need some environmental factors in order to attain maturation and the cooperative effect of glucocorticoids and brain extracts which we observed is in agreement with the recent finding of Kamatsu et al.[14]. These authors showed a marked increase in GS activity in a highly enriched K^+-containing medium, a condition which is found in the neuronal environment.

1 Martinez-hernandez, A., Bell, K.P., and Norenberg, M.D., Science *19* (1977) 1356.
2 Norenberg, M.D., J. Histochem. Cytochem. *27* (1979) 756.
3 Tardy, M., Fages, C., Rolland, B., Bardakdjian, J., and Gonnard, P., Experientia *37* (1981) 19.
4 Bardakdjian, J., Tardy, M., Pimoule, C., and Gonnard, P., Neurochem. Res. *4* (1979) 519.
5 Pishak, M.R., and Phillips, A.T., Analyt. Biochem. *94* (1979) 82.
6 Pettmann, B., Delaunoy, J.P., Courageot, J., Devilliers, G., and Sensenbrenner, M., Devl Biol. *75* (1980) 278.
7 Ramon Y Caja, in: Histologie du système nerveux de l'homme et des vertébrés, vol.2, p.859. A. Maloine, Paris 1909.
8 Varon, S.S., and Somjen, G.G., Neurosci. Res. Prog. Bull. *17* (1979) 19.
9 Vernadakis, A., and Braggarnold, E., Adv. Cell Neurobiol. *1* (1980) 256.
10 Hallermayer, K., J. Neurochem. *37* (1981) 43.
11 Juurlink, B.H.J., Schousboe, A., Jørgensen, O.S., and Hertz, L., J. Neurochem. *36* (1981) 136.
12 Pishak, M.R., and Phillips, A.T., J. Neurochem. *34* (1981) 866.
13 Patel, J., Hunt, A., and Tahourdin, C.S.M., 8th Meeting Int. Soc. Neurochem. Nottingham 7-11 September 1981; abstracts, p.174.
14 Kanamatsu, T., Ason, H., Takayi, Y., and Hirano, S., 8th Meeting Int. Soc. Neurochem., Nottingham 7-11 September 1981; abstracts, p.138.

Monovalent cation conductance in liposomes induced by ionophore A23187[1]

Kathryn S. Pyant and G.P. Brierley

Department of Physiological Chemistry, College of Medicine, Ohio State University, Columbus (Ohio 43210, USA), 24 September 1981

Summary. In the absence of divalent cations, ionophore A23187 supports low rates of monovalent cations loss ($Na^+ > K^+$) from unilamellar liposomes containing the sulfate salts. Monovalent cation efflux is optimal when a pH gradient (interior alkaline) is applied. The maximum observed rate of 0.56 ngion $K^+ \cdot min^{-1} \cdot nmole^{-1}$ A23187 is insufficient to account for the rates of K^+ efflux induced by the ionophore in mitochondria (150 ngion $K^+ \cdot min^{-1} \cdot nmole^{-1}$ A23187). These studies therefore support the concept that A23187 induces loss of K^+ from mitochondria by removal of regulating divalent cations from an endogenous K^+/H^+ exchanger.

The carboxylic acid ionophore A23187 has been shown to activate K^+/H^+ exchange in liver and heart mitochondria[2-6]. This ionophore is known to deplete mitochondrial Mg^{2+} and Ca^{2+} to very low levels[7-9]. It has been proposed that A23187 activates an endogenous K^+/H^+ exchange component present in the mitochondrial membrane which is normally maintained in an inactive form by matrix Mg^{2+}, Ca^{2+}, or both[2-4,10]. Other studies with mitochondria and model systems[5,6] have concluded that A23187 itself can promote K^+/H^+ exchange, especially in the presence of a transmembrane pH gradient. A strong argument against this interpretation is provided by the observation that once divalent cations have been removed from mitochondria, wide variations in the amount of A23187 present do not affect either the rate of net K^+ efflux[3,4] or the steady-state level of K^+ in liver mitochondria treated with valinomycin[3] However, as Dordick et al.[3] have pointed out, even a small direct contribution of A23187 to K^+/H^+ exchange would complicate the interpretation of the kinetics of K^+ efflux on the putative mitochondrial K^+/H^+ exchanger. For this reason we have used a liposome model to re-examine the question of whether A23187 promotes a direct K^+/H^+ exchange. This system is free of proteins which may contribute to uniport or exchange reactions in native membranes and it also permits imposition of an artificial pH gradient of known magnitude.

Materials and methods. Unilamellar liposomes were prepared and characterized essentially as described by Johnson et al.[11]. Asolection (Associated Concentrates, Inc., Long Island, N.Y.) was sonicated at 25 mg/ml in 0.1 M K^+ or Na^+ sulfate containing Tris (10 mM, pH 8.7), EGTA (25 μM), and EDTA (25 μM). All of the solutions and liposome preparations used in this study contained this level of divalent cation-chelators in order to avoid possible interaction of A23187 with adventitious Ca^{2+} or Mg^{2+}. The resulting suspension of vesicles was passed over Sephadex G-50 equilibrated with tetramethylammonium (TMA)-sulfate (0.1 M), Tris, and the chelators in order to remove extravesicular K^+ or Na^+. Vesicle concentration was established by phosphorous analysis[12].

The efflux of K^+ at 25 °C from the liposomes was followed with a K^+ electrode (Beckman 39047) connected by a choline chloride salt bridge to a reference electrode as previously described[4]. Efflux of Na^+ was recorded in the same way using a Beckman Na^+-selective electrode.

Results and discussion. When liposomes containing K_2SO_4 (0.1 M, pH 8.7) are added to a medium of TMA-sulfate (0.1 M, pH 8.7), there is an initial electrode response due to the small amount of extravesicular K^+ which is present (fig. 1). After this deflection, the electrode shows that there is only a very slow leak of K^+ from these liposomes at 25 °C and that the K^+ can be released by disrupting the vesicles with Triton X-100 (fig. 1). Addition of the monovalent cation/H^+ exchanger nigericin[13] results in a rapid and nearly complete release of the sequestered K^+ (fig. 1). The rate of K^+ release is proportional to the concentration of nigericin added in the concentration range from 5×10^{-11} M to 2×10^{-10} M and a plot of K^+ released $\cdot min^{-1}$ vs nmole nigericin is linear with the slope of this plot corresponding to 460 ngion $K^+ \cdot sec^{-1} \cdot nmole^{-1}$ of nigericin. Addition of valinomycin to provide an exogenous uniport pathway for K^+ efflux[12] results in only a small increase in the rate of K^+ loss from the vesicles (fig. 1). Increasing the H^+ conductivity of the liposomes by addition of high concentrations of an uncoupler (m-chlorocarbonylcyanidephenylhydrazone, CCP, in figure 1) also does not affect the release of K^+ from these liposomes, either in the absence of a pH gradient (as shown in figure 1) or when the vesicles are sus-

pended at pH 5.8 ($\Delta pH = 2.9$, not shown). However, addition of an uncoupler greatly accelerates the valinomycin-dependent efflux of K^+ (fig. 1). These results establish that the liposomes have a very low intrinsic permeability to K^+ and that, even in the presence of an exogenous K^+ uniporter, K^+ efflux is limited by a diffusion potential unless a pathway for charge compensation by H^+ influx is provided. The system therefore provides an assay for K^+/H^+ exchange activity.

Addition of increasing amounts of A23187 to liposomes containing K_2SO_4 (0.1 M, pH 8.9) and suspended in TMA-sulfate (0.1 M, pH 5.76) results in increasing rates of K^+ efflux (fig. 2A). The ionophore has no effect at 20 nmoles $\cdot$ mg^{-1} phospholipid and below, but above this threshold produces K^+ efflux which is linear with the concentration of A23187 (fig. 2B). Under these conditions the rate of A23187-dependent K^+ release averages 0.56 ngion $K^+ \cdot min^{-1} \cdot nmole^{-1}$ A23187. In the absence of a pH gradient (interior and exterior both pH 8.7) this rate declines to 0.07 ngion $K^+ \cdot min^{-1} \cdot nmole^{-1}$. A23187 does not increase the rate of K^+ release in the presence of valinomycin as does the proton-conducting uncoupler CCP (fig. 1). These studies establish that A23187, like nigericin, supports an exchange of K^+ for H^+ across the liposome membrane, but that A23187 is 5×10^3-fold less effective than nigericin under optimal conditions.

Ionophore A23187 supports Na^+ efflux from liposomes at much higher rates than those for K^+ efflux (2.4 ngion $Na^+ \cdot min^{-1} \cdot nmole^{-1}$, fig. 2B). The efflux of Na^+ is not as dependent on the pH gradient as is the K^+ efflux (data not shown). Ionophore A23187 is known to promote Ca^{2+} efflux from liposomes[14] and the addition of either Ca^{2+} or Mg^{2+} (in excess of the amount of chelator routinely present in our protocols) inhibits the efflux of both Na^+ and K^+ from vesicles in the presence of A23187. Similar results to those shown in figures 1 and 2 are obtained when liposomes prepared from mitochondrial phospholipids are substituted for the asolectin vesicles.

Pfeiffer and Lardy[5] reported that A23187 forms a weak complex with Na^+ and an even weaker one with K^+ at alkaline pH. They suggested that the alkaline interior pH of the mitochondrion would promote the formation of such adducts and lead to K^+ loss by direct K^+/H^+ exchange on the ionophore[5]. The present results confirm that A23187 may indeed provide such a pathway under the proper conditions, but they also strongly suggest that K^+ loss by this mechanism is not rapid enough to account for the observed efflux of K^+ induced by A23187 from mitochondria. This process reaches rates of 150 ngion $K^+ \cdot min^{-1} \cdot mg\ protein^{-1}$ in the presence of as little as 1 nmole A23187[3]. For such an efflux of K^+ to depend directly on A23187, a turnover of 150 ngion $K^+ \cdot min^{-1} \cdot nmole^{-1}$ A23187 would be required. The K^+ gradient (ca. 200 mM) and the ΔpH (as much as 3 pH units) imposed on the liposomes in the present study are comparable to or exceed those which might be encountered under the conditions in which K^+ efflux was measured in liver and heart mitochondria[3-6]. The fact

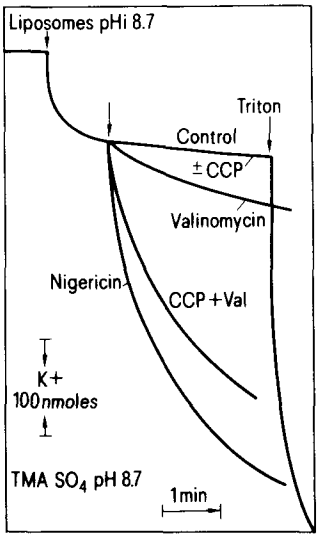

Figure 1. Ionophore mediated efflux of K^+ from liposomes. Phospholipid vesicles were prepared as described under 'Materials and methods'. Potassium sulfate (0.1 M, pH 8.7) was trapped inside the vesicles and 50 µM (EDTA-EGTA) was present throughout. The liposome suspension (100 µl) was added to 7 ml of tetramethylammonium$^+$ sulfate (0.1 M, pH 8.7) and the release of K^+ was monitored using a K^+ electrode. Nigericin and valinomycin were present where indicated at 0.6 nmol/mg phospholipid and m-chlorocarbonylcyanidephenylhydrazone, CCP, at 6.0 nmoles/mg phospholipid.

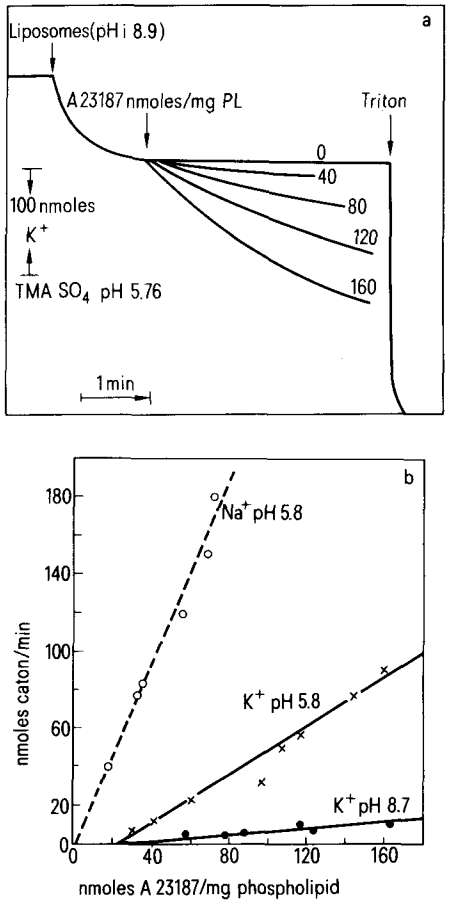

Figure 2. *a* A23187 mediated efflux of K^+ from liposomes. K_2SO_4 (0.1 M, pH 8.7) was trapped inside vesicles and the suspending medium was tetramethylammonium$^+$ sulfate (0.1 M, pH 5.8). Reaction conditions were as described in fig. 1. A23187 was added to the indicated final concentrations (nmole $\cdot$ mg^{-1} phospholipid, PL). Nigericin, a known K/H exchanger produces an identical series of plots in the concentration range from 7×10^{-11} M to 2×10^{-10} M.

b Comparison of rate of Na^+ vs K^8 efflux mediated by A23187. The vesicle internal pH was 8.75 and the suspending medium was either pH 5.8 or pH 8.7 as indicated. Initial rates were estimated from records such as those shown in (a).

that the A23187-dependent K^+ conductance seen in the present studies is 2 orders of magnitude less than that encountered in mitochondria strongly supports our previous contention that it is the removal of divalent cations from the matrix by the ionophore which activates an endogenous K^+/H^+ exchanger[2-4,10]. For most of the studies in which A23187 was used to activate mitochondrial K^+/H^+ exchange, less than 1 nmole A23187 was added per mg protein[3,4,15]. This corresponds to about 4 nmoles · mg^{-1} mitochondrial phospholipid and is therefore well below the threshold for direct K^+ transport found in the present study. The chances of A23187 contributing directly to Na^+/H^+ exchange in biological membranes are considerably greater than those for K^+/H^+ exchange. The transport of Ca^{2+} on A23187 is inhibited by Na^+[16] and it has been reported recently[17] that A23187 can transfer both Na^+ and K^+ across the chloroplast envelope, but only Na^+ through thylakoid membranes. The present studies suggest that such a discrepancy could be explained by the presence of an endogenous K^+ exchanger in one of the membranes.

1 These studies were supported in part by United States Public Health Services Grant HL09364.
2 J. Duszynski and L. Wojtczak, Biochem. biophys. Res. Commun. *74*, 417 (1977).
3 R.S. Dordick, G.P. Brierley and K.D. Garlid, J. biol. Chem. *255*, 10299 (1980).
4 G-Y. Shi, D.W. Jung, K.D. Garlid and G.P. Brierley, J. biol. Chem. *255*, 10306 (1980).
5 D.R. Pfeiffer and H.R. Lardy, Biochemistry *15*, 935 (1976).
6 R.F. Kauffman, R.W. Taylor and D.R. Pfeiffer, J. biol. Chem. *255*, 2735 (1980).
7 P.W. Reed and H.A. Lardy, J. biol. Chem. *247*, 6970 (1972).
8 D.R. Pfeiffer, S.M. Hutson, R.F. Kauffman and H.A. Lardy, Biochemistry *15*, 2690 (1976).
9 J.P. Wehle, M. Jurkowitz, K.M. Scott and G.P. Brierley, Archs Biochem. Biophys. *174*, 2690 (1976).
10 K.D. Garlid, J. biol. Chem. *255*, 11273 (1980).
11 S.M. Johnson, A.D. Bangham, M.W. Hill and E.D. Korn, Biochim. biophys. Acta *233*, 820 (1971).
12 P.S. Chen, T.Y. Toribara and H. Warner, Analyt. Chem. *28*, 1756 (1956).
13 B.C. Pressman, A. Rev. Biochem. *45*, 501 (1976).
14 M. Deleers and W.J. Malaise, Biochem. biophys. Res. Commun. *95*, 650 (1980).
15 D.W. Jung, G-Y. Shi and G.P. Brierley, Archs Biochem. Biophys. *209*, 356 (1981).
16 E. Couturier, M. Deleers and W.J. Malaise, Cell Calcium *1*, 171 (1980).
17 G. Ben-Hayyin and S.H. Krause, Archs Biochem. Biophys. *202*, 546 (1980).

Changes in activity levels of AMP deaminase and adenosine deaminase in the Indian apple snail, *Pila globosa* (Swainson) during starvation and aestivation stress

K. Srinivasa Moorthy, B. Kasi Reddy, C. Sreeramulu Chetty[1] and K.S. Swami

Department of Zoology, S. V. University, Tirupati-517502 (India), and The University of Mississippi Medical Center, 2500, North State Street, Department of Neurology, Jackson (Mississippi 39216, USA), 20 October 1981

Summary. Activity levels of AMP deaminase and adenosine deaminase have been studied in hepatopancreas, foot and mantle tissues of *Pila globosa* with reference to starvation and aestivation. The activity levels of both enzymes were decreased in all the tissues of aestivating snails while in starved animals AMP deaminase activity was increased, in contrast to the decreased adenosine deaminase activity.

Aestivation is an adaptation of animals to water scarcity in the environment during which significant changes in the pattern of nitrogenous excretary products can be expected[2]. The successful survival of *Pila globosa* during aestivation is primarily due to the excretion of nitrogenous waste products in the form of uric acid[3], though this snail is undoubtedly ammonotelic in its active life. Problems relating to different aspects of aestivation have received considerable attention in former years[4-6]. A decrease in ammonia and urea levels and accumulation of uric acid in the tissues and body fluid during aestivation was reported[7-9]. In the present study the activities of AMP deaminase and adenosine deaminase, the main enzymes responsible for the production of ammonia were estimated during starvation and aestivation, in order to elucidate their role in the diversion of ammonotelism to urecotelism.

Collection and maintenance of *Pila globosa* and the method for introducing aestivation has been discussed

Activity levels of AMP deaminase and adenosine deaminase in selected tissues of normal, starved and aestivated *Pila globosa*

Sample	Tissue	AMP-deaminase			Adenosine deaminase		
		Normal	Starved	Aestivated	Normal	Starved	Aestivated
1	Hepatopancreas	45.3±3.21	51.6±3.9	11.4±1.0	18.2±1.7	14.3±1.3	6.8±0.9
	% change		+12.1	−75.3		−21.3	−62.6
	p-values		$p<0.05$	$p<0.001$		$p<0.001$	$p<0.001$
2	Foot	55.6±3.5	62.3±4.7	23.5±1.3	17.3±1.3	15.1±1.1	7.2±0.7
	% change		+10.7	−57.7		−13.6	−58.4
	p-values		$p<0.005$	$p<0.001$		$p<0.005$	$p<0.001$
3	Mantle	40.2±3.0	45.3±4.0	20.9±2.1	13.1±1.1	11.7±0.9	6.9±0.6
	% change	+11.3	+11.3	−48.1		−10.7	−47.3
	p-Values		$p<0.01$	$p<0.001$		$p<0.025$	$p<0.001$

Values expressed in μmoles of ammonia formed per mg protein/h. p-Values indicate levels of significance. Values are means ± SD of 6 observations.