Question 1: If you could place microelectrodes into both a presynaptic and a postsynaptic neuron, how would you determine whether the synapse between them was chemically or electrically mediated?

Answer: A microelectrode placed in the presynaptic and postsynaptic neuron would show different results for electrical and chemical transmission. For electrical transmission, the two electrodes would show identical or similar changes in electrical activity, *i.e.*, the action potential in the presynaptic membrane would produce an action potential in the postsynaptic membrane. Chemically mediated synapses operate differently. An action potential in the presynaptic membrane causes neurotransmitter release in the synaptic cleft, and the postsynaptic membrane might respond to the neurotransmitter with changes in conduction, but not an action potential. An action potential is generated in the postsynaptic neuron of a chemically mediated synapse only if the whole neuron is sufficiently depolarized. This action potential would not be evident in a postsynaptic dendrite or cell body (typical synaptic sites); you would have to record for the axon of the postsynaptic neuron.

Question 2: List the criteria that are used to determine whether a chemical serves as a neurotransmitter. What are the various experimental strategies you could use to show that ACh fulfills the criteria of a neurotransmitter at the neuromuscular junction?

Answer: Three certain criteria must be met for a molecule to be considered a neurotransmitter.

1) The molecule must be synthesized and stored in the presynaptic neuron.

2) The molecule must be released by the presynaptic axon terminal upon stimulation.

3) The molecule, when experimentally applied, must produce a response in the postsynaptic cell that mimics the response produced by the release of the neurotransmitter from the presynaptic

neuron. Immunohistochemistry shows where specific molecules are localized, and *in situ* hybridization shows where specific mRNA transcripts for specific proteins are located. These methods could be used to demonstrate the presence of ACh in the presynaptic terminal at the neuromuscular junction. It would be useful to show that the synthesizing enzyme is present as well.

Question 3: What are three methods that could be used to show that a neurotransmitter receptor is synthesized or localized in a particular neuron?

Answer: Three methods are used to study the receptors of various neurotransmitters: neuropharmacological analysis of synaptic transmission, ligand-binding methods, and molecular analysis of receptor proteins. Neuropharmacological analysis studies the actions of different drugs. Ligand-binding methods can be used to identify the location of receptors by labeling ligands that bind to them, such as specific agonists, antagonists, or chemical neurotransmitters. Molecular analysis studies the protein molecules and the subunits that form the neurotransmitter receptors, such as transmitter-gated ion channels and G-protein-coupled receptors. This method may also be used to examine the genes that encode these proteins and the consequences of altering the genes or the gene products.

Question 4: Compare and contrast the properties of (a) AMPA and NMDA receptors, and (b) $GABA_A$ and $GABA_B$ receptors.

Answer: (a) AMPA and NMDA are glutamate receptor subtypes; both are activated by glutamate, but the drug AMPA acts only on the AMPA receptor and the drug NMDA acts only on the NMDA receptor. AMPA and NMDA are chemical agonists used to differentiate the glutamate receptor subtypes. Their antagonists can also distinguish receptor subtypes, for

example, the antagonist for AMPA is CNQX and the antagonist for NMDA is AP5. The differences in the receptors are related to slight differences in the protein. An important property of the NMDA receptor is that it is only active in the presence of glutamate *and* sufficient depolarization in the postsynaptic neuron.

(b) $GABA_A$ and $GABA_B$ are GABA receptor subtypes; both respond to GABA but muscimol is the agonist for the $GABA_A$ receptor, and the agonist for $GABA_B$ is baclofen. The antagonist for $GABA_A$ is bicuculline whereas the antagonist for $GABA_B$ is phaclofen.

Question 5: Synaptic inhibition is an important feature of the circuitry in the cerebral cortex. How would you determine whether GABA or Gly, or both, or neither, is the inhibitory neurotransmitter of the cortex?

Answer: Synaptic inhibition is represented by inhibitory postsynaptic potentials in the postsynaptic neuron of an inhibitory synapse. To determine whether GABA or Gly or both are inhibitory neurotransmitters, you could record IPSPs in response to GABA or Gly application in an *in vitro* preparation. You might also examine the nature of the postsynaptic receptors. Both GABA and Gly receptors gate a chloride channel, which when opened, would help hyperpolarize the postsynaptic cell and make that neuron less likely to fire an action potential.

Question 6: Glutamate activates a number of different metabotropic receptors. The consequence of activating one subtype is the *inhibition* of cAMP formation. A consequence of activating a second subtype is *activation* of protein kinase C. Propose mechanisms for these different effects.

Answer: The subtype of glutamate metabotropic receptor that inhibits cAMP formation may activate G_i. This is the mechanism used by the NE receptor subtype called α_2, which inhibits adenylyl cyclase and, consequently, inhibits cAMP formation. The other subtype of glutamate metabotropic receptor might activate a G-protein that stimulates the enzyme phospholipase C (PLC). PLC splits the membrane phospholipids PIP_2 into two parts: DAG and IP_3. DAG stays in the plane of the membrane and activates the downstream enzyme protein kinase C (PKC). (IP_3, on the other hand, diffuses away and causes organelles to discharge their calcium stores.)

Question 7: Do convergence and divergence of neurotransmitter effects occur in single neurons?

Answer: Diverging neurotransmitter effects are represented by the multitude of consequences a single neurotransmitter may have because it affects many different receptor subtypes in postsynaptic neurons. This effect may occur in a single neuron that possesses G-protein-coupled receptors with two or more intracellular functions or, potentially, single neurons that elaborate different types of receptors in different parts of the neuron. Convergence occurs when several transmitters affect a single effector system. This can occur in a single cell at the level of the G-protein, the second messenger cascade, or the type of ion channel. Neurons integrate divergent and convergent signaling systems, resulting in a complex map of chemical effects.

Question 8: Ca^{2+} ions are considered to be second messengers. Why?

Answer: Ca^{2+} ions are considered to be second messengers because elevations of Ca^{2+} ions in the cytosol can have widespread and long-lasting effects on the neuron. An example of this is

Ca^{2+} activation of the enzyme calcium-calmodulin-dependent protein kinase (CaMK), which is important in molecular mechanisms of memory.