Supporting Methods

Calculations and Statistics. Analysis of the time course of substrate accumulation was based on a one-compartment model described in ref. 1. Analysis of saturation curves and calculations of K_i values are reported in ref. 2. Fitted parameters such as K_m and K_i are given as geometric mean with a 95% confidence interval. Arithmetic means are given with SEMs.

The efflux of ET (Fig. 5) was described with a simple exponential: rc = 100% * ($e^{-k * t}$). The efflux of stachydrine had to be modeled with two coupled differential equations: $dA/dt = B * k_{21} - A * k_{12}$ and $dB/dt = A * k_{12} + C * k_{32} - B * (k_{21} + k_{23})$, where A and B represent two distinct intracellular states of stachydrine and C represents extracellular stachydrine. Velocity of uptake of ET as a function of Na^+ concentration (Fig. 6) could be described with a modified Hill function: $v = V_0 + V_{lim}/(1 + K * c_{Na^+}{}^{-h})$, where h is the Hill coefficient and c_{Na^+} is the sodium concentration. For stachydrine, a model of competitive inhibition was used: $v = V_0/(1 + c_{Na^+}/K_i)$. Velocity of uptake as a function of pH (Fig. 7) could be described with $v = V_{max} * (10^{-pH}/(10^{-pH} + K_a)) * S/(K_m + S)$, where K_a is the acid dissociation constant and S is the substrate concentration. This function is based on the assumptions that (*i*) only V_{max} is affected by pH changes ($v = V_{max} * \alpha * S/(K_m + S)$ and (*ii*) only the protonated transporter (TH^+) is active in transport ($K_a = H^+ * T/TH^+$; $\alpha = TH^+/(TH^+ + T) = H^+/(H^+ + K_a)$).

Drugs. Radiotracers (all from ARC, St. Louis) were as follows: 3-*O*-acetyl-L-carnitine (^{3}H, 2.96 kBq/pmol; ART-739), L-carnitine (^{3}H, 2.96 kBq/pmol; ART-739), glycine betaine (^{14}C, 1.85 Bq/pmol, ARC-1432), L-histidine (^{3}H, 1.48 kBq/pmol, ART-234), 4-hydroxy-L-proline (^{3}H, 0.555 kBq/pmol, ART-231), L-proline (^{3}H, 3.33 kBq/pmol, ART-475), and tetraethylammonium (^{14}C, 2.04 Bq/pmol, ARC-577).

Unlabeled compounds were as follows: betonicine (0605, Extrasynthese, Genay, France), γ-butyryl betaine hydrochloride (403245, Sigma-Aldrich), L-(+)-ergothioneine (F-3455, Bachem), *N*-methyl-L-proline monohydrate (306843, Sigma-Aldrich), stachydrine hydrochloride (0604, Extrasynthese), 1-methyl-4-phenylpyridinium iodide (D-048, Sigma-Aldrich). All other chemicals were at least of analytical grade.

1. Russ, H., Gliese, M., Sonna, J. & Schömig, E. (1992) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **346,** 158-165.
2. Schömig, E., Babin-Ebell, J. & Russ, H. (1993) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **347,** 379-383.