

Department of Pharmacology
Qualifying Examination (Part I)
December 13 & 14, 2005

Please remember that this is a closed-book examination. You must be prepared to answer 4 of the 7 questions. Although not necessary, you may prepare written answers, overhead figures, or any type of materials that you think might be useful in the presentation of your answers. You may bring such preparation materials with you to the examination. The oral examination itself will not extend beyond two hours.

If you have any questions regarding the examination, please contact Joey Barnett at:

936-1722 (w)
385-4396 (h)
300-9569 (c)

BEST WISHES FOR YOUR SUCCESSFUL COMPLETION OF THE EXAMINATION!

You have identified a novel protein, Corban, that is expressed only in cardiac myocytes. The protein is predicted to pass through the membrane a single time and contain a large extracellular domain. You generate a knockout mouse and find that *Corban* null mice have hypertension. The blood pressure of wildtype and null animals in both normal and high salt is depicted in Figure 1. You perform a comprehensive survey of the factors known to regulate blood pressure and obtain the data shown in Figure 2 for Atrial Natriuretic Peptide (ANP).

Figure 1

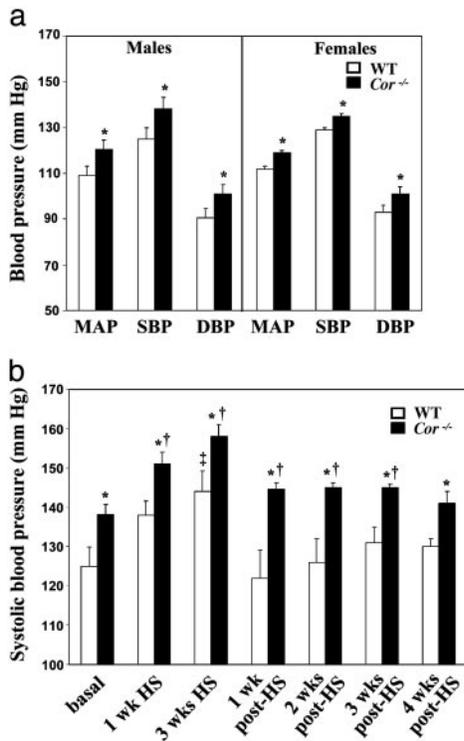


Figure 1. Hypertension in *Cor*^{-/-} mice. (a) When fed with a standard salt diet, *Cor*^{-/-} mice (filled bars) demonstrated elevated blood pressure compared with WT mice (open bars). The data represent averages over 12 hour light/dark cycles. n=5 for each genotype and sex. *, P<0.01 vs. WT in the same group, by Student's t test. MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure. (b) SBP increased further in *Cor*^{-/-} mice with 3 weeks of high dietary salt (HS). SBP increased in WT mice but never reached the levels in *Cor*^{-/-} mice treated similarly. After reverting to standard diet (post-HS), SBP normalized in WT mice within 1 week but remained higher in *Cor*^{-/-} mice even after 3 weeks of normal diet. n=5 for each genotype. *, P<0.05 when comparing *Cor*^{-/-} mice to similarly fed WT mice at the same time point; †, P<0.05 when comparing *Cor*^{-/-} mice to *Cor*^{-/-} mice fed with standard diet; ‡, P<0.05 when comparing with WT mice fed with standard diet. Statistical analysis was performed by using ANOVA and least square difference.

Figure 2

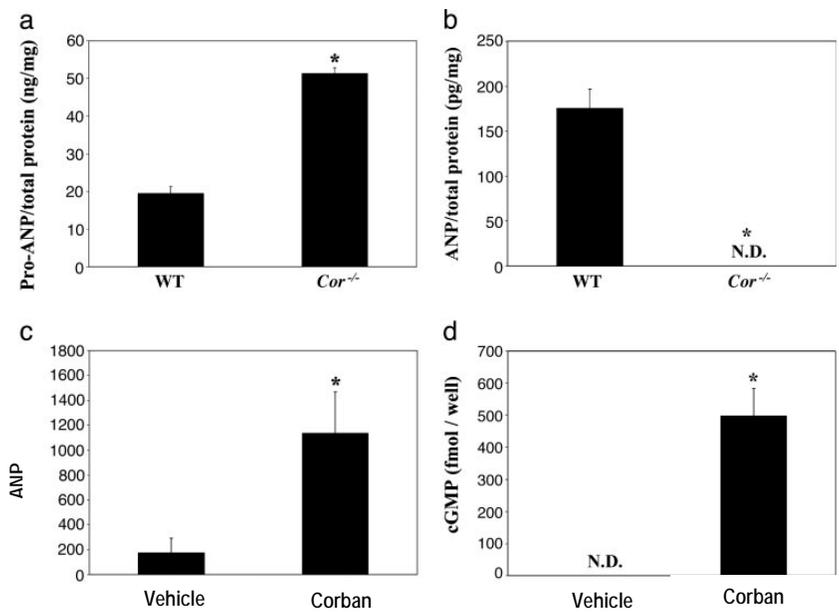


Figure 2. Pro-ANP and ANP levels in *Cor*^{-/-} mice. Pro-ANP (a) and ANP (b) levels in atrial extracts from WT and *Cor*^{-/-} mice were determined by HPLC, Western, and ELISA analyses. (c) i.v. injection of the soluble extracellular domain of Corban (EKsolCorban) in *Cor*^{-/-} mice results in a significant transient increase in total plasma pro-ANP and ANP antigen levels as compared with injection with vehicle. (d) A concomitant increase in plasma ANP activity was observed, as measured by a cell-based cGMP-stimulating activity assay. *, P<0.01 vs. WT or vehicle-treated controls, by Student's t-test. ND, not detectable.

Interpret the data in Figure 2. Outline a hypothesis as to the function of Corban and a series of in vitro and in vivo experiments to test this hypothesis.

Your laboratory has recently generated strains of mutant mice in which the expression of either Gq or G₁₁ have been selectively ablated using homologous recombination in embryonic stem cells. Mice homozygous for a null allele of the gene encoding G₁₁, *gna11*, did not show any phenotypic abnormalities. These defects were relatively mild when one considers the number of potential physiologic systems affected by a loss of G₁₁ signaling, and probably reflects the high functional redundancy of Gq and G₁₁, which share 88% amino acid sequence identity. Indeed, mice lacking both Gq and G₁₁ die at day 10.5 of embryogenesis. To circumvent this embryonic lethality, you took advantage of the Cre/loxP system to generate a mouse line which allows conditional, tissue-specific inactivation of the Gq gene, *gnaq*, in constitutively G₁₁-deficient mice. These mutant animals, lacking G₁₁ expression in all tissues and lacking Gq expression solely in neurons, demonstrated decreased rates of growth (Fig. 1A,B), somatotroph hypoplasia (1C) and decreased growth hormone levels in isolated plasma samples (Fig. 1D).

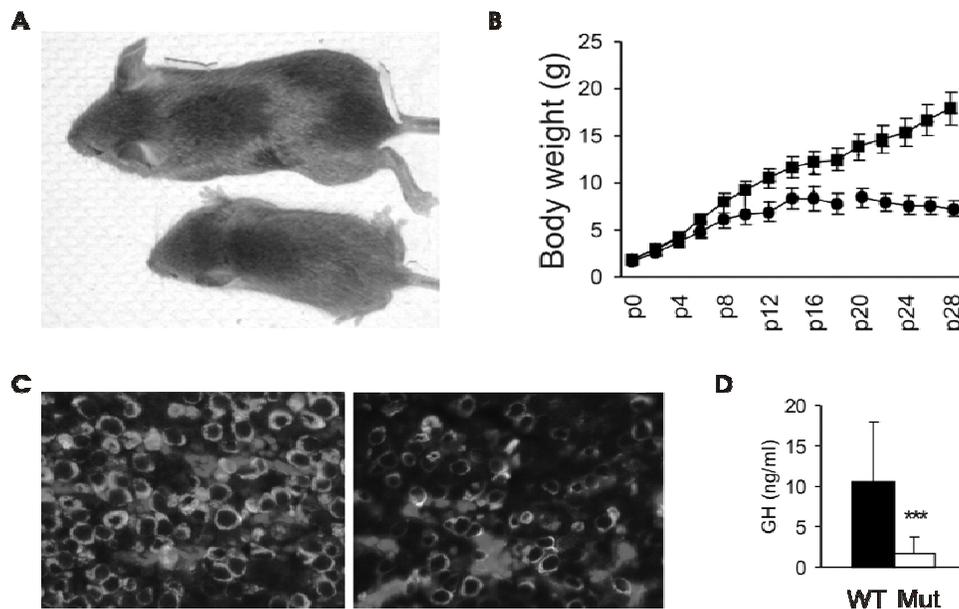


Figure 1. Phenotypic comparisons of wild-type mice and mutant animals lacking Gq in neuronally-derived cells and G11 in all tissues. A) Reduced body length of a mutant mouse (bottom) compared to a control littermate (top) at postnatal day 20. **B)** Postnatal growth rate analysis of wild-type (■) and mutant (●) mice (mean ± SEM; n = 8). **C)** Immunofluorescence staining for GH in the anterior pituitary of 15-day-old control (left) and mutant (right) mice. **D)** Plasma levels of GH in wild-type (WT) and mutant (Mut) mice at postnatal day 15 (mean ± SEM; n = 6).

- 1) Given your knowledge of the regulation and biosynthesis of growth hormone, develop a hypothesis for the specific component(s) of the GH pathway that could be affected by this loss of neuronal Gq signaling. Be prepared to explain the interrelationship between the various components of the GH pathway and how your proposed defect explains the observed phenotypic alterations.
- 2) Devise an experimental strategy to confirm your hypothesis.
- 3) Provide a strategy for cloning the Gq-coupled receptor that is affecting the GH pathway in these mutant animals.

In the kidney, renal perfusion pressure affects the synthesis and release of renin from the juxtaglomerular cells of the kidney, with an increase of blood pressure inhibiting and a decline of blood pressure stimulating the renin system. This homeostatic feedback circuit is called the "renal baroreceptor mechanism" is depicted in Figure 1.

Figure 1

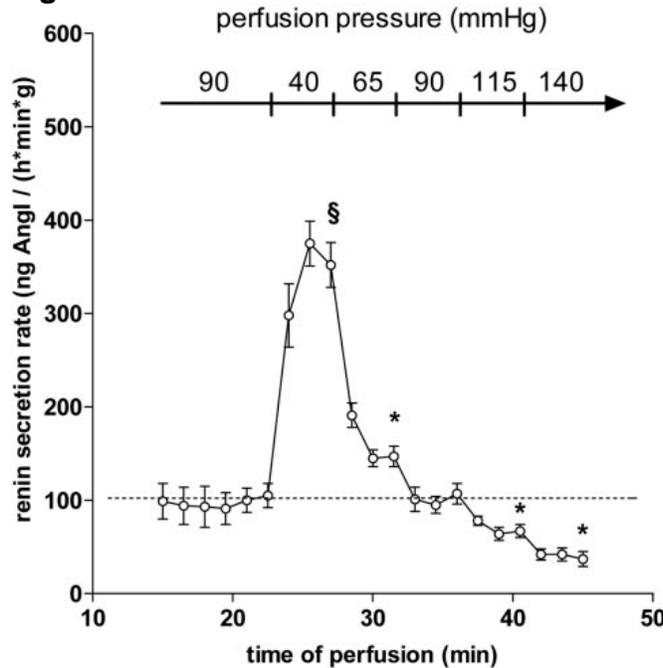


Figure 1. Effects of renal perfusion pressure on renin secretion rates of isolated perfused mouse kidneys. After a control period (perfusion pressure 90 mm Hg) the perfusion pressure of isolated perfused kidneys from C57BL/6 mice (n=5) was switched to 40, 65, 90, 115, and 140 mm Hg in a stepwise fashion. * $P < 0.05$ vs 90 mm Hg; § $P < 0.001$ vs 80 mm Hg.

Figure 2

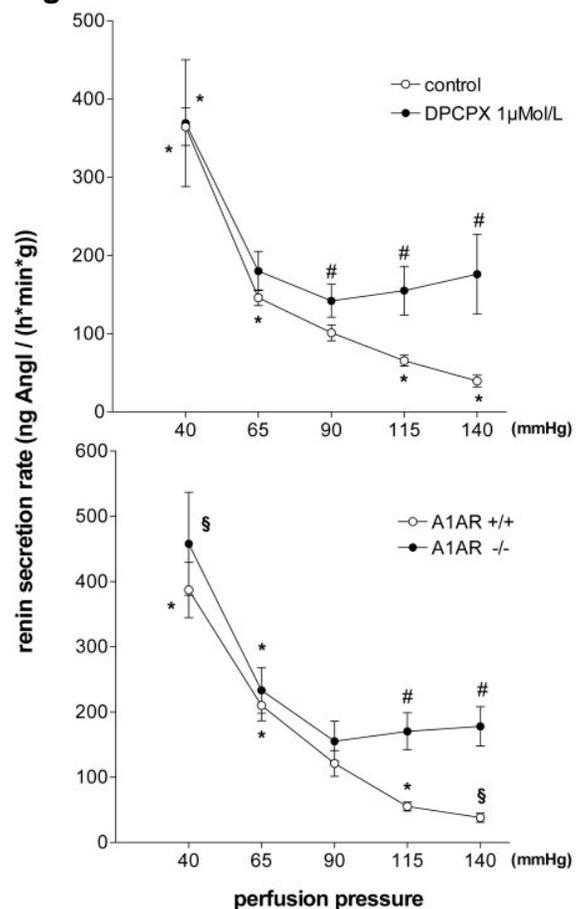


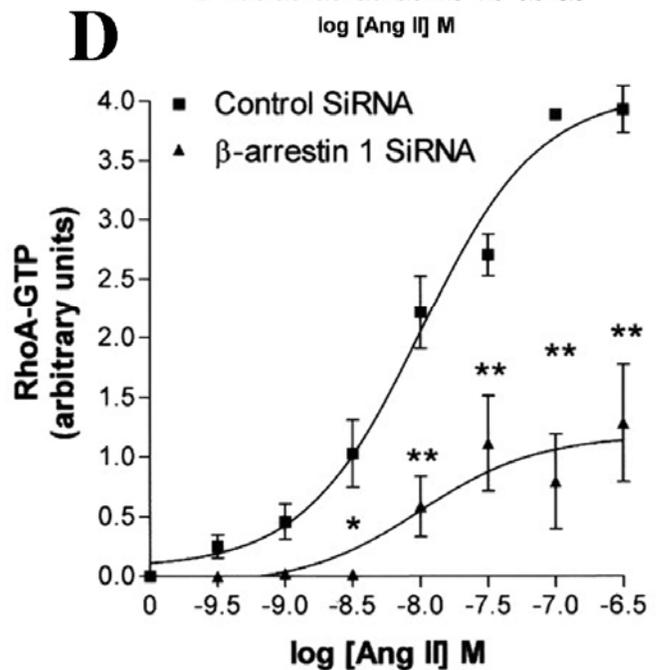
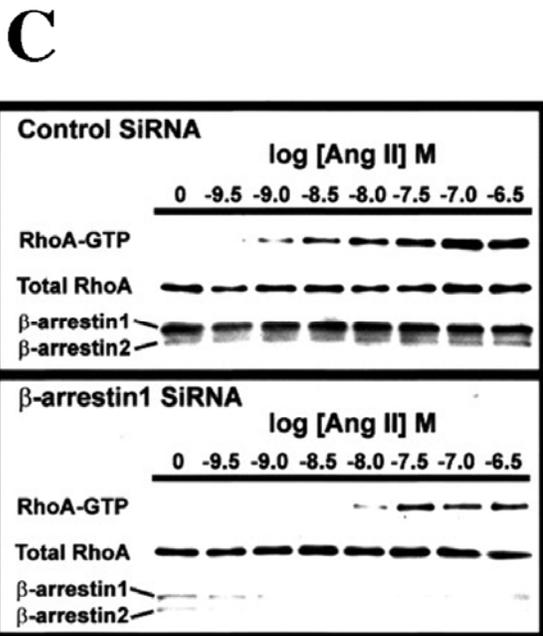
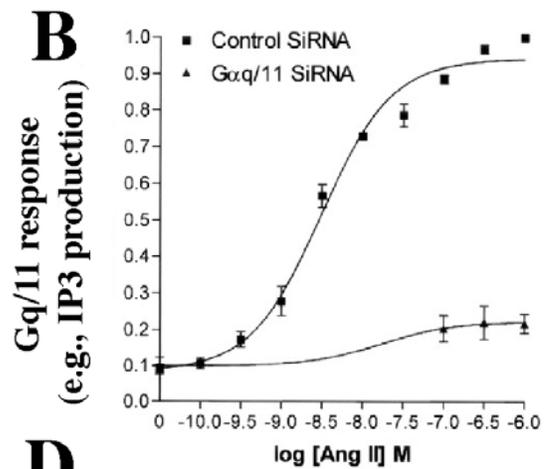
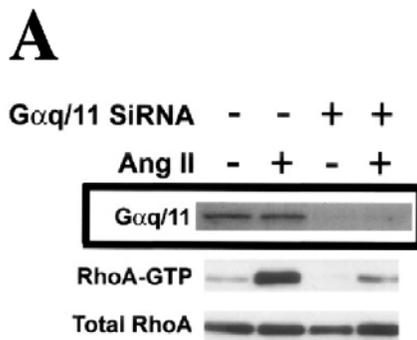
Figure 2. Role A1 adenosine receptors (A1ARs) in the pressuredependent regulation of renin secretion rates of isolated perfused kidneys. Perfusion pressure was changed in a stepwise manner from 40 mm Hg to 140 mm Hg in isolated kidneys from C57Bl/6 mice under concomitant application of DPCPX 1 $\mu\text{mol/L}$ (upper panel, black circles, n=5) or in isolated kidneys from A1 adenosine receptor knockout mice (A1AR^{-/-}, lower panel, black circles, n=7). Vehicle-treated kidneys (upper panel, white circles, n=5) or kidneys from A1AR wild-type mice (lower panel, white circles, n=7) served as controls, respectively. * $P < 0.05$ vs 90 mm Hg; § $P < 0.001$ vs 90 mm Hg; # $P < 0.05$ vs control/A1AR^{+/+} at same perfusion pressure.

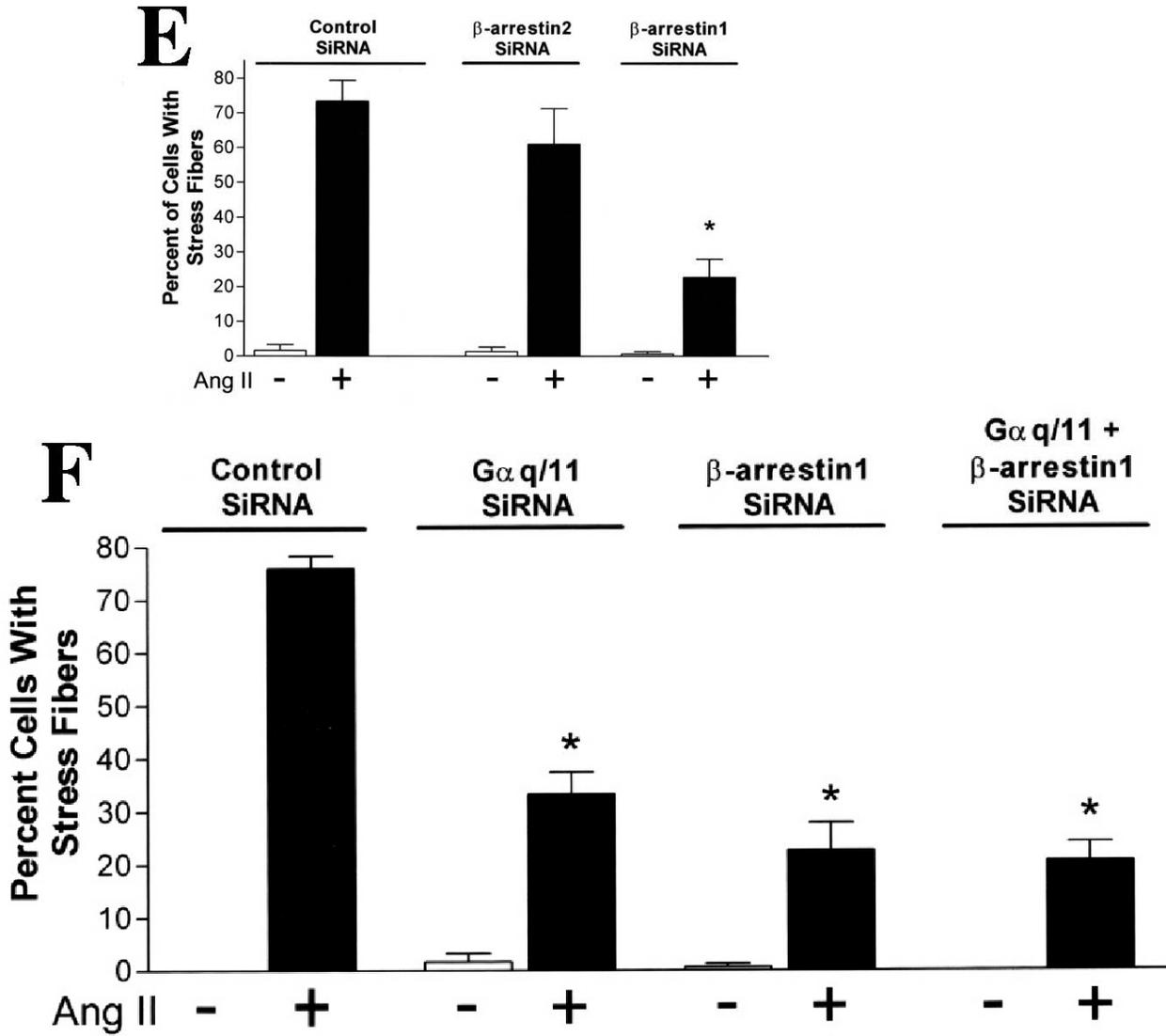
Examination of the effect of perfusion pressure on renin secretion rate in isolated, perfused kidneys harvested from wild type animals, wild type animals treated with DPCPX (an adenosine type I receptor antagonist) or wild type animals and A1 adenosine receptor (A1AR) null animals is depicted in Figure 2.

How would this mechanism regulate blood pressure in the intact animal? Explain the data in Figure 2 and propose a hypothesis as to the role of A1AR in the "renal baroreceptor mechanism". Outline both in vitro and in vivo experiments to test this hypothesis.

You are studying angiotensin II (Ang II)-induced formation of stress fibers. You know that the mammalian cells you are using express angiotensin receptor AT1A (which is a typical GPCR). Stress fiber formation is known to be mediated by a small G protein RhoA.

For the experiments presented below you've used RNAi knockdown of the signaling proteins that may be involved in stress fiber formation. The following figures summarize your data on Ang II-induced signaling, including RhoA activation. Note that RhoA-GTP is the active form of RhoA; β -arrestin1 and β -arrestin2 (a.k.a. arrestin2 and arrestin3) are two non-visual arrestin that share about 70% homology; panels A and C show Western blot data for total and active RhoA, panel C also shows Westerns for β -arrestins 1 and 2 to demonstrate successful knockdown of β -arrestin1, and no change in β -arrestin2 expression, although the latter may be hard to notice).





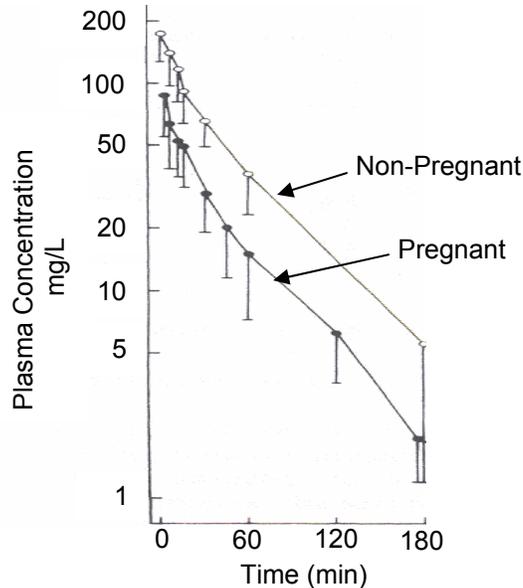
- How would you test whether Ang II acts via angiotensin receptor AT1A:
 - in these cells.
 - in another cell type that does not express endogenous AT1A receptors.
- If you find that AT1A is involved, propose two models that explain your findings.
- Propose alternative experimental approaches to test the role of β -arrestins in Ang II-induced stress fiber formation:
 - in cells that express endogenous AT1A receptor.
 - in cells that do not.
- Propose likely signaling cascade downstream of $G\alpha$ q/11. How would you test the involvement of these signaling proteins in stress fiber formation?

Treatment of PC12 cells with nerve growth factor (NGF) leads to differentiation of the cells with a distinct morphology reminiscent of neurite extension. You are asked to dissect the signaling pathways responsible for NGF-induced differentiation. In general, NGF activates members of the TRK family that belongs to receptor protein tyrosine kinases (RPTK). Activated TRK initiates at least three distinct downstream signaling pathways leading to activation of phospholipase C-gamma (PLC-gamma), MAPK and phosphoinositol-3-kinase (PI3K).

1. What are the molecular mechanisms by which PLC-gamma, MAPK and PI3K are switched on by TRK?
 - a. What happens when PLC-gamma becomes active and what are the downstream events following activation of PLC-gamma?
 - b. What are proteins involved in the MAPK pathway?
 - c. What are substrates of PI3K? What is the outcome following activation of PI3K?

2. Each of the three down-stream pathways singularly or in combinations may be essential for NGF-dependent neurite extension in PC12 cells. Design at least two independent strategies to investigate the contribution of each pathway in ligand-dependent neurite outgrowth.

The pharmacokinetics of the antibiotic drug Piperacillin were assessed in pregnant and non-pregnant women after intravenous bolus administration. The non-pregnant and pregnant subjects did *not* serve as their own controls.



Pharmacokinetic analyses of Piperacillin (mean \pm S.D.) were performed:

Parameter	Non-Pregnant (n=5)	Pregnant (n=8)	p-value ⁺
Dose (mg)	4000	4000	
AUC (mg·min/L)	7407 \pm 805	2666 \pm 375	p<0.01
t _{1/2} (min)	53 \pm 5	47 \pm 10	p>0.05 n.s.
A _{U∞} (mg)	3625 \pm 230	3600 \pm 340	p>0.05 n.s.

⁺ comparison between non-pregnant and pregnant women, Student's t-test

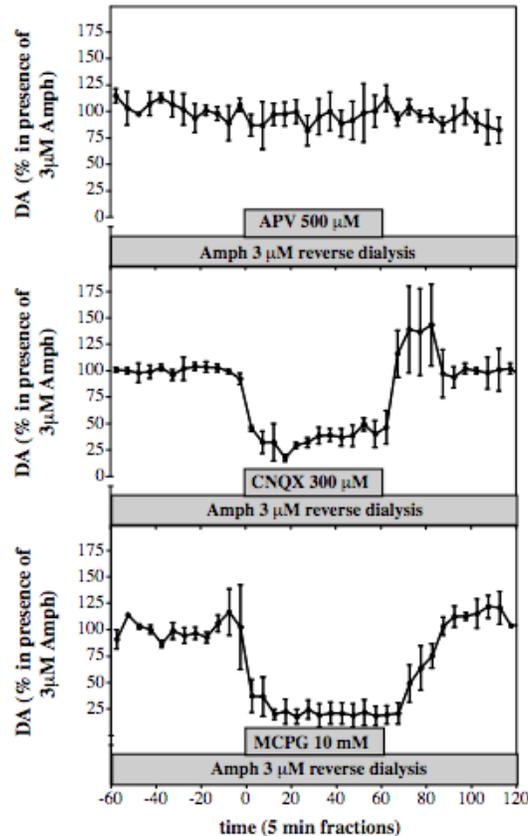
AUC = area under the plasma concentration-time curve from time = 0 to time = ∞

t_{1/2} = half-life

A_{U∞} = amount of unchanged drug excreted in urine from time = 0 to time = ∞

- Q1. How do you interpret the pharmacokinetic differences between pregnant and non-pregnant women?
- Q2. What mechanisms are possibly involved in the differences in plasma drug concentrations between pregnant and non-pregnant women? Support your answer with relevant equations.
- Q3. How would you design in vitro and in vivo experiments to test your mechanistic hypotheses?

In examining the interactions between glutamate and dopamine (DA) signaling in the nucleus accumbens, you generate the following data of the effects of glutamate receptor antagonists on amphetamine (AMPH)-evoked DA release using dialysis techniques in an anesthetized animal in vivo. APV is an NMDAR antagonist, CNQX is an AMPA/kainate receptor antagonist and MCPG is an mGLUR antagonist. AMPH alone causes a doubling of baseline DA efflux, here normalized to 100% at the start of the experiment.



1. Describe the mechanism of AMPH action in triggering DA release and whether in your model release is vesicular or nonvesicular. For the mechanism you propose, describe pharmacological, toxin and lesion approaches you could use to validate how DA release is being triggered by AMPH, release dependence on neural excitability and the cellular origin of DA projections.
2. Reserpine or alpha-methylparatyrosine (AMPT) injections of animals 24 hrs prior to the same experiment results in a loss of AMPH evoked DA release whereas L-DOPA administration leads to an enhancement of AMPH effects. Propose mechanisms by which these agents are impacting AMPH evoked DA release.
3. Describe the structures of glutamate receptors involved in modulation of AMPH evoked DA release and what properties of these receptors might be conducive to tonic support for AMPH evoked DA release. Where do the data provided indicate these receptors are located (pre or postsynaptically?) and suggest a model by which endogenous glutamate signaling in the nucleus accumbens is influencing AMPH-evoked DA release. How might your model be tested?