Department of Pharmacology

Qualifying Examination (Part I)

June 21-24, 2005

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:

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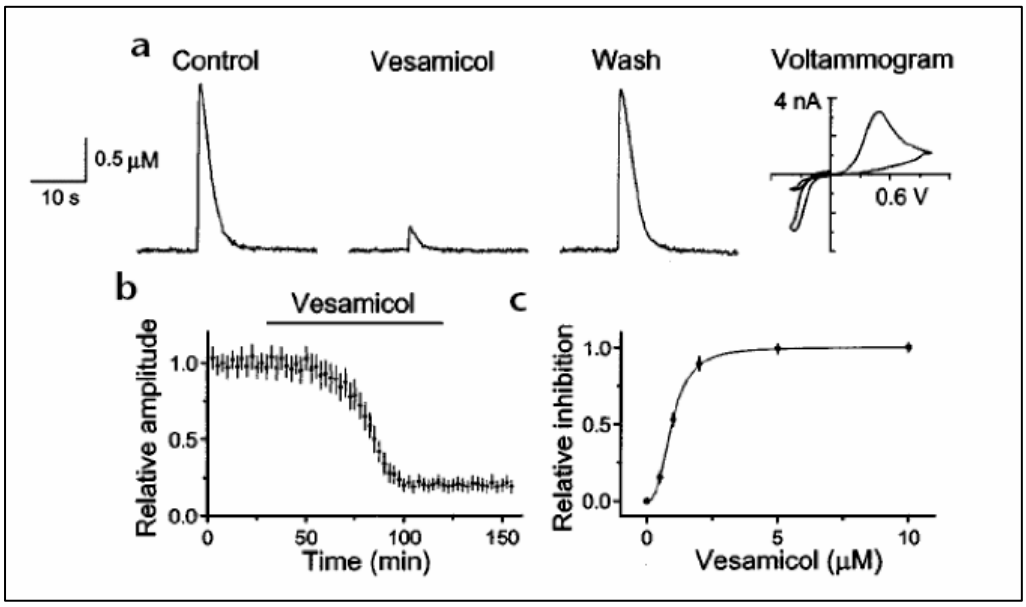
BEST WISHES FOR YOUR SUCCESSFUL COMPLETION OF THE EXAMINATION!

Question 1:

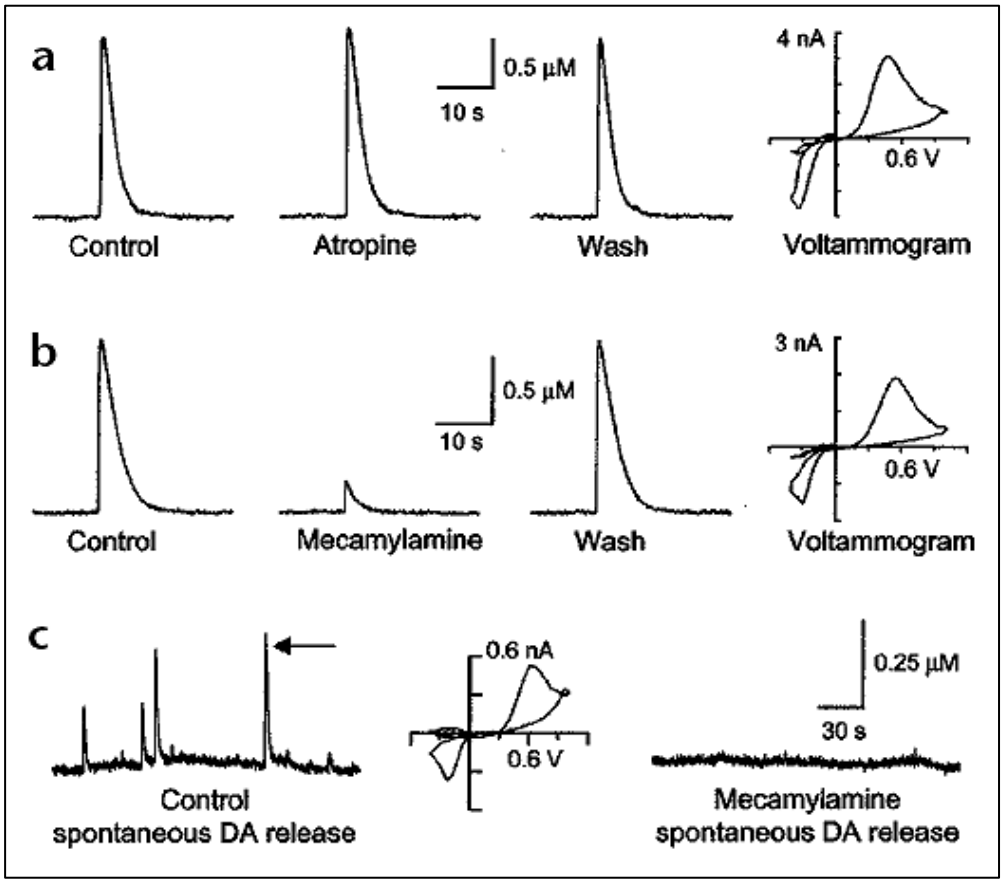
Nicotine is believed to exert its addictive effects, in part, through modulation of central dopamine (DA) release. Investigators exploring the central actions of nicotine on DA release have obtained the following data using ventral striatum brain slices and carbon fiber, voltammetric recordings.

Figure 1



In Fig. 1 A) brief (10 msec) electrical stimulation of slices in vitro is used to trigger release of DA (far right; cyclic voltammagram used to identify DA) in the presence or absence of the vesicular acetylcholine (ACH) transporter (VACHT) inhibitor vesamicol (2 µM, bath applied 2hrs prior to recording). In B and C, summary data for the effects of vesamicol are presented as a function of time and dose. Vesamicol is known to have a Ki for VACHT inhibition of ~1µM.

Figure 2



In Fig. 2, studies conducted as in Fig. 1 are repeated in the presence of the muscarinic antagonist A) atropine (1µM) or B) the nicotinic receptor antagonist mecamylamine (1µM). In C) drug effects on spontaneous DA release are recorded.

1) In addition to the cyclic voltammagram, what pharmacological/cellular manipulations could you use to prove that the voltammetric signals represent endogenous DA?

2) What do these data suggest about the role of ACH in supporting evoked DA release in the striatal slice preparation? Are these findings relevant to nicotine addiction?

3) What other molecular targets linked to ACH synthesis, release and inactivation could you manipulate to test a role for endogenous ACH in DA release?

4) Propose a synaptic model that could account for the actions of both vesamicol and mecamylamine on DA release. How would your model explain why the effect of vesamicol is delayed?

5) Describe the structure of receptors likely involved in ACH action on DA release. Define signaling pathways and targets by which ACH receptor activation could regulate DA release? How could these pathways be tested *in vitro* and *in vivo*.

Question 2:

Signals mediated by G-protein-linked receptors display agonist-induced attenuation and recovery involving both protein kinases and phosphatases. Many second messenger-dependent kinases and phosphatases are localized to subcellular structures via their interactions with A kinase-anchoring proteins (AKAPs). In contrast, the GRKs are recruited to the plasma membrane following agonist stimulation where they bind to and phosphorylate target GPCRs. A novel AKAP (AKAP-VU) was recently identified as an interacting partner for the β2-adrenergic receptor (β2-AR). To investigate the affects of GRK2, protein phosphatase 2B (PP2B) and AKAP-VU on β2-AR desensitization and resensitization, a group of investigators performed the following experiments.

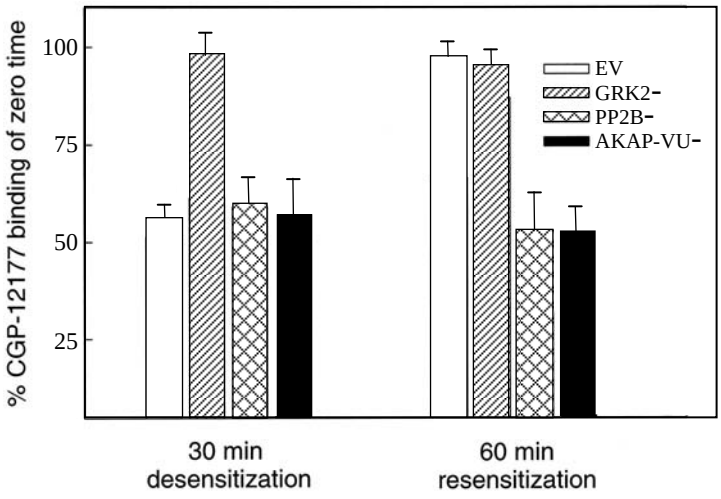


Fig. 1. Stable transfectants of human epidermoid carcinoma cells (A431) harboring an empty vector (EV) or a vector engineered to suppress the expression of GRK2 (GRK2-) PP2A (PP2B-) or AKAP-VU (AKAP-VU-) were studied 30 min following a challenge with 10 μM isoproterenol (desensitization) and again 60 min following a complete wash-out of agonist (resensitization). Receptor binding was assayed using trititated CGP-12177 a hydrophilic ligand for the β2-AR. The values presented are mean values +/- S.E. from at least three separate experiments.

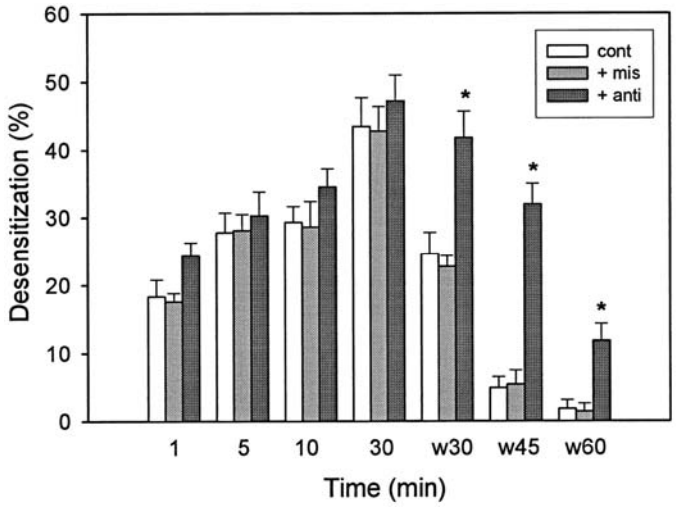


Fig. 2. A431 cells were treated with oligodeoxynucleotides antisense (*anti*) or missense (*mis*, same composition in scrambled order) to gravin (an AKAP family member) or with vehicle alone (*cont*). Treated cells were challenged with 10 μM isoproterenol to induce desensitization, which was monitored at 1, 5, 10, and 30 min after challenge. Fractions of the desensitized cells (treated 30 min with isoproterenol) were washed free of the agonist and the recovery from desensitization monitored at 30 (*w30*), 45 (*w45*), and 60 (*w60*) min after wash-out. The results are displayed as mean values +/- S.E. (n=4).

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

2. Design two independent experiments to test your hypothesis.

Question 3:

In rat PC12 cells, treatments with nerve growth factor (NGF) lead to differentiation of the cells manifested as neurite outgrowth. It is known that PC12 expresses three distinct yet related NGF receptors, trkA, trkB and trkC. You are assigned to investigate the role of each receptor isoform in NGF-dependent neurite outgrowth.

1. Select one receptor homodimer or/and heterodimer as the functional receptor in NGF-dependent differentiation of PC12 cells.
2. Design a series of experiments to support your hypothesis.

Your colleagues who study neuroblastomas in human ask you to investigate if mutations in the trkA receptor lead to neuroblastomas. You were given biopsy samples of tumors from three unrelated patients and by PCR you obtained the cDNA of trkA from these tumors. Nucleotide sequencing analysis revealed that about 50% of the cDNA contain wild-type trkA sequence and 50% contains mutations in the tyrosine kinase domain.

3. Why do these mutations act in a dominant manner as patients are heterozygote for the trkA mutation?
4. Describe the structure of tyrosine kinase and how mutations affect the kinase activity.

Question 4:

You have recently identified a strain of mice demonstrating physiological characteristics of Cushing's syndrome including excess fat accumulation, muscle atrophy, thin skin and alopecia. Hormone analyses of wild-type and mutant animals are indicated below:

	CRH (pg/ml)	ACTH (pg/ml)	Corticosterone (μg/ml)
mutant	1.2±0.6	11±4	452±23
wild-type	15±3	125±7	50±4

Given the elevated level of glucocorticoid production, you ask a trained pathologist to examine the adrenal glands from wild-type and mutant mice, but no obvious differences are detected.

A) Given your knowledge of the hypothalamic-pituitary-adrenal (HPA) axis, explain the regulatory interrelationships between the hormones indicated above and how the observed changes in mutant mice would lead to the physiological alterations characteristic of Cushing's syndrome.

B) Based upon the available data, propose two independent hypotheses that could explain the defect in mutant mice resulting in this Cushing's syndrome-like phenotype and propose a series of experiments that could be used to test your hypotheses.

C) Based on your hypotheses, propose potential strategies that could be used to treat this condition and ameliorate the phenotypic alterations.

Question 5:

The pharmacokinetics of Drug A were assessed during continuous oral administration of 500 mg once daily in 14 healthy subjects. Plasma drug levels were determined on the first dose (Day 1) and after 10 days of treatment.

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

Parameter	Day1	Day 10	p-value[+]
AUC (ng·hr/mL)	1696 ± 178	285 ± 119	$p<0.001$
CL/F (L/hr)	304 ± 75	729 ± 29	$p<0.05$
$t_{1/2}$ (hr)	2.9 ± 0.2	2.5 ± 0.02	$p>0.05$

[+] comparison between Day 1 and Day 10 with 2-tailed, paired t-test
AUC = area under the plasma concentration-time curve
CL = systemic clearance
F = bioavailability
$t_{1/2}$ = half-life

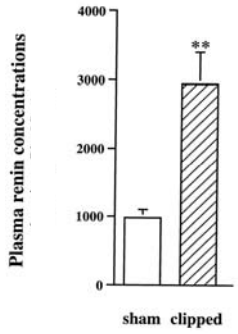
1. How do you interpret the pharmacokinetic differences between Day 1 and Day 10?

2. What mechanisms are possibly involved in the differences in AUC?

3. How would you design in vitro and in vivo experiments to test your mechanistic hypotheses?

Question 6:

A recent study by O. B. Juan Kenobe *et al*., examined mice in which one renal artery had been surgically constricted 4 weeks before. The degree of constriction was not severe enough to cause death of renal tissue (i.e., infarction). Blood pressure and circulating levels of renin were measured. The animals with the "clipped" renal artery exhibit a 20 mmHg greater mean arterial blood pressure than control (sham surgery) mice. Circulating levels of renin are illustrated in the figure below.



1. Explain the likely pathophysiology of elevated blood pressure in the "clipped mice". Emphasize differences between the clipped and unclipped kidneys especially with regard to glomerular hemodynamic parameters, GFR, tubuloglomerular feedback and Na balance.

2. Predict the effects of treatment with pharmacological agents that impact the renin-angiotensin-aldosterone system. Again, emphasize differences between the clipped and unclipped kidneys.

Question 7:

A screen for polymorphisms in the coding region of candidate genes in human heart failure identified an alteration in the β_1-adrenergic receptor at nucleotide 1165 that results in either an Arg or Gly at position 389. Dr. Ima Hart and colleagues generated transgenic mice that overexpress these two forms using the α-myosin heavy chain promoter that restricts expression of the transgene to ventricular muscle. Strains were chosen for comparison that displayed similar levels of total receptor expression (1.0±0.17 and 1.2±0.21 pmol receptor per mg protein) at both 3 and 6 months.

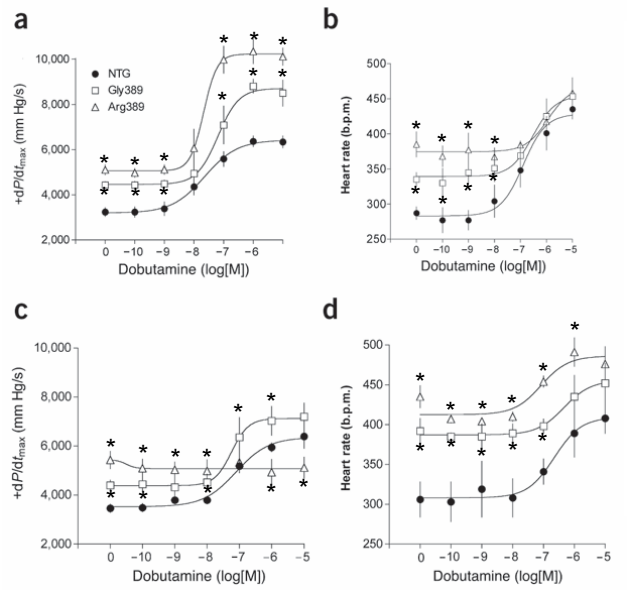


Fig. 1. Isolated heart preparations were prepared from transgenic animals at 3 and 6 months of age and the contractile and heart rate response to the β_1-adrenergic receptor agonist determined. (**a**,**b**) Work-performing heart studies at 3 months of age measuring contractility ($+dP/dt_{max}$) **a**) and heart rates (**b**). (**c**,**d**) Studies of contractility at 6 months of age (Arg389 mice when compared to NTG ($P < 0.01$, $n = 5$)). (**c**) and heart rate (**d**) Arg389, but not Gly389, β_1-AR transgenic mice (*$P < 0.01$ for comparison to NTG animals, $n = 5$ in all groups). NTG, nontransgenic. Symbols in **b**−**d** are same as in **a**.

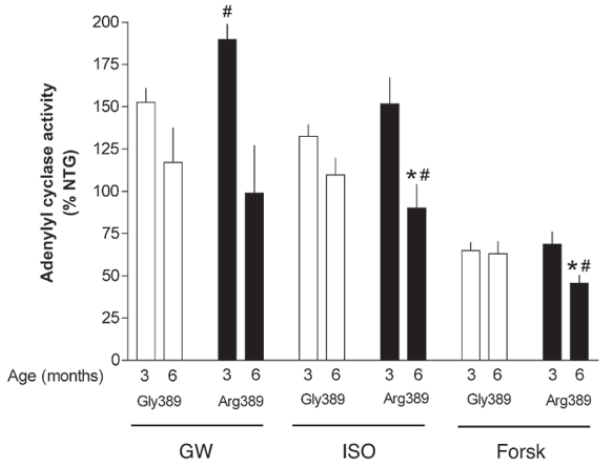


Fig. 2. Adenylate cyclase activity in ventricular membranes from Arg 389 and Gly 389 mice as a precentage of adenylate cyclase activity in NTG membranes. (**a**) Adenylate cyclase activity stimulated by agonist GW805415 (GW), isoproterenol (ISO), or. forskolin (Forsk). *, $P < 0.02$ for 3 months compared with 6 months, within genotype; #, $P < 0.02$ between genotypes of the same age (n = 5−9). NTG, nontransgenic. Data expressed as percent of nontransgenics.

Analysis of hearts at 6 months reveal marked sign of heart failure in ARG389 animals.

Please address the following points:

1) Describe the data presented in Figures 1 and 2.
2) Outline a hypothesis to explain these data in both 3 month and 6 month old mice
3) Outline a series of experiments to address your hypothesis *in vitro* and *in vivo*.
4) Why do these animals develop heart failure?