

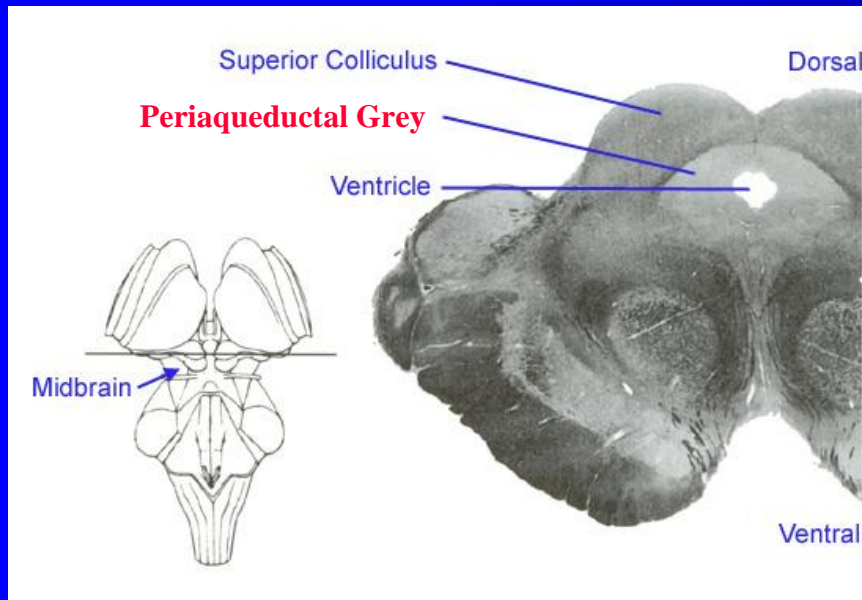
# cADP-Ribose-mediated $\text{Ca}^{2+}$ Signaling in Morphine-induced Tolerance

Mark Bradham Brewster

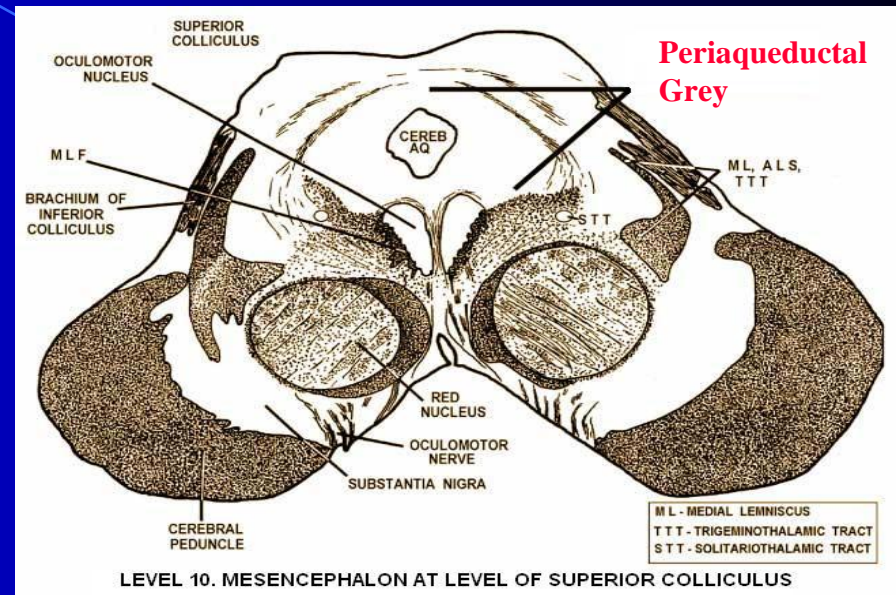
Dept. of Pharmacology & Toxicology

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# Background: Periaqueductal Grey (PAG)



<http://www.colorado.edu/epob/epob3730rlynch/image/figure12-6.jpg>



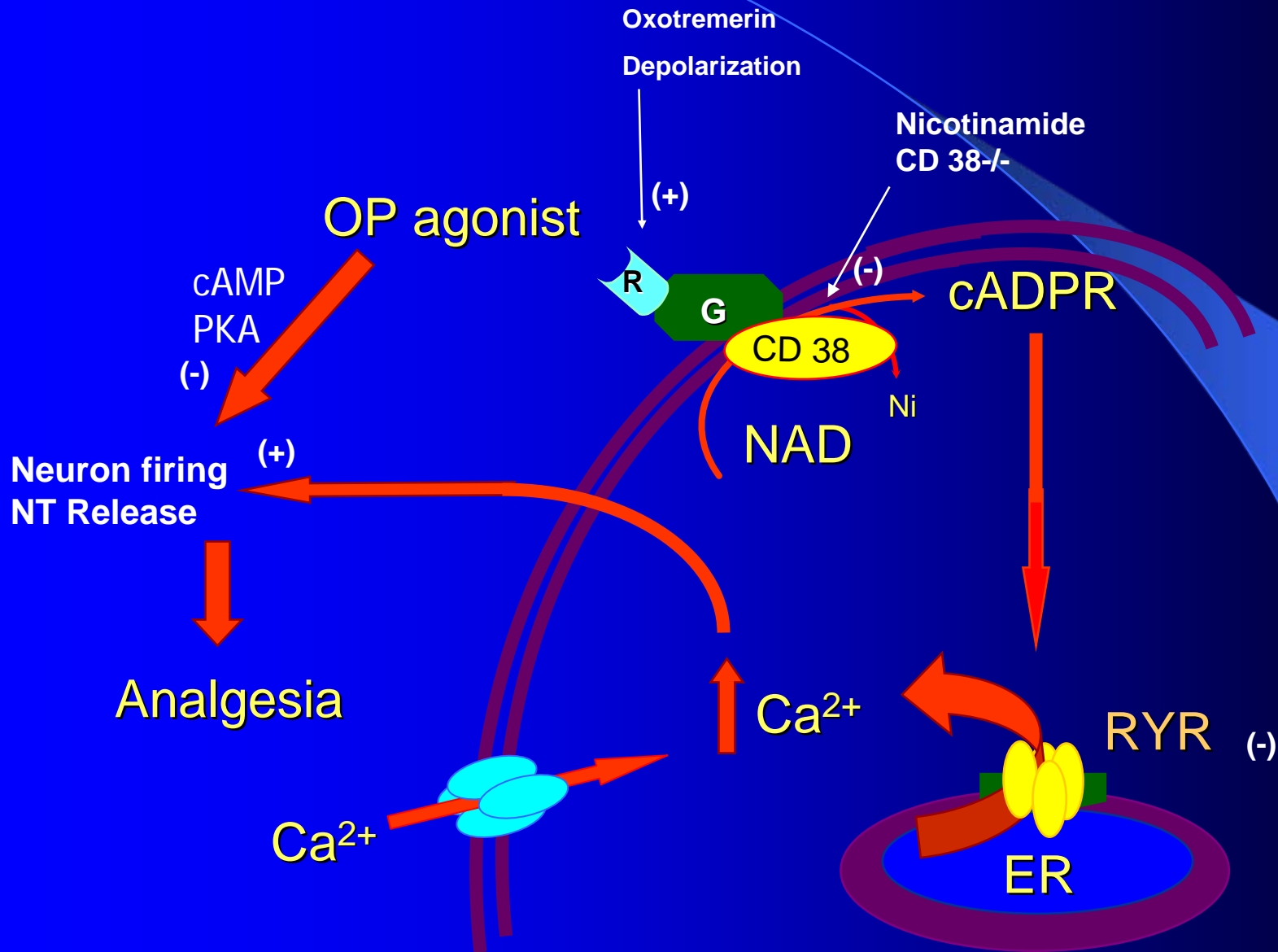
<http://www.neuroanatomy.wisc.edu/virtualbrain/BrainStem/24PAG.html>

- Efferent nociceptive trunk between brain and spinal chord
- Opiates acting on PAG induce analgesic effects by inhibiting nociception

## Background: Known Effect of Morphine on PAG

- Morphine increases intracellular  $\text{Ca}^{2+}$  in PAG
- Increased intracellular  $\text{Ca}^{2+}$  invokes analgesia
- Analgesia caused by PAG inhibition of pain signal transmission

# Role of cADPR in Morphine-Induced Tolerance



# Hypothesis

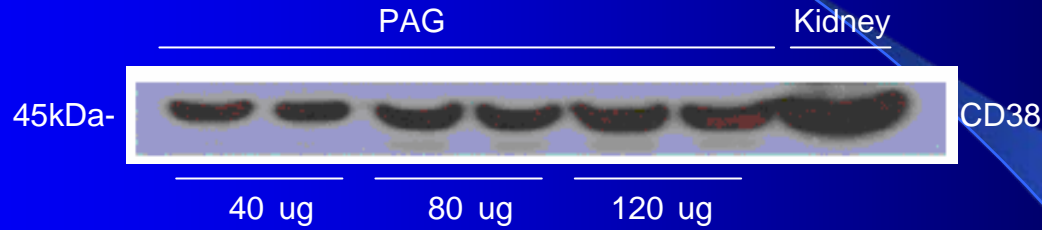
- cADPR-mediated  $\text{Ca}^{2+}$  signaling plays an integral role in morphine-induced tolerance

# Questions

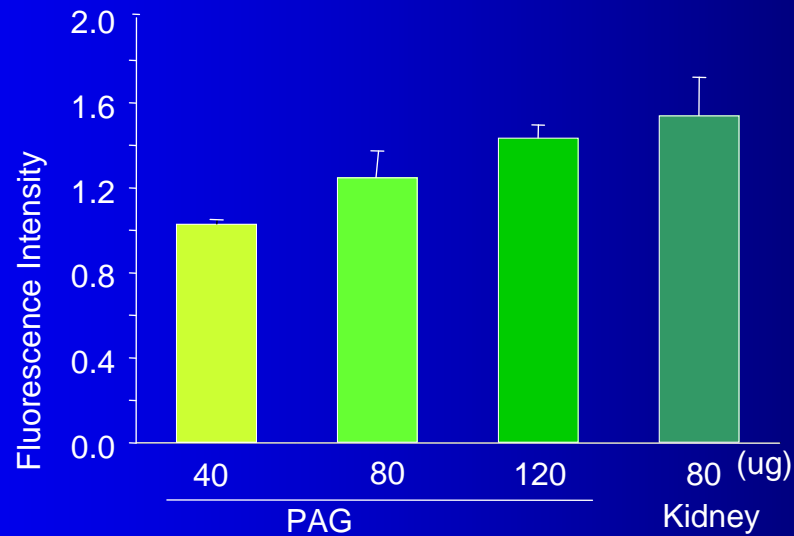
- Is CD38 present in PAG? (WB, RT-PCR)
- Is CD38 functioning in PAG? (HPLC)

# Western Blot Analysis of CD38 in PAG

A

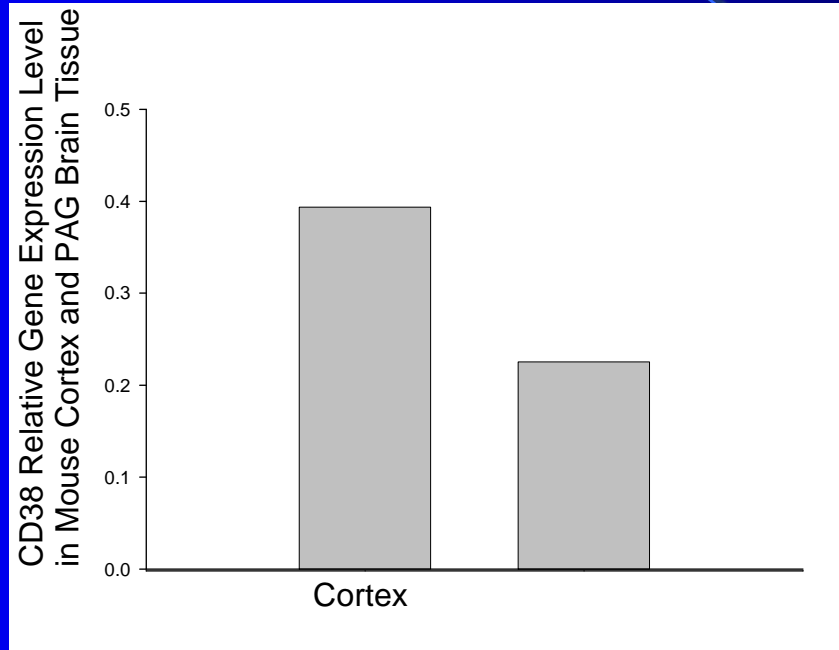


B



# Gene Expression Level of CD38 in PAG

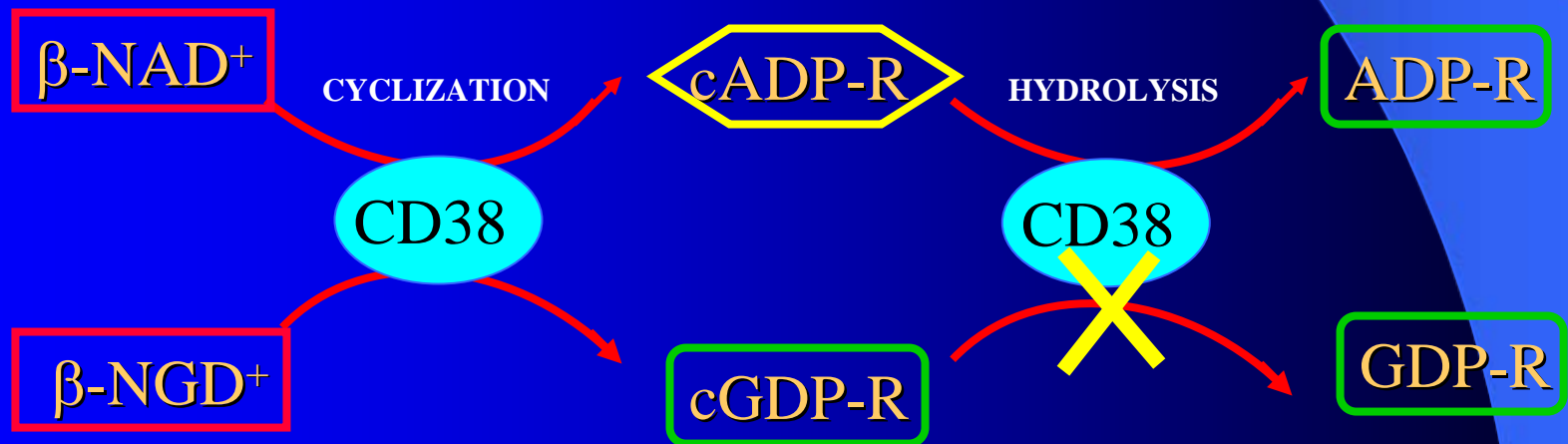
A





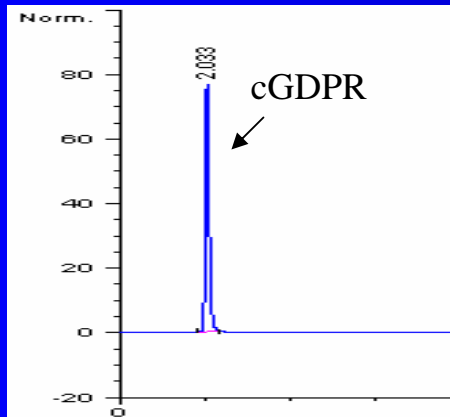
# Measurement of CD38 Cylase Activity in Mouse PAG Membrane

- CD38 is bifunctional enzyme as cylase and hydrolase
- Post-cyclization cADPR is further reduced to ADP-ribose
- cGDPR production is used to calculate cyclase activity

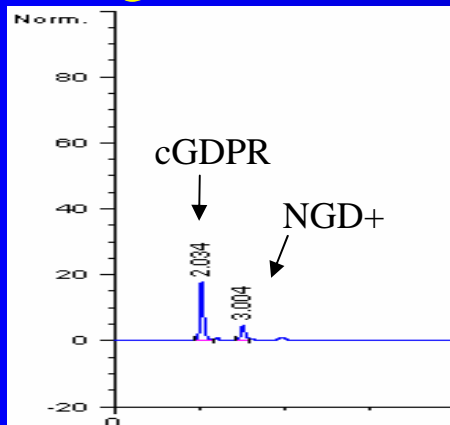


# CD38 is functioning in Mouse PAG Membrane

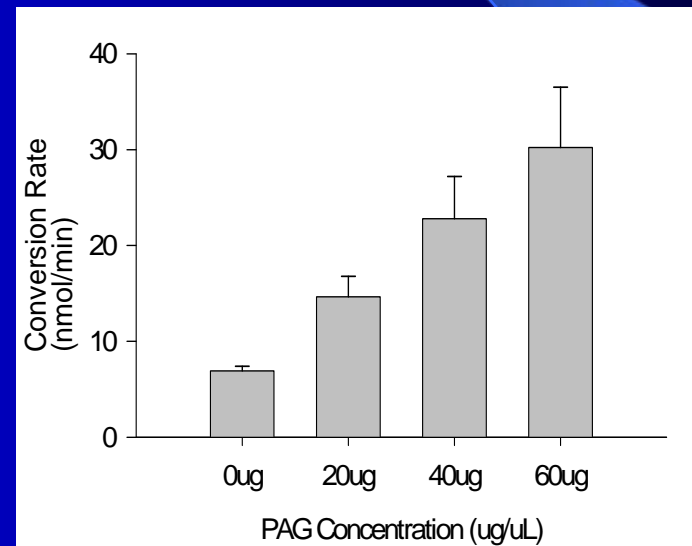
## A: Standard



## B: 40ug PAG



C

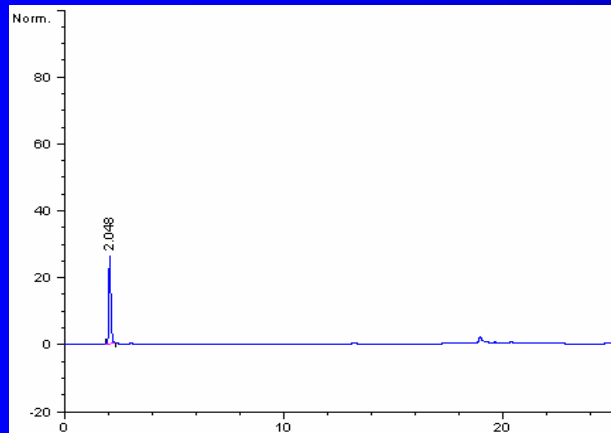


# Protocol:

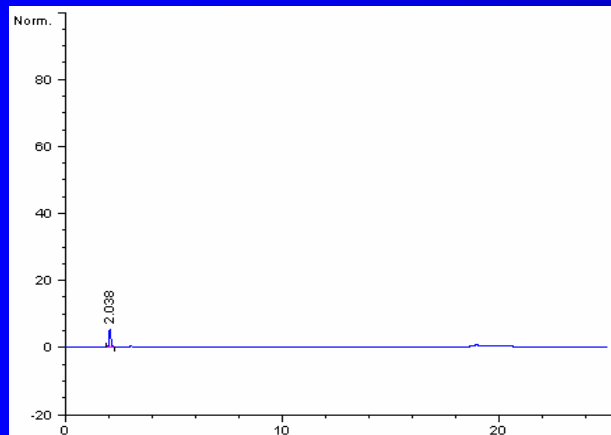
- 3 groups of mice: Untreated, Morphine treated, Naloxone treated (n=5 in each)
- PAG homogenates were incubated with 1mM  $\beta$ -NGD+ for 2 hours at 37C
- Measured cGDPR production by HPLC analysis.

# Effect of Morphine on CD38 Activity in Mouse PAG

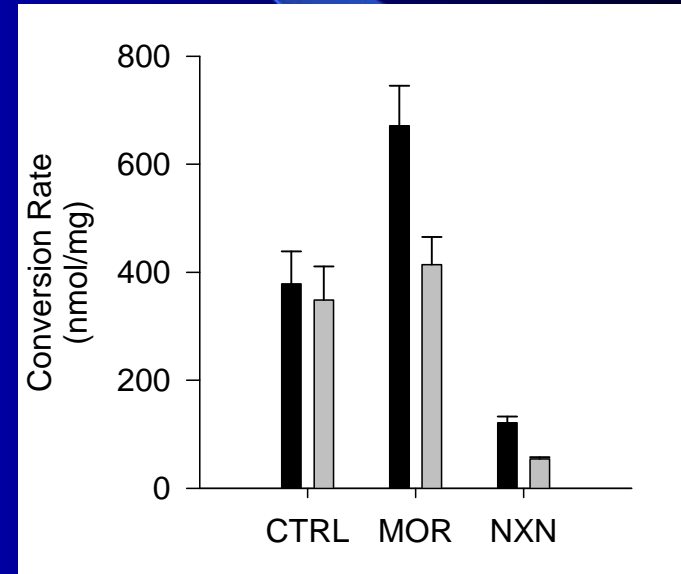
A



B



C



## Summary

- CD38 present in PAG
- Higher levels of CD38 present in cortex than PAG, but higher CD38 activity in PAG than cortex
- CD38 activity in PAG when incubated with NGD
- Morphine increases enzymatic activity of CD38 in PAG

## Future Direction

- 1.  $\text{Ca}^{2+}$  Assay: Study effects of different pharmacological interventions on morphine-induced  $\text{Ca}^{2+}$  increase
- 2. Study the effects of these inhibitors on morphine tolerance

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