Fluorometric Assay of Verapamil in Biological Fluids and Tissues

R. G. McALLISTER ˣ **and S. M. HOWELL**

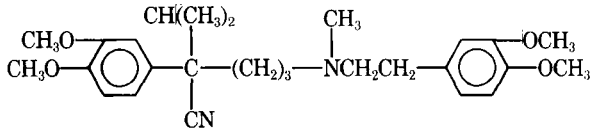
Abstract □ Verapamil concentrations in blood, urine, or tissue homogenates may be measured fluorometrically in the 0.1–10-μg/ml range after extraction into heptane and back-extraction into acid; recovery rates are above 87%. The drug apparently is eliminated rapidly in guinea pigs, with a plasma half-life of 45 min.

Keyphrases □ Verapamil—fluorometric analysis, biological fluids and tissues □ Fluorometry—analysis, verapamil, biological fluids and tissues □ Antiarrhythmic agents—verapamil, fluorometric analysis, biological fluids and tissues

Verapamil, 5-[(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile (I), has been widely used outside the United States as an antianginal (1, 2) and antiarrhythmic (3–5) agent. Although not available for clinical use in this country, verapamil is being intensively studied because of its ability to suppress inward calcium currents in cardiac and other excitable tissues (6–8). Such "slow" calcium currents may be involved in fatal arrhythmias occurring during myocardial ischemia or infarction and associated with sudden cardiac death (9).

Although the mechanism of verapamil's action at the cellular level in terms of excitation–contraction coupling and competitive antagonism of calcium-induced effects has been studied *in vitro* (5, 9, 10), little information is available regarding the pharmacological disposition of the drug, either in animal species or in humans. McIlhenny (11) studied ^{14}C-verapamil in rats and dogs and reported that it was rapidly metabolized to *O*- and *N*-dealkylated forms and eliminated primarily in feces.

Slow channel depolarization phenomena may be studied by determining the effects of different antiarrhythmic agents on calcium-dependent currents (12). There is, therefore, considerable interest in correlating tissue concentrations of verapamil with the electrophysiological effects observed. Present studies in these laboratories involve the use of guinea pigs pretreated with verapamil and studied at varying times after drug administration. To determine the kinetics of verapamil in experimental animals and to correlate tissue drug levels with measured effects, a spectrophotofluorometric assay procedure that permits determination of verapamil levels in the 0.1–10-μg/ml range was developed and is reported here.



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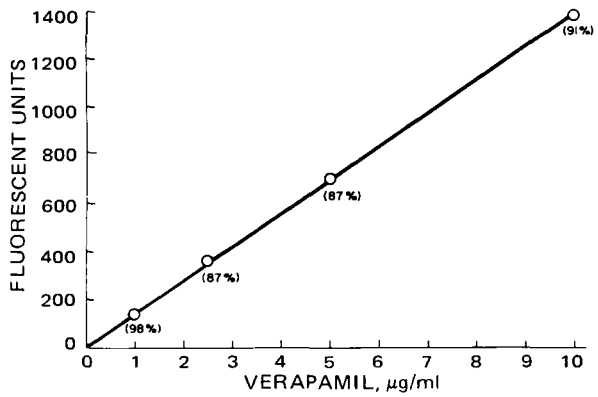

Figure 1—*Standard curve obtained by assay of control guinea pig liver homogenate to which known quantities of verapamil had been added. Similar results were obtained in plasma, serum, urine, and heart homogenates.*

EXPERIMENTAL

The assay procedure for verapamil is identical for plasma, serum, urine, or tissue homogenates. To prepare tissue homogenates, the liver and heart were removed from guinea pigs and mice immediately after sacrifice. Then a sufficient volume of 0.1 *N* HCl was added to give an approximate concentration of 75 mg/ml for liver and 500 mg/ml for heart. The tissues were then homogenized in hydrochloric acid by a motor-driven pestle[1] within a prefitted glass container.

An external standard solution of verapamil was prepared at a concentration of 100 μg of base/ml in 0.1 *N* HCl. To prepare internal standards, blank samples of body fluids or tissue homogenate aliquots of 4.75 ml were combined with 0.25 ml of the standard solution. Both external and internal standards showed a linear correlation between fluorescence and drug concentration in the 1.0–10-μg/ml range (Fig. 1).

To 5 ml of homogenate or body fluid in a 45-ml centrifuge tube were added 1.0 ml of 1 *N* NaOH and 10 ml of heptane (analytical

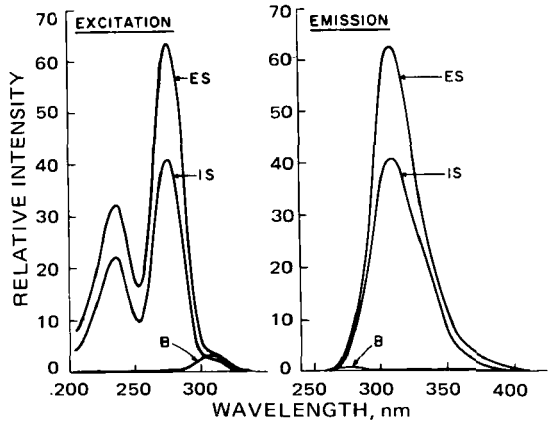

Figure 2—*Excitation and emission spectra of verapamil in external (ES) and internal (IS) standards at concentrations of 5 and 3.5 μg/ml, respectively. Fluorescence of the internal blank (B) was negligible.*

[1] Coated with Teflon.

Table I—Fluorescent Verapamil Concentrations in Guinea Pig Plasma and Tissue (Mean ± *SE*) after 30-mg/kg Injection

Minutes after Injection	n^a	Plasma Verapamil, μg/ml	Micrograms of Verapamil per Milligram of Tissue	
			Heart ($\times 10^{-3}$)	Liver ($\times 10^{-2}$)
30	5	1.98 ± 0.29	7.78 ± 1.27	4.27 ± 0.87
60	3	1.96 ± 0.41	7.73 ± 1.22	4.67 ± 1.22
90	3	1.18 ± 0.22	5.63 ± 1.74	2.40 ± 0.46
120	3	0.71 ± 0.14	1.53 ± 0.28	2.27 ± 0.33

[a] Number of different guinea pigs studied. From each animal, samples of plasma and tissue homogenate were assayed in triplicate.

grade). Each sample was shaken vigorously for 15 min in the upright position and then centrifuged for 10 min at 1800 rpm. Prior to shaking liver homogenates, a sufficient quantity of sodium chloride was added to make a saturated solution to assist in emulsifying the supernatant fatty layer.

After centrifugation, 7 ml of the heptane layer was transferred to a clean 45-ml centrifuge tube containing 5 ml of 0.1 *N* HCl. Each tube was vigorously shaken for 15 min and centrifuged at 1800 rpm for 10 min. The supernatant organic phase was aspirated and discarded. Two milliliters of the aqueous phase was used for spectrophotofluorometric[2] analysis at an excitation wavelength of 275 nm and an emission wavelength of 310 nm (Fig. 2).

The true final concentration of internal standards was calculated, and the spectrophotofluorometer reading was related to that of the corresponding external standard to find the percent recovery. Values ranged from 87 to 98%. Concentrations of unknown samples were calculated by comparison to the readings obtained for internal standards.

In the present series of experiments, guinea pigs were pretreated with intraperitoneal injections of verapamil hydrochloride, 30 mg of base/kg. They were sacrificed at varying periods after injection, and verapamil concentrations in plasma and tissue homogenates were measured (Table I).

RESULTS AND DISCUSSION

Consistently reproducible readings were obtained on multiple samples of control homogenates of the liver and heart, as well as plasma, to which known quantities of verapamil had been added (Fig. 1). Six replicate determinations at each concentration showed a standard deviation no greater than 2% at any point. Subsequent work demonstrated a continuing linear relationship between fluorescence and drug concentration to a lower limit of 0.1 μg/ml.

Verapamil was administered to guinea pigs, and drug concentrations were determined in the plasma, heart, and liver. The fluorescence spectra obtained after extraction were identical with that found for pure verapamil (Fig. 2). This finding does not eliminate the possibility of a metabolite containing the fluorophore being extracted and measured with the parent drug. Therefore, the quantity measured in these *in vivo* experiments is termed "fluorescent verapamil."

In the 200–300-g guinea pigs given verapamil, 30 mg/kg ip, fluorescent plasma verapamil levels demonstrated a first-order decline with an apparent half-life of 45 min (Table I). The drug was concentrated in both heart and liver, with a similar rate of elimination. Disappearance rates of fluorescent verapamil were studied in other groups of guinea pigs given 50- and 20-mg/kg injections. At the former dosage, half of the animals died within 30 min, at the latter dosage, elimination rates similar to those already noted were seen.

These preliminary studies demonstrated a rapid and reproducible assay for fluorescent verapamil in tissue homogenates and plasma; the drug appears to follow first-order kinetics at the dose level used, and it is rapidly eliminated from both plasma and tissues.

Preliminary tissue concentration–electrophysiological effect studies indicate that the ability of verapamil to block calcium-dependent membrane phenomena is apparent when measurable quantities of the drug have vanished[3]. Thus, concentrations of physiologically active material may be below the level of detection of this assay, or much of the drug's effects may be attributable to active metabolites not measured in the procedure reported here. Both possibilities are currently being investigated.

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[2] Aminco-Bowman spectrophotofluorometer, American Instrument Co.

[3] B. Surawicz, S. Imanishi, and R. G. McAllister, unpublished observations.