

vesicles for reconstitution assays has been described^{10,20–22}. Briefly, the internal pH of the vesicles was set at 6.0 by dialysis. The final uptake solution contained 1 mM ²²Na⁺, 250 mM mannitol, 30 mM potassium gluconate, 1 μg ml⁻¹ valinomycin and 50 mM Tris/MES at either pH 6.0 or 8.0. Sodium uptake was determined by application of the reaction mix to 1 ml Dowex 50X8 (Tris), 100-mesh columns and rapid elution with vacuum suction with 1 ml 300 mM mannitol (pH 8.0) at 0°C. Under these voltage-clamped conditions, the proton-stimulated component of sodium uptake was taken as a measure of Na⁺/H⁺ exchange rate.

Quantification of Na⁺/H⁺ exchanger activity by cytosolic pH measurement. The intracellular pH (pH_i) of small groups of cells was determined by microphotometry of the fluorescence emission of the pH-sensitive dye, 2',7'-bis-(2-carboxyethyl)-5-(and 6)carboxyfluorescein, using dual wavelength excitation^{15,16}. Cells grown to 70–80% confluence on glass coverslips were concurrently loaded with 25 mM NH₄Cl and 2 mg ml⁻¹ of the acetoxymethyl ester precursor of 2',7'-bis-(2-carboxyethyl)-5-(and 6)carboxyfluorescein in PBS for 10 min at 37°C. Where indicated, 10 μM forskolin or 50 μM isoprenaline was also added. Acid loading was accomplished by washing cells with a Na⁺-free medium containing (in mM): 117 N-methyl-D-glucammonium chloride, 1.66 MgSO₄, 1.36 CaCl₂, 5.36 KCl, 25 HEPES, 5.55 glucose, pH 7.5, 290 ± 10 mosM. Sodium-dependent pH_i recovery was then initiated by perfusing the cells with a Na⁺-rich solution composed of (in mM): 117 NaCl, 1.66 MgSO₄, 1.36 CaCl₂, 5.36 KCl, 25 HEPES, 5.55 glucose, pH 7.5. To measure fluorescence, the coverslip was placed into a holding chamber attached to the stage of a Nikon Diaphot TMD inverted microscope equipped with a Nikon Fluor 40x/1.3 N.A. oil-immersion objective. Clusters of 6–12 cells were selected for analysis with an adjustable diaphragm. Excitation light provided by a Xenon lamp was alternately selected using 495 ± 10 nm and 445 ± 10 nm filters at a rate of 50 Hz and then reflected onto the cells by a 510-nm dichroic mirror. Emitted light was directed to the photometer through a 530 ± 30 nm band-pass filter. Photometric data were acquired at 10 Hz using a 12-bit A/D board (Labmaster, National Instruments) interfaced to a Dell 486 computer and analysed with Felix software (Photon Technologies). Calibration of fluorescence intensity with pH_i was done in the presence of 5 mM nigericin in high-K⁺ medium (140 mM KCl, 20 mM HEPES, 1 mM MgCl₂, 5 mM glucose) as described^{15,16}.

Received 1 December 1997; accepted 19 January 1998.

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Acknowledgements. We thank J. Shannon for peptide sequencing; T. Kurose for the β₂ receptor tail GST fusion protein construct; N. Freedman for the Flag-tagged wild-type β₂ receptor construct; J. Raymond for advice; G. Irons, D. Steplock and K. Tate for technical assistance; and D. Addison and M. Holten for help in preparing the manuscript. This work was supported in part by grants from the NIH to R.J.L. and E.J.W. and from the Duke Comprehensive Cancer Center to S.S.; C.W.C. is the recipient of a clinician–scientist award from the Department of Medicine at the University of Toronto; A.C. is a recipient of a postdoctoral fellowship from the Heart and Stroke Foundation of Canada; and S.G. is an international scholar of the Howard Hughes Medical Institute.

Correspondence and requests for materials should be addressed to R.J.L. (e-mail: lefko001@mc.duke.edu).

corrections

Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein

Stefan Brocke, Koenraad Gijbels, Mark Allegretta, Iris Ferber, Christopher Piercy, Thomas Blankenstein, Roland Martin, Ursula Utz, Nathan Karin, Dennis Mitchell, Timo Veromaa, Ari Waisman, Amitabh Gaur, Paul Conlon, Nicholas Ling, Paul J. Fairchild, David C. Wraith, Anne O'Garra, C. Garrison Fathman & Lawrence Steinman

Nature **379**, 343–346 (1996)

In this Letter, “96P” indicates a proline at residue 96, and not phenylalanine as published. □

A new pattern for helix–turn–helix recognition revealed by the PU.1 ETS-domain–DNA complex

Ramadurgam Kodandapani, Frédéric Pio, Chao-Zhou Ni, Gennaro Piccialli, Michael Klemsz, Scott McKercher, Richard A. Maki & Kathryn R. Ely

Nature **380**, 456–460 (1996)

In the legend to Fig. 3, lines 11 and 12 should read: “Arg 232 (NH1) makes two hydrogen bonds with G9(06) and G9(N7).” In panel b, a dashed line indicating a hydrogen bond to base A10 should be deleted. The coordinates for the complex remain unchanged as originally submitted to the Brookhaven Protein Data Bank under accession number 1 pue. □