Kinetics of Drug–Drug Interactions: Biliary Excretion of Iodoxamic Acid and Iopanoic Acid in Rhesus Monkeys

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Abstract □ The dynamic method originally developed for studying the capacity-limited kinetics of the cholecystographic agents iodoxamic acid and iopanoic acid was applied to study the *in vivo* interactions of these two compounds following coadministration in the monkey. Results indicate that these interactions are complex. The compounds appear to compete for plasma protein binding sites as well as for binding sites on intrahepatic proteins. The biliary excretion data apparently fit the "ligand exclusion" model in which iopanoic acid acts as an inhibitor and competes with iodoxamic acid for binding to either of two identical sites within the liver. This competition probably is the rate-limiting step in the liver's overall elimination of these radiographic contrast agents.

Keyphrases □ Drug–drug interactions—pharmacokinetics, iodoxamic acid, iopanoic acid, biliary excretion, rhesus monkeys □ Pharmacokinetics—drug–drug interactions, iodoxamic acid, iopanoic acid, biliary excretion, rhesus monkeys □ Cholecystographic agents—iodoxamic acid, iopanoic acid, interactions, biliary excretion, pharmacokinetics, rhesus monkeys

In cholecystography, the degree of biliary system opacification depends directly on the concentration and the amount of iodine excreted in the bile (1). Therefore, a reasonable approach to increasing the degree of opacification would be to increase the cholecystographic agent dose. This approach, however, is limited by the fact that most cholecystographic compounds are handled in the liver by saturable processes, *e.g.*, by binding to the intrahepatic binding proteins—the so-called Y- and Z-proteins (2, 3); above a certain dose level, these processes become saturated and the excretion rate does not increase. High doses would also produce excessively high blood levels of the compound and add stress to eliminating organs, such as the kidney, which could lead to systemic toxicity (4, 5).

An alternative approach to increasing the biliary iodine concentration would be to coadminister more than one compound. If, however, the compounds share hepatic elimination mechanisms, coadministration might result in competition for the binding sites of the intrahepatic (Y and Z) proteins or for other carrier systems responsible for hepatic uptake, biotransformation, and biliary excretion of the compounds. Therefore, biliary excretion of two or more compounds may be no greater than that for a single agent.

Reported here is an *in vivo* study of the interaction of two cholecystographic agents, iodoxamic acid and iopanoic acid, coadministered to rhesus monkeys. The capacity-limited biliary excretion of these two compounds in dogs was reported previously (6, 7). During these studies, TLC confirmed that iodoxamic was excreted in the bile unchanged, while iopanoic acid was 98% metabolized to the glucuronide. At the same time, the blood concentrations contained only intact drugs with both agents. They, therefore, represent an interesting combination of cholecystographic agents with which to study drug interactions.

THEORETICAL

One or two identical binding sites may exist on the enzyme or the carrier for a substrate. This condition produces a competitive situation if the binding of an inhibitor to one of the sites prevents the substrate from binding to the site(s). This competition may also distort the substrate binding sites by the binding of the inhibitor or from mutual steric hindrance. Alternatively, the inhibitor may have a binding site that overlaps or utilizes part of each substrate binding site. The equation of the ligand exclusion model (8) can be applied to describe this latter system:

$$v = \frac{\overline{V}_m(S/K_m + S^2/K_m^2)}{1 + 2S/K_m + S^2/K_m^2 + I/K_i} \quad \text{(Eq. 1)}$$

where v is the enzymatic reaction rate, $\overline{V}_m$ is the maximum reaction rate when all binding sites are saturated, K_m is the Michaelis–Menten constant for the substrate, S is the substrate concentration, K_i is the Michaelis–Menten constant for the inhibitor, and I is the inhibitor concentration.

In the absence of the inhibitor ($I = 0$), Eq. 1 can be simplified by multiplying the numerator and the denominator by K_m^2:

$$v = \frac{\overline{V}_m[(K_m)(S + S^2)]}{K_m^2 + 2[(K_m)(S + S^2)]} \quad \text{(Eq. 2)}$$

$$v = \frac{(\overline{V}_m)(S)(K_m + S)}{(K_m + S)^2} \quad \text{(Eq. 3)}$$

$$v = \frac{\overline{V}_m S}{K_m + S} \quad \text{(Eq. 4)}$$

Therefore, in the absence of the inhibitor, Eq. 1 reduces to the Michaelis–Menten equation (Eq. 4), and the v *versus* S plot is the usual hyperbola. In the presence of the inhibitor, the v *versus* S plot becomes sigmoidal.

EXPERIMENTAL

Materials—Studies were performed on one male (Monkey A) and one female (Monkey B) rhesus monkey, 9.1 and 4.5 kg, respectively. Both monkeys had been used previously for exp ents (6, 7) in which iodoxamic acid or iopanoic acid alone had been infused. Thus, each monkey served as its own control. The monkeys were restrained in plastic chairs and had, as previously described (9), a T-tube in the common bile duct for bile collection and another T-tube in the duodenum for bile salts recycling. Both monkeys had been cholecystectomized.

^{125}I-Labeled iodoxamic acid[1] was used. Its radiopurity was >99% by TLC. For infusion, the ^{125}I-iodoxamic acid was diluted with normal saline to 26.5 and 28.4 m*M*, as determined according to its iodine content by fluorescent excitation analysis (10).

The iopanoic acid infusion solution was prepared by dissolving 913 mg of iopanoate sodium[2] in 10 ml of water. The resultant solution was filtered through a membrane filter[3] and diluted to 100 ml with normal saline.

Piston-type infusion pumps[4] were used for infusing the solutions. An automatic γ-counter[5] was used for determining ^{125}I-radioactivity. A multiple equilibrium dialysis system[6] was used for plasma protein binding studies.

[1] Iodine-125 meglumine iodoxamate, E. R. Squibb and Sons, Princeton, N.J.
[2] Sterling-Winthrop Research Institute, Division of Sterling Drug, Rensselaer, N.Y.
[3] Millex disposable filter unit, Millipore Corp., Bedford, Mass.
[4] Lamda pump and driver, serial 1300 and 1301, Harvard Apparatus Co., Millis, Mass.
[5] Auto-gamma-scintillation spectrometer, model 1185, Searle Analytical, Des Plaines, Ill.
[6] Dianorm, Innorativ-Medizin AG, CH-Esslingen, Switzerland.

0022-3549/79/1100-1430$01.00/0

Table I—Protein Binding of Iodoxamic Acid and Iopanoic Acid in Monkey A [a]

Minutes	$C_{b_{IDX}}$, μM	$C_{f_{IDX}}$, μM	$C_{f'_{IDX}}$, μM	$C_{b_{IPA}}$, μM	$C_{f_{IPA}}$, μM	$C_{f'_{IPA}}$, μM
90	217	29.8	76.2	160	1.27	1.13
106	97	9.6	34.2	92	0.27	0.80
121	43	3.1	14.1	39	0.02	0.51
151	17	0.84	5.5	16	0.002	0.11
181	13	0.56	3.6	8.7	—[b]	—[b]
211	11	0.46	3.3	2.6	—[b]	—[b]
241	10	0.40	3.0	0.5	—[b]	—[b]
286	8.7	0.33	2.7	0.3	—[b]	—[b]

[a] $C_{b_{IDX}}$ and $C_{f_{IDX}}$ are the blood and unbound plasma concentrations, respectively, of iodoxamic acid in the absence of iopanoic acid; $C_{f'_{IDX}}$ is the unbound concentration of iodoxamic acid in the presence of iopanoic acid; $C_{b_{IPA}}$ and $C_{f_{IPA}}$ are the blood and unbound iopanoic acid concentrations in the absence of iodoxamic acid, respectively; and $C_{f'_{IPA}}$ is the unbound iopanoic acid concentration in the presence of iodoxamic acid. The samples were collected in the presence of heparin, which was not evaluated for its effect on the protein binding. [b] Below HPLC assay sensitivity.

Procedure—The dynamic infusion method used for studying the capacity-limited kinetics of iodoxamic acid and iopanoic acid (6, 7) was used. One infusion study was performed in Monkey A. Iopanoic acid was infused at the rate of 1.09 μmoles/kg/min. After 30 min, ^{125}I-iodoxamic acid was infused at the rate of 1.60 μmoles/kg/min. Ninety minutes after the first infusion, both infusions were terminated.

Another infusion study was performed in Monkey B. Iopanoic acid was infused at the rate of 0.73 μmole/kg/min for 150 min; 90 min after the initiation of the iopanoic acid infusion, ^{125}I-iodoxamic acid was infused at the rate of 1.20 μmoles/kg/min for 60 min.

Generally, except for ascertaining lag times, bile samples were collected for both monkeys every 5–15 min for 285 min. Sodium taurocholate[7] (1% in water) was infused into the duodenum at the rate of 5.56 μmoles/kg/min 1 hr before and throughout both experiments to replace the bile salts lost due to bile collection and to maintain as much as possible a constant bile flow (11, 12).

To determine the lag time between the presence of contrast material in the biliary canaliculi and that at the biliary sampling site, bile samples were also collected every 2–4 min during the first 30 min of each experiment. The lag time was estimated by plotting the cumulative amount of iopanoic acid excreted in the bile during the first 30 min against time and extrapolating the initial linear portion to the abscissa (Fig. 1). The lag time varied from 2 to 7 min. After correcting for the lag time, the biliary excretion rates were plotted for the midpoint of each collection period.

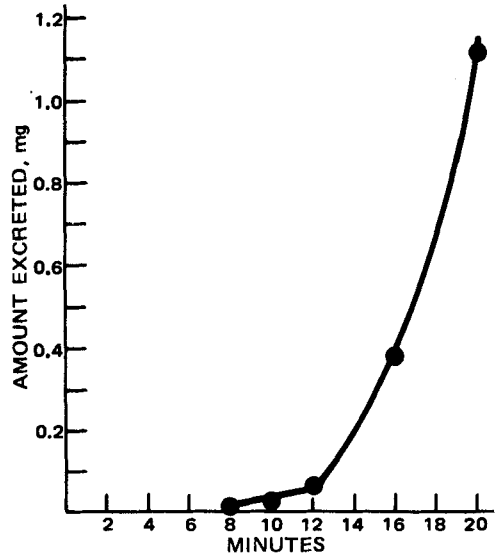

Figure 1—*Cumulative iopanoic acid excreted in the bile* versus *time during the first 30 min of infusion at 1.09 μmoles/kg/min into Monkey A.*

[7] ICN Pharmaceuticals, Cleveland, Ohio.

Table II—Protein Binding of Iodoxamic Acid and Iopanoic Acid in Monkey B [a]

Minutes	$C_{b_{IDX}}$, μM	$C_{f_{IDX}}$, μM	$C_{f'_{IDX}}$, μM	$C_{b_{IPA}}$, μM	$C_{f_{IPA}}$, μM	$C_{f'_{IPA}}$, μM
151	187.9	25.0	85.40	136.48	0.52	1.46
171	52.6	3.82	23.65	49.87	0.14	0.90
191	25.0	1.27	10.51	15.75	0.03	0.45
226	14.5	0.57	5.25	5.25	0.008	0.34
250	12.0	0.43	4.73	4.20	0.005	0.24
280	9.6	0.31	3.81	5.79	—[b]	—[b]
310	8.3	0.25	3.68	4.20	—[b]	—[b]
353	7.6	0.22	3.02	7.35	—[b]	—[b]

[a,b] See Table I.

Blood samples were collected at appropriate time intervals such that the total amount of blood taken did not exceed 20 ml/study.

For each blood and bile sample, the total iodine concentration was determined by fluorescent excitation analysis (10). The iodoxamic acid concentration in each sample was determined by counting the ^{125}I-radioactivity. The ^{125}I-iodoxamic acid iodine concentration was subtracted from the total iodine concentration to obtain the iopanoic acid concentration in each bile and blood sample.

For each animal, whole blood samples were used for equilibrium dialysis studies of each drug alone (6, 7) and in the presence of the other compound. The samples were taken from whole blood obtained during the interaction studies. The proportion of unbound iodoxamic acid was determined by counting ^{125}I-radioactivity in the protein-free buffer; the proportion of unbound iopanoic acid was determined using the high-pressure liquid chromatographic (HPLC) method described previously (7). The resultant unbound concentrations are plotted on the same graphs as those for biliary excretion rates of the two acids (Fig. 2).

RESULTS AND DISCUSSION

Goergen *et al.* (13) coadministered iodipamide, a compound chemically similar to iodoxamic acid (4, 5), and iopanoic acid in dogs and found that iodipamide biliary concentration decreased to ~12% of the control level.

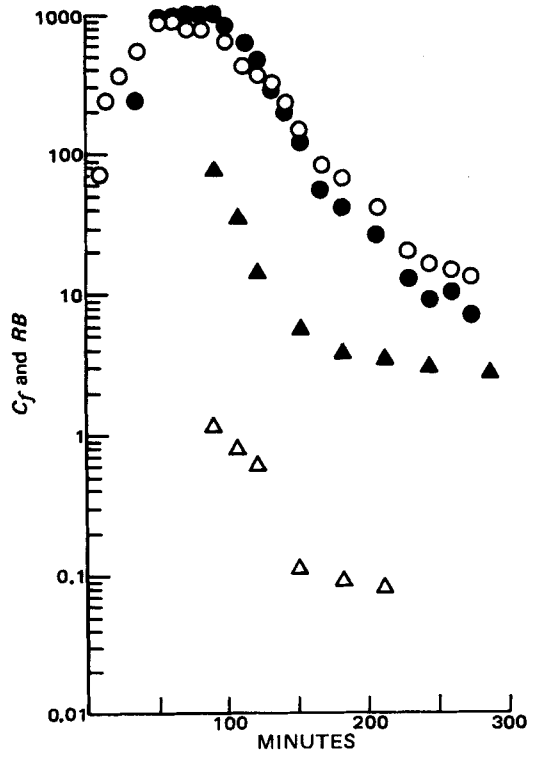

Figure 2—*Semilog plot of the time course of biliary excretion rate* (RB, *in nanomoles per kilogram per minute*) *of iodoxamic acid* (●) *and iopanoic acid* (○) *and the unbound plasma concentration* (C_f, *micromolar*) *of iodoxamic acid* (▲) *and iopanoic acid* (△) *in Monkey A. The same results were found for Monkey B.*

Table III—Results of the Coadministration Experiment in Monkey B [a]

$C_{f'IDX}$, μM	$C_{f'IPA}$, μM	RB, μmole/kg/min	RB', μmole/kg/min	Residual, μmole/kg/min
5.05	0.32	0.056	0.087	0.031
6.40	0.38	0.082	0.108	0.026
8.62	0.41	0.123	0.155	0.032
10.62	0.44	0.196	0.193	−0.003
14.10	0.58	0.293	0.234	−0.059
26.19	0.95	0.459	0.369	−0.090
66.47	1.33	0.618	0.707	0.089

[a] $C_{f'IDX}$ and $C_{f'IPA}$ are the unbound plasma concentrations of iodoxamic and iopanoic acids, respectively; RB is the biliary excretion of iodoxamic acid; and RB' is the computer-fitted biliary excretion rate of iodoxamic acid according to Eq. 1. Residual is the difference between RB and RB'.

These results were confirmed when Moss *et al.* (14) administered a bolus dose of iopanoic acid to dogs being infused with iodipamide and showed that the total biliary iodine concentration was reduced, although the blood iodine concentration was increased. In this latter study, however, biliary excretion of each agent was not measured.

In a recent study (15), iopanoic acid was administered into the duodenum of dogs either simultaneously or 1 hr after an intravenous iodipamide infusion. Biliary iodipamide excretion was diminished concomitantly with an increased urinary iodipamide output.

Both iodoxamic acid and iopanoic acid bind readily to plasma proteins and have low blood clearances (6, 7, 16). Therefore, a displacement of plasma protein binding by one of these compounds may alter the elimination of the other. The coadministration studies described earlier did not assess this factor. Neither has the site nor the type of the interactions been evaluated.

The present investigation of protein binding (Tables I and II) shows that unbound concentrations of either iodoxamic acid or iopanoic acid, when infused in the presence of the other (the "competitor"), were greater than that when either acid was infused alone (6, 7) (11 of 12 instances for Monkey A and in all instances for Monkey B). These results suggest that the two compounds compete for binding sites in the proteins. Because of insufficient data, no attempts were made to determine the type of competition.

Furthermore, in both monkeys, the biliary excretion rate of each drug was lower when coadministered than when administered alone (6, 7); this finding confirms previous reports (13–15) that the biliary excretion of

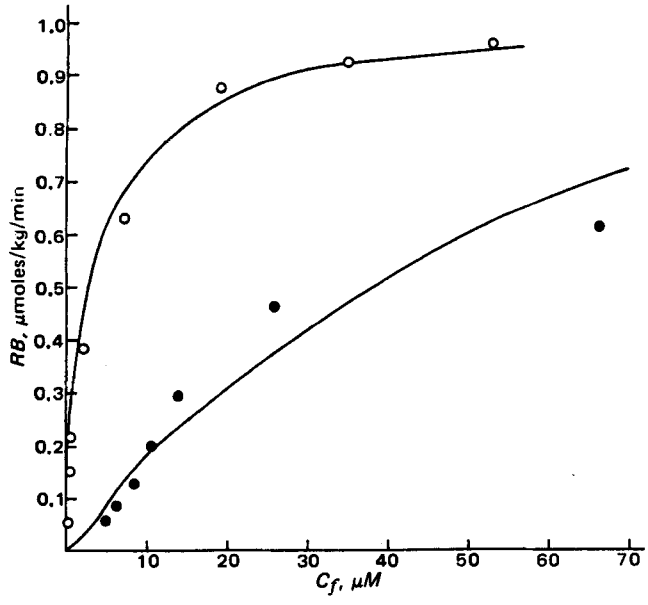

Figure 3—*Relationship between the biliary excretion rate* (RB) *and the unbound plasma concentration* (C_f) *of iodoxamic acid in the absence* (○) *and in the presence* (●) *of iopanoic acid in Monkey B. The upper solid line represents the computer-fitted values according to Eq. 4. The lower solid line represents the computer-fitted values according to Eq. 1.*

Table IV—Michaelis-Menten Parameter for Biliary Excretion of Iodoxamic and Iopanoic Acids in Monkey B

Parameter	Iodoxamic Acid	Iopanoic Acid	Combination Experiment [a]
$\overline{V}_m$, μmoles/kg/min	1.01 (0.07) [b]	1.1 (0.04)	1.01 (0.08)
K_m μM	3.5 (1.0)	0.22 (0.02)	3.5 (13.0)
K_i, μM	—	—	0.08 (0.49)

[a] $\overline{V}_m$ and K_m are fixed at iodoxamic values, and K_i is allowed to float. [b] Numbers in parentheses represent standard error of the mean.

one cholecystographic agent is reduced when coadministered with the other. In addition, the lower rate of iodoxamic acid was comparable to that of iopanoic acid (Fig. 2).

Finally, in both experiments, when the compounds were coadministered, even though the biliary excretion rates for both compounds were comparable, unbound concentrations of iopanoic acid were much lower than those of iodoxamic acid (Fig. 2 and Tables I and II). These findings suggest that iopanoic acid has a higher affinity than iodoxamic acid for the intrahepatic binding proteins (*e.g.*, Y- and Z-proteins) responsible for hepatic uptake or biliary excretion. Furthermore, relative to control values, when the compounds were administered concomitantly, competition for the intrahepatic uptake or biliary excretion between these two compounds led to lower overall excretion of both acids, with a greater reduction of iodoxamic acid excretion, despite the increased unbound plasma concentration.

In the present investigation, the "inhibitor" (iopanoic acid) was infused virtually to steady state. However, when the substrate (iodoxamic acid) infusion was started, protein binding equilibria were disturbed and the binding of both drugs was shifted. This displacement also affected the uptake or biliary excretion of both drugs, which is dependent on the free drug. After a period, the infusion of one or both compounds was discontinued. Safety considerations prevented a steady-state experiment relative to both drugs. Because the potential for drug accumulation was not known, it was feared that the animal might be killed if the infusion was continued.

After correction for the lag time, the midpoints of biliary excretion no longer coincided with the blood sampling times. Therefore, an INTERPOLATE program of the PROPHET computer system[8] was used to determine the unbound concentrations of iodoxamic acid present in the blood at the midpoints of biliary excretion. A graph (Fig. 3) of these data for Monkey B shows that the relationship between the biliary excretion rates of iodoxamic acid and its unbound concentrations during the coadministration reveals a curve that appears sigmoidal rather than hyperbolic, as was the case in the control study of the compound infused alone (6). A sigmoid curve was also demonstrated in the experiment for Monkey A. At the lowest biliary excretion rate (almost zero value), considerable unbound iodoxamic acid was still in the plasma.

Because the relationship between the biliary excretion rate (RB) and unbound concentration of iodoxamic acid (C_f) is sigmoidal rather than hyperbolic in the presence of the inhibitor, iopanoic acid, one of the following types of enzyme inhibition probably takes place: (*a*) ligand exclusion, (*b*) a two-substrate system forming a ternary complex, or (*c*) an activator of an allosteric enzyme mimicing the substrate and excluding the inhibitor (8). Only the ligand exclusion model does not require a sigmoidal relationship between the rate of enzymic reaction (v) and the substrate concentration (S) *in the absence* of the inhibitor (8). According to this model, in the absence of an inhibitor, a hyperbolic v *versus* S relationship exists (see *Theoretical*), which was the case for iodoxamic acid.

In both monkeys, a hyperbolic curve was obtained for iodoxamic acid in the absence of an inhibitor and a sigmoidal curve was obtained in the presence of iopanoic acid serving as the inhibitor. The relationships between the biliary excretion rate and the plasma unbound iodoxamic acid concentration in the absence and in the presence of the inhibitor in Monkey B are shown in Fig. 3 (Table III). The PROPHET computer system program "Hyperbolic" was used with the data weighted using the reciprocal of the variances. Both $\overline{V}_m$ and K_m were fixed at the values listed for iodoxamic acid, and K_i was at the $\overline{V}_m$ value of iopanoic acid (Table IV). Under such constraints, the data could not be fitted to Eq. 1. However, when K_i was allowed to float, results were obtained (column

[8] PROPHET system is a specialized computer resource developed by the Chemical/Biological Information Handling Program of the National Institutes of Health. A detailed description of the system's features appeared in *Proc. Natl. Compt. Conf. Exposition*, **43**, 457 (1974).

4 in Table IV). With the parameter estimates, the computer-fitted excretion rate values (RB') and residual values listed in columns 4 and 5 of Table III were obtained. These values indicate that the interaction between iodoxamic acid and iopanoic acid in the liver can be described by the ligand exclusion model. In such a model, iopanoic acid acts as an inhibitor and competes with iodoxamic acid for binding to either of two identical sites within the liver, which presumably is the rate-limiting step in the liver's overall elimination of these radiographic contrast agents. However, because of the large standard error of the mean of the estimated values of K_m and K_i, the fit may be fortuitous. Further studies will be required to investigate this tentative conclusion.

Presumably, the experimental method and the data analysis used in the present investigation can be applied to the study of the interactions between other compounds (*e.g.*, indocyanine green, sulfobromophthalein, propranolol, lidocaine, and propoxyphene) that have high hepatic excretion. To apply this approach, one must have assay procedures for each compound independently and be able to measure the unbound concentrations in plasma water.

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ACKNOWLEDGMENTS

Supported in part by U.S. Public Health Service Grants NIGMS 16-496 and GM 00728 and in part by the University of California Patent Fund.

Antibody Specificity Studies for Reserpine, Its Metabolites, and Synthetic Reserpine Congeners: Radioimmunoassay

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Received February 6, 1979, from the *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.* Accepted for publication May 24, 1979.

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Abstract □ Progress in the development of radioimmunoassay techniques for reserpine and related compounds is reported. A conjugate of reserpine with human serum albumin was prepared, involving linkage at the indole nitrogen atom of reserpine. Injection of the purified conjugate into sheep elicited antibodies of high titer, which bound reserpine selectively. Tritiated reserpine was employed in the procedure, and dextran-coated charcoal was utilized to separate free and bound forms of the drug. Antibodies exhibited a selectivity for reserpine and did not cross-react significantly with major human metabolites. Cross-reactivity of antibodies with other reserpine derivatives (*i.e.*, syrosingopine, deserpidine, and rescinnamine) also was investigated. A stable tritiated or radioiodinated reserpine derivative of high specific activity is being sought to improve assay sensitivity for use in bioequivalence and bioavailability studies. In the absence of any extraction or concentration procedures, at least a 10-fold increase in immunoassay sensitivity would be required to follow reserpine levels in humans given normal doses of the drug. The methods show promise also for the assay of reserpine derivatives such as deserpidine, which exhibits cross-reactivity to reserpine antibodies.

Keyphrases □ Reserpine—antibody specificity studies, metabolites, synthetic congeners, radioimmunoassay □ Radioimmunoassay—analysis, reserpine, metabolites, synthetic congeners □ Antihypertensive agents—reserpine, radioimmunoassay, antibody specificity studies, metabolites, synthetic congeners

Levy *et al.* (1) reported the development of a sensitive and specific radioimmunoassay for the antihypertensive agent reserpine, with a sensitivity of 15 ng/ml in rat plasma and no interference from major metabolites. Application of the method in human bioavailability and bioequivalence studies, without preliminary extraction procedures, has been limited by sensitivity. This limitation results from the instability of tritiated reserpine of high specific activity. Experience in this laboratory with reserpine immunoassay development has been entirely analogous, but the method holds promise if a suitable tritiated or iodinated reserpine analog can be prepared.

Earlier reserpine assays, which were reviewed recently (2, 3), are useful for certain applications but involve time-consuming separations and extractions and have inadequate sensitivity and specificity for reserpine determinations in plasma or urine. A recent method (4) is reportedly sensitive to 200 pg/ml and, perhaps, to 50 pg/ml with less confidence. However, this procedure is indirect, involving extraction followed by TLC and a fluorometric densitometer scan. Recovery during extraction may be variable and must be established.

The purposes of this report are to compare specificities of antiserums produced in these laboratories during immunoassay development with the work of Levy *et al.* (1) and to discuss the potential utility of reserpine antiserums in the immunoassay of commercially available reserpine analogs such as deserpidine and rescinnamine.

0022-3549/79/1100-1433$01.00/0