Estimation of Pharmacological, Biophasic, and Biological Half-Lives of Quinidine in Rabbits

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Abstract □ Pharmacological response intensities were measured from a lengthening of QT intervals and corresponding RR intervals following slow and rapid intravenous infusion of quinidine gluconate to rabbits for the purpose of determining the kinetics of pharmacological response. Measurements of the lengthening of the QT interval were corrected for heart rate, not according to Bazett's formula as has been done in the past but using a bisegmented linear relationship which more accurately relates the two variables. The temporal variation in response intensities was additionally used to quantify biophasic drug levels. This was accomplished by the use of a dose-effect curve, which served as a calibration curve to provide graphically the relationship between response and the amount of drug directly responsible for eliciting the response. Plasma concentrations of quinidine were also determined following intravenous dosing. Semilogarithmic time plots of response intensities, derived biophasic drug levels, and plasma concentrations were best fitted with similar biexponential relationships from which half-lives of 24.1–33.6 min were calculated. The results indicated that once the drug reaches the biophase, binding as well as debinding is instantaneous, and that the rate-determining step to decline in response is removal of drug from the biophase. It was further reasoned that the biophase is located within tissues kinetically indistinguishable from plasma. Distribution to a pharmacologically inert depot compartment was also apparent from the results.

Keyphrases □ Quinidine—estimation of pharmacological, biophasic, and biological half-lives, rabbits, pharmacological response intensities, ECG monitoring □ Half-lives, pharmacological, biophasic, and biological—estimation, quinidine, rabbits □ Pharmacological response intensities—monitored by ECG, estimation of pharmacological, biophasic, and biological half-lives of quinidine, rabbits □ Pharmacokinetics—estimation of pharmacological, biophasic, and biological half-lives of quinidine, rabbits, pharmacological response intensities, ECG monitoring □ Biological half-life—quinidine, rabbits □ Biophase distribution—quinidine, rabbits

The usefulness of quinidine in maintaining normal sinus rhythm following conversion from refractory cardiac arrhythmias is often limited by the difficulty of determining an adequate dosage and/or a suitable dosing interval. To provide some means of estimating dosages and dosing regimens without risking the unexpected development of toxicity, clinicians have sought a relationship between plasma drug levels and the therapeutic effect. Qualitatively, the therapeutic effect as measured by changes in QRS duration or heart rate-corrected QT intervals has been shown to correlate with plasma quinidine concentrations (1). However, a more quantitative correlation has not been possible because of the large degree of scatter generally displayed by ECG measurements. This is due in part to the difficulty in making precise measurements (1) and in part to a poorly defined baseline or reference response from which subsequent measurements influenced by drug must be compared.

This report shows that once a stable baseline is established for pharmacological response, it is possible to quantify the response intensities associated with quinidine for the purpose of estimating the kinetics of pharmacological response, calculating biophasic drug levels, and comparing these processes to plasma levels of drug as a function of time in rabbits following intravenous dosing.

METHODS

A commercially obtained quinidine gluconate solution[1] was injected slowly over 1 min in the marginal ear vein of each of two rabbits; this procedure will be referred to as rapid intravenous dosing. The doses in terms of quinidine base were 0.31, 0.62, 1.24, 2.5, 3.75, 5.0, and 6.23 mg/kg of body weight. Quinidine prolongs the QT interval of the ECG at the time the antiarrhythmic effect of the drug is observed (2); it follows then that QT is the most relevant parameter to quantitate. In addition to QT intervals, the cycle length RR was measured to correct for changes in QT dependent on RR but independent of drug concentrations. This was accomplished through the use of Eq. 1:

$$QT = (bRR + a)Z + (1 - Z)QT_B \qquad \text{(Eq. 1)}$$

When determined for individual subjects before dosing, the QT interval is related to RR according to the bisegmented linear relationship expressed as Eq. 1 and shown in Fig. 1, where a and b represent the intercept and slope of the diagonal linear segment, respectively, and QT_B represents the baseline value of QT independent of RR. The parameter Z is referred to as a "dummy variable" and is defined as:

$$Z = \begin{cases} 1, & \text{if } RR \leqslant E \\ 0, & \text{if } RR > E \end{cases} \qquad \text{(Eq. 2)}$$

Point E represents the intersection or join point of the two linear segments; QT and RR were measured directly from the stored sweep displayed by an oscilloscope[2] equipped with a dual-time base[3] consisting of two sweep modes and a high gain differential amplifier[4].

To measure the ECG intervals, a precordial-type lead was placed over the anterior aspect of the rabbit's chest with the indifferent lead positioned over the caudal region of the lower back. The rabbits were placed in a suitable restrainer to minimize postural effects on the measurements. Disposable silver electrodes[5] coated with a small quantity of electrolyte paste were affixed to the exposed skin. Although various formulas (3–6) have been used to correct QT for the cycle length, including the commonly used square-root relationship (7), it was found that Eq. 1 more closely described the relationship between QT and RR for individual data in a number of species including humans[6]. The procedure consisted of first recording QT–RR data at relatively slow heart rates by permitting the animal to remain inactive in the restrainer. Measurements corresponding to faster heart rates were obtained by removing the animal from the restrainer and with electrodes left in place inducing exercise in a treadmill fashion. Measurements were taken as soon as the rabbit was returned to the

[1] Eli Lilly and Co.
[2] Model 5103N, Tektronix, Inc., Beaverton, Ore.
[3] Model 5B12N, Tektronix, Inc., Beaverton, Ore.
[4] Model 5A22N, Tektronix, Inc., Beaverton, Ore.
[5] Electronics for Medicine, Inc., White Plains, N.Y.
[6] R. D. Schoenwald and V. E. Isaacs, to be published.

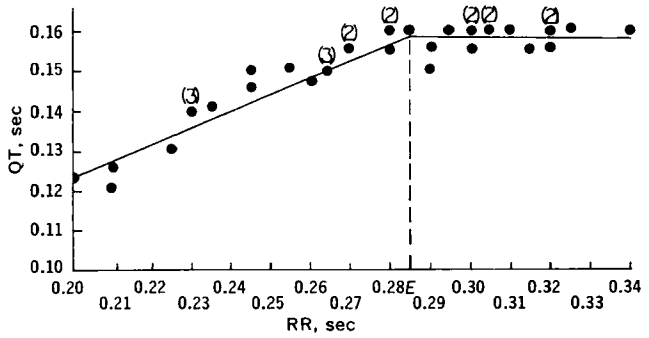

Figure 1—*Bisegmented linear relationship between the experimentally obtained QT and RR for a single rabbit. The numbers in parentheses represent the number of replications recorded at that point. The line denotes the computer least-squares line of best fit, which is represented by* $QT = (0.44 + 0.0355)Z + (1 - Z)0.158$. *The join point is symbolized by* E.

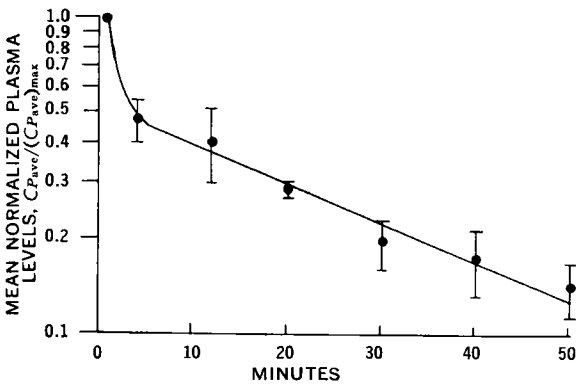

Figure 2—*Time course of mean normalized plasma levels of quinidine. The computer least-squares line of best fit is* $C_{Pave}/(C_{Pave})_{max} = 3.57e^{-0.535t} + 2.01e^{-0.0288t}$. *Each point is an average of one determination made on each of two rabbits; the vertical bars represent 1* SD.

restrainer. A few repetitions of this exercise produced a full range of QT–RR measurements so that predosing parameter values could be reliably obtained. Curve-fitting programs were written in FORTRAN IV for the digital computer[7] to calculate the parameter values of Eq. 1.

To ascertain the join point E, calculations were made by computer in which values of E were chosen in a successive manner, beginning with the third lowest RR value and proceeding to the maximum RR value. A sum of squares solution was written for Eq. 1 as outlined by Hudson (8) for fitting segmented curves whose join points are estimated from the data. By this approach, a least sum of squares is calculated for every possible join point. The line of best fit is determined by the lowest sum of squares computed, and the parameter values calculated for a, b, and QT_B were taken to describe the data best. Each set of data points was given equal weight; however, when duplicate points were recorded, they were included in the calculations, thereby indirectly weighting that particular determination by the number of replications recorded for that point. The primary advantage in using Eq. 1 to correct QT for heart rate over other formulas is that QT_B represents a reference point independent of RR, which permits greater precision to be obtained in the individual case because of the closer fitting relationship. The QT–RR relationship was well defined just before dosing and was assumed to apply for the duration of the experiment[8]. A new QT–RR relationship was determined just before the administration of each dose.

Following dosing, the response measurements, QT and RR, were recorded in triplicate at 1, 2, 3, 6, 9, 11, 13, and 15 min and every 5 min thereafter until measurements returned to the baseline value, QT_B. For most determinations, gain settings were chosen that permitted one or two RR intervals to be enlarged in sweep A whereas the QT interval corresponding to the first cycle in sweep A was enlarged in sweep B. The position control was adjusted so previous sweeps could be stored; with this procedure, two or three sets of sweeps could be displayed in a matter of seconds. This method of recording the ECG allowed for the simultaneous display of two or three tracings, each representing essentially the same point in time; in addition, it minimized the chances of mistaking a T wave for a partially merged T plus U complex and permitted measurements of the QT interval to within 0.005 sec. When an irregular baseline or artifact occurred or if U wave interference was suspected, the measurement was discarded.

Additional measurements of response were performed on two rabbits (4.30 and 4.25 kg) during the time course of a slow intravenous infusion of a 2.5-mg/ml solution of quinidine prepared from the commercial quinidine gluconate solution and distilled water. The solution was infused using a syringe pump[9] at a zero-order rate of 0.0247 ml/min (0.0145 mg/kg/min).

Nonweighted fits to all experimental data used in this report were obtained[10]. Beginning with an initial set of parameter values, the program obtains a least-squares fit to experimental data by means of stepwise Gauss–Newton iterations (9). Exponential relationships were fit to a summation of exponential terms. To obtain initial estimates, data were plotted as a function of time on semilogarithmic paper. By using back-extrapolation procedures (10), the curves were resolved into the appropriate number of exponential components to yield values for the equation parameters A_i's and m_i's. For the determination of plasma levels of quinidine, each of the two rabbits received a 3.75-mg/kg dose in separate experiments. Following dosing to the marginal vein of the left ear, venous blood samples were collected from the marginal vein of the right ear at 1, 5, 12, 20, 30, 40, and 50 min. Response was monitored only to ascertain the point in time when drug effects had dissipated. Quinidine concentrations were determined by the method of Cramer and Isaksson (11), in which quinidine is first extracted into benzene and finally transferred to sulfuric acid before fluorometric[11] determinations are made.

THEORETICAL

A method was developed by Smolen and Schoenwald (12) and applied to the mydriatic response intensity of tropicamide to quantitate the amount of pharmacologically active drug in the biophase following ophthalmic administration (13, 14). The results indicate that the drug was passively absorbed across the cornea as well as surrounding peripheral tissues, *i.e.*, nasolacrimal duct and scleral tissue, following dosing to rabbits. The bioavailability of tropicamide from the ophthalmic, oral, and intramuscular routes of administration was calculated entirely from pharmacological data and determined to be a function of pH and vehicle adjuvants. This method offers an *in vivo* approach to quantifying drug levels from pharmacological response intensities by the use of a dose–effect curve, which serves as a calibration curve to provide graphically the relationship between pharmacological response and the amount of drug directly responsible for eliciting the response. This method obviates the necessity for deriving the-

[7] CDC digital computer, Computer Science Center, University of Washington, Seattle, Wash.

[8] An attempt was made to determine QT changes solely as a function of RR during steady-state biophasic drug levels (slow intravenous infusion of quinidine), but interpretation was difficult because of fluctuating response values, presumably due to changing biophasic drug levels induced by exercise. This was attempted on two occasions, but a great deal of scatter resulted. The E value as well as QT_B was estimated to be the same as that determined before dosing, but values of a and b could not be reliably calculated. The effect of exercise on k_{ij} values is not well known; therefore, changes in parameter values could reflect changing biophasic drug levels and not necessarily a changing QT–RR relationship. Therefore, it was assumed that whatever stimuli were responsible for QT changing in response to RR, *e.g.*, simultaneous or separate positive and negative chronotropic effects of quinidine, the QT–RR relationship and the parameters a, b, QT_B, and E remained constant during the experiment.

[9] Harvard Apparatus Co., Millis, Mass.

[10] By use of the BMDX 85 FORTRAN IV computer program written for the CDC 6400.

[11] Flurospec, SF-100, Baird-Atomic Inc., Bedford, Mass.

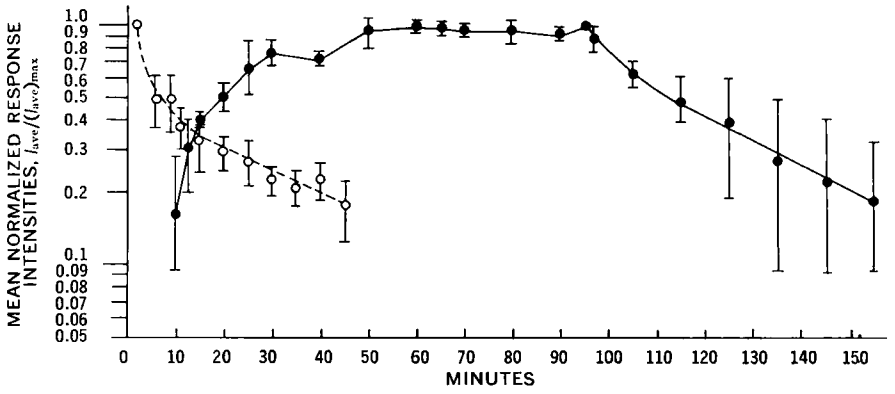

Figure 3—*Time course variation in response intensities following rapid (○) and slowly infused (●) intravenous doses of quinidine. Each point represented by open circles is an average of eight determinations for two rabbits given four doses of 2.5, 3.75, 5.0, and 6.25 mg/kg. The dashed line is a computer least-squares line of best fit represented by* $I_{ave}/(I_{ave})_{max} = 1.32e^{-0.461t} + 0.495e^{-0.0241t}$. *The solid line is a computer least-squares line of best fit for a slowly infused dose of quinidine of 0.0145 mg/kg/min to each of two rabbits for the postinfusion data beginning at* $t = 95$ *min and represented by* $I_{ave}/(I_{ave})_{max} = 0.224e^{-0.247(t-95)} + 0.777e^{-0.0240(t-95)}$. *The vertical bars represent 1* SD.

oretical relationships between pharmacological response and body drug concentration and concerns itself with the compartment of most interest, the biophase.

Expressions were derived previously (12, 15) that describe the relationship between response intensities and the quantity of drug in the biophase, Q_B (Eq. 3), as well as the time course variation of proportional biophasic drug levels, $f(I)$ (Eq. 4):

$$Q_B = f(I)\beta_{t_{max}} \qquad \text{(Eq. 3)}$$

$$f(I) = \frac{f(I)_{max}}{\beta_{t_{max}}} \sum_{i=1}^{n} A_i e^{-m_i t} \qquad \text{(Eq. 4)}$$

The values of $f(I)$ are obtained from the dose–effect curve by finding the experimentally observed response intensity value (I) on the ordinate and directly reading the corresponding abscissa value in milligrams per kilogram. In this way, I values as a function of time can be converted into the time course of proportional biophasic drug levels. The parameter $f(I)_{max}$ is a constant equal to the dose when the drug is given intravenously. The A_i's and m_i's are equation parameters evaluated from the data; n denotes the number of compartments included in the model. The constant $\beta(t_{max})$ equals the summation, $A_i e(-m_i t_{max})$; its value is unity for any model in which the biophase is kinetically indistinguishable from the central systemic compartment. Equation 4 assumes that the kinetics of drug disposition are first order and that the response is instantaneous once drug enters the biophase.

RESULTS AND DISCUSSION

Time Course of Plasma Levels of Quinidine following Rapid Intravenous Dosing—Figure 2 depicts the time course of logarithmic quinidine concentrations in plasma as a function of a 3.75-mg/kg rapid intravenous dose to two rabbits. Because the data points could be best represented by a well-fit biexponential relationship, the kinetics of quinidine distribution and elimination may be characterized by a two-compartment linear model with elimination most likely occurring from the central compartment. The latter log linear segment of the computer least-squares line of best fit yielded a biological half-life of 24.1 min.

Although the double-extraction fluorometric assay of Cramer and Isaksson (11) was reported by Resnekov *et al.* (16) to be more specific in terms of measuring unchanged quinidine than other often used assays (17–19), it is not presently known to what degree metabolic derivatives, probably possessing different pharmacokinetic properties from the parent compound, contribute to the assumed serum concentrations of quinidine. Consequently, with the specificity of the assay in question, a two-compartment linear model can only be assumed. In addition, the model is only assumed to be correct for the length of time the data were collected.

Time Course of Pharmacological Response Intensities—To evaluate the response characteristics of quinidine in individual rabbits as a function of time following intravenous dosing, it was necessary to correct measured QT intervals, in which the corresponding RR values were less than or equal to the join point E. This was achieved by the following relationship:

$$QT_t = \frac{QT_B}{(b\,RR + a)} QT_t' \qquad \text{(Eq. 5)}$$

where QT_t represents the ECG QT calculated as a function of time; and a, RR, and b are as previously defined. The symbol QT_t' represents the uncorrected ECG QT measured as a function of time following dosing. The ratio (≥ 1) of $QT_B/(b\ RR + a)$, when multiplied by QT_t', compares all QT_t measurements from a common reference point, QT_B, independent of RR; therefore, QT_t in effect takes on theoretically corrected values that would have been generated if QT_B and any RR greater than E had been the actual baseline values.

When Eq. 5 was applied to ECG measurements made before dosing or when the effect of quinidine on QT was no longer apparent at large values of t, QT_t was within the 95% confidence limits of QT_B. During the time course of effect, QT_t was significantly greater than QT_B; therefore, the fractional increase in response in

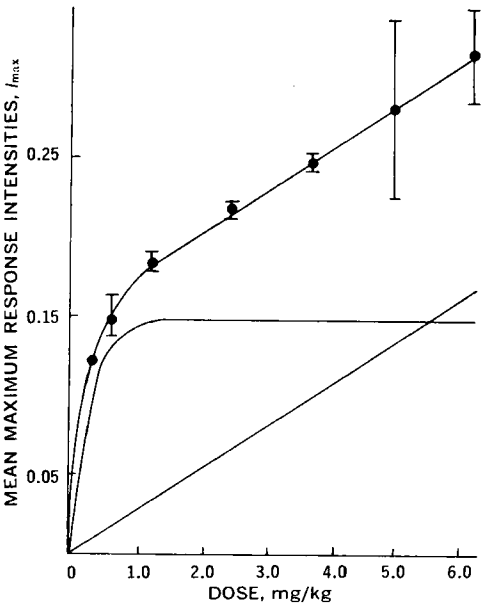

Figure 4—*Relationship between response intensities of quinidine calculated from QT lengthening at maximum intensities and the corresponding rapid intravenous dose of quinidine. The experimental points are averages of two determinations and computer fitted to a sum of two components classical receptor theory and intrabiophasic partitioning. The vertical bars represent 1* SD. *The resolution of the dose–effect curve into each component is shown separately.*

Table I—Equation Parameters[a] Characterizing the Kinetics of Pharmacological Response, Derived Biophasic Drug Levels and Plasma Concentrations of Quinidine following Slow and Rapid Intravenous Infusions to Two Rabbits

Type of Data[b]	Mode of Administration	A_1	m_1[c]	A_2	m_2[c]	A_3	$t_{1/2}$[d]
Pharmacological	Rapid intravenous[e]	1.32	0.461	0.495	0.0241	—	28.8
	Slow intravenous, postinfusion[f]	0.224	0.247	0.777	0.0240	—	28.9
Plasma	Rapid intravenous[f]	3.57	0.535	2.01	0.0288	—	24..1
Biophasic	Rapid intravenous[e]	3.48	0.687	0.127	0.0265	—	26.2
	Slow intravenous, postinfusion[f]	0.809	0.177	0.170	0.0206	—	33.6
	Slow intravenous infusion[f]	0.0016	0.648	1.39	0.0239	1.16	29.0

[a] Determined from nonlinear, least-squares, unconstrained computer fits to the experimentally obtained data. [b] All data were normalized by dividing each value by the maximum value recorded; therefore, A_i's are dimensionless. [c] Expressed as minutes^{-1}. [d] Calculated from the equation $t_{1/2} = 0.693/m_2$; expressed in minutes. [e] Parameters were computed from an average of eight determinations, four doses given to each of two rabbits. [f] Parameters were determined from an average of two determinations, one dose given to each of two rabbits.

reference to QT_B was calculated according to Eq 6:

$$I_t = \frac{QT_t - QT_B}{QT_B} \qquad \text{(Eq. 6)}$$

where I_t represents the intensity of response attributed to quinidine as a function of time for the rapid intravenous doses (Fig. 3). The response intensities obtained from each rabbit and at each dose were normalized with respect to maximum values at each time following dosing. The normalized response intensities were then averaged for each rabbit and dose. The computerized least-squares line of best fit was best represented by a biexponential relationship from which the latter log linear region of the curve yielded a pharmacological half-life of 28.8 min. The response intensities averaged for each rabbit but expressed separately for each dose could be fit by biexponential relationships producing pharmacological half-lives of 29.9, 38.4, 37.9, and 43.9 min for doses of 2.5, 3.75[12], 5.0, and 6.25 mg/kg, respectively. Response intensities significantly greater than baseline values were observed for 50–60 min following injection with the exception of the lower doses, 0.31, 0.62, and 1.24 mg/kg, which rapidly dissipated to predosing values within 15–30 min and were, therefore, not included in the averaging of data. Maximum response intensities for these lower doses, however, were used in establishing the dose-effect relationship.

The purpose of averaging the individual data was to obtain a more reliable fit to the data than might be obtained from treating the data from each dose separately. Justification for averaging response intensities over all doses comes from the fact that not only could the normalized response intensities for each dose be fit by a biexponential relationship but that pharmacological half-lives showed no apparent trend with dose. The rapid development of the maximal response, 2.17 ± 0.98 min, following dosing indicates the rapidity with which quinidine can enter the biophase and bind to receptors to elicit a response; consequently, the biophase can be kinetically assigned to the same compartment as the plasma. Under these conditions, $\beta(t_{max})$ for quinidine can be assigned a value of unity. The initial decline of plasma concentration for the rapid intravenous dosing was calculated to be approximately the same as the corresponding decline of response intensities as judged by the ratio of m_1 for each determination, 1.16 (Table I). Once equilibrium is reached, the continued monoexponential declines in response intensities and plasma levels nearly parallel one another. This would suggest that removal of drug from the biophase and not debinding of drug from receptor sites is the rate-determining step to decline in response. Garrett *et al.* (20) showed, by analog computer simulations, that the apparent half-life calculated from the decline in response may approach the half-life determined from plasma level measurements provided the number of receptor sites is relatively large, the affinity of drug for sites is low, and the rates of binding as well as debinding are large. These postulates, as far as can be ascertained from the data, are in agreement with the observed results.

[12] Calculated for one rabbit only; the other rabbit died of unknown causes before the dose could be administered.

The pharmacological response intensities attributed to quinidine in this study are a consequence of quinidine as well as any pharmacologically active metabolites; however, Conn (21) reported that antiarrhythmic activity in the rabbit is mainly the result of the cellular effects of unchanged quinidine. He found that dihydroquinidine, a contaminant in levels of 5–9% in commercial quinidine preparations (22) and also detected in rabbit urine[13] (21), was the only metabolite to produce significant antiarrhythmic activity. For the purpose of presenting new information and due to the lack of specific knowledge to the contrary, the percentage of contaminants was considered to be negligible in this report.

Figure 3 graphically demonstrates the logarithmic change in response intensities during the time course of the slow intravenous infusion of quinidine as well as the decline in response intensities following termination of the infusion after 95 min. The latter log linear decline in response intensities yielded a pharmacological half-life of 28.9 min, decidedly similar to the rapid intravenous results.

Intravenous Dose-Effect Curve—Figure 4 represents the dose-effect curve constructed from I values recorded at t_{max} for the corresponding rapid intravenous dose. The curve represents a summation of two components, hyperbolic and linear. The hyperbolic component is suggestive of a dose-effect relationship in which the "occupancy assumption" applies; that is, one drug molecule combines with one receptor site, response is proportional to the concentration of bound sites, and a negligible fraction of total drug is combined so that the response intensities can be related to total biophasic drug levels in preference to unknown free drug levels (23). The linear component of the dose-effect curve suggests a second set of receptor sites exhibiting a different affinity for drug in which partitioning of quinidine occurs under apparent nonsaturable conditions over the dose range studied; additionally, the possibility of one class of sites exhibiting mutual interaction should not be excluded. According to these assumptions, the response intensities can be related to Q_B using Eq. 7:

$$I = \frac{I_{max} Q_B^n}{K_D + Q_B^n} + P Q_B^n \qquad \text{(Eq. 7)}$$

where K_D represents the quantity of drug in the biophase at one-half of I_{max} for the hyperbolic component of the dose-effect curve, P represents the proportionality constant for the linear component, and n is a constant value which is 1 for a hyperbolic relationship. As n becomes greater than 1, the curve becomes more sigmoidal, implying multiple and cooperative binding sites for drug (24). Substituting dose [$\equiv f(I)_{max}$] for Q_B and assuming $\beta(t_{max})$ to be equal to 1.0, the values of I_{max} = 0.151 mg/kg, K_D = 0.116 mg/kg, n = 0.981, and P = 0.0281 mg/kg were determined from computer calculations and used in generating the theoretically based line in Fig. 4. The closeness of n to unity suggests that occupation theory is correct in describing the hyperbolic component of the dose-effect curve.

[13] Percentage not specified.

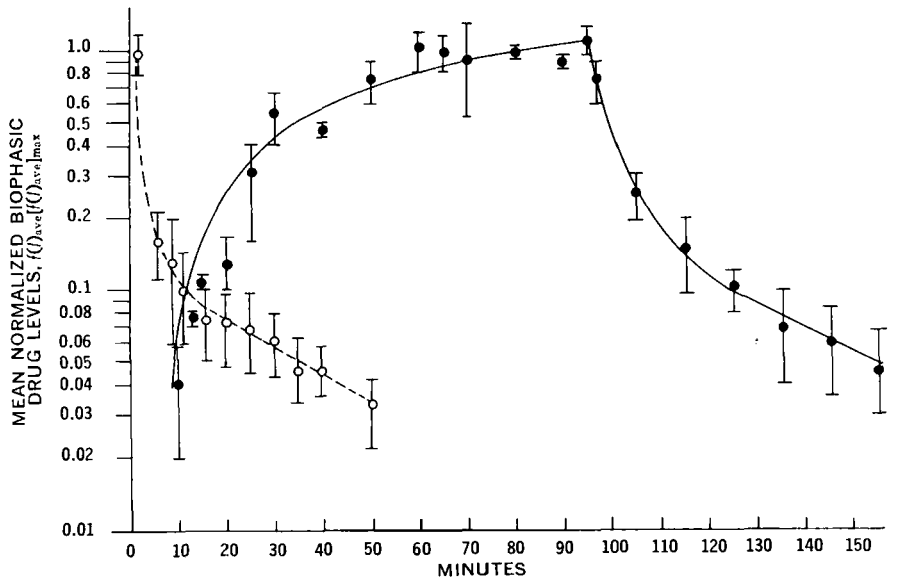

Figure 5—*Time course variation in biophasic drug levels following rapid* (○) *and slowly infused* (●) *doses of quinidine. Each point represented by open circles is an average of eight determinations calculated from response intensities measured in two rabbits given four doses of 2.5, 3.75, 5.0, and 6.25 mg/kg. The dashed line is a computer least-squares line of best fit represented by* $f(I)_{ave}/[f(I)_{ave}]_{max} = 3.48e^{-0.687t} + 0.127e^{-0.0265t}$. *The solid line is a computer least-squares line of best fit for a slowly infused dose of quinidine of 0.0145 mg/kg/min to each of two rabbits. The postinfusion data beginning at* $t = 95$ *min are represented by* $f(I)_{ave}/[f(I)_{ave}]_{max} = 0.809e^{-0.177(t-95)} + 0.170e^{-0.0206(t-95)}$. *The computer line of best fit for the infusion data up to and including* $t = 95$ *min was represented by* $f(I)_{ave}/[f(I)_{ave}]_{max} = 1.16 - 0.0016e^{-0.468t} - 1.39e^{-0.0239t}$. *The vertical bars represent 1* SD.

Time Course of Biophasic Drug Levels—Figure 5 depicts the time course of fractional amounts of quinidine in the biophasic compartment of the body. For the rapid intravenous dosing, the averages of eight determinations obtained for two rabbits and four doses are shown; the average of a single-infusion run administered to each of two rabbits is also shown. Biophasic half-lives were calculated from the slopes of the latter linear segments of the semilogarithmic plots for the rapid and postinfusion data, yielding values of 26.2 and 33.6 min, respectively. The independence of these values on dose is indicated by their similarity. Additionally, a lack of trend in half-lives was observed for the rapid intravenous doses of 2.5, 3.75, 5.0, and 6.25 mg/kg, corresponding to 22.6, 10.3, 42.3, and 37.5 min, respectively, which justified the averaging of the normalized data. A confirmation in biophasic half-life was further demonstrated from the calculation of the half-life from the infusion data up to 95 min using Eq. 8:

$$Q_B = f(I) = A_3 - A_1e^{-m_1t} - A_2e^{-m_2t} \qquad \text{(Eq. 8)}$$

Equation 8 corresponds to a two-compartment model where the A_i's and m_i's are equation parameters and the m_i's have the same meaning as in the rapid intravenous case (25). As a result of the kinetic characteristics of quinidine, $f(I)$ is considered to be directly equal to Q_B. From a least-squares computerized fit to the infusion data shown in Fig. 5, an m_2 value of 0.0239 was calculated to yield a biophasic half-life of 29.0 min.

In terms of ideal compartment theory, the drug is instantaneously dispersed within a body compartment so that the drug within these tissues can be considered as kinetically homogeneous. Therefore, once pseudoequilibrium is reached, as in the case of a multicompartment model, the monoexponential decline of drug sampled from any region of the compartment will be identical. In the case of quinidine, the rapid development of a maximum response indicates for all practical purposes an instantaneous dispersion within the central compartment of which the biophase is a part. In addition, the similar biological and biophasic half-lives calculated directly from plasma concentrations and indirectly from response intensities, respectively, suggest a kinetically homogeneous relationship between plasma and biophase. A rapid distribution phase, kinetically similar to that of the plasma data, was apparent with biophasic drug levels and represents equilibration into a pharmacologically inert depot compartment.

SUMMARY

In summary, pharmacological, biological, and derived biophasic half-lives were found to be similar following rapid and slow intravenous infusions. From direct measurements of response intensities, a maximum response was observed to be immediate following the rapid intravenous injection of quinidine. Although an insufficient number of replications was performed to draw firm conclusions, certain postulates were considered to be consistent with the observed results. The results indicate that the rate-determining step to decline in response is removal of drug from the biophase and that binding as well as debinding is instantaneous once drug reaches the biophase. Moreover, the biophase is located within tissues from which drug is in rapid instantaneous equilibration with plasma concentrations. Distribution to a pharmacologically inert depot was also apparent from the results. A comparison of the kinetics of pharmacological response to derived biophasic drug levels and plasma concentrations as determined by computerized fits of the data is summarized in Table I.

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Synthesis of Substituted Anilino-[3-methoxy-4-(4-arylthiosemicarbazidocarbonylmethyleneoxy)]benzylidenes: Correlation between Anticonvulsant Activity and Monoamine Oxidase Inhibitory and Antihemolytic Properties

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Abstract □ Several substituted anilino-[3-methoxy-4-(4-arylthiosemicarbazidocarbonylmethyleneoxy)]benzylidenes were synthesized and characterized by their sharp melting points, elemental analyses, and IR spectra. Their ability to inhibit monoamine oxidase and to afford protection against hypoosmotic hemolysis in dog erythrocytes was found to bear no relationship with their anticonvulsant activity against pentylenetetrazol-induced seizures in mice.

Keyphrases □ Anilino-[3-methoxy-4-(4-arylthiosemicarbazidocarbonylmethyleneoxy)]benzylidenes, substituted—synthesis, characterization, monoamine oxidase, antihemolytic, and anticonvulsant activity □ Anticonvulsant activity—substituted benzylidenes, relationship to monoamine oxidase inhibitory and antihemolytic activity □ Antihemolytic activity—substituted benzylidenes against hypoosmotic hemolysis, relationship between monoamine oxidase inhibitory activity—substituted benzylidenes, relationship between antihemolytic and anticonvulsant activity

Hydrazine derivatives (1) and semicarbazides and thiosemicarbazides (2–4) have been shown to inhibit monoamine oxidase [EC 1.4.3.4 monoamine: O_2 oxidoreductase (deaminating)]. Monoamine oxidase inhibitors have also been shown to possess pronounced anticonvulsant properties (5). Evidence has been put forward to demonstrate that various drug responses are presumably mediated due to alterations in the physical properties of cell membrane and drug receptor interactions (6, 7). Studies with various central nervous system depressants, tranquilizers, and biogenic amines (8–11) revealed the ability of these compounds to stabilize the red blood cell membrane. Furthermore, substituted benzylidenes have been reported to possess psychotropic properties (12). These observations led to the synthesis of substituted anilino-[3-methoxy-4-(4-arylthiosemicarbazidocarbonylmethyleneoxy)]benzylidenes. The ability of these benzylidenes to inhibit monoamine oxidase and to exhibit membrane-stabilizing properties was investigated. The anticonvulsant activity of these compounds against pentylenetetrazol-induced seizures was also determined in an attempt to correlate their anticonvulsant activity with their monoamine oxidase inhibitory property and their ability to afford protection against hypoosmotic hemolysis using dog red blood cells.

The various substituted benzylidenes were synthesized by following the methods outlined in Scheme I.

EXPERIMENTAL[1]

Chemistry—Substituted anilines (I) on condensation with vanillin (II) gave substituted anilino-(3-methoxy-4-hydroxy)benzylidenes (III and IV). Treatment of these compounds with ethyl chloroacetate resulted in the formation of substituted anilino-[3-methoxy-4-(ethoxycarbonylmethyleneoxy)]benzylidenes (V and VI) which, on treatment with hydrazine hydrate, gave substituted anilino-[3-methoxy-4-(hydrazinocarbonylmethyleneoxy)]benzylidenes (VII and VIII). These benzylidenes, on further treatment with aryl isothiocyanates, yielded substituted anilino-[3-methoxy-4-(4-arylthiosemicarbazidocarbonylmethyleneoxy)]benzylidenes (IX–XXIV).

Substituted Anilino-(3-methoxy-4-hydroxy)benzylidenes (III and IV)—A mixture of 0.1 mole of substituted aniline (I) and 0.1 mole of vanillin (II) in ethanol, together with a few drops of acetic acid, was refluxed for 4 hr. The solid mass, which separated on cooling, was filtered, dried, and recrystallized from ethanol (Table I).

Substituted Anilino-[3-methoxy-4-(ethoxycarbonylmethyleneoxy)]benzylidenes (V and VI)—To a solution of 0.08 mole of III and IV in dry acetone were added 0.08 mole of ethyl chloroacetate and 0.08 mole of anhydrous potassium carbonate, and the reaction mixture was refluxed on a steam bath for 15 hr. The reaction mixture was filtered and excess acetone was removed by distillation. The crude product, which separated on cooling, was filtered, dried, and recrystallized from ethanol (Table I).

[1] Analyses for carbon, hydrogen, and nitrogen were performed by the Central Drug Research Institute, Lucknow, India. Melting points were taken in open capillary tubes and are uncorrected.