Department of Pharmacology

Qualifying Examination (Part I)

December 16, 2004

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

Dopamine (DA) neurons of the ventral tegmental area (VTA) are known to receive projections from the cortex and midbrain. VTA slices are placed in an electrophysiological recording chamber and tyrosine hydroxylase (TH) positive neurons recorded with whole cell patch clamp techniques. A stimulating electrode in the slice triggers the excitatory postsynaptic currents (EPSCs) seen in Figure 1, recorded in the presence of the GABA receptor antagonist picrotoxin. The AMPA receptor antagonist DNQX and NMDA receptor antagonist APV are used to infer components of the evoked response.

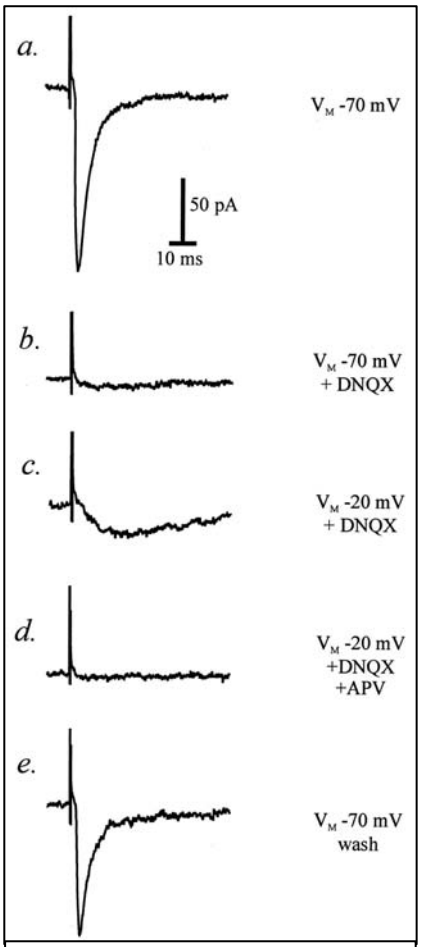


Figure 1

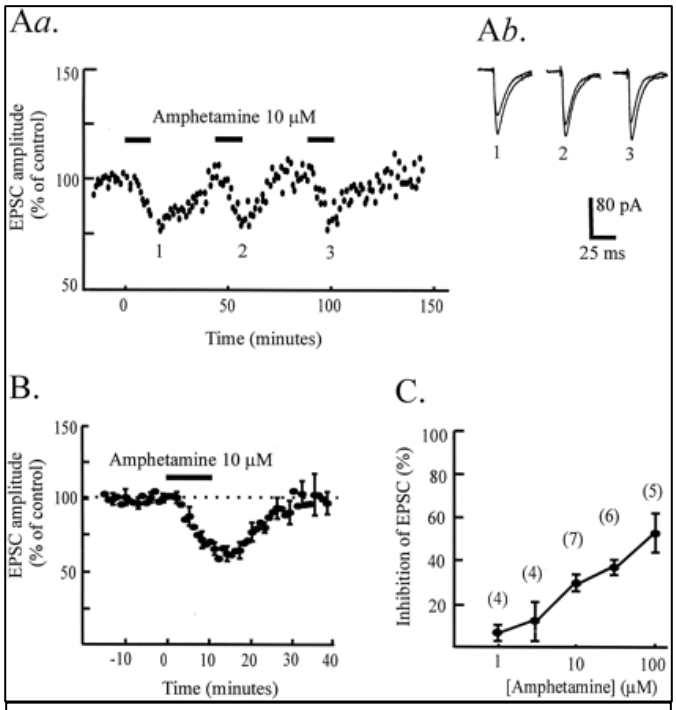


Figure 2

A) Describe what the studies with DNQX and APV reveal regarding receptors supporting synaptic transmission onto VTA neurons. Describe why a difference exists for responses when the VTA neuron is held at -70 mV versus -20 mV, in the presence of DNQX.

Cells are held at –70mV and D-amphetamine (AMPH) is applied to VTA slices during stimulation and a suppression of EPSC amplitude recorded in VTA neurons is observed (Figure 2a-d).

Question 1 (continued)

Although exogenously applied DA mimics the effect of AMPH, pretreatment of slices with various DA receptor antagonists (e.g. SCH23390, sulpiride) or the TH inhibitor AMPT fails to block the amphetamine effect (Figure 3A-C). In contrast, reserpine pretreatments completely block the effects of AMPH as does fluoxetine (Prozac™) and MDMA ("ecstasy") mimics the effects of AMPH.

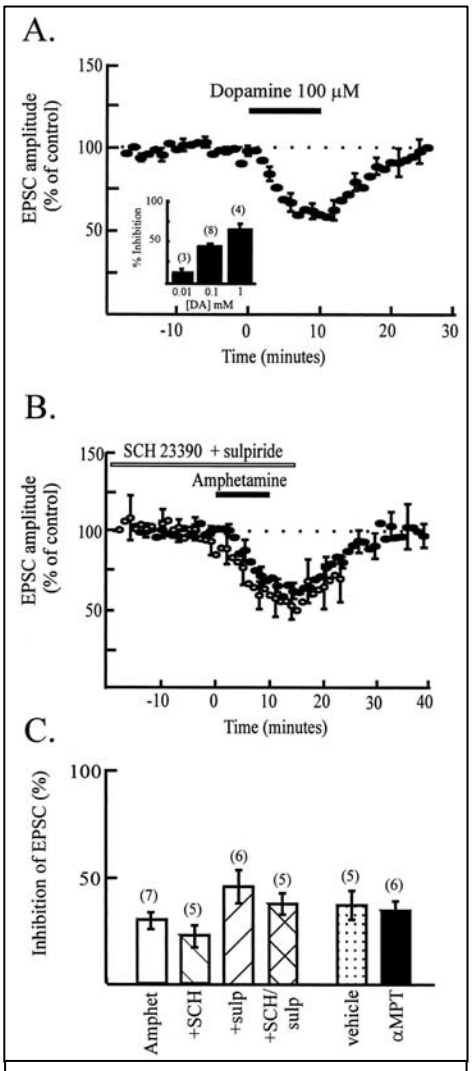


Figure 3

B) Propose targets for AMPH action, diagramming possible synaptic inputs onto VTA neurons that can explain your hypothesis. How might you test your hypothesis for a target for AMPH action?

C) Describe a mechanism within VTA cells or within excitatory terminals by which electrically evoked EPSC amplitude is reduced by AMPH treatment.

Question 2

Tubuloglomerular feedback (TGF) in an intrinsic renal mechanism for modulating glomerular filtration rate. There has been much debate about the soluble mediator of TGF with the two lead candidates: adenosine and ATP. The role of adenosine in mediating TGF is strongly supported by pharmacological and genetic evidence. Recent studies implicate ATP release by macula densa cells as an important mediating event in TGF. Reconciling these two ideas has been difficult.

German investigators recently published data obtained from mice homozygous for deletion of *CD73* encoding ecto-5'-nucleotidase, an enzyme responsible for the conversion of extracellular adenosine monophosphate (AMP) to adenosine. They examined TGF responses in wildtype (+/+) and knockout (–/–) mice (**Figure 1**).

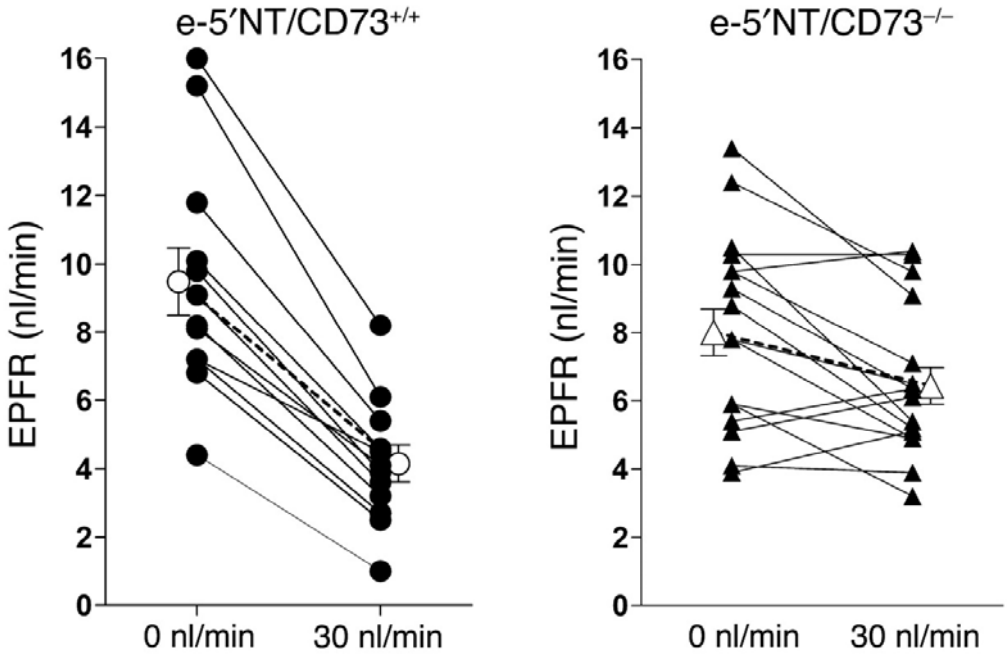


Figure 1 – TGF responses in wildtype (e-5'NT/CD73 +/+) and knockout (e-5'NT/CD73 –/–) mice in response to changes in perfusion rate of thick ascending limb of the loop of Henle (0 vs 30 nl/min). EPFR = early proximal tubular flow rate (a proxy for GFR). Mean ± SEM are provided as open circles for wildtype mice, and open triangles for knockout mice.

A. Explain the observation illustrated by data in Figure 1.

B. Formulate a hypothesis to account for these observations.

Question 2 – cont'd.

These investigators also examined the response of afferent arterioles from these mice to adenosine application (**Figure 2**).

C. Develop a simple model to explain the observations in Figure 1 and 2. By what approaches can you test your model.

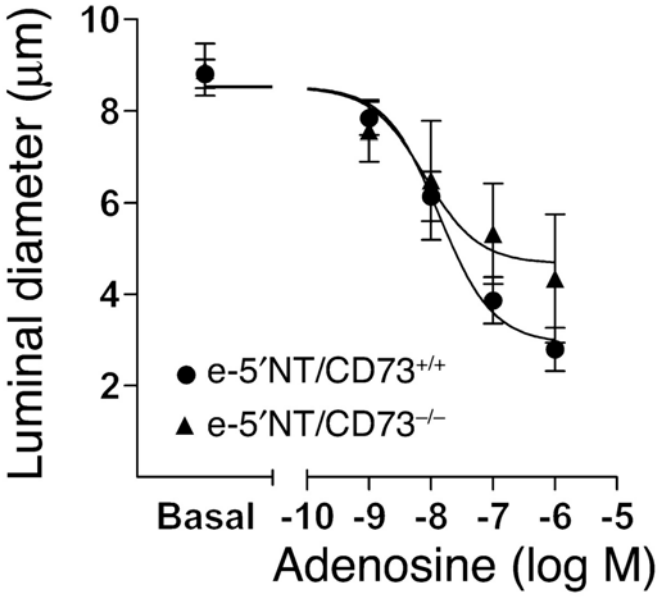


Figure 2 – Changes in afferent arteriolar diameter in response to adenosine. Differences between the two data sets are **not** statistically significant.

Question 3

After perusing through the last three years of *Nature*, you notice two articles describing homozygous mutant strains of mice with what appear to be identical phenotypes. These two mouse strains [shorty (*srt*), and tiny (*tny*)] are congenic (have identical genes except for the mutation) and are one-quarter the size of control animals. You decide that there must be a relationship between these strains and that examining the molecular basis of dwarfism might represent a good dissertation project. You contact the two labs that originally described these mice and ask that homozygous breeding pairs for each strain be sent to you at your laboratory address. Upon arriving at Vanderbilt, a thoughtless worker in the animal care facility inadvertently put both strains in the same cage. The F1 progeny resulting from this slight mishap are all of normal size. Further interbreeding of your F1 mice gives rise to a large F2 colony of animals that are either normal or one-quarter sized in a ratio of 1:1.

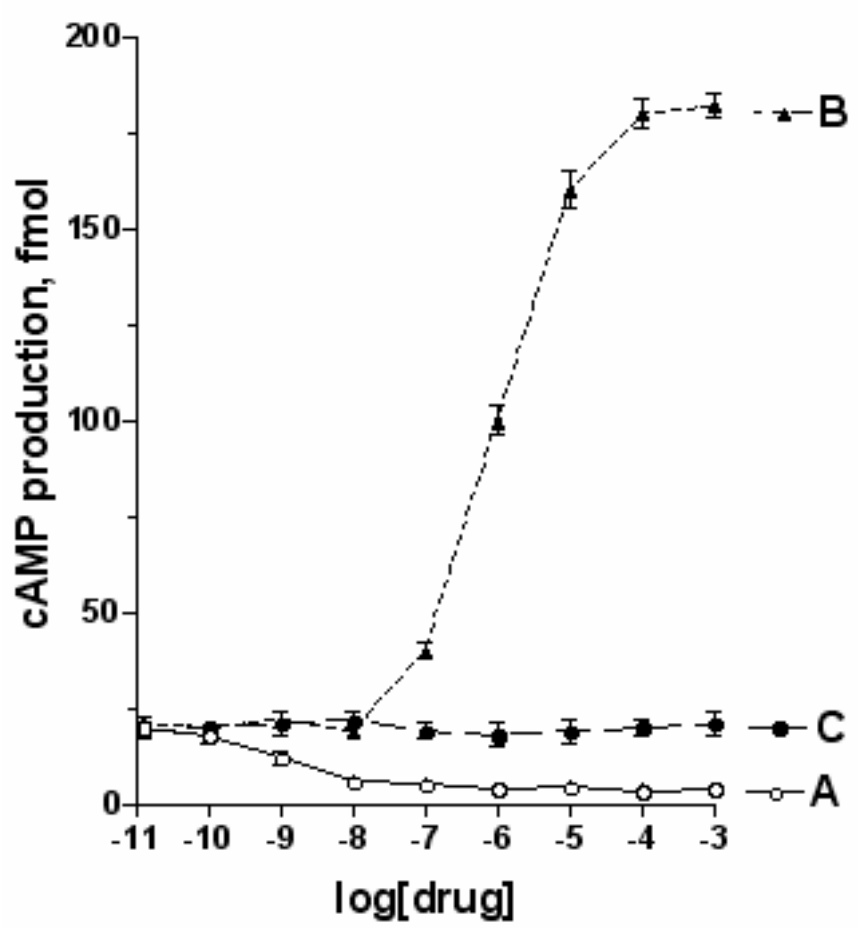
1) Based on this information alone, develop a hypothesis concerning the nature of these mutations and the relationship between them.

After working on this project for several months, a new article appears in *Science* demonstrating that homozygous *srt* mice expressing a growth hormone transgene are normal in size, while homozygous *tny* mice expressing the same transgene are not rescued by this genetic manipulation.

2) Develop a hypothesis for candidate gene(s) containing the *srt* and *tny* mutations and devise experiments to test it.

Question 4

You transfected cells that do not express endogenous β2-adrenergic receptor (β2AR) with an expression construct encoding wild type β2AR. The graph below depicts dose-response curves you have obtained with intact cells for three chemically related compounds, A, B, and C.



1. What working hypotheses regarding the pharmacological properties of compounds A, B, and C can you propose based solely on the data in this graph?
2. How can you ascertain that the effects you observe are mediated by β2AR? Suggest at least two independent approaches using the same cAMP production assay as readout and any additional compounds you may need.
3. Can you compare the potency of these compounds? Based on the data, what can you say about the affinity of A and B?
4. Using the same assay as a readout and any additional compounds you may need, how can you test whether compound C acts on the same receptor as compounds A and/or B? Suggest at least two independent methods.
5. If you find that compound C acts on the same receptor as B, how can you determine the affinity of C for the receptor using the same assay as readout?

Question 5

Both EGFR1 and EGFR4 are expressed in primary cultures of skin epithelial cells, based on Western blotting. When these cells were treated with NRG (neuregulin), cell proliferation (based on MTT assay) was observed. However, when treated with TGF-alpha (transforming growth factor-alpha), epithelial cells differentiated to become keratinocytes.

Based on your understanding of EGFR signaling, (A) propose TWO different mechanisms by which two different ligands exert two distinct effects (i.e. growth or differentiation). Please include in your discussion how each receptor (homodimers or heterodimers) is likely to be involved in controlling growth and differentiation of epithelial cells, (B) based on your hypothesis, design a series of experiments to address the role of each EGF receptor (homodimers or heterodimers) in regulation of growth or differentiation.

Question 6

At BIGPHARMA Ltd. you are part of a team evaluating the longterm behavioral effects of a 5HT2B agonist in mice. Surprisingly, you notice that after a few weeks of daily administration, mice develop labored respiration and do not exercise. Within two weeks of this observation the mice begin to die. The pathology report indicates pronounced myocardial thickening (hypertrophy).

You harvest 5 vehicle and 5 drug injected animals and obtain the data shown in Table 1 below.

Table 1

Group	Heart weight (mg)	Heart/body weight
Vehicle Injected	8.4 ± 0.4	6.2 ± 0.2
Drug Injected	13.7 ± 0.7*	9.6 ± 0.5*

*p<0.05 vehicle versus drug injected

A representative heart from each group above is depicted in Figure 1 below.

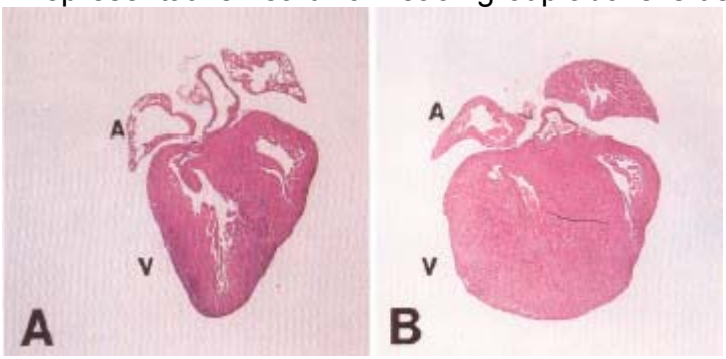
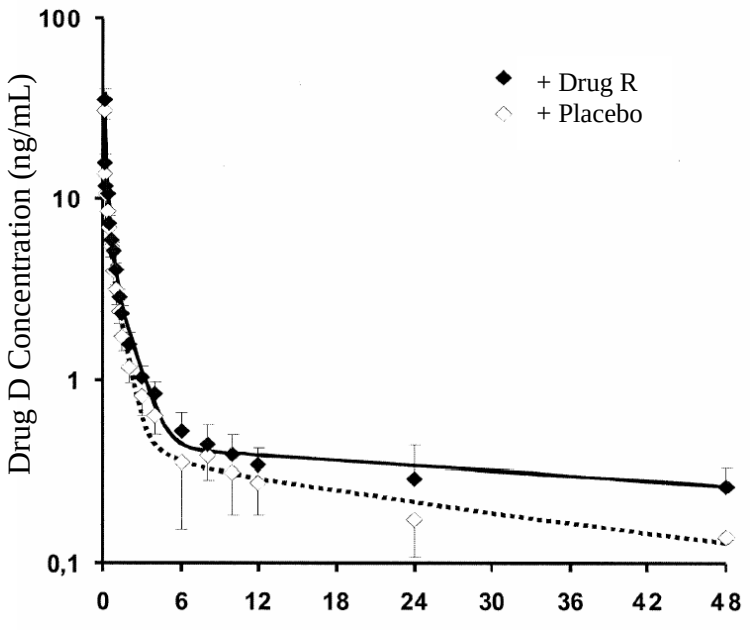


Figure 1. Cross section of 12 week old mice after administration of vehicle (A) or Drug (B) for six weeks. small A, atria; small V ventricle.

Outline two hypotheses to explain these results and both in vivo and in vitro experiments to test your hypotheses.

Question 7

The pharmacokinetics of Drug D (0.5mg) given intravenously was studied in individuals given concomitant oral administration of a placebo or Drug R pre-dosed to steady-state. The average plasma disappearance plot of unchanged Drug D is shown as follows:



Pharmacokinetic analysis of the Drug D data (mean ± S.D.) were performed:

Parameter	Placebo	Drug R	p-value[+]
AUC (ng.hr/mL)	22 ± 7	41 ± 17	p<0.01
CL (mL/min)	409 ± 30	238 ± 29	p<0.001
Vd_{ss} [#] (L)	255 ± 47	451 ± 60	p<0.001
$t_{1/2}$ (hrs)	16 ± 3	41 ± 9	p<0.01
Ae_{∞}* (ug)	273 ± 25	295 ± 33	p=0.15

[#] steady-state volume of distribution
* amount excreted unchanged in urine from time 0 to infinity
[+] comparison between Placebo and Drug R with Wilcoxon test

A. How do you interpret the pharmacokinetic differences between the two groups?
B. What mechanisms are possibly involved in the differences in clearance and volume of distribution between the two groups?
C. How would you design in vitro and in vivo experiments to test your mechanistic hypotheses?