5-HT2C Receptors and NMDA Interact In Situ via a Src Kinase Pathway to Enhance Spinal Cord Motoneuronal Activity

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Introduction

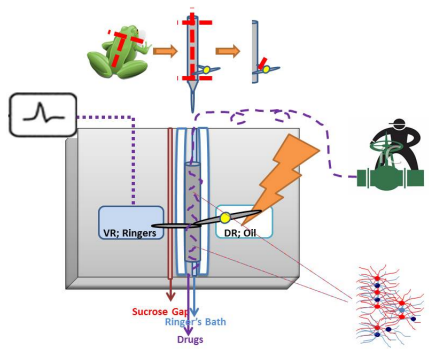
Spinal cord motoneurons express many serotonin (5-hydroxytryptamine; 5-HT) receptors that are activated when 5-HT is released from the descending tracts of the raphe nuclei. 5HT2C is one of 14 known 5-HT receptors and is shown here to interact with N-methyl- D-aspartate (NMDA) to enhance motoneuronal activity. Previous findings have shown that 5-HT2A and 5-HT2B receptors mediate activity via G-protein and Ca2+ pathways, respectively. Here we provide evidence that SRC tyrosine kinase mediates 5HT2C receptor enhancement of NMDA depolarization of spinal cord motoneurons using in situ electrophysiological recording of frog motoneuron membrane potentials.

Methods

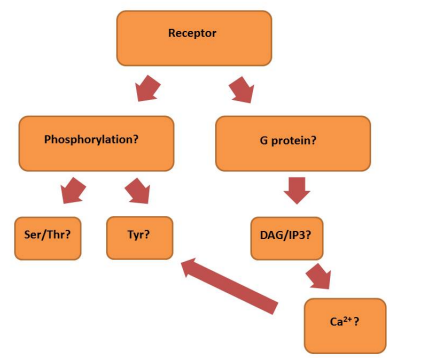
A rana pipien frog (30-50g) was anesthesized by cooling. After the frog was decapitated, a spinal cord laminectomy and spinal cord hemisection was performed, preserving the IXnth dorsal and ventral root. The spinal cord is then transferred to a sucrose gap chamber where it is perfused with Ringer's solution and pharmacological agents. Motoneuronal depolarization was recorded from the ventral root after stimulating the dorsal root ganglion.

Acknowledgements

Thanks to Dr. John C. Hackman for his mentorship and guidance.

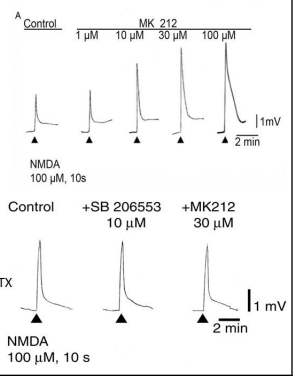


Laminectomy & Sucrose Gap Chamber



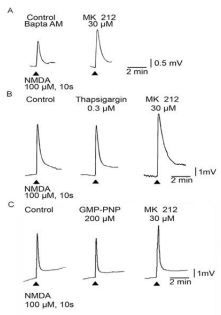
1. 5-HT2C Enhances Motor Activity

- 5-HT$_{2C}$ **Agonist MK212 increases** the NMDA-evoked motoneuron depolarizations in a dose-dependent manner.
- 5-HT$_{2C}$ **Antagonist SB 206553 blocks** the *MK212* induced increases of NMDA responses. TTX but no Mg^{2+} present.



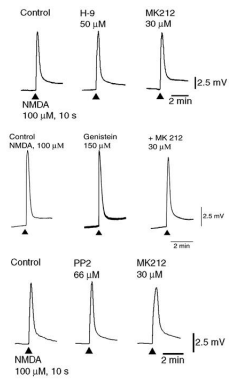
2. G-protein & Ca2+ Interactions

- **Bapta AM** / 0 Ca2+, **Thapsigargin**, or G-protein **antagonist GMP-PNP**
  - **-Neither reduce** MK 212 mediated increases in NMDA-induced motoneuronal depolarization in the frog spinal cord.



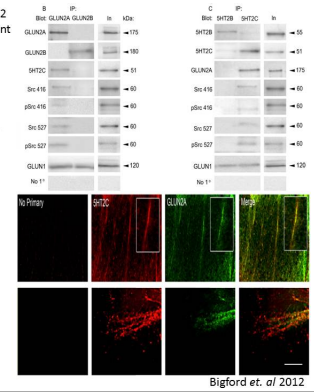
3. SRC Kinase

- Protein Kinase **Antagonist H-9 blocks** the *MK212* evoked enhancement of NMDA responses.
- Tyrosine Kinase **Inhibitor Genistein blocks** the MK212-induced increase in NMDA responses.
- **SRC Kinase Antagonist PP2 blocks** the *MK212* induced increase in NMDA responses.



4. Multiprotein Complex

- In **spinal neuronal cultures**, MK212 induced and increase in the amount of SRC phosphorylation.
  - 5-HT$_{2C}$ and TK inhibitors attenuated phosphorylation
- **Co-immunoprecipitation** experiments revealed NMDA subunit GluN2A, 5-HT$_{2C}$ receptors, and SRC form a **multi-protein complex** in synaptosomes
- 5-HT$_{2C}$ and GLUN2A **co-localize** in spinal cord tissue and spinal neuronal cultures



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Results

5-HT2C receptor activation enhances NMDA-induced motoneuron depolarization. G proteins and Ca2+ related mechanisms are not involved with 5-HT2C receptors. 5-HT2C activation & potentiation of NMDA motoneuron depolarization is blocked by general protein kinase inhibition. 5-HT2C activation & potentiation of NMDA motoneuron depolarization is blocked by SRC protein kinase inhibition.

Conclusion

Our work shows that unlike other 5-HT2 receptors (5HT2A and 5HT2B), the 5HT2C interaction with NMDA is independent of G-proteins and Ca2+. Instead, electrophysiological experiments demonstrated that the 5HT2C agonist MK 212 enhances NMDA motoneuronal depolarization produced by activation of 5HT2C receptors. Furthermore, we were able to show the MK212 enhancement is mediated by a tyrosine kinase pathway, particularly the SRC kinase. This was substantiated with spinal neuronal cultures and co-immunoprecipitation experiments which revealed a protein association between the NMDA subunit GluN2A, 5HT2C receptors, and SRC in both mammalian and amphibian spinal neurons and mammalian synaptosomes. There is a distinct multiprotein complex in the spinal cord by which 5-HT and NMDA coordinate intracellularly, via a SRC tyrosine kinase pathway, to mediate functional activity.