

Discovery of Nitric Oxide and its Role in Vascular Disorders and Drug Development

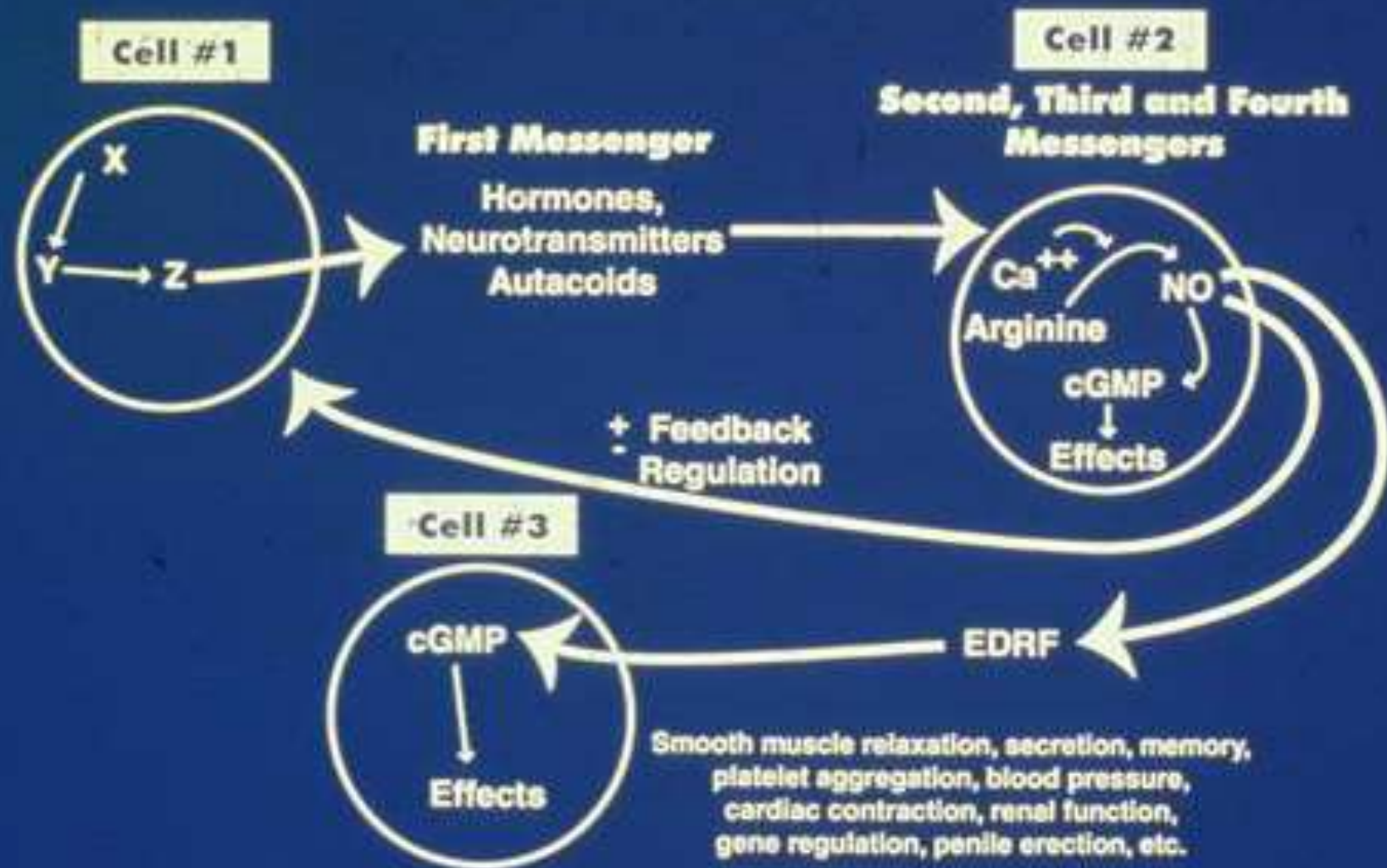
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**THE UNIVERSITY
of TEXAS**
HEALTH SCIENCE CENTER
at HOUSTON

The Nitric Oxide and Cyclic GMP Cell Signaling Pathway (Cells talking to each other)



Some sources of Nitric Oxide and Some of its Effects

Some Sources Of Nitric Oxide

Natural substance
In the body

Drugs

Car Exhaust

Cigarette Smoke

Fires

Other combustible
materials



Some effects

Ozone depletion and earth warming

Free radical interactions

Numerous biological effects
(both beneficial & deleterious)

Blood pressure

Memory

Stroke

Diabetes

Esophagitis

Anti-bacterial

Platelet aggregation

Arthritis

Inflammation

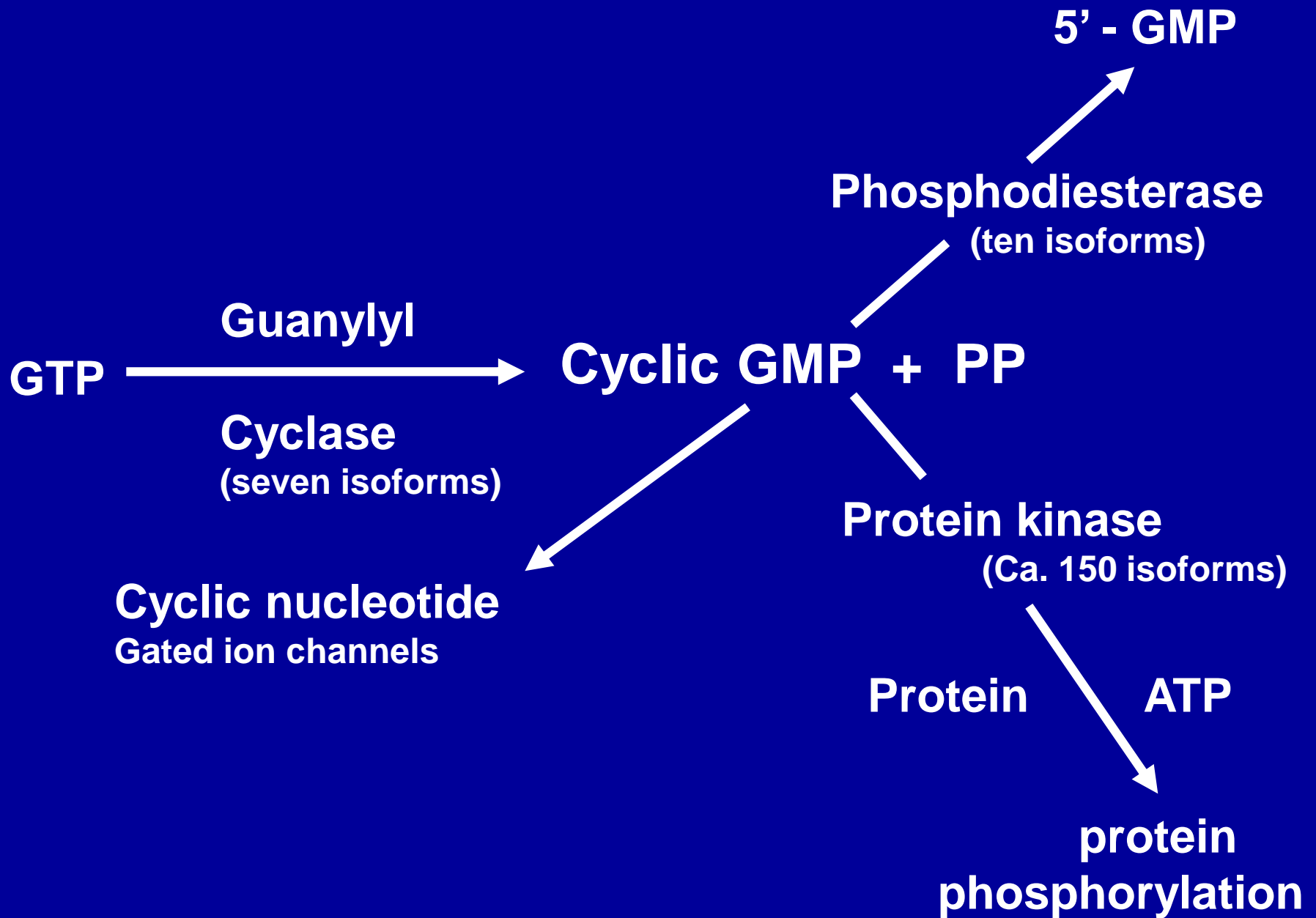
Angina

Heart contraction

Penile erection

Gene regulation

Etc.



**How do hormones, neurotransmitters, etc. regulate guanylyl cyclase and increase cyclic GMP synthesis?
What is the molecular mechanism?**

What are some biological effects of cyclic GMP?

Guanylyl cyclase activity in cytosolic and particulate fractions of homogenates with different properties

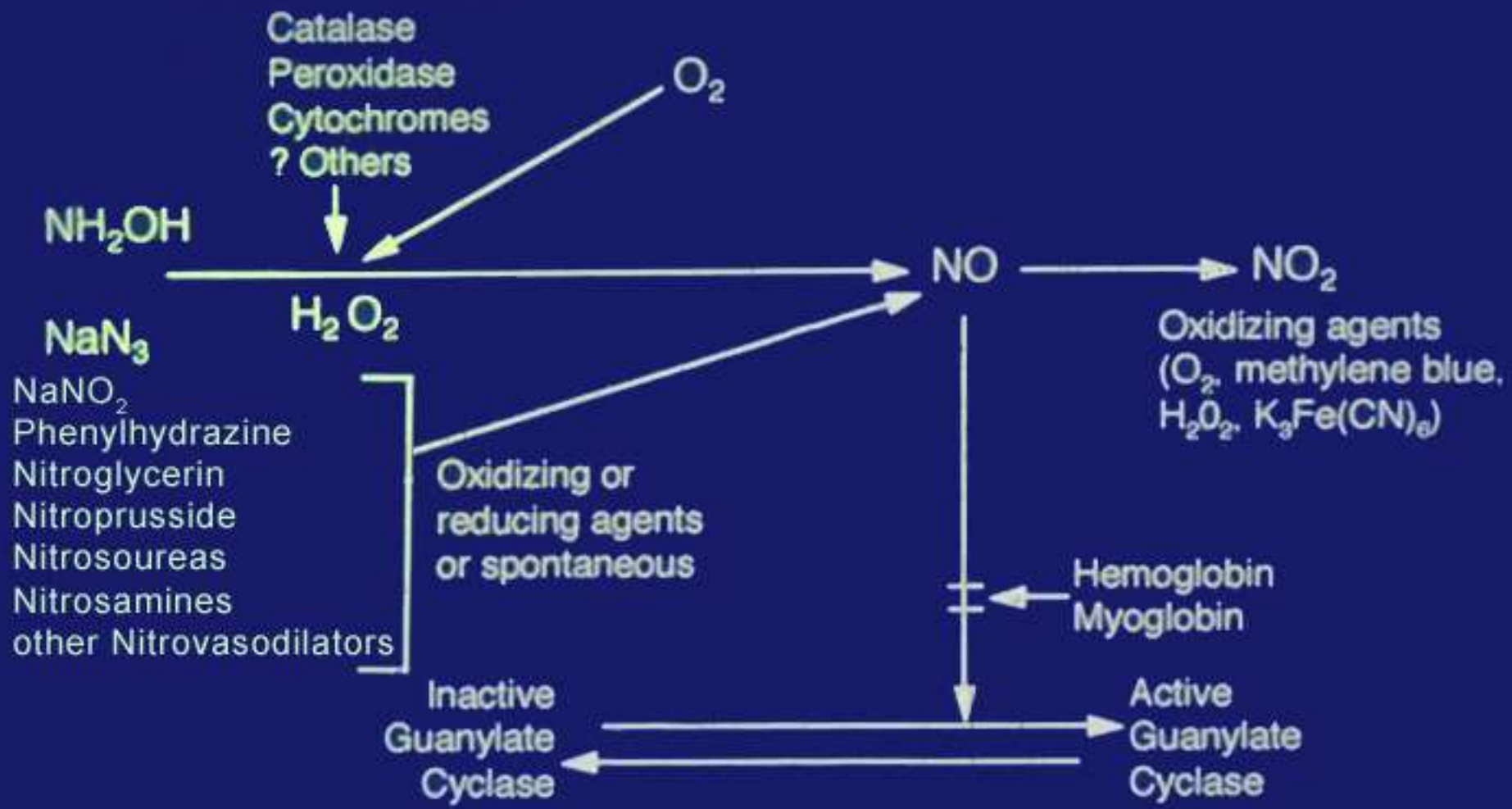
	<u>Soluble</u>	<u>particulate</u>
Ca ⁺⁺	stimulates	inhibits
ATP, IC ₅₀	0.4mM	>1mM
GTP, Hill coef.	1.0	1.74
Detergent	stimulates 50-100%	stimulates 300%

From Kimura & Murad, Metabolism 24, 439, 1975

Effects of NaN₃ on soluble guanylyl cyclase from rat liver, heart and cerebral cortex

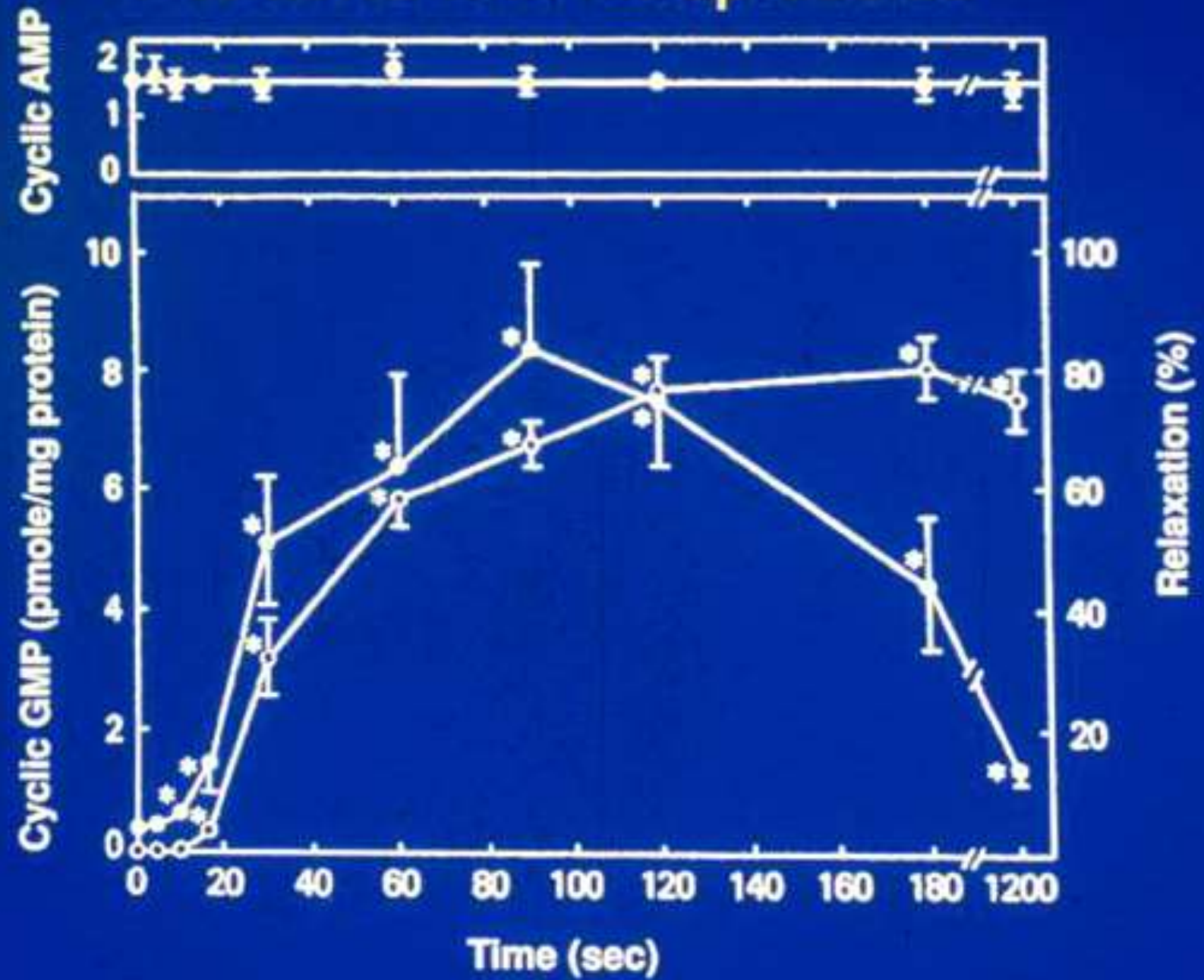
Enzyme	Cyclic GMP		Ratio +N ₃ /-N ₃
	-NaN ₃	+NaN ₃	
Liver	38.8	595.4	15.3
Heart	23.0	23.2	1.0
Cerebral Cortex	46.0	42.0	0.9
Liver + heart	27.3	23.1	0.8
Liver +cerebral cortex	23.0	899.0	39.1

from Kimura, Mittal & Murad, J. Biol. Chem. 250, 8016, 1975



Murphy et al, Adv. Cyclic Nucleotide Res. 9, 145, 1978; Adv. in Pharmacol. & Therap. 3, 123, 1978.

Rat aorta with nitroprusside

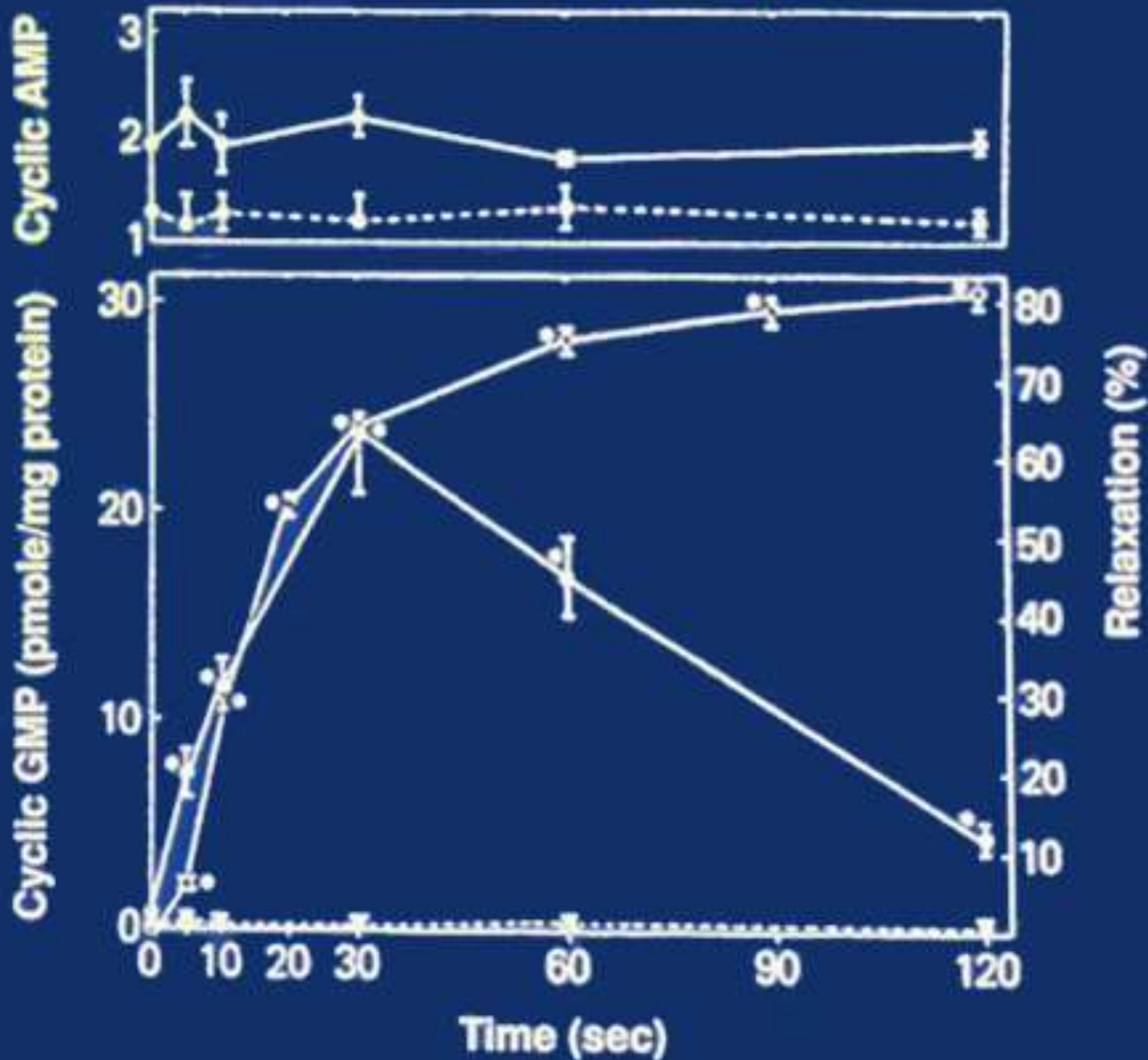


Guanylate Cyclase: Activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin.

Murad, et al, Adv. Cyclic Nucleotide Res. 9, 145-58, 1978

“....the scheme proposed may prove to be the mechanism of action this class of agents (nitrovasodilators).....Perhaps the effects of various hormones and other agents on cyclic GMP accumulation are attributable to altered Redox and nitric oxide formation from endogenous precursors.”

Rat aorta with acetylcholine



Nitrovasodilators



NO



**Endothelial dependent
vasodilators**



EDRF



Guanylyl cyclase



Cyclic GMP



PKG



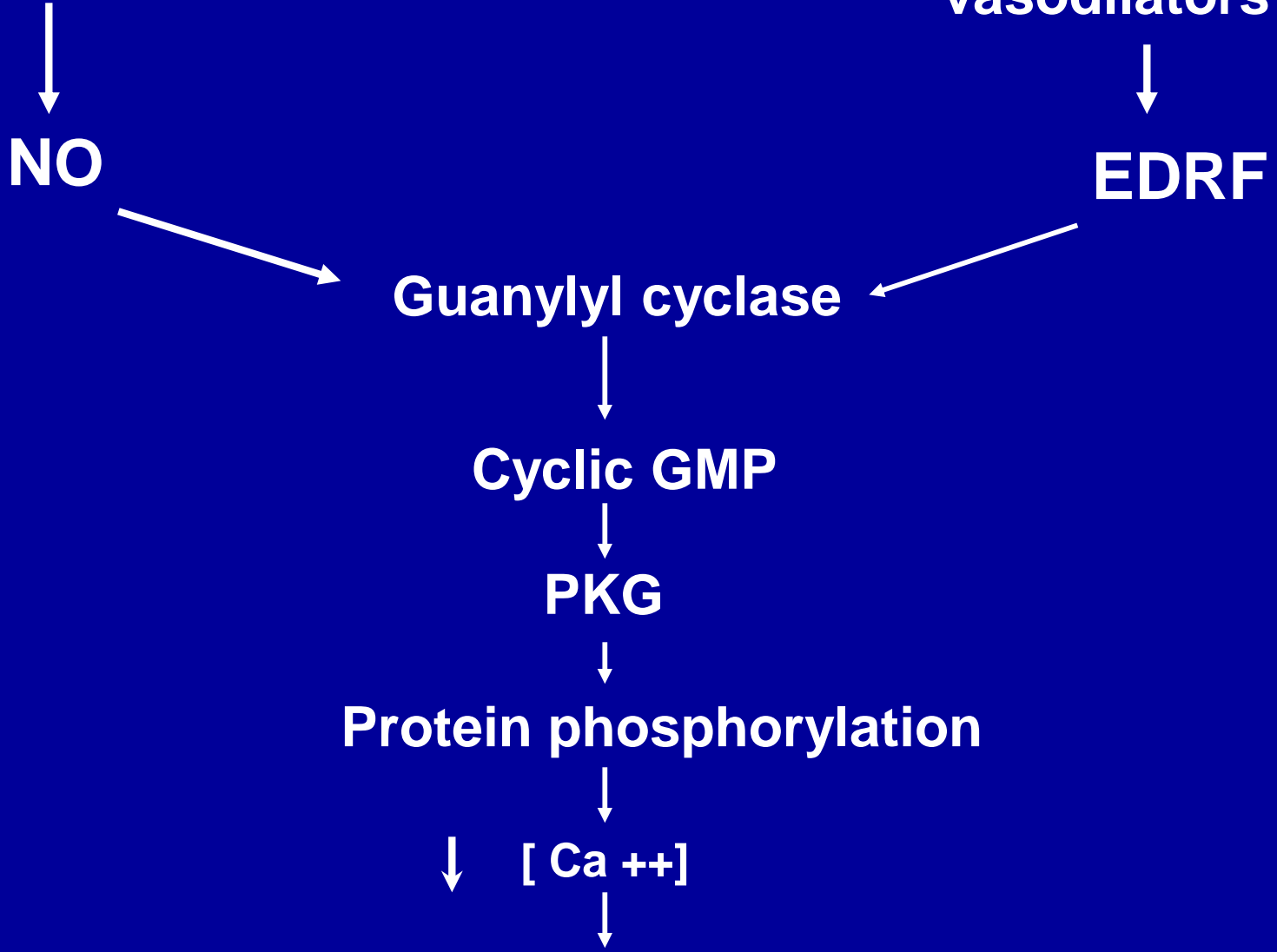
Protein phosphorylation



↓ [Ca ++]



Smooth muscle relaxation

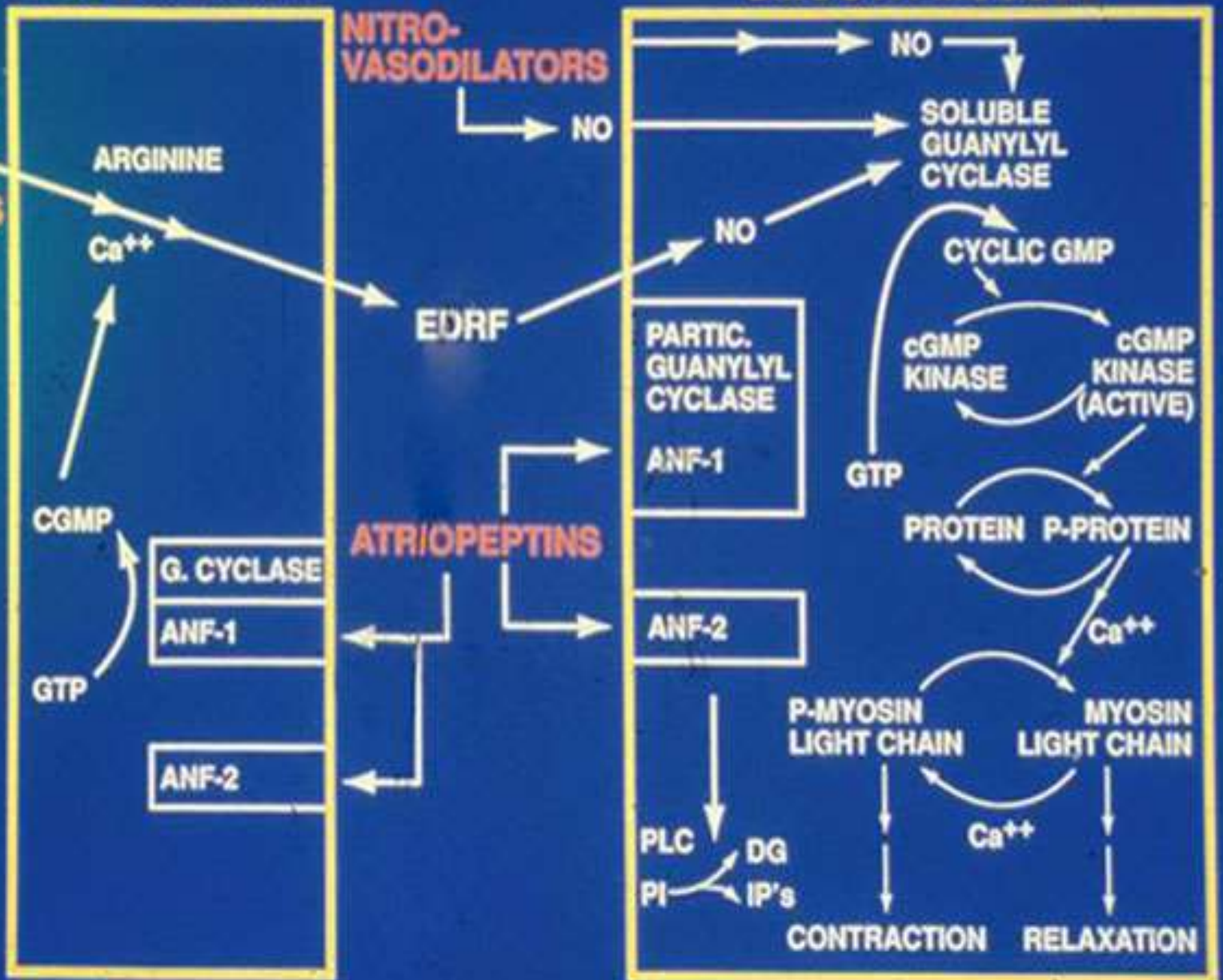


ENDOTHELIUM

SMOOTH MUSCLE

ENDOTHELIUM DEPENDENT VASODILATORS

- ACETYLCHOLINE,
- HISTAMINE,
- THROMBIN,
- ATP,
- ETC.



Deguchi T., Yoshiako M. L-arginine identified as an endogenous activator for soluble guanylate cyclase from neuroblastoma cells. J. Biol. Chem. 257, 10147, 1982.

Hibbs J, Traintor R, Varrin Z. Macrophage cytotoxicity: Role for L-arginine deminase and imino nitrogen oxidation to nitrate. Science 235, 473, 1987.

NOS-1 (155kD)

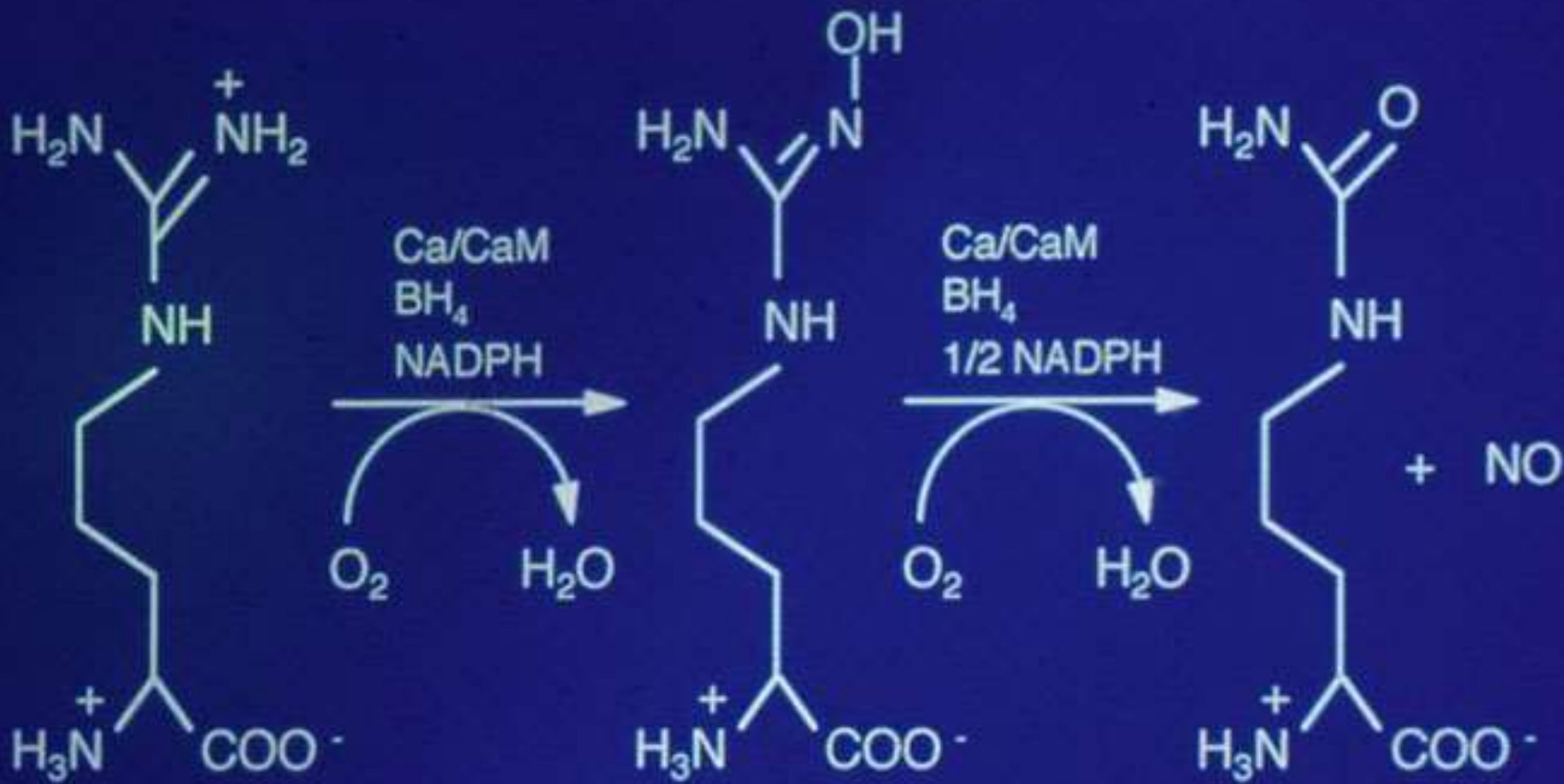
neuronal, brain, Type I-NOS; central and peripheral neurons, NANC neurons, islets, endometrium, skeletal muscle, etc.

NOS-2 (125kD)

inducible, Type II-NOS; macrophage, liver smooth muscle, endothelium, heart, etc; effects of LPS, cytokines, and glucocorticoids

NOS-3 (135kD)

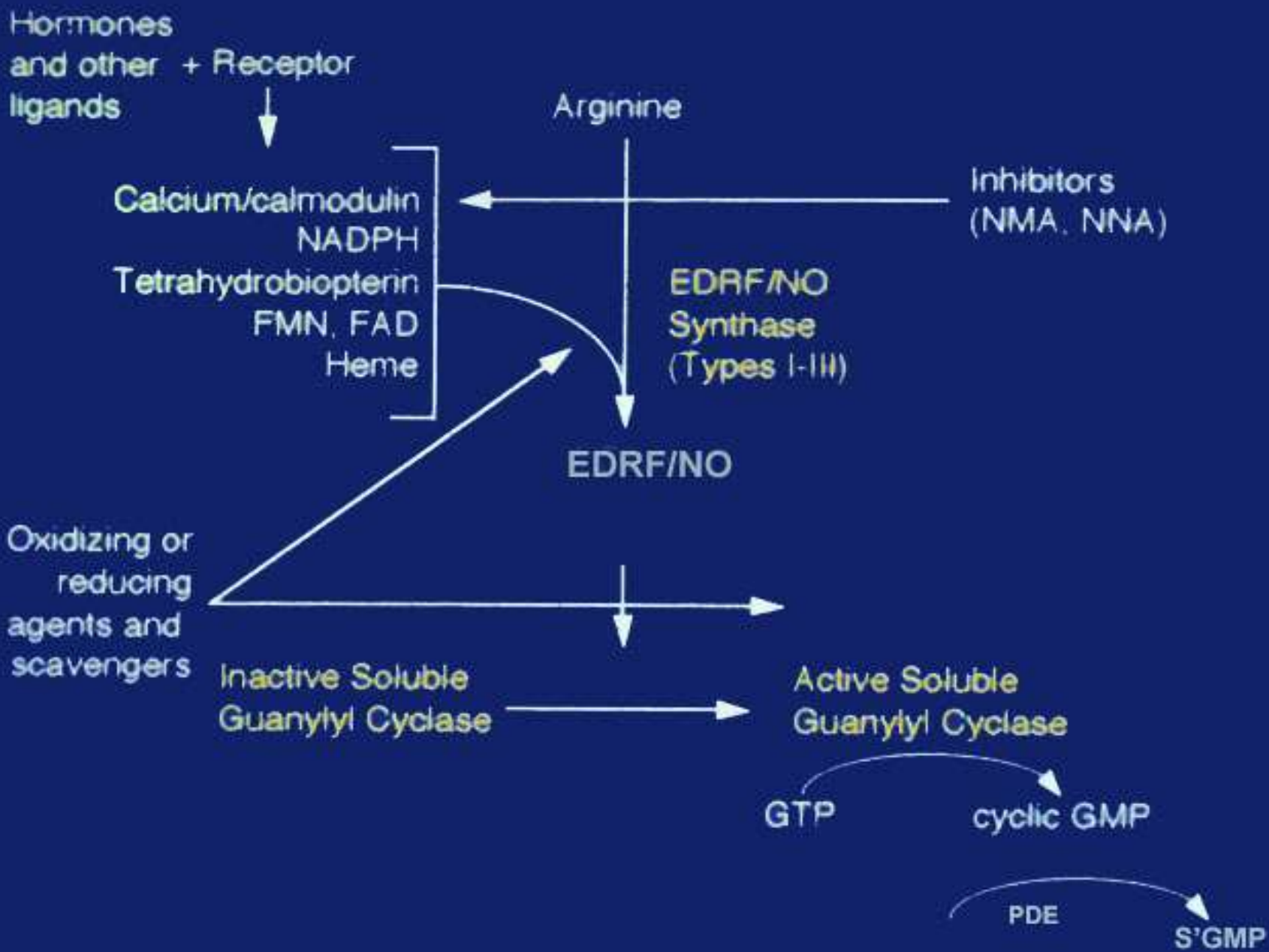
endothelial, Type III-NOS; endothelium, brain, heart, etc; acylation, phosphorylation

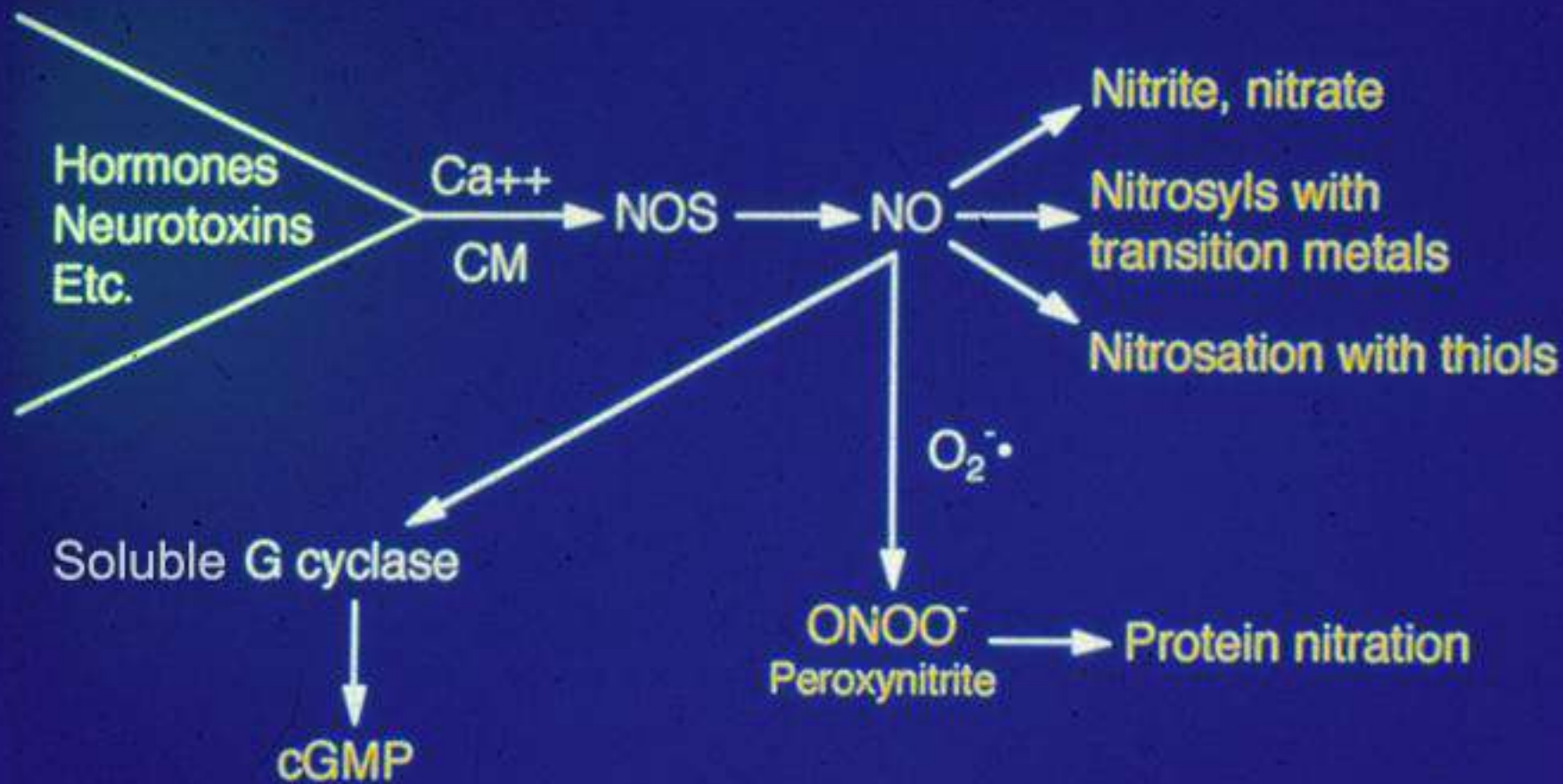


L-arginine

N-hydroxy-L-arginine

L-citrulline





Endothelial Dysfunction

(Hypertension, Diabetes, Atherosclerosis, Tobacco use)

- elevated Asymmetric Dimethyl Arginine (ADMA)
- elevated Reactive Oxygen Species (ROS)
- decreased NOS cofactors
- decreased NOS activity
- decreased NO production
- increased peroxynitrite formation with increased removal of NO

Possible role of L-arginine and antioxidant supplements

Processes and Diseases with NO Participation

- **Neurotransmission, memory, stroke**
- **Glaucoma and neural degeneration**
- **Vasodilation, blood pressure, blood flow**
- **Pulmonary hypertension**
- **Penile erection**
- **Angiogenesis, wound healing**
- **Atherogenesis**
- **Inflammation, arthritis, nephritis, colitis, etc**
- **Cytotoxicity tissues, pathogens, tumors**
- **Asthma**

Processes and Diseases with NO Participation

- **Tissue transplantation**
- **Septic shock, dialytic hypotension**
- **Platelet aggregation**
- **Gastrointestinal motility**
- **Hormone secretion**
- **Gene regulation**
- **Hemoglobin delivery of oxygen**
- **Stem cell proliferation and differentiation**
- **Bronchodilation**