Inhibitory synapses in the auditory system -part II-
Outline

- Revision of the basics
- CCC-family (Cl⁻-cotransporters)
  - Na⁺-coupled
  - K⁺-coupled
- Research on KCC2
- Summary
- References
Basics

- 30% of neurons are inhibitory
- activity important for synaptogenesis
- rats & mice as model organisms: inhibitory MNTB/LSO projection → easy to investigate
- Shift from depolarization to hyperpolarization (D/H-shift) at P8, hearing onset at P12
- electrophysiological research showed that $[\text{Cl}^-]_i$ is responsible for D/H-shift
Age-related changes of $E_{Cl}$ ($E_{Gly}$)

A

B

C

$E_{Gly} = -38.4$ mV

$E_{Gly} = -62.4$ mV

$E_{Gly} = -79.7$ mV
Development of $E_{Cl}$ and $V_{rest}$ in LSO neurons
Age-dependent $[\text{Cl}^-]_i$

$E_{\text{Cl}} > V_{\text{rest}} \rightarrow$ efflux of Cl$^-$

$E_{\text{Cl}} < V_{\text{rest}} \rightarrow$ influx of Cl$^-$
CCC-family

- Cation-coupled cotransporters
- Secondary active, electroneutral
- Two branches:
  - $\text{Na}^+$-coupled (NCC, NKCC1, NKCC2)
  - $\text{K}^+$-coupled (KCC1, KCC2, KCC3, KCC4)
Na\textsuperscript{+}-coupled

- driving force: inward-directed Na\textsuperscript{+} gradient
- transport of Cl\textsuperscript{-} into the cell
  - increase of [Cl\textsuperscript{-}]\textsubscript{i}
  - depolarization
NKCC1

- major candidate for inward-directed Cl⁻-transport
- expression analyses: absent in SOC in neonatals, slightly increase with age

→ not responsible for high [Cl⁻]ᵢ in immature LSO-neurons
Further candidates

- AE3: HCO$_3^-$/Cl$^-$ exchanger
- GAT-1: Na$^+$/Cl$^-$-dependent $\gamma$-aminobutyric acid (GABA) transporter

$\rightarrow$ Cl$^-$ inward transporter remains unknown at present
K\(^{+}\)-coupled

- driving force: outward-directed K\(^{+}\) gradient
- transport of Cl\(^{-}\) out of the cell
  - decrease of \([\text{Cl}^{-}]_i\)
  - hyperpolarization
Characteristics of KCC2

- neuron specific
- responsible for decreasing $[\text{Cl}^-]_i$, consequently the D/H-shift
- regulation of $[\text{Cl}^-]_i$ in mature LSO neurons
Expression of KCC2

- expressed during both phases (e.g. P1 and P16)
  - evidenced by Western Blot analyses
Immunohistochemistry

- translocation of KCC2
  - < P8: located in soma, neuropil, plasma membrane (?)
  - > P8: located predominantly in plasma membrane

→ 1st step to activation of KCC2
Features of KCC2 -/-

- knock-down mice with >95% reduction of protein expression
- phenotype of homocgygous:
  - seizures during first postnatal week
  - die between P10 & P16
- phenotype of heterocgygous:
  - indistinguishable from WT until P7-8
No difference between KCC2<sup>+/+</sup> & KCC2<sup>-/-</sup> @ P3

→ KCC2 is not an active Cl<sup>-</sup> transmembrane transporter in neonatal LSO neurons
Comparison of KCC2\(^{+/+}\) & KCC2\(^{-/-}\) @ P12
Differences between KCC2\(^{+/+}\) & KCC2\(^{-/-}\) @ P3 & P12
Summary

- D/H-shift due to changes in $[\text{Cl}^-]_i$
- high $[\text{Cl}^-]_i$ in immature neurons:
  - inward-directed $\text{Cl}^-$ transporter remains unknown
- low $[\text{Cl}^-]_i$ in mature neurons:
  - KCC2 is a outward-directed $\text{Cl}^-$ transporter
References

- Ehrlich et al. (1999): Shift from depolarizing to hyperpolarizing glycine action in rat auditory neurones is due to age-dependent Cl⁻ regulation. J Physiol 520: 121-137.