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Structural effects in drug distribution: comparative pharmacokinetics of apomorphine analogues

A. M. BURKMAN, ROBERT E. NOTARI AND W. KENT VAN TYLE

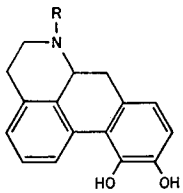
Divisions of Pharmacology and Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, U.S.A.

A two compartment open model, modified to include brain as a third compartment, was found to describe the pharmacokinetics of three aporphines in mice. Data include whole animal analysis, brain analysis and evaluation of stereotypical gnawing behaviour. The relative potencies of the derivatives, based upon brain content, were found to be apomorphine = 1, norapomorphine = 0·06 and *N*-n-propylnorapomorphine = 0·39. The potency of the *N*-n-propyl analogue is less than that previously reported on an intraperitoneal (i.p.) dosage basis (1·14). The ability to distribute into the brain, based on the entire time course for drug in that compartment, appears to be *N*-n-propylnorapomorphine >apomorphine >norapomorphine. The values for $t_{\frac{1}{2}}$ in mice are 29 min for the *N*-n-propyl derivative, 20 min for norapomorphine and 47 min for apomorphine which was previously reported to be 8·5 min based upon the inappropriate assumption of a one compartment model. The successful pharmacokinetic analysis was combined with the evaluation of structural effects on drug distribution and its resultant influence on pharmacokinetic response for the three closely related derivatives to demonstrate the applicability of the previously described whole animal pharmacokinetic treatment (Notari, Burkman & Van Tyle, 1974).

The rational design and evaluation of new drug analogues require a comprehensive interpretation of the effects of molecular modification on both the pharmacokinetic behaviour and the pharmacological response. It is well recognized that observed differences in the potencies of a series of closely related chemical structures may be due not only to variations in drug-receptor interactions but also to differences in the distribution of the drugs to the site of action. The time course for a drug at its site of action is influenced by many processes including the rate of elimination of drug from the body, the distribution of drug to tissues containing the site and the distribution of drug to various other tissues. It is therefore necessary to define the detailed pharmacokinetic time course for all compartments containing drug concomitant with an evaluation of the pharmacological response in order to assess the overall effect of structural differences in a series of analogues.

Previous publications have emphasized the growing need for suitable methodology to simultaneously evaluate structural effects on pharmacokinetic and pharmacological responses (Notari, 1973). Time-dependent measurements of a biological response to a drug, along with measurement of the drug's concentration in the anatomical region of its site of action, represent one approach to delineating these effects. Such studies may be carried out by analysis of whole animal homogenates together with analysis of specific organs or tissue homogenates as a function of time. Equations

and methods for developing pharmacokinetic models from such data have been evaluated (Notari, Burkman & Van Tyle, 1974). By choosing appropriate analogues the pharmacological response may also be evaluated and its relation to the model may then be examined.



Norapomorphine	R = —H	253·3 mol wt base
Apomorphine	R = —Me	267·3 „
N-n-Propylnorapomorphine	R = n—Pr	295·4 „

Criteria for choosing ideal analogues for such studies were previously defined (Notari & others, 1974). Three aporphine analogues (norapomorphine, apomorphine and *N*-n-propylnorapomorphine) were chosen as being reasonably good models relative to those criteria. By describing the time course for each in brain, blood, tissues and metabolic pool, the effects of the *N*-substituent groups on the relative potencies have been defined. Whole animal pharmacokinetic methods have been successfully applied in this evaluation and the results obtained provide insights into aporphine pharmacology that would not be apparent in the absence of kinetic data. The relative potency in terms of brain concentration has been assessed and the kinetics controlling availability of each analogue to the brain itself have been quantitatively defined. In this way both the effect of structure on biological response in brain and on the ability to gain access to the brain have been examined.

METHODS

Animals. Adult male albino mice (ICR strain, Harlan Industries, Inc., Cumberland, Indiana) weighing 18–39 g were used. The animals were housed in plastic cages (28 cm × 18 cm × 13·5 cm) and were maintained at a controlled environmental temperature of 22 ± 1° and conditioned to a 12–12 h light-dark cycle. Rodent chow (Ralston Purina Co.) and fresh water were continually available except just before and during an experimental run.

Assays of brain and whole animal drug content. A modification of the fluorometric procedure for the determination of apomorphine described by Van Tyle & Burkman (1971, 1972) was adopted for the three compounds in brain. The entire brain (400–500 mg) was homogenized in HCl (3 ml; 0·1M) and ethyl acetate (1 ml) (Spectroquality, Matheson Coleman Bell) in a Virtis "45" homogenizer equipped with Pyrex microhomogenization flasks. The homogenate was quantitatively transferred to a 10 ml volumetric flask and made up to 10 ml with 0·1M HCl. An aliquot (4 ml) was transferred to a 35 ml glass centrifuge tube and shaken for 2–3 min with ethyl acetate (5 ml). After centrifugation for 10 min and the removal of the ethyl acetate wash by aspiration, the pH was adjusted to 6·5—7·5 with sodium bicarbonate solution (1 ml; 0·5M) and the sample extracted with ethyl acetate (2 × 5 ml). The fluorescence of the pooled extract was determined in an Aminco-Bowman Spectrophotofluorometer at the excitation and emission wavelengths appropriate for the aporphine. A quinine sulphate standard (1 μg ml^{-1} of quinine sulphate in 0·1M sulphuric acid) was used to adjust the instrument to a constant sensitivity.

A modification of the above procedure was employed for the assay of the three compounds in the whole animal. The sample for assay was prepared by homogenizing the animal in an Osterizer blender with sufficient 0·1M HCl to make a 1:10 w/v dilution of the tissue and chilling the homogenate to 4° to await assay. The following procedure was then employed: An aliquot (4 ml) was transferred to a 35 ml glass centrifuge tube, HCl (4 ml; 0·1M) and ethyl acetate (10 ml) were added and the homogenate was shaken for 2–3 min. After centrifugation the ethyl acetate was removed by aspiration. The homogenate was then adjusted to pH 6·5–7·5 with sodium bicarbonate solution (2 ml; 0·5M) and extracted with ethyl acetate (10 ml) and the fluorescence of the extract measured.

The linear relation between fluorescence intensity and aporphine concentration was evaluated by similarly assaying tissue homogenates containing known amounts of the aporphine. Calibration curves were prepared to represent the range of tissue concentrations. Each was constructed from 5 datum points, each point representing 4–12 tissue samples.

The brain standards were prepared from homogenized whole brains of untreated animals to which HCl (1 ml; 0·1M) containing a known amount of the aporphine base was added just before the final addition of sufficient 0·1M HCl to make a total volume of 10 ml. Standards were otherwise identical to samples of brain to be assayed. The mean fluorescence of homogenate samples containing no aporphine was subtracted from the fluorescence of standard samples as a fluorescence blank correction.

Whole animal standards were similarly prepared. One ml of the 0·1M HCl containing known concentrations of the aporphine was transferred to a volumetric flask and sufficient homogenate from one untreated animal was added to make a total of 50 ml. Four ml replicates of this standard were assayed. The mean fluorescence of samples containing no aporphine was subtracted from the fluorescence of standard samples.

Assay specificity. Two methods were used to assess the selectivity of the extraction and assay procedures. In one case excitation and emission spectra were obtained for each of the three derivatives in both whole animal and brain homogenates following administration of the compound to the animals. These spectra were compared with those obtained by identical workup of samples prepared by addition of the derivative to homogenates from untreated animals.

The possibility of metabolic interconversion of administered derivative to a fluorometrically indistinguishable metabolite was ruled out using thin-layer chromatographic (t.l.c.) analysis. Apomorphine and *N*-n-propylnorapomorphine may potentially undergo *N*-dealkylation to yield norapomorphine whereas *N*-methylation of norapomorphine would form apomorphine.

The R_F value and minimum detectable level were determined for each of the three derivatives, solutions of which were prepared in whole animal homogenates by adding sufficient compound to make a final concentration of 0·1 mg ml^{-1} of the base. The samples were then extracted as described and increasing amounts of the ethyl acetate extract were spotted on t.l.c. plates (silica gel 0·25 mm, 5 × 20 cm, Brinkman Industries, Westbury, N.Y.) beginning with 10 μl and increasing in 10 μl increments up to 60 μl. The plates were developed using methanol: acetone, 1:1, and spots visualized by ultraviolet light.

To test for interconversion, each of the three derivatives was administered intravenously (i.v.) to groups of three animals in doses of 50 mg kg^{-1}. At a time corresponding to the estimated half-life for each compound, the animals were killed, homogenized and a 4-ml aliquot extracted. The ethyl acetate extract was shaken with HCl (4 ml; 0·1M) and the organic phase discarded. The aqueous phase was rapidly frozen in an acetone-dry ice bath and lyophilized (Virtis Model 10–145 MR–BA lyophilization apparatus, Gardner, N.Y.). The residue was dissolved in 100 μl of pH 7 McIlvaine buffer (McIlvaine, 1921) and the aporphines were extracted into ethyl acetate (100 ml). The ethyl acetate phase was spotted on t.l.c. plates, developed and visualized as described previously. Reference standards containing 10 μg ml^{-1} of the HCl salt, prepared in homogenate, were extracted, lyophilized and chromatographed adjacent to the sample extracts.

Time course in brain. The dose was selected to produce stereotyped gnawing behaviour in 100% of the animals, without producing overt toxic effects, and to maintain a duration of response of at least 40–50 min. Doses used were (mg kg^{-1}): norapomorphine HCl, 20; apomorphine HCl, 10; *N*-n-propylnoraporphine HCl, 10. A solution of each compound was prepared in normal saline to maintain a constant ratio of injection volume to body weight. Solutions were injected *via* the lateral tail vein in a volume of 0·1 ml of solution per 10 g weight. Solutions were prepared just before use. A group of randomly selected animals was used to determine each brain time profile following the administration of the aporphine. The animals were weighed to the nearest gram, injected with drug, then individually confined in wide-mouth glass gallon jars or 1000 ml glass beakers. At a preselected time after injection, the animal was decapitated and the entire brain was removed, weighed and homogenized. Saline-treated animals were assayed at identical time intervals. A mean blank fluorescence was subtracted from the fluorescence intensity reading for each aporphine-treated animal. The mean concentration of aporphine base in brain tissue $\pm$ standard error was determined for each elapsed time interval and the percent of the administered dose was then calculated. 154 mice were used to establish the brain concentration-time profiles of the three aporphines.

Time course in whole animal. The preparation of injection solutions, the route of administration and the volume of injected solution were the same as described for the brain determinations. For the estimation of the aporphine content in the whole animal, groups of randomly selected mice were weighed, injected with requisite volumes of solution and at preselected times homogenized. Saline-treated controls were similarly treated. 124 mice were used.

The aporphine content was expressed as a fraction of the amount present at the time of injection. To determine a zero time value, a group of animals was weighed and killed by cervical dislocation. They were then injected intraperitoneally with requisite amounts of the aporphine, homogenized and the homogenate assayed.

Pharmacokinetics. The fraction of the intravenous dose remaining in the whole animal and in the brain was calculated as a function of time. The mean value of six or more mice was used for each time interval. The whole-animal data were evaluated by nonlinear regression analysis based on a two-compartment model (Notari & others, 1974). The values for the constants k_{12}, k_{21} and k_2 and their standard deviations were determined for each derivative. These values were used to generate time profiles for the fraction of drug in the following compartments: whole animal,

plasma (B), tissue (T) and glucuronide formation (C) using the model defined previously (Notari & others, 1974).

The brain data were incorporated into the above model by assuming that drug supply to brain occurred *via* perfusing blood rather than from the tissue compartment of the two-compartment model. There are several studies in the literature that support this assumption (see Rall, 1971). The experimental values for the fraction of apomorphine or norapomorphine in the brain as a function of time were fit by analog computer using k_{13} and k_{31} as the adjustable parameters in the model

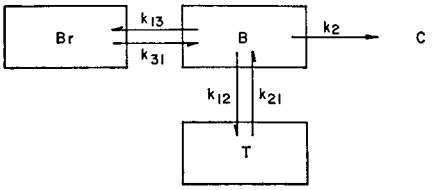


FIG. 1. General model used to describe the pharmacokinetics of the three apomorphine derivatives. B is the blood compartment, T is tissue compartment and C is the sum of elimination by all routes (Notari & others, 1974) with the addition of a brain compartment (Br) for which independent data were obtained.

shown in Fig. 1. Since the relative fraction in the brain was insignificant compared to the rest of the animal (apomorphine was less than 3% at its maximum and norapomorphine less than 1%), it was possible to include brain data after establishing values for k_2, k_{12} and k_{21}. *N*-n-Propylnorapomorphine showed a maximum brain fraction of 5%. However, its time profile can be described by a single proportionality constant relating it to plasma (rather than by k_{13} and k_{31}). *N*-n-Propylnorapomorphine brain fractions were linearly related to the fraction of drug in the plasma over the entire experimental time (1 to 50 min). Therefore, it was also possible to relate its brain time profile to plasma after k_2, k_{12} and k_{21} had been evaluated.

Relative potency. The EC50 or median effective brain concentration was defined as that concentration of aporphine in brain which would be expected to produce stereotypical gnawing behaviour in 50% of a population of animals. The determination is based upon the assumption that each animal has a threshold above which brain concentrations of the various norapomorphine congeners must rise to produce the gnawing syndrome. Further, it is assumed that in a population these threshold values are log normally distributed. Thus, as the brain concentration of the aporphine falls due to its removal by metabolism and elimination, the concentration will reach a level at which 50% of the animals will still be responding and 50% will not.

Groups of animals were randomly selected for analysis of drug in brain at various time intervals. Just before death the number of gnawing animals was recorded. The logarithm of brain concentration $\pm$ standard error was plotted against the percent of animals responding. The data were tested for significant regression and the least squares regression equation was calculated. The brain concentration corresponding to a 50% response and its 95% confidence interval were estimated from the regression equation. EC50 values were expressed in terms of nanomoles of aporphine base per gram of brain tissue.

RESULTS

Assays of brain and whole mouse content. The excitation/emission maxima determined in ethyl acetate were as follows: norapomorphine, 284/368 nm; apomor-

phine, 282/368 nm; and *N*-n-propylnorapomorphine, 284/368 nm. Their similarity suggests that the catechol-like structure is necessary for fluorescence and that the *N*-substituent has no effect on the fluorescence characteristics of the compounds.

Standard curves were prepared by the addition of each of the compounds to either brain or whole animal homogenate. The relation of fluorescence intensity to aporphine concentration is linear over the concentration range investigated. The least squares regression equations for each standard are summarized in Table 1. When the intercept of the least squares line was found not to differ significantly from zero, a new slope for the standard curve was calculated having a zero intercept (Steel & Torrie, 1960). The fluorescence intensities of the homogenate blanks were used to correct sample fluorescence for that of non-aporphine origin. Average blank values of 0·36 ± 0·01 fluorescence units for control brains and 2·10 ± 0·07 for whole animals were established.

Table 1. *Summary of least squares regression equations for standard curves.*

Compound and Tissue	Slope* (±s.e.)	Intercept
Norapomorphine		
Brain	15·366 (0·300)	—0·460†
Whole animal	10·756 (0·246)	0
Apomorphine		
Brain	22·899 (0·260)	0
Whole animal	19·825 (0·163)	0
N-n-Propylnorapomorphine		
Brain	14·365 (0·471)	0
Whole animal	13·059 (0·341)	—0·415†

* Relative fluorescence units per concentration unit (μg ml^{-1}).

†Intercept is significantly different from zero ($P < 0{\cdot}05$).

Assay specificity. A comparison of the spectrum of the authentic compound with that of the extract of the drug-treated animal in all cases revealed no significant differences between spectra. All spectra were of the same form as that described by Van Tyle & Burkman (1971). However, the fluorescence blank for whole-animal homogenate is appreciably greater than that of brain.

Since *N*-substituted aporphines might be *N*-dealkylated to norapomorphine and norapomorphine might be methylated to apomorphine, whole-animal extracts were examined for the presence of fluorescent metabolic interconversion products. Thin-layer chromatograms were made of extracts of homogenates from animals which had received apomorphine derivatives. With each of the three compounds there were no detectable spots for the potential fluorescent metabolites. In contrast, the administered compound was always found as a readily visible fluorescent spot. Based upon the minimum detectable levels, any fluorescent metabolites that might be undetected would represent less than 10% of the total aporphine content of the sample extract. Adler (1963) has shown that only 2% of a 5 mg kg^{-1} dose of morphine is *N*-demethylated in mice and there is no evidence to suspect substantial *N*-dealkylation of apomorphine.

Pharmacokinetics. Nonlinear regression analysis based on equations for a two compartment model was applied to the whole animal data in the manner previously described (Notari & others, 1974). Each experimental point used in the regression

Table 2. *Pharmacokinetic parameters for aporphine congeners in mice as determined by whole animal analyses following intravenous administration.*

	a (±s.d.)	α* (±s.d.)	b (±s.d.)	β* (±s.d.)	k_{12}* (±s.d.)	k_{21}* (±s.d.)	k_2* (±s.d.)
Nor-apomorphine	0·374 (0·137)	0·229 (0·112)	0·645 (0·141)	0·0345 (0·0076)	0·0597 (0·0401)	0·0637 (0·0349)	0·0940 (0·0142)
Apomorphine	0·835 (0·088)	0·135 (0·020)	0·138 (0·091)	0·0148 (0·015)	0·0214 (0·0106)	0·0263 (0·0183)	0·129 (0·009)
N-n-Propyl-nor-apomorphine	0·567 (0·187)	0·158 (0·057)	0·441 (0·191)	0·0237 (0·013)	0·0414 (0·0222)	0·0359 (0·0261)	0·0961 (0·010)

* Units are min^{-1}.

was the average value for six or more animals. The data were analysed twice for each derivative. In one case the adjustable parameters were a, α, b, β and in the second case the values for k_{12}, k_{21} and k_2 were iterated. Results are listed in Table 2.

The values obtained for the pharmacokinetic parameters indicate that the analyses are in the region of maximum expected reliability for the whole animal method (Notari & others, 1974). The ideal b/a range was suggested to be 0·1 to 10. Here the b/a ratios are nearly optimal as the value for norapomorphine is 1·7, apomorphine 0·2 and *N*-n-propylnorapomorphine 0·8. An optimum value of unity would indicate that half of the observable change in whole animal analysis is due to the alpha phase and half due to the beta phase. The relative values for k_{12}, k_{21} and k_2 are roughly 1:1:5 for apomorphine and 1:1:2 for the other derivatives. The previous investigation on whole animal methodology demonstrated that rate constants having these ratios were successfully estimated to within 1·0 to 5·9% of their true values.

The calculated values for k_{12}, k_{21} and k_2 (listed in Table 2) were used to generate the time course for fractions of drug in each compartment (whole animal, blood, tissue and metabolites). Typical examples showing computer-generated whole

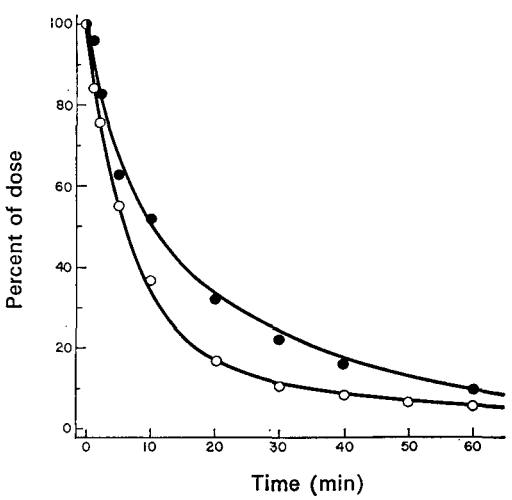


FIG. 2. Computer-generated curves and experimental values for whole animal assays as a function of time for apomorphine (○) and norapomorphine (●). The values for k_{12}, k_{21} and k_2 in Table 2 were used for the computer simulation.

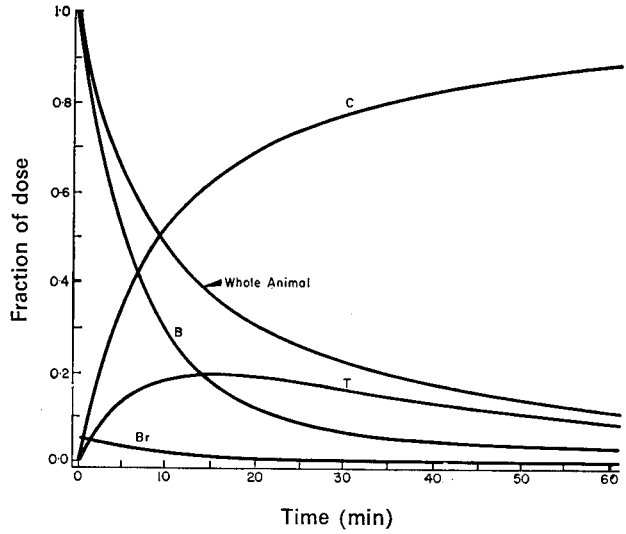


FIG. 3. Computer-generated curves for each compartment in Fig. 2 plus the whole animal simulation for *N*-n-propylnorapomorphine. The values for k_{12}, k_{21} and k_2 are found in Table 2. The brain compartment was related to blood by the constant 0·0544.

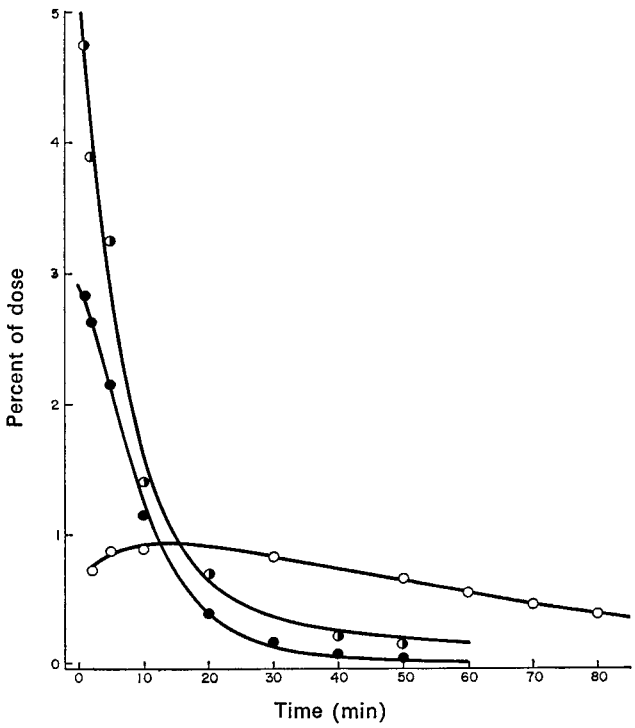


FIG. 4. Computer-generated curves and experimental values comparing time course for the three derivatives in brain. The brain compartment for *N*-n-propylnorapomorphine (◑) is related to the blood compartment by the constant 0·0544. The values employed for k_{13} and k_{31} (in min^{-1}) are: $7·3 \times 10^{-3}$ and 0·258 for apomorphine (●); $6·8 \times 10^{-4}$ and $1·7 \times 10^{-2}$ for norapomorphine (○). For additional values see Table 2.

animal curves for apomorphine and norapomorphine are compared to experimental values for whole animal assays in Fig. 2. Independent analyses for the time course of each derivative in brain allowed inclusion of a separate brain compartment as illustrated in Fig. 1. Data representing the fraction of each drug in brain as a function of time (plotted on expanded scale) were simulated by curve fitting on an analog computer. Satisfactory parameters relating brain levels to plasma levels were determined for each derivative. This allowed definition of all the compartments (plus whole animals) in Fig. 1 as a function of time. A typical description is shown in Fig. 3 for the *N*-n-propyl derivative. These profiles allow a complete comparison of the detailed distribution patterns of the three derivatives.

Fig. 4 shows the computer-generated time profiles for each drug in the brain compartment together with their experimentally determined values. It is obvious that the three derivatives differ in their ability to distribute from plasma into brain. *N*-n-propylnorapomorphine appeared to achieve equilibrium between brain and plasma immediately after intravenous injection. Linear regression of % dose in brain as a function of % dose in plasma showed that the brain content was 0·0544 times the plasma content over the entire experimental course of 1 to 50 min ($r = 0{\cdot}993$*). The highest observed value for brain content of the *N*-n-propyl derivative was 4·7% of the dose at 1 min which is the highest value for the three analogues.

Apomorphine showed a maximum brain content of 2·8% at 1 min. Brain content was linearly related to plasma content over the range of 5 min to 50 min ($r = 0{\cdot}999$). The 1 and 2 min values were not linearly related and were omitted from the least squares regression which indicated a brain/blood (Br/B) ratio of 0·0436.

The norapomorphine time profile showed the slowest brain distribution pattern and the lowest peak value. The maximum value of only 0·89% occurred between 5 and 10 min. In order to observe a disappearance curve the experimental time range was increased to include values from 2 to 80 min. A constant ratio of Br/B = 0·11 was observed for the region 50 to 80 min ($r = 0{\cdot}998$). However, contrary to the other derivatives, the intercept value was not zero but equal to 0·21% brain content when plasma becomes zero. The plasma content used for this regression is roughly 1 to 5% which is very low and also of limited range compared to the other derivatives. (The *N*-n-propyl derivative was linear over the plasma range of 3 to 82%; apomorphine was linear from 1 to 49%.) The values for Br/B may therefore be less reliable for norapomorphine. It is clear, however, that the rate of brain distribution is greatly reduced. A constant Br/B ratio is not achieved until the animal has nearly eliminated all of the drug (if it is achieved at all). The Br/B ratio is misleadingly higher than the other derivatives in that it occurs at very low plasma levels. The positive value calculated for brain content in the absence of plasma values could reflect some degree of "binding" in the brain although the limited range of values for the regression makes the observation unreliable.

Estimation of EC50 and relative potency values. A positive correlation between the brain concentration of apomorphine and the percentage of animals exhibiting gnawing behaviour has been previously reported (Van Tyle & Burkman, 1972). The EC50 values of apomorphine, determined at two dose levels, 2 and 10 mg kg^{-1}, were not significantly different. The data for these two determinations were pooled and the resultant EC50 and its 95% confidence interval is 0·32 $\mu g\ g^{-1}$ (0·30—0·35).

* Correlation coefficient.

This value has been converted to nanomoles of apomorphine per gram of brain for comparison of relative potencies.

Positive correlations between the concentration of *N*-n-propylnorapomorphine and norapomorphine in brain and the percent of animals exhibiting gnawing behaviour were also established. The EC50 values and their 95% confidence limits were determined from the regression equations for each line and were converted to nanomoles of compound per gram of brain tissue. Table 3 summarizes the potencies relative to apomorphine. Based upon the EC50 values, the relative value for norapomorphine is 0·06. This value is essentially the same as that determined by Koch, Cannon & Burkman (1968) based upon intraperitoneal doses. In contrast, *N*-n-propylnorapomorphine has a relative potency of 0·39, while based upon the intraperitoneal route (Koch & others, 1968) it is 1·14.

Table 3. *Brain concentration and biological activity of aporphine congeners.*

Compound	EC50 and 95% CL* nM g^{-1}	RP†	GD50±s.e.‡ μM kg^{-1}	
Norapomorphine	21·05 (20·97—21·13) n=40§	0·06	213±18 n=40	0·04
Apomorphine	1·20 (1·12—1·31) n=36	1·00	8·4±4·6 n=24	1·00
N-n-Propylnor-apomorphine	3·05 (2·78—3·73) n=30	0·39	7·5±0·7 n=40	1·14

* Median effective brain concentration and 95% confidence limits in mice.

† Potency relative to apomorphine.

‡ Median gnawing dose in mice (Koch & others, 1968).

§ Number of mice contributing to the dose-effect line from which the EC50's (or GD50's) were interpolated.

DISCUSSION

The unique combination of whole animal pharmacokinetics, analysis of drug content in the brain and quantitative evaluation of stereotypical gnawing provides information that has enabled us to compare both the potency and distribution of three closely related aporphines in mice. This investigation demonstrates the applicability of previously described methods (Notari & others, 1974) and clearly illustrates the interdependency of each of the three types of data (gnawing activity, brain concentrations and whole animal pharmacokinetics) required to assess structural effects on distribution and response. Interpretation of any one of these results is incomplete without the remaining two. For example, the relative potencies of the aporphines based on brain concentration is shown to differ from that previously based upon i.p. doses. Comparison of the time course for the three analogues in brain clearly shows how the brain distribution patterns account for this observed difference in potency. But this does not explain *why* the brain time courses differ between the three analogues since the time profile is kinetically related to the plasma time course in each case and this in turn is affected by the rates of metabolism and tissue distribution. Thus, only through the examination of the entire pharmacokinetic analysis can one interpret the brain time course and the response data to define the impact of *N*-substitution. This successful analysis of three aporphines, in precisely the manner described, provides such a demonstration of the interdependence of the

three types of data. In addition, the use of whole animal pharmacokinetics has established a more appropriate two compartment model for apomorphine distribution in mice. The biological $t_{\frac{1}{2}}$ determined in this study is therefore much longer than previous literature values which were estimates based on a one compartment model.

Selection of drugs. Three aporphine analogues, shown in Fig. 1, were selected for study based on their qualifications relative to previously described criteria (Notari & others, 1974). These compounds appear to satisfy the requirements in the following ways. They elicit a readily observable and quantifiable stereotypical gnawing behaviour in mice in doses well below the minimum toxic levels (Koch & others, 1968; Van Tyle & Burkman, 1972, 1973). Although the evidence is largely circumstantial, the site of action of these compounds is presumed to be the corpus striatum (Ernst & Smelik, 1966; Ernst, 1967). Despite uncertainties in defining a *specific* locus of action, the central stimulating activity can be considered to reside in the brain which can be easily removed intact from small animals for independent assay.

Previous comparisons of the potencies of these aporphine derivatives were based upon intraperitoneal doses administered to mice (Koch & others, 1968). The evaluation of distribution characteristics thus provides information permitting a comparison of potencies based on concentration at the locus of action (*i.e.*, the brain) to those based upon the administered dose. The influence of distribution on the relative potencies of the analogues may then be assessed.

The pharmacokinetic method based on intact drug content in whole animal homogenates is most applicable when the compounds studied are eliminated solely by metabolism or when unaltered drug content of bladder urine is accountable. Apomorphine is subject to *in vivo* glucuronidation in mice (Kaul & Conway, 1971) and it seems reasonable to suggest that the other closely related aporphines studied are also metabolized, at least in part, to 10- and 11-glucuronides. Since glucuronides have not been shown to influence fluorescence (and fluorescent metabolites were absent or insignificant in concentration) the assay method is believed to be sufficiently specific for intact drug. Small amounts of unaltered drug that may be present in the urinary bladder are judged to be insignificant and do not compromise the analyses.

Mice have proved to be eminently suitable for the gnawing behaviour evaluations and the chemical assays. Many animals can be employed for each determination with resultant increase in statistical validity while allowing convenience and economy.

The derivatives can be administered by rapid intravenous injection of solutions of the soluble salts. This eliminates potential problems associated with differences in rates and amounts released from the site of injection. For example, there is evidence that bioavailability of normorphine following intraperitoneal injection in rats is less than that of morphine (Johannesson & Milthers, 1962).

Finally, several papers have appeared wherein time profiles for apomorphine in mice (homogenized whole animals) have been reported (Kaul, Brochmann-Hanssen & Way, 1961; Kaul & Conway, 1971). These studies suggested the potential utility of whole animal kinetics for identifying parameters of apomorphine pharmacodynamics. Their data, however, were not used to determine suitable compartment models. The data reported in the earlier papers are not amenable to suitable analysis because of the inappropriate time intervals chosen for the experimental determinations and the lack of data describing the *beta* or elimination phase of the time-concentration profile.

Pharmacokinetics of blood/brain distribution. The time course for the three aporphine analogues in mice is described by a two compartment open model based upon pharmacokinetic analysis of intact drug content in whole animal homogenates. Since the brain contains the site of action for stereotypical gnawing behaviour, the time profile for each derivative in brain was defined and the pharmacological response was measured simultaneously. Although the brain contains a minor fraction of the administered dose, its independent assay allows the development of a general model to describe all three analogues as shown in Fig. 2. The time course for drug in brain is thus included as a means of examining the effect of structure on the distribution to the site and the resultant pharmacological effects.

The relative potencies reported here are based upon concentrations of drug determined from the time course of drug in whole brain. Although these results do not necessarily reflect the potencies at the "receptor site" level, they do define the relative amounts of each derivative required in the target organ to produce equivalent effects. The primary questions, then, to which we may address ourselves are (a) how much drug is needed in the brain in order to provide a defined biological response and (b) what is the fate of the remaining drug?

The order of relative ability of the drugs to distribute from blood into brain appears to be *N*-n-propylnorapomorphine > apomorphine > norapomorphine (Fig. 4). The *N*-n-propyl derivative equilibrates between blood and brain extremely rapidly (<1 min). Apomorphine brain distribution is also rapid (within 5 min). Norapomorphine did not exhibit a constant Br/B ratio until 50 min and since the apparent validity of the ratio is equivocal (see Results section), equilibrium may not have been achieved at all. The difference in the shapes of the three time profiles in Fig. 4 illustrates one significant advantage of this kinetic approach. Among the three analogues, norapomorphine is unique in its brain time course. Furthermore, it does not reflect the plasma time profile as do the other two compounds. This observation could not be predicted *a priori*. Thus, Fig. 4 clearly illustrates how the evaluations of relative potencies based upon dose may yield time-dependent results, whereas those based on the concentration of drug in the target organ may not since they are time-independent.

The relative ease with which the analogues having different *N*-substituents distribute into brain (n-propyl > methyl > hydrogen) might have been predicted *a priori* based on the assumption that an increased oil/water partition coefficient in a closely related series would increase permeation of the brain. This simplistic approach ignores the kinetic aspects and considers only the thermodynamics of the system. If the analogues were administered by constant intravenous infusion to achieve equal steady-state blood levels, then simple partition coefficient values might predict distribution ratios. However, in the case of a single dose, the brain concentration will be dependent upon the blood concentration since transport must occur from blood to brain and cerebrospinal fluid (Rall, 1971). Inasmuch as the time course for drug in blood is not constant but continually changing, it will influence the brain concentration at any given time. Considering the simple illustration in Fig. 2, there are three possible reasons for brain time profiles to differ when comparing two analogues. They are: ability to permeate brain (or transfer between blood and brain), relative amounts in blood and tissue (or drug available for transfer from blood to brain) and rates of loss from body (again influencing blood content).

When two analogues have brain levels that are linearly related to plasma levels

over all times, the *ratios* of brain concentrations to blood concentrations (Br/B) might be expected to correlate with partition coefficients since this *ratio* may not be kinetically controlled. *N*-n-Propylnorapomorphine appears to equilibrate immediately between brain and blood and the ratio of Br/B is constant over the entire time course. The time course for this derivative in brain therefore mirrors that of blood which may be described by a biexponential curve with exponents of α and β.

Apomorphine also distributes quite rapidly to brain and achieves a constant ratio within 5 min. Once this distribution ratio has become constant, the time course in brain also mirrors that of the blood and may again be described by a biexponential equation with exponents α and β. Since the blood level time profiles for these drugs are somewhat similar, a direct comparison of their absolute brain levels (at post distribution times) yields results that are similar to those obtained by comparing their Br/B distribution ratios. For example, both the brain content and the Br/B ratio for *N*-n-propylnorapomorphine is 1·62 times the corresponding values for apomorphine when compared during the time period of 5 to 40 min wherein distribution of both is complete. However, this is not the case for norapomorphine. Comparison of this derivative with the others gives time-dependent results. For example, the ratios of brain concentration of the n-propyl derivative to norapomorphine are 3·7 (5 min), 1·6 (10 min) and 0·19 (50 min).

Thus, two of the derivatives behave in a manner which might be predicted from partition coefficient theory. However, such agreement would be fortuitous without knowledge of the kinetic behaviour controlling the brain to plasma dynamics. The norapomorphine results illustrate the complexity of such comparisons. The time profile for this derivative in brain appears to be kinetically controlled with no firm evidence that equilibrium is operative. Comparisons of brain concentrations for this derivative with either of the other two will yield time-dependent results. Thus, an equilibrium value, such as a partition coefficient, would not be a rational parameter to invoke when the distribution of one of the compounds is kinetically controlled.

Relative potency. The relative potencies for the three derivatives were calculated by correlation of brain concentration with observed response. The results are given in Table 3 where literature values based upon the intraperitoneal dose are also listed for comparison. Apomorphine has been set at a value of 1 for both cases in this comparison. It is now apparent that the *N*-n-propyl derivative appears to be more potent than apomorphine in the dosage-based comparison because of its increased distribution to the brain. This is illustrated in Fig. 5. When the responses based on brain content are used as the basis for comparison the *N*-n-propyl derivative is 0·39 as effective as apomorphine. Conversely, the relative potency of norapomorphine remained constant regardless of the basis for comparison. This agreement is probably fortuitous in view of the dramatic difference in the time course for norapomorphine in brain relative to the other analogues.

It might be argued that the time course for drug content in total brain would not provide an adequate assessment of relative potency if regional differences in drug content existed throughout the brain itself. There have been reports illustrating such regional differences in time profiles of structurally related analogues. A recent study examined ten regions of rat brain at four time intervals after intravenous injection of four closely related phenothiazines (Sanders, 1973). While three of the analogues were strikingly similar, the fourth showed deviations which were attributed

to differences in chemical structure. However, there is virtually no information regarding organ distribution of the aporphines examined in this study. Veit (1935) reported differences in apomorphine content of various regions of the central nervous system (cns) following subcutaneous administration of large doses to monkeys and cats. Within 60 to 70 min all cns areas were found to contain approximately the same amounts of apomorphine. The equilibration in cns areas would be expected to occur more rapidly following intravenous injection than that observed by Veit following subcutaneous administration. Although the significance of regional differences in brain distribution of aporphines is yet undefined the present data do, nevertheless, establish the relative potencies in the target organ itself for the first time.

Half life. Biological half-life is defined by the equation

$$t_{\frac{1}{2}} = 0{\cdot}693/\beta$$

where β represents the negative value of the slope of the first-order plot for a one-compartment model or the slope of the terminal portion of the plot for a two-compartment model. Substituting for β from Table 2 yields $t_{\frac{1}{2}}$ values of 20 min for norapomorphine, 47 min for apomorphine and 29 min for *N*-n-propylnorapomorphine. The $t_{\frac{1}{2}}$ value for apomorphine does not agree favourably with those previously reported. Kaul & others (1961) estimated the $t_{\frac{1}{2}}$ for apomorphine loss from mice to be 8·5 min based on whole animal analysis. The first-order plot used for this estimate included only four points all taken during the first 10 min after intravenous injection. That report predates publication of two compartment model analysis (Riegelman, Loo & Rowland, 1968) which has now become commonplace and defines $t_{\frac{1}{2}}$ as a function of the elimination or *beta* phase of the time profile curve (Notari, 1971). In the case of apomorphine the *beta* phase does not begin until 30 min after intravenous injection as is evidenced by constant ratios of T/B at that time. The slope of the first-order plot of whole animal data for the first 10 min is a function of both α and β (Notari & others, 1974). When the data for the initial slope were treated in a manner similar to that previously published by Kaul & others (1961), a $t_{\frac{1}{2}}$ value of 7·32 min (95% confidence limits, 6·77 to 7·96) is obtained in good agreement with the 8·5 min value. However, the value is without significance since it is a hybrid involving all of the constants in Fig. 2.

Kaul & Conway (1971) have examined glucuronidation rates of apomorphine in mice in a similar fashion. Data used for comparing disappearance rate constants following various pretreatments covered only 8 to 16 min following administration. Again, the $t_{\frac{1}{2}}$ was reported as 8 min, but this reflects distribution of apomorphine together with the contribution from metabolism. The rate constant, k_2, in Fig. 2 would provide the most sensitive method for comparing metabolism. For the three analogues given in Table 2 the metabolic constant for apomorphine (0·129 min^{-1}) is significantly different from that of *N*-n-propylnorapomorphine (0·0961 min^{-1}) but no other statistically significant differences were observed between k_2 values.

Summary

This study using whole animal analysis, specific organ analysis and pharmacological response on three closely related aporphines illustrates the utility of the previously reported technique (Notari & others, 1974) for evaluating structural effects on kinetics and biological response. Detailed assessment of the time profiles has provided pharmacokinetic models to explain biphasic disappearance of the derivatives from

mice. The apparent $t_{\frac{1}{2}}$ values previously reported for apomorphine in mice probably represent both alpha and beta phase kinetics since distribution would not be expected to be complete during the time periods used in those studies. The relative potency of *N*-n-propylnorapomorphine, based on brain concentrations, is less than that previously reported using the dose administered intraperitoneally for the comparison. This is attributed to a decreased potency but increased brain distribution for the *N*-n-propyl derivative relative to apomorphine. The three analogues also illustrate potential difficulties in using non-kinetic methods to compare distribution to a specific organ. While *N*-n-propylnorapomorphine and apomorphine demonstrate time courses in brain that appear similar to those calculated for blood (after a 2–5 min distributive phase for the apomorphine case) the norapomorphine pattern is unique among the three, passing through a maximum value but paralleling neither blood nor tissue. The information gained through the combined approach thus provides insights into the disposition of the analogues that would not be possible through study of either pharmacological response or pharmacokinetics alone.

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