The Dopamine Transporter: More Exciting than Housekeeping

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Dopaminergic Synapse

Presynaptic cell

Postsynaptic cell

DA

MGluR

D2

D1

α1

GIRK

2 Na⁺ Cl⁻
Currents are activated at lower dopamine concentrations than are required for transport.

\[ K_{0.5} = 37 \pm 5 \text{ nM} \]
\[ V_{\text{max}} = 38 \pm 1 \text{ pA} \]
Low DA concentrations increase firing

Ingram, et al., 2002
DAT-mediated chloride current is excitatory in cultured midbrain DA neurons.

Ingram, et al., 2002
Proteins that regulate intracellular Cl⁻
Whole-cell patch clamp recordings from DA neurons

Raclopride
Sulpiride
Prazosin
TTX

biocytin

Prasad and Amara, 2001
Amphetamines activates a DAT-mediated current at low concentrations.

Watts, Fyfe and Ingram, unpublished data.
DA neurons make glutamatergic autapses in culture and amphetamine increases AMPA currents

Ingram, et al., unpublished data
mbYFPQS localizes to the membrane of cultured midbrain neurons

Watts, Jimenez and Ingram, unpublished data.
Amphetamine stimulates a dose-dependent change in mbYFPQS fluorescence in both soma and dendrites of DA neurons.

Watts and Ingram, unpublished data.
KCC2 Expression is different in cultures and slices

Jamieson and Ingram, unpublished data.
Baculoviral Transfections

Watts and Ingram, unpublished data.
Summary

- DAT-mediated chloride current may alter excitability of DA neurons and integration of synaptic activity.

- The current may be activated selectively (relative to transport) by low DA and amphetamine concentrations suggesting a role in increasing release of DA.

- The amphetamine-mediated current is dose-dependent in both cultures and slices of midbrain neurons but is inhibited at high amphetamine concentrations (20 µM).

- The mbYFPQS is a sensitive tool to measure intracellular chloride concentrations ($K_{50} = 30$ mM) and is useful for monitoring changes in intracellular chloride concentrations in dendrites.

- Amphetamine can increase influx and efflux of chloride in separate cells in culture suggesting that there are different $E_{Cl}$ in DA neurons. These results are supported by the presence of KCC2 in a subpopulation of DA neurons in culture. Therefore the physiological relevance of DAT-mediated currents will have to focus on DA neurons in substantia nigra slices.
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Activity-Dependent Intracellular Chloride Accumulation and Diffusion Controls GABA<sub>A</sub> Receptor-Mediated Synaptic Transmission

Peter Jedlicka,1,2* Thomas Deller,1 Boris S. Gutkin,3,4,5 and Kurt H. Backus2

Jedlicka, et al., 2010
Dopamine Neurons Mediate a Fast Excitatory Signal via Their Glutamatergic Synapses

Nao Chuhma, Hui Zhang, Justine Masson, Xiaoxi Zhuang, David Sulzer, Rene Hen, and Stephen Rayport

The Journal of Neuroscience, January 28, 2004 • 24(4):972–981
DA transport by DAT is slow

- In midbrain neurons it takes 1.3 sec for one DAT to move one DA molecule.
- Uptake by DAT is insensitive to physiological changes in membrane potential.

Prasad & Amara, 2001
Vesicular Dopamine Release Elicits an Inhibitory Postsynaptic Current in Midbrain Dopamine Neurons

Michael J. Beckstead, David K. Grandy, Kevin Wickman and John T. Williams
Cl⁻ regulation in neurons

DAT

NKCC

NDCBE

ClC-2

GABA_A

GABA