Department of Pharmacology

Qualifying Examination (Part I)

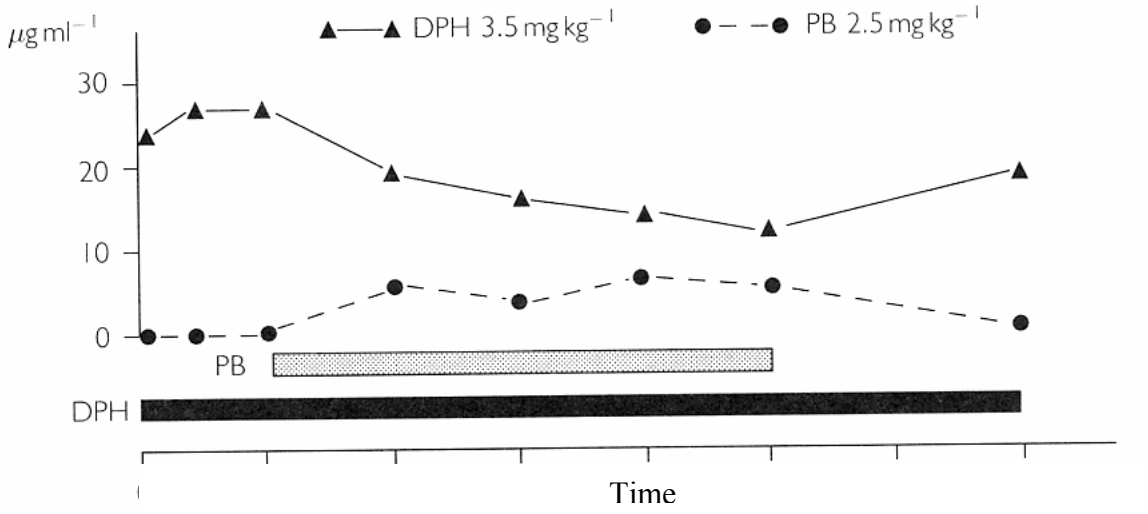
June 25-27, 2003

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.

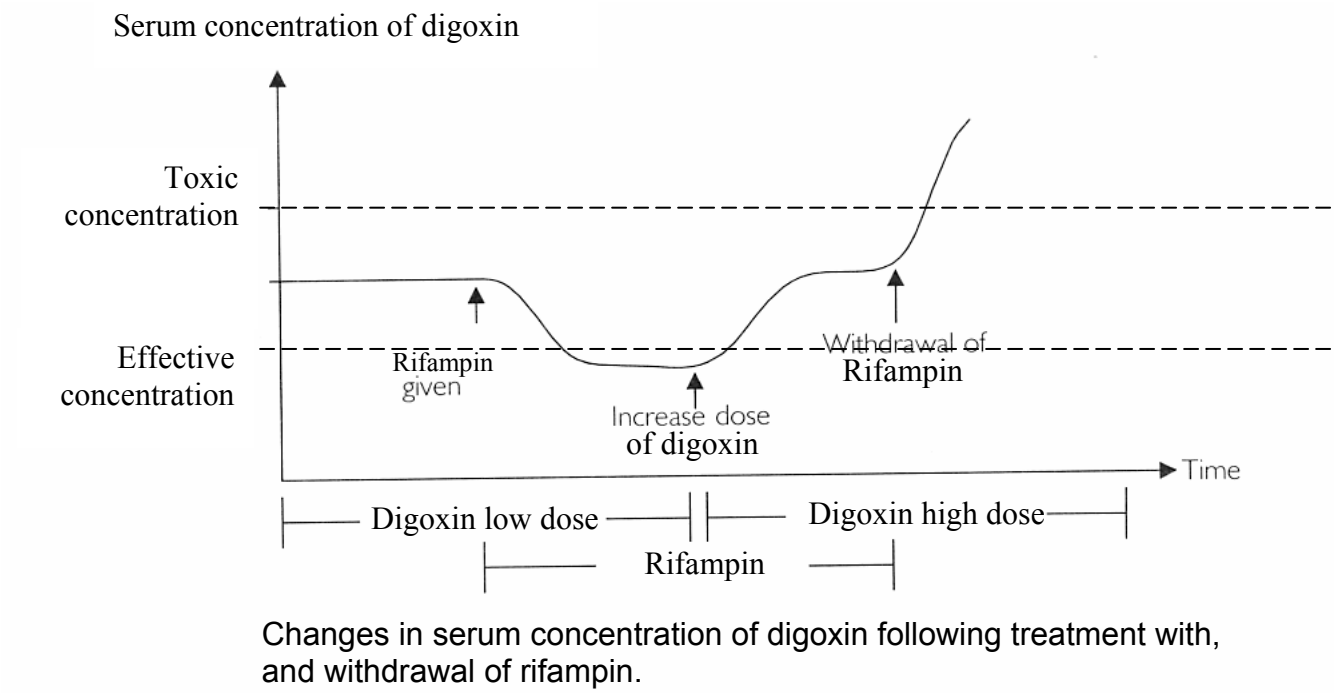
If you have any questions regarding the examination, please contact Joey Barnett at 936-1722 (w) or 385-4396 (h).

BEST WISHES FOR YOUR SUCCESSFUL COMPLETION OF THE EXAMINATION!

Question #1:



Effect of phenobarbital (PB) treatment on the steady-state serum concentration of phenytoin (DPH) in man.



Changes in serum concentration of digoxin following treatment with, and withdrawal of rifampin.

The figures show the effects of oral dosing with phenobarbital on the plasma levels of phenytoin (top panel), and rifampin on those of digoxin (bottom panel). In both findings, the time scales are on the order of several weeks.

1. How do you interpret the two sets of results?

2. How would you go about testing your hypotheses utilizing both *in vivo* and *in vitro* approaches?

Question #2:

Familial hyperkalemic hypertension (a.k.a. Gordon's syndrome) is an autosomal dominant disease featuring severe hypertension, elevated plasma potassium concentration (ie. hyperkalemia) and normal renal function. Mutations in two related kinase genes, *WNK1* and *WNK4* (named for "with no lysine" at a key catalytic residue) have been found responsible. Mutations in WNK1 are predicted to increase expression levels of this gene, while WNK4 mutations are predicted to cause loss-of-function. Both kinases are expressed in the distal portions of the nephron (distal convoluted tubule through the collecting duct) but their protein substrates are not known.

Recently, a group of investigators at Yale examined the functional effects of WNK1, WNK4, and the unrelated SGK kinase on the Na-Cl co-transporter (NCC) heterologously expressed in *Xenopus* oocytes. Data from their experiments is provided below.

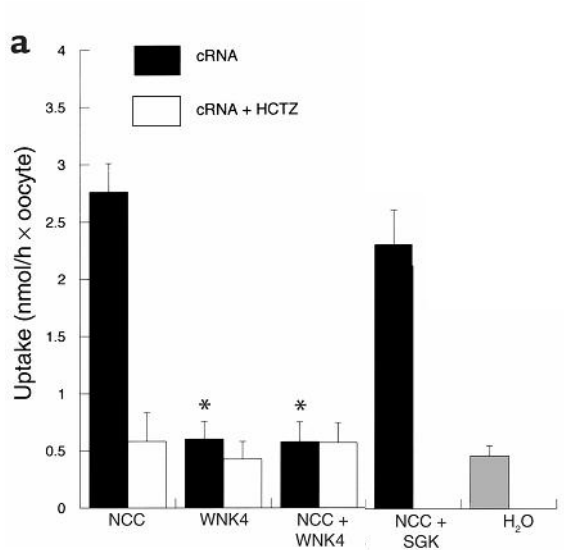


Figure 1 - Sodium (^{22}Na) uptake in oocytes expressing the Na-Cl co-transporter (NCC) with or without different kinases. Uptake assays were performed in the absence (filled bars) or presence (open bars) of hydrochlorthiazide (HCTZ). * indicates $p < 0.05$ in comparisons with NCC alone (no statistics were performed on the HCTZ data).

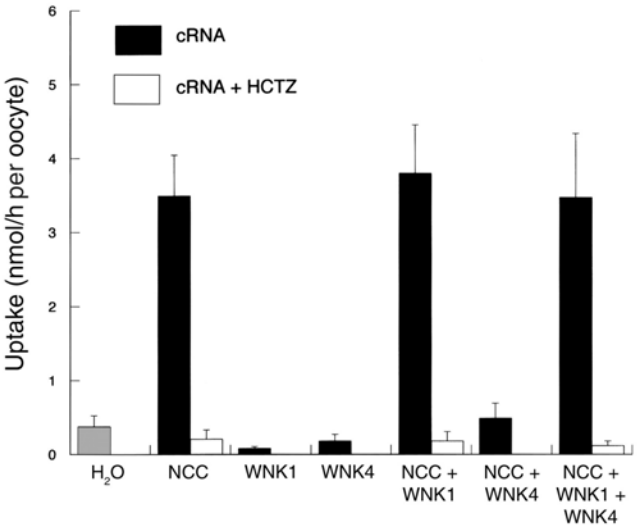


Figure 2 - Sodium (^{22}Na) uptake in oocytes expressing the Na-Cl co-transporter (NCC) with or without different kinases. Experimental conditions are identical to Figure 1.

Co-expression of NCC with mutant WNK4 alleles found in Gordon's syndrome patients produces ^{22}Na uptake similar to NCC alone (data not shown).

1. Develop at least two hypotheses to explain how gain of function mutations in WNK1 and loss of function mutations in WNK4 both cause hypertension. Explain how you might test these ideas.

2. What therapeutic intervention might be beneficial to patients with this disease?

Question #3:

The functions of G protein-coupled receptors (GPCRs) such as the β_2 adrenoreceptor (β_2AR) are highly regulated by their agonist-stimulated phosphorylation by both second messenger-stimulated kinases (PKA and PKC) and the specialized G protein-coupled receptor kinases (GRKs). Recently, studies have revealed that phosphorylation by PKA of some stimulatory guanine nucleotide regulatory protein (G_s)-coupled receptors not only decreases their coupling to G_s but leads to the activation of the extracellular signal-regulated kinases ERK1/2. To investigate the mechanisms of PKA-mediated switching of β_2AR coupling from G_s to activation of ERK1/2, a group of investigators performed the following experiments.

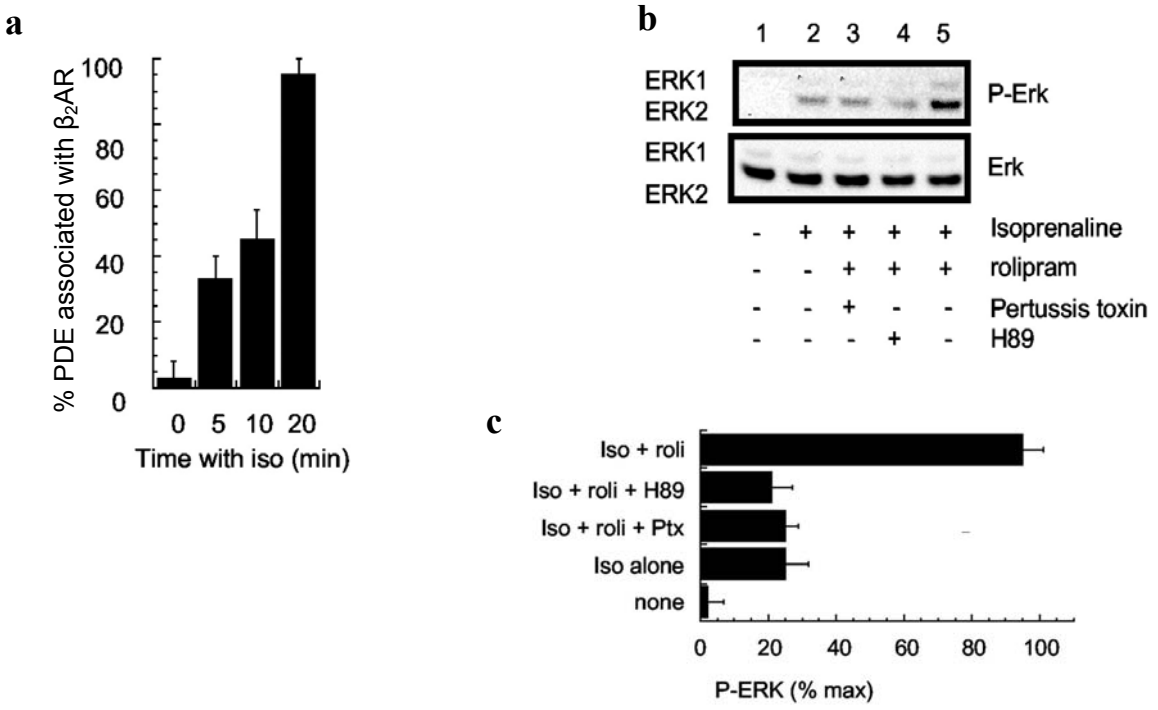


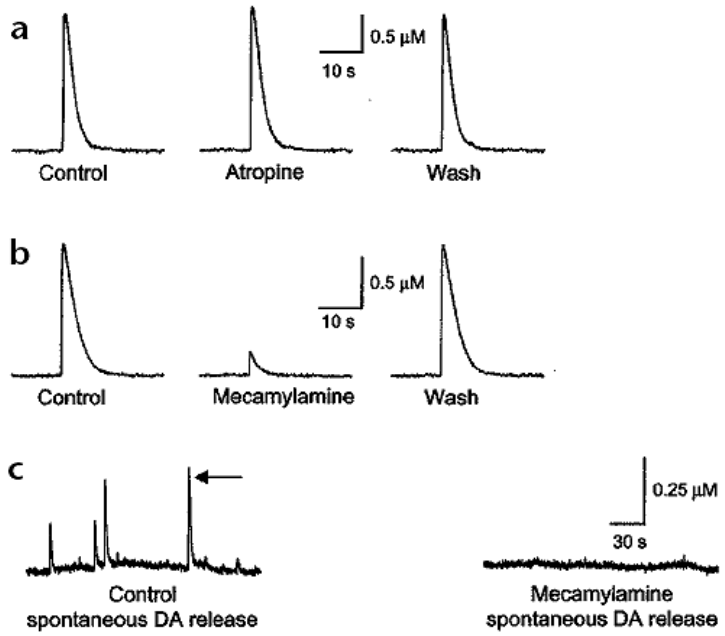
Fig. 1. β_2AR-mediated activation of ERK in primary smooth muscle myocytes. (*a*) Cells were challenged as indicated with isoprenaline (iso, 10 μM) and harvested, and the immunopurified β_2AR was immunoblotted for phosphodiesterase 4D (PDE4D). (*b*) Myocytes were pretreated with 25 ng/ml pertussis toxin, 10 μM rolipram (a selective PDE4D inhibitor), or 1 μM H89 (a selective PKA inhibitor), and then challenged with the 10 μM isoprenaline for 10 min before Western blotting for ERK and P-ERK. (c) Quantification of the data shown in *b* for three experiments with means ± SD

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

2) Design two independent experiments to test your hypothesis.

Question #4:

The following voltammetric data were acquired in rat striatal slices assessing the impact of cholinergic signaling on evoked dopamine (A, B) and spontaneous (C) release. In panels a and b, release of DA is triggered electrically in the slice and measured in the presence or absence of atropine (muscarinic antagonist) versus mecamylamine (nicotinic antagonist).



From these data and your knowledge of anatomy/pharmacology, propose a model describing:

1) where on the DA neuron that acetylcholine is acting to trigger this effect,

2) what type of receptor and signaling event is supporting acetylcholine actions,

3) if slices are pretreated with a cholinesterase inhibitor, the electrically evoked DA increase disappears. Why?

4) how might these responses look if the preparation was derived from slices pre-treated with reserpine, cocaine or using tissue from animals administered 6-hydroxydopamine into the striatum one week earlier.

Question #5:

Many receptor protein tyrosine kinases (RPTK's) are involved in growth and differentiation of cells during development. Aberrant expression of some RPTK's also leads to carcinogenesis and tumor formation. Thyroid tumors have a drastic increase in tyrosine phosphorylated proteins when compared to normal thyroid tissues.

Please address the following to investigate the involvement of a specific RPTK in thyroid tumor formation.

(1) Design at least two strategies to identify candidate RPTK in thyroid tumors.

(2) Design experiments to establish that a specific candidate RPTK is critically involved in thyroid tumorigenesis.

Question #6:

Initial examination of the phenotype of an RGS2 (regulators of G-protein signaling 2) null mouse yield the following data.

Figure 1

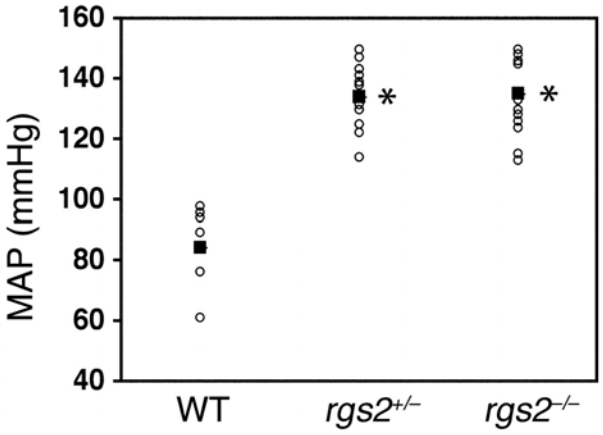


Figure 1. Blood pressures of wild-type and RGS2-deficient mice. Mean Arterial Pressures (MAPS) (mmHg; open circles) of anesthetized male wild-type, *rgs2*$^{+/-}$, and *rgs2*$^{-/-}$ mice with heart rates greater than 330 beats per minute are shown. The mean blood pressure for each genotype is indicated (filled squares). *$P < 0.0005$ relative to wild-type mice.

Figure 2

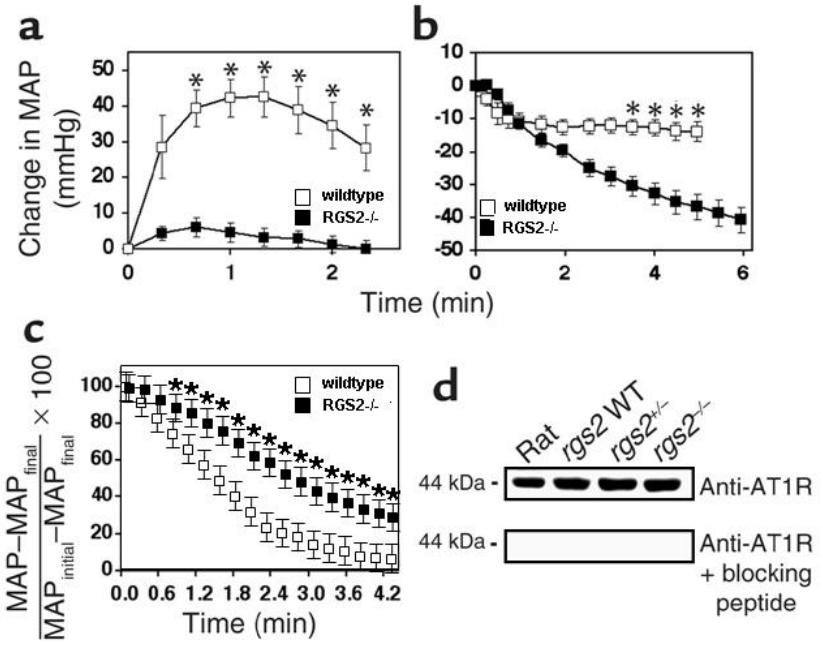


Figure 2. Blood pressure responses following challenge with angiotensin II or angiotensin receptor 1(AT1) blockade in wild-type and RGS2-deficient mice. (**a**) Time courses of blood pressure increase (change in MAP ± SEM, mmHg) following intraarterial administration of pressor doses of angiotensin II to RGS2-deficient mice and wild-type mice. (**b**) Time courses of blood pressure decrease (change in MAP ± SEM, mmHg) following AT1 receptor blockade with candesartan in wild-type and *rgs2*$^{-/-}$ mice. (**c**) Time course of blood pressure decrease [(MAP – MAP_{final})/($MAP_{initial}$ – MAP_{final})] ± SEM following AT1 receptor blockade in wild-type and *rgs2*$^{-/-}$ mice following pretreatment with a pressor dose of angiotensin II to elevate blood pressures to similarly high starting values (systolic blood pressure = 160–170 mmHg). The results shown in each panel are representative of more than ten experiments performed with each genotype. The changes in blood pressure for wild-type and *rgs2*$^{-/-}$ mice were compared at each time point. Statistically significant differences are indicated (*P < 0.01). (**d**) Western blot analysis of AT1 receptors in aortic extracts. AT1 receptor expression in aortae from wild-type rats and *rgs2*$^{+/+}$ (WT), *rgs2*$^{+/-}$, and *rgs2*$^{-/-}$ mice is indicated (upper panel). Antibody specificity was verified by probing of an identical blot with primary antibody that first had been incubated with blocking peptide (lower panel).

Figure 3

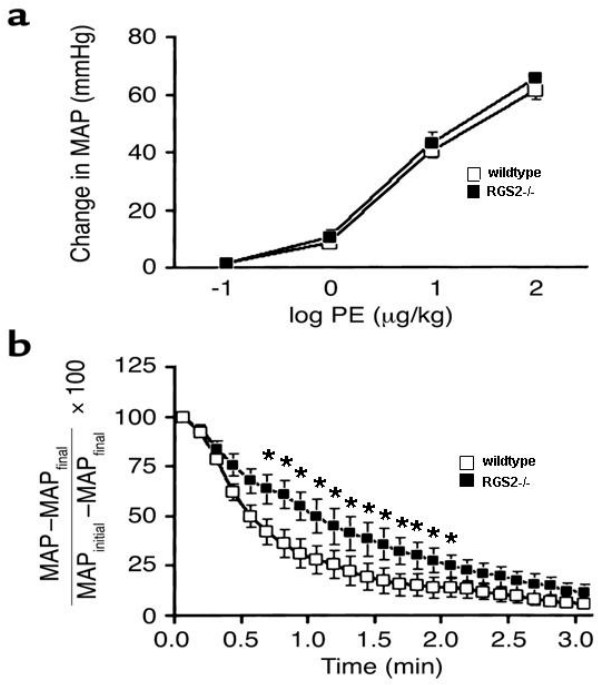


Figure 3. Vasoconstrictor dose-response relationships and kinetics of blood pressure decline in wild-type and *rgs2*$^{-/-}$ mice. (**a**) Dose-response relationships for blood pressure increase (change in MAP) upon challenge with increasing doses of the α-adrenergic vasoconstrictor phenylephrine (PE) in wild-type and *rgs2*$^{-/-}$ mice that had been pretreated with candesartan to decrease initial blood pressures to similar low basal levels. (**b**) Rates of blood pressure decrease [(MAP – MAP_{final})/($MAP_{initial}$ – MAP_{final})] ± SEM following challenge with a pressor dose of phenylephrine in wild-type and *rgs2*$^{-/-}$ mice pretreated with candesartan to decrease blood pressure to low basal levels. Statistically significant differences are indicated (*P < 0.01).

Outline a hypothesis to explain the consequences of the loss of RGS2 on vascular reactivity and tone. Design additional experiments to test this hypothesis in either intact animals or cultured vascular smooth muscle cells from WT and null mice.

How does the changes in vascular reactivity noted relate to the alterations in mean arterial blood pressure in intact mice (Figure 1)?

What is the significance of the heterozygous phenotype?

Question #7:

Your laboratory has recently received a strain of mice from a collaborator that demonstrates sleepiness, decreased heart rate, increased body weight, and indications of myxedema. Since your graduate mentor was expecting to receive wild-type mice from the collaborator, he is at a loss to explain the abnormal phenotype. You hypothesize that the collaborator sent the wrong cage of mice to you and that the mouse strain that was received has an abnormal thyroid status. To support your hypothesis, you perform radioimmunoassays to assess the levels of TRH in portal capillary blood and TSH, T_3 and T_4 levels in the systemic circulation.

Mouse #	TRH (nM)	TSH (nM)	T3 (nM)	T4 (nM)
1	17.6	1.2	0.3	14
2	12.9	2.2	0.5	9
3	14.2	0.9	0.3	11
Normal Range	3.0-5.5	7.3-12.1	1.1-3.1	70-160

1) Solely on the basis of the data presented above, provide **two** distinct hypotheses that could explain both the observed phenotype in the suspected mutant mice and the corresponding blood hormone levels.

To further examine these animals, you develop a bioassay in which portal capillary blood extracts from wild-type and "suspected mutant" mice are applied to a pituitary thyrotrope cell line and assessed for increases in phosphoinositide hydrolysis (helpful info: the TRH receptor couples to G_q/G_{11}) as shown below:

2) How do these new data affect the hypotheses that you presented in response to Question 1? Design a series of additional experiments that you would perform to determine the precise molecular nature of the defect in the suspected mutant animals.

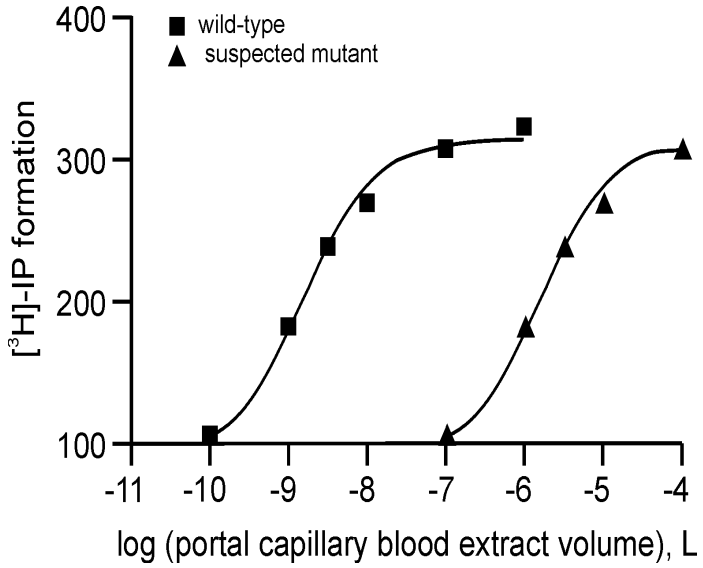


Figure 1. Functional analysis of TRH receptor signaling in a pituitary thyrotrope cell line in response to portal capillary extracts.

3) What effect would exogenous TSH administration have upon the phenotype of the suspected mutant mice as well as upon the circulating levels of TRH, T_3 and T_4?