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FATTY ACIDS MODULATION OF DRUG BINDING TO PLASMA PROTEINS

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INTRODUCTION

After reaching the circulation, most drugs are extensively and reversibly bound to different blood constituents including cells and plasma proteins (Jusko and Gretch 1976; Koch-Weser and Sellers 1976). For the majority of drugs, binding to serum albumin is the most important (Koch-Weser and Sellers 1976; Vallner 1977; Müller and Wollert 1979). The binding to plasma proteins can influence the action of drugs in the body since it is the unbound drug concentration that is in equilibrium with the sites of action. In addition, it can also influence the fate of the drug in the body since only the unbound (free) drug is available to sites of biotransformation within the liver, or for filtration by the kidney (Koch-Weser and Sellers 1976; Müller and Wollert 1979). In many clinical situations, the free plasma concentration of certain drugs is an index of the intensity of drug action and can be used as a guide to optimal drug dosage (Greenblatt, Sellers,

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and Koch-Weser 1982). The relative affinities and capacity of these various processes compared to plasma binding determine the ultimate importance of drug binding to plasma proteins (Koch-Weser and Sellers 1976). There are large intra- and inter-individual variations in drug binding (Abel 1979; Abel et al. 1979; Naranjo et al. 1980a). Various factors affecting drug binding in vitro and in vivo have been identified in recent years. These observations have contributed to a better understanding of the kinetic and dynamic implications of plasma protein binding. The clinical relevance of these observations, however, still remains controversial (Sellers 1979; Sellers et al. 1982).

Free fatty acid (FFA) concentrations in serum or plasma change in a variety of physiological, pathological, or pharmacologically induced conditions. These variations in fatty acids have been associated with concomitant changes in drug binding (Birkett, Myers, and Sudlow 1977; Birkett, Myers, and Hagedorn 1979; Naranjo, Sellers, and Khouw 1980; Naranjo et al. 1980b,c; Sellers et al. 1980b; Ridd et al. 1982, 1983; Nau, Luck, and Kuhnz 1984). In this chapter, we will review the current knowledge with respect to the mechanisms of fatty acid effects on drug binding in vitro and in humans, the conditions under which these changes occur in humans, as well as the potential pharmacokinetic and clinical consequences of these variations.

FATTY ACIDS BINDING TO HUMAN SERUM ALBUMIN

Fatty acids interact mainly with drug binding to serum albumin (Sjödin 1977; Birkett, Myers, and Sudlow 1977; Birkett, Myers, and Hagedorn 1979). Fatty acids seem to have a differential effect depending on the site of drug binding (Sellers et al. 1980b,c). Thus, increases and decreases in drug binding have been reported (Naranjo, Sellers, and Khouw 1980b; Naranjo et al. 1980c; Sellers et al. 1980b,c). Since effects on drug binding seem to be more marked and consistent for those drugs binding to sites I and II of the human serum albumin (HSA), we will concentrate on those studies. A brief review of the location of drug binding sites in HSA is, therefore, pertinent.

The HSA molecule consists of a single peptide chain of 585 amino acid residues which is formed into 9 double loops or subdomains by paired disulfide (Behrens, Spiekerman, and Brown 1975; Geisow 1977). The covalent structure of HSA consists of three domains, each formed by three loops. A number of sites specifically binding drugs and endogenous ligands have been described in HSA

(Fehske, Müller, and Wollert 1981; Müller, Fehske, and Schläfer 1984). These are the warfarin or site I (Sudlow, Birkett, and Wade 1975, 1976; Sjöholm et al. 1979), indole and benzodiazepine binding site or site II (Müller and Wollert, 1975a,b; Sudlow, Birkett, and Wade 1975, 1976; Sjöholm et al. 1979), and the digitoxin (Lukas and de Martino 1969; Sjöholm et al. 1979), bilirubin (Jacobsen 1969; Gray and Stroupe 1978) and fatty acids (Ashbrook et al. 1975) binding sites.

Fatty acids interact with different sites of HSA molecule, but effects seem to be more marked and consistent for those drugs binding to sites I and II. Variations in fatty acid concentrations can be associated with an enhanced or reduced binding of drugs to HSA, where both competitive and allosteric effects have been reported (Birkett, Myers, and Hagedorn 1979). Nevertheless, since the binding of fatty acids is remote from both the warfarin and the diazepam binding sites (Fehske, Müller, and Wollert 1981), fatty acid effects on drug binding are most likely explained by allosteric effects (Spector and Santos 1973; Sjödin 1977; Birkett, Myers, and Sudlow 1977; Birkett, Myers, and Hagedorn 1979; Wilting et al. 1980). The long-chain fatty acids (which predominate in human blood) binding site is probably located in the third domain of the HSA structure around amino acid residue 422 (Fehske, Müller, and Wollert 1981). This binding site is possibly located very close to the benzodiazepine binding site because of the profound allosteric changes between both sites (Birkett, Myers, and Sudlow 1977; Sjödin 1977). This site is also distinctively different from the one binding medium chain fatty acids (Koh and Means 1979).

The intimate mechanism of the allosteric changes induced by fatty acids is unknown. In addition, even though results are usually consistent for probes such as warfarin (site I) and benzodiazepine (site II), these findings may not be generalizable to other drugs bound to the same ligand binding site of HSA (Kiem, Fehske, and Müller 1984). For example, recent experiments in vitro did not always find similar results for drug binding site I (warfarin and azapropazone) or site II (diazepam and flurbiprofen). These findings could suggest that the determinant of the direction and magnitude of the FFA-induced allosteric changes could be the drug-binding site combination instead of the binding site per se (Müller, Fehske, and Schläfer 1984).

FATTY ACIDS AND DRUG BINDING: IN VITRO STUDIES

A series of in vitro studies were performed to determine fatty acid effects on drug binding. As expected from the different binding

TABLE 18.1
In Vitro Studies Showing an Effect of Fatty Acids on Drug Binding to HSA

Drug Probe	Fatty Acids Added	Effects on Drug Binding to HSA	Reference
Diazepam	Laurate	↓	Tutsumi et al. 1975
Warfarin	Oleic and palmitic acids		Wilding et al. 1977
	-FFA/alb. < 3.1	↑	
	-FFA/alb. > 3:1	↓	
Diazepam	Oleic acid	↓	Sjödin 1977
Warfarin	Palmitic acid	↑	Nilsen et al. 1977
Diazepam	Oleic acid	↓	Wong and Sellers 1979
Diazepam	Oleic acid	↓	Patwardhan et al. 1980
Chlordiazepoxide		↓	
Propranolol		↓	
Diazepam	Oleic and palmitic acids	↓	Ridd et al. 1982, 1983
Diazepam	Palmitic and oleic acids	↓	Sellers et al. 1980b, 1982
Desmethyldiazepam		↓	
Chlordiazepoxide		↓	
Oxazepam		↓	
Lorazepam		↓	
Clobazam		↓	
Flunitrazepam		↓	
Alprazolam		↓	
Bromazepam		↓	
Warfarin		↑	
Propranolol		↓	

Notes:
↑ = increase
↓ = decrease

characteristics of drugs and from the variety of drug binding sites, variations in susceptibility to these effects has been observed. However, the studies show very consistent results concerning the fatty acids modulation for drugs binding to sites I and II (Table 18.1).

In vitro, fatty acids displace diazepam from human serum albumin (Tsutsumi et al. 1975; Sjödin 1977; Wong and Sellers 1979; Patwardhan et al. 1980; Ridd et al. 1982) even at FFA/albumin molar ratios as low as 0.21 to 1.7:1 which are the ratios observed in humans (e.g., in late pregnancy) (Sjödin 1977; Ridd et al. 1982). Fatty acids show differences in potency with respect to this effect (palmitic > oleic > lauric) (Sellers et al. 1980b). Interestingly, augmentation of diazepam binding can occur when diluted albumin concentrations are used (Wong and Sellers 1979). The susceptibility of particular benzodiazepines is directly proportional to their initial degree of binding (Sellers et al. 1982). Moderate elevations of FFA/albumin molar ratios (< 3:1) increase the affinity of warfarin (Nilsen,

Storstein, and Jacobsen 1977; Wilding, Feldhoff, and Vessel 1977; Sellers et al. 1982) whereas FFA/albumin molar ratios > 3:1 diminish warfarin affinity to albumin (Wilding, Feldhoff, and Vessel 1977). The binding of propranolol to HSA is decreased by FFA (Patwardhan et al. 1980; Sellers et al. 1982).

VARIATIONS OF FATTY ACID CONCENTRATIONS IN HUMANS AND EFFECTS ON DRUG BINDING TO PLASMA PROTEINS

The concentrations of FFAs are increased or decreased in a number of physiological, pathological, and pharmacologically induced conditions (Table 18.2). A series of studies have been conducted for assessing variations in plasma drug binding associated with these changes in fatty acid concentrations. Since most studies have been conducted with drug probes for binding site I (e.g., warfarin) or site II (e.g., diazepam), we will concentrate on these studies. Most studies show consistent results concerning the modulation by FFA of drug binding to sites I and II. For example, fatty acids generally decrease diazepam binding (Naranjo, Sellers, and Khouw 1980b; Naranjo et al. 1980c; Sellers et al. 1980b) and increase warfarin binding (Nilsen, Storstein, and Jacobsen 1977; Routledge et al. 1979; Naranjo et al. 1980b, c; Sellers et al. 1980b; Abel et al. 1982). Furthermore, variations in diazepam and warfarin binding are reciprocal (Naranjo et al. 1980b, c; Sellers et al. 1980b). Variations in FFA seem to account for 50 to 75 percent of the variability in diazepam and warfarin binding (Naranjo et al. 1980b, c; Sellers et al. 1980b; Ridd et al. 1983).

Physiological Changes in Fatty Acid Concentrations

Meals

Fasting is associated with an increase in fatty acids and their concentrations decrease significantly after eating a meal (Barter, Carroll, and Nestel 1971; Schlierf and Dorow 1973). Thus, meal-induced variations in fatty acids provide an appropriate physiological model for assessing the effects of these variations on drug binding. Meal-induced decreases in FFA are associated with concomitant increases in diazepam free fraction (Naranjo, Sellers, and Khouw 1980b) and decreases in warfarin free fraction (Sellers et al. 1980b; Abel et al. 1982). Meal-induced fluctuations in diazepam free fractions are associated with reciprocal changes in total diazepam concentrations (Naranjo et al. 1980a). Variations in FFA correlated

TABLE 18.2
Conditions Associated with Variations in Fatty Acid Concentrations in Humans and Concomitant Changes in Drug Binding

Condition	Effect on Fatty Acids	Drug Probe	Effects on Drug Binding	Reference
I. Physiological Changes				
a) Meals				
Pre-prandial	↑	Diazepam	↓	Naranjo et al. 1980b
		Warfarin	↑	Sellers et al. 1980b
		Propranolol	Unaffected	Naranjo et al. 1982b
Post-prandial	↓	Diazepam	↑	Naranjo et al. 1980b
		Warfarin	↓	Sellers et al. 1980b
		Propranolol	Unaffected	Naranjo et al. 1982b
b) Exercise	↑	Phenytoin	Unaffected	Borgå et al. 1978
		Warfarin	↑	
c) Late pregnancy	↑	Diazepam	↓	Kuhnz and Nau 1983
		N-desmethyldiazepam	↓	
	↑	Diazepam	↓	Ridd et al. 1982
d) Neonate	↑	Diazepam	↓	Nau et al. 1983
		N-desmethyldiazepam	↓	
		Warfarin	↑	
		Diazepam	↓	Ridd et al. 1983
	↑	Diazepam	↓	Nau et al. 1984
		N-desmethyldiazepam	↓	
e) Ethnicity: Kutchin Athapaskan Indians	↑	Diazepam	↓	Abel et al. 1982
		Warfarin	↑	
II. Pathological Changes				
a) Myocardial infarction	↑	Diazepam	↓	Sellers et al. 1980b
b) Alcohol withdrawal syndrome	↑	Diazepam	↓	Sandor et al. 1983
		Warfarin	↑	
III. Pharmacological Changes				
a) Heparin	↑	Diazepam	↓	Desmond et al. 1980
				Naranjo et al. 1980c
		Chlordiazepoxide	↓	Desmond et al. 1980
		Oxazepam	↓	
		Lorazepam	↓	
		Warfarin	↑	Nilsen et al. 1977
			↑	Routledge et al. 1979
			↑	Naranjo et al. 1980c
b) Caffeine	↑	Diazepam	↓ (n.s.)	Patwardhan et al. 1980
		Chlordiazepoxide	↓ (n.s.)	

Notes:
n.s. = not significant
↑ = increase
↓ = decrease

positively with increases in diazepam free fractions ($r = 0.89$, $p < 0.001$) (Naranjo, Sellers, and Khouw 1980b) and negatively with decreases in warfarin free fraction ($r = -0.65$, $p < 0.01$) (Sellers et al. 1980b). Reciprocal variations in diazepam free fraction and warfarin free fraction correlated ($r = -0.59$, $p < 0.01$). The reciprocal changes of total plasma diazepam concentrations and diazepam free fraction have been recently confirmed by other investigators (Ridd et al. 1982; Nakano et al. 1984). Interestingly, meal-induced decreases in FFAs have no detectable effect on serum propranolol binding, which possibly indicates a differential susceptibility to FFA effects depending on the characteristics of drug binding (Naranjo, Sellers, and Khouw (1982b). Propranolol binds lipoproteins and α_1-acid glycoprotein (α_1-AGP) in addition to serum albumin (Sager, Nielsen, and Jacobsen 1979), whereas diazepam and warfar are principally bound to albumin (Abel 1979; Abel et al. 1979).

Exercise

A rise in FFA induced by exercise has been described (Carlson and Pernow 1961). Thus, the influence of these variations in FFA on drug binding has been studied in rats (Gugler, Shoeman, and Azarnoff 1974) and humans (Borgå, Juhlin-Dannfeldt, and Dahlqvist 1978). In rats, rises in FFA were associated with concomitant increases in phenytoin and warfarin free fractions (Gugler, Shoeman, and Azarnoff 1974); however, the results in humans with the same drugs were different. In humans, FFA concentrations in plasma increased two- to three-fold in the post-exercise period and peaked 6 to 17 minutes after the end of exercise. The peak FFA/albumin molar ratios ranged from 1.8 to 4.2. Phenytoin binding was unaffected, whereas warfarin binding increased (Borgå, Juhlin-Dannfeldt, and Dahlqvist 1978). Thus, species differences do exist and results from animals cannot be extrapolated to humans.

Late Pregnancy and Neonatal Period

Variations in drug binding during late pregnancy, labor, and neonatal period have been extensively studied (Dean et al. 1980; Dvorchick 1982; Kanto 1982; Nau et al. 1982; Ridd et al. 1982, 1983). In pregnant women, a marked increase in FFA and diazepam free fraction have been observed during late pregnancy, labor, or prior to Cesarean section (Ridd et al. 1982, 1983; Kuhnz and Nau 1983; Nau, Luck, and Wegener 1983; Nau, Luck, and Kuhnz 1984). Increases in diazepam free fraction peak at delivery or within four hours post-partum. The time course of increases in diazepam free fraction and FFA are parallel and changes correlate ($r = 0.64$, $p < 0.01$) (Ridd et al. 1982). Interestingly, variations in diazepam free fraction in parturients are also associated with reciprocal

changes in total diazepam concentrations (Ridd et al. 1982). A change which is similar to the meal-induced reciprocal fluctuations in diazepam total concentration and diazepam free fraction previously described (Naranjo et al. 1980a). Neonates also show an increase in diazepam and N-desmethyldiazepam free fractions associated with elevations in FFA (Nau, Luck, and Wegeuer 1983; Nau, Luck, and Kuhnz 1984; Ridd et al. 1983). These variations in drug binding are also temporarily and quantitatively correlated with variations in FFA. For example, variations in FFA and diazepam free fraction correlated highly ($r = 0.87$, $p < 0.001$) (Ridd et al. 1983). During the first day of life, diazepam and desmethyldiazepam free fractions doubled and subsequently slowly returned to normal levels within one week (Nau, Luck, and Kuhnz 1984).

Ethnicity

The concentrations of FFA and the associated variations in warfarin and diazepam binding were also determined in a sample of 37 Kutchin Athapaskan Indians (Abel et al. 1982). The Kutchin Athapaskans constitute the northernmost Indian settlement in Canada and they have remained biologically isolated. In these Indians, diazepam free fractions were higher than in other groups and varied directly with FFA concentrations ($r = 0.65$, $p < 0.001$). In addition, the high FFA concentrations were correlated with lower warfarin free fractions ($r = -0.43$, $p < 0.01$). Warfarin free fraction and diazepam free fraction correlated inversely ($r = -0.33$, $p < 0.05$) (Abel et al. 1982).

Pathological Changes in Fatty Acid Concentrations

Fatty acids also increase in a number of pathological conditions such as in acute myocardial infarction and in the alcohol withdrawal syndrome. In 15 post-myocardial infarction patients, diazepam free fraction and FFA increased and variations correlated ($r = 0.42$, $p < 0.01$) (Sellers et al. 1980b). Similarly, in 15 patients in acute alcohol withdrawal, FFA and diazepam free fraction increased, whereas warfarin free fraction decreased while subjects were symptomatic and values normalized one week later. However, changes in fatty acids and variations in diazepam and warfarin free fractions did not correlate (Sandor et al. 1983).

Pharmacologically Induced Changes in Fatty Acid Concentrations

Heparin

Free fatty acids increase after heparin administration and concomitant variations in drug binding have been described (Nilsen, Storstein, and Jacobsen 1977; Routledge et al. 1979; Wood, Shand, and Wood 1979; Desmond et al. 1980; Naranjo et al. 1980c). Heparin activates lipoprotein lipase (Olivecrona et al. 1977), and since variations in drug binding are temporally and quantitatively associated with changes in FFA, it was stated by some that this was the only mechanism responsible for heparin-induced drug binding changes (Giacomini et al. 1980a,b). However, heparin-induced variations in drug binding can also occur even though activation of lipoprotein lipase has been prevented by the prior injection of protamine (Naranjo, Khouw, and Sellers 1982a), or the addition of a mixture of protamine/ethylene diamine tetraacetic acid (EDTA) (Brown et al. 1981), or of paraoxon (Schultz et al. 1983) to the blood samples immediately after collection. These maneuvers attenuate, but do not suppress completely, the heparin-induced changes in drug binding (Brown et al. 1981; Naranjo, Khouw, and Sellers 1982a; Schultz et al. 1983). The mechanisms of non-fatty acids modulated variations in drug binding may be complex including factors such as formation of heparin complexes with Ca^{++} and α_1-AGP, as well as possible pH-dependent variations in albumin conformation (Naranjo, Khouw, and Sellers 1982a). Also, a direct displacing effect by heparin for drugs such as diazepam and warfarin has been observed (Naranjo, Khouw, and Sellers 1982a). These findings, therefore, strongly suggest that the practice of using heparin locks for collecting blood samples in pharmacokinetic studies should be abandoned to prevent artifacts. It is also important to remark that heparin-induced drug binding changes are highly variable and dependent on a variety of factors such as feeding state of subjects, biological activity of heparin lot, and drug binding characteristics (Naranjo et al. 1980c).

Heparin-induced increases in FFA correlated with increases in diazepam free fraction ($r = 0.73$, $p < 0.001$) and decreases in warfarin free fraction ($r = -0.74$, $p < 0.001$). Reciprocal variations in diazepam and warfarin also correlated ($r = -0.48$, $p < 0.01$). Similar findings have been reported by others for diazepam (Desmond et al. 1980) and warfarin (Nilsen, Storstein, and Jacobsen 1977; Routledge et al. 1979). However, reported correlations between FFA and changes in propranolol free fractions vary widely with r values ranging from as low as $r = 0.22$ (n.s.) (Naranjo, Khouw, and Sellers 1982a) to as high as $r = 0.99$ ($p < 0.001$) (Wood, Shand,

and Wood 1979). However, since meal-induced variations in FFA did not correlate with changes in propranolol free fraction ($r = 0.18$, n.s.) (Naranjo, Sellers, and Khouw 1982b), and since heparin-induced variations in propranolol binding can occur even in the absence of changes in FFA (Naranjo, Khouw, and Sellers 1982a), the association is perhaps only temporally, but not necessarily causally related.

Caffeine

Fatty acids also increase after caffeine administration. Caffeine stimulates lipolysis in adipose tissue via activation of cyclic-adenosine monophosphate (AMP) (Patwardhan et al. 1980). However, despite these changes, no significant variations in diazepam, chlordiazepoxide or propranolol free fraction were detected (Patwardhan et al. 1980). A closer inspection of these data indicates that variations in the binding of these drugs did occur, even though they did not reach statistical significance. In the same study, the in vitro addition of oleic acid increased significantly the free fraction of the three drugs (Patwardhan et al. 1980).

PHARMACOKINETIC IMPLICATIONS OF VARIATIONS IN DRUG BINDING

Theoretically, the free drug plasma concentration of drugs will correlate with the intensity of drug action and could be used as a guide to optimal drug dosage. However, most assay techniques in current use measure the total (free plus bound) plasma drug level rather than the concentration of unbound drug. Use of total, rather than unbound drug concentrations to adjust drug dosage will not lead to important clinical errors if the free fraction does not vary appreciably within and among individuals (Greenblatt et al. 1982). If free fraction does not vary among or within individuals this suggests that the binding affinity is constant, since free fraction is proportional to the dissociation constant for binding at low drug concentrations. In this case, the concentration of unbound drug in serum or plasma is always proportional to the total concentration. However, large intra- and inter-individual variations in the extent of binding are quite common with several drugs (*e.g.*, benzodiazepines) (Sellers et al. 1982). These may lead to misinterpretation of total drug concentrations and to important pharmacokinetic errors (Greenblatt et al. 1982; Sellers et al. 1982).

The free fraction (α) of a drug in plasma is that proportion of the total concentration which is not bound to protein. Free fraction can range from 0 to 1.0 and is calculated as follows:

$$\alpha = \frac{\text{unbound drug concentration}}{\text{total drug concentration}}$$

The unbound or free concentration (C_f) is the absolute level (measured in units of amount/volume) of unbound drug in plasma. C_f is determined by the rate at which the drug reaches the systemic circulation, and by the ability of the clearing organ (usually liver or kidney) to remove or convert the drug via biotransformation and/or excretion. This capacity for elimination of the unbound drug is called unbound or free drug clearance (Cl_f). If a drug dose (D) is given repeatedly at regular time intervals (τ), and if the entire dose reaches the systemic circulation (bioavailability F = 1.0), then the average value of C_f at steady-state is:

$$C_f = \frac{D}{\tau} \cdot \frac{1}{Cl_f} \qquad \text{Equation 1)}$$

Free fraction (α) does not appear in this equation, and therefore C_f, the most important determinant of the intensity of a drug's effect, does not depend on the extent of protein binding. The reason for this is that α and C_f are determined by two different and independent physiologic processes. Alpha depends on the physicochemical interaction of the drug with protein, whereas C_f depends on the balance between the rate at which the drug enters the body and the rate of elimination of unbound drug biotransformation or excretion.

Total concentration (C_t) relates to free concentration only as a dependent variable as follows:

$$C_t = \frac{C_f}{\alpha}$$

Equation 1 is the proper expression of steady-state concentrations of relevance despite the more familiar

$$C_t = \frac{D}{\tau} \cdot \frac{1}{Cl_t} \qquad \text{Equation 2)}$$

For drugs such as diazepam, which is highly bound, the apparent influence of protein binding on total diazepam clearance using Equation 2 will depend on the relative magnitude of average differences among subjects in binding, and inter-subject differences in free clearance (Greenblatt et al. 1980). Although 40 percent of

females have higher free fractions than males (Abel et al. 1979), clearance of diazepam in females is lower (MacLeod et al. 1979). This confirms that inter-subject differences in intrinsic hepatic clearance rather than free fraction account for a larger part of the inter-subject variation in body clearance of total diazepam (Greenblatt et al. 1980).

Many sources incorrectly suggest that an increase in α necessarily leads to an increase in C_f, and therefore, to a more intense therapeutic effect, or a greater likelihood of toxic actions (Sellers 1979). In fact, it is clear from the above that a change in α, whatever the reason, has no effect on steady-state C_f as long as D, τ, and unbound clearance remain the same, and the volume of drug distribution is large (Greenblatt, Sellers, and Koch-Weser 1982).

The potential kinetic implications of variations in drug binding are illustrated by recent observations that diazepam and N-desmethyldiazepam concentrations fluctuate widely within and between days (Naranjo et al. 1980a). These fluctuations of parent drug and metabolite follow a characteristic temporal pattern and are correlated with each other in time and size. These fluctuations in diazepam and N-desmethyldiazepam are associated with reciprocal variations in diazepam free fractions (Naranjo et al. 1980a). Meal-induced variations in fatty acids appear to modulate these fluctuations, since meal-induced decreases in FFA and diazepam free fraction are highly correlated ($r = 0.87$, $p < 0.001$) (Naranjo, Sellers, and Khouw, 1980b). Therefore, when FFA concentrations decrease after a meal, the binding of diazepam increases (i.e., free fraction decreases) causing diazepam in the tissues to enter the intravascular compartment and consequently total plasma diazepam concentration rises (Naranjo, Sellers, and Khouw 1980b). Diurnal variations in drug concentrations result in variation in the area under the curve (AUC) of total plasma drug concentration versus time. Consequently, pharmacokinetic parameters dependent on calculation of AUC of total drug will be affected dependent on the choice of sampling times. For example, plasma diazepam concentration may increase two-fold after a meal in normal volunteers under highly controlled conditions (Naranjo et al. 1980a). Sequential sampling every day in such individuals (e.g., at 0800 h) would result in a calculated AUC approximately 50 percent of that obtained sampling 1 to 3 h after a meal (e.g., between 0900 and 1100 h). Thus, the apparent volume of distribution ($dose/\beta \cdot AUC_0 \rightarrow \infty$) and clearance ($dose/AUC_0 \rightarrow \infty$) would be overestimated (Naranjo et al. 1980a). Previously unrecognized diurnal variations in drug concentration may be a factor contributing to apparent inter-investigator variation in results which is usually ascribed to inter-patient variation in pharmacokinetics.

Alterations in α are most likely and of greatest importance for those drugs that are extensively bound to plasma proteins. For a drug that is 99 percent bound ($\alpha = 0.01$), a small absolute change in α from 0.01 to 0.02 will cause C_t to fall by 50 percent. For a drug that is less extensively bound (for example, $\alpha = 0.20$) the same absolute change (from 0.20 to 0.21) is inconsequential and produces an undetectable change in C_t. Thus, a knowledge of α for a drug can predict the likelihood of major kinetic problems (Greenblatt, Sellers, and Koch-Weser 1982; Sellers et al. 1982).

POSSIBLE CLINICAL IMPLICATIONS

The clinical implications of variations in drug binding remain controversial (Sellers 1979; Sellers et al. 1982). Inter-subject differences in protein binding of drugs which are restrictively eliminated, may result in differences in acute clinical effects after single and multiple dosing. Distribution of drug is more rapid in persons with higher free fractions, and peak and valley free drug concentrations over a dosing interval will be more extreme (Levy 1976). Perhaps the occasional cardiopulmonary arrest after rapid intravenous diazepam is explained by these changes (Sellers et al. 1982).

Variations in drug binding associated with changes in fatty acids with potential clinical relevance have been detected in a few instances. For example, marked increases in fatty acid concentrations after birth may result, in the neonate, in increased free fractions of diazepam and desmethyldiazepam (Nau, Luck, and Wegeuer 1983). Because of immature neonatal hepatic elimination capacity, these elevated free fractions may result in elevated free concentrations of the two compounds, which may explain the excessive sedation and respiratory depression observed clinically in some neonates receiving diazepam (Nau, Luck, and Kuhnz 1984).

Since diurnal fluctuations in diazepam free concentration after single dose, and variation in total concentration also occur in individuals receiving diazepam chronically, clinical variation in the effect of diazepam may occur (Naranjo et al. 1980a). Such circadian variation in clinical response to diazepam has been occasionally observed (Baird and Hailey 1972; Nicholson and Stone 1978). However, the wide margin of safety of diazepam and the development of acute and chronic tolerance probably protect most patients from serious clinical consequences.

Finally, the marked variations in fatty acids and drug free fraction associated with heparin administration, may result in elevated free plasma concentrations of propranolol and diazepam during cardiac catheterization (Wood et al. 1980).

These examples may indicate that variations in drug binding induced by changes in FFA concentrations might, in fact, have some clinical importance and are not simply of special interest to some few clinical pharmacological researchers.

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