Department of Pharmacology
Qualifying Examination (Part I)
December 17-20-2001

***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

Natriuretic peptide is released from the heart in response to stimuli, such as stretch, and causes vasodilation in the kidney and natriuresis. The targeted disruption of natriuretic peptide receptor A in the mouse kidney results in the physiological changes noted below.

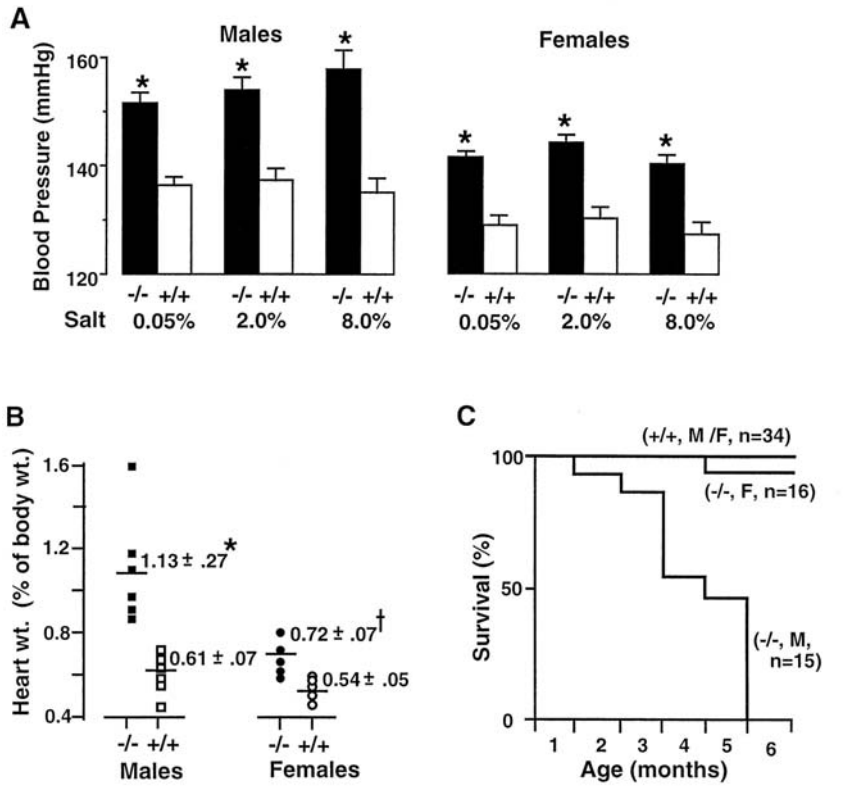


FIG. 1. Blood pressures, heart weights, and survivals of mutant and wild-type mice. *(*A) Blood pressures as a function of *Npr1* genotype and dietary salt. Mean blood pressures measured by a noninvasive computerized tail-cuff method in conscious young adult mice aged 95–115 days are shown for male *(n* 512 per genotype) and female *(n* 5 20 per genotype) *Npr1* 2y2 (solid bars) and 1y1 (open bars) animals on a low (0.05% NaCl), intermediate (2% NaCl), or high (8% NaCl) salt diet. SEMs are indicated above the bars. p, *P* , 0.001 vs. wild-type controls by ANOVA. *(*B) Heart weights as a percent of total body weight of 3-month-old mice. Individual values are represented as squares (males) and circles (females), and mean values are indicated by horizontal lines. p, *P* , 0.001 and †, *P* , 0.01 vs. wild-type controls by two-tailed *t* test. *(*C) The percentage survival of male (M) and female (F) *Npr1* 2y2 animals and wild-type (1y1) controls as a function of age.

1. Describe the data and propose a model for the actions of natriuretic peptide on blood pressure and myocardial function.

2. Males and Females differ in their response to the loss of natriuretic peptide receptor A. You hypothesize that estrogen is protective or testosterone exacerbates the phenotype. Outline experiments to test each hypothesis.

Question #2

A recent study by O. B. Juan Kenobe *et al*., directly examined the effects of angiotensin II on glomerular afferent arteriolar vascular responses. These investigators used an *in vitro* preparation consisting of a single afferent arteriole and its intact glomerulus together with the adherent portion of the thick ascending limb and early distal convoluted tubule. The afferent arteriole was cannulated with a perfusion pipette through which they perfused oxygenated tissue culture media containing 5% albumin. The tubule was similarly cannulated and perfused with a buffered solution containing variable levels of NaCl ("Low NaCl" = 5 mM NaCl; "High NaCl" = 79 mM NaCl) with or without angiotensin II (Ang II) or Losartan (Los). The following data figures were generated:

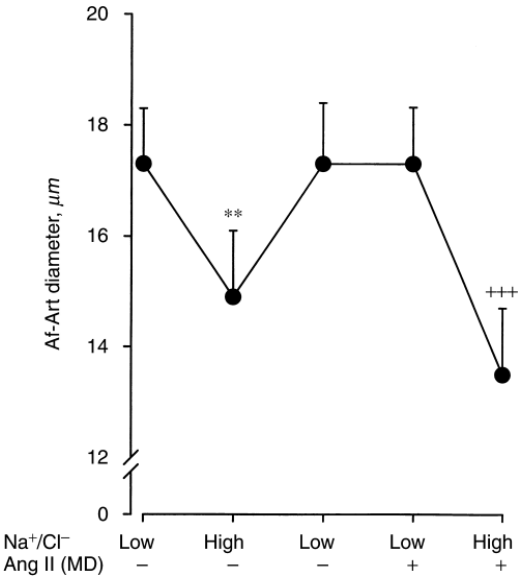


Figure 1

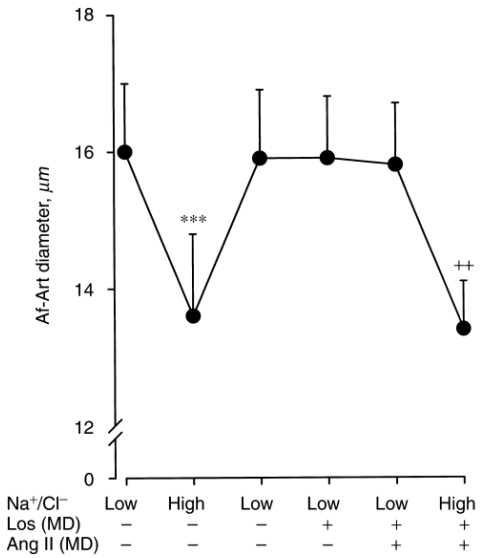


Figure 2

In each experiment, the diameter of the afferent arteriole was monitored by videomicroscopy and plotted on the *y*-axis. The *x*-axis shows the various experimental maneuvers employed (high or low NaCl tubule perfusion; etc.). The investigators tested the effects of perfusing the tubule (luminal delivery) with Ang II and Losartan.

1. Please explain the results illustrated by Figures 1 and 2.

2. What effect will these maneuvers have on renin secretion?

3. If these observations accurately reflect the *in vivo* response to changing tubular fluid NaCl concentration, how does this impact on the physiological adaptation of glomerular filtration rate to extracellular volume depletion? In other words, does this effect of Ang II on the response of the afferent arteriole contribute to restoring GFR in volume depletion or is counterproductive. Explain your answer.

Question #3

Figure 1 below depicts the concentration-effect curves for a series of novel compounds "triangle", "square" and "diamond", that you have developed, on basal adenylyl cyclase activity CHO cells transfected with an "orphan" GPCR, GPR666 cDNA.

1. Which compound has the highest potency? Which has the highest affinity?

2. Provide two distinct and disparate explanations for the effect of these compounds on adenylyl cyclase activity.

3. Design experiments that would allow you to distinguish between these hypotheses.

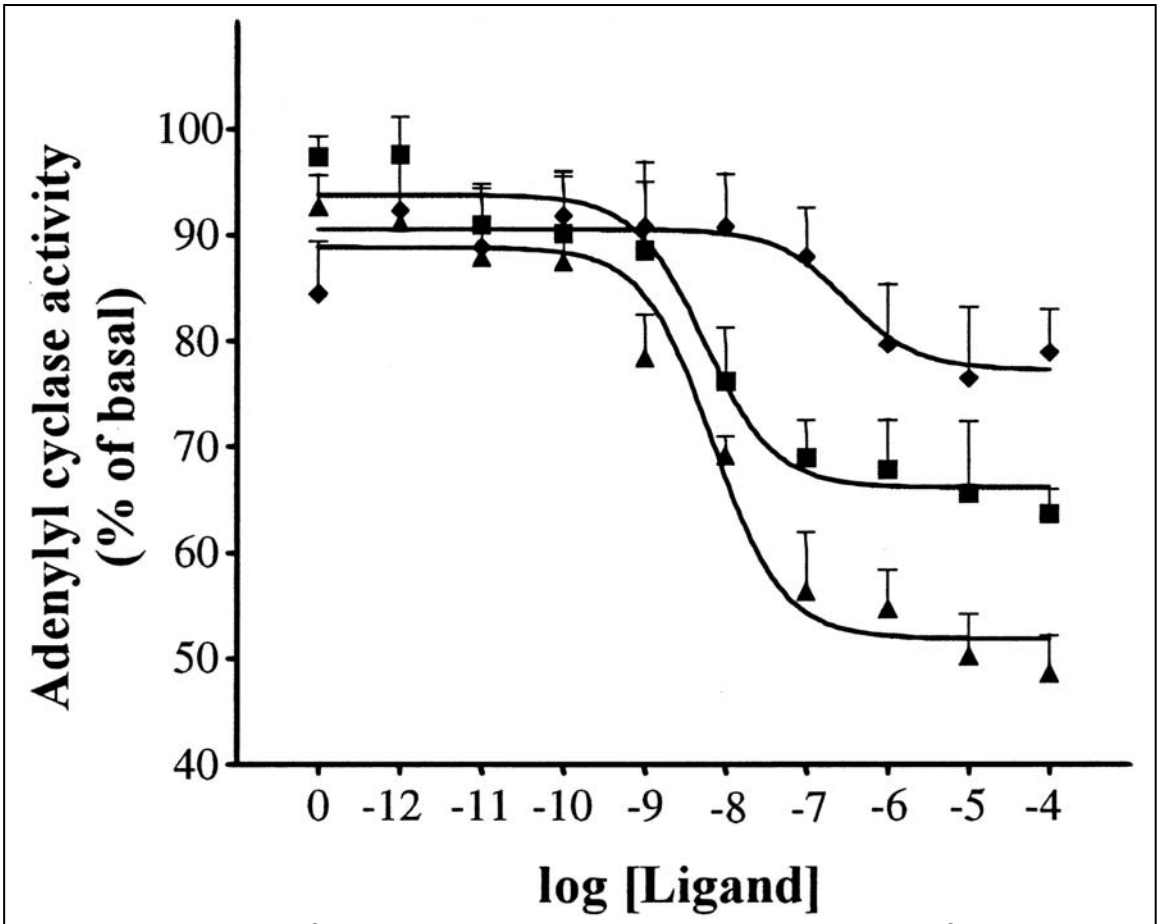


Figure 1. Inhibition of adenylyl cyclase activity in membranes derived from CHO cells expressing GPR666. Adenylyl cyclase activity was determined from cells incubated in the presence of increasing concentrations of each of the three structurally related ligands "triangle", "square" and "diamond".

Question #3 (continued)

You perform further experiments on GPR666 transfected cells using radiolabeled ligands. In some cases you co-transfect a plasmid expressing $G_{s\alpha}$. In each case you obtain results similar to those shown in Figure 2 below.

1. How might you interpret these results?

2. How would you test your hypothesis?

3. How does this impact your initial hypotheses for the mechanism of action of the "triangle", "square" and "diamond", drugs?

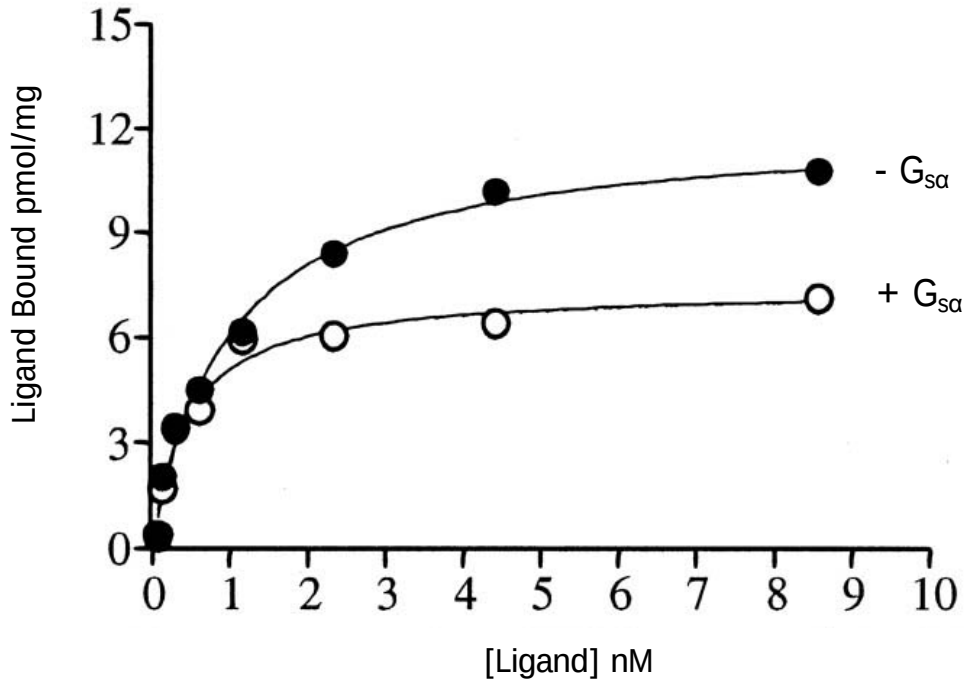
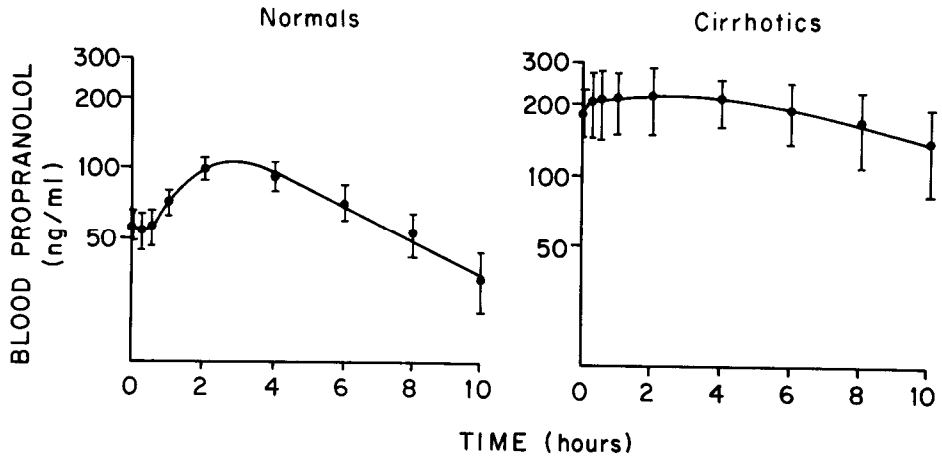


Figure 2. Saturation binding isotherm for radiolabeled ligands with (open circle) or without (filled circles) co transfection of $G_{s\alpha}$.

Question #4

The following data were obtained by oral administration of propranolol to normal volunteers, and to cirrhotic patients



1. Interpret the differences in the drug concentration-time profiles between the two groups.

2. What possible changes associated with the disease of cirrhosis can account for the observed changes in the handling of propranolol?

3. How would the results differ if the same dose of propranolol were administered intravenously to the two groups of subjects?

4. a. How would you assess drug metabolizing activity in a sample of liver tissue?
   b. If such samples were available from the two groups above, what type of results would you predict for the biotransformation of propranolol *in vitro*?

Question #5

A recently formed pharmaceutical company, Chubby-R-Us, initially focused its research efforts upon the growing problem of human obesity by attempting to identify new compounds that can modulate the central regulation of feeding behavior. Unfortunately, oral administration of one of their newest test compounds significantly increased food intake in rodents. Not deterred by this minor setback, Chubby-R-Us changed it research direction to focus upon that small percentage of Americans that are chronically underweight and have difficulty in maintaining their body mass.

Additional dose-response analyses of their new drug revealed that when administered at levels five-fold higher than those shown to affect food uptake, experimental animals demonstrated weakness, sluggishness and eventually died within about 6 weeks. A more detailed analysis of these mice revealed the fasted, mean blood values indicated below:

Laboratory test	Treated animal value	Normal range
Na^+	112 mmol/L	137-145 mmol/L
Cl^-	81 mmol/L	98-107 mmol/L
K^+	5.4 mmol/L	3.5-5.0 mmol/L
Glucose	90 mg/dL	150-200 mg/dL
Corticosterone	<0.2 µg/dL	AM 6.2-29.1 µg/dL
		PM 3.0-17.3 µg/dL
ACTH	1745 pg/mL	9-52 pg/mL

ACTH, adrenocorticotropic hormone; Cl^-, chloride; K^+, potassium; Na^+, sodium

1. Based upon <u>all</u> of these observations, develop a hypothesis regarding the physiological changes in response to your compound and propose a mechanism for its action.

2. Describe a series of experiments by which to confirm your proposed mechanism of action.

Question #6

Several studies have shown neuroprotection from transient cerebral ischemia by administration of the a-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor antagonist NBQX immediately after ischemic injury. You have recently developed an animal model of transient cerebral ischemia consisting of a bilateral occlusion of the cerebral vessels for 10 minutes and then assess the extent of neuronal damage 10 hours after the ischemic insult by measuring the number of necrotic cortical neurons. After occlusion of the cerebral vessels in two strains of mice (DBA1 and DBA2) for 10 minutes, followed immediately by administration of vehicle or the AMPA receptor antagonist, NBQX, you obtain the results indicated below:

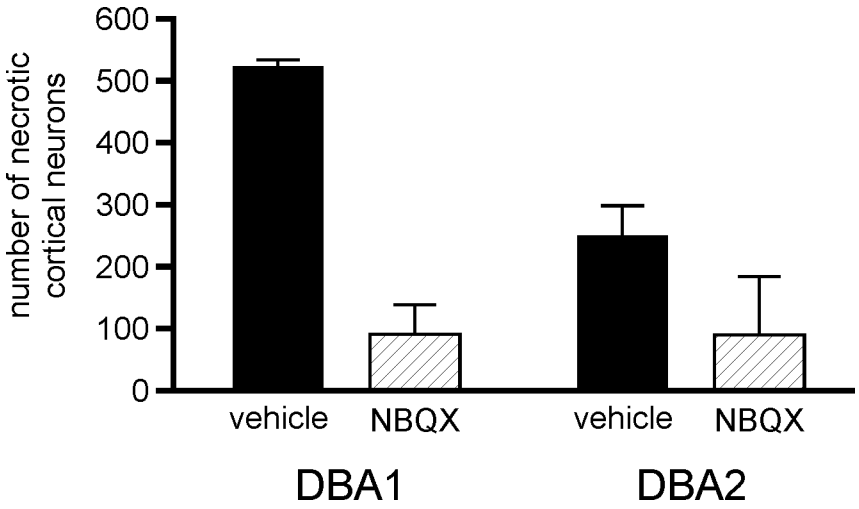


Figure 1. Neurotoxicity responses of DBA1 and DBA2 mice to transient cerebral ischemia.

1) Outline the proposed cellular pathway(s) resulting in hypoxia/ischemia-dependent neurotoxicity and detail the role of glutamate neurotransmission in this pathophysiological process.

2) Ignoring any anatomical differences between the two mouse lines, develop a hypothesis to explain the strain-specific differences observed in your experimental data by identifying at least two specific cellular targets that could explain the differential ischemic responses. Provide experimental strategies by which you could test whether such targets are responsible for the effects seen in DBA1 versus DBA2 animals.

Question #7

Your laboratory is focusing upon a mutant strain of mouse that is resistant to cholera toxin-induced diarrhea. Positional cloning studies have revealed that this toxin-resistance is due to a serine to alanine mutation in an intestinal chloride channel that is normally expressed in epithelial cells in the crypts of Lieberkühn. Upon isolation of the cDNA encoding this novel chloride channel (chlor1), you begin to further characterize its properties by transfecting an expression plasmid containing the full-length chlor1 cDNA into two different mouse epithelial cell lines (referred to as M12 and M29) and measuring current at a series of test voltages between –60 and +10 millivolts using patch clamp recording. Western blotting analysis with a chlor1-specific antiserum demonstrated expression of similar levels of chlor1 protein in both transfected cell lines. Results from the patch-clamp recording experiments are indicated below:

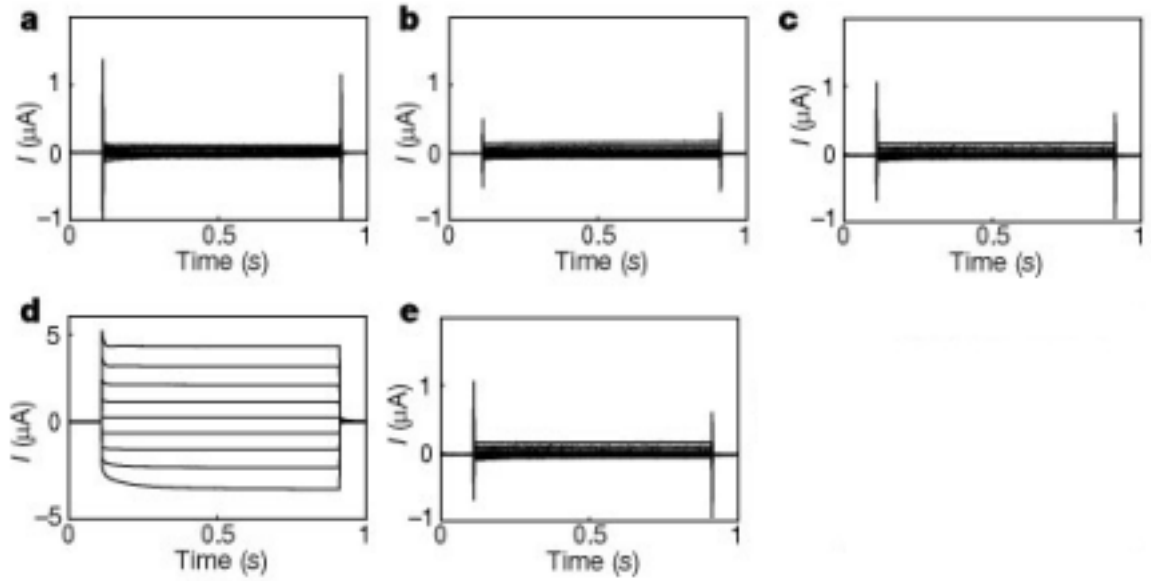


Figure 1. Patch-clamp recording of chlor1 current in mouse epithelial cell lines.
a) Parental (non-transfected) M12 cells; b) Parental (non-transfected) M29 cells; c) M12 cells transfected with 5 μg of chlor1 expression vector; d) M29 cells transfected with 5 μg of chlor1 expression vector; e) M29 cells transfected with 5 μg of chlor1 expression vector + 10^{-7}M chloride channel blocker.

1) Based upon the data presented in Figure 1, please provide **two** distinct hypotheses that could explain these experimental results.

2) Design experimental strategies by which to test your hypotheses.