OVERVIEW OF NEUROPHARMACOLOGY

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Introduction

For some, the word “pharmacology” may stir sensations of interest, excitement, and enthusiasm. However, for many, the topic brings to mind memories of attempts to learn seemingly endless lists of medications as well as vague visceral sensations of unpleasantness. While I may find myself leaning more towards the former category, I’m not a neuropharmacologist. My research background is in neurophysiology and my clinical interests are in pediatric epilepsy and sleep (this may explain why many of my examples below will involve anticonvulsants!). In general, pharmacology is the study of how the body acts on drugs and how they act on the body. Neuropharmacology – as we will use the term here – is a chance to discuss both the function of the nervous system as well as the ways in which drugs affect that functioning.

Pharmacology can be divided into pharmacokinetics – the study of drug absorption, distribution, biotransformation, and excretion – and pharmacodynamics – the study of how drugs work. Approaching this later category will require reviewing the basic signaling systems of the nervous system since these are the primary targets of the majority, though by no means all, of the medications prescribed by neurologists. Before turning to neurophysiology and its disruption by drugs, we’ll first review some basic topics in pharmacokinetics focusing specifically on concepts with clinical utility. Over the course of your clinical career you will be faced with many new drugs and incorporation of these agents into your practice will require some understanding of these principles.

Pharmacokinetics

Pharmacokinetics deals with the way in which drugs become biologically active, get to their sites of action, and are then eliminated. As a privileged site protected by the Blood Brain Barrier (BBB) the Central Nervous System is unique with regards to drug distribution. Brain capillary endothelial cells are tightly linked to each other in a way which significantly limits the distribution of water-soluble molecules from the plasma into the parenchyma. Note that highly lipid-soluble molecules and gasses are not limited by this barrier and that the entry of such drugs is a function of cerebral blood flow. While peri-capillary glia are closely associated with the endothelial basement membrane and are important for filtering substances from the CNS into the blood, they are not an important barrier to material flowing from the plasma into the parenchyma.

Key pharmacokinetic principles for our purposes include:

- Distribution
  - Bioavailability
  - Volume of distribution
    - Lipid solubility
    - Protein binding
- Clearance
  - Biotransformation
  - Elimination kinetics
- Bioequivalence

Bioavailability

Bioavailability, the extent to which a drug reaches its site of action, is particularly important when dealing with centrally-acting agents. If a drug such as an anticonvulsant is administered orally, it must be absorbed from the gastrointestinal tract into the vascular space then pass from the plasma into the CNS. Along the way to the cerebral circulation, it must not be substantially degraded by the liver. This is the “first-pass effect” in which enterically-absorbed molecules pass first through the portal circulation and are acted upon by the liver. If the avidity of the liver for the substance is great and it acts to transform the molecule into inactive metabolites then the substance will be rendered non-bioavailable (at least with oral absorption). One of the limitations of dihydroergotamine in its utility for treating migraine pain is a high first pass effect leading to poor oral bioavailability which necessitates parenteral administration. Many other neuro-active substances suffer from a similar limitation. Such factors affect the available routes of administration for drugs - particularly whether or not

enteral administration is possible. Even more specifically, enterally administered drugs are often absorbed from a particular segment of the GI tract and are best absorbed at a certain pH. Bypassing, for example, the stomach with a tube into the duodenum or jejunum, may significantly limit absorption of a drug designed to be absorbed in the acidic environment of the stomach. Similarly, co-administration of proton pump inhibitors, H2 blockers, or antacids can limit absorption. Warfarin, for example, is not well absorbed via jejunostomy tubes. Of course, avid uptake by the liver is very useful for drugs requiring bio-activation (those given as a pro-drug which is metabolized into the active compound) as will be discussed below.

Volume of distribution

While it is a bit of a confusing concept, volume of distribution is a convenient way to describe the amount of drug which remains in the plasma rather than passing into other tissues. Mathematically, the Volume of Distribution (Vd) is the amount of drug (D) in the body divided by the concentration of the drug in the blood or plasma (C): Vd=D/C. Note that if the drug remains entirely within the vascular space compartment then the Vd will be the volume of the blood or plasma:

1000 mg of Jim-314, a theoretical drug which stays entirely in the vascular space, is given and results in a blood concentration of 0.18 mg/mL.

Vd = D/C = 1000/0.18 = 5555 mL = 5.6 L

5.6 L is the approximate blood volume of a 70 kg adult male.

However, if the drug in question preferentially distributes to fat, for example, the plasma concentration will be very low and hence the Vd will be much larger than the blood or plasma volume:

100 mg of pentobarbital, which is highly lipid soluble, is given intravenously resulting in a blood concentration of 0.002 mg/mL.

Vd = D/C = 100/0.002 = 50,000 mL = 50 L

Another way to think of this is: in how much blood would the administered amount of medication have to be dissolved in order to result in the achieved final blood concentration. In the case of lipophilic pentobarbital, it would take about 10 times the normal blood volume of an adult 70 kg male to achieve the final concentration. This indicates that pentobarbital preferentially ends up outside the vascular space. In addition to lipophilicity, active transport into another compartment can underlie a large volume of distribution. For example, lamotrigine levels in the CSF are significantly higher than those in the plasma – which likely reflects active transport of lamotrigine into the CSF (lamotrigine's Vd is approximately 80 L in adults).

The extent to which a drug is bound to plasma proteins significantly effects the Vd since this binding largely prevents the drug from escaping the vascular space. Phenytoin is not very water soluble – which should result in a very high volume of distribution. However, consider the typical clinical scenario in which a 300 mg dose results in a blood level of about 15 µg/mL (0.015 mg/mL):

Vd = D/C = 300/0.015 = 20,000 mL = 20 L

Phenytoin is approximately 90% bound to plasma albumin (as well as other plasma proteins to a lesser extent). This keeps the total plasma concentration of phenytoin higher than it otherwise would be without this protein-binding and decreases the Vd. As you might imagine, measuring the *Free Phenytoin* blood concentration results in a much higher Vd than does the Total Phenytoin blood concentration. Another example would be a patient in liver failure with decreased protein production resulting in decreased protein binding.

Note that interactions between drugs can certainly change Vd. For example (picking on phenytoin again), valproic acid displaces phenytoin from its protein binding sites thereby increasing the *Free Phenytoin* blood concentration, which causes the volume of distribution of free phenytoin to decrease. However, the Vd of total phenytoin increases since more of the non-protein bound phenytoin can now leave the vascular space. Another example would be a patient in liver failure with decreased protein production or renal failure with protein wasting resulting in decreased protein binding.

Finally, values for Vd are typically normalized by dividing by body weight (generally in kilograms). One would therefore expect, based simply on this identity, that children would tend to exhibit a higher apparent (weight-normalized) Vd than adults (given that children usually weigh less than adults) and this is generally the case.

Biotransformation

As mentioned above, some drugs require modification before they become bioactive (that is, they are administered as pro-drugs such as fosphenytoin) and the majority of drugs are metabolically modified in some way in the process of elimination. This diverse set of biochemical processes are referred to as biotransformation. While most drugs undergo some form of modification, some - like gabapentin - do not. These processes largely occur in the liver and involve a wide variety of biochemical processes such as oxidation, reduction, and hydrolysis (together historically called “Phase 1” reactions with most catalyzed by the Cytochrome P-450 superfamily) and conjugation of the drug to another molecule such as an acetyl or methyl group or glutathione (called “Phase 2” reactions). For example, phenytoin undergoes aromatic hydroxylation, diazepam undergoes deamination, carbamazepine undergoes hydrolysis, and clonazepam is acetylated. Phase 2 reactions are very important for clearing many hydrophobic compounds since the conjugation reaction tends to produce a more water soluble (and therefore more easily transported and eliminated) compound.

Focusing on enzymes involved in Phase 1 reactions, a seemingly bewildering protean protein heap have been described and then named using a non-intuitive nomenclature system. In brief, members of the cytochrome P-450 superfamily all have names beginning with “CYP”. The letters and numbers which follow then refer to the family, subfamily, and gene of the particular enzyme – with the grouping based on primary structure (amino acid sequence similarity). So CYP2C19 is a cytochrome p-450 enzyme from family 2, subfamily C, and gene 19. Overall, 12 enzymes seem particularly important for drug metabolism in humans and one (CYP3A4) is involved in the metabolism of approximately half of all clinically used medications.

There is great genetic variability in the expression levels of these enzymes as well as many clinically-significant polymorphisms. Pharmacogenomics refers to the attempt to understand the genetic factors which may predict how a patient will handle a given medication or combination of medications. Current clinical applications are mostly to be found in psychiatry, oncology and in retrovirology. Yet genetic influences on the metabolism of mediations commonly used by neurologists are increasingly well recognized. For example, clinically significant polymorphisms have been recognized in both the cytochrome P-450 enzymes metabolizing Warfarin, as well as in its target (vitamin K 2,3-epoxide reductase). Such variability directly affects the dose needed to produce a certain INR. Clopidogrel is a pro-drug which requires enzymatic conversion to an active metabolite. Patients with lower metabolic activity of this crucial enzyme (CYP2C19) produce lower blood levels of the active metabolite and therefore are less likely to benefit from treatment with clopidogrel. Desmethylclobazam – a long half-life bioactive metabolite – is responsible for much of the somnolence associated with clobazam and the ratio of clobazam to desmethylclobazam is determined partly by CYP2C19 isoform.Though not widely used at present, it is likely that an ever-expanding number of pharmacogenomic tests will be available to clinicians as they try to select the best medication for their patient.

Biotransformation also represents a significant locus of drug-drug interactions. In general, cytochrome P-450 enzymes are inducible: that is, their expression can be increased by exposure to substrate. For example, carbamazepine is metabolized by CYP3A4 and induces increased expression of this enzyme which therefore decreases plasma levels of the parent drug. This auto-induction necessitates continued increases in dosage (approximately every week) until the process plateaus in about a month. Drugs can also increase the metabolism of other compounds which are processed by the same CYP enzyme. Estrogen and progesterone are both metabolized, in part, by CYP3A4 which means that induction of that enzyme reduces blood levels achieved by oral contraceptives – an important consideration in women taking hormonal contraceptives in combination with anticonvulsants such as carbamazepine. Drugs can also inhibit the metabolism of other compounds – either by competing for binding sites on the same enzyme or by altering the activity of the enzyme (competitive inhibition vs noncompetitive inhibition). Inhibition at the level of gene expression may also occur. Valproic acid decreases the clearance of lamotrigine thereby increasing plasma levels of the later. Because of this, one must titrate dose of lamotrigine upwards much more slowly in patients on valproic acid than those on other anticonvulsants or on lamotrigine alone. As more drugs are added to the neurologist’s pharmaceutical armamentarium, the potential for these drugs to interact grows increasingly rapidly. Indeed, given the vast number of potential interactions wise clinician’s will take advantage of the ready availability of electronic means to search for such interactions.

Elimination

The kidney is the final pathway for the excretion of most ingested and injected (but not inhaled) drugs – either as the unchanged parent compound or as biotransformed metabolites. Clearance is the pharmacokinetic expression of elimination and is important not only as the final disposition of the administered compound but also

in determining what dose should be given. To a first approximation, the steady state concentration of a drug is a function of dose, bioavailability, and clearance. Usually clearance is a “first order” process in which a given fraction of drug is eliminated per unit time. Some drugs, however, exhibit “zero order” elimination kinetics in which case a set amount of drug is eliminated per unit time regardless of the blood level. Phenytoin is notorious for, amongst other features, changing from first order to zero order elimination kinetics within the therapeutic range. The most likely mechanism underlying this change is saturation of the enzyme system responsible for phenytoin metabolism. To return to the concept of pharmacogenomics, this “inflection point” in the dose-clearance relationship is not the same for every patient and seems to be a reflection of, in part, genetic variability. It is important to monitor plasma levels when titrating phenytoin upwards.

Elimination half-life is defined as the amount of time it takes for the concentration of a drug to be reduced by half. While half-life is usually conveyed as a single number, it is important to remember that the elimination of a compound is often a rather non-linear affair and one may have different half-lives over time. It is also important to keep in mind factors which may alter half-life and therefore change optimal dosing strategies – given that approximately 4 half-lives are required to reach a steady-state concentration. In order to be eliminated a drug must be accessible to the enzyme system which metabolizes it (or to be filtered via the kidney). This means that volume of distribution – reflective of the amount of drug in plasma – is an important determinant of half-life. In this way, factors such as protein binding and lipid solubility again come into play. Also, elimination half-life can change with age due to a wide variety of factors. For example, phenobarbital’s very long half life in adults (about 100 hours) is even longer in neonates but is somewhat shorter and more variable in children. Illness can change both clearance as well as volume of distribution and therefore significantly affect half-life and this must be kept in mind when initiating or modifying therapy in hospitalized patients.

Bioequivalence

The concept of pharmacological bioequivalence underlies a current controversy in clinical epilepsy: are generic antiepileptics pharmacokinetically similar enough to be safely substituted one for another. In order to be approved a generic compound must have pharmacokinetic properties which are between 80 and 125% of the pre-existent brand-name drug. Pharmacodynamic equivalence is generally less of an issue given that the proposed generic compound is structurally identical to the brand name drug. Perhaps the most important pharmacokinetic principle in demonstrating equivalence is to compare the relationship between plasma drug level and time – often referred to as the Area Under the Curve (AUC). Of course, a tall skinny curve and a short broad curve can have similar areas so the AUC must be qualified by parameters such as the maximal concentration and the time to maximal concentration. Indeed, the more specific definition of bioequivalence for a generic drug is that the 90% confidence interval of the log-transformed ratios of AUC and Cmax should fall between 80% and 125% of that of the brand name preparation. Whether or not a 20-25% difference in pharmacokinetic properties makes a difference in therapeutic efficacy remains a matter of some debate. However, the issue is even more complicated in the modern marketplace with multiple approved generic formulations. In this case a patient may be switched from one generic to another generic which – although neither is more than 25% different from the brand name – may be well over 25% different from each other. This was well illustrated in a study by Krauss and colleagues (Annals of Neurology (2011); 70:221-228) who obtained (via a Freedom of Information Act petition) pharmacokinetic data (the Abbreviated New Drug Application (ANDA)) for generic antiepileptic drugs and analyzed simulated switches between these compounds. They found that 2% of generic pairs differed by more than 25% from each other (though not from the brand name medication). Since the generic formulation provided to patients can vary between refills, this may theoretically cause problems for patients whose epilepsy is under fragile control.

Pharmacodynamics

After this brief overview of pharmacokinetics, we’ll spend the rest of our time focused upon pharmacodynamics – the mechanism(s) of action of the drugs which we use to treat our patients. To begin with, we’ll review synaptic function and then turn to how these processes and pathways can be altered for therapeutic benefit in the treatment of epilepsy, movement disorders, and a variety of psychiatric conditions. The synapse is where communication or signaling occurs and the signals are, for the most part, neurotransmitters (we’re ignoring direct electrical synaptic connections via gap junctions as well as the effect of local electrical fields on the activity of neurons – “ephaptic effects” – since those are less important for our present pharmacological purposes). The basic synapse is composed of a pre-synaptic neuron, a post-synaptic neuron, and surrounding glial cells.

Pre-synaptic neurons contain:

- Enzymatic machinery to make neurotransmitter
- Packaging machinery to incorporate neurotransmitter and other factors which are co-released with the neurotransmitter (ions such as zinc, for example) into vesicles
- A mechanism to couple incoming excitatory signals (an action potential) to vesicular release of neurotransmitter. This step depends on calcium influx leading to the fusion of vesicles with the presynaptic membrane and subsequent release of neurotransmitter into the synaptic cleft.
- Receptors for neurotransmitters that are released. In pre-synaptic neurons, these regulate the release rate of the neurotransmitter; stimulation of the pre-synaptic receptor typically decreases subsequent release.
- Machinery to re-cycle both vesicles and neurotransmitters and protein scaffolding to hold them in place

Post-synaptic neurons contain:

- A signal transduction mechanism. Receptors for neurotransmitters receive the pre-synaptic signal and translate it into changes in post-synaptic neuronal activity. Receptors are of two types (true also for pre-synaptic neurons), ionophoric and metabotropic (G protein coupled) receptors. Ionophores include ligand-gated receptors that bind neurotransmitter, thereby changing shape and opening an ion-selective pore that causes excitation or inhibition. G protein coupled receptors bind neurotransmitters and thereby activate second messenger systems that affect neuronal activity via second messengers themselves or protein phosphorylation that leads to specific alterations in cell metabolism, structure and/or function.
- Ion-selective voltage-gated channels that open or close based on the voltage across the membrane and that allow excitation (influx of calcium or sodium) or inhibition (efflux of potassium). Excitation and inhibition summate with a resultant change in the probability that the neuron will fire an action potential.

The role of glia:

- Structural support for the synapse as well as synapse development
- Maintaining ionic homeostasis via ion uptake and buffering through the glial syncitium
- Inactivation of neurotransmitters via transporters or enzymatic breakdown. Neurotransmitters may also simply diffuse away, a slower process.
- Release of growth factors and neuromodulatory substances (“gliotransmission”).

THE VARIETY OF NEUROTRANSMITTERS

A large number of molecules have been postulated to be chemical transmitters. Those molecules that are established neurotransmitters have met the following four experimental criteria:

- Synthesized in the neuron.
- Present in the presynaptic terminal and released in amounts sufficient to exert its postulated action on the postsynaptic neuron or effector organ.
- When applied exogenously in appropriate concentrations, the substance exactly mimics the action of the endogenously released transmitter.
- A specific mechanism exists for removing the substance from its site of action.

Although neurons are diverse and highly specialized, a general observation is that a *neuron makes use of the same transmitter substance(s) at all of its synapses.* This is known as Dale's principle. It is important to note that this does not restrict a neuron to making only one neurotransmitter. Indeed, many neurons release two or more substances or "cotransmitters" from their synapses (see below).

There are two major classes of neurotransmitters. The first class consists of small molecules, in many cases amino acids or derivatives. These small molecule transmitters are made by enzymes within the presynaptic terminals and are subject to rapid production and turnover, with reuptake being the major inactivation mechanism. The transmitters may be recycled into new vesicles for additional rounds of synaptic transmission. Many are released with other active molecules; for example, zinc is released with amino acid transmitters and can substantially affect the activity of ionophore receptors.

Small Molecule Transmitters:

- Acetylcholine
- Catecholamines
    - Dopamine
    - Norepinephrine
    - Epinephrine

- Serotonin
- Histamine
- Excitatory Amino Acids
    - Glutamate
    - Aspartate
- Inhibitory Amino Acids
    - Gamma-aminobutyric acid (GABA)
    - Glycine

The second major class of neurotransmitters is neuroactive peptides of which more than 50 pharmacologically active molecules have been described. Many exist in synaptic vesicles together with small molecule transmitters or ions that are "cotransmitters." For example, the peptide CRGP (calcitonin gene-related peptide) is released with acetylcholine by lower motor neurons at the neuromuscular junction. Neuroactive peptides are produced in the cell body by gene transcription and translation followed by proteolysis and other processing steps in the ER and Golgi apparatus. Once formed, these peptides are packaged into vesicles and transported by fast axonal transport to synaptic terminals.

A third class of neurotransmitters does not fit the major criteria, but can still be considered neurotransmitters under certain conditions. These include nitric oxide, eicosanoids, and adenosine. Such neurotransmitters are not stored in vesicles but are synthesized upon demand, either in the synapse (adenosine) or in the presynaptic terminal and passively diffuse across the synaptic membranes (NO and eicosanoids). Endocanabinoids are also non-peptidergic neuromodulatory molecules which can act in a retrograde (post-synaptic to pre-synaptic) manner (as can eicosanoids like arachidonic acid). Other non-canonical neuromodulators include molecules of the immune system such as TNFα and growth factors such as TGF-β1. Such molecules serve as a linkage point between processes such as inflammation and development and synaptic function.

SIGNAL TRANSDUCTION AND NEUROTRANSMITIER RECEPTORS

As mentioned, there are two general classes of neurotransmitter receptors, the ligand-gated ion channel receptors that subserve fast signal transduction, and the G protein coupled receptors that subserve slower transduction. In the ligand-gated ion channel receptors, binding of neurotransmitter results in a conformational change that allows the selective flow of ions across a previously established gradient. Depending on the specific channel and the ions that flow, this may constitute an excitatory (i.e. depolarizing) or inhibitory (hyperpolarizing) event.

Binding of a ligand to a G protein coupled receptor initiates a series of events that result in generation of "second messengers" within the cell. These events include binding of GTP to a receptor associated G-protein which in turn activates an "Effector" that is responsible for generation of the second messenger.

Examples of Some G Protein Coupled Receptors

Neurotransmitter	Norepinephrine	Acetylcholine
Receptor	Beta-adrenergic Receptor	Muscarinc Receptor
Transducer	Gs	Go
Primary Effector	Adenyl Cyclase	Phospholipase C
Second Messenger	cAMP	IP3 and DAG
Secondary Effector	cAMP-Dependent Kinase	Calcium release, Protein Kinase C

The elaboration of second messengers can bring about a large variety of effects ranging in onset from seconds to hours. Sometimes these second messengers act directly on ion channels. For example, light activates the production of cGMP in photoreceptor cells which in turn directly opens cation-selective channels. In other instances, these second messengers activate protein kinases which results in

phosphorylation of cellular proteins as diverse as the neurotransmitter receptor itself (this is one mechanism used to desensitize many G protein- linked receptors) and transcription factors. In this latter case "neurotransmission" results in changes in gene expression.

Some neurotransmitters can act at multiple different receptor sites. Glutamate, the major excitatory neurotransmitter in the CNS, acts at both ligand gated (NMDA, AMPA) and G protein coupled ("metabotropic") receptors. Similarly, GABA, the major inhibitory neurotransmitter acts at both ligand gated ($GABA_A$, "$GABA_c$") and G protein-coupled $GABA_B$ receptors.

NEUROTRANSMITTERS & RECEPTORS, AND THEIR ACTIONS

In the sections that follow, each class of neurotransmitter will be described with respect to 1) production; 2) mechanisms for removal from 3) localization and primary actions; 4) receptors; and in most cases, 5) specific examples related to clinical uses.

Acetylcholine

Structure, Production, and Inactivation.

- **Choline acetyltransferase** acts on choline and acetyi-CoA in the nerve terminal to produce acetylcholine (ACh):
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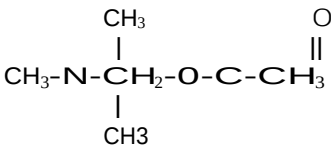


- ACh is packaged in specialized small vesicles that accumulate near the active zone of the nerve terminal.
- **Acetylcholinesterase** rapidly degrades Ach in the synaptic cleft; this is a major regulator of signaling duration. Some nerve gases and insecticides act by blocking this enzyme, causing death by tetanic paralysis; milder forms are useful in myasthenia gravis and dementia).
- Choline is rapidly taken up by the nerve terminal and utilized in the production of new transmitter

Anatomical Localization, Receptors, and Actions.

- Neurons that use acetylcholine as a neurotransmitter are known as cholinergic neurons. A wide variety of cholinergic neurons exist both centrally and peripherally. Those neurons arising centrally and synapsing peripherally use Ach.
- ACh is the primary neurotransmitter for all lower motor neurons, including those of the brainstem cranial nerve nuclei and the ventral horn of the spinal cord.
- These cells innervate skeletal muscle and the synapse where ACh is released is the **neuromuscular junction (NMJ).**
- This highly specialized synapse has large amounts of acetylcholinesterase so that rapid and dynamic changes in signaling can take place.
- The receptors at the NMJ are known as **nicotinic receptors** (nicotine is an agonist at these receptors). These receptors are fast ligand-gated ion channels that upon activation lead to depolarization at the NMJ. In turn, this causes a massive and coordinated release of calcium in the muscle cells resulting in contraction.
- Nicotinic receptors are part of a larger family of ligand-gated receptors that are comprised of 5 subunits, with each type of subunit having multiple variants. The binding of agonist induces a conformational change such that the ionophore opens and (in this case) Na+ rushes in to depolarize the cell.
- Preganglionic cell bodies of the sympathetic and parasympathetic systems, residing in the intermediolateral column of thoracic spinal cord and in brainstem and sacral spinal cord, respectively, are also cholinergic.
- The postsynaptic receptors in autonomic ganglia are classified as nicotinic, but arepharmacologically distinct from nicotinic receptors at the NMJ.

- All postganglionic parasympathetic nerves innervating the viscera (i.e. heart, bronchi, gastrointestinal tract, bladder, eye, and exocrine glands) and postganglionic sympathetic nerves that innervate sweat glands (all other postganglionic sympathetics rely on norepinephrine, see below) are cholinergic.
- The postsynaptic receptor for acetylcholine in these nerves is a G-protein coupled receptor known as the **muscarinic receptor** (muscarine is an agonist). These receptors mediate slow (seconds), modulatory responses at parasympathetic neuroeffector synapses. These include slowing of the heart; smooth muscle constriction in bronchi, stomach, intestines, bladder, and eye; and stimulation of exocrine gland secretion.

Central cholinergic pathways include:

- Cell bodies in the ventral forebrain represented by the septum, diagonal band of Broca, and nucleus basalis (of Meynert) that project to hippocampus, interpeduncular nuclei, and neocortex, respectively. These projections are important for cortical activation and memory processing.
- Cell bodies in the brainstem tegmentum (midbrain and pons) that innervate the hypothalamus and thalamus. These pathways activate thalamocortical centers involved in arousal and REM sleep.
- Short interneurons located primarily in the striatum, but also found scattered in cortex and other areas. In the striatum these interneurons participate in control of movement.
- The great majority of central acetylcholine receptors are muscarinic, but *several subtypes* exist, each associated with a different effector protein.

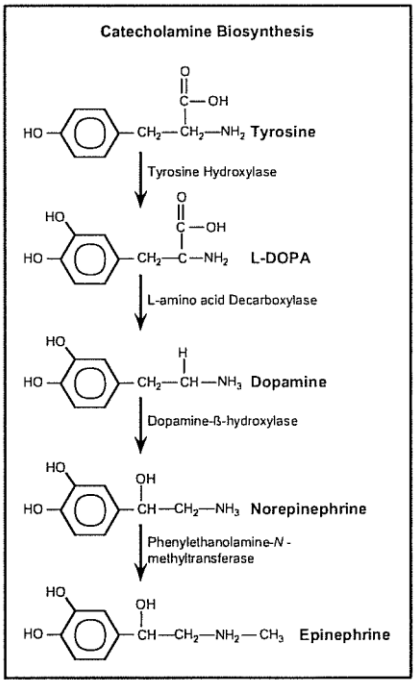
Clinical Relevance.

- Inhibitors of acetylcholinesterase can increase the amount of neurotransmitter at the neuromuscular junction and are useful in the treatment of **myasthenia gravis,** an autoimmune disorder where patients harbor antibodies to acetylcholine receptors and present with weakness characterized by extreme and early muscle fatigability. If given in proper amounts, acetylcholinesterase inhibitors can produce some improvement in muscle strength. They were often used (less so currently) in diagnosing the condition (Tensilon test).
- Acetylcholinesterase inhibitors that preferentially act in the CNS have been found to be helpful in symptomatic improvement of memory function in Alzheimer's disease.
- Drugs that block muscarinic receptors (e.g. atropine) can alleviate tremor of Parkinson's disease, presumably by counterbalancing some of the effects of decreased striatal dopamine (the primary neurochemical deficit in this disease).
- An effective treatment for dystonia (abnormal and often disabling contraction of muscles) is the injection of botulinum toxin into affected muscle groups. In correct doses, this potent toxin provides a prolonged partial weakening of muscles by directly inhibiting the release of acetylcholine from nerve terminals.

Catecholamines and other Amine Transmitters

Structure, Production, and Inactivation.

- The catecholamine transmitters, dopamine, norepinephrine, and epinephrine, are all derived from the amino acid tyrosine in a common pathway (Figure), but the sites and actions of each are unique.
- Tyrosine hydroxylase is the first and rate-limiting enzyme in the catecholamine biosynthetic pathway.
- Levels of active enzyme are modulated by phosphorylation and feedback inhibition by endproducts.
- The specific neurotransmitter made depends on the enzymes that a neuron contains.
    - **Dopaminergic neurons** have L-aromatic amino acid decarboxylase, but lack the remaining enzymes in the pathway.
    - **Noradrenergic neurons** are those cells that have the capacity to synthesize norepinephrine
    - **Adrenergic neurons** of the brain and adrenal gland medullary cells convert about 80% of norepinephrine to epinephrine.
- Each of the catecholamines is stored in specific granules at the nerve terminal.
- Catecholaminergic transmission is terminated by active high affinity reuptake of released transmitter into the nerve terminal and by enzymatic degradation.
- Specific transporters terminate neurotransmission and help recycle catecholamines.
- *Cocaine* blocks catecholamine reuptake, leading to prolonged activation of central receptors. Various therapeutic drugs have this action also; some are relatively specific for particular catecholamines
- The enzymatic degradation of catecholamines involves two types of enzymes: **monoamine oxidases** and **catechol-0-methyltransferase (COMT).** Monoamine oxidases are located in mitochondria and are responsible for inactivating catecholamines that are free in the terminal after reuptake. COMT is a cytoplasmic enzyme found in a wide variety of cell types



Dopamine:
The actions of these two degradative enzymes lead to the principal metabolites of the catecholamines. These metabolites, including homovanillic acid (arising from dopamine), 3-methoxy-4-hydroxy- phenylethyleneglycol (arising from norepinephrine in the CNS), and vanillylmandelic acid (from norepinephrine in the periphery), can be measured in the blood or urine as indicators of catecholamine production.

Dopaminergic neurons are of three major types.

- **Interneurons** with very short axons are located in peripheral autonomic ganglia and in the retina and olfactory bulb. These interneurons modify sensory input by inhibiting their target neurons. An important example is lateral inhibition in the retina which is a critical and early step in visual image processing.
- Neurons with intermediate-length axons located in the **tuberoinfundibular area** and in the **lateral hypothalamus.** The tuberoinfundibular neurons are responsible for inhibiting the production of prolactin by the anterior pituitary (and are the inadvertent target for antipsychotics that block dopamine receptors; galactorrhea is side effect). Dopamine reaches the anterior pituitary via the portal vasculature, so it behaves as a local neurohormone rather than at a synaptic cleft. Many pituitary tumors produce prolactin and symptomatic relief as well as reduction in tumor size can be accomplished by treatment with bromocriptine, a drug with dopaminergic properties.
- Neurons in the midbrain ventral tegmentum and substantia nigra with long axonal projections. The dopaminergic neurons of the **ventral tegmentum** project to many parts of the limbic system, including the nucleus accumbens, olfactory tubercule, septum, amygdala, and frontal and cingulate cortex. This mesolimbic system is associated with mood alterations and cognitive function. The release of dopamine in the nucleus accumbens generates positive reinforcing feelings and it is here that the stimulant and addictive properties of cocaine (blocks dopamine reuptake) and amphetamines (enhances dopamine release) appear to reside. The nigrostriatal system, with cell bodies in the **substantia nigra** provides dopaminergic input into the caudate and putamen. This input modulates the motor planning and execution functions of the basal ganglia. Loss of these cell bodies is a key feature of Parkinson's disease.

Dopamine receptors fall into five classes:

- D1-5 all G-protein coupled receptors, with differing actions and divided into D1-like family and the D2-like family.
- D1 (high in striatum) and D5 receptors have a lower affinity for dopamine and binding activates adenylyl cyclase, stimulating cAMP formation
- D2-4 receptors have a high affinity for dopamine and they inhibit adenylyl cyclase and cAMP production.
- The D2 receptor is primarily responsible for inhibiting sympathetic ganglion activity, prolactin secretion, and acetylcholine release from striatal neurons.

Norepinephrine and Epinephrine

- In the periphery, norepinephrine is the primary neurotransmitter of postganglionic sympathetic fibers.
- Centrally, norepinephrine containing neurons are found in the **locus ceruleus, located** in the dorsal pons, and in the **lateral tegmental nuclei,** located in the pons and medulla. Projections from the locus ceruleus are highly branched and innervate regions controlling responses to external stimuli, including cerebral cortex, hippocampus, thalamus, cerebellum, and sensory and motor nuclei of the brain stem and spinal cord. Areas involved in autonomic and neuroendocrine control, such as the hypothalamus, are innervated by projections from the lateral tegmental nuclei. These two systems work together in response to challenging internal or external stimuli to provide activation of the sympathetic system (lateral tegmental projections) and arousal and attention (locus ceruleus).
- Epinephrine containing neurons are restricted to the brain stem and are found in **lateral and dorsal tegmental nuclei.** These cell bodies send axons to the hypothalamus, the locus ceruleus, and the intermediolateral cell column in the spinal cord. These latter neurons are the cell bodies for the preganglionic sympathetic nerves.
- In the periphery, epinephrine is released by adrenal medullary chromaffin cells in response to sympathetic stimulation. The main role of epinephrine in the CNS is thought to be related to regulation of autonomic and hypothalamic function.
- The terminals of noradrenergic and adrenergic neurons are very different from the discrete synapses of many other neurotransmitters. They exist as boutons (swellings) located all along the length of the axon, with each bouton representing a site of transmitter release. This arrangement serves to diffusely "bathe" postsynaptic targets with transmitter.

- There are two major subdivisions of adrenergic receptors, α- and β -adrenergic receptors, each with pharmacologically distinct receptor subtypes, all G protein coupled.
- α1 acts postsynaptically via the activation of phospholipase C eventually leading to calcium release and activation of protein kinase C
- α_2 downregulates cAMP and thus is inhibitory (sites of action include central neurons and presynaptically on sympathetic and parasympathetic nerves)
- β -adrenergic receptors are coupled to G proteins (Gs) that increase levels of cAMP.

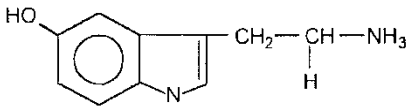
Many useful drugs have been developed as agonists or antagonists of these receptors:

- Clonidine, a specific a_2 receptor agonist, is useful for high blood pressure because it decreases sympathetic norepinephrine release.
- β_1 receptors are located in heart and mediate increased heart rate in response to sympathetic stimulation; antagonists are useful in the treatment of angina.
- β_2 receptors are found in smooth muscle including bronchial smooth muscle; beta-2 agonists are used to treat asthma, for example

The symptoms of Parkinson's disease may be effectively treated, most successively in the early stages, by providing the immediate precursor of dopamine: L-DOPA. This compound readily crosses the blood-brain-barrier and is processed to dopamine by the remaining dopaminergic nerve terminals in the striatum (arising from substantia nigra). Because L-OOPA is rapidly broken down in the periphery, it is always given with a second compound, **carbidopa,** which serves to block enzymatic degradation and does not gain access to the central nervous system.

Serotonin

Serotonin is an indole amine (See figure below) which is synthesized from the amino acid tryptophan by enzymes similar to those involved in catecholamine biosynthesis. Tryptophan hydroxylase is the first enzyme in the process; its activity can be regulated by second messenger systems but not by end product inhibition. This means that as more tryptophan enters the brain (e.g. due to diet or nutritional supplementation), more serotonin is produced.



Serotonin

The major pathways for removal of serotonin from the synapse are similar to those for catecholamines: reuptake into the nerve terminal and degradation by monoamine oxidase The major end product of serotonin degradation is 5-hydroxyindole acetic acid (5-HIAA).

Serotonergic neurons are located in the **Raphe nuclei** in the midline reticular formation of the brain stem.

The rostral Raphe in the upper pons and midbrain send projections to the basal ganglia, thalamus, hypothalamus, limbic system, and cortex. Caudal Raphe nuclei in the lower pons and medulla project to the medulla and spinal cord.

Serotonin receptors are diverse and include 3 major families:

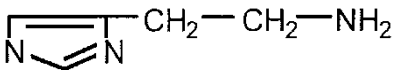
- 5-HT_1 receptors (of which there are at least 3 varieties) are predominantly inhibitory and decrease levels of cAMP
- G protein coupled 5-HT_2 receptor activates phosphoinositol metabolism and causes depolarization of cortical neurons
- 5-HT_3 receptor is a ligand-gated ion channel that causes depolarization

Serotonergic projections are widespread, but are relatively poorly understood. In the cortex and thalamus they are believed to be involved in sensory processing and regulation of the sleep-wake cycle. Serotonin is likely to be important for homeostasis via influences on autonomic centers and the spinal projections are involved in

central mediation of pain sensation. Serotonin clearly plays a significant role in mediation of mood as demonstrated by the effectiveness of serotonin reuptake inhibitors such as fluoxetine in the treatment of depression. Monoamine oxidase inhibitors may be helpful also.

Histamine

Although the case for histamine serving as a neurotransmitter is less well established than for other molecules mentioned here, it is produced by select groups of neurons and has specific effects in the CNS. Histamine is produced from the amino acid histidine:



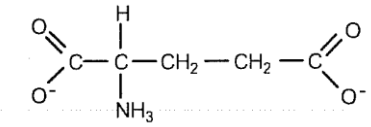
- Cell bodies for histamine lie in the lateral tuberomammillary nucleus of the hypothalamus and project widely to cerebral cortex, limbic system, hypothalamus, basal ganglia, brain stem and spinal cord.
- Histamine receptors of several types are widespread in the periphery where they mediate a variety of responses. Centrally, the H1 histamine receptor is a G-protein coupled receptor that activates phospholipase C resulting in eventual release of calcium and activation of protein kinase C. The sedative effects of anti-histamines result from blockade of these central receptors suggesting that histamine plays a role in arousal.

Excitatory Amino Acids

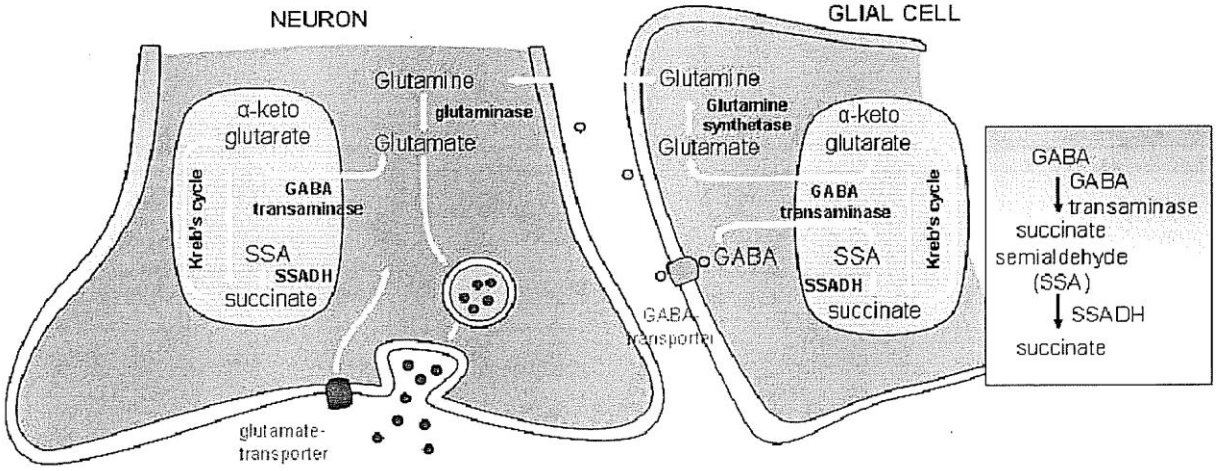
Structure, Production, and Inactivation

The amino acid **glutamate** is the major excitatory transmitter in the brain. Virtually all fast excitation in the CNS is mediated by glutamate. The related amino acid aspartate may also serve in fast excitation, but its role *in vivo* is less well-defined.

Glutamate is produced from alpha-ketoglutarate, an intermediate formed during the metabolism of glucose in the Kreb's cycle. It can also be synthesized from glutamine or aspartate.



Glutamate is rapidly removed from the synapse by active transport by both neurons and astrocytes. Astrocytes may be particularly important in this regard; they have a high affinity glutamate transporter and contain high levels of an enzyme called glutamine synthetase which converts glutamate to glutamine. Glutamine produced by astrocytes can be taken up by neurons which convert it back to glutamate. This recycling pathway is critical to effective use of glutamate as a neurotransmitter since high or persistent extracellular levels of glutamate can lead to neuronal death by overactivation of receptors. This phenomena is known as **excitotoxicity** and will be described in more detail below.



This illustrates both the recycling of glutamate, but also the use of GABA to help make glutamate.

Anatomical Localization, Receptors, and Actions.

Nearly every efferent (outgoing) system as well as all of the major excitatory projections between brain areas utilize glutamate. Examples of these **glutamatergic** pathways include corticostriatal, corticothalamic, corticobulbar, and corticospinal (e.g. upper motor neurons); intrahemispheric and interhemispheric association pathways; hippocampal circuits; primary afferents and somatosensory and special senses pathways (e.g. visual, auditory, and olfaction); cerebellar afferents; and excitatory interneurons.

Four different glutamate receptors have been well characterized:

- Three (NMDA, AMPA and kainate) are ligand-gated ion channels and are named after specific agonists. The 'family tree' consists of groups of subunits that combine in groups of five to produce a ligand gated receptor-ionophore (channel).
- Activation of AMPA and kainate receptors opens a cationic channel that depolarizes the postsynaptic membrane. Depolarization may initiate an action potential by activating voltage- dependent Na^+ channels and further increases the influx of calcium by activating voltage- dependent Ca^{2+} channels.
- When activated, NMDA receptors become permeable to potassium, sodium and calcium.
  - The receptor is relatively slow to open and close
  - The receptor requires concomitant binding of glycine for maximal activity
  - The ionophore is ligand gated but also voltage-sensitive. Mg^{2+} blocks the channel pore at resting membrane potentials; when the cell is depolarized the Mg^{2+} block is relieved and the NMDA ionophore is free to pass more ionic current. These unique properties endow this receptor with a specific "integrator" function since several conditions must be met before it opens.
- The metabotropic receptor is coupled by a G protein to phospholipase C and other effectors.

Either directly or indirectly, activation of glutamate receptors leads to increases in intracellular calcium. Calcium has many effects within the cell including activation of a wide variety of enzymes, including those responsible for generation of molecules with additional modulatory roles (e.g. arachidonic acid and its metabolites; nitric oxide), and activation of specific kinases and transcription factors. Thus, glutamate subserves excitatory transmission, but is an important signaling molecule that under appropriate conditions can lead to long-lasting changes in synaptic efficiency (such as that known as long-term potentiation (LTP), a model of memory).

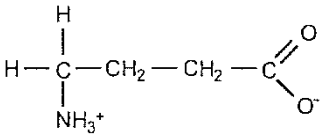
Clinical Relevance.

- Glutamate is involved in nearly all major excitatory synapses in the brain.
- Overall inhibition of the system is not well-characterized, but antagonists such as ketamine or phencyclidine (PCP) are anesthetics and cause hallucinations- these will be discussed in later lectures.
- High levels of glutamate or specific glutamate agonists (e.g. NMDA or kainate) lead to specific neuronal death in a calcium dependent manner; this is known as **excitotoxicity.**
- During brain ischemia (stroke), high levels of glutamate accumulate in the affected area due to
- massive release from presynaptic stores. Selective NMDA antagonists have been shown to be neuroprotective in animal models of stroke, indicating that excitotoxicity is a major contributor to the injury. Unfortunately, clinical trials for treatment of stroke with glutamate antagonists have not shown significant benefit; however, these drugs are being considered for other conditions.
- Excitotoxicity may play a role in neurodegenerative diseases, particularly Huntington's disease.

Inhibitory Amino Acids

Structure, Production, and Inactivation.

- Gamma-aminobutyric acid (GABA) is the principle inhibitory neurotransmitter of the central nervous System



- Glycine is the inhibitory neurotransmitter in some brainstem and spinal cord pathways.

- GABA is produced at the nerve terminal by the decarboxylation of glutamate. The enzyme is called glutamic acid decarboxylase (GAD).
- The mechanism for inactivation at the synapse is rapid and highly efficient reuptake by both neurons and astrocytes.
- GABA is shunted back to the Kreb's cycle by two enzymatic reactions that produce ATP and recycle GABA back to its precursor, glutamate (in astrocytes this is glutamine which is released and taken up by neurons that then convert it to glutamate). This cycle is called the GABA shunt (see initial figure).
- Glycine is a simple amino acid that is primarily produced in neurons by breakdown of serine. Like GABA. the primary mechanism for inactivation is by Na+ dependent reuptake.

Anatomical Localization, Receptors, and Actions.

•GABA is abundant in the central nervous system.

Immunohistochemical localization of GAD provides a tool for identification of neurons with the capacity to make GABA.

GABA is utilized by two major classes of neurons:

- Small inhibitory interneurons with localized connections throughout the CNS
- Major inhibitory pathways including cerebellar Purkinje cell projections to deep cerebellar nuclei and GABA-ergic projections from the striatum to the substantia nigra and globus pallidus.

Neurons that utilize glycine are predominately located in the brain stem and spinal cord. The best understood are the Renshaw cells that serve in a feedback loop to inhibit motor neuron activity. The Renshaw cell circuitry illustrates one example of how inhibitory interneurons provide hyperpolarizing input to neurons that are also receiving excitatory input. Their function has been likened to the brake pedal of an automobile with glutaminergic input serving as the gas pedal. Rapid excitation-inhibition coupling provides a necessary mechanism for sensory discrimination and fine motor control.

The importance of inhibitory synapses can best be appreciated by the consequences of using antagonists. Strychnine is a glycine receptor antagonist that causes severe tetanic convulsions of skeletal muscle. GABA antagonists such as picrotoxin result in immediate seizures and rapid death.

The inhibitory actions of GABA are mediated through two types of receptors:

- $GABA_A$ and $GABA_C$, ligand-gated channels for chloride ions. $GABA_C$ is found in the retina only and has similar but not identical properties to the GABAA receptor.
- $GABA_B$, a G-protein coupled receptor that inhibits the formation of cAMP or activates voltage-dependent K+ channels (and thus the closing of calcium channels)
- The net effects of these GABA-A and GABA-B channels are rapid hyperpolarization and slow synaptic inhibition, respectively.

The GABAA receptor has binding sites for a number of useful pharmacological agents. Benzodiazepines such as diazepam augment chloride conductance through the channel and are used as anti-anxiety drugs, sedatives, and anti-seizure medications.

Other sedatives such as barbiturates and alcohol also act on GABAA receptors.

GABAA receptors also have modulatory sites for steroid hormones and anesthetics such as propofol.

Like GABAA, the glycine receptor found in brain stem and spinal cord is a ligand-gated chloride channel. As already mentioned, the rat poison strychnine is an antagonist of this receptor.

Neuroactive Peptides

Structure, Production, and Inactivation.

- Neuroactive peptides represent a diverse group of molecules that are derived from larger protein precursors by specific cleavage. Unlike small molecule neurotransmitter, the precursor proteins are translated, cleaved, and post-translationally modified in the cell body.

- The neuroactive peptides are packaged in specialized secretory granules which are then transported to axon terminals by fast axonal transport. Neuroactive peptides are often released as "cotransmitters" with small molecule neurotransmitters (see below)
- These peptides are inactivated by extracellular peptidases.
- Many of the neuroactive peptides were first characterized in gut and endocrine tissues where they play a number of diverse modulatory roles. A partial list of neuroactive peptides:

Some Families of Neuroactive Peptides:

Family	Representative Members
Opioid	opiocortins, enkephalins, dynorphin
Neurohypophyseal	vasopressin, oxytocin, neurohypophysins
Tachykinins	substance P, bombesin, substance K
Secretins	secretin, glucagon, vasoactive intestinal peptide
Insulins	insulin, insulin-like growth factors 1 and 2
Somatostatins	somatostatin, pancreatic polypeptide
Gastrins	gastrin, cholecystokinin
Others	angiotensin II, bradykinin, calcitonin gene-related peptide, neuropeptide Y

Anatomical Localization, Receptors, and Actions.

- Neuroactive peptides are widely dispersed in the central nervous system and in autonomic nerves in the periphery. The greatest diversity is found in the hypothalamus, followed by amygdala, autonomic nuclei, pain-modulating centers of brain stem and spinal cord, and to a lesser degree, in the basal ganglia and cortex.
- Neuroactive peptides all work through G-protein coupled receptors which are at least as diverse as the peptides themselves. In general these function as relatively slow, but long-lasting modulators of synaptic function, either pre- or post-synaptically.
- Because of the high affinity of these receptors, neuroactive peptides are active at very low concentrations.
- Their receptors share the property of desensitization (reduced activity) in the continued presence of peptides.

Hypothalamic Neuroactive Peptides.

- The hypothalamus contains the greatest concentration of neuroactive peptides. These peptides exert potent effects on endocrine and autonomic centers and are essential in homeostatic control. Three major functional categories exist
- Magnocellular hypothalamic neurons in the supraoptic and paraventricular nuclei release oxytocin and vasopressin in the general circulation.
- Hypothalamic neurons projecting to the median eminence secrete modulators of anterior pituitary function into the portal circulation. These include thryotrophin-releasing hormone, corticotrophin-releasing factor (CRF), gonadotrophin-releasing factor, growth hormone- releasing factor, and somatostatin
- Other hypothalamic neurons project diffusely to autonomic and limbic areas of the brain. These peptides include CRF and somatostatin, indicating multiple functions for these molecules.

Central Modulation of Pain Reception.

- Unmyelinated primary nociceptive afferents contain the neuroactive peptides substance P and calcitonin gene-related peptide (CGRP). These peptides mediate central transmission of nociceptive information. They also are released at the sensory nerve terminals in the periphery where they influence vasomotor and permeability changes in response to injury. This process is known as neurogenic inflammation.
- In the spinal cord, peptides referred to as opioid peptides play major roles in central modulation of analgesia. These peptides include the enkephalins, J3-endorphin, and dynorphin. The well-known analgesic effects of opioid compounds at the spinal and supraspinal levels are via agonist interactions with receptors for these peptides.

Much of the pharmacokinetic material above was partially adapted, with kind permission from Dr Robert Gross, from the 2007 AAN Neuropharmacology course syllabus based on noted developed by M. Kerry O'Banion MD PhD.

Suggested additional reading:

For general information please see recent editions of The Biochemical Basis of Neuropharmacology, Cooper, Bloom, Roth (eds.); or Principles of Neuroscience, Kandel, Schwartz, Jessel (eds.). Good man and Gilman's The Pharmacological Basis of Therapeutics has an excellent section on neuropharmacology.

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