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Why do plants make drugs for humans?

5

A Project Funded by a NIDA Science Education Drug Abuse Partnership Award

Module 5: Why do plants make drugs for humans?

Description of the module

What do cocaine, nicotine, caffeine, THC (tetrahydrocannabinol), morphine, and aspirin have in common? They all come from plants. Why would plants make these drugs? How do the compounds get out of the plant to cause actions in the body? Plants are the oldest and most widely used source of medicinal drugs. Even today, many drugs are still extracted from plants for use as therapeutic agents or for non-medicinal purposes. And, based on knowledge we have gained about drugs derived from plants, pharmaceutical companies can develop new drugs synthetically that have better efficacy and fewer side effects.

This module will illustrate some basic plant biology, human biology, and chemistry principles by discussing several properties of drugs obtained from plants. Topics include: 1) a discussion of natural selection, 2) a description of the plant classification of Angiosperms and the difference between monocots and dicots, 3) a description of the alkaloid class of chemicals, 4) types of chemical bonds that enable drugs to bind to their targets (proteins such as receptors, enzymes or transporters), and 5) the role of enzymes in metabolism.

Learning objectives:

1. Understand basic plant structure
2. Understand plant classification
3. Understand acid-base chemistry
4. Understand the structural difference between polar and non-polar compounds
5. Understand atomic structure and bonding forces
6. Understand protein function (e.g. enzymes and receptors)
7. Understand that the brain has special areas for different functions and behaviors

This module integrates information from the following areas:

botany, biology, chemistry, medicine, and business marketing strategy

Student Handout

What do nicotine, caffeine, cocaine, morphine, THC (tetrahydrocannibinol, the active ingredient in marijuana) and salicylate have in common? They all come from plants! The chemicals found in plants can have medicinal and non-medicinal uses. For example, the bark of the willow tree contains salicylate, the precursor to aspirin, and the foxglove plant makes digoxin, which is used to treat heart arrhythmias. The non-medicinal use of drugs such as nicotine, caffeine, cocaine, etc. stems from their ability to make a person "feel good". These drugs have psychoactive effects in the brain, although they can also have non-psychoactive effects in other parts of the body as well.

1. Provide the name of the plants that contain the following drugs: nicotine, cocaine, morphine, THC and salicylate.

2. What is a psychoactive property?

3. Why would plants make compounds that have psychoactive properties?

The characteristics of the chemicals found in plants have an important impact in how they are handled by the body. One of the most common forms of active compounds contained in plants is called alkaloids. Nicotine, caffeine, cocaine and morphine are all alkaloids. Alkaloids are synthesized in a plant cell and then stored in vacuoles. In contrast, compounds like THC are not alkaloids; instead they are more like oils.

4. What is an alkaloid? Describe the typical characteristics of its chemical structure.

5. What aspect of the chemical structure of THC gives it its oily character?

6. In what kind of plants are alkaloids found? Where in the plant are alkaloids found?

7. Describe the structure of a vacuole. What does it do inside the cell?

In order to be consumed by humans, the drugs contained in plants need to be released from the plant cells. For medicinal or non-medicinal use, drugs are often extracted chemically. But in the case of non-medicinal use, drugs are also obtained by smoking the plant (e.g., tobacco, marijuana, opium) (see Module 1), smoking the extracted compound (e.g., crack cocaine), or chewing the dried leaves (e.g. chewing tobacco or coca leaves). The extraction of drugs, especially alkaloids, from plants is based on their chemical properties (acid-base characteristics) and their solubility in water versus an organic solvent.

8. In what form, charged or uncharged, does an alkaloid exist in the plant? (Hint: if it's in a vacuole, it's dissolved in water).

9. To chemically extract a drug from the plant, it must be in its non-polar (uncharged) form. Would one add an acid or a base to do this? Draw an equilibrium reaction of an alkaloid such as morphine in an acidic and in a basic medium. What is involved in the extraction process?

10. If a plant is smoked to release a drug, more drug will be volatilized in the smoke if the drug is in its non-polar form. In fact, tobacco companies play a "chemical trick" to increase the nicotine in the smoke by keeping the nicotine in the cigarette in its non-polar form. What do the tobacco companies add to the tobacco to do this? Why does this work? How does this help the tobacco companies to sell more cigarettes?

Once these drugs get into the body, they travel through the bloodstream and they are delivered to tissues, including the brain. To produce their effects, drugs like nicotine, morphine, cocaine, caffeine, and even aspirin bind to specific proteins located on cell membranes or inside cells. These proteins include enzymes, receptors, and transporters.

11. Why would the body have protein targets for drugs that are found in plants?

12. What is an enzyme? What is a receptor? What is a transporter? Indicate which of these proteins is a target for nicotine, cocaine, morphine, caffeine, THC and aspirin.

The binding of the drug to the protein involves several types of forces, including electrostatic forces, hydrogen bonds, and van der Waals forces. There are a few examples of covalent interactions between a drug and its target, but this is rare. One example is nerve gas (see Module 4).

13. How do electrostatic forces, hydrogen bonds and van der Waals forces help the drug to bind to the protein? When the drug molecule approaches the protein, which force occurs first? Which force contributes most to the stability of the interaction?

14. Why is it uncommon to find drugs that interact with proteins in a covalent manner?

When the drug binds to the protein target, it causes a change in the shape ("conformation") of the protein. This usually causes something to happen in the cell that then leads to the actual biological response.

15. The conformation of the receptor for nicotine changes when nicotine binds to it. What happens next? What is the biological response that is produced?

The last question is a bit tricky. The biological response that is produced will depend on where in the body the receptors for nicotine are found. Receptors for nicotine (also called acetylcholine receptors because acetylcholine, found in the body, binds to them) are found on neurons in many parts of the brain and on muscle cells (also see Module 4). In neurons, nicotine causes electrical impulses to be generated, causing release of neurotransmitters from neuron terminals. In muscles, nicotine causes contraction of the muscle.

16. Where are the protein targets for cocaine found? List 3 biological responses produced by cocaine, depending on where it binds to its target.

17. Where are the protein targets for aspirin found? List 3 biological responses produced by aspirin in three areas of the body.

Plants are excellent sources of drugs

Many plants contain chemicals that can be used as drugs. For example, cocaine is derived from the coca plant (*erythroxylon coca*), nicotine from the tobacco plant (*nicotiana tabacum*), THC (9-tetrahydrocannibinol) from the marijuana plant (*cannabis sativa*), morphine from the opium poppy plant (*papaver somniferum*), and salicylic acid (the precursor to aspirin) from the bark of the willow tree (*salix nigra*) (**Figure 1**). In many cases, the active ingredient in the plant affects behavior or mood; this effect in the brain is called a **psychoactive** effect. Cocaine, nicotine, THC and morphine all have psychoactive properties. On the other hand, aspirin, like salicylic acid, does not have psychoactive properties; its analgesic effect (reduction of pain) does not involve changes in behavior or mood. Similarly, digoxin, the active ingredient in the foxglove plant, is still used today to treat heart rhythm disturbances and congestive heart failure, but it has no psychoactive properties.

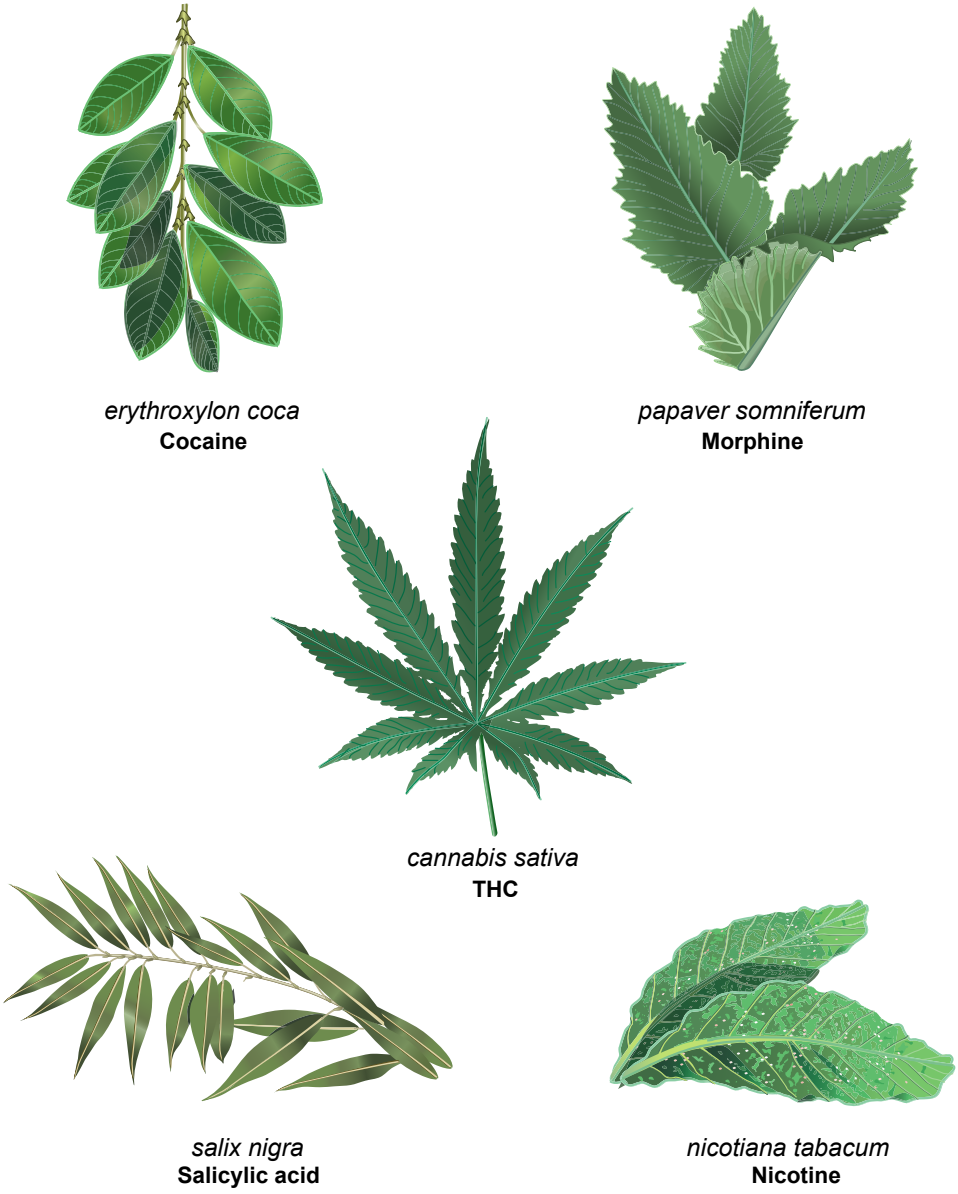

Figure 1. The leaf structure is shown for several plants that contain compounds used as drugs.

Why Do Plants Make Drugs?

Why would plants make drugs that are used by humans? More specifically, why would plants make drugs at all? Perhaps this may be explained by **natural selection**. The concept of natural selection was proposed by Charles Darwin in the late 1800's as a cornerstone of his theory of evolution. The theory of natural selection stated that organisms survive by passing on traits that are desirable and promote survival. In the case of plants, they need to ward off predators such as insects to avoid being eaten. Thus, plants developed three types of defenses against predators; 1) nutritional, 2) physical, and 3) chemical. A nutritional defense produced by plants is to contain low nitrogen levels or an unfavorable balance of amino acids, making the metabolism difficult if the insect eats the plant. Second, plants can have physical characteristics (e.g. thorns) that make them difficult to hold, manipulate, and consume by insects. Third, and most relevant to our discussion, a plant can harbor chemicals to ward off insects. For example, a plant can produce substances that result in adverse physiological effects in the insect, such as a bitter taste or even poisoning. Nicotine, contained in the tobacco plant, is an excellent insecticide, producing death in insects by paralyzing their muscles (it would do this in humans, too, if they are exposed to high enough concentrations). Cocaine, contained in the coca plant, kills insects by inhibiting their feeding (a similar anorexic effect of cocaine in humans is well-established).

Why would plants make compounds that are psychoactive? While these compounds can make humans "feel good", they may serve completely different functions for the plant. In some cases, these compounds act as insecticides, but in many cases, no known function of these compounds exists for the plant. Many of the compounds in plants have similar structures to those chemicals (especially neurotransmitters) found in humans. Humans who seek psychoactive properties from these plants have contributed to Darwinian "survival of the fittest". Over decades and centuries, man has selectively cultivated the plants with the most desirable properties. A good example is the high potency of marijuana today compared to that available in the 1960's.

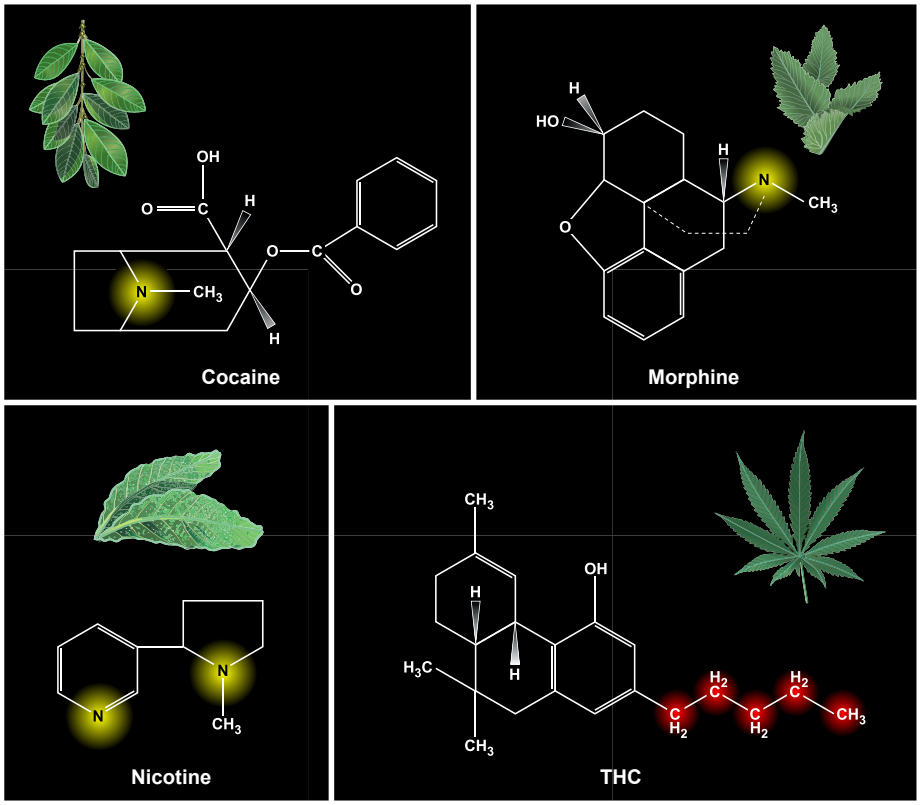

Figure 2. The chemical structure is shown for several alkaloids found in plants. Alkaloids contain a nitrogen atom, which gives the compound a basic structure. THC is not an alkaloid—note that it doesn't contain a N atom.

What types of drug-derived chemicals are found in plants?

One of the most common groups of chemicals that has medicinal properties found in plants is the **alkaloids**. Alkaloids are natural substances that react like bases – like alkalis—and they are bitter, probably to make the plant less palatable to **herbivores** (see above). Alkaloid concentrations are often highest in the most vulnerable tissues or the outermost parts of the plant, such as the external layers of the bark, stems, roots, or the seed **tegument**. **Figure 2** shows the chemical structure of some common alkaloids and the plants from which they are obtained. Notice in each example, that the alkaloid contains a nitrogen atom, which provides the basic character (i.e., the N atom can bind to additional H atoms). Although included here, THC is not an alkaloid. It does not have any N atoms, but instead it has a multi-carbon chain that gives it an oily character. Similarly, lipids contain chains of C atoms, providing their **hydrophobic** (water-fearing) or oily character.

Alkaloids are found typically in flowering plants called **angiosperms.** Angiosperms, defined as "seed in a vessel," are a type of vascular plant with its seed enclosed by an ovary wall. Angiosperms are one the most successful land plants and they produce much of our food, as well as many of our psychoactive drugs. About 10-15 percent of Angiosperms produce alkaloids. The Angiosperms can be sub-divided into **monocots** and **dicots**. Monocots have embryos bearing only one seed leaf (known as a **cotyledon**); they have parallel-veined leaves, 3-petaled (or multiples of 3) flowers, and scattered vascular bundles that run through out the plant (examples include corn, grains, lilies, irises, and grasses). The vascular bundles contain cell structures called **xylem**, which transports water and salts, and **phloem**, which transports sugars and other metabolites. (Non-vascular plants lack water- and food-conducting tissues, so they do not have true leaves, stems, and roots--e.g. mosses, liverworts, and hornworts). An example of a monocot is shown in **Figure 3**. This monocot is called *Dioscorea*, or the wild yam. The compounds of interest found in this plant (and others in the *Dioscorea* genus) include diosgenin, a steroid-like compound that provides the starting material to make progesterone used in birth-control pills. In fact, if it weren't for the Mexican yam, there would be a severe shortage of birth-control pills!

Figure 3. The leaf and flower of a monocot (*Dioscorea* or the wild yam) is compared to a dicot (*papaver somniferum* or opium poppy). Dicots, but not monocots, contain alkaloids.

In contrast, dicots have embryos bearing two cotyledons, net-veined leaves, 4- or 5-petaled flowers, and vascular cylinders arranged in concentric rings within the plant stem (examples include woody or herbaceous plants such as oak, apple and cherry trees, potatoes, tomatoes, and cacti). In Figure 3, an example of a dicot is shown—the opium poppy. Also referred to as a eudicot, the opium poppy's leaf looks more like a monocot. Alkaloids are found more commonly in the dicots, although monocots contain many compounds that have poisonous qualities.

Alkaloids are synthesized at a specific site in a plant such as the growing root, **laticiferous cells**, and **chloroplasts**. After they are synthesized, the alkaloids are transported to a storage site, usually a **vacuole** located in the cytoplasm of the cell (**Figure 4**). Vacuoles are bubble-like compartments that are surrounded by membranes. Vacuoles are a prominent feature of plant cells and often become very large, occupying up to 90% of the total volume of a plant cell. These structures contain a watery solution called “cell sap”, consisting of a variety of nutrients and waste. Many compounds, including amino acids, sugars, and alkaloids, are dissolved in the cell sap of the vacuole. The watery environment of the vacuole enables the storage of compounds that can be dissolved in water. Alkaloids can exist in both a water-soluble (**polar** or charged) form and in a more lipid-soluble (**non-polar** or uncharged) form, depending on the pH of the environment. The equilibrium reaction showing the conversion of an alkaloid such as cocaine from the polar to the non-polar form in an aqueous medium depending on the pH is shown in **Figure 5** (also see Module 1). Because the pH within the vacuole is relatively acidic, the excess of H atoms will shift the equilibrium to more polar form; the alkaloid will gain H atoms (they form bonds with the N) and it becomes charged or polar. (If the vacuole pH were more basic, the alkaloid would be more non-polar (uncharged or **lipophilic**) and it would not be able to be dissolved well in the water milieu of the vacuole.)

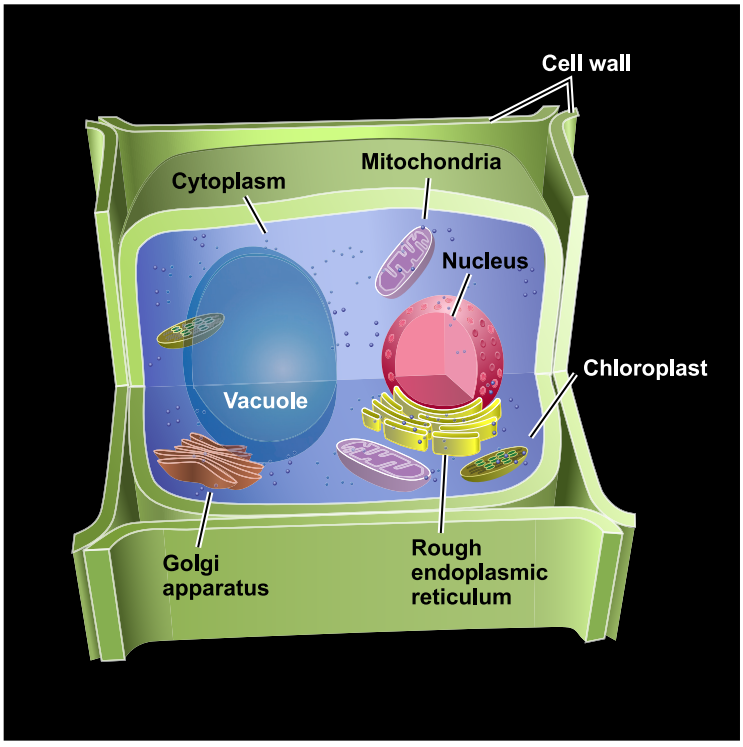

Figure 4. The basic structure of a plant cell is shown. Note the large size of the vacuole relative to other cytoplasmic structures. The vacuoles contain alkaloids dissolved in an acidic environment. After Davidson (micro.magnet.fsu.edu/cells/plantcell.html).

There are several other groups of compounds in plants that have medicinal properties. These include glycosides, saponins (soap-like steroids), and oils. A famous glycoside is digitalis (or digoxin), found in the foxglove plant. It is still used today in the treatment of heart arrythmias and congestive heart failure. Salicylates (the precursor to aspirin), from the bark of the willow tree, are aromatic acids. THC (its medicinal use is hotly debated), obtained from the cannabis plant, falls into the category of an oil—it is very lipid soluble.

How do active compounds get out of the plants?

Basically (no pun intended!), there are several ways to get alkaloids out of the plants. The most efficient ways are to extract the drug chemically or burn the leaves, releasing the drug into the smoke. Both methods have been used for hundreds or thousands of years, and they are still used today. The efficiency of extraction by either method depends on the extent to which the drug exists in a polar vs non-polar form. Let's focus on chemical extraction first. The extraction process consists of drying and crushing the plant. Then the crushed particles are mixed with an alkaline aqueous solution--this shifts the equilibrium in favor of the uncharged form (non-polar) of the alkaloid (this is known as the "free base"). Remember, the alkaloid exists mainly in the charged form while it is stored in the vacuoles. Figure 5 shows how the addition of an acid or a base shifts the equilibrium between the charged and uncharged forms of cocaine. Because the free bases are now soluble in organic solvents, they can be extracted from their watery environment with an organic solvent such as ethyl acetate or chloroform. Once the organic solvent is evaporated, there is a dry residue consisting of the basic alkaloids. Alkaloids in the free base form have a distinct advantage over the charged form (known as the acid salts because they donate a H^+). They can be smoked, and that gets them to the brain faster, producing their psychoactive effects faster (see Module 1).

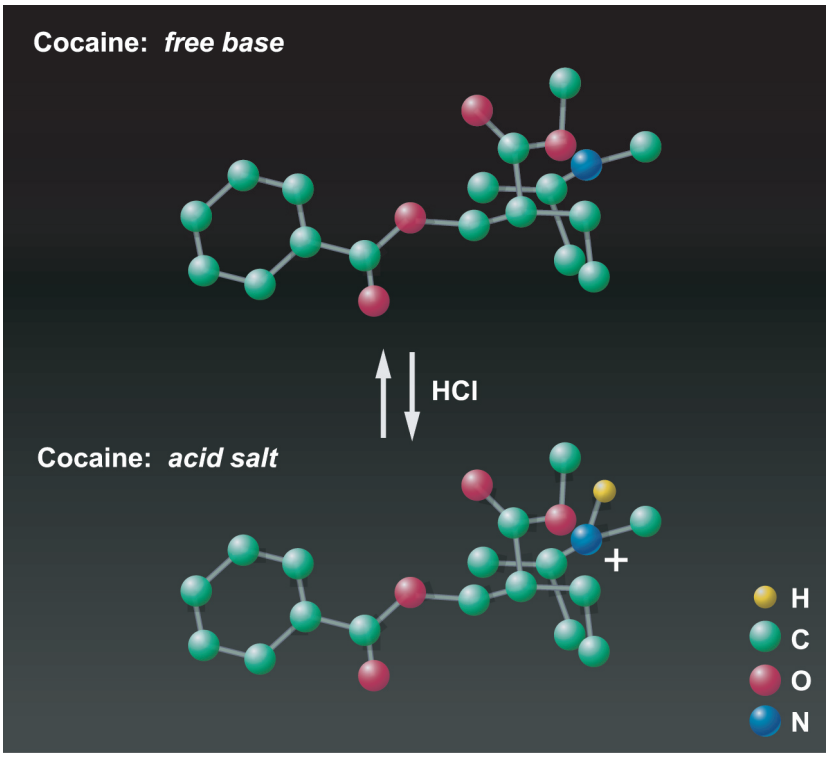

Figure 5. Treating the cocaine free base with HCl generates the charged form of cocaine, cocaine hydrochloride or the 'acid salt'. Conversely, treating the acid salt with a base such as sodium bicarbonate (baking soda) yields the free base form of cocaine. Note: only the extra H is drawn in.

Smoking the dried and crushed leaves extracts alkaloids using similar chemical logic. First, only the free base form (non-polar) of the alkaloids, such as cocaine, morphine or nicotine, can be volatilized and released into the smoke. The free base form of alkaloids volatilizes at a lower temperature than that required to volatilize the charged form or the acid salt. (As a general rule, ionic compounds, such as the acidic or basic salt, have high boiling points, while covalent compounds have a low boiling point). Thus, the charged form must be heated so high, that the molecules are destroyed. Although the alkaloids exist predominantly in the polar form (within the vacuoles), there is a small amount of non-polar alkaloid that can be volatilized. The chemists in tobacco companies have taken advantage of this property of free bases. They have manipulated tobacco chemically to increase the rate and extent by which nicotine can be extracted from the burning tobacco leaves into the smoke. As discussed above, the polar form of nicotine (within the tobacco leaves) includes an extra H^+ bound to the N atom. The tobacco companies add a base (ammonium hydroxide) to the tobacco leaves--this removes the extra proton and creates a non-polar form of nicotine (see Figure 5 for example), thus facilitating its volatilization and release from the cigarette into the smoke. In this way, the tobacco companies can sell more cigarettes. There is more nicotine released per gram of tobacco, so less tobacco is needed to provide the blood level of nicotine that the smoker requires to get an effect from a single cigarette. Therefore, for every bushel of tobacco harvested, more cigarettes can be manufactured and sold.

Some plants that contain non-polar compounds can be smoked to release their active ingredients without all these chemical tricks. Marijuana is a good example. Its active ingredient is THC. As discussed earlier, THC, a **cannabinoid,** is not an alkaloid. It does not form salts in an aqueous solution (i.e., it's not soluble in water). Rather, THC is an oily substance; it is non-polar and extremely lipid soluble (lipophilic). Because it exists predominantly in a non-polar form within the cannabis plant, it is easily released into the smoke produced by burning the marijuana.

How do chemicals derived from plants produce biological effects?

Most drugs, including those derived from plants, interact with cells in a specific way to produce their effects. These cellular interactions may occur between the drug and molecular targets inside the cell or on the cell membrane. The active compounds derived from plants usually mimic or block the effects of chemicals in the body that interact with these same cellular targets. Drugs do not produce effects on all tissues of the body; drugs act at some sites to produce biologic effects, yet at other sites, drugs produce no biologic response—it will depend on where the targets are located. The ability of drugs to produce a biological effect is dependent on its binding to protein targets such as enzymes, receptors, or transporters. Proteins are large complex molecules made up of one or more chains of amino acids (**Figure 6**). When a drug binds to an enzyme, receptor, or transporter, it triggers a sequence of cellular events that produces a specific function. All drug interactions require a degree of specificity – that is, the binding of a drug to an enzyme, receptor, or transporter requires the drug to have a specific shape and specific chemical properties to produce its effects.

Some examples of the interactions between drugs derived from plants and their protein targets are given here. The first is a drug-enzyme interaction. An **enzyme** is a protein that facilitates a biochemical reaction. An enzyme acts as a catalyst and it binds to one or more of the reactants called a **substrate** (often a drug) to produce a product. In general, enzymes and drugs combine in a reversible fashion (see below). Aspirin (acetylsalicylic acid) is one example of a drug that produces its effects by combining with an enzyme. The enzyme **cyclooxygenase** (or COX) helps generate chemicals (called **prostaglandins**) that cause pain. Aspirin binds to the COX and in this case prevents the enzyme from helping generate the pain-producing chemicals. The COX is also present in the hypothalamus, an area of the brain that controls body temperature and appetite (among other things!). When aspirin reaches the hypothalamus, it prevents the synthesis of prostaglandins there, too, reducing a fever. Cyclooxygenase helps generate prostaglandins in the stomach to protect it against too much acid. Some people have

stomach upset or even stomach bleeding (or ulcers) when they take aspirin because it has prevented the COX enzyme from generating the protective chemicals there.

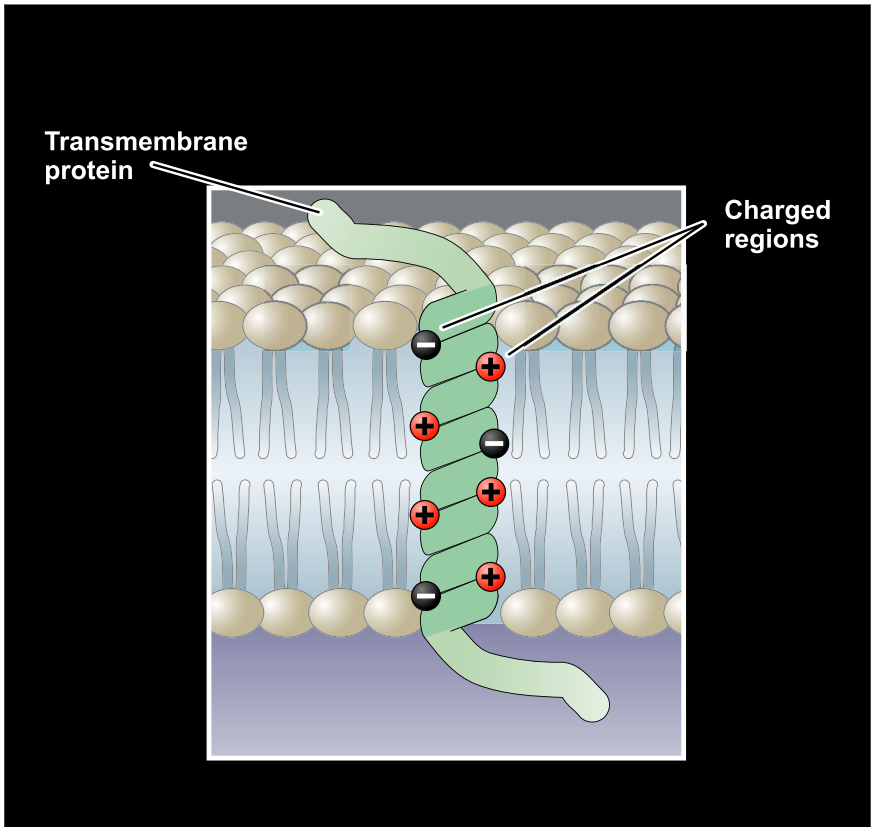

Figure 6. The helical nature of a protein is shown within a cell membrane. Proteins are macromolecules made of chains of amino acids. Some amino acid residues have positive charges and some have negative charges.

Enzymes are also important in the body because they convert active drugs into inactive compounds or other active compounds (this usually occurs in liver cells). For example, enzymes help to convert aspirin into salicylic acid by hydrolysis, which results in the aspirin molecule being cleaved by the addition of a water molecule (see Module 4 about hydrolysis). Salicylic acid also has analgesic activity, but it is more toxic than aspirin. Luckily, it doesn't stay around in the body very long because it undergoes a reaction with another enzyme that helps convert it into an inactive compound or a **metabolite**, which is excreted into the urine. Other examples of drugs metabolized by enzymes to produce active metabolites include heroin and codeine. Both of these opiate compounds is metabolized by enzymes to morphine, which produces analgesia and euphoria. However, in the case of codeine, there is only a small amount of morphine generated—enough for analgesia and a bit of sedation, but not enough to cause euphoria. Whenever morphine is produced, it is metabolized eventually by additional enzymes to inactive compounds for excretion.

Nicotine and THC are examples of drugs that produce biological effects by binding with receptors. Nicotine binds to nicotinic acetylcholine receptors and THC binds to cannabinoid receptors. A **receptor** is a specialized protein—it is a macromolecule that can be present on the cell membrane, in the cytoplasm (see Module 6) or on membranes of cytoplasmic organelles. Drug-receptor interactions require a great degree of specificity to produce a biologic effect—much like a specific key that fits a lock. For example, nicotine binds to acetylcholine receptors of the nicotinic type in the central nervous system (i.e., the brain and spinal cord) (**Figure 7**), producing a variety of effects (see Module 4). The location of acetyl-

choline receptors dictates the type of effect that is produced when nicotine binds to them. The binding of nicotine to receptors in the forebrain (in a small area called the **nucleus accumbens**), a brain area associated with reward, contributes to the pleasurable effects of the drug. The binding of nicotine to receptors in the hypothalamus, decreases appetite. In contrast, nicotine binding to receptors in the brain stem, which is responsible for basic physiological functions, often results in nausea and vomiting during the early stages of smoking. High concentrations of nicotine can affect brainstem structures responsible for respiration, causing respiratory arrest. Acetylcholine receptors are also present outside the central nervous system, on muscles and on nerves that connect with blood vessels and the heart. When nicotine binds to these peripheral receptors on muscles, it makes them contract. When binding to receptors near the blood vessels and the heart, nicotine can increase the blood pressure and heart rate. This is the basis for heart disease in smokers.

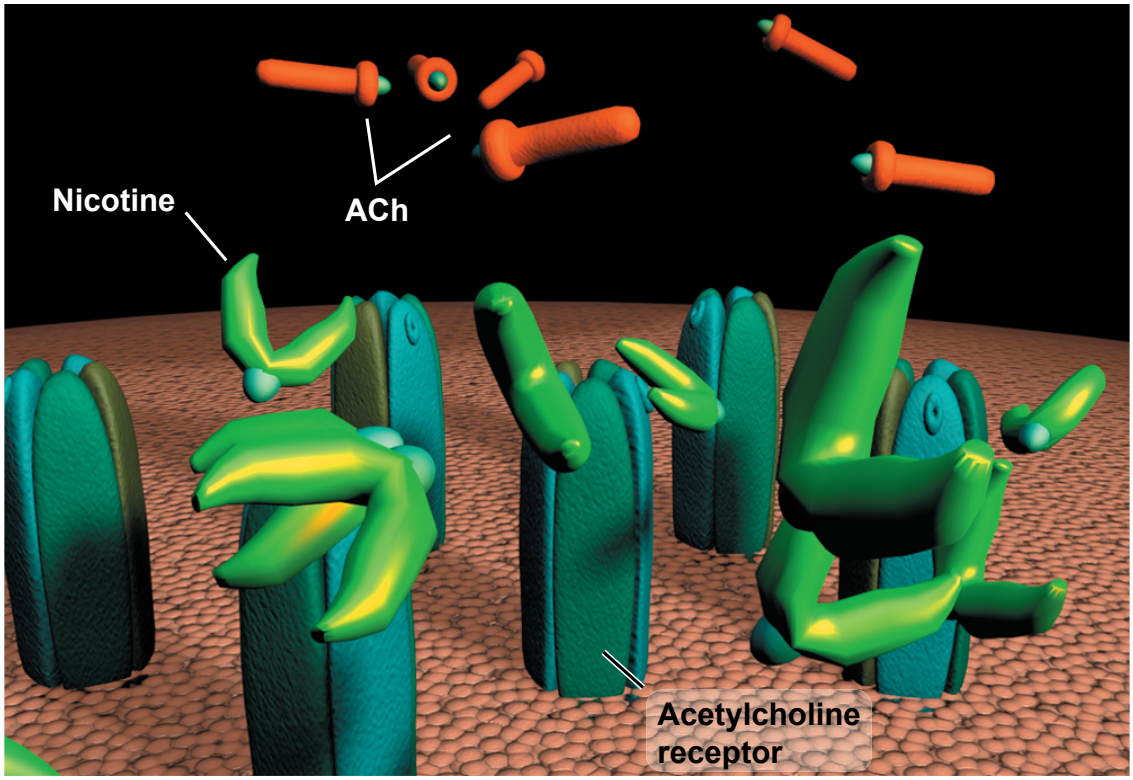

Figure 7. When nicotine binds to an acetylcholine receptor the receptor conformation changes, creating a channel for sodium ions to enter into the cell. This produces a cellular response such as nerve firing or muscle contraction, depending where the receptors are located. Taken from: *Animated Neuroscience and the Actions of Nicotine, Cocaine and Marijuana in the Brain* (Gross de Núñez and Schwartz-Bloom)

THC exerts its effects by binding to specific receptors as well. These "cannabinoid" receptors respond to a chemical naturally found in the body called **anandamide**. Scientists are still studying what anandamide actually does in the body, and it appears to be important in producing some analgesia, increasing appetite and reducing vomiting. (Recent studies indicate anandamide may also regulate memory!) The receptors for anandamide and for THC have been found in several areas of the brain, including the limbic system, a region of the brain that regulates mood, the cerebral cortex, a large outer region of the brain that is a target for the psychoactive effects of marijuana, the hypothalamus, important in regulating appetite, and the hippocampus, which is important in learning and memory. THC acts in these areas to elevate mood, cause perceptual distortions, increase appetite, and impair short-term memory (this is especially important because it disrupts learning). However, THC has very low toxicity, defined as its ability to produce death, because few cannabinoid receptors are present in brainstem areas that control respiration.

Unlike nicotine and THC, which bind to receptors, cocaine acts by binding to plasma membrane **transporters.** These proteins transport the neurotransmitters **dopamine**, **norepinephrine**, and **serotonin** into nerve terminals after their release into the synaptic space (**Figure 8** demonstrates dopamine transport). Cocaine binds to these transporters and blocks the transport of dopamine and norepinephrine back into the neuron (**Figure 8**). Thus, there is more dopamine or norepinephrine in the synaptic space, producing greater biologic effects. Similar to nicotine, the effects of cocaine depend on the type and location of the transporter to which the drug binds. Some dopamine transporters are located in the same forebrain area as the nicotine receptors—the nucleus accumbens. When cocaine prevents these transporters from working, the pleasurable effects of cocaine are produced. When cocaine prevents norepinephrine and dopamine transporters from working in the hypothalamus, it reduces the appetite. Norepinephrine transporters are located in nerves that connect with blood vessels and the heart. Block of these transporters results in more norepinephrine to bind to its targets, causing a sharp rise in blood pressure and heart rate.

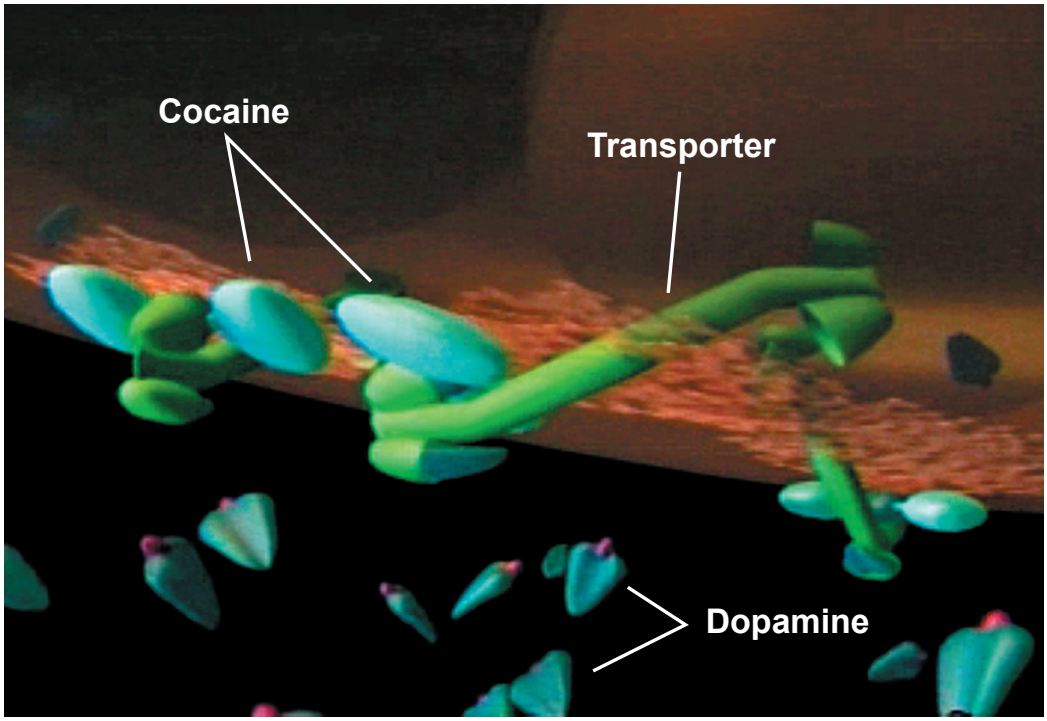

Figure 8. The neurotransmitter dopamine is transported back into the neuron by proteins (transporters) on nerve terminals. Cocaine binds to the transporter as well, blocking the transport of dopamine. This causes dopamine levels to rise in the synaptic space. Taken from: *Animated Neuroscience and the Actions of Nicotine, Cocaine and Marijuana in the Brain* (Gross de Núñez and Schwartz-Bloom)

How does a drug interact with its target? It's all in the chemistry!

To bind to an enzyme, receptor or transporter, a drug must have a specific structure to "fit" into the protein. In addition, the protein exists in a conformation or 3-D shape that will allow bonds to form between the protein and the drug. For a drug to have an effect, it must be attracted to its target. Let's use a receptor as the example for this discussion. The drug is attracted to its receptor by intermolecular forces. After these forces attract the drug to its receptor, they are also important in keeping it attached to the receptor

for a sufficient period of time to initiate the biological changes within the organism. The forces that are important in the binding of drugs to receptors include, electrostatic attractions and **van der Waals forces** (e.g., **hydrogen bonds** and dipole-dipole forces) (Figure 9).

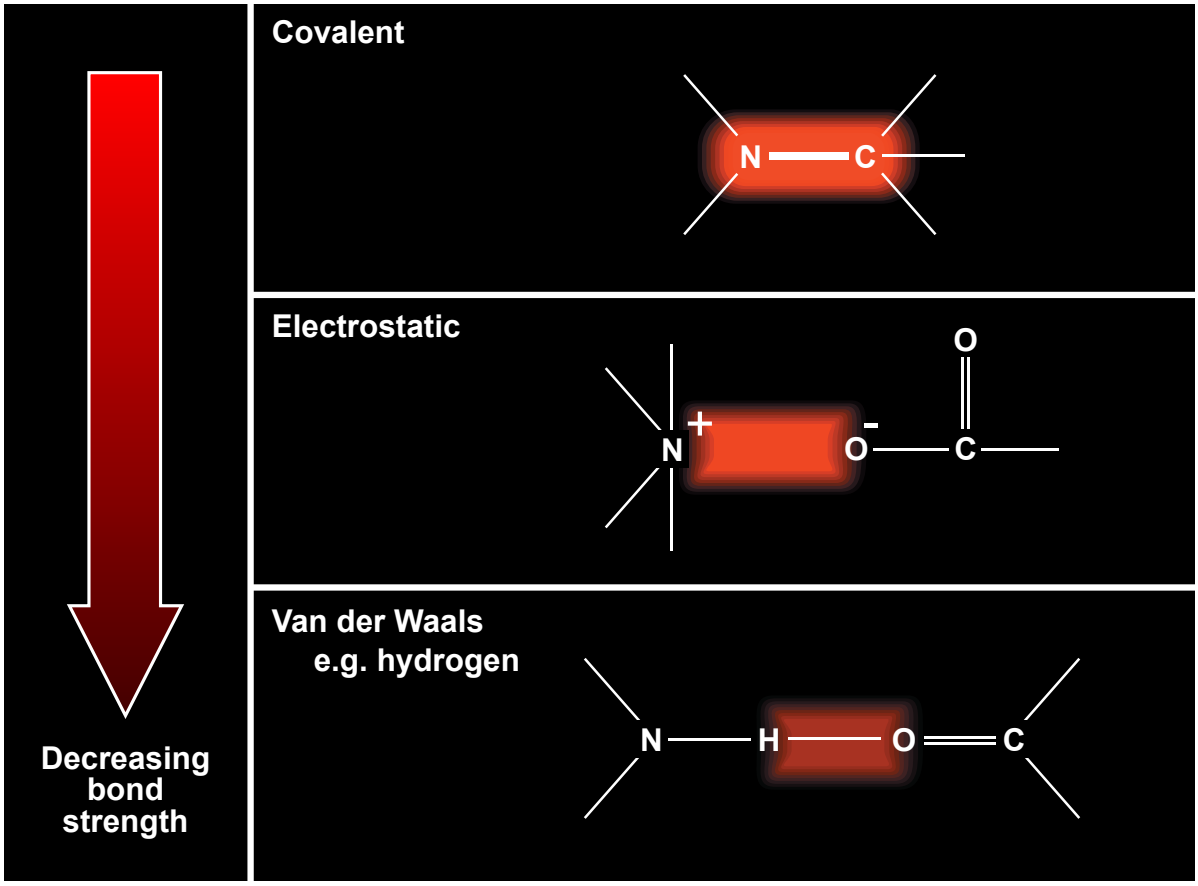

Figure 9. Examples of intermolecular forces or bonds are shown for the interaction between a drug and its target.

A brief review of atomic structure will help in this discussion of the chemical bonds formed between drugs and their targets. The atom contains an internal nucleus with a positive electric charge. The nucleus is surrounded by electrons with a negative charge. Enough electrons are present to counterbalance the positive charge of the nucleus so that typically, the entire atom is electrically neutral. All atoms seek to reach chemical stability by giving up, taking on, or sharing electrons. This occurs in an atom-to-atom bond, (an intramolecular force) or between molecules (an intermolecular force). Intermolecular forces that between molecules are the hallmark of drug-receptor interactions. A review of the types of intramolecular and intermolecular forces is found in the Appendix.

Consider nicotine as an example for a drug-receptor interaction. Once the nicotine is in the body (blood-stream), it travels to tissues where it comes into contact with cells. The first process to occur in the binding of nicotine to the acetylcholine receptor is an electrostatic attraction between the nicotine and receptor molecules. The electrostatic force between oppositely charged atoms pulls the two molecules together from some distance away. All proteins are covalent molecules and most are polar; they have a slightly positive charged region and a slightly negative charged region. (This depends on the particular amino acids that are present.) Most drugs also have a positive or negative charge. Look at the nicotine molecule in Figure 2. It can exist in its charged form when the N atoms form one more H bond (N atoms can form 4 bonds altogether). Although the electrostatic attraction has sufficient strength to allow the drug to interact with its receptor, the attraction is too weak to keep the nicotine bound to its receptor long enough to initiate a

biological event—in this case the opening of an ion channel (Figure 7). So, as the nicotine and receptor molecules come closer together, van der Waals forces contribute to keeping the drug-receptor combination stable. Van der Waals forces are attractive forces between neutral atoms or groups. Although these are weak forces, they operate at close range and they are strongest when molecules are close together. One of the strongest types of van der Waals forces is the hydrogen bond. Hydrogen bonds occur between an H and two strongly negatively-charged groups (e.g., N, O, F). A single hydrogen bond is weaker than electrostatic forces, but when several hydrogen bonds occur simultaneously, they can increase the strength and stability of a drug-receptor interaction substantially. The van der Waals forces may seem insignificant because of their weak character, but actually, they provide the final critical component for the stability of the drug-receptor interaction. The combined effort of all of these forces allows the drug-receptor binding to be reversible. This is the most common situation for drug-target interactions and it is highly desirable!

In rare cases, covalent interactions can occur between a drug and its target. **Covalent bonds** are formed when a pair of electrons is shared between two atoms. For example, nerve gas has a phosphorus atom that reacts with an oxygen atom on the enzyme acetylcholinesterase (see Module 4). By sharing a pair of electrons, a new molecule is formed via a covalent interaction. The interaction is very strong, leading to irreversible binding between a drug and its target. This usually results in a sustained biological effect that cannot be altered. Clearly this would not be advantageous (imagine an overdose—how could we undo it?) and most drugs do not form covalent interactions with their targets. Sometimes we want an irreversible effect, such as in the case of penicillin or anti-cancer drugs, which have covalent interactions with bacterial enzymes or DNA, respectively.

Glossary

alkaloid –an organic compound of natural origin. It contains a nitrogen atom and it is usually basic. Most alkaloids have marked pharmacological properties.

anandamide –a chemical produced by the body that has properties similar to THC from the marijuana (cannabis) plant

angiosperm – a type of flowering vascular plant that has water- and food-conducting tissues. Its seed is enclosed by an ovary wall. Angiosperms include the monocots and dicots.

cannabinoids – a group of active compounds in the cannabis plant. They are lipophilic molecules that act on cells in the central nervous system to produce short-term memory loss, increased appetite and euphoria.

chloroplast – a specialized organelle in green algae and plants that contains chlorophyll (a light absorbing pigment). It performs photosynthesis.

cotyledon – the primary leaves of an embryo that are present in the seed (also known as a seed leaf). It is a stored food source for the embryo.

covalent bond – a type of bond that forms by the sharing of electrons between two atoms. Covalent binding between a drug and its target is very strong and it occurs rarely in drug-receptor interactions.

cyclooxygenase – an enzyme responsible for the oxidation of fatty acids to form prostaglandins (chemical compounds that have numerous effects in many tissues). It is inhibited by aspirin to reduce pain.

dicot – a type of angiosperm defined by flowering plants with embryos having two cotelydons, net-veined leaves, 4- or 5-petaled flowers, and concentric vascular cylinders.

dopamine – a neurotransmitter (chemical messenger) in the catecholamine family that affects brain processes involved in movement, emotion, pain, and pleasure.

enzyme – a protein that catalyzes the rate at which a reaction occurs. It binds to one of the reactants (a substrate) to cause a change in the reactant's structure, facilitating the reaction.

herbivore – an organism that feeds on plants.

hydrogen bond – occurs between two strongly negatively charged ions. A type of van der Waals force. When several occur simultaneously, they are responsible for increasing the stability of a drug-receptor interaction.

ion – an atom, radical, or molecule that has gained or lost one or more electrons. Therefore it acquires a net negative or positive charge.

laticiferous cells – secretory cells.

metabolite – a product resulting from the chemical breakdown (metabolism) of a parent compound. It can be biologically active or inactive.

monocot – type of angiosperm defined by flowering plants with embryos having a single cotelydon, parallel-veined leaves, 3-petaled flowers, and scattered vascular bundles.

natural selection – theory of evolution proposed by Charles Darwin stating that organisms survive by passing on traits that are desirable and promote survival.

non-polar – a chemical property of a substance that indicates an even distribution of charge within the molecule. A non-polar or non-charged compound mixes well with organic solvents and lipids but not with water.

norepinephrine – a neurotransmitter (chemical messenger) in the catecholamine family that mediates chemical communication in the sympathetic nervous system. It is responsible for the physiologic response to a stressful challenge (the 'flight or fight' response).

nucleus accumbens – an area of the forebrain that is important in the rewarding or pleasurable effects of drugs.

phloem – tissue in vascular plants that is responsible for the transport of sugar and other metabolites.

polar –a chemical property of a substance that indicates an uneven distribution of charge within the molecule. A polar substance or drug mixes well with water but not with organic solvents and lipids. Polar or charged compounds do not cross cell membranes (lipid) very easily.

prostaglandins – a family of compounds that are involved in several aspects of reproductive function, cardiovascular function, smooth muscle contraction and pain. They are generated by the oxidation of fatty acids with the help of cyclooxygenase.

psychoactive – a compound that produces its effects in the brain to cause changes in behavior and mood.

receptor – a protein to which hormones, neurotransmitters and drugs bind. They are usually located on cell membranes and elicit a function once bound.

serotonin – a neurotransmitter (chemical messenger) that helps to regulate sleep, mood, and learning.

substrate – a molecule to which an enzyme act binds.

tegument – a natural outer covering.

tetrahydrocannabinol (THC) – one of the main psychoactive compounds (there are hundreds) in the *Cannabis sativa* plant (marijuana). It binds to the THC receptor in the brain to cause loss of short-term memory and increased appetite.

transporter – a protein that usually exists within a membrane to transport a compound (either large or charged) across the membrane to the other side.

vacuole – a watery sac-like compartment in a plant cell that stores alkaloids. It is responsible for "holding" various nutrients and wastes, as well as dissolving amino acids and sugars.

van der Waals forces – attractive forces between neutral atoms or groups on molecules (e.g. hydrogen bonds, dipole-dipole and dispersion forces). These forces are activated when the drug and receptor molecules are close together.

xylem – tissue in vascular plants that is responsible for the transport of water and salts.

Module 5: Supplemental Classroom Activities

"Spectrophotometric Analysis Of Salicylate In Plant Tissues"

***The Challenge*:**

After reading about salicylates in plants, formulate a testable hypothesis in which the measurable variable is the difference in salicylate content. Design an experiment to test the hypothesis.

***Objectives*:**

1. Use of a dichotomous key to identify plants
2. To determine the amount of salicylate present in plant tissue using UV-visible absorption spectroscopy or colorimeter
3. To compare various plant tissues with respect to the amount of salicylate present
4. To design and carry out a scientific investigation

***Standards and Skills*:**

AA1-4, AD1-4, AC1, CA21, CA22, CA26, CB32, CC10, CC14, CC15, CE10-14, CE20, CF14, CG21, CG22

***Science Concepts*:**

Plants produce a great variety of secondary compounds, many of which afford the plant a degree of protection from insects and other herbivores. Salicylic acid, a precursor of acetylsalicylic acid (aspirin), is produced by many species of Angiosperms. [When the salicylic acid is mixed with a base in an aqueous medium, it exists as a charged compound, or a salt—it is then called salicylate, usually in the sodium salt form.] It is stored in large amounts in the leaves and bark of Willows (Salicaceae) and Birches (Betulaceae) and serves to protect these plants from insect attack. In Potato and Tobacco (Solanaceae), the amount of salicylic acid present in the plant is normally low, but increases to high levels in response to attack by pathogens. These high levels of salicylic acid trigger systemic-acquired resistance (SAR) in these plants, enabling the plant to fight off infections. Potato and Tobacco plants can be made to produce protective levels of salicylic acid by either simply scratching the leaves or spraying the plant with arachidonic acid or benzaldehyde—a sort of plant vaccination. Interestingly, a derivative of salicylic acid, methyl salicylate, is released into the air by virus-infected Tobacco plants. This vapor actually activates increased production of salicylic acid in neighboring uninfected plants, rendering them more resistant to viral attack.

The use of salicylic acid as an analgesic has a long history. More recently it has been shown to have anticoagulant properties, making it a useful drug for helping to prevent heart attacks and strokes. It has been reported that strict vegetarians have markedly higher levels of salicylic acid in their blood than do non-vegetarians, even some of those who take a daily aspirin tablet. Some have suggested that the high levels of salicylic acid explain the lower rates of heart attacks and some cancers among vegetarians compared to that of the general population.

However, some people have sensitivity to salicylates. For this reason there is concern that agronomists trying to increase the levels of salicylic acid in plants may actually create plants that can be toxic to a small percentage of the population. Sensitivity to salicylate may produce some of the following symptoms:

Anaphylaxis (rare)	Asthma
Breathing difficulties	Changes in skin color
Congestion	Fatigue
Headaches	Hyperactivity

Itchy skin, rash, or hives	Itchy, watery, or swollen eyes
Stomach aches or upsets	Wheezing

Salicylates have been added to a variety of household products. Examples of some products that may contain salicylate compounds are:

Acne products	Breath savers
Bubble baths	Cosmetics
Fragrances and perfumes	Gums - mint flavored
Hair shampoos, conditioners, or sprays	
Herbal remedies	Lipsticks
Lotions	Lozenges
Mouth washes	Muscle pain creams
Razors with aloe strips	Shaving creams
Skin cleansers or exfoliants	Sun-screens or tanning lotions
Toothpastes	Wart or callus removers

***Review of Basic Concepts*:**

- Chemistry students selecting plants for testing are reminded that the easiest ways to distinguish monocots from dicots is to study the veins in the leaf. Dicots have branched veins, while monocots typically have parallel leaf (although, there are exceptions—for example, the opium poppy is a dicot, but its leaf resembles that of a monocot). More dicots have been found to contain alkaloids than monocots.
- Compounds that are highly polar in nature are soluble in polar solvents such as water but are not soluble in nonpolar solvents, or lipids. The converse is also true; most substances that are nonpolar will dissolve in nonpolar solvents but not in polar solvents or aqueous solutions.

***Procedure*:**

Collect the plant materials to be tested. Identify the plant and determine if it is a monocot or dicot. Dry the tissue*, grind, and collect ~0.5 grams. Add 10 ml of 0.25 M NaOH and let stand 10 minutes. This process will extract the salicylate into the aqueous solution. While extracting the salicylate, prepare the standard curve.

** Fresh sample can be used. To use plants containing chlorophyll, prepare the blank from the stock plus Trinder's Solution.*

Standard curve preparation:

Materials: Spec 20, cuvettes (6), 100 ml of 0.01 M sodium salicylate, 10-ml volumetric flask, distilled water, Trinder's Reagent, micropipettes.

1. Using a volumetric flask, mix the following salicylate standards, place in cuvette:

Blank: 7.5 ml Trinder's, fill to volume with water.
1.50 mM: 7.5 ml Trinder's, 1.50 ml of 0.01 M salicylate, water to volume.
1.25 mM: 7.5 ml Trinder's, 1.25 ml of 0.01 M salicylate, water to volume.
1.00 mM: 7.5 ml Trinder's, 1.00 ml of 0.01 M salicylate, water to volume.
0**.75 mM**: 7.5 ml Trinder's, 0.75 ml of 0.01 M salicylate, water to volume.

0.**50 mM**: 7.5 ml Trinder's, 0.50 ml of 0.01 M salicylate, water to volume.

2. Record the absorbance for each concentration with the Spec 20 set at 545 nm. Zero each time with the blank and start with 0.50 mM and work up to 1.5 mM.

3. Graph absorbances, determine the best fit line

Plant tissue analysis:

1. Pack a disposable pipette with a small amount of glass wool. Filter the solution.
2. Place 0.50 ml of filtered solution in flask, add 7.5 ml Trinder's and water to make 10 ml of solution.
3. Record absorbance with Spec 20 or colorimeter, remembering to zero with a blank each time (see step 1 in standard curve procedure).
4. Calculate the amount of salicylate in the sample.

***Teacher Notes*:**

You may use colorimeters instead of Spec20s. If you use a colorimeter, set the wavelength to GREEN.

1. The possible plant-related studies using this basic approach are numerous. Students could sample a wide range of plants in order to simply see if salicylic acid is present. It is likely that there are some common species that have not been sampled. (eg. Dandelions). Another possibility would be to monitor the salicylic acid levels of a single plant throughout the growing season. Different tissues on the same plant could be examined, such as new bud, old leaves, etc. Comparisons between related taxa would be interesting, such as Thyme, Mint, Oregano, and Tarragon. With respect to health issues, the amount of salicylic acid in raw, boiled, baked, and fried sweet potatoes might be a nice study. Does cooking affect the levels of salicylic acid?

2. Standard curve data are supplied below for absorbances at 530 nm and 545 nm. The regression line derived from the 545 nm data appears to be slightly better when the formula for the regression line is: y = 0.51 x. To determine salicylate concentration from absorbances: salicylate conc. = absorbance / 0.51

4. The procedure with respect to plant tissue seems to be more robust. Measurable amounts of salicylate in 0.5 g of fresh tarragon have been obtained after just 5 minutes of reaction with 0.25 M NaOH.

Solutions

Trinder's Reagent:

0.40 g of Iron (III) nitrate 9-hydrate in 1.2-ml of 12 M HCl. Add water to 100 ml.
0.01 M sodium salicylate
0.16 g sodium salicylate. Add water to 100 ml
or….0.14 g salicylic acid + 0.04 g NaOH. Add water to 100 ml.

References:

Blacklock, C. J. et al. 2001. Salicylic acid in the serum of subjects not taking aspirin. Comparison of salicylic acid concentrations in the serum of vegetarians, non-vegetarian, and patients taking low dose aspirin. J. Clin. Pathol. 54:553-555.

Delaney, T. P. et al. 1994. A central role of salicylic acid in plant disease resistance. Science 266: 1247-1250.

Ruuhola, T. 2001. Dynamics of salicylates in willows and its relation to herbivory. 138 pp. PhD dissertation, University of Joensuu, No. 8. (Accessed online)

Websites:
For quite literally everything about aspirin, from basic chemistry to clinical uses:
http://www.inchem.org/documents/pims/pharm/aspirin.htm

***Optional Activities*:**
The student may write an introductory paper focusing on the following points.

- Historical significance of acetylsalicylic acid (aspirin)
  - http://crystal.biol.csufresno.edu:8080/projects/27.html
  - http://www.angelfire.com/ok4/Aspirin/page1.html
  - http://inventors.about.com/library/inventors/blaspirin.htm
- Where does acetylsalicylic acid (aspirin) come from?
  - http://www.didyouknow.cd/aspirin.htm
  - http://sres.anu.edu.au/associated/fpt/nwfp/aspirin/aspirin.html
- How acetylsalicylic acid (aspirin) works
  - http://biz.howstuffworks.com/aspirin.htm?printable=1

Resources

The following resources provide supplemental information that pertains to the topic in this module.

Spinella, Marcello. (2001). The Psychopharmacology of Herbal Medicine: Plant Drugs that Alter Mind, Brain, and Behavior. The MIT Press, Cambridge, MA.

JA Nathanson, EJ Hunnicutt, L Kantham and C Scavone (1993) Cocaine as a naturally occurring insecticide. Proc. Natl. Acad. Sci. *USA* 90:9645-9648.

RR Levine, CA Walsh and RD Schwartz-Bloom. Pharmacology: Drug Actions and Reactions. 6th Edition, Parthenon Publishing (London). 2000

RD Schwartz. "*The Brain and the Actions of Cocaine, Opiates and Marijuana*", Teaching Packet for Neuroscientists, Produced for NIDA, 1995. http://www.nida.nih.gov/Teaching/Teaching.html

G Gross de Núñez and RD Schwartz-Bloom. "*Animated Neuroscience & The Actions of Nicotine, Cocaine and Marijuana in the Brain*", 3-D Computer-Animated Video, SAVANTES, Durham, NC © 1997. Films for the Humanities and Sciences, Princeton, NJ. http://www.films.com

A review of different types of alkaloids from various plants is found at the following websites. However, not all of the pharmacological information is correct.
http://waynesword.palomar.edu/chemid2.htm#alkaloids
http://waynesword.palomar.edu/ww0703.htm

Reference to diosgenin in Dioscorea (yams) is found as well at:
http://waynesword.palomar.edu/plsept96.htm