0031-6997/82/3401-0109$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 34, No. 1
Printed in U.S.A.



# Antibodies as Specific Antagonists of Toxins, Drugs, and Hormones*

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## Introduction

NINE decades ago, von Behring demonstrated that antibodies specific for the exotoxins produced by *Clostridium tetani* and by *Corynebacterium diphtheriae*, respectively, prevented the deleterious effects of these exotoxins in experimental animals (2). Antibodies have subsequently been used therapeutically, both in human and in veterinary medicine, to prevent the deleterious effects of these and other toxins, including snake and insect venoms (43, 44, 74), plant toxins (40), other bacterial exotoxins and, more recently, bacterial endotoxin (73) (table 1). It is now well established that some antibodies to biologically active mammalian proteins, particularly enzymes, and cell membrane receptors are capable of inhibiting the corresponding enzymes (15, 21, 48) or of blocking specific cell membrane receptor sites (33, 41, 59); similarly, some antibodies to peptide hormones specifically antagonize the physiological effects of the corresponding hormones, as demonstrated both in vivo and in vitro (6, 13, 27, 30, 32, 37, 38, 46, 66, 71, 72). Such antibodies to mammalian proteins and peptide hormones have been employed in experimental animals but not used therapeutically in man. Representative examples of proteins and peptides that can be inactivated or inhibited by specific antibodies are listed in table 1. The properties of antibodies to biologically active proteins and polypeptides have been described elsewhere (14) and will not be dealt with in this review. It should be noted, however, that in some instances specific antibodies may not only fail to inactivate an enzyme or peptide hormone but may actually potentiate or prolong its effects, presumably either by causing alterations in structural configuration or by preventing enzymatic degradation (15, 30); such antibody-mediated enhancement of the biological effects of an antigen is not frequently encountered, but its occasional occurrence must be kept in mind.

Antibodies to biologically active haptens are also capable, in many instances, of inhibiting the physiological or pharmacological effects of the corresponding haptens. Thus, antibodies are capable of inactivating steroid hormones (22, 45, 49, 50), prostaglandins (24), catecholamines (65), serotonin (23, 52, 55), melatonin (51), histamine (19), pyridoxal (67), biotin (3), and various drugs (4, 10, 12, 20, 26, 31, 68, 69, 70), carcinogens (17, 54), and environmental toxins (39, 42, 60) (table 2). In specifically binding such biologically active substances, antihapten antibodies often interfere with the metabolism or excretion of the corresponding low molecular weight compounds, thus elevating blood levels and prolonging in vivo half-lives (4, 9–12, 25, 31, 36, 53, 58).

Antibodies have been elicited to numerous low molecular weight toxins and carcinogens by conjugating them as haptens to antigenic protein carriers and immunizing experimental animals with the resulting hapten-protein conjugates (7, 8). Although used principally in the development of specific immunoassays, it is clear that many such antibodies are capable of neutralizing the corresponding toxins (17, 39, 42, 54, 60). More extensive experience, however, has been obtained with antibodies to hormones and drugs. Hence, this review will deal with the experimental use of such antibodies, their mechanism of action, their effects on hapten disposition in vivo, and their clinical use. Finally, the possible applicability of such an immunochemical approach to the prevention of reversal of the deleterious effects of environmental toxins will be briefly considered.

## Experimental Effects of Antibodies

Antibodies are capable of blocking the pharmacological (4, 10, 12, 20, 26, 31, 68, 69, 70) and toxic (10, 16, 56) effects of various drugs. Antibodies are also capable of preventing the lethal effects of various low molecular weight toxins (39, 60). However, in general, such immunopharmacological studies have shed little light on the mechanism of drug or toxin action. In contrast, antibodies to hormones have served as powerful experimental reagents in determining whether specific in vivo or in vitro phenomena are mediated, in whole or in part, by the corresponding hormones. For example, such antibodies have been used to establish a role for angiotensin in certain forms of experimental hypertension (13), establish a causal relationship between the midcycle rise in estrogens and luteinizing hormone release in the primate (22), provide evidence that circulating somatostatin plays a prominent role in the stress-induced inhibition of growth hormone secretion in the rat (66), and provide evidence for a role of melatonin and/or N-acetylserotonin in the maintenance of basal blood prolactin levels (51). Conversely, biologically active antibodies to hormones have also been used to exclude a role for the correspond-

* This work was supported by the U. S. Public Health Service research grant HL 10608.

TABLE 1
*Examples of antibody neutralization of biologically active molecules*

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|---|
| Bacterial exotoxins |
| Clostridial |
| *Corynebacterium diphtheriae* |
| Bacterial endotoxins |
| Gram-negative sepsis |
| Plant toxins |
| Ricin |
| Venoms |
| Snake |
| Insect |
| Enzymes |
| Soluble |
| Renin |
| Membrane-bound |
| Na,K-ATPase |
| Membrane receptors |
| Acetylcholine |
| Prolactin |
| Insulin |
| Peptide hormones |
| Hypothalamic |
| Angiotensin |
| Bradykinin |
| Pituitary |
| Glucagon |
| Lymphokines |
| Gastrointestinal |
| Insulin |

TABLE 2
*Examples of neutralization of small biologically active molecules by antibodies*

| |
|---|
| Hormones |
| Steroid hormones |
| Prostaglandins |
| Catecholamines |
| Serotonin |
| Melatonin |
| Drugs |
| Digitalis glycosides |
| Barbiturates |
| Morphine |
| Nalorphine |
| Mescaline |
| Macromycin |
| Environmental toxins |
| Carcinogens |
| Kepone |
| Paralytic shellfish poison |
| Other |
| Histamine |
| Pyridoxal |
| Biotin |

ing hormone in certain experimental phenomena. For example, the failure of antiserum to growth hormone to inhibit the lactogenic effect of certain human plasma specimens has been used to exclude the possibility that growth hormone, rather than prolactin, was exerting the observed effect (27); similarly, the failure of antiglucagon serum to lower basal blood glucose levels has provided evidence that the absolute concentration of plasma glucagon is of minor importance for the maintenance of basal blood glucose in the rat (37).

Although numerous examples of the ability of antibodies to prevent drug, hormone, or toxin action have been reported, relatively few examples of the immunochemical reversal of the action of such substances have been reported. The ability of antibodies to reverse the pharmacological and toxic effects of cardiac glycosides has, however, been extensively studied by Butler et al. (10). These studies will be dealt with in some detail. In our initial in vivo studies, we had found that 10 rabbits that had been immunized with digoxin-albumin conjugates exhibited no electrocardiographic abnormalities during two hours of continuous monitoring after an i.v. dose of digoxin (0.6 mg/kg) that killed, within a two-hour period, all normal control nonimmunized rabbits and rabbits immunized with albumin alone (56). We next turned our attention to the study of the effect of digoxin-binding antibodies on established digoxin intoxication in nonimmunized dogs, an experimental model more closely related to clinical digoxin toxicity in man. In this study, 17 dogs were given 0.09 mg of digoxin per kg i.m. once daily for three days. All 17 animals developed vomiting, weakness, lethargy, and, within one to three hours after the last dose, a digoxin-toxic arrhythmia; the most common disturbances were ventricular ectopic activity, frequently associated with periods of ventricular tachycardia, and varying degrees of heart block, often complete. Of nine control dogs, none was in normal sinus rhythm after six hours of monitoring and all were dead within 48 hours after the final digoxin dose. In contrast, in eight dogs that received antidigoxin antibodies two to three hours after the final dose of digoxin, six were in normal sinus rhythm and all eight were clinically improved within six hours; none of these eight dogs died and normal sinus rhythm was restored in all within 48 hours (57). Other workers have also found that antibodies to digitalis glycosides are capable of preventing or reversing advanced digitalis intoxication in experimental animals (16, 18, 34, 47, 53, 61).

## Mechanism of Action of Antidigoxin Antibodies

Three possible mechanisms for the immunological reversal of digoxin action were considered: 1) the binding of digoxin in the extracellular fluid, causing a decrease in the effective free extracellular drug concentration and creating a concentration gradient, thereby promoting the egress of digoxin from cells; 2) a direct interaction between digoxin and specific antibody at the cell membrane level, resulting in the inactivation and/or dissociation of the cardiac glycoside; and 3) the entry of antibody into cells to bind digoxin intracellularly. Since immunoglobulins do not ordinarily enter nonphagocytic cells, the third mechanism was considered to be highly unlikely.

To learn about the mechanism by which antibodies exerted this action, the effects of antibodies on cellular digoxin concentration and action were studied in human erythrocytes (28). When antidigoxin serum was added to red cells that had been previously incubated for three hours with [$^3$H]digoxin ($1.53 \times 10^{-7}$ M), there was a rapid and almost complete removal of intracellular digoxin, the concentration of which fell from 23 to 0 pmoles/ml within 10 minutes; since repetitive in vitro washing of the red cells produces a similar effect on intracellular (but not on membrane-bound) digoxin, it seems likely that antibodies remove intracellular digoxin by lowering the effective extracellular concentration of free drug. Antibodies also removed membrane-bound digoxin from red cells, but at a much slower rate; removal was not complete after five hours. Since excess ouabain, another cardiac glycoside, displaces membrane-bound digoxin at a rate very similar to that of displacement by antibody, we have suggested that antibodies bind the membrane-bound fraction in the extracellular space immediately after dissociation of digoxin molecules from membrane binding sites. We suspect that these antibodies, like ouabain, lower membrane digoxin concentrations by preventing reassociation of recently dissociated digoxin molecules rather than by directly removing them in some manner from membrane receptor sites (28).

In the red cells that had been incubated for three hours with digoxin in the above study, monovalent cation transport had been inhibited, reflecting a well-known pharmacological action of cardiac glycosides; red cell uptake of potassium ion was inhibited by digoxin from 2.4 mmoles/liter of red cells per hour in untreated cells to 0.93 mmoles/liter of red cells per hour in digoxin-treated cells. After addition of antidigoxin serum, restoration of potassium transport was gradual but it was only partial after five hours, and the restoration was temporally associated with the immunological removal of the membrane-bound fraction of digoxin. These observations, like other pharmacological studies, indicate that there are at least two fractions of red cell digoxin, only one of which, viz. the membrane-bound fraction, is responsible for the pharmacological effects of the glycoside. On the basis of these experiments, we concluded that antibodies reversed the effects of digoxin by removing it from cells by two mechanisms: 1) binding the drug in the extracellular fluid, lowering the effective concentration of free drug, and creating a concentration gradient, thereby promoting the egress of intracellular digoxin; and 2) binding recently dissociated digoxin molecules and thereby preventing their reassociation with membrane receptors (28).

Additional evidence that inactivation of digitalis by antibody does not occur at the cellular receptor was provided by Gold and Smith (29). These workers demonstrated that antibodies to ouabain reverse the positive inotropic effect of this cardiac glycoside on isolated cat papillary muscle in vitro. Having demonstrated that this reversal was more rapid (mean time for half-reversal, 124 minutes) than that observed in the red cell studies, Gold and Smith (29) went on to show that subsequent addition of excess ouabain to the muscle bath produced an appropriate restoration of the positive inotropic effect of the drug. Because of this lack of alteration of response to subsequently added drug, it seems unlikely that a stable antibody-hapten complex forms at the glycoside receptor site. This inference is supported by evidence that the dominant chemical determinants of both cardiac activity and antibody binding (62) reside in the C and D rings and unsaturated lactone portions of the molecule; this makes simultaneous receptor and antibody binding of glycoside unlikely (10).

## Effects of Antibodies and Fab Fragments on Drug Pharmacokinetics

It has been known for 25 years that antibodies may prolong the in vivo half-time of peptide hormones (5). More recently, evidence has been obtained that specific antibodies increase blood concentrations and prolong the in vivo half-time of morphine (4, 36), nalorphine (31), digitalis glycosides (9, 10, 53, 58), and barbiturates (11, 12, 25). The findings are particularly striking in rabbits actively immunized with digoxin-protein conjugates. When a single dose of [$^3$H]digoxin was administered i.v. to these animals, the presence of antibodies resulted in a 100-fold increase in serum digoxin concentration at 12 hours (8300 ng/ml vs. 87 ng/ml in control rabbits), decreased urinary digoxin excretion and a 21-fold prolongation of the serum half-life of digoxin to 2.5 months (vs. 3.4 days in control animals). Without further immunization, significant serum concentrations of digoxin (in the 85 ng/ml range) were observed one year after the drug was given (58).

When digoxin-treated dogs are passively immunized with sheep antidigoxin antibodies, a rise in serum digoxin (from 4–7 to 180–220 ng/ml) is observed, as digoxin is presumably removed from tissues. Urinary digoxin excretion also falls to negligible levels. Since dogs eventually catabolize the sheep antidigoxin antibodies and are unable to replace them, as do actively immunized rabbits, serum digoxin levels eventually fall; the timing and rate of digoxin release vary greatly in different individual dogs (9). The released digoxin is, of course, pharmacologically active and certainly has a toxic potential if a sufficient quantity is released in a brief period.

Monovalent Fab fragments, produced by papain treatment of bivalent antidigoxin antibodies (9, 18, 61), also remove digoxin from tissues. However, because of their smaller molecular size, Fab fragments are excreted by the kidney and thereby enable the predictable, rapid excretion of digoxin in a bound, pharmacologically inactive form (9); in the case of the more slowly excreted digitoxin, urinary excretion of glycoside after Fab treatment is considerably more rapid than in control animals (53). Fab fragments also diffuse more rapidly into the extracellular space and, in a study by Lloyd and Smith

(47), were capable of more rapid restoration of sinus rhythm in digoxin-toxic dogs with ventricular tachycardia (mean, 36 minutes) than were intact antidigoxin antibody molecules (mean, 85 minutes). Fab fragments have an additional advantage over intact immunoglobulin in that they are significantly less immunogenic than the parent antibody molecules (64).

On the basis of our in vitro and in vivo studies, we believe that digoxin-specific antibodies and their Fab fragments act as follows: 1) Following i.v. administration, antibodies and Fab fragments initially bind intravascular digoxin. 2) They then diffuse into the interstitial space, Fab diffusing more rapidly than immunoglobulin, binding free interstitial digoxin. 3) The decrease in the extracellular digoxin concentration promotes egress of free intracellular digoxin into the extracellular fluid, where it is also bound. 4) The antibodies and Fab fragments bind digoxin molecules which have freshly dissociated from membrane receptors and prevent their reassociation. This is the slowest, but most important, step as it is correlated with the reversal of glycoside action; this step occurs more rapidly in the myocardium than in red blood cells. 5) The rise in the extracellular concentration of bound digoxin is reflected in an increased bound (pharmacologically inactive) serum digoxin level. 6) The subsequent disappearance of digoxin is largely dependent on the metabolic fate of the Fab or antibody, with rapid urinary excretion in the case of Fab and with slower and less predictable excretion in the presence of intact antibody.

## Clinical Experience

Although there has been extensive clinical experience with the administration of antibodies to macromolecular toxins, clinical experience with antibodies to low molecular weight drugs, hormones, and toxins has been quite limited and data are available on less than 20 patients with advanced digitalis intoxication treated with digoxin-specific Fab or $(Fab')_2$ fragments (1, 35, 62a, 63). In all instances in which they have been administered to severely toxic patients, fragments of digoxin-specific antibodies have rapidly reversed toxic rhythm and conduction disturbances (complete heart block; persistent ventricular tachycardia and/or fibrillation); in hyperkalemic patients, the serum potassium has fallen rapidly (63). Clinical effects have been noted as soon as one hour after Fab administration and have usually been complete within four to six hours. The effects of Fab fragments on digoxin pharmacokinetics in man have been similar to the effects of these fragments on digoxin pharmacokinetics in dogs. There have been no allergic or other untoward reactions to Fab fragments to date (1, 62a, 63).

## Applicability to Environmental Toxins

The populations of industrialized nations are being exposed to increasing numbers of environmental toxins, including carcinogens. Although active immunization with protein-toxin conjugates is theoretically possible, it would be impractical as a general measure because the number of toxic compounds is great and the risk of the population at large to any specific toxin is small. Even if consideration is limited to individuals at high risk for a specific toxin, active immunization would not be advisable until more experimental work is carried out. There is the hazard of enzymatic or hydrolytic release of active toxin from the immunogenic conjugate. In addition, it is likely that the antibodies elicited by active immunization would prolong the half-life of toxin, after exposure; in this instance, potentiation, rather than protection, might ensue. If one could selectively induce secretory antibodies to toxins to be released into the gastrointestinal and respiratory tracts, prolongation of the half-life would not be a concern; however, such selective induction of secretory antibodies is not practical at this time. One would also need to determine what antibody concentration confers a protective effect and to determine whether periodic "booster" immunizations are required; boosting would increase the risk of sensitization to the carrier protein.

Passive immunization with antibodies to toxins or with Fab fragments of such antibodies is possible at this time. Although considerably less hazardous than active immunization, passive immunization to most toxins is also not practical at this time; antibodies are generally not available and, when available, little is known about their efficacy. In the case of low molecular weight toxins, Fab fragments are preferred over intact immunoglobulin molecules because they are less immunogenic, have a larger volume of distribution and may facilitate excretion of the toxin in a bound, inactive form; when available, affinity-purified specific Fab fragments are preferred because the foreign protein dosage is considerably less than the dosage of Fab fragments prepared from total immunoglobulin preparations. The Fab fragments should have a high affinity for the toxin. Before use, it must be established that the Fab fragments inhibit, and do not potentiate, the toxin. The absence of any toxic effect of the Fab fragments should be demonstrated in animals. The use of Fab fragments should initially be considered only in the case of toxins which exert undesirable effects with total body stores of 10 mg or less, because 10 mg of a toxin of molecular weight of 500 is stoichiometrically equivalent to 1 g of purified specific Fab fragments; the production of larger individual doses of specific Fab fragments would be logistically difficult at present. Animal Fab fragments have sufficiently little immunogenicity to permit their safe clinical use this time. Human Fab fragments would probably persist longer and their use would virtually eliminate the risk of sensitization to foreign proteins. However, the clinical use of such Fab fragments will require refinement of currently available methodology for the in vitro induction and production (by cell culture and/or by peptide synthesis) of human antibodies to low molecular weight toxins.

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