Department of Pharmacology

Qualifying Examination (Part I)

December 11 & 12, 2003

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.**

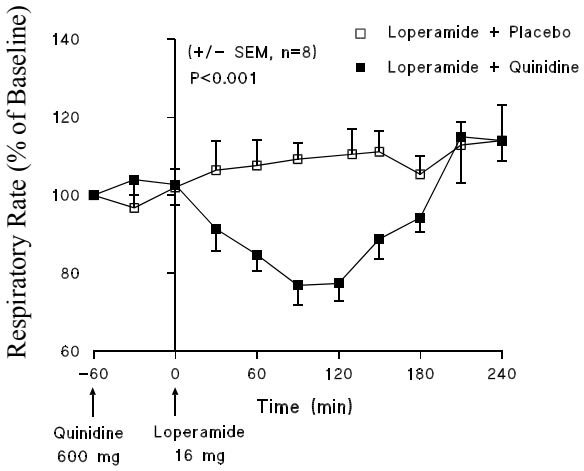
If you have any questions regarding the examination, please contact Joey Barnett at 936-1722 (w) or 385-4396 (h).

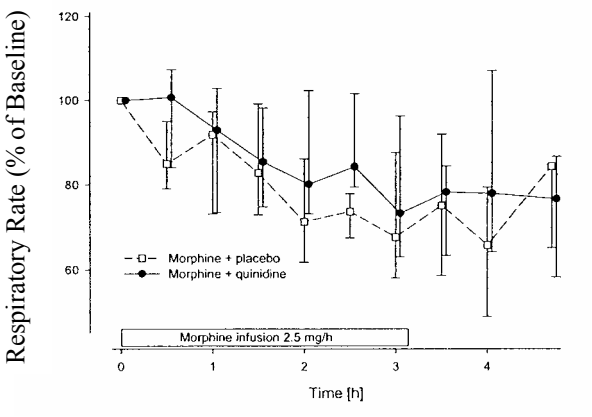
BEST WISHES FOR YOUR SUCCESSFUL COMPLETION OF THE EXAMINATION!

Question 1 – Qualifying Examination, December 2003

Describe the structure of a prototypical EGF receptor. Describe the cellular events that lead to activation of EGFR. How would you detect or assay activation of EGFR2 (ERBB2) in Hek293 cells (which endogenously express both EGFR2 and EGFR1)? Please include experimental details.

Question 2 – Qualifying Examination, December 2003





The above figures indicate the effect of oral pretreatment with placebo or quinidine – a CYP3A substrate - on respiratory responsiveness following either oral loperamide (left panel) or intravenous morphine (right panel) in human subjects. Loperamide (Imodium®), when used for the treatment of diarrhea, has no central effects despite being an opioid.

1. How do you interpret this data?

2. What mechanisms are possibly involved?

3. How would you test your hypotheses?

Question 3 – Qualifying Examination, December 2003

Synthetic agonists of the peroxisomal proliferator activated receptor, subtype gamma (PPAR-gamma) such as rosiglitazone (RGZ) are highly beneficial in the treatment of type II diabetes mellitus. However, they are also associated with fluid retention and edema, potentially serious side effects of unknown origin.

In a set of recent experiments, RGZ (94 mg/kg diet) was fed to male, non-diabetic, Sprague-Dawley rats (~270g) for 3 days. Mean arterial blood pressure (MAP), measured by radiotelemetry, was decreased significantly in rats fed RGZ, relative to control rats. Change in MAP from baseline was –3.2 ± 1.2 mm Hg (i.e. decreased MAP) in rats fed high-dose RGZ versus +3.4 ± 0.8 for rats fed control diet. RGZ did not affect feed or water intake, but rats treated with high-dose RGZ had decreased kidney weight (–9%), and decreased creatinine clearance (a measure of GFR) (–35%). Figure 1 depicts the responses to treatment.

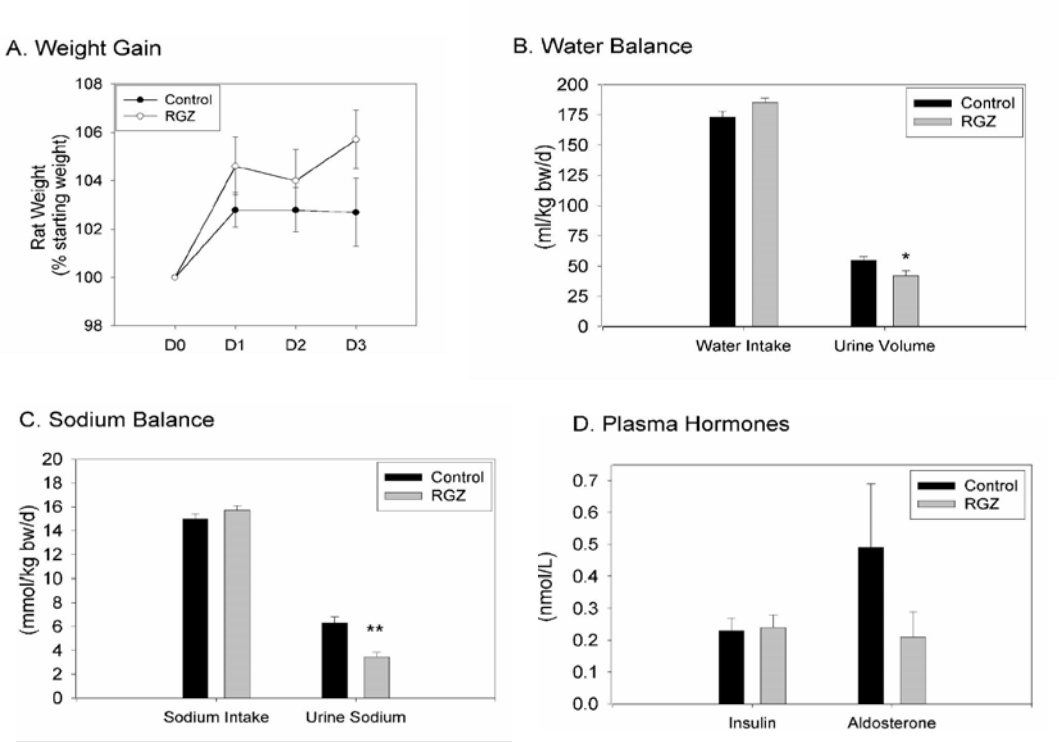


Figure 1 - Responses to RGZ treatment. In panel A, the x-axis is labeled in days after onset of treatment (D0 is baseline). In panels B and C, * indicates $P < 0.05$ and ** indicates $P < 0.01$. No other comparisons were statistically significant. All data are mean ± SEM.

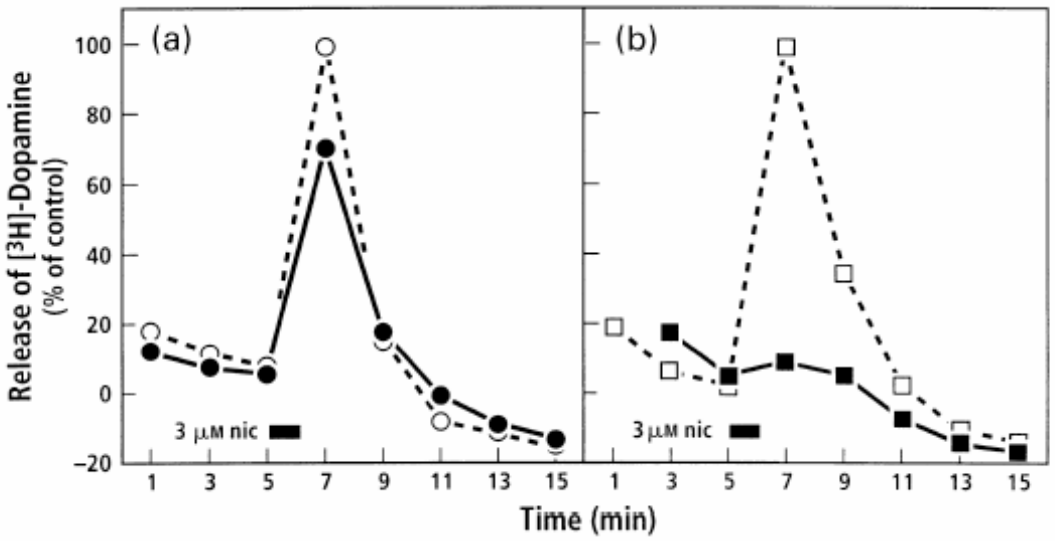
1. Develop a general hypothesis from the kidney perspective to explain the action of RGZ on body weight.

2. Explain step-by-step the renal responses to reduced blood pressure under normal physiological conditions, and compare this with the responses exhibited by the RGZ treated animals.

3. In the absence of a significant increase in plasma aldosterone, what mechanisms could account for reduced urinary Na excretion in the RGZ treated rats?

Question 4 – Qualifying Examination, December 2003

Nucleus accumbens synaptosomes loaded with [3H]dopamine are subjected to two conditions illustrated in panels a) and b) below. Synaptosomes are first preincubated for 5 minutes with either no drug (dotted line), a muscarinic antagonist (panel a, solid circles) or a nicotinic antagonist (panel b, solid squares). Nicotine is then added to the synaptosomes 5 minutes after starting *in vitro* perfusion of the synaptosomes and the extent of [3H]dopamine released is analyzed as a function of time.



A) What does the data indicate in terms of the type of receptors supporting nicotine action? What is the likely neurotransmitter acting on these receptors?

B) Propose two mechanisms by which the application of nicotine elevates extracellular DA? Propose tests that can distinguish among possible mechanisms?

C) Amphetamine triggers a similar rise in extracellular DA but this effect is not blocked by the nicotinic antagonist. Why is this?

Question 5 – Qualifying Examination, December 2003

Homer proteins are thought to function as scaffolding proteins that bind G protein-coupled receptors (GPCRs) and inositol 1,4,5-triphosphate (IP_3) receptors; however, their role in calcium signaling *in vivo* is unclear. Characterization of calcium signaling in pancreatic acinar cells from Homer2$^{-/-}$ and Homer3$^{-/-}$ mice revealed that Homer3 had no discernible role in calcium signaling. In contrast, Homer2 was found to play a role in tuning the intensity of calcium signaling by GPCRs to regulate the frequency of intracellular calcium oscillations. To further investigate the molecular mechanisms responsible for this phenomenon, a group of investigators performed the following experiment.

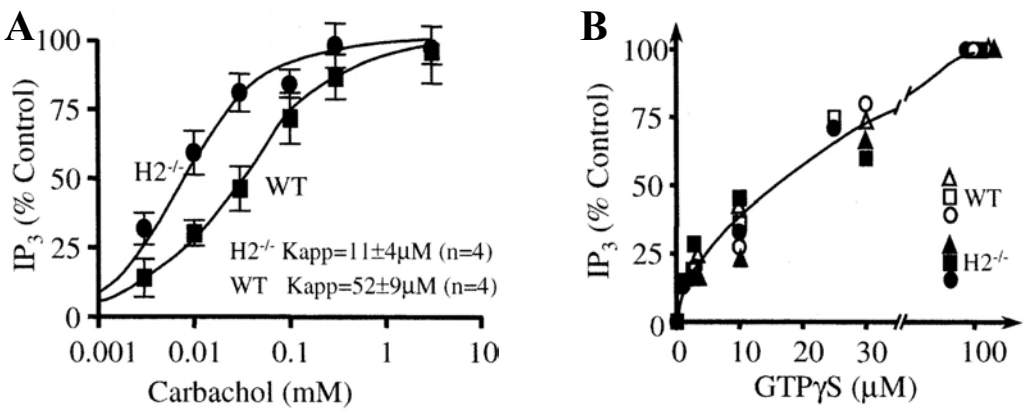


Figure 1. **PLCβ activity in agonist and GTPγS stimulated cells from WT and Homer2$^{-/-}$ cells.** (A) Intact cells from WT (squares) or Homer2$^{-/-}$ (circles) were stimulated with various concentrations of carbachol, the reactions stopped, and IP_3 was extracted and measured. The figure shows the mean +/- SEM of four experiments performed in duplicates with four different cell preparations. (B) Cells from three WT (open symbols) and three Homer2$^{-/-}$ (closed symbols) mice were permeabilized and stimulated with the indicated concentration of GTPγS and then assayed for IP_3 levels.

The investigators also demonstrated that the effects observed in the experiment depicted above were ***not*** due to aberrant localization of IP_3Rs in cellular microdomains or changes in IP_3R channel activity. Since carbachol stimulates IP_3 production in pancreatic acinar cells, the investigators performed the following experiments.

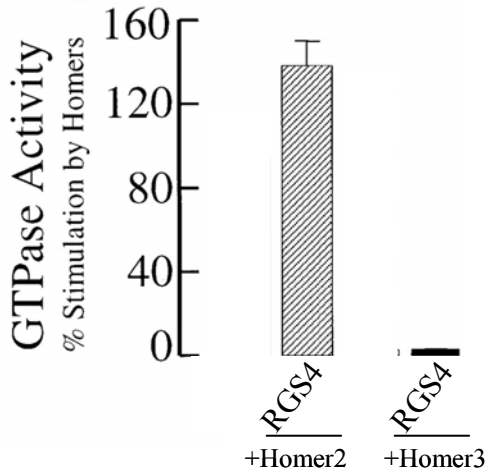


Figure 2. **Effect of Homers on RGS4 GTPase activity.** Recombinant, purified M1 receptor, and $G\alpha_q\beta\gamma$ heterotrimer were reconstituted into liposomes and used for measurements of RGS4 GTPase activity. GTPase activity was measured in the absence and presence of 2 nM RGS4, and in the absence and presence of 20 nM Homer 2 or 3. GTPase activity was initiated by stimulation with 1 mM carbachol. Stimulation by Homers was calculated as a percentage of the activity measured in the absence of Homers 2 and 3 and the presence of RGS4.

1) Develop a hypothesis to explain these data. Describe how the results of these experiments support your hypothesis.

2) Design two independent experiments to test your hypothesis.

Question 6 – Qualifying Examination, December 2003

The data below were obtained from transgenic mice that overexpress the β1-adrenergic receptor in cardiac myocytes only (TG-M, transgenic mice male; TG-F, transgenic mice female; WT, wildtype mice).

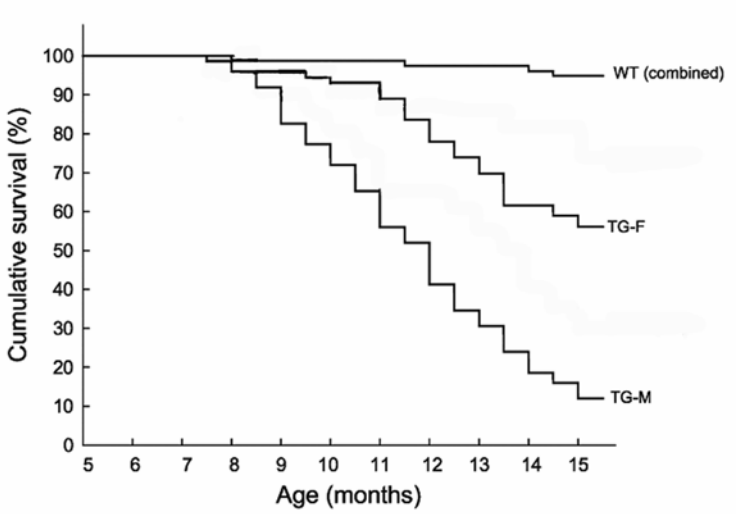


FIG. 1. Survival curves of male and female β1-AR TG and WT littermate mice. Survival was significantly better in female (n=73) than male (n=75) TG mice at 15 mo of age (*P*<0.001). The average age of premature deaths due to cardiac reasons (up to 15 mo) was similar in male and female TG mice (52±12 *vs.* 49±10 wk; mean±SD; *P*<not significant). The survival rates were identical in WT mice with (n=75; 37 males and 38 females) and therefore were combined for clarity. Note that premature deaths due to cardiac reasons started at about 8 mo in both TG groups.

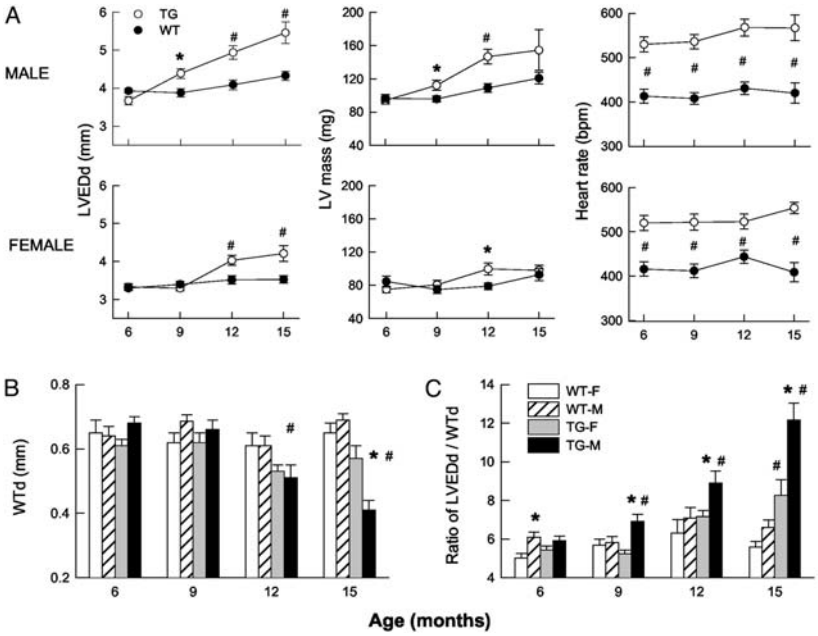


FIG. 2. Changes in LVEDd, LV mass, heart rate, anterior WTd, and the ratio of LVEDd/WTd in male and female TG and WT mice from 6–15 mo of age. All parameters were obtained by echocardiography. The group sizes were 25 for each gender in WT mice. For transgenics, there were 63 males and 41 females at 6 mo of age. By 15 mo, the group sizes were reduced to 9 and 20, respectively. Values are means ± SEM. *, *P* < 0.05 and #, *P* < 0.01 for TG *vs.* WT of the same gender for panel A. *, *P* < 0.05 male *vs.* female in the same genotype; #, *P* < 0.01 TG *vs.* WT of the same gender for panels B and C. (Note, LVEDd, Left Ventricular End Diastolic diameter; LV, Left Ventricle; WTd, Wall Thickness at diastole)

1. Describe the data.

2. State a hypothesis concerning how β1 adrenergic receptor overexpression might signal the cardiac changes noted. State a hypothesis concerning the effect of gender. How might you test your hypotheses?

Question 7 – Qualifying Examination, December 2003

You have recently identified a family with Pendred syndrome (PS), an autosomal recessive disease characterized by a profound sonsorineural hearing loss and a thyroid goiter resulting from an apparent lack of iodine organification. Further analysis of the thyroid glands from these patients have indicated that the peroxidase and iodinase activities as well as both thyroglobulin biosynthesis and sodium/iodide symporter activity are normal in affected individuals. Perchlorate, which inhibits the function of the sodium/iodide symporter, causes the leakage of any free iodide back into the bloodstream. Thus, in PS patients, the partial release of radiolabelled iodide during a perchlorate discharge test indicates that iodide uptake by the thyrocyte is normal, but that there is a defect in its transport across the apical membrane or its incorporation into thyroglobulin. You hypothesize that a novel iodide transporter is required for the movement of iodide across the apical membrane of follicular cells into the colloid and that affected individuals may have a defect in the gene encoding this transporter protein.

1) Describe the cellular and biochemical processes involved in the synthesis of the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3).

2) a) Provide a detailed cloning strategy by which you might isolate the cDNA encoding this novel iodide transporter. Please be sure to provide sufficient experimental detail to insure that the examination committee understands the rationale for the experiments that you propose.

b) Propose a series of experiments to insure that the cDNA that you have isolated encodes the suspected mutant transporter.