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Volume-activated fluxes of [^{3}H] taurine, $^{96}Rb^+$ and $^{125}I^-$ in rat brain endothelial cells

M.A. Barrand, M.E. Hickman, C.E. Jackman, C. Littlewood, J. Mitchell, C.J. Taylor, C. Wrann and S.B. Hladky

Department of Pharmacology, Cambridge University, Cambridge CB2 1PD, UK

Secretion of fluid by the endothelial cells of the blood–brain barrier requires transfer of solutes and water across the luminal and abluminal membranes of the cells, exposing them to osmotic challenge. These cells should therefore have means to regulate their volume. Swelling of rat brain endothelial cells induces a large increase in $^{125}I^-$ permeability and Cl^- conductance, which is inhibited by antagonists of phospholipase A_2 and 5-lipoxygenase (von Weikersthal *et al.* 1997, 1999). We report here properties of the volume-activated efflux of [^{3}H] taurine, $^{86}Rb^+$ and $^{125}I^-$ from these cells.

Brain endothelial cells from rats killed in accordance with UK legislation were grown as adherent monolayers, loaded with $^{125}I^-$, [^{3}H]-taurine or $^{86}Rb^+$ for 30 min and washed with efflux buffer (mM: 82 NaCl, 4 KCl, 0.3 $CaCl_2$, 0.6 $MgSO_4$, 1.1 $NaHPO_4$, 0.6 KH_2PO_4, 10 Hepes, 6 glucose, 90 mannitol, adjusted to pH 7.4 with NaOH). Efflux at 37 °C was measured by replacing the extracellular buffer once each minute. After three isotonic replacements cells were exposed to hypotonic solution (omission of mannitol). Maximum activation of efflux was achieved in 1–2 min. For I^- the rate constant increased from 0.20 ± 0.03 to 0.84 ± 0.09 min^{-1} (mean ± S.E.M., $n = 9$) as previously reported. For taurine the rate constant increased from 0.032 ± 0.003 to 0.40 ± 0.04 min^{-1} (mean ± S.E.M., $n = 18$); for Rb^+ from 0.048 ± 0.003 to 0.079 ± 0.005 min^{-1} ($n = 12$). After the peak the efflux rate constants decreased over several minutes as if the volume decrease accompanying the efflux of KCl and organic anions removed the stimulus for increased efflux.

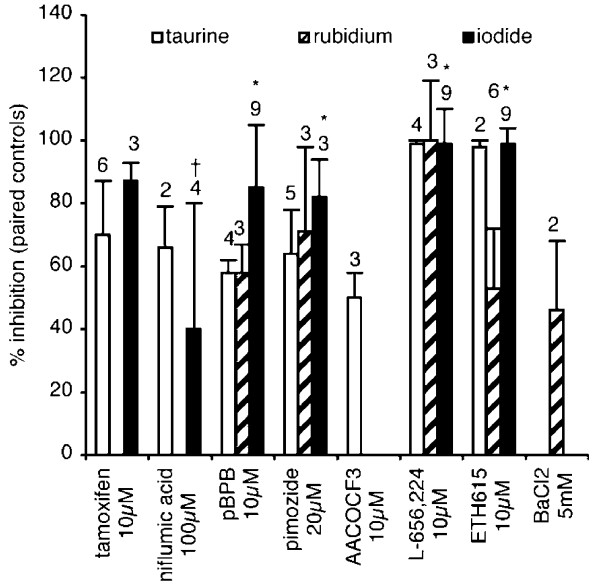

Figure 1. Inhibition of the peak increase in efflux rate by various inhibitors as a percentage of paired controls. Data are shown as means ± S.E.M. with the number of experiments indicated. †In two experiments with niflumic acid inhibition of I^- efflux was > 60 %, whilst in two others no effect was seen. *Data from von Weikersthal *et al.* (1997).

Inhibition of peak efflux (see Fig. 1) by PLA_2 inhibitors pBPB (4-bromophenacyl bromide), pimozide and AACOCF3 (arachidonyl trifluoromethyl ketone) and by 5-lipoxygenase inhibitors ETH615 (4-[2-quinolylmethoxy]-*N*-[3-flurobenzyl]-phenylaminomethyl-4-benzoic acid; Leo Pharmaceuticals, Ballerup, Denmark) and L-656,224 (7-chloro-2-[(4-methoxyphenyl)methyl]-3-methyl-5-propyl-4-benzofuranol; Merck Frosst, Canada) suggests that a product of 5-lipoxygenase is important in coupling volume increase to activation of efflux.

von Weikersthal SF *et al.* (1997). *Biochim Biophys Acta* **1325**, 99–107.

von Weikersthal SF *et al.* (1999). *J Physiol* **516**, 75–84.

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All procedures accord with current UK legislation.