0031-6997/82/3401-0073$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 34, No. 1

Printed in U.S.A.

Disposition of Drugs in Man by Radioimmunoassay

SYDNEY SPECTOR

Roche Institute of Molecular Biology, Nutley, New Jersey

THERE are two important roles that drugs play: for the clinician they are therapeutic agents; for the pharmacologist they can serve as keys to unlock the fundamental mechanisms underlying diseases that they ameliorate. If the correlation is made that a drug is affecting a particular physiological system and thereby achieving clinical efficacy, it would be very nice to demonstrate a relationship between plasma concentrations and therapeutic action. All too often it is difficult to show the existence of such a simple relationship; also, there is a great variability in responses of patients to a drug. As our analytical methods become more sensitive and improve our ability to follow the disposition of a drug, we can achieve a better understanding of this variability. We can monitor differences in drug absorption, distribution, and elimination as a consequence of sex, genetic composition, and the complexity of the disease itself. Also, with the increased sensitivity from today's analytical methods, we can follow plasma content of the parent compound and its metabolites and the disposition of the drug in the presence of other drugs of interest, i.e., whether transport or metabolism is facilitated or inhibited. A complicating factor in the effort to develop a nice relationship between plasma levels and effects is that many drugs bind to plasma proteins; consequently the concentration of the free drug in plasma is not always proportional to the total drug present.

Since the pharmacological effects of the drug may be determined by the concentration of the free drug at its physiological site, it is important to measure the free drug in the plasma rather than the total drug (which is free and bound).

In the last analysis, the critical question the clinician asks is: How does the patient respond to a particular dose of a drug? Despite such a pragmatic view, one still attempts to relate clinical improvement with plasma drug concentrations. If one attempts to generate relationships between plasma content of a drug and response to that compound, sensitive analytical methods are necessary. The analytical methods can also be applied to study the pharmacokinetics of a drug after either an acute dose of the steady state levels during treatment. The pharmacologists have many methods at their disposal. One of the methods that possesses sensitivity, specificity, and ease of operation is radioimmunoassay. Since the assay requires the development of an antibody and because most drugs are unable to stimulate antibody production by themselves because of their size, the drug must be coupled to a larger molecular weight substance. Radioimmunoassay has been developed for many drugs (2).

There are idiosyncratic responses with many drugs. Jaffe and Martin (5) comment that opioid analgesics and antagonists produce a wide spectrum of effects including allergic phenomena occasionally. Dr. Elliot Vesell and I followed the disposition of morphine by radioimmunoassay in 10 normal men. We found a multiphasic decay curve. Within the first 10 minutes after the i.v.

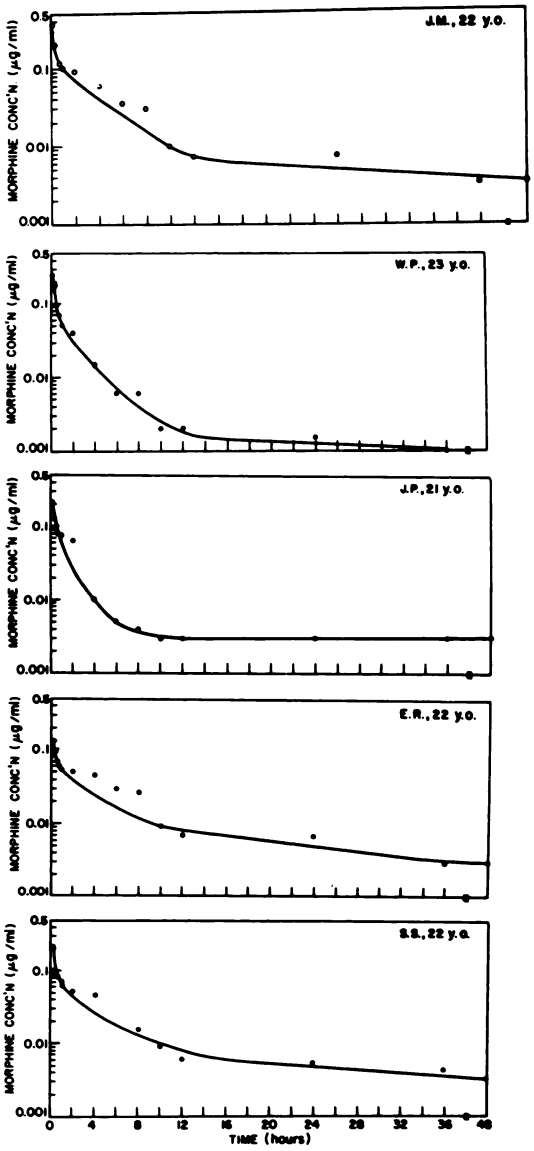


FIG. 1. Disappearance of morphine in serum of five subjects during 48 hours after a single i.v. injection of morphine, 10 mg/70 kg.

administration of 10 mg of morphine per 70 kg of body weight there occurred a very rapid decline. The next few hours also showed a rapid decline, but not as precipitous a fall, with a half-life of 2 to 3 hours. However, there was a very slow decline in 9 out of 10 subjects from 10 to 48 hours postinjection; the decline was slow up to 96 hours. Figure 1 shows the decline in 5 of the 10 subjects. A possible explanation for the prolonged presence of morphine in blood is that after i.v. administration a small amount of morphine may be bound to albumin. This raises the question whether the morphine bound to albumin is immunogenic and the allergic reactions seen in a small percentage of the population are the result of IgE antibody formation. Prolonged concentrations of morphine in the blood may have clinical implications; toxicity due to drug accumulation in the blood may result from repeated administration of morphine and, as Vesell and Page (12) pointed out, this possibility is more likely to occur in people with slow metabolism or in patients receiving multiple drug therapy. One might also speculate that the deaths associated with overdose of the opiate alkaloids by addicts could be a consequence of an anaphylactic response. Those who die from "overdose" usually are not naive drug takers; however, they have been self-administering the narcotic under septic conditions and thus one can suspect that the drug is being administered in the presence of an adjuvant that would promote the immunogenicity of the bound drug. The renal, pulmonary, and cardiac symptomatology of those who die from "overdose" would also suggest anaphylactoid symptomatology.

There have been many approaches to detoxify patients addicted to opiates. The antihypertensive drug clonidine is currently used to detoxify opiate addicts without eliciting opiate withdrawal signs and symptoms (4). It has

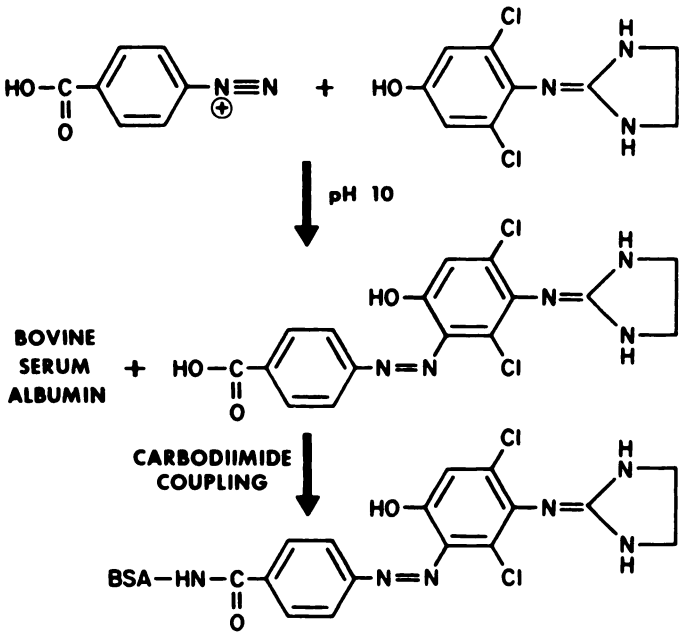


FIG. 2. Procedure for the preparation of clonidine immunogen.

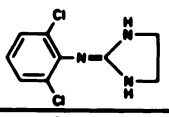
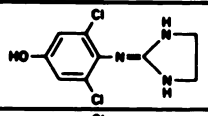
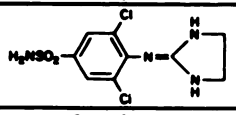
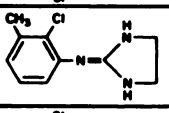
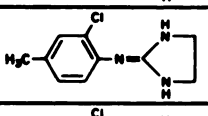
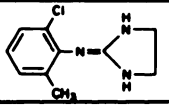
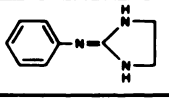
COMPOUNDS	MODIFICATIONS TO PHENYL RING	IC_{50} (nM)
CLONIDINE		26.5
4-HYDROXY CLONIDINE		19
Ro21-4254		194
ST-608		96
ST-375		150
ST-93		90
SKF-34429		>5000

FIG. 3. Cross reactivity of clonidine analogs for antibody binding site. The IC50 represents the concentration of unlabeled compound inhibiting binding of [^{3}H]clonidine to the antiserum by 50%.

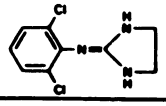
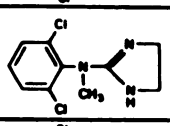
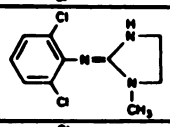
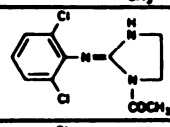
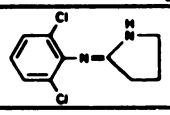
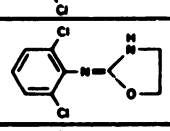
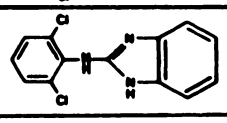
COMPOUNDS	MODIFICATIONS TO IMIDAZOLINE RING	IC_{50} (nM)
CLONIDINE		26.5
ST-1444		2200
SKF-38391A		>5000
SKF-34874		553
SKF-34531		1052
SKF-35246A		>5000
SKF-35066		>5000

FIG. 4. Crossreactivity of clonidine analogs for antibody binding site.

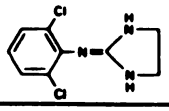
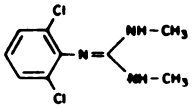
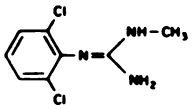
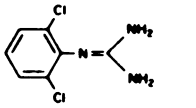
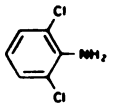
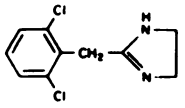
COMPOUNDS	CLONIDINE DERIVATIVES	IC_{50} (nM)
CLONIDINE		26.5
SKF-34688		>5000
SKF-36597		1760
SKF-37295		>5000
2,6-DICHLORO ANILINE		>5000
SKF-34689		>5000

FIG. 5. Crossreactivity of clonidine analogs for antibody binding.

been suggested by Gold et al. (4) that the basis for the clinical efficacy of clonidine is that it acts at the α_2 site in the locus coeruleus and reduces the increased noradrenergic neuronal activity seen during opiate withdrawal. Aghajanian (1) found that although clonidine and the opiates may not act at the same receptor within the locus coeruleus, both drugs have similar depressant effects on locus coeruleus activity. He speculated that the parallel but independent effects at this site might possibly provide a basis for the α_2 agonist, clonidine, to suppress certain symptoms of opiate withdrawal. With the antibodies one could also localize clonidine and its metabolite in the central nervous system by immunocytochemical techniques.

Radioimmunoassays have proved capable of determining picogram amounts of psychotropic drugs such as morphine (10), barbiturates (3), benzodiazepines (8), desmethylimipramine (11), and reserpine (7). Jarrott and I (6) developed a radioimmunoassay for clonidine for pharmacokinetic studies. By the procedure outlined in figure 2, an antibody for clonidine was developed that could be used for immunocytochemical studies to localize its site of action. The antibody that was generated showed a high specificity for substituted phenyliminoimidazolidine compounds, and an opening on the imidazolidine ring or a substitution on an imidazolidine N or imino N produced no crossreactivity (figs. 3–5). A known metabolite of clonidine, 4-hydroxyclonidine, can be measured by this assay (9). However, a simple benzene solvent extraction of plasma before radioimmunoassay allows the selective assay of clonidine.

Our studies indicated that 60 minutes after the administration of clonidine, the metabolite 4-hydroxyclonidine could be detected in the plasma. Since 4-hydroxyclonidine is as potent an α-adrenoreceptor agonist as clonidine, it would be interesting to determine whether the suppressive effects of clonidine on opiate withdrawal are due to the parent drug or to the metabolite.

Just as the development of antibodies against hormones had an impact on endocrinology, so too will the development of antibodies against drugs impact on pharmacology.

Summary

The disposition of morphine and clonidine was investigated by means of radioimmunoassay. After the administration of a single i.v. dose of morphine (10 mg/70 kg) to 10 men who had not received other drugs for two weeks preceding the study, a multiphasic decline in serum concentrations of morphine occurred. Detectable blood concentrations of morphine, or a metabolite, or of both, persisted for up to 96 hours after a single i.v. dose.

The radioimmunoassay for clonidine is capable of detecting as little as 10 pg of clonidine. The antibody fails to bind 2,6-dichlorophenylguanidine, a known metabolite of clonidine, while the other metabolite, 4-hydroxyclonidine, was as potent as clonidine in displacing labeled clonidine from the antibody. The disposition of clonidine in plasma and tissues could be monitored by radioimmunoassay.

REFERENCES

1. AGHAJANIAN, G. K.: Tolerance of locus coeruleus neurons to morphine and suppression of withdrawal response by clonidine. Nature (Lond.) **276:** 186–188, 1978.
2. BUTLER, V. P.: The immunological assay of drugs. Pharmacol. Rev. **29:** 103–184, 1978.
3. FLYNN, E. J., AND SPECTOR, S.: Determination of barbiturate derivatives by radioimmunoassay. J. Pharmacol. Exp. Ther. **181:** 547–554, 1972.
4. GOLD, M. S., POTTASH, C. A., SWEENEY, D. R., AND KLEBER, H. D.: Opiate withdrawal using clonidine. J. Am. Med. Assoc. **243:** 343–346, 1980.
5. JAFFE, J. H., AND MARTIN, W. R.: Opiod analgesics and antagonists. *In* The Pharamcological Basis of Therapeutics, 6th ed., ed. by A. G. Gilman, L. S. Goodman, and A. Gilman, pp. 494–534, Macmillan Publishing Co. New York, 1980.
6. JARROTT, B., AND SPECTOR, S.: Disposition of clonidine in rats as determined by radioimmunoassay. J. Pharmacol. Exp. Ther. **207:** 195–202, 1978.
7. LEVY, A., KAWASHIMA, K., AND SPECTOR, S.: Radioimmunoassay for desmethylimipramine. Psychopharmacol. Commun. **1:** 421–429, 1975.
8. PESKAR, B., AND SPECTOR, S.: Quantitative determination of diazepam in blood by radioimmunoassay. J. Pharmacol. Exp. Ther. **186:** 167–172, 1973.
9. REHBINDER, D., AND DECKERS, W.: Intersuchung zur Pharmakokinetik und zum Metabolismus des 2-(2,6-Dechlorophenglamine) 2-Imidazolin-Hydrochlorid (ST 155). Arzneim.-Forsch. **19:** 169–176, 1969.
10. SPECTOR, S., AND PARKER, C. W.: Radioimmunoassay. Science **168:** 1347–1348, 1970.
11. SPECTOR, S., SPECTOR, N., AND ALMEIDA, M.: Radioimmunoassay for desmethylimipramine. Psychopharmacol. Commun. **1:** 421–429, 1975.
12. VESELL, E. S., AND PAGE, J. G.: Genetic control of drug levels in man: Phenylbutazone. Scinece **159:** 1479–1480, 1968.