Department of Pharmacology
Qualifying Examination (Part I)
June 17-19, 2002

***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 material 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) or 385-4396 (h).

GOOD LUCK!

Question #1

Your research team at Paythroughthenose Pharmaceuticals has identified a cDNA expressed exclusively in cardiac myocytes that is predicted to encode a transmembrane protein that you believe to be a receptor. You quickly clone the gene and generate a genetically modified mouse in which expression is ablated (i.e., knockout) as well as a transgenic animal that overexpresses the protein only in cardiac myocytes. Both sets of animals are viable and are interbred to yield an animal that is null at the targeted locus and that expresses the transgene only in the heart. A close examination of the hearts are performed. Comparisons of the wildtype (WT), transgenic (TG), null, and null+TG result in the following data.

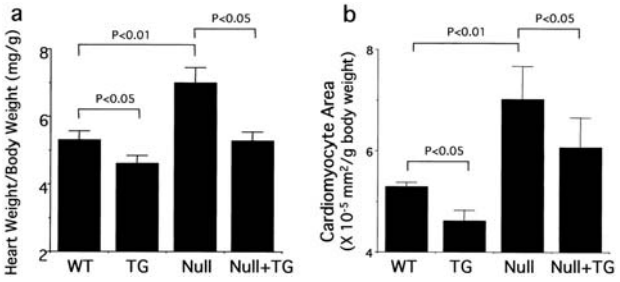


Figure 1 Comparison of (a) heart weight to body weight ratio and (b) area of individual myocytes corrected for body weight between experimental groups.

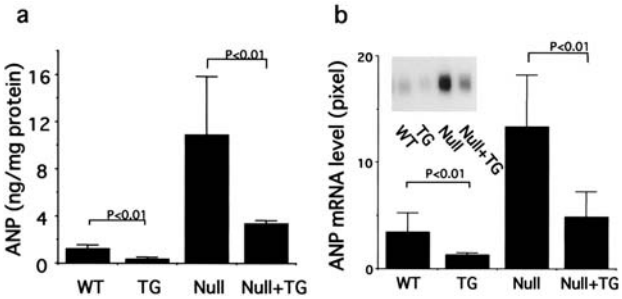


Figure 2 Expression of Atrial Naturietic Peptide (ANP) (a marker of heart failure) (a) protein (b) mRNA in each experimental group.

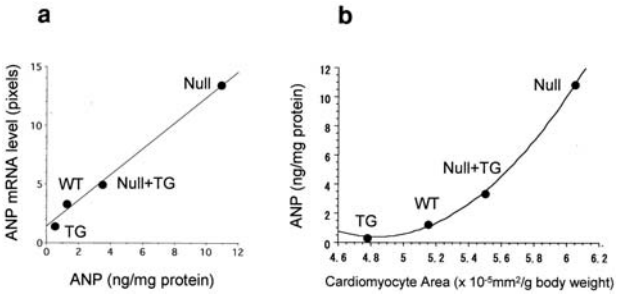


Figure 3 Relationship of (a) ANP mRNA and protein expression and (b) ANP expression and cardiac myocyte area to each experimental group.

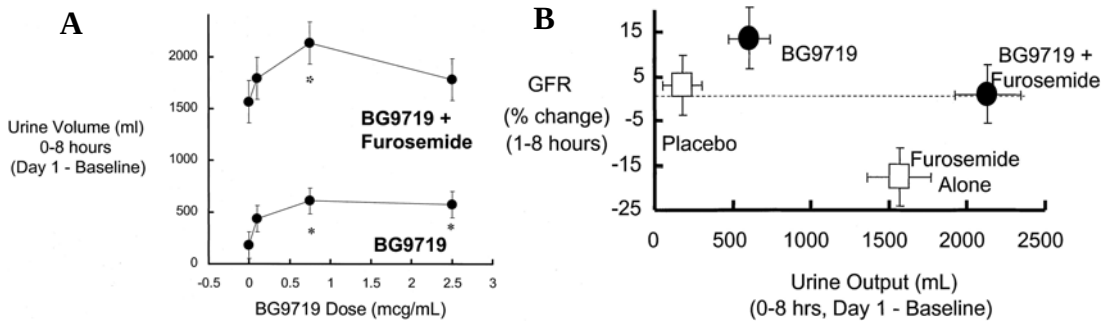
In data not shown, an *in vitro* model using purified mouse myocytes in culture (harvested from WT, TG, null, and TG+null animals) produced similar results for both ANP production and myocyte area.

Describe the data and outline a hypothesis concerning the role of this putative receptor.

Design an experimental strategy to identify the putative ligand for this receptor.

Question #2

Chronic congestive heart failure (CHF) is often associated with impaired renal function. Several of the drug treatment regimens for CHF including furosemide, digitalis and angiotensin converting enzyme inhibitors. A recent study examined the effects of BG9719, an investigational A1 adenosine receptor antagonist, on renal function during treatment of CHF with a single bolus IV injection of furosemide. The following data were obtained in CHF patients. No changes in blood pressure or heart rate were observed during the entire study.

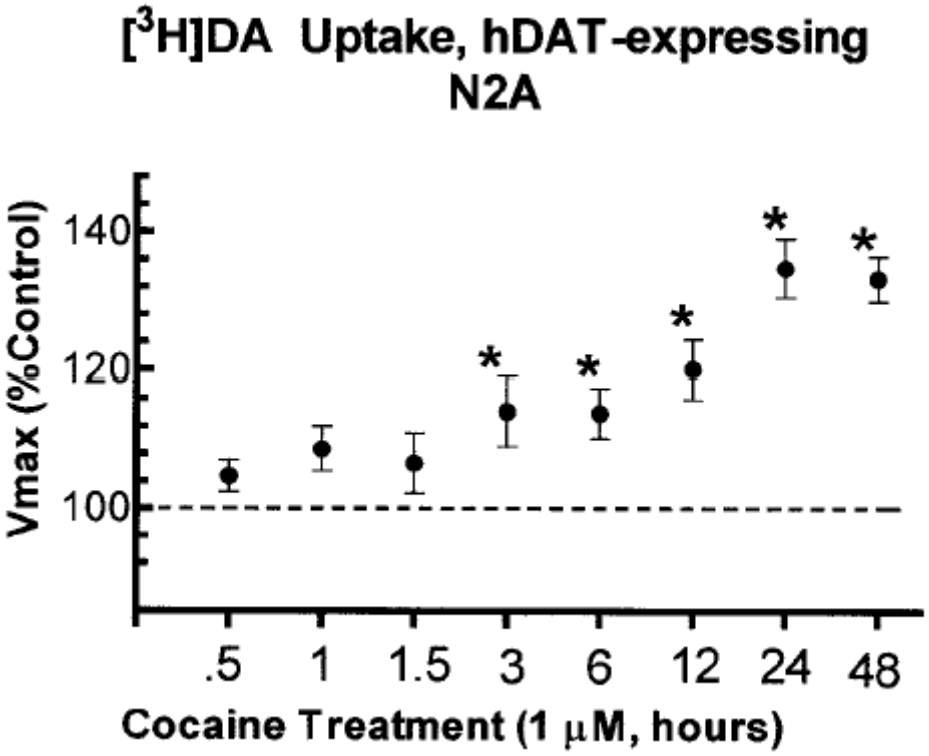


Questions:

1. Interpret the results depicted in the two graphs.
2. Propose a mechanism to explain dose-dependent effects of BG9719 on furosemide induced changes in urine output. How would you test your hypothesis?
3. Predict the effects of an angiotensin II receptor blocking drug on GFR levels in patients with CHF. Explain and defend your answer.

Question #3

Shown below is the uptake of ^{3}H-dopamine in primary cultures of striatal neurons that express the dopamine transporter. Cells were incubated with 1 μM cocaine for the times indicated prior to the measurement of ^{3}H-dopamine uptake. The asterisks (*) indicate that these values are significantly different from striatal neurons incubated with vehicle alone. Propose two possible explanations for the increase in maximum velocity (Vmax) and design experiments to test these hypotheses.



Question #4

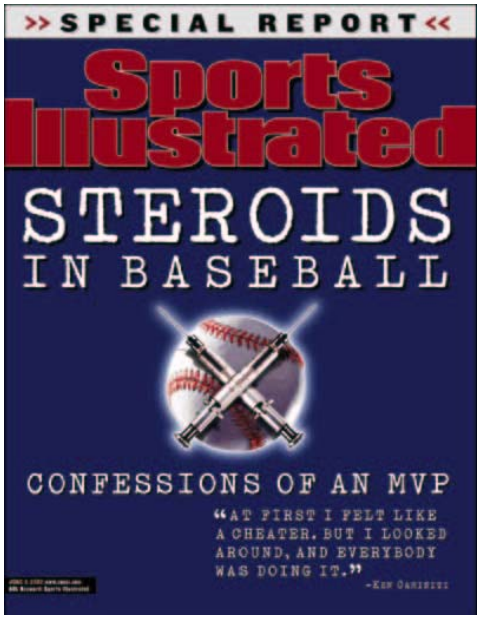
A cDNA encoding a novel receptor protein tyrosine kinase (RPTK), nPTK, was isolated and expression was noted to be upregulated in renal carcinoma in mice. You are asked to design experiments to investigate:

(1) the signal transduction process mediated by nPTK, and

(2) the involvement of nPTK in carcinogenesis in whole animals and tissue culture.

Question #5

In the most recent issue of *Sports Illustrated*, former major-leaguer Ken Caminiti detailed his steroid use while winning the National League Most Valuable Player award in 1996. He estimated that at least half of the players in baseball use some sort of performance enhancing drug. In 1998, while attempting to beat Roger Maris' record of 61 home runs in a season, baseball player Mark McGwire revealed that he routinely took androstenedione to improve his batting performance. Due to pressure from both the media and baseball fans, Bud Selig, the commissioner of Major League Baseball, develops a strategy by which to stop androstenedione abuse by developing an androstenedionespecific antagonist that could be administered to all of the players in their drinking water. To generate such an antagonist, Bud Selig calls upon you to evaluate a series of compounds that could serve such a function. Testing one of these compounds (HR-RBI) provides the competition binding data provided below:



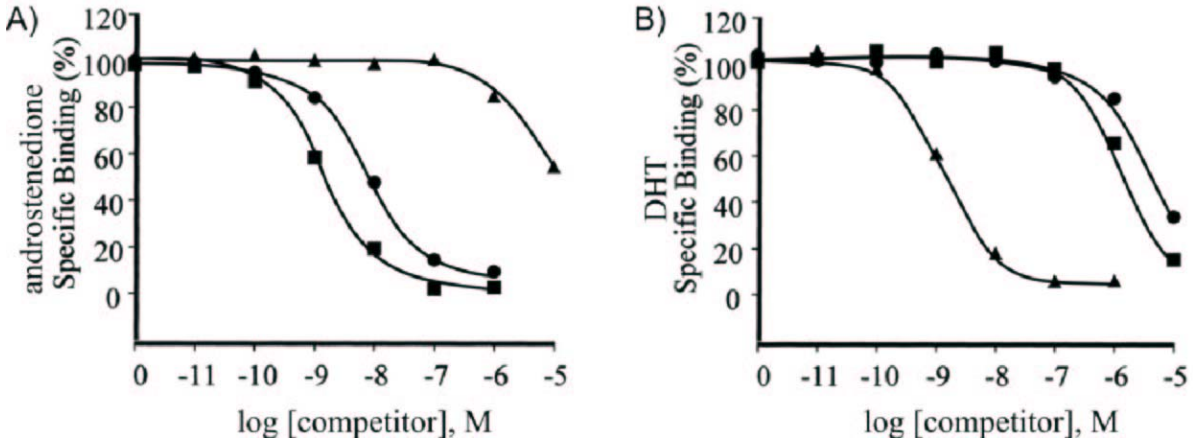


Figure 1. Competition binding analysis using purified, recombinant androgen receptor and HR-RBI („A), androstenedione (ƒþ), and dihydrotestosterone (DHT; •) with either [3H]-androstenedione (A) or [3H]-dihydrotestosterone (B) as radioligands. Values are expressed as percent specific binding and a theoretical Langmuir binding isotherm has been fitted to the experimental data.

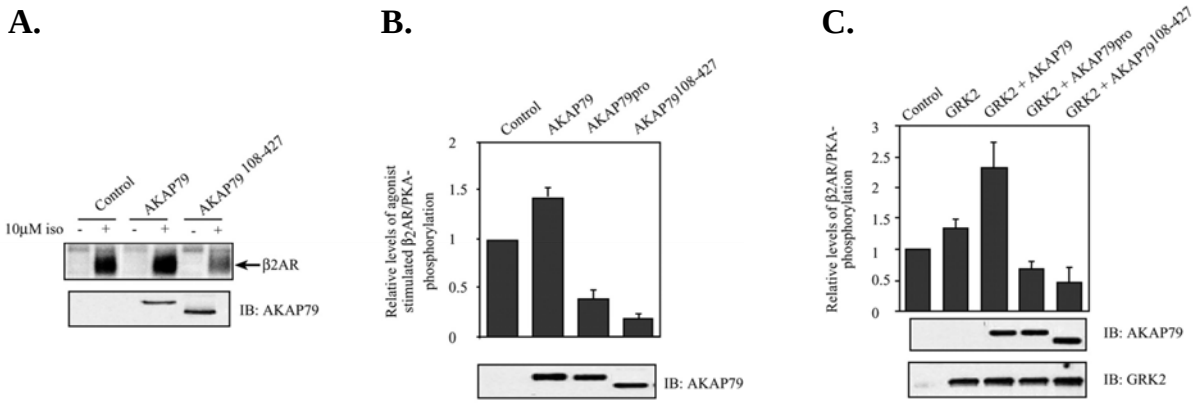
In addition to competition binding studies, you also examine the effect of HR-RBI treatment on muscle development by administering HR-RBI orally to control mice and mice simultaneously treated with androstenedione. Before you have an opportunity to assess alterations in muscle mass however, both groups of animals demonstrate a drastic reduction in extracellular fluid and blood volume, decreases in plasma sodium levels and increases in plasma potassium levels resulting in death within 3 days to 2 weeks.

1) Develop a hypothesis for the unexpected effects of HR-RBI and devise experimental strategies to test your ideas.

2) Other than the competition binding studies that you performed, what additional analyses would have been useful to more fully characterize a putative androstenedione-specific antagonist for the treatment of anabolic steroid abuse?

Question #6

The β2 adrenergic receptor (β2AR) undergoes desensitization by a process involving its phosphorylation by both protein kinase A (PKA) and G protein-coupled receptor kinases (GRKs). The second messenger-activated kinases are constitutively localized to subcellular structures, including the β2AR, via their interactions with A kinase-anchoring proteins (AKAPs). In contrast, the GRKs are recruited to the plasma membrane where they bind to and phosphorylate target GPCRs following agonist stimulation. To investigate the effects of PKA anchoring on β2AR phosphorylation, a group of investigators performed the following experiments.



A, HEK293 cells overexpressing FLAG-β2AR were transiently transfected with mammalian expression plasmids encoding wild-type AKAP79 or AKAP79$^{108\text{-}427}$ (a mutant that binds PKA but lacks the receptor and membrane-targeting domain) and incubated in [^{32}P]orthophosphate. Cells were stimulated with 10 μM isoproterenol (*iso*) for 5 min, and FLAG-β2AR was immunoprecipitated and resolved by SDS-PAGE. *Upper panel* shows a representative autoradiogram (n = 4) with basal (-) and isoproterenol-stimulated (+) β2AR phosphorylation in transfected cells as indicated. AKAP79 and AKAP79$^{108\text{-}427}$ expression was detected in cell lysates by immunoblotting (*IB, lower panel*). ***B,*** FLAG-β2AR/PKA$^-$ is a **mutant receptor lacking all PKA phosphorylation sites**; previous studies have demonstrated that this mutant receptor **can not** be phosphorylated by PKA. HEK293 cells expressing FLAG-β2AR/PKA$^-$ were transiently transfected with AKAP79, AKAP79pro (a mutant that is targeted to the plasmid membrane and binds to the β2AR, but is unable to bind to the PKA regulatory subunit), or AKAP79$^{108\text{-}427}$, and isoproterenol-stimulated phosphorylation of β2AR/PKA$^-$ was measured as described earlier. The *graph* shows the mean levels of phosphorylation of β2AR/PKA$^-$ relative to control transfected cells. AKAP79, AKAP79pro, and AKAP79$^{108\text{-}427}$ expression were detected in cell lysates by immunoblotting (*lower panel*). ***C,*** HEK293 cells expressing β2AR/PKA$^-$ were transiently transfected with GRK2, AKAP79, AKAP79pro, and AKAP79$^{108\text{-}427}$ as indicated. Isoproterenol-stimulated phosphorylation of β2AR/PKA$^-$ was measured as described earlier. Expression of AKAP79, its mutants, and GRK2 was confirmed by immunoblotting (*lower panels*).

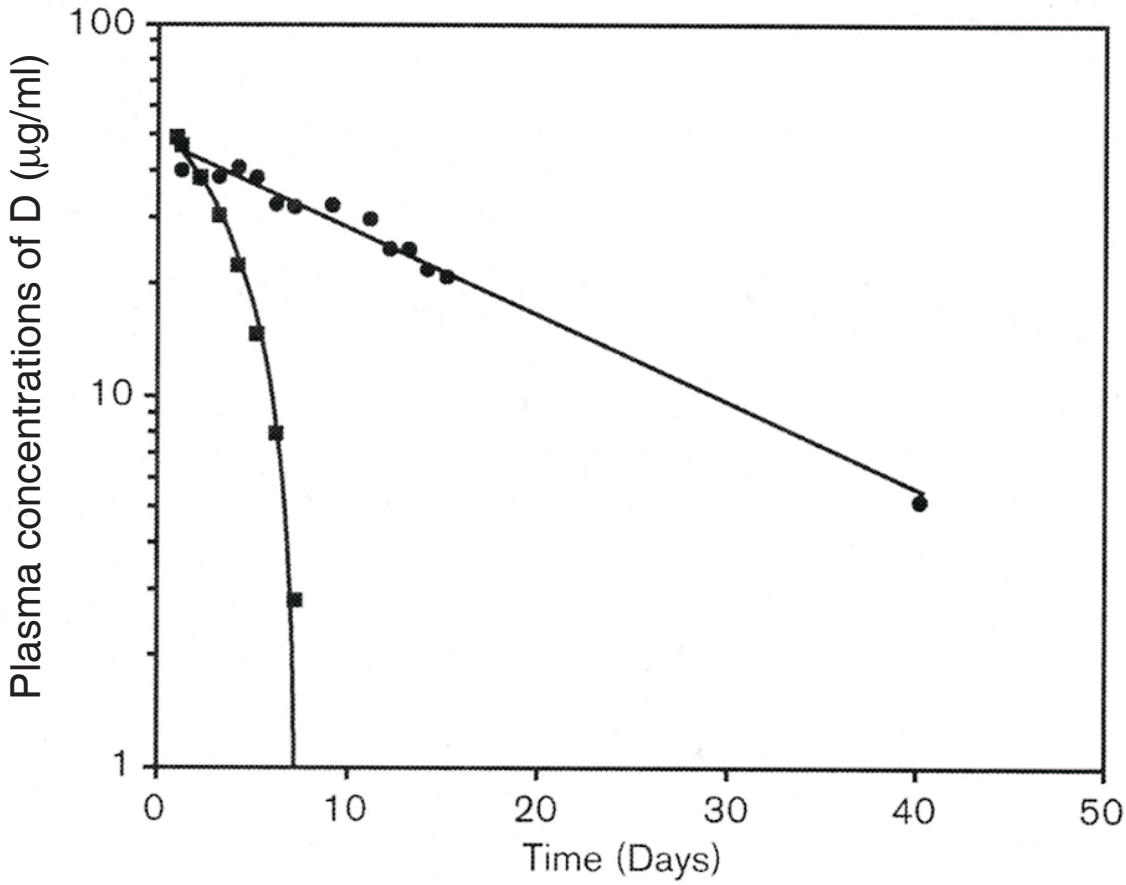
1) How would you interpret these data? Develop a hypothesis to explain these results.

2) Design two independent experiments to test your hypothesis.

Question #7

The Figure depicts the profile of drug D plasma levels in two subjects given a 300mg oral dose of D in a Phase I clinical trial.

Fig. 1



1. Discuss possible explanations for the differences in profiles in the two subjects.

2. How would you determine a mechanistic basis for these different profiles, making use of both *in vitro* and *in vivo* tests.