Biochimica et Biophysica Acta, 946 (1988) 261–269
Elsevier

BBA 74251

Effects of L-alanine on membrane potential, potassium (^{86}Rb) permeability and cell volume in hepatocytes from *Raja erinacea*

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(Received 1 July 1988)
(Revised manuscript received 20 September 1988)

Key words: L-Alanine; Membrane potential; Potassium permeability; Cell volume; Hepatocyte; (Fish liver)

Isolated hepatocytes from the elasmobranch *Raja erinacea* were examined for their regulatory responses to a solute load following electrogenic uptake of L-alanine. The transmembrane potential (V_m) was measured with glass microelectrodes filled with 0.5 M KCl (75 to 208 MΩ in elasmobranch Ringer's solution) and averaged -61 ± 16 mV (S.D.; $n = 68$). L-Alanine decreased (depolarized) V_m by 7 ± 3 and 18 ± 2 mV at concentrations of 1 and 10 mM, respectively. V_m did not repolarize to control values during the 5–10 min impalements, unless the amino acid was washed away from the hepatocytes. The depolarizing effect of L-alanine was dependent on external Na^+, and was specific for the L-isomer of alanine, as D- and β-alanine had no effect. Hepatocyte V_m also depolarized on addition of KCN or ouabain, or when external K^+ was increased. Rates of $^{86}Rb^+$ uptake and efflux were measured to assess the effects of L-alanine on Na^+/K^+-ATPase activity and K^+ permeability, respectively. Greater than 80% of the $^{86}Rb^+$ uptake was inhibited by 2 mM ouabain, or by substitution of choline$^+$ for Na^+ in the incubation media. L-Alanine (10 mM) increased $^{86}Rb^+$ uptake by 18–49%, consistent with an increase in Na^+/K^+ pump activity, but had no effect on rubidium efflux. L-Alanine, at concentrations up to 20 mM, also had no measurable effect on cell volume as determined by ^3H_2O and [^{14}C]inulin distribution. These results indicate that Na^+-coupled uptake of L-alanine by skate hepatocytes is rheogenic, as previously observed in other cell systems. However, in contrast to mammalian hepatocytes, V_m does not repolarize for at least 10 min after the administration of L-alanine, and changes in cell volume and potassium permeability are also not observed.

Introduction

Transport of L-alanine across the basolateral plasma membrane of the liver cell is believed to be the rate-limiting and regulated step in the metabolism of this important amino-acid substrate for the liver [1–3]. Uptake of L-alanine by hepatocytes, including hepatocytes from the skate *Raja erinacea* [4], is concentrative, and occurs predominantly by an electrogenic Na^+-cotransport system [5]. Studies by Kristensen [6] and Kristensen and Folke [7] demonstrate that the cotransport of Na^+ and L-alanine by rat hepatocytes leads to an increase in cell volume, presumably as a result of the increase in solute load. The mammalian hepatocyte compensates by increasing K^+ permeability and Na^+/K^+-ATPase activity, thereby restoring cell volume and the sodium gradient. Addition of 10 mM L-alanine to rat or

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mouse hepatocytes also results in a transient depolarization, followed by a sustained hyperpolarization, which is attributed primarily to a change in K^+ permeability [8–10]. Thus, mammalian hepatocytes appear to regulate their osmotic balance during organic solute transport by eliminating intracellular ions, particularly K^+. The present study examines this phenomenon in hepatocytes from a lower vertebrate, *Raja erinacea*, to determine whether similar regulatory mechanisms exist in response to a solute load. Previous studies from this laboratory have demonstrated that these cells transport L-alanine primarily by a Na^+-dependent process, and are able to concentrate this amino acid at least 16-fold when incubated in the presence of 0.5 mM L-alanine [4].

Materials and Methods

Materials and animals. Hepatocytes were isolated from male skates (*Raja erinacea*; 0.7–1.2 kg body weight), that were obtained from Frenchman's Bay in Maine, and maintained for up to 4 days before use in large tanks equipped with flowing 15°C sea water at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine. [*Carboxyl*-^{14}C]Carboxylinulin, [^{3}H]H_2O and ^{86}RbCl were obtained from New England Nuclear, Boston, MA. Collagenase (Type I) deoxyribonuclease II, L-alanine, D-alanine, β-alanine, ouabain and aminooxyacetic acid were obtained from Sigma, St. Louis, MO.

Hepatocyte isolation. The methods for isolating skate hepatocytes, as well as their morphologic and biochemical characterization are described in detail elsewhere [4,11]. In brief, skates were anesthetized with pentobarbital sodium, 5 mg/kg, administered via the caudal vein. During surgery the animals' gills were superfused with running sea water. A glass cannula was inserted into the portal vein, the liver was removed from the abdominal cavity, and placed in a water-cooled perfusion dish (15°C). A non-recirculating perfusion with oxygenated and heparinized Ca^{2+}/Mg^{2+}-free elasmobranch Ringer's solution was then started immediately. After 10 min of single-pass perfusion, a recirculating perfusion was started with a collagenase solution (0.07–0.1% of Sigma collagenase No. C-0130, in elasmobranch Ringer's containing (mM), 270 NaCl, 4 KCl, 3 $MgCl_2$, 0.5 Na_2SO_4, 1 KH_2PO_4, 8 $NaHCO_3$, and 350 urea). The collagenase perfusion was continued for 10–15 min, at which time the liver was raked free of connective tissue, in a solution of elasmobranch Ringer's solution containing deoxyribonuclease II. The resulting cell suspension was filtered through 60 μm nylon mesh and centrifuged at $250 \times g$. The cells were resuspended in either normal elasmobranch Ringer's solution, or a sodium-free medium (see below), and centrifuged at $250 \times g$. The washed hepatocytes were resuspended at a concentration of 20–40 mg wet wt./ml (approx. $(2–4) \cdot 10^6$ cells/ml), in one of two different incubation buffers: (1) normal elasmobranch Ringer's, or (2) a sodium-free medium that was prepared by isosmotic substitution of NaCl and $NaHCO_3$ with the respective choline salts, and by replacing Na_2SO_4 with $MgSO_4$ ($MgCl_2$ was decreased to adjust the Mg^{2+} concentration). Where indicated, the medium also contained 2 mM aminooxyacetate and/or 2 mM ouabain. The cells were preincubated at 15°C in 50 ml polypropylene tubes, under an atmosphere of 99% O_1/1% CO_2 for 20–30 min before the start of each experiment.

Electrical measurements. Aliquots of the final cell suspension, 100 μl containing approx. 10^6 cells, were added to 35-mm tissue culture dishes containing glass coverslips plus 2 ml of modified elasmobranch Ringer's solution comprising additions of Hepes buffer (Na^+ salt, 5 mM) and adjusting pH to 7.6 by adding 1 M HCl (1.5 ml/l Ringer's). Hepatocytes adhered to the coverslips at 13°C for approx. 4 h prior to electrophysiologic measurements. In this preparation, 3–4 hepatocytes often remained attached to one another and surrounded a bile canaliculus, thus retaining the same structural polarity as in the intact liver [11].

Microfilament capillary glass (1.2 mm o.d., 0.68 i.d., A-M Systems, Everett, WA) was cleaned by boiling for 15 min in 0.5 l of distilled water containing three drops of detergent (Liquinox), followed by a 1 h tap-water rinse. The capillaries were then boiled again for 15 min in distilled water, and dried at 90°C in a forced-air oven. Micropipettes were drawn from the capillary tubing by a horizontal puller (Industrial Sci. Assoc., Ridgewood, NY). Microelectrodes were prepared

by filling the micropipettes with 0.5 M KCl. Tip-resistances in Ringer's solution ranged from 100 to 240 MΩ, but averaged 120 MΩ. An Ag|AgCl half-cell in an agar (4% in Ringer's solution) bridge was used as a reference electrode. Microelectrodes were connected by an Ag|AgCl half-cell to a high-input impedance (above 10^{11}) preamplifier with unit gain (KS700 Dual-Probe, World Precision Instr., New Haven, CT). DC voltages were recorded with a digital voltmeter and a strip-chart recorder (Western Graphtec). Estimated cell input resistance was monitored continuously during most recordings by passing intermittent current (0.1 nA, 300 ms duration) through the recording microelectrode. A bridge circuit with a variable resistance in the preamplifier provided the means to compensate for the microelectrode resistance.

Micropuncture technique. Micromanipulators with remote joystick controls (Brinkmann) were mounted to the stage of an inverted microscope (Olympus IMT). A microelectrode was positioned manually immediately above the cell. The microelectrode was advanced axially until it touched the cell surface. The latter was characterized either by an increase in input resistance or by a 5–10 mV shift in offset voltage. Impalement of the cell was achieved by lightly tapping the table which supported the microscope. Valid recordings of membrane potential (V_m) were characterized by (1) a sudden increase in electronegative voltage on impalement, (2) an increase in input resistance, (3) 1 min of stable intracellular recording, and (4) return of trace to baseline when the microelectrode was withdrawn from the cell.

Coverslips containing adhered hepatocytes were placed into an acrylic chamber on the microscope stage. Cells were superfused continuously by gravity at 3.5 ml/min with elasmobranch Ringer's solution modified as above. In some experiments, cells were superfused with a Na^+-free Ringer's solution made by substituting equivalent amounts of choline chloride, choline HCO_3 and $MgSO_4$. Na-Hepes was not added and pH was maintained at 7.6 by gassing the solution with 99% O_2/1% CO_2. Ringer's solution was cooled prior to entering the cell chamber as it ran through the coil of a Graham condenser, whose jacket was flushed continuously with 12–13°C sea water, resulting in solution temperatures of 16–17°C in the chamber. Effects of drugs, amino acids, and altered ionic composition of the Ringer's solution on V_m were determined by switching from control to experimental Ringer's solution while continous intracellular recordings of V_m were obtained.

Rubidium uptake and efflux. Potassium flux measurements were made using $^{86}Rb^+$ as a tracer for K^+. $^{86}Rb^+$ uptake experiments were initiated by the addition of tracer concentrations of $^{86}RbCl$ (0.2 μCi/ml) to the incubation mixtures kept at 15°C, under an atmosphere of 99% O_2/1% CO_2. The polypropylene incubation tubes were agitated periodically to maintain the cells in suspension. Aliquots of the cell suspensions (0.3 ml) were removed at appropriate time intervals, and added to 1.5 ml polypropylene microfuge tubes containing 1.1 ml of ice-cold incubation medium. This suspension was immediately centrifuged at room temperature for 10 s at 10000 × *g* in a Beckman Microfuge B. An aliquot of the supernatant was removed for scintillation counting, and the rest aspirated with a Pasteur pipette. The residual fluid on the surface of the cell pellet was removed by gently touching the top of the pellet with a strip of filter paper, as previously described [12]. The microfuge tubes were cut with a razor blade just above the cell pellets, and the pellets transferred to glass scintillation vials. The pellets were extracted by vigorous mixing in the presence of 0.2 ml of 4% 5-sulfosalicylic acid, and dissolved in 6 ml of Opti-Fluor (Packard Instrument Co.) Radioactivity in the pellet extracts, and in aliquots of the incubation media and wash solutions was determined by scintillation spectrometry. Rubidium uptake is expressed as the ratio of dpm $^{86}Rb^+$ in the intracellular and extracellular water spaces.

For the ^{86}Rb efflux experiments, the cells were preloaded with rubidium for 1.5 h, centrifuged for 2 min at 250 × *g*, and resuspended in either choline Ringer's, or in normal Ringer's containing either (a) 10 mM L-alanine, (b) 10 mM L-alanine plus 2 mM aminooxyacetic acid, (c) 2 mM ouabain, or (d) hypotonic media (40% H_2O). Efflux was terminated at various time intervals by centrifugation of a 0.3 ml aliquot for 10 s at 10000 × *g*. The supernatant was separated from the cell pellet as described above.

Cell volume measurements. The intracellular water space was determined as the difference be-

tween the ^3H_2O and [^{14}C]inulin distribution spaces. Cells were incubated in media containing both ^3H_2O (0.4 μCi/ml) and [^{14}C]inulin (0.2 μCi/ml): 0.3 ml aliquots of the cell suspensions were removed at various time intervals, and centrifuged for 10 s at $10000 \times g$. ^{3}H and ^{14}C distribution spaces were calculated by dual-label counting, after appropriate correction for quenching and channel overlap. For each experiment, quenching was estimated by adding an equivalent cell pellet containing no radioactivity (prepared at the beginning of the experiment) to an aliquot of the incubation solution.

Results

Hepatocyte membrane potential – effects of L-alanine

Valid recordings of V_m were obtained in 41% of impalement attempts, and V_m averaged -61 ± 16 mV (S.D.; $n = 68$) ranging from -30 to -100 mV. A frequency distribution for these V_m is shown in Fig. 1. Estimated cell input resistance ranged from 75 to 208 MΩ and averaged 139 ± 36 MΩ (S.D.; $n = 68$).

Effects of metabolic inhibitors on the apparent V_m were used to verify that microelectrodes were inside of cells when positioned in the cell clusters. KCN (10^{-4} M) decreased V_m by 13 ± 4 mV ($n = 3$) within 2 min. Ouabain (10^{-3} M) decreased V_m by 9 ± 1 mV ($n = 4$) over a period of 20–30 min.

To establish further the validity of our V_m recordings, we measured the effects of increasing external KCl concentration (equivalent KCl substitution for external NaCl) on V_m, assuming that K^+ conductance contributes to the V_m of skate

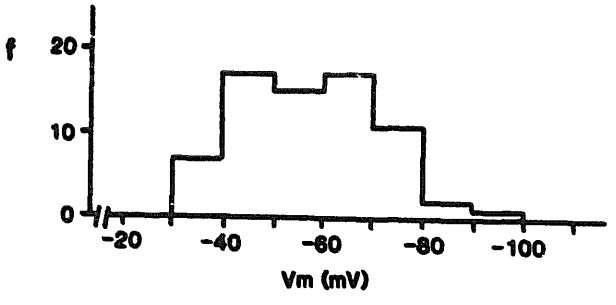

Fig. 1. A frequency distribution of skate (*R. erinacea*) hepatocyte V_m. V_m were measured in isolated cells that had attached to glass coverslips for approx. 4 h.

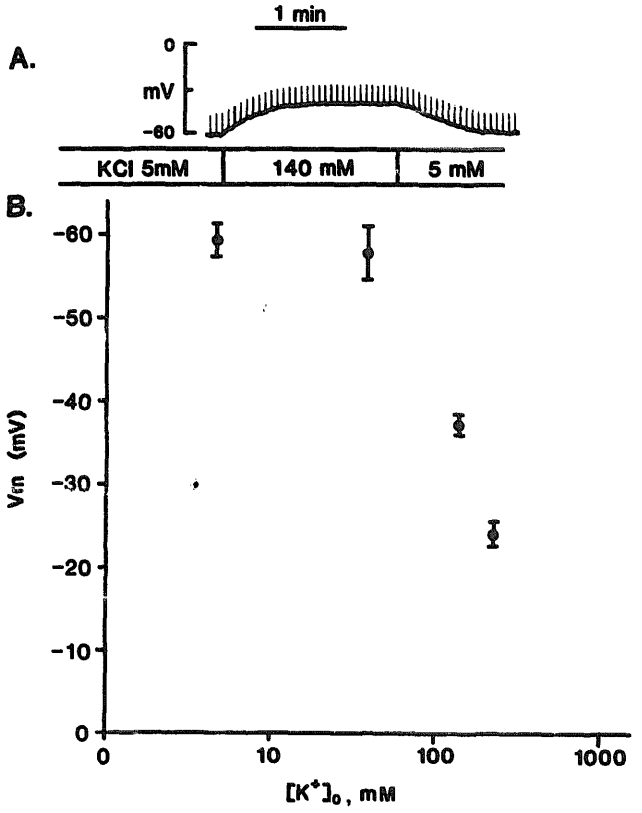

Fig. 2. (A) A representative continuous recording of V_m during three-step protocol of superfusing cells with control medium, with experimental medium (high K^+), and again with control medium. $[K^+]_o$ was increased by substituting external NaCl with an equivalent concentration of KCl. (B) Skate hepatocyte V_m as a function of $\log_{10}$ $[K^+]_o$. Each point is the mean ± S.E. of at least three measurements. $[K^+]_o$ were 4, 50, 140 and 230 mM.

hepatocytes. Effects of these ion substitutions on hepatocyte V_m were measured while continuously maintaining intracellular recordings (Fig. 2A). V_m decreased to a steady state in response to increasing external K^+ to 140 mM and repolarized to control V_m when the external K^+ concentration was restored to 5 mM. V_m plotted as a function of the $\log_{10}$ $[K^+]_o$ is shown in Fig. 2B. These increases in $[K^+]_o$ had no measurable effect on microelectrode junction potentials recorded when the ion substitution was performed with the microelectrode in the bath.

The effect of L-alanine on skate hepatocyte V_m is illustrated in Fig. 3 (top traces). At a concentration of 10 mM, L-alanine immediately decreased V_m by 18 ± 2 mV (from -90 ± 12 to -72 ± 13 mV; mean ± S.D. of 13 measurements in two cell preparations), an effect that was dose-dependent,

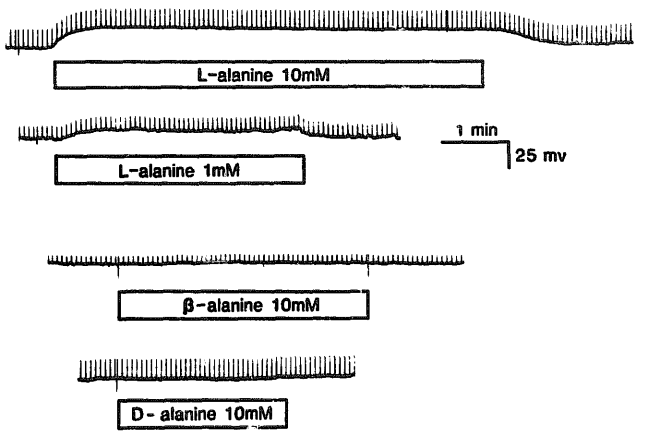

Fig. 3. Effects of various alanine analogues on skate (*R. erinacea*) hepatocyte V_m. Time and voltage bases apply to all traces. Vertical deflections result from intermittent current (0.1 nA, 300 ms) to measure input resistance.

since 1 mM L-alanine decreased V_m by only 7 ± 3 mV (from -70 ± 20 to -63 ± 20 mV; mean ± S.D. of five measurements in two cell preparations) (Fig. 3). With alanine present in the superfusion media, V_m repolarized toward control value only by 1–2 mV (Fig. 3). This small repolarization value is much less than occurs in mammalian hepatocytes, where V_m often repolarizes to values more electronegative than the control V_m, while extracellular L-alanine is present continuously [8–10]. When Na^+ was substituted with choline in the elasmobranch Ringer's, L-alanine had no effect on V_m ($V_m = -35 \pm 11$ mV remained unchanged with 10 mM alanine, $n = 5$). Thus, the alanine-induced depolarization requires the presence of Na^+ in the external media.

Neither D-alanine nor β-alanine (10 mM) altered skate hepatocyte V_m (Fig. 3, bottom traces). In contrast, L-glutamine, which inhibits L-alanine transport in skate hepatocytes [4], decreased V_m by 5 ± 3 mV (from -52 ± 7 to -47 ± 7 mV; $n = 5$, traces not shown).

Rubidium ($^{86}Rb^+$) fluxes

Fig. 4 illustrates the time-course of $^{86}Rb^+$ uptake by skate hepatocytes. Uptake was measured in normal elasmobranch Ringer's and in media where the Na^+ was replaced with choline$^+$, for (a) control cells, (b) cells incubated with 10 mM L-alanine, and (c) cells incubated with 2 mM ouabain. The results are expressed as the ^{86}Rb concentration ratio between the intracellular and extracellular water spaces. Uptake in Na^+-Ringer's was 3–4-fold higher than in choline$^+$-Ringer's (Fig. 4A). The distribution ratio was approx. 14 after 4 h of incubation in Na^+-Ringer's, and was still increasing, suggesting that the isotope had not reached electrochemical equilibrium over this time interval. Cells incubated with 10 mM L-alanine accumulated more $^{86}Rb^+$ than control cells (Fig. 4B); the distribution ratio was 20.8 after 4 h of incubation in Na^+-Ringer's. In contrast, cells treated with ouabain (2 mM) accumulated only small quantities of $^{86}Rb^+$ (Fig. 4C). Ouabain inhibited the uptake of rubidium in both Na^+- and choline$^+$-containing media. Since a small amount of Na^+ was still present in cell suspensions incubated in choline-Ringer's (no more than 5 mM), some of the $^{86}Rb^+$ taken up in choline media may have also been dependent on Na^+-coupled mechanisms. This residual Na^+ probably originated from both the release of intracellular Na^+ during the incubations, and as an extracellular contaminant during the cell-washing procedure.

To distinguish whether L-alanine stimulated $^{86}Rb^+$ uptake by enhancing Na^+ influx, and

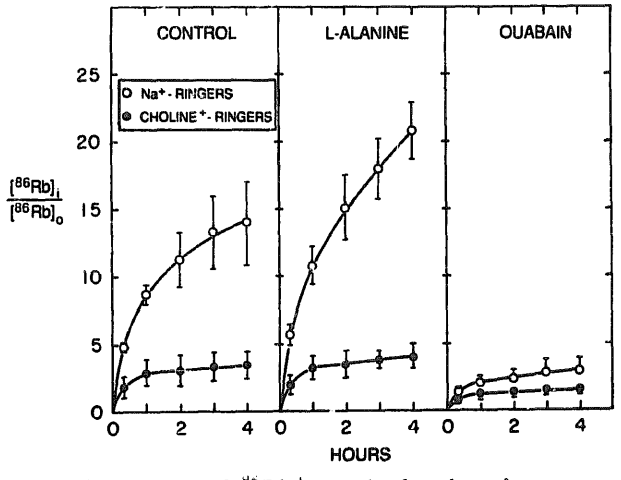

Fig. 4. Time-course of $^{86}Rb^+$ uptake by skate hepatocytes. Cells were incubated in either normal Na^+-Ringer (○) or in media where choline$^+$ replaced the Na^+ (●), for (A) control cells, (B) cells incubated with 10 mM L-alanine, and (C) cells incubated with 2 mM ouabain. The cells were preincubated with the indicated concentrations of L-alanine and ouabain for 30 min prior to the addition of $^{86}Rb^+$. Results are expressed as the ^{86}Rb concentration ratio between the intracellular and extracellular water spaces. Values shown are means ± S.D. of four experiments, each performed in duplicate.

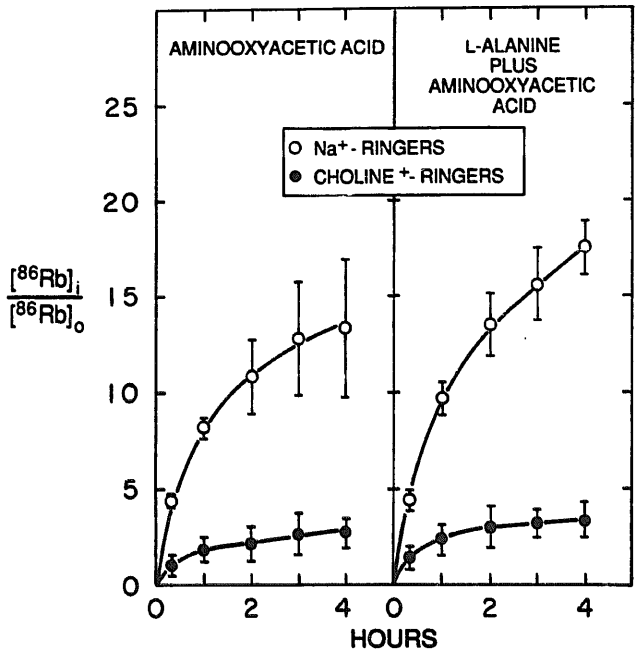

Fig. 5. Effects of L-alanine on the time course of $^{86}Rb^+$ uptake by skate hepatocytes treated with 2 mM aminooxyacetic acid, an inhibitor of alanine oxidation. For explanatory information see legend to Fig. 4. Values are means ± S.D. of three cell preparations, each performed in duplicate.

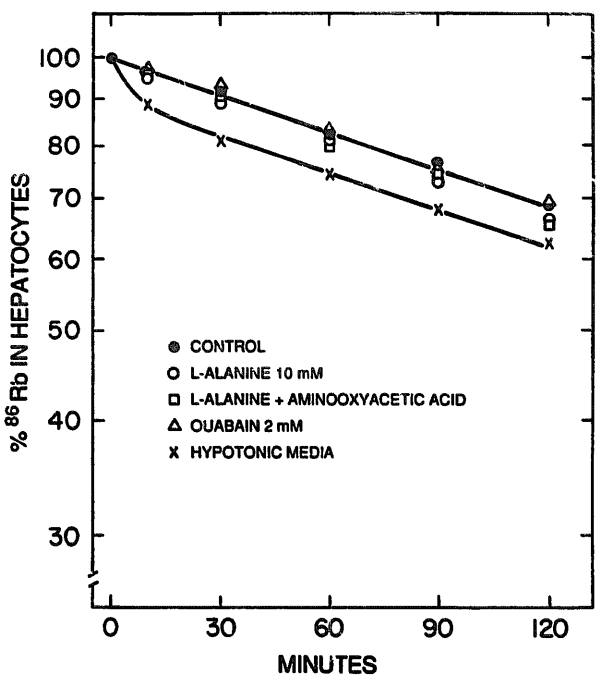

Fig. 6. Time-course of $^{86}Rb^+$ efflux. Efflux was measured in control skate hepatocytes (●; $n = 4$ cell preparations, each performed in duplicate) and hepatocytes that received either 10 mM L-alanine (○, $n = 4$), 10 mM L-alanine plus 2 mM aminooxyacetic acid (□, $n = 3$), 2 mM ouabain (△, $n = 4$) or 40% H_2O (×, hypotonic media; $n = 2$). These additions were made at time zero, and rubidium content of the hepatocytes measured over the subsequent 120 min.

thereby stimulating Na^+/K^+-ATPase activity, or by serving as an oxidative substrate for the liver, the experiments presented in Fig. 4A and B were repeated with cells that were incubated with 2 mM aminooxyacetic acid, an inhibitor of L-alanine oxidation (Fig. 5). L-Alanine enhanced $^{86}Rb^+$ influx even in the presence of aminooxyacetate; the rubidium distribution ratios after 4 h in Na^+-Ringer's were 13.5 and 17.5 for control and L-alanine-treated cells, respectively. These data suggest that the increase in $^{86}Rb^+$ influx was not due to the oxidation of L-alanine, but was probably secondary to the increase in intracellular Na^+ resulting from Na^+-alanine cotransport.

TABLE I

EFFECTS OF L-ALANINE, OUABAIN, AND A Na^+-FREE MEDIUM ON VOLUME OF ISOLATED SKATE HEPATOCYTES

The intracellular water space was determined as the difference between the ^3H_2O and [^{14}C]inulin distribution spaces, in cells continuously exposed to both isotopes in the incubation media. L-Alanine or Ringer's for control was added at 30 min, while ouabain or choline$^+$-Ringer's was added at the start of the incubation. Values are means ± S.D. of the number of cell preparations indicated, each performed in duplicate.

	n	Time (min):	Relative volume 0	10	30	40	60	90	120	150
Control	4		100	95.2±5.6	95.4±3.6	90.6±10.5	94.7±11.0	91.9±12.8	86.1±11.0	83.6±14.0
L-Alanine 20 mM	3		100	96.9±2.8	94.6±3.5	94.2± 6.7	89.3± 4.7	93.6± 8.5	81.2± 9.4	76.0±14.6
Ouabain 2 mM	4		100	100.1±3.3	102.3±8.2	–	96.7± 8.6	95.8±11.0	91.5±12.1	91.7±15.9
Choline$^+$-Ringer's	3		100	99.3±8.0	99.6±6.9	–	91.7±14.4	92.8±18.6	82.7± 4.0	80.9± 2.9

Efflux of $^{86}Rb^+$ from skate hepatocytes was a first-order process (Fig. 6). 30–35% of the rubidium was released over a 2 h interval, and this rate of release was unaffected by ouabain or L-alanine (Fig. 6). This latter observation is in contrast to findings in rat hepatocytes, where L-alanine increases K^+ permeability 3–4-fold [6]. Hepatocytes exposed to hypotonic media released ^{86}Rb slightly faster for the first 30 min, but then ^{86}Rb efflux rate was the same as controls for the subsequent 90 min (Fig. 6).

Cell volume

The effects of ouabain and Na^+-free medium on cell volume are illustrated in Table I. Neither of these treatments had any effect on cell volume, suggesting that, as in other cells, mechanisms in addition to the Na^+/K^+-ATPase pump account for the short-term regulation of cell volume by skate hepatocytes [13]. However, in contrast to mammalian hepatocytes [7,14], L-alanine also had no effect on cell volume (Table I). Other studies have shown that skate hepatocytes swell rapidly when exposed to hypotonic media, and that the cells return slowly towards their original volume over a 1 h interval [15].

Discussion

Uptake of L-alanine and Na^+ by a variety of cell types activates mechanisms that regulate their osmotic and electrical equilibrium [6,7,10,14,16, 17]. In response to the cotransport of Na^+ and alanine, cells must compensate for both solute load and increased positive charge to maintain their volume and membrane electric potential, respectively. Although the mechanisms are not well understood, recent studies in rat hepatocytes suggest that the two processes are closely interrelated [7,14]. When rat hepatocytes are incubated with 10 mM L-alanine, there is an immediate decrease in membrane potential, and an increase in cell sodium and cell volume. Subsequent increases in K^+ permeability and Na^+/K^+-ATPase pump activity repolarize the membrane potential, and provide partial compensation for the increased osmotic load due to the uptake of Na^+ and alanine. These homeostatic mechanisms act in concert to reestablish the Na^+ electrochemical gradient during Na–alanine cotransport, while restoring cell volume and membrane voltage. Studies in rat hepatocytes and *Necturus* enterocytes show that the membrane potential actually repolarizes to or above control values after the L-alanine-induced depolarization, even if the amino acid remains in the incubation media [6,10,16]. Although the primary stimulus for this increase in K^+ permeability has not been defined, recent studies by Kristensen and Folke [7] and Bakker-Grunwald [14] suggest that changes in cell volume may be the primary stimulus for the activation of the K^+ channels, rather than to a direct effect of Na^+ and alanine, or to an effect of membrane depolarization on K^+ channels.

The present findings in skate hepatocytes demonstrate that the concentrative uptake of L-alanine by these cells also is associated with membrane depolarization. However, in contrast to rat hepatocytes, skate liver cells remain depolarized for at least 5–10 min in the presence of L-alanine, and exhibit no measureable change in cell volume or significant increase in K^+ ($^{86}Rb^+$) permeability. These findings suggest that electrogenic alanine transport elicits a counter-balancing volume regulatory response that is independent of K^+ loss, or alternatively that cell volume is not increased significantly during alanine uptake in this elasmobranch cell. Since skate hepatocytes exhibit a volume regulatory decrease following exposure hypotonic media [15] that cannot be explained by the observed increase in K^+ efflux (Fig. 6), these cells probably regulate cell volume differently from mammalian hepatocytes. This interpretation is consistent with previous studies showing that many lower vertebrates regulate cell volume primarily by adjusting intracellular organic solute (e.g., amino acid) rather than salt concentrations [18]. Indeed, previous studies by King et al. [19] and Boyd et al. [20] indicate that skate hepatocytes are capable of adjusting their intracellular amino-acid concentrations in response to an osmotic stress; however, neither the mechanism nor the principal amino acids involved in this process has been identified.

The dissociation between the effects of L-alanine on membrane potential and its effects on cell volume and K^+ ($^{86}Rb^+$) permeability in skate hepatocytes indicates that these events are not necessarily coupled. Thus, membrane depolariza-

tion is not sufficient to induce an increase in K^+ permeability or cell volume. These observations therefore indirectly support the hypothesis proposed by Kristensen and Folke [7] and Bakker-Grunwald [14] that the L-alanine-induced change in K^+ permeability in rat hepatocytes is due primarily to a change in cell volume.

The different responses of skate and rat hepatocytes to the concentrative uptake of L-alanine also may be due in part to the kinetics of the uptake mechanisms [4,21]. Although the liver cells of both species concentrate L-alanine approx. 15–20-fold when incubated in low concentrations of L-alanine (0.5 mM), the rates of uptake differ markedly. Skate hepatocytes incubated at 15°C require nearly 2 h to establish this gradient [4], while in rat hepatocytes incubated at 37°C alanine reaches its steady-state distribution in only 10 min [21]. These different transport rates have obvious implications with regard to the mechanisms that these cells must employ to compensate for the increased influx of Na^+ and L-alanine. For example, although in the present studies we noted only a 1–2 mV repolarization of V_m over a 5–10 min interval following the L-alanine-induced depolarization (18 mV; Fig. 3), this time interval may not be sufficient to allow recovery of V_m. Similarly, since Na^+ and L-alanine are taken up relatively slowly by skate hepatocytes, changes in cell volume may not be detectable, since volume regulatory mechanisms may have sufficient time to compensate for the increased osmotic load.

L-Alanine-induced membrane depolarization in skate hepatocyte was dependent on external Na^+ and was specific for the L-isomer of alanine, as D- and β-alanine had no effect. These observations are consistent with our previous findings that the uptake of L-alanine by skate hepatocytes was largely Na^+-dependent, and was not inhibited by D- or β-alanine [4]. In contrast, L-glutamine, an amino acid that inhibits L-alanine uptake in skate hepatocytes, also depolarized the membrane potential.

The plot of V_m vs. $\log_{10}[K^+]_o$ shows that V_m decreases with increasing $[K^+]_o$, thereby substantiating the validity of the potential recordings, and showing that K^+ conductance contributes significantly to skate hepatocyte V_m. However, the curvilinear relationship between V_m and $[K^+]_o$ indicates that, while K^+ conductance contributes to V_m, it is not a Nernstian function, as would be expected of a K^+-selective membrane (Fig. 2). Thus, membrane conductances of other ions, notably Na^+ and/or Cl^-, also may contribute to V_m. The decrease of V_m in response to KCN reflects an energy dependent transport process, as does the decrease following exposure to ouabain.

The flat or platykurtic frequency distribution of V_m (Fig. 1) is consistent with a distributed population of cells with different electrical properties. It is possible that hepatocyte V_m is heterogeneous and differs among cells as a function of the cells' different metabolic states within the hepatic lobule. There is evidence that heterogeneity exists among hepatocytes within the hepatic lobule with respect to biochemical pathways and cytochemical markers. V_m also might vary with position of the microelectrode within cell compartments. Most often, it is assumed that the microelectrode is positioned within the cytoplasm, but skate hepatocytes contain a large number of lipid vacuoles that constitute a substantial portion of the cell volume [22]. These organelles might also have a transmembrane potential and, if impaled, would be in series with the membrane V_m and yield a larger range for V_m values.

Acknowledgements

We thank Phoebe and Annie Boyer for technical assistance. The work was supported in part by United States Public Health Service Grants DK-39165 and ES-01248 to N. Ballatori, by grants from the Lucille P. Markey Charitable Trust and the Kroc Foundation to R.W., NIH grants DK-25636, DK-17433 and DK-34989 to J.L.B., and ES-03828 to the Mt. Desert Island Biological Laboratory.

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