (6)
Ca^{++}-free	8.40 ± 2.0 (3)	72.7 ± 0.46 (3)

*Cells were loaded with ^{3}H-taurine and superfused as described in Materials and Methods with isosmotic medium or with a medium of reduced osmolarity (0.5). The ionic composition was modified in both isosmotic and hyposmotic media. Na^+ and Cl^- in the experimental solutions were replaced by the corresponding salts of choline and gluconate. Calcium-free medium contains no calcium and 200 μM EGTA. Results are means ± S.E.M. of the number of experiments indicated in parentheses.

TABLE II. Effect of Inhibitors of Ionic Fluxes and Other Compounds on ^{3}H-Taurine Release Stimulated by Hyposmolarity

Drug	Concentration	^{3}H-taurine release (%) Isosmotic	Hyposmotic
Control		9.1 ± 0.8	68.0 ± 7.1
TEA	15 mM	5.4 ± 1.0	74.3 ± 0.1
Barium	15 mM	10.3 ± 0.8	67.1 ± 0.4
Gadolinium	10 μM	3.3 ± 0.3	69.6 ± 3.4
DIDS	100 μM	10.5 ± 2.6	50.2 ± 1.4
Quinidine	75 μM	9.2 ± 1.6	66.1 ± 12.0
Bumetanide	20 μM	8.8 ± 0.8	69.4 ± 4.8
	100 μM	10.9 ± 0.8	65.7 ± 3.0
Furosemide	1 μM	9.2 ± 0.4	66.3 ± 5.9
Pimozide	3 μM	8.5 ± 1.5	69.2 ± 5.2
Trifluoperazine	10 μM	9.8 ± 0.8	64.6 ± 12.1
Colchicine	0.5 mM	16.0 ± 3.0	69.5 ± 3.0
Cytochalasin B	10 μM	6.3 ± 1.3	68.5 ± 2.1

Cells were incubated with the drugs during 15 min at the end of the loading period and in the media during the whole superfusion period. When solvents other than water were used, controls were exposed to the same amount of solvent. Results are means ± S.E.M. of 3–38 experiments.

TABLE III. Effect of Decreased Osmolarity on FAA Content of Rabbit Lymphocytes

Amino acid	Concentration (nmoles/mg protein) Isosmotic	Hyposmotic[a]
Taurine	314.8 ± 26.3	134.6 ± 25.4
Glycine	22.9 ± 1.9	10.4 ± 1.0
β-Alanine	77.2 ± 4.2	39.0 ± 1.6
α-Alanine	12.2 ± 1.4	7.7 ± 0.5
Glutamic acid	8.2 ± 2.6	3.6 ± 0.4
Serine	7.0 ± 1.0	4.9 ± 0.7
Valine	4.6 ± 1.5	4.1 ± 0.9
Phenylalanine	2.6 ± 0.6	2.5 ± 0.3
Histidine	1.6 ± 0.4	1.2 ± 0.1

[a]Cells were incubated in isosmotic medium or in medium with reduced osmolarity (50%) at 37°C during 8 min. After incubation, cells were centrifuged and washed and FAA extracted with 70% ethanol. FAA content was measured by reversed-phase HPLC. Results are means ± S.E.M. of four separate experiments.

6.0–7.8. Taurine release induced by hyposmotic conditions examined at temperatures of 15°C and 25°C was not different from that observed at 37°C. At 4°C, the volume-sensitive release of taurine was markedly inhibited to only 20% of control at 37°C (results not shown).

The influence of the cytoskeleton on the volume-sensitive release of ^{3}H-taurine was examined by exposing cells to colchicine (0.5 mM) or cytochalasin B (10 μM). None of these drugs affected ^{3}H-taurine efflux (Table II).

The effects of compounds affecting ionic fluxes activated during the regulatory volume process on the release of ^{3}H-taurine evoked by hyposmolarity were examined in order to investigate a possible link between these two processes. Table I shows that the inhibitors of K^+/Cl^- cotransport furosemide and bumetanide had no effect on the volume-sensitive release of ^{3}H-taurine. From inhibitors of Cl^- channels, DIDS (100 μM) reduced ^{3}H-taurine release by 26%, whereas gadolinium had no effect. The K^+ channel blockers, tetraethylammonium (TEA), barium, and quinidine all failed to modify ^{3}H-taurine release (Table II). In complementary experiments, potassium channels were activated in isosmotic conditions by increasing intracellular calcium concentration with the ionophore A23187 [12]. Taurine release was not stimulated under these conditions (results not shown). Pimozide and trifluoperazine did not affect ^{3}H-taurine release.

Taurine was the most abundant free amino acid in rabbit lymphocytes and accounts for 70% of the total FAA pool (Table III). Other components of the FAA pool include glutamic acid, glycine, serine, α- and β-alanine, and histidine (Table III). Following stimulation with a hyposmolar medium, taurine content in cells fell from 314.8 to 134.6 nmoles/mg, i.e., a reduction of more than 58% (Table III). Similar decreases were observed in the intracellular concentration of glycine, glutamate, and β-alanine, whereas the concentration of α-alanine, serine, and histidine decreased only by 37%, 30%, and 13%, respectively. The concentration of valine, phenylalanine, and other non-identified FAA was unaffected by decreases in osmolarity (Table III). The concentration of FAA and taurine found in the extracellular medium after the hyposmotic stimulus was practically equivalent to that lost from the cellular pool.

DISCUSSION

Results of the present study showed that rabbit lymphocytes possess the mechanisms for cell volume regulation that have been described in other blood cells, including human lymphocytes [12,23]. Cell swelling in rabbit lymphocytes exposed to hyposmotic conditions is very rapid, and volume restoration is also rapid, somewhat faster than in human lymphocytes. Rabbit lym-

phocytes respond to the hyposmotic challenge by a massive release of FAA, particularly taurine, which is the predominant component of the FAA pool. The time course of taurine release closely follows that of the volume regulatory process. The magnitude of taurine release is proportional to the intensity of the stimulus and is quite sensitive, responding to small changes in osmolarity.

In most cells with the ability for volume adjustment, a loss of K^+ and Cl^- largely contributes to cell volume recovery in response to hyposmolar stimulus [1,13]. As previously mentioned, in many cells of marine invertebrates and vertebrates and in avian and mammalian tissues, FAA contribute also to the volume regulatory process. A possible link between the volume-sensitive release of FAA and the ionic fluxes activated by hyposmolarity has not been investigated. The results of the present study suggest that FAA efflux and K^+ conductance associated to volume recovery are unconnected events since anyone of the blockers of K^+ fluxes affected FAA release. The complementary observation that activating K^+ fluxes in isosmotic conditions did not elicit FAA release further supports this notion. A possible connection between Cl^- movements and FAA efflux is suggested by the inhibitory effect of DIDS, a blocker of Cl^- channels, and of the omission of Cl^-, on the volume-sensitive release of taurine. Taurine release evoked by hyposmolarity in rabbit lymphocytes seems unrelated to intracellular calcium concentration or to calcium-dependent transduction mechanisms. This is in contrast to that observed in Ehrlich ascites tumor cells, in which release of FAA stimulated by hyposmolarity is inhibited by anticalmodulin drugs [25].

The mechanism of taurine and FAA release associated with volume regulation is still unclear. Taurine, as many amino acids, enters the cell by a Na^+-dependent, energy-mediated, specific, and saturable transport system. This mechanism apparently is not involved in the release of taurine evoked by hyposmolarity since taurine efflux is not affected by removal of Na^+ and the release of endogenous taurine is not stimulated by an increase in external taurine concentration ([25,26] and the present results). In flounder and eel red cells, a Na^+-independent, non-saturable process of taurine accumulation is highly activated in hyposmolar conditions. These observations suggest that a simple leak pathway, independent of the saturable uptake system, is responsible for the net loss of taurine during the regulatory volume decrease. This seems to be the mechanism also in rabbit lymphocytes as suggested by the Na^+ and temperature independence of the taurine release process. The inhibitory effect of very low temperatures on the volume-sensitive efflux of taurine may be due to changes in the fluidity of membrane constituents that could affect diffusional processes.

FAA and taurine contribute only a minor fraction to volume restoration in hypotonically swollen lymphocytes since the loss of FAA is 54% of an endogenous pool of approximately 45 mM. It has been assumed that in human lymphocytes K^+ and Cl^- are the only osmotically active solutes involved in volume regulation [27], but the present observations on the magnitude and the temporal course of taurine release in hyposmotically challenged rabbit lymphocytes suggest a contribution, although modest, of this organic compound to the regulatory process. Early observations of Hoffmann and Handel [28] have also pointed to the involvement of FAA, particularly taurine and glycine, in volume regulation in Ehrlich ascites cells. Moreover, Deutsch and Lee [23] have stressed the requirement of an efflux of osmolytes other than K^+ and Cl^- to underlie regulatory volume decreases in lymphocytes, particularly in conditions of large reductions in osmolarity. This consideration is based on the amount of chloride $[Cl]_i$ in lymphocytes being entirely exhausted under conditions of large decreases in osmolarity in which, however, a regulatory volume decrease still occurs. Taurine and other FAA may then contribute to cell volume adjustment in these stringent situations.

ACKNOWLEDGMENTS

This research was supported in part by grants PCALCNA 050281 (CoNaCyT) and PRDCyT (OEA). The authors wish to thank the help provided by Ms. Claudia Díaz in the initial part of this study.

REFERENCES

1. Lauf PK: J Membr Biol 88:1–13, 1985.
2. Macknight AD: Renal Physiol Biochem 3–5:114–141, 1988.
3. Spring KR, Ericson AC: J Membr Biol 69:167–176, 1982.
4. Ussing HH: Acta Physiol Scand 114:363–369, 1982.
5. Spring KR: Fed Proc 44:2526–2529, 1985.
6. Beck FK, Dörge A, Thurau K: Renal Physiol Biochem 5:174–186, 1988.

7. Kregenow FM: Annu Rev Physiol 43:493–505, 1981.
8. Cala PM: Mol Physiol 4:33–52, 1983.
9. Hoffmann EK, Lambert IH, Simonsen LO: Renal Physiol Biochem 3–5:221–247, 1988.
10. Olson J, Holtzman D: Brain Res 246:273–279, 1982.
11. Kimelberg HK, Frangakis MV: Brain Res 361:125–134, 1985.
12. Grinstein S, Dupré A, Rothstein A: J Gen Physiol 79: 849–868, 1982.
13. Eveloff SL, Warnock DG: Am J Physiol 252:F1–F10, 1987.
14. Pierce SK, Greenberg MJ: J Exp Biol 57:681–692, 1972.
15. Fincham DA, Wolowyk MW, Young JD: J Membr Biol 96:45–56, 1987.
16. Fugelli K, Thoroed SM: J Physiol 374:245–261, 1986.
17. Shihabi ZK, Goodman HO, Holmes RP, O'Connor ML: Comp Biochem Physiol 92A:545–549, 1989.
18. Roy G, Sauvé R: J Membr Biol 100:83–96, 1987.
19. Pasantes-Morales H, Schousboe A: J Neurosci Res 20: 505–509, 1988.
20. Fukuda K, Hirai Y, Yoshida H, Nakajima T, Usui T: Clin Chem 28:1758–176, 1982.
21. Vinton NE, Laidlaw SA, Ament ME, Kopple JD: J Clin Nutr 44:398–404, 1986.
22. Grinstein S, Rothstein A, Sarkadi B, Gelfand EW: Am J Physiol 246:204–215, 1984.
23. Deutsch C, Lee SC: Renal Physiol Biochem 3–5:260–276, 1988.
24. Boyum A: Scand J Clin Lab Invest 21:77–83, 1968.
25. Lambert IH: Mol Physiol 7:323–332, 1985.
26. Lambert IH: Mol Physiol 6:233–246, 1984.
27. Grinstein S, Dixon J: Physiol Rev 69:417–481, 1989.
28. Hoffmann EK, Handel KB: J Comp Physiol 108:279–286, 1976.