Does Nitric Oxide Regulate Serotonin Firing in the Brain?

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Background

- Serotonin (5-HT) is a chemical neurotransmitter released from neurones that are located in a section of the brain called the dorsal raphe nucleus (DRN). The production of 5-HT is catalysed by the enzyme tryptophan hydroxylase (TPH).
- Dysfunction of the 5-HT system is implicated in neuropsychiatric disorders such as anxiety and depression.
- The DRN also contains neurones which express the enzyme neuronal nitric oxide synthase (nNOS) which catalyses the synthesis of nitric oxide (NO). nNOS is found co-localised with TPH in some neurones in the DRN.
- Both anatomical and behavioural evidence suggest that 5-HT and NO interact however the interactions that occur are unknown.

Aim

- To determine the effects of modulators of nitric oxide signalling on the 5-HT neuronal firing in the DRN using in vitro electrophysiology. Also to look at the distribution of TPH and nNOS in the DRN using Immunohistochemistry.

Methods

In vitro Electrophysiology

- Immunohistochemistry was used to determine the effect of DEA-NONO, a drug which releases NO, on 5-HT cell firing.
- Extracellular recordings were carried out on rat brain slices that were placed in a continuously perfused chamber of oxygenated artificial cerebral spinal fluid (ACSF).
- Phenylephrine (PE) was added to the ACSF to stimulate the 5-HT neurones in the brain slice to fire action potentials.
- Recordings were taken from 5-HT neurones and non-5-HT neurones in the DRN and surrounding areas.
- DEA-NONO was added to the neurones and the response to this drug was measured.

Immunohistochemistry

- Immunohistochemistry was used to determine the distribution of TPH and nNOS in the DRN of the rat
- Brain sections containing the DRN were incubated with primary antibodies which bind to TPH and nNOS.
- Fluorescently labelled secondary antibodies were used to visualise the primary antibody.
- Sections were mounted on microscope ides and examined using a fluorescence microscope.

Results – Electrophysiology

- 5-HT and non 5-HT cell responses to DEA-NONO vary.
- Fig.1. shows a common 5-HT cell inhibitory response to DEA-NONO and Fig.2. shows no response on the addition of DEA-NONO to a non 5-HT cell.
- Most of 5-HT cell responses to DEA-NONO are inhibitory (52%).
- The majority of the non 5-HT cell responses to DEA-NONO are either inhibitory, or show no response.
- Compared to the 5-HT cells, fewer of the non-5-HT cells responses to DEA-NONO are inhibitory
- Very few cells, both 5-HT and non 5-HT, show both an excitatory and inhibitory response to DEA-NONO. These differing responses were dependant on the dose of DEA-NONO administered.

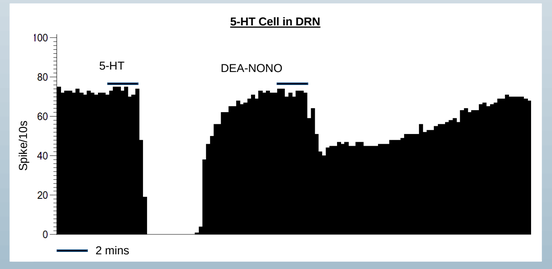


Fig.1. Presumed 5-HT neurone in the DRN of a rat, note inhibitory response to 5-HT, showing an inhibitory response to DEA-NONO.

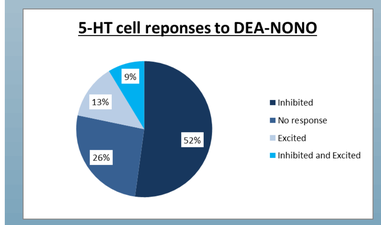


Fig.2. The variety of responses observed from presumed 5-HT cells in the DRN to DEA-NONO.

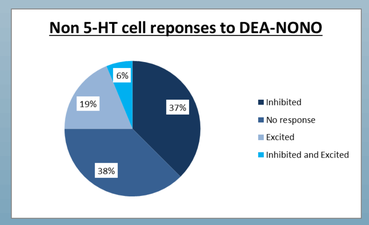


Fig.3. The variety of responses observed from presumed non 5-HT cells in the DRN on the addition of DEA-NONO.

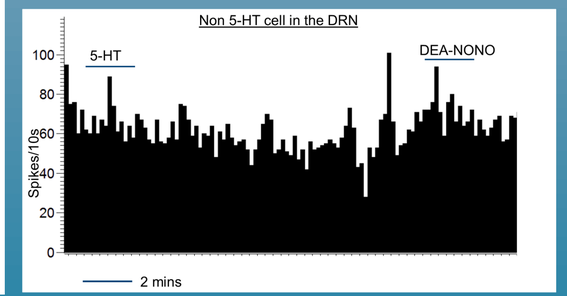
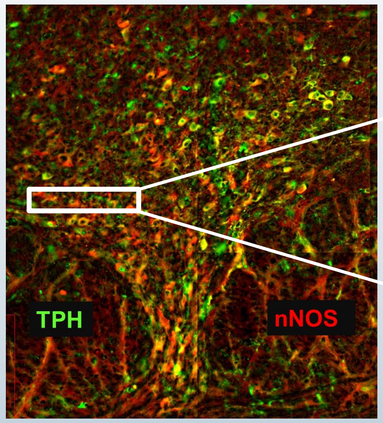


Fig 4. Presumed Non 5-HT neurone in the DRN of a rat, note the lack of response to 5-HT, showing no response on the addition of DEA-NONO.



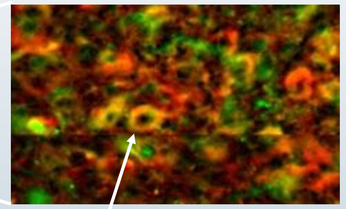


Fig.5. Mosaic showing immunofluorescence staining of the cells present in the DRN of a rat.

Results – Immunohistochemistry

- Cells in the DRN that produce TPH only are stained green and cells that produce nNOS only are stained red.
- 5-HT neurones that express nNOS appear orange.
- Co-localisation of TPH and nNOS occurs in the DRN of a rat.

Discussion and Conclusion

- 5-HT cells and non 5-HT cells in the DRN both respond to the NO modulator drug DEA-NONO.
- Cells that express both TPH and nNOS enzymes suggest that NO and 5-HT are co-localised.
- The responses of 5-HT cells and non 5-HT cells can be dose dependant, or can differ due to the whereabouts of the cell in the DRN.
- The variation of 5-HT cell responses and non 5-HT responses to DEA-NONO in the DRN are similar.
- A possible limitation to the results would be the number of cells sampled in the DRN, further studies could be done using a larger number of cells.
- The results obtained are similar to results from a previous study on the co-localisation of TPH and nNOS in the raphe nucleus (Lu Y., et al., 2010).
- Further studies could be done by measuring the responses of cells using different NO modulator drugs.
- Further research could also be done to examine the mechanisms of how NO regulates the 5-HT system.

References

- Lu Y., Simpson K., Weaver K. and Lin R., (2010) “Co-expression of nitric oxide in the raphe complex: cortical vs subcortical circuit.” Anatomical record 296(11): 1954 - 1965

Acknowledgements

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