The P450 enzyme system: A key to understanding the metabolism of drugs

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There is currently much research in progress on the metabolism and the detoxification and excretion of drugs. The P450 microsomal enzyme system in the liver seems to be the dynamic entity rendering our anesthetic agents and accessory drugs reversible and excretable. In this article, the author attempts to express a complex process in an understandable fashion.

Terminology

Cytochrome—any of a class of hemoproteins whose principal biologic function is electron transport by virtue of a reversible valency change of its heme iron cytochromes, which are widely distributed in animal and plant tissues, and are distinguished according to their prosthetic group as a, b, c and d.

Enzyme—protein catalyst responsible for driving chemical reactions within the cell.

Coenzyme—compound bound lightly to an enzyme.

Prosthetic group—nonprotein bound very tightly to the enzyme protein.

Hydroxylation—a process by which any of a group of enzymes (oxidoreductases, also called hydroxylases) bring about the coupled oxidation of two donors, with incorporation of oxygen into one of the donors.

Mixed function oxidases—cytochromes b5 and P450.

NADP—nicotinamide adenine dinucleotide phosphate, a coenzyme required for a limited number of reactions and similar to NAD except for the inclusion of three phosphate units; called also TPN (triphosphopyridine nucleotide) and Warburg’s enzyme.

NADPH—reduced NADP, required by microsomal enzymes.

Oxidation—loss of an electron.

Reduction—gain of an electron.

Ribosome—intracellular ribonucleoprotein particles concerned with protein synthesis. Ribosomes of nerve cells are called Nissl bodies.

Within the human cell, lying in the cytoplasm, is a network of tubular and flat vesicles constructed of lipid, two-layer protein substances called the endoplasmic reticulum. For the most part, this network of little “canals” surrounds the nucleus, although some of the “canals” also lie as isolated structures in the cytoplasm. In some cells (for example, liver cells), the total surface area of this network can be as much as 30-40 times as great as the cell membrane area. The space inside the tubules and vesicles is filled with a fluid that is different from the fluid outside the endoplasmic reticulum.2

Large numbers of small granular particles called ribosomes are attached to the outer surfaces of many parts of the endoplasmic reticulum. The ribosomes are composed mainly of ribonucleic acid (RNA), which functions in the synthesis of proteins.

Part of the endoplasmic reticulum has no attached ribosomes and is called the smooth endoplasmic reticulum. It serves in the synthesis of lipid substances and in many enzymatic processes. It contains the enzymes that control glycogen breakdown when glycogen is to be used for energy and synthesizes glycoproteins.

More importantly for anesthetists, the smooth endoplasmic reticulum contains a vast number of enzymes that are capable of detoxifying substances that are damaging to the cell, such as drugs. The smooth endoplasmic reticulum achieves detoxification in a number of ways, including coagulation, oxidation, hydrolysis and conjugation.
Enzyme-substrate complexes

The striking characteristics of enzymes are their catalytic power and specificity. Enzymes accelerate reactions by factors of at least a million. For example, each enzyme molecule can hydrate 10^6 molecules of CO_2 in one second. Enzymes are highly specific—both in the reaction catalyzed and in their choice of reactants (called substrates). The degree of specificity for a substrate is usually high and is sometimes virtually absolute.

E (Enzyme) + S (Substrate) → ES Complex

Enzyme-substrate (ES) complexes have been directly visualized by electron microscopy and x-ray crystallography. Spectrophotometry is likewise useful in identifying them. Spectrophotometers are instruments for detecting and measuring the degree of absorption of radiant energy.

The liver plays a key role in the detoxification of many substances, both endogenous (such as hormones) and exogenous, (such as drugs and ingested substances). This is accomplished by numerous mechanisms, including oxidation, reduction, sulfoxidation, deamination, and dealkylation, which reduce pharmacological activity of these compounds. The reactions involve several enzymatic systems, including the mixed function oxidases (which are cytochromes b_5 and P450) and glutathione S-aryl transferase (cytoplasmic).

Cytochromes are hemoproteins. The P450 cytochromes are microsomal; that is, they are submicroscopic particles. Conjugation reactions of these enzyme systems with glucuronic acids, sulfate, taurine and glycine produce water-soluble derivatives from less soluble substrates so that these detoxified substances can be excreted in bile and urine.

Differential centrifugation of a liver homogenate (tissue that is finely shredded and mixed) prepared with an isotonic sucrose solution yields a fraction called the microsomes. This fraction contains particles together with endoplasmic reticulum.

In 1958, a novel hemoprotein was observed in liver preparations. It shows a cytochrome b-type spectrum, with an absorption maximum at 420 nm (Soret band). In the Fe^{2+} (ferric) form, it complexes with carbon monoxide (CO) to absorb at 450 nm. The new hemoprotein was termed cytochrome P450; the P means that its CO complex peaks at 450 nm spectrophotometrically. Investigations of this new entity revealed it to be the terminal component in discrete multi-enzyme systems, funneling electrons initially from NADH or NADPH, eventually to molecular oxygen, and reducing and activating it so that one oxygen atom ends up in water and the other undergoes oxygen transfer to substrate. Equivalent amounts of drug, O_2, and NADPH are utilized during the oxidative reaction. Once the activated O_2-cytochrome P450-substrate complexes are formed, they rearrange themselves to form oxidized cytochrome P450 and oxidized products. In the sense that cytochrome P450 binds to oxygen at the ferrous porphyrin site of the enzyme, it resembles hemoglobin and myoglobin, the reversible O_2-binding heme proteins.

The P450 enzyme system

The reaction involving the binding of the substrate with the oxidized form of cytochrome P450 causes small but significant changes in the absorbance spectrum of the enzyme, indicating a lack of substrate specificity, a highly unique feature contrary to the behavior of all other enzymes in the body. Under maximal induction, up to 10% of the microsomal proteins of the liver can be of the P450 variety. Cytochrome b_5 does not react directly with oxygen. The heme is buried in the interior of the protein, presumably inaccessible to oxygen.

In adrenal cells, cholesterol is converted into steroid hormones—aldosterone, hydrocortisone and corticosterone—by a series of P450 mediated hydroxylation steps.

Within the liver, the most important organ for the metabolism of anesthetic agents, the enzymes are located mainly in the endoplasmic reticulum of the hepatic parenchymal cells. Most of the enzymes catalyzing oxygenations are associated with the endoplasmic reticulum, (the microsomal fraction), and are commonly known as the hepatic microsomal drug-metabolizing enzymes.

The highest content of P450 is found in the liver, with much smaller amounts found in the kidney, lung, intestinal mucosa and skin. The kidney contains only one-seventh the amount found in the liver. Since the liver is six times greater in weight than the kidneys, the latter contributes only 2.3% of the total hydroxylation of a drug in the body.

Cytochromes P450 and b_5 were first demonstrated in human hepatic microsomes in 1969. A family of P450-type cytochromes is found in animal cells, but initially they were refractory to extensive purification, so that a bacterial P450 system was studied, notably pseudomonas camphor hydroxylase. Human liver contains less cytochrome P450 than livers of other species.

The microsomal hydroxylase located in the
endoplasmic reticulum of liver cells belongs to the group of mixed function oxidases that utilizes two electrons: one reduces one atom of oxygen to water, and the second oxygen atom is incorporated into the substrate.

Many lipid-soluble drugs can accelerate their own metabolism and also the metabolism of other compounds by stimulating the drug-hydroxylating enzyme systems in the endoplasmic reticulum of the liver. This phenomenon is termed enzyme induction. There is a corresponding increase in the actual amount of enzymes present, which have been shown to represent new synthesis. Induction of the microsomal enzymes involves increases in the biosynthesis of both heme and protein.

After a single injection of phenobarbital, the induction of P450 is maximal in 24 hours. The newly formed enzyme has the same half-life (about one day) as normal cytochrome P450. Allobarbital and other barbiturates containing allyl (C=C-C) groups cause destruction of P450, probably due to the action of a metabolite.

Paralleling the increased microsomal activity is a significant increase in smooth endoplasmic reticulum. As this growth becomes obvious, the liver itself enlarges. The increase in metabolizing activity appears to be due to the increased quantity of enzymes rather than to any change in the oxidative systems.

Drug metabolism

More than 200 substances, including barbiturates, polycyclic hydrocarbons, and polychlorinated insecticides, have been found to enhance the metabolism of drugs by the cytochrome P450 system. Ethanol, which is metabolized by both the microsomal and the mitochondrial enzymes, is a potent inducer in man. Enzyme induction is the most likely explanation for the reduction in the plasma half-life of warfarin, phenytoin, and tolbutamide seen in chronic alcoholics. Inducing action is dose-dependent. Some drugs are inducers only in high, nearly toxic doses.

Enzyme induction caused by cigarette smoking may also accelerate the formation of other toxic metabolites which can cause methemoglobinemia.

Inhalation anesthetics, except for nitrous oxide and cyclopropane, are capable of stimulating drug metabolism. In general, such stimulation resembles the induction seen with phenobarbital, except for methoxyflurane. Some drugs are not metabolized, since they possess no sites susceptible to attack by drug-metabolizing enzymes. Penicillin and tetracycline, for example, are eliminated by the kidney. Premature infants and individuals with liver disease may have a deficiency of the microsomal enzymes. Some drugs inhibit the drug-metabolizing enzymes.

Renal excretion is an important mechanism with limited efficiency. Here, the fate of the drug is largely determined by its degree of ionization at urinary pH and by its lipid solubility. Highly ionized drugs do not cross the barrier. A highly lipid-soluble drug such as thiopental is completely reabsorbed during transit through the kidney tubules. A poorly lipid-soluble drug is eliminated almost unchanged in the urine.

Summary

A variety of mechanisms is available to counter any invasion of the body by drugs or other foreign substances. These include delivery to body tissues such as adipose tissue which defers the degradation process; biotransformation mediated by enzymes located primarily, but not exclusively, in liver microsomes; and excretion via the kidneys, lungs, biliary tract, secretory glands and organs.

It is important to realize that biotransformation is not necessarily detoxification or degradation. Several other pathways are possible, such as transforming an inert parent compound into a pharmacologically active metabolite; and converting an active compound into another substance of similar or greater activity.

REFERENCES


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