Internalization, Dimerization, and Activation of CD38 during mNOX Activation:

O_2^- and Ca^{2+} Signaling in Coronary Arterial Smooth Muscle

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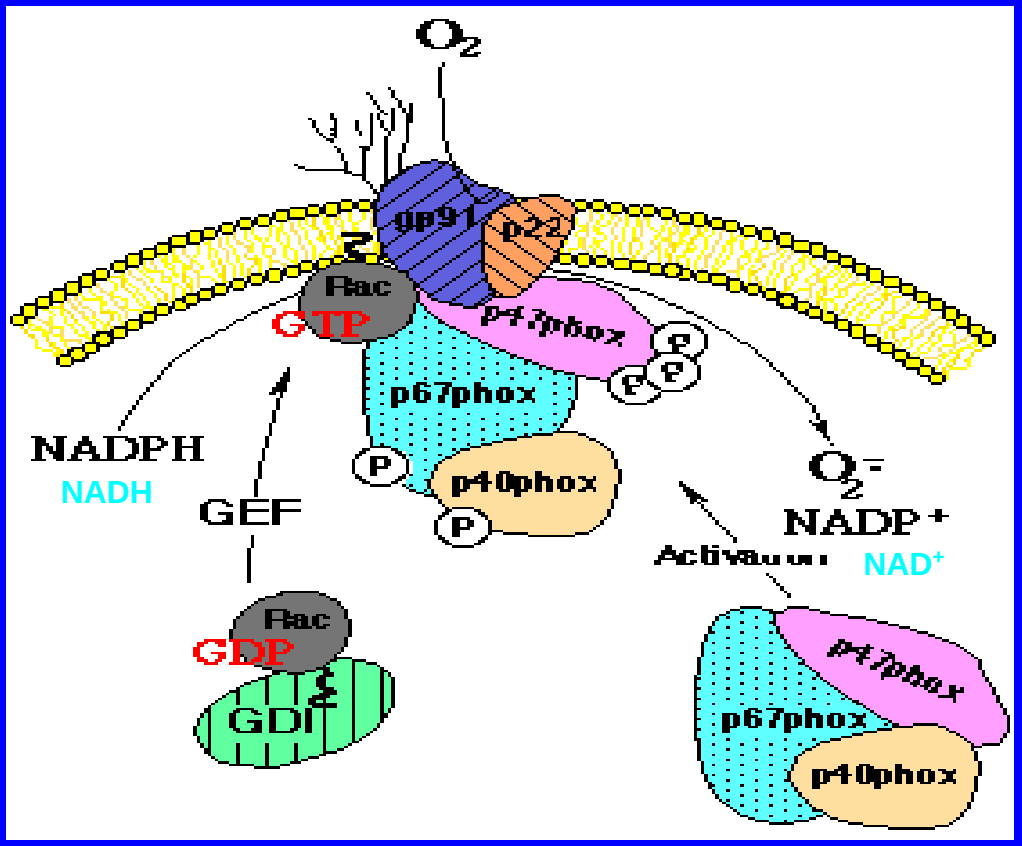
Redox Signaling under Physiological Conditions

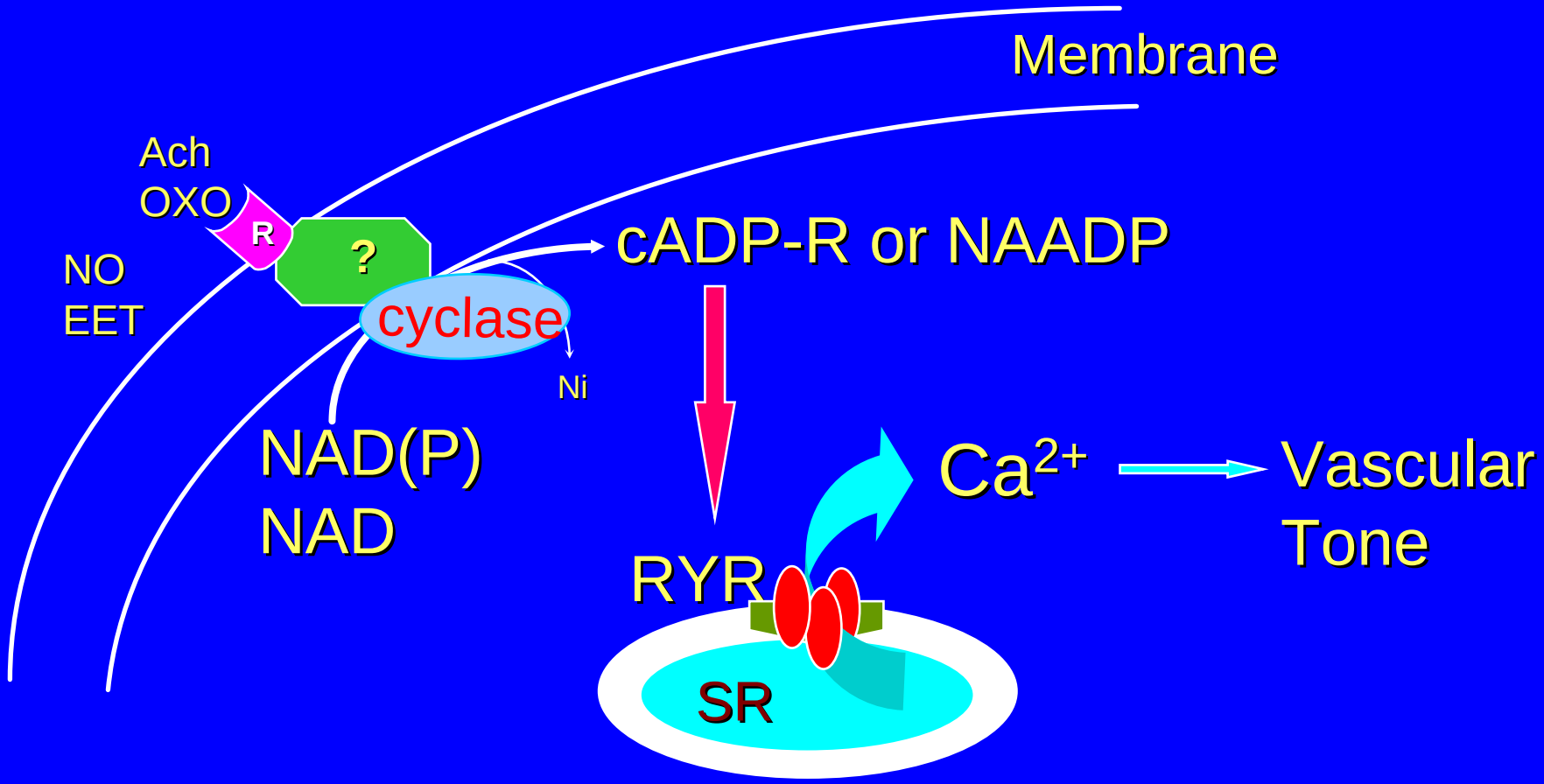
- Redox-mediated signaling is emerging as a fundamental regulatory mechanism in cell biology.
- Many cellular proteins, such as transcription factors, receptors, enzymes and ion channels are sensitive to reactive oxygen species (ROS).

NAD(P)H Oxidase: A Major Enzyme Mediating $O_2^{\cdot-}$ Production and Redox Signaling in Vessels



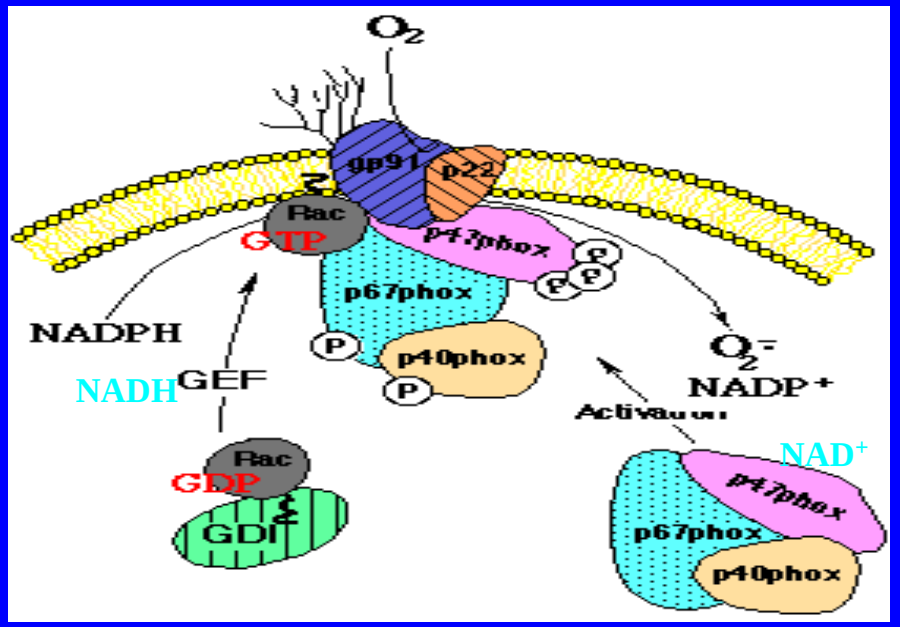
Modified based on www-dsv.cea.fr/thema/bbsi/vengl/taoxyd.htm

cADP-R-mediated Ca^{2+} Mobilization in the Control of Vascular Tone

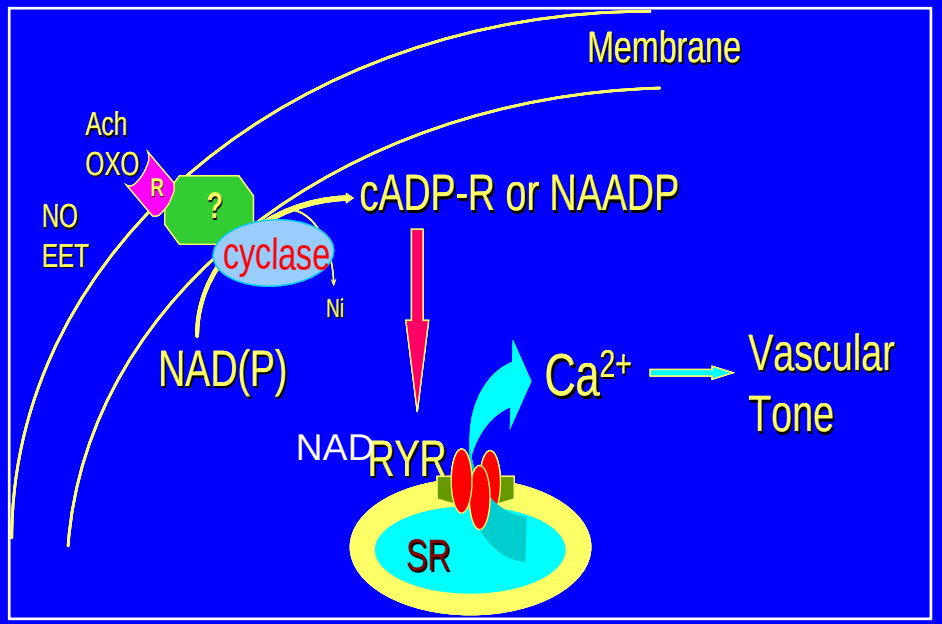


Linkage of NAD(P)H Oxidase-Mediated Redox Signaling to cADP-R-Ca^{2+} Signaling

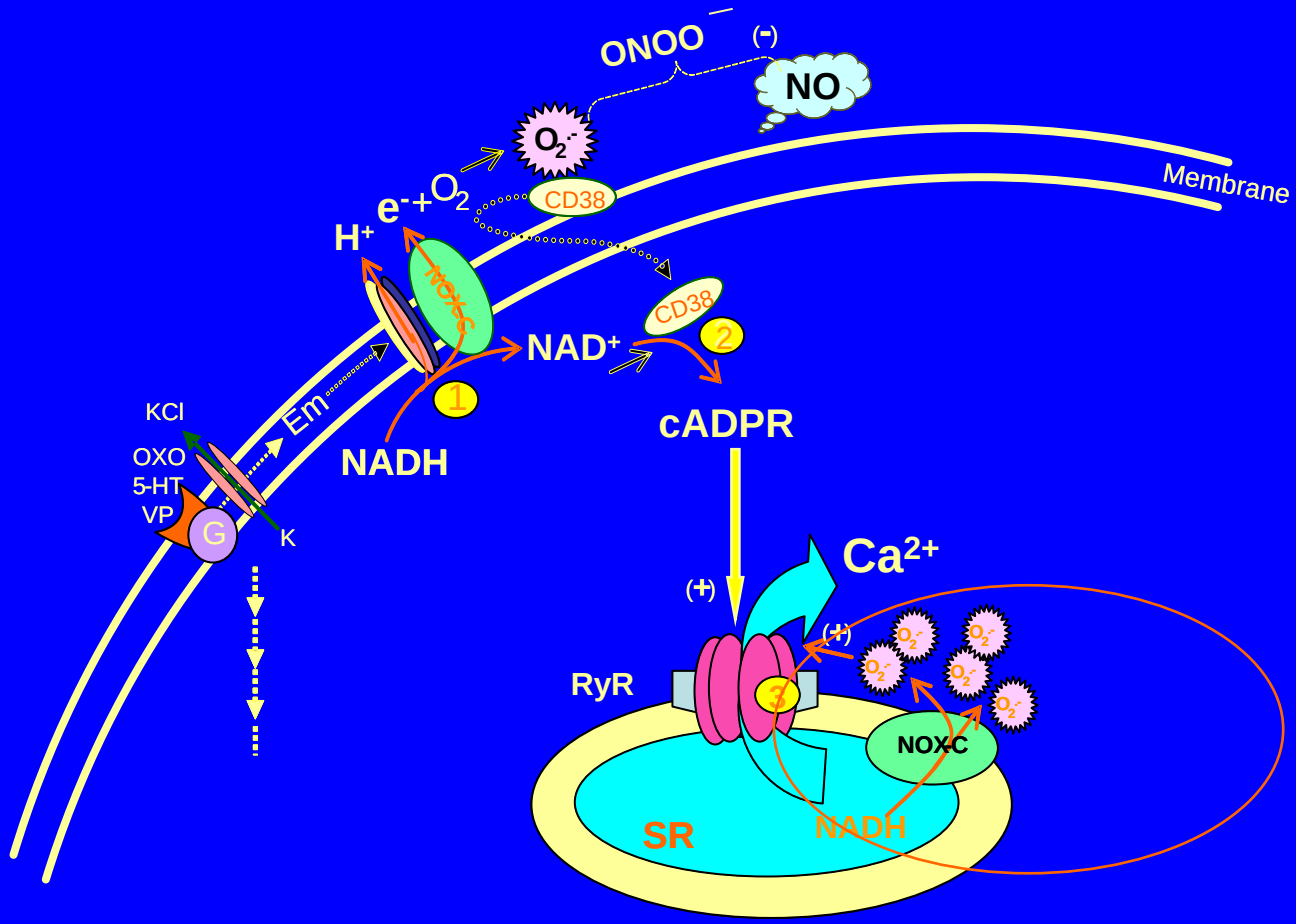
NAD(P)H Oxidase-Mediated Redox Signaling



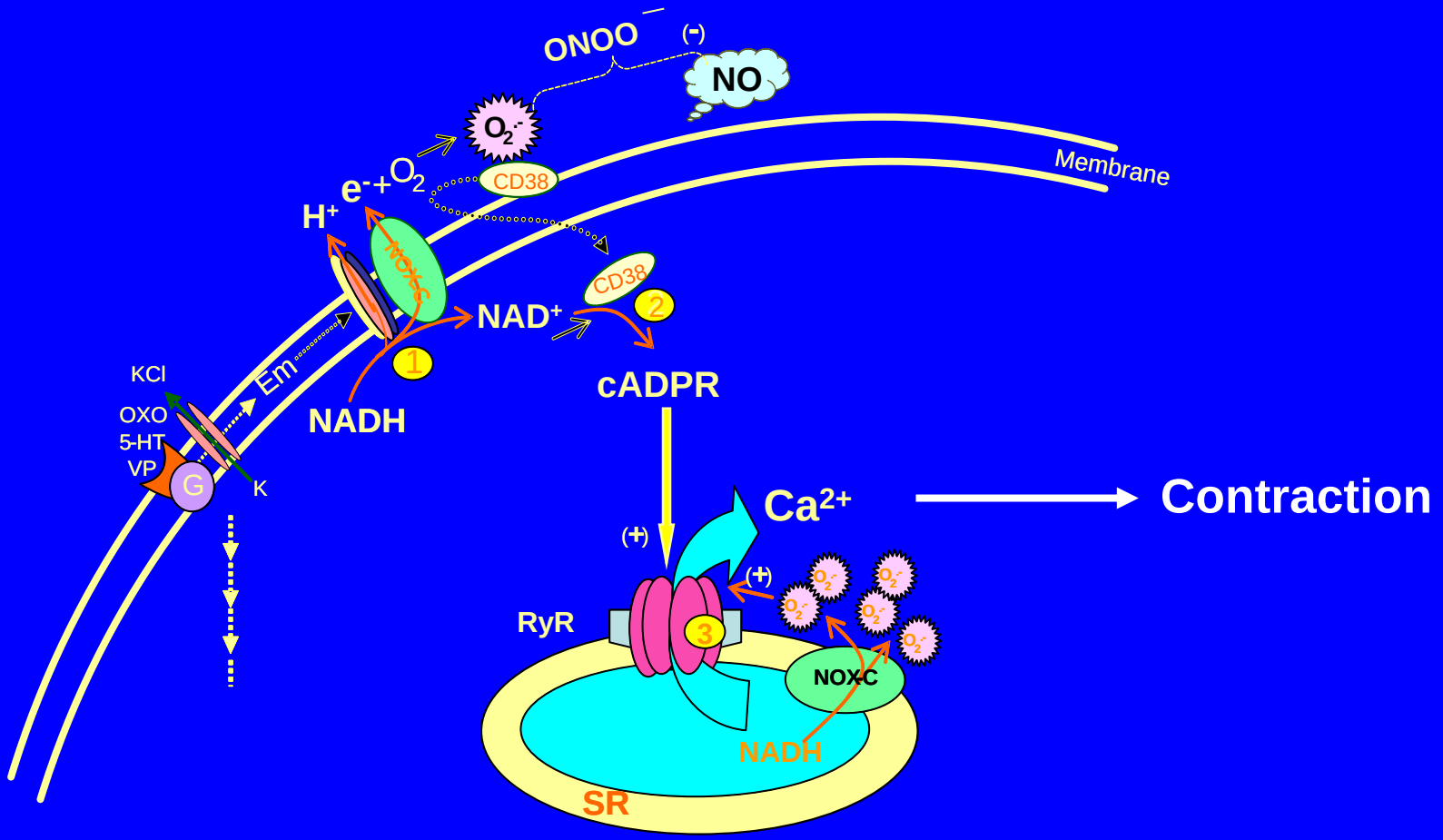
cADP-Ribose-Mediated Ca^{2+} Signaling



Revised Hypothesis: Redox Amplification of Ca^{2+} Signaling



Revised Hypothesis: Redox Amplification of Ca^{2+} Signaling



How do we test our hypothesis?

Experimental Design

- Coronary arterial myocytes (CAMs) from freshly dissected bovine coronary arteries (BCA) were homogenized and ultracenterfuged into cytosol and microsome.

- mNOX was stimulated by incubating the BCA microsome at 37^0C at differing times, to find an optimal peak in incubation time for stimulation, in different agonists such as oxotremorine (OXO) and later xanthine/xanthine oxidase (X/XO).

- In separate groups, NOX inhibitors DPI, apocynin (Apo), and SOD, along vehicle were incubated at 37^0C for differing times to find an optimal peak in incubation time for inhibition.

- These samples are then detected by HPLC (Fluorescence)

Anticipated Results

- Increased O_2^- production and cGDPR expression in the BCA microsome with the addition of the agonist OXO in comparison with control homogenate.

- Decreased O_2^- production and cGDPR expression with the addition of NOX inhibitors DPI and Apo to OXO treated homogenase in comparison to addition of OXO alone.

HPLC
High Performance Liquid Chromatography

- HPLC detectors pass a beam of light through a column effluent as the fluid passes through a low-volume flow cell. Variations in light intensity are recorded and a chromatograph is generated.

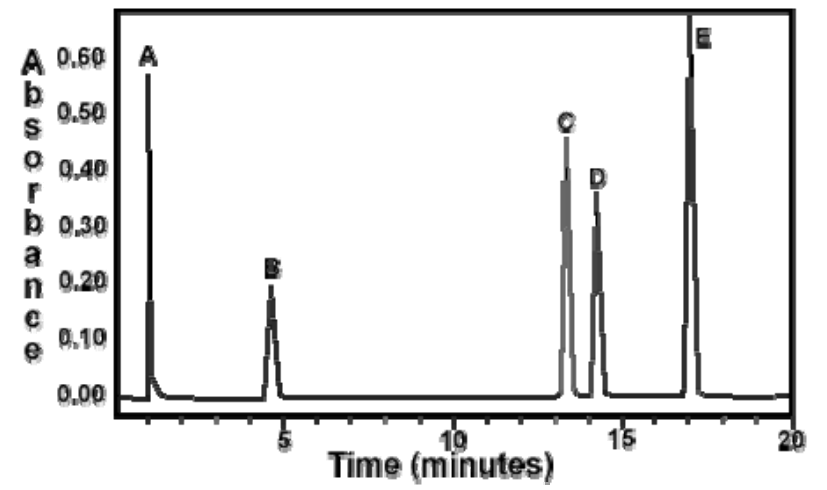
- HPLC detectors use several detection methods. Ultraviolet (UV) detectors measure the ability of a sample to absorb light at one or more wavelengths. Light scattering detectors nebulize the effluent, vaporize the solvent, and then detect droplets in a light scattering cell.

- The fluorescence detector is one of the most sensitive LC detectors and for this reason is often used for trace analysis.

HPLC
High Performance Liquid Chromatography

- Utilizes special instruments designed to separate, quantify and analyze components of a chemical mixture.
- Samples of interest are introduced to a solvent flow path; carried through a column packed with specialized materials for component separation; and component data is obtained through the combination of a detection mechanism coupled with a data recording system.

HPLC
High Performance Liquid Chromatography

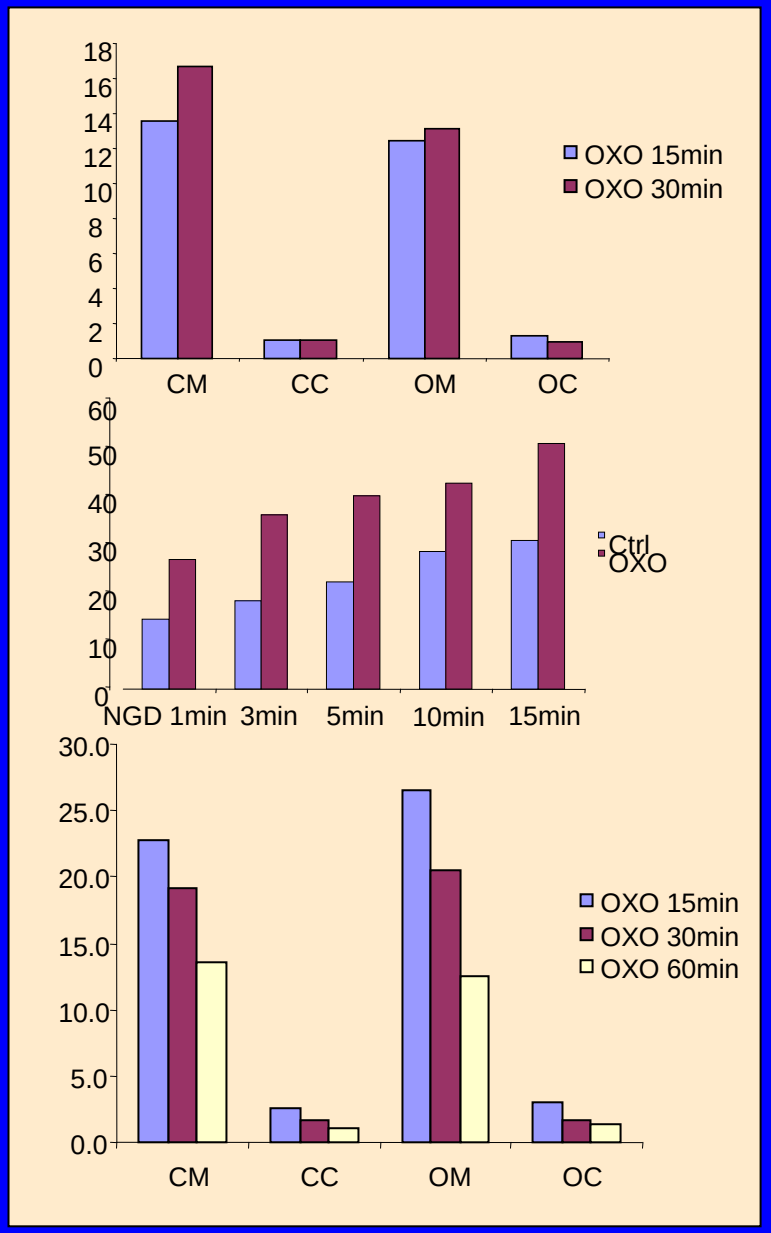


In this chromatogram, there are five types of molecules. The molecules at the peak labeled “A” are probably the smallest molecules, because they took the shortest time amount of time to go through the HPLC. The molecules at the peak labeled “E” are probably the largest.

The amount of time it takes for a molecule to run through the HPLC is called its retention time. These peaks are differentiated by retention time along the X-axis and standards are run to establish an expected retention time for each molecule.

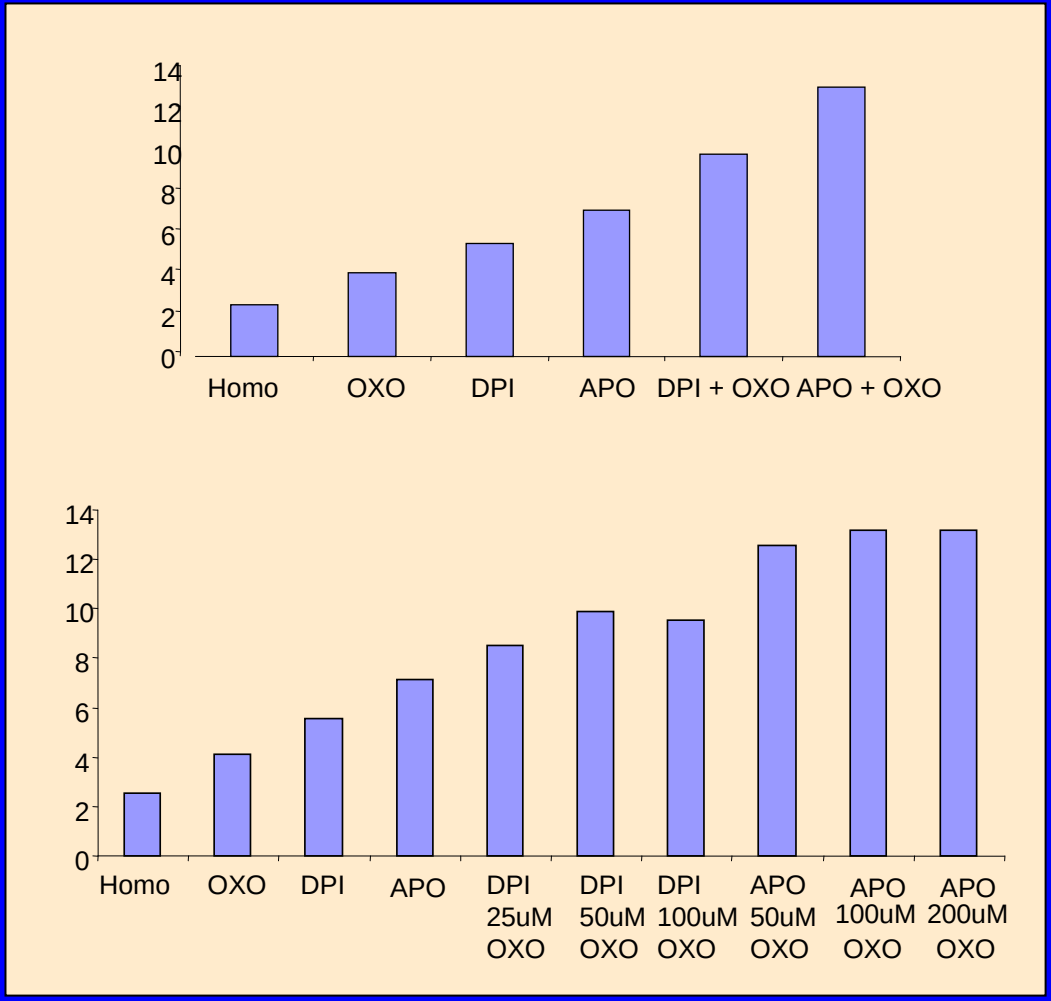
cGDPR Production after Incubation

- The first part of the protocol was finding optimal incubation times for the agonists being added and whether to use microsome or cytosol
- Microsome was found to be much more efficient
- Optimal incubation times for NGD (1uM) and OXO(50uM) were found to be 5mins and 15min, respectively.
- From previous protocol, 15mins was determined to be the optimal incubation time for DPI(50uM) and Apo(100uM).



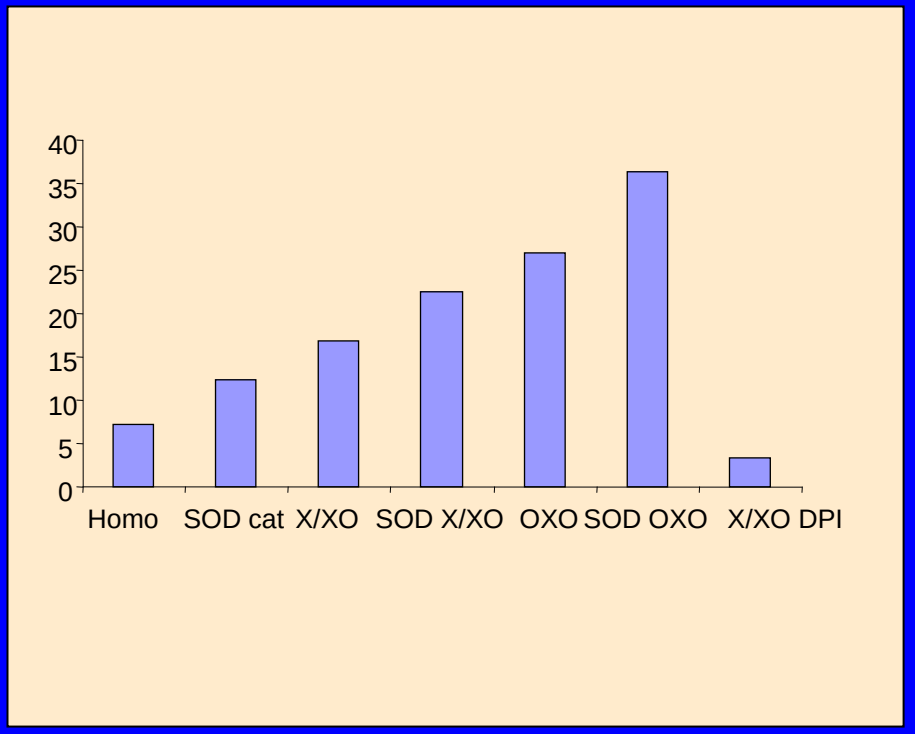
cGDPR Production after Incubation

- After a total of 5 experiments with 2 samples each, the inhibitors consistently raised the expression of cGDPR.
- Even with differing concentrations DPI and Apo the data remained consistent



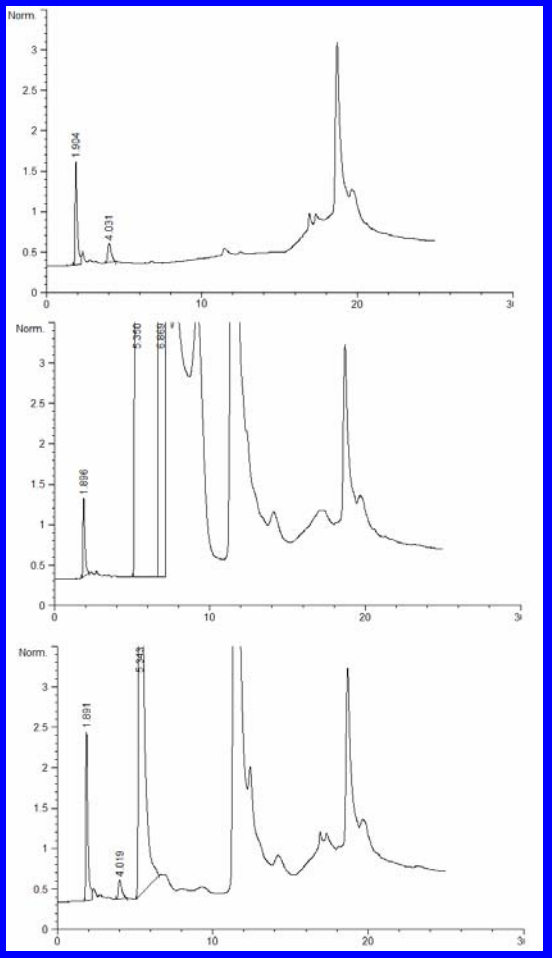
cGDPR Production after Incubation

- BCA microsome was then incubated with SOD and X/XO
- While there were SOD problems, the results found with X/XO were very interesting



cGDPR Production after Incubation

- Xanthine/xanthine oxidase was causing significant extra peaks
- Incubated alone, xanthine did not do much
- X/O however created large peaks
- When combined together, some of these peaks were inhibited, which would be interesting to investigate further



Conclusions

- Oxotremorine was found to increase the expression of cGDPR when homogenate was incubated at 37^0C for 15mins.
- DPI and Apo were not found to inhibit the expression of cGDPR when combined with OXO. There may be different pathways involved or possibly toxicity effects.
- The results from X/XO yielded results that may prove interesting in further studies

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