Biochimica et Biophysica Acta 858 (1986) 181–187
Elsevier

BBA 73126

Differential effects of transmembrane potential on two Na^+-dependent transport systems for neutral amino acids

M. Valdeolmillos, J. García-Sancho * and B. Herreros

Departamento de Fisiología y Bioquímica, Facultad de Medicina, 47005-Valladolid (Spain)

(Received December 19th, 1985)

Key words: Amino acid transport; Membrane potential; Na^+ dependence; (Ehrlich ascites tumor cell, Human erythrocytes)

The effects of changes of membrane potential on amino acid transport through systems A, ASC and L was investigated in the Ehrlich cell and the human erythrocyte. Changes of membrane potential were produced by incubating cells whose K^+ permeability had been increased, either by valinomycin or by activation of Ca^{2+}-dependent K^+ channels, in medium containing different K^+ concentrations. The changes in membrane potential were followed by measuring the distribution ratio reached by lipophilic indicators. Transport through Na^+-dependent system A was sensitive to the membrane potential, the rate of amino acid uptake increasing 2.2–3.1-times for each 60 mV-hyperpolarization. The Na^+-dependent system ASC was insensitive to membrane potential. The Na^+-independent system L was not directly affected by membrane potential, but the steady-state accumulation of system L substrates was increased by hyperpolarization.

Introduction

It is generally accepted that Na^+-dependent amino acid transport systems are coupled to the electrochemical gradient of Na^+ to produce accumulation of the substrate [1]. Since membrane potential contributes substantially to this gradient its modification should affect amino acid transport. Such an effect has been reported many times [2–12]. However, the relationship between membrane potential and every particular transport system has not been investigated.

In this paper we have studied the effects of membrane potential on amino acid transport by three different transport systems. The transport systems studied included the two principal Na^+-dependent pathways for neutral amino acid transport in the Ehrlich cell, namely systems A and ASC, and the Na^+-independent system L [13,14]. The differential study of systems A and L was facilitated by the use of specific substrates for these pathways, but no model substrate is available for system ASC at present. We took advantage of the lack of transport system A activity in the human erythrocyte [15] to study system ASC in isolation from the other Na^+-dependent transport system. Our results indicate that transmembrane potential strongly affects amino acid uptake through transport system A, but does not modify transport through system ASC. The initial rate of uptake of a model substrate of the Na^+-independent transport system L was not modified by changes of the membrane potential, but the steady-state accumulation increased on hyperpolarization of the cells.

* To whom correspondence should be addressed.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; TPP, tetraphenylphosphonium; TPMP, triphenylmethylphosphonium; DIDS, 4,4-diisothiocyanostilbene-2,2'-disulphonic acid; BCH, 2-aminobicyclo(2,2,2)heptane-2-carboxylic acid.

Methods

Methods for collecting and handling of the Ehrlich cells were as described by Inui and Christensen [16]. The cells were first incubated at 5% cytocrit and 37°C for 30 min in standard medium. Then they were washed twice with ice-cold 0.15 M choline chloride solution and resuspended in the same solution at 50% cytocrit. The amino acid uptake experiments were started by adding 0.3 ml of the 50% cell suspension to 2.7 ml of incubation medium containing the test amino acid and tracer amounts of the appropriate ^{14}C-isotope. After a measured interval at 37°C the incubation period was terminated by dilution with ice-cold medium and centrifugation as described before [16,17]. The processing of the cell pellets and supernatant solutions [17] and the counting of radioactivity [18] have been described previously. The values of total cell water and extracellular space in the cell pellet reported previously were used for calculations [19]. The standard incubation medium contained (mM): NaCl, 135; KCl, 1; $CaCl_2$, 0.5; $MgSO_4$, 1.4; Na^+-Hepes, 20 (pH 7.4). The Na^+-free medium had the same composition except that NaCl and Na^+-Hepes were replaced by choline chloride and Tris-Hepes respectively. For the experiments in which different K^+ concentrations were used (Fig. 1) the incubation medium had the following composition (mM): NaCl, 120; KCl + choline chloride, 30; $CaCl_2$, 0.5; $MgSO_4$, 1.4; Na^+-Hepes, 20 (pH 7.4). In the experiments designed to measure the uptake of the norbornane amino acid (BCH), a model substrate for system L [20], the endogenous amino acid pool was first depleted by three consecutive 15-min incubations in standard medium [21]. This manoeuvre reduced the endogenous amino acid pool, measured by high-pressure liquid chromatography after dansylation of the cell extracts [22], to about 1/3 of the original value. Additional incubations in standard medium did not deplete the endogenous amino acid pool further.

Membrane potential was estimated from the distribution of either TPP [1,23] or TPMP [24]. The design of the experiments was similar to that described above for measurement of amino acid uptake except that 1 μM TPP or 4.4 μM TPMP and tracer amounts of the corresponding ^{14}C-isotope were added to the medium. In the case of TPMP, 3.3 μM tetraphenylboron was also added to accelerate the distribution of the lipophilic cation [24]. The incubation was terminated by a 30-s centrifugation at 12000 $\times$ g of 1-ml aliquots of the cell suspension, and the ^{14}C content of the supernatant and the extracts of the cell pellets was determined as described previously. In some experiments the cells were treated with rotenone, an irreversible inhibitor of mitochondrial respiration [25], in order to avoid mitochondrial accumulation of the lypophylic cations. This manoeuvre systematically decreased the distribution ratio reached by the lipophilic indicator, but the results were not qualitatively different from those from untreated cells.

Red cells were obtained from fresh blood drawn into heparin. The cells were washed twice with a solution containing 75 mM NaCl, 75 mM KCl, 0.1 mM EGTA and 10 mM Tris-HCl (pH 7.7), and once more with standard incubation medium containing (mM): NaCl, 150; KCl, 5; $CaCl_2$, 0.5, $MgCl_2$, 0.2; Tris-Hepes, 10 (pH 7.5). Then the cells were suspended at 10% haematocrit in standard incubation medium, and DIDS was added to give a final concentration of 20 μM. After a 10 min incubation at room temperature, the cells were sedimented by centrifugation and washed twice with Na^+-free medium. Na^+-free medium had the same composition as the standard incubation medium except that NaCl was replaced by choline chloride. The cell pellet was resuspended at 10% haematocrit either in standard incubation medium or in Na^+-free medium and transfered to a 37°C bath. Valinomycin, 2 μM, or A23187, 10 μM, were added to aliquots of the cell suspensions and the experiment was started by addition of either [^{14}C]alanine, 0.1 mM, or [^{14}C]TPP, 1 μM. After a measured period the incubation was terminated either by centrifugation at 12000 $\times$ g for 30 s (TPP uptake experiments) or by dilution with ice-cold medium and centrifugation over dibutylphthalate oil as described previously (alanine uptake experiments, Ref. 26). The original volume of cells was estimated from haemoglobin measurements in aliquots of the cell suspension [27].

The ^{14}C-isotopes of alanine, TPP and TPMP were purchased from Amersham International, plc. BCH and [^{14}C]BCH (b $\pm$ isomer) were generous

gifts of Dr. H.N. Christensen, The University of Michigan, Ann Arbor, MI, U.S.A. DIDS was obtained from Calbiochem.-Behring Corporation and the ionophore A23187 from Boehringer-Mannheim. Other chemicals were obtained either from E. Merck, Darmstadt or from Sigma Chemical Co. Ltd., London.

Results

The effects of changing the extracellular K^+ concentration on the uptake of TPMP and α-aminoisobutyric acid by the Ehrlich cell in the presence and in the absence of propranolol are shown in Fig. 1. Propranolol was reported previously to increase the membrane permeability to K^+ by activation of the Ca^{2+}-dependent K^+ channels [19]. The TPMP distribution ratio was little modified by the external K^+ in the controls without propranolol. This finding is consistent with the evidence suggesting that the membrane potential in the Ehrlich cell is not a K^+ diffusion potential [28,29]. Following treatment with propranolol the TPMP distribution ratio was increased by decreasing K^+ concentration indicating membrane hyperpolarization (Fig. 1a). A decrease of external K^+ from 30 to 10 mM increased the distribution ratio of TPMP 2.9-times, a value which is 96% of that expected for a pure diffusion potential for K^+. At lower K^+ concentrations the changes of membrane potential, estimated from TPMP distribution, were smaller than those predicted from the Nernst equation, suggesting that the contribution of other diffusing ions to the membrane potential became significant. At about 20 mM K^+ the distribution of TPMP was not affected by propranolol. In these cells the internal concentration of K^+ was about 90 mM, a value that was somewhat lowered due to the previous treatment with rotenone (see Methods). Under these conditions the equilibrium potential for K^+, and hence the probable value of the membrane potential in the cells treated with propranolol, would be about −39 mV. This value is similar to that reported by other authors [23,30–33] and considerably lower than that of −53 mV, which would be estimated by applying directly the Nernst equation to the distribution ratio of TPMP, suggesting that the indicator is bound to the intracellular content to some degree. The treatment with propranolol in a medium containing 1 mM K^+ increased the distribution ratio reached by TPMP by about 4-times relative to the control. This corresponds to a hyperpolarization of 36 mV.

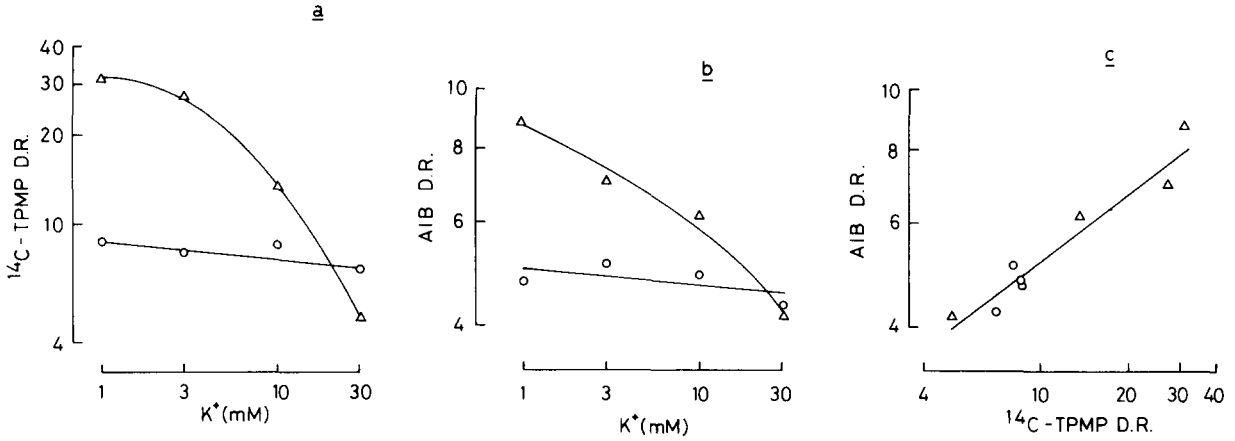

Fig. 1. Effects of the external K^+ concentration on the uptake of [^{14}C]TPMP (a) and α-aminoisobutyric acid (AIB) (b) by the Ehrlich cell in the presence (△) and in the absence (○) of 0.5 mM propranolol. The uptake was measured during 8 min at 37°C in cells which had been previously treated with rotenone (100 μg/l). The concentration of α-aminoisobutyric acid was 1 mM and that of TPMP 4.4 μM. Tetraphenylboron was added to a final concentration of 3.3 μM. Preliminary experiments showed that a steady-state distribution ratio of [^{14}C]TPMP was reached and that the uptake of α-aminoisobutyric acid was linear during the 8-min incubation period chosen for the experiments. The uptake is expressed as the distribution ratio (D.R.) (cpm per ml cell water/cpm per ml incubation medium) reached by the end of the incubation period. Note the logarithmic scale on the ordinate. Panel c shows the correlation between the uptakes of α-aminoisobutyric acid and TPMP in double-logarithmic plot.

Fig. 1b shows the uptake of α-aminoisobutyric acid, a model substrate for system A [34], measured in parallel experiments. The effects of external K^+ were similar to those described above for TPMP. The correlation between the distribution ratio reached by TPMP and the uptake of α-aminoisobutyric acid was linear when a double-logarithmic plot was made (Fig. 1c). These results indicate that amino acid transport via system A is strongly dependent on membrane potential.

Table I summarizes the effects of propranolol on the uptake of alanine by the Ehrlich cell. This amino acid is a good substrate for both systems, A and ASC [34,35]. The uptake of alanine was increased 37% by propranolol. In the presence of excess methyl α-aminoisobutyric acid, which inhibits competitively the transport through system A [34,36], propranolol did not modify the uptake of alanine. The membrane potential was assessed in parallel experiments using TPP. The treatment with propranolol increased the distribution ratio reached by TPP by about 2.9-times (28 mV hyperpolarization), and this effect was not significantly modified by the presence of excess methyl α-aminoisobutyric acid (data not shown). The third line in Table I shows the uptake of alanine in Na^+-free medium. Under these conditions the contribution of both transport systems, A and ASC, is eliminated, and this allows the contribution of each system to be calculated. In the control without propranolol 16% of the total uptake was Na^+-independent, and 50 and 34% took place through systems A and ASC, respectively. The uptake through system A was increased by 72% as a result of the hyperpolarization, without significant modification of the other components of the uptake.

The human erythrocyte shows Na^+-dependent transport of alanine which takes place through system ASC [15,37,38]. Since system A is not present in these cells [15], the activity of system ASC can be directly estimated from the Na^+-dependent uptake of alanine, without the need to use inhibitors of the transport system A. Fig. 2 shows the effect of hyperpolarization on the uptake of alanine by erythrocytes. Hyperpolarization was produced by increasing the membrane permeability to K^+ of cells incubated in low-K^+ medium. The increase of K^+ permeability was accomplished either by treatment with the K^+-selective ionophore valinomycin or with the divalent cation ionophore A23187 and Ca^{2+}, which activates Ca^{2+}-dependent K^+ channels [39]. The cells were previously treated with DIDS to decrease the permeability to Cl^- [40] in order to slow the net loss of K^+ produced by the treatment with ionophores. Results in cells not treated with DIDS were similar to those shown in Fig. 2. The upper panel of Fig. 2 shows that the cell/medium TPP distribution ratio increased with treatment with either valinomycin or A23187 + Ca^{2+}. This demonstrates that hyperpolarization of the cells has been accomplished. The uptake of alanine, however, remained unmodified by the hyperpolarization even though a large part of it was Na^+-dependent (lower panel of Fig. 2). This confirms the observation described above for the Ehrlich cell, that the Na^+-dependent system ASC is not sensitive to membrane potential.

The effects of membrane potential on the Na^+-independent system L were studied in the Ehrlich cell using the norbornane amino acid (BCH), a model substrate for this transport system [20]. Treatment with propranolol did not modify the uptake of BCH in Na^+-free medium. In Na^+-containing medium the uptake of BCH was not significantly modified by propranolol during the first

TABLE I

DEPENDENCE ON Na^+ AND SENSITIVITY TO *N*-METHYLAMINOISOBUTYRIC ACID OF THE STIMULATION OF THE UPTAKE OF [^{14}C]ALANINE BY PROPRANOLOL (0.5 mM) BY THE EHRLICH CELL

The uptake of alanine (100 μM) was studied during 1 min at 37°C and it is expressed in mmol/kg cells. Other details are as in Fig. 1. Each value is the mean of two individual values differing by less than 10%. The uptake of [^{14}C]TPP was measured in parallel experiments with the same batch of cells before and after the addition of propranolol. The distribution ratios reached by the lipophilic cation in Na^+-containing, (Na^+ + *N*-methylaminoisobutyric acid)-containing and Na^+-free media were 32, 32 and 40, respectively, and they were increased to 85, 75 and 175 after the addition of propranolol.

Condition	Control	Propranolol
Na^+-containing medium	0.93	1.27
+25 mM methyl α-amino-isobutyric acid	0.47	0.48
Na^+-free medium	0.15	–

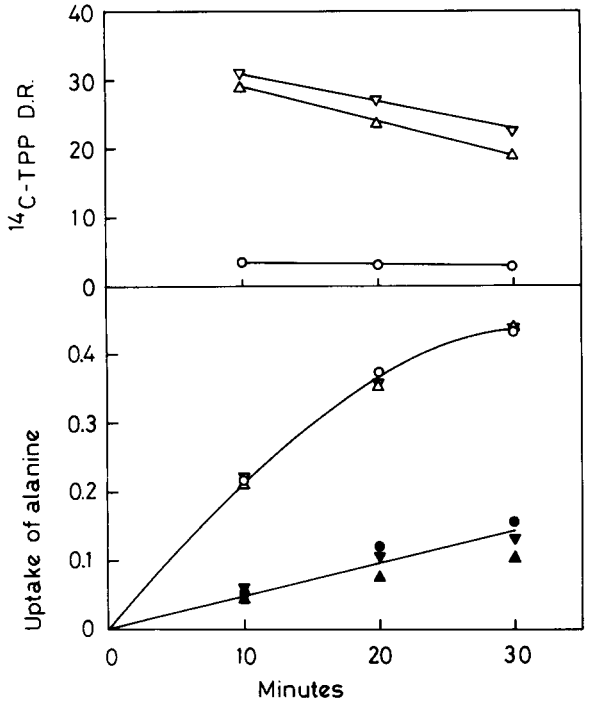

Fig. 2. The effects of increasing the membrane permeability to K^+ on the uptake of [^{14}C]TPP, 1 μM (upper panel) or [^{14}C]alanine, 100 μM (lower panel) by the human red cell. The cells were treated with DIDS prior to the uptake experiment as described in Methods, and resuspended at about 20% haematocrit and 37°C in either Na^+-containing (open symbols) or Na^+-free (closed symbols) medium. The concentration of K^+ was 5 mM in both media. Either 5 μM valinomycin (inverted triangles) or 10 μM A23187 (triangles) was added about 1 min before starting the experiment. No additions were made to the control (circles). The experiment was started at time zero by the addition of either alanine or TPP, and samples were taken after different incubation periods as shown. The uptake is expressed as the distribution ratio (D.R.) (cpm per ml original cells/cpm per ml medium) reached by the end of the incubation period.

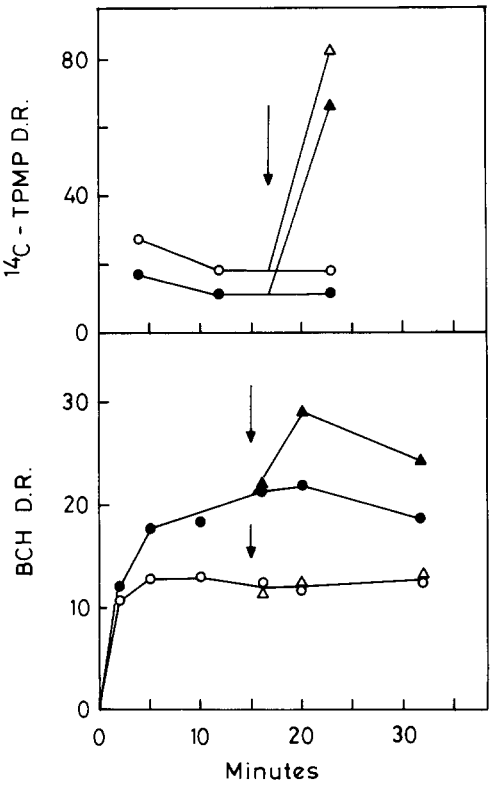

Fig. 3. Effects of propranolol on the uptake of TPMP (upper panel) and BCH (lower panel) by the Ehrlich cell. The cells were depleted of endogenous amino acids prior to the experiment as described in Methods. At zero time the cells were resuspended either in Na^+-containing (closed symbols) or Na^+-free (open symbols) medium containing 100 μM [^{14}C]BCH. At the time marked by the arrow, propranolol, 0.5 mM, was added to aliquots of the cell suspensions (triangles). Other details are as in Fig. 1. D.R., distribution ratio.

2 min of incubation. However, if the incubation period was prolonged, the steady-state level reached by BCH was higher in propranolol-treated cells (data not shown).

Fig. 3 shows the result of a typical experiment in which the addition of propranolol was delayed by 15 min with respect to the beginning of the incubation with BCH. The steady-state level of BCH reached before the addition of propranolol was systematically higher in Na^+-containing than in Na^+-free medium. The addition of propranolol increased the distribution ratio reached by BCH in Na^+-containing medium, but had no effect in Na^+-free medium (lower pannel of Fig. 3). The upper pannel of Fig. 3 shows that a membrane hyperpolarization, demonstrated by the increase of the distribution ratio reached by TPMP, was accomplished by propranolol treatment in both media. Fig. 4 compares the uptake of BCH and the effect of propranolol in Na^+-containing medium with and without *N*-methylaminoisobutyric acid, added in excess to saturate transport system A [34,36]. The distribution of BCH reached at the steady-state was smaller when the medium contained *N*-methylaminoisobutyric acid. On the other hand, the presence of *N*-methyl-

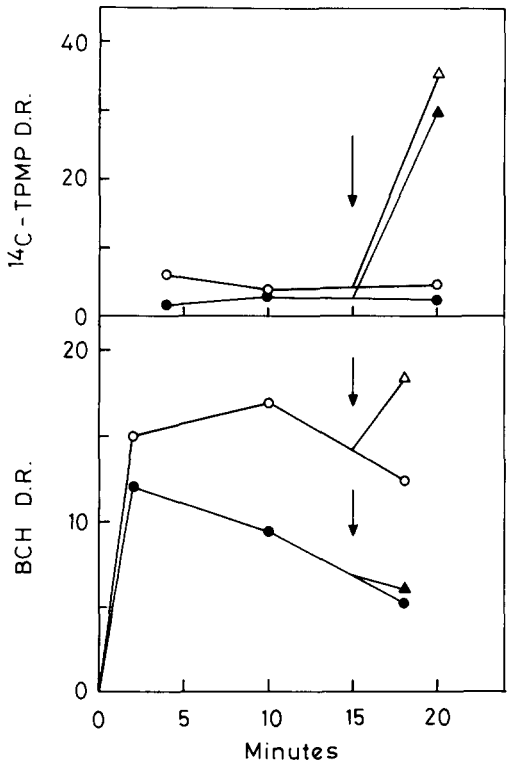

Fig. 4. Effects of propranolol on the uptake of TPMP (upper panel) and BCH (lower panel) in the presence (closed symbols) or in the absence (open symbols) of 25 mM *N*-methylaminoisobutyric acid. The incubation medium contained Na^+ in all cases. The time of addition of propranolol is marked by the arrow. Other details as in Fig. 3. D.R., distribution ratio.

aminoisobutyric acid prevented the effect of propranolol on the accumulation of BCH. The upper part of Fig. 4 shows that propranolol increased the distribution ratio reached by TPMP, and hence hyperpolarized the membrane, to about the same degree both in the presence and in the absence of *N*-methylaminoisobutyric acid.

Discussion

The finding that changes of the extracellular K^+ concentration do not modify the membrane potential in the Ehrlich cell supports the recent proposal that membrane potential in these [29] and other cells [41] could depend more on the activity of electrogenic pumps than on the passive diffusion of ions. It is worth noting that the activation of the Ca^{2+}-dependent K^+ channels by propranolol shifts membrane potential towards a K^+ diffusion potential in the Ehrlich cell and could, therefore, be used as a tool to change the membrane potential as shown here. Attempts to achieve the same result with valinomycin were, in our hands, much less reliable. The activation of Ca^{2+}-dependent K^+ channels, by the joint use of the ionophore A23187 and Ca^{2+} and by the treatment with valinomycin, seemed to be equally reliable manoeuvres to achieve membrane potentials set by the diffusion of K^+ ions in the human red cells.

Only one of the transport systems for amino acids studied here, the Na^+-dependent transport system A, was affected by changes in membrane potential. Hence this transport pathway would mediate the increases of amino acid transport observed with hyperpolarization [2–12] and the rheogenic effects of amino acid transport reported previously [21,28,31,42,43]. From the data of Fig. 1c and Table I it can be estimated that the uptake of α-aminoisobutyric acid and the *N*-methylaminoisobutyric acid-sensitive uptake of alanine would increase 2.2- and 3.3-times, respectively, by increasing the membrane potential by 60 mV.

Changes in membrane potential did not modify amino acid uptake through the Na^+-dependent system ASC in the Ehrlich cell and the human erythrocyte (Fig. 2 and Table I). This finding is consistent with the previous observation that, although amino acid uptake via system ASC increased the influx of $^{22}Na^+$, this was balanced by an increase of Na^+ efflux so that the cell level of Na^+ did not change with amino acid accumulation [35]. The insensitivity to membrane potential should be expected if system ASC operated by compulsory exchange, as proposed by Christensen and coworkers [35,44–46].

The initial rate of uptake of BCH, a model substrate for the Na^+-independent system L [20], was independent of membrane potential. However, under steady-state conditions the accumulation of BCH was increased by membrane hyperpolarization when the experiments were performed in Na^+-containing medium. The removal of Na^+ or the addition of excess of *N*-methylaminoisobutyric acid prevented the effects of membrane potential on the transport of BCH, suggesting that the interaction takes place through

transport system A. Two alternative possibilities could explain these findings. Either BCH retains some residual reactivity for system A [34], or the internal amino acid pool, which seems impossible to eliminate, remains higher in the hyperpolarized cells due to the increased reuptake via system A of the amino acids which leak from the cells; the endogenous amino acids could then exchange for extracellular BCH allowing a higher level of this amino acid to be reached in the steady-state. In any case it is clear that none of these alternatives include direct effects of membrane potential on the Na^+-independent system L.

Acknowledgements

We wish to thank Drs. D.A. Eisner and G.L. Smith for helpful comments on an early draft of the manuscript. Financial support from the Spanish Fondo Nacional para el Desarrollo de la Investigacion Cientifica (Proyecto No. 2873/83) is gratefully acknowledged.

References

1 Geck, P. and Heinz, E. (1981) in Membrane Transport (Bonting, S.L. and De Pont, J.J.H.H.N., eds.), pp. 285–310, Elsevier/North-Holland, Amsterdam
2 Gibb, L.E. and Eddy, A.A. (1972) Biochem. J. 129, 979–981
3 Reid, M., Gibb, L.E. and Eddy, A.A. (1974) Biochem. J. 140, 383–393
4 Eddy, A.A. and Philo, R. (1975) in Amino Acid Transport and Uric Acid Transport (Silvernagl, S., Lang, F. and Greger, R., eds.), pp. 27–33, Georg Thieme Verlag, Stuttgart
5 Colombini, M. and Johnstone, R.M. (1974) J. Membrane Biol. 28, 315–334
6 Lever, E. (1977) J. Supramol. Struct. 6, 103–124
7 Johnstone, R.M. (1978) Biochim. Biophys. Acta 512, 550–556
8 Johnstone, R.M. and Laris, P.C. (1980) Biochim. Biophys. Acta 599, 715–730
9 Kilberg, M.S. and Christensen, H.N. (1980) Membrane Biochem. 3, 155–168
10 Burckhardt, G. and Pietrzyk, C. (1980) Biochim. Biophys. Acta 60, 403–414
11 Heinz, A., Sachs, G. and Schafer, J.A. (1981) J. Membrane Biol. 61, 143–153
12 Valdeolmillos, M., García-Sancho, J. and Herreros, B. (1982) Biochim. Biophys. Acta 689, 177–179
13 Oxender, D.L. and Christensen, H.N. (1963) J. Biol. Chem. 238, 3686–3699
14 Christensen, H.N., Liang, M. and Archer, E.G. (1967) J. Biol. Chem. 242, 5237–5246
15 Al-Saleh, E.A. and Wheeler, K.P. (1982) Biochim. Biophys. Acta 684, 157–171
16 Inui, Y. and Christensen, H.N. (1966) J. Gen. Physiol. 50, 203–224
17 García-Sancho, J., Sanchez, A. and Christensen, H.N. (1976) Biochim. Biophys. Acta 464, 295–312
18 García-Sancho, J. and Herreros, B. (1975) Biochim. Biophys. Acta 406, 538–552
19 Valdeolmillos, M., García-Sancho, J. and Herreros, B. (1982) Biochim. Biophys. Acta 685, 273–278
20 Tager, H.S. and Christensen, H.N. (1972) J. Am. Chem. Soc. 94, 968–972
21 Laris, P.C., Bootman, M., Perhadsingh, H. and Johnstone, R.M. (1978) Biochem. Biophys. Acta 512, 397–414
22 Osborne, H.N. (1974) Microchemical Analysis of Nervous Tissue, Pergamon Press, Oxford
23 Heinz, E., Geck, P., Pietrzyk, C. and Pfeiffer, B. (1977) in Biochemistry of Membrane Transport (Semenza, G. and Carafoli, E., eds.), pp. 236–249, Springer-Verlag, Berlin
24 Cheng, K., Hasper, H.C., Vallano, M.L., Osotimehim, B. and Sonenberg, M. (1980) J. Membrane Biol. 56, 191–201
25 Slater, E.C. (1967) Methods Enzymol. 10, 48–56
26 Ferreira, H.G. and Lew, V.L. (1976) Nature 259, 47–49
27 Tiffert, T., García-Sancho, J. and Lew, V.L. (1984) Biochim. Biophys. Acta 773, 143–156
28 Heinz, E., Geck, P. and Pietrzyck, C. (1975) Ann. N.Y. Acad. Sci. 264, 428–441
29 Bashford, C.L. and Pasternak, C.A. (1984) J. Membrane Biol. 79, 275–284
30 Laris, P.C., Pershadsingh, H.A. and Johnstone, R.M. (1976) Biochim. Biophys. Acta 436, 475–488
31 Phylo, R.D. and Eddy, A.A. (1978) Biochem. J. 174, 811–817
32 Smith, T.C. and Robinson, S.C. (1980) Biochim. Biophys. Res. Commun. 95, 722–727
33 Heinz, A., Jackson, J.W., Richey, B.E., Sachs, G. and Shafer, J.A. (1981) J. Membrane Biol. 62, 149–160
34 Christensen, H.N. (1975) Biological Transport, 2nd Edn., W.A. Benjamin Inc., Reading, MA
35 Wheeler, K.P. and Christensen, H.N. (1967) J. Biol. Chem. 242, 3782–3788
36 Christensen, H.N., Oxender, D.L., Liang, M. and Vatz, K.A. (1965) J. Biol. Chem. 240, 3609–3616
37 Young, J.D., Wolowyk, M.W., Jones, S.E.M. and Ellory, J.C. (1979) Nature 279, 800–802
38 Young, J.D., Jones, S.E.M. and Ellory, J.C. (1980) Proc. R. Soc. Lond. Ser. B 209, 355–375
39 Lew, V.L. and Ferreira, H.G. (1976) Nature 263, 336–338
40 Fortes, P.A.G. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 175–195, Academic Press, New York
41 Bashford, C.L. and Pasternak, C.A. (1985) Biochim. Biophys. Acta, 817, 174–180
42 Johnstone, R.M. (1979) J. Biol. Chem. 254, 12479–12483
43 Johnstone, R.M. and Laris, P.C. (1980) Biochim. Biophys. Acta 599, 715–730
44 Thomas, E.L. and Christensen, H.N. (1971) J. Biol. Chem. 246, 1682–1688
45 Koser, B.H. and Christensen, H.N. (1971) Biochim. Biophys. Acta 241, 9–19
46 Christensen, H.N. (1972) in Na-linked Transport of Organic Solutes (Heinz, E., ed.), pp. 161–167, Springer-Verlag, Berlin