Relationships Between Partition Coefficients and Cholinesterase Inhibition of Carbamoylpiperidinoalkanes

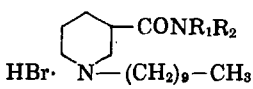
By RONALD P. QUINTANA

Partition coefficients (benzene/water) of members of a series of carbamoylpiperidinodecanes have been determined. A parallel was observed between this property and the ability of the compounds to inhibit human plasma pseudo-cholinesterase.

In previous communications (1–3), the relationships existing between selected physicochemical properties (*i.e.*, surface activity, electric moments) of some carbamoylpiperidinoalkanes and their inhibitory effect upon isolated human plasma pseudo-cholinesterase have been reported.

The observation of Thomas and Marlow (4) that the lipophilic–hydrophilic ratio of members of a homologous series of compounds is an important factor affecting inhibitory characteristics has prompted the inclusion of partition-coefficient determinations in our program relating physicochemical properties with biochemical response. Therefore, the partition coefficients (benzene/water) of a series of nine carbamoylpiperidinodecanes and one related pyridinium analog which was available were determined.

Table I.—Inhibition of Isolated Human Plasma Pseudo-Cholinesterase and Partition Characteristics of Mono(carbamoylpiperidino)decanes



Compd.	—NR_1R_2	Human Plasma Cholinesterase Inhibition ($I_{50} \pm$ S.E.)[a]	Relative Inhibitory Activity	Relative Partition Coefficient	Partition Coefficient (Benzene/Water) ($\pm\sigma$)[b]
I	$-NH_2$	$(6.23 \pm 0.155) \times 10^{-5}$ *M*	1.00	1.00	0.03 ± 0.01
II	$-NHCH_3$	$(3.48 \pm 0.105) \times 10^{-5}$ *M*	1.79	2.33	0.07 ± 0.01
III		$(2.57 \pm 0.125) \times 10^{-5}$ *M*	2.42	6.33	0.19 ± 0.01
IV	$-N(CH_3)_2$	$(2.17 \pm 0.10) \times 10^{-5}$ *M*	2.87	6.67	0.20 ± 0.01
V	$-NHC_2H_5$	$(1.371 \pm 0.007) \times 10^{-5}$ *M*	4.54	12.67	0.38 ± 0.03
VI		$(0.766 \pm 0.0085) \times 10^{-5}$ *M*	8.13	16.33	0.49 ± 0.00
VII	$-N(C_2H_5)_2$[c,d]	$(0.527 \pm 0.011) \times 10^{-5}$ *M*	11.82	52.67	1.58 ± 0.03
VIII		$(0.318 \pm 0.0195) \times 10^{-5}$ *M*	19.59	60.33	1.81 ± 0.03

[a] Summarized from a paper by Beasley and co-workers (7). [b] Standard deviation. [c] Values obtained for the corresponding 4-carbamoyl substituted derivative, 1-decyl-4-(*N*,*N*-diethylcarbamoyl)piperidine hydrobromide (IX) are: $I_{50} \pm$ S.E., $(2.65 \pm 0.18) \times 10^{-5}$ *M* (7); relative inhibitory activity, 2.35; relative partition coefficient, 5.67; partition coefficient $\pm \sigma$, 0.17 ± 0.04. [d] Values obtained for the corresponding pyridinium analog, 1-decyl-3-(*N*,*N*-diethylcarbamoyl)pyridinium bromide (X) are: $I_{50} \pm$ S.E., $(0.365 \pm 0.000) \times 10^{-5}$ *M* (9); relative inhibitory activity, 17.07; relative partition coefficient, 0.00; partition coefficient $\pm \sigma$, 0.00 ± 0.00.

EXPERIMENTAL

Materials.—All of the compounds employed in this investigation were of analytically pure grade. Their preparation and properties were previously reported (5–7).

Method.—An accurately weighed 250-mg. sample of each compound was partitioned between 25 ml. of benzene (Eastman 777) and 25 ml. of distilled water contained in a vessel fastened to a Burrell Wrist-Action shaker. The benzene–water system, chosen for convenience, has been employed by others (*e.g.*, 8) in correlations of physicochemical properties with biological activity. The vessel was agitated for 2 hr. in an air-conditioned room (22–26°), then allowed to stand until the benzene and water phases separated cleanly (*i.e.*, 1.5 hr.). A 10-ml. aliquot of the benzene layer was withdrawn and evaporated in a tared beaker on a steam bath. The residue was dried further for 6 hr. *in vacuo*. The weight of compound in 25 ml. of benzene was determined and corrected by means of a blank determination; the weight of compound remaining in the aqueous phase was calculated by difference. In each case, four independent determinations were used to calculate an average value for the partition coefficient.

RESULTS AND DISCUSSION

Relationships between partition characteristics and cholinesterase inhibition are given in Table I. While no completely quantitative relationship between the two parameters was found to exist, it is significant that cholinesterase inhibition increases in exactly the same order as the partition coefficient.

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The results obtained for the series of carbamoylpiperidinodecanes thus seem to substantiate the observation of Thomas and Marlow (4) and also suggest that this property, in the case of variations around the carbonyl function (2, 3, 7), may be more critical in effecting inhibitory action than the surface activity of the compounds (2).

The fact that such relationships do not hold outside the scope of a particular series is shown by results obtained with the pyridinium analog (X). While this compound is an extremely potent inhibitor, it has a partition coefficient of zero.

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Technique Utilizing Frog Immersed in Drug Solution to Study Drug Absorption Rates

By CLYDE W. WHITWORTH

The frog has been used to study the absorption of drugs from solution. The results so far indicate compliance with the pH-partition theory of passive absorption of weak electrolytes.

SIMPLE *in vivo* and *in vitro* processes are needed to study the effect of the many physicochemical factors involved in drug absorption. The objective of this study was to determine the usefulness of the frog in such studies.

EXPERIMENTAL

Ten frogs (*Rana pipiens*) were used in each determination. Each of the frogs was placed in 500 ml. of drug solution contained in glass animal jars of about 2500-ml. capacity. Separate tests indicated that the rate of evaporation of solvent was constant and thus had no appreciable effect on the relative results obtained. Samples of the drug solution were analyzed spectrophotometrically at 20-min. intervals over a 2-hr. period, and the amount of drug absorbed was determined from the amount remaining in solution.

Aqueous solutions of salicylic acid and sodium salicylate in the concentration of 2.5×10^{-4} moles/L. were utilized. Analyses were made in a Beckman DU spectrophotometer at a wavelength of 297 mμ. There is excellent compliance to the Beer-Lambert law with the concentration of drugs used.

The drug solutions were assayed directly without having to perform extracting procedures. The frogs were rinsed with distilled water prior to each experiment and were also forced to urinate by slight squeezing. Preliminary experimentation showed that the frogs excreted no interfering substances. The solution was returned to the container after analysis to try to maintain volume.

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RESULTS AND DISCUSSION

Shore *et al.* (1) have supplied evidence that certain drugs cross the intestinal epithelium chiefly in their nonionized form and that their ions penetrate little. Various investigators (2–5) have found oil soluble drugs penetrate the skin more readily. Until now, results indicate conformity to the pH-partition theory of passive drug absorption, as seen in Fig. 1.

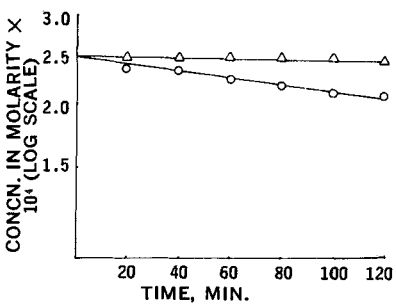

Fig. 1.—Plot of average salicylate concentrations in solution over 2-hr. period when 10 frogs each were placed in 500 ml. of aqueous drug solution. Key: △, sodium salicylate; ○, salicylic acid.

It is also apparent from Fig. 1 that the data exhibit characteristics of a first-order absorption rate.

Statistical analysis of the data by means of the Student-Fisher *t* test at 95% confidence level indicate the differences in absorption of the two forms of salicylic acid are significant. Standard deviations indicate highly reproducible results.

On the basis of the results so far, it appears that the frog may be useful for studying the passive absorption of certain drugs from solutions. The author is presently using the technique to study the effect of various additives on drug absorption.

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