Regulation of early neurite morphogenesis by the Na\(^+\)/H\(^+\) exchanger NHE1

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Supplementary Figure Legends
**Supplementary Figure 1.** Functional characterization of NHE1 mutants expressed in NHE-deficient PS120 cells. Sub-confluent PS120 cells were co-transfected with pEGFP-C1 (Clontech) and plasmids containing cDNA encoding full-length HA-tagged NHE1, NHE1-KR/A or NHE1-E266I at a ratio of 1:1. Shown are the recoveries of pHi, expressed as BCECF-derived background-subtracted emission intensity ratio ($BI_{488}/BI_{452}$) values, from 20 mM $NH_4^+$-induced internal acid loads recorded simultaneously from EGFP-expressing cells (green traces) and adjacent non-EGFP-expressing cells on the same coverslips (red traces). Cells co-transfected with EGFP and either HA-tagged full-length NHE1 (a) or HA-tagged NHE1-KR/A (b) exhibited cariporide-sensitive recoveries of pHi from imposed internal acid loads, whereas cells expressing HA-tagged NHE1-E266I (c) and untransfected cells under all conditions did not. Records are representative of 3 - 4 independent experiments for each treatment group.

**Methodological details.** Forty-eight h after transfection, coverslips with cells attached were placed in a temperature-controlled recording chamber at 37°C and superfused with a pH 7.35 HEPES-buffered medium. EGFP-expressing cells were identified by direct visual inspection (x40 objective) during illumination at 488 nm and regions of interest were positioned on these cells and adjacent non-fluorescing cells in the same field. Superfusion was then interrupted, the output of the 100 W mercury arc lamp was attenuated electronically, neutral density filters were placed in the light path and 2.5 μM BCECF-AM was added to the recording chamber. After 10 min, BCECF-AM was washed off by superfusion for at least 15 min prior to the start of the records shown,
which were obtained on an Atto Bioscience imaging system (BD Biosciences, Rockville, MD). Under the conditions employed, BCECF-derived fluorescence emission intensities at each excitation wavelength (488 and 452 nm) were >20 times greater than the respective signals derived from EGFP and no changes in $BI_{488}/BI_{452}$ ratio values were observed in response to internal acid loads imposed on cells transfected with EGFP but not loaded with BCECF.
**Supplementary Figure 2.** Quantification of the effects of over-expression of full-length NHE1 and NHE1 mutants on the numbers and cumulative lengths of primary neurites and neurite branches in PC12 cells. Cells were either untransfected (Con) or transiently transfected with HA-tagged full-length NHE1 (NHE1), NHE1 lacking ion transport (E266I) or NHE1 lacking ERM binding (KR/A) and differentiated for 72 h with 50 ng mL\(^{-1}\) NGF in the absence (open bars) or continuous presence (grey bars) of 1 μM cariporide. Experiments were conducted in parallel, all measured values are per cell, error bars represent SEM and \(n\) values are shown in the columns. *, \(P<0.05\); **, \(P<0.01\); ***, \(P<0.001\); n.s., not significant \((P>0.05)\).
Supplementary Figure 3. Effects of over-expressing NHE1-Δ556-564 on neurite outgrowth in NGF-differentiated PC12 cells.  

**a**, Compared to untransfected NHE-deficient PS120 cells (red trace), cells on the same coverslip co-transfected with pEGFP-C1 and HA-tagged NHE1-Δ556-564 at a ratio of 1:1 (green trace) exhibited slow cariporide-sensitive pHᵢ recoveries from 20 mM NH₄⁺-induced internal acid loads. The record is representative of 4 independent experiments. See Supplementary Figure 1 for methodological details.  

**b**, Representative PC12 cells were either untransfected (Con) or transiently transfected with HA-tagged NHE1-Δ556-564 and differentiated for 72 h with 50 ng mL⁻¹ NGF. Untransfected and transfected cells were labeled with Alexa Fluor 488-conjugated phalloidin and anti-HA antibody, respectively. Scale bar, 20 μm.  

**c**, Quantification of neurite outgrowth in untransfected PC12 cells (Con) and PC12 cells over-expressing HA-tagged full-length NHE1 or NHE1-Δ556-564, in the absence (open bars) or continuous presence (grey bars) of 1 μM cariporide. Experiments were conducted in parallel with those the results of which are presented in Figure 2. All measured values are per cell, error bars represent SEM and n values are shown in the columns. *, P<0.05; **, P<0.01; ***, P<0.001; n.s., not significant (P>0.05).
**Supplementary Figure 4.** Quantification of the effects of NHE1 inhibitors on the numbers and cumulative lengths of primary neurites and neurite branches in E16 WT mouse neocortical neurons. Neurons were cultured for 72 h under control conditions (Con; 0.1% DMSO) or in the continuous presence of 1 µM cariporide, 100 µM cariporide or 1 µM EIPA, as indicated on the figure. Experiments were conducted in parallel, all measured values are per cell, error bars represent SEM and n values are shown in the columns. *, \( P<0.05 \); **, \( P<0.01 \); n.s., not significant (\( P>0.05 \)).
**Supplementary Figure 5.** Quantification of the effects of over-expression of full-length NHE1 on the numbers and cumulative lengths of primary neurites and neurite branches in E16 WT mouse neocortical neurons. Neurons were either untransfected (Con; open bars) or transiently transfected with HA-tagged full-length NHE1 (NHE1) and cultured for 72 h in the absence (open hatched bars) or continuous presence (grey hatched bars) of 1 μM cariporide (car). Experiments were conducted in parallel, all measured values are per cell, error bars represent SEM and n values are shown in the columns.

*, P<0.05; **, P<0.01; ***, P<0.001; n.s., not significant (P>0.05).
Supplementary Figure 6. Quantification of the effects of cariporide on the numbers and cumulative lengths of primary neurites and neurite branches in P0.5 NHE1+/+ neocortical neurons treated with netrin-1, BDNF or IGF-1. Neurons were cultured for 72 h under control conditions (Con) or in the continuous presence of 100 ng mL⁻¹ netrin-1, 50 ng mL⁻¹ BDNF or 50 ng mL⁻¹ IGF-1, in the presence of either cariporide vehicle (0.1% DMSO; open bars) or 1 μM cariporide (grey bars). Experiments were conducted in parallel, all measured values are per cell, error bars represent SEM and n values are shown in the columns. *, P<0.05; **, P<0.01; n.s., not significant (P>0.05).
Supplementary Figure 7. Quantification of the effects of netrin-1, BDNF and IGF-1 on the numbers and cumulative lengths of primary neurites and neurite branches in P0.5 NHE1−/− neocortical neurons. Neurons were cultured for 72 h in the absence (Con) or presence of 100 ng mL−1 netrin-1, 50 ng mL−1 BDNF or 50 ng mL−1 IGF-1, in the presence of either cariporide vehicle (0.1% DMSO; open bars) or 1 μM cariporide (grey bars). Experiments were conducted in parallel, all measured values are per cell, error bars represent SEM and n values are shown in the columns. *, P<0.05; **, P<0.01; n.s., not significant (P>0.05).
**Supplementary Figure 8.** Quantification of the effects of netrin-1 on the numbers and cumulative lengths of primary neurites and neurite branches in NHE1⁻/⁻ neurons over-expressing full-length NHE1 or NHE1-E266I. P0.5 NHE1⁻/⁻ neocortical neurons were either untransfected or transiently transfected with HA-tagged full-length NHE1 (NHE1⁻/⁻ + NHE1) or the HA-tagged ion translocation-deficient mutant NHE1-E266I (NHE1⁻/⁻ + E266I) and cultured for 72 h in the absence or presence of 100 ng mL⁻¹ netrin-1, as indicated on the figure, and in the absence (open bars) or presence (grey bars) of 1 μM cariporide. Experiments were conducted in parallel, all measured values are per cell, error bars represent SEM and n values are shown in the columns. *, P<0.05; **, P<0.01; n.s., not significant (P>0.05).