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Enhancement of Gastrointestinal Absorption of a Quaternary Ammonium Compound by Trichloroacetate

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Abstract □ The hypothesis that ion-pair formation and transport across the lipid gastrointestinal barrier is an important mechanism for drug absorption has been examined using a quaternary ammonium compound, isopropamide, as the cationic component and trichloroacetate (TCA) as the anionic component of the ion pair. Studies of the effect of trichloroacetate on the partitioning of isopropamide between water and nonaqueous phases such as chloroform and *n*-octanol demonstrated that a highly lipid-soluble isopropamide-TCA ion pair can dissolve in such nonaqueous phases. Mydriasis tests in mice demonstrated that both the rate and efficiency of oral absorption of isopropamide are increased when it is administered in combination with 10-fold or 50-fold molar excesses of trichloroacetate in solution. Although the specific mechanism by which absorption is enhanced has not been determined, evidence is presented which clearly indicates that TCA, an anion capable of forming a lipid-soluble ion pair with isopropamide, significantly enhances the pharmacologic response of orally administered isopropamide.

Keyphrases □ Isopropamide absorption—trichloroacetate effect □ Ion-pair formation—isopropamide, trichloroacetate □ Partition coefficients—isopropamide □ Mydriasis, mice—absorption, dose-response indicator □ Colorimetric analysis—spectrophotometer

Current concepts regarding the mechanism by which drugs are absorbed suggest that the barrier membrane exhibits properties of a lipid-like substance (1). Many drugs are assumed to be absorbed passively across this type of membrane. The pH-partition hypothesis (2) predicts that for acidic and basic drugs, absorption will be markedly influenced by the pH at the absorption site, since the pH at the site determines the fraction of the drug present as the unionized, lipid-soluble form.

These concepts of factors influencing drug absorption have been valuable despite the general recognition that there are important deviations from them. For example, quaternary ammonium compounds are completely ionized at all pH values, yet some of these compounds are absorbed appreciably (3). Also, some simple weak organic acids, such as salicylate, are absorbed rapidly and efficiently from the intestine when the pH is such that the acid is virtually completely ionized (2).

If these drugs are not absorbed by active transport processes, then it appears that adjunctive substances may play a role in their facilitated transport. Such adjunctive substances might combine with the drug to make it more lipophilic and, in this manner, facilitate diffusion of the drug across a lipid-like membrane. These substances might exist naturally in or near the absorption site or could be administered with the drug. It was the purpose of this investigation to test the hypothesis that some adjunctive substances combine with ionic drugs to form ion pairs which are lipophilic and might be transported across the gastrointestinal membrane. This report deals with the effect of trichloroace-

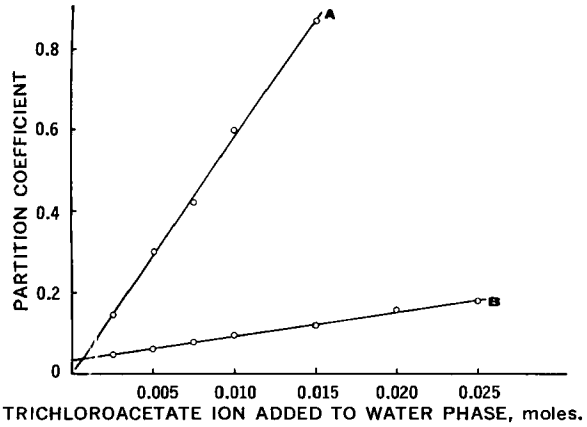

Figure 1—*Plot showing the effect of trichloroacetate anion on the n-octanol–water and chloroform–water partition coefficients for 0.001 M isopropamide. Key: A, n-octanol–water; B, chloroform–water.*

tate on the partition coefficient of isopropamide in chloroform–water and *n*-octanol–water systems and on enhancement of mydriatic activity in mice when excess trichloroacetate is administered orally along with isopropamide.

EXPERIMENTAL

Materials—Isopropamide iodide[1]; absolute ethanol; all other materials were reagent grade.

Apparatus—Thirty-six tube rocking device (4); pH meter[2]; spectrophotometer.[3]

Determination of Chloroform–Water Partition Coefficients—Isopropamide iodide was initially dissolved in chloroform and equilibrated against 0.1 *M* citrate buffer (pH 6.00) containing various concentrations of trichloroacetic acid. After equilibration, both phases were analyzed for isopropamide.

Determination of *n*-Octanol Alcohol–Water Partition Coefficient—Isopropamide iodide was initially dissolved in 0.1 *M* citrate buffer (pH 6.00) containing various concentrations of trichloroacetic acid and equilibrated against *n*-octanol. The aqueous phases were analyzed for isopropamide before and after equilibration and the partition coefficients calculated by difference.

Analysis of Isopropamide in Water or Chloroform—A modification of the method described by Santoro (5) was employed throughout. An aliquot of either the aqueous phase or the chloroform containing at least 0.1 mg. of isopropamide was placed in a 125-ml. separator. To this was added 30 ml. of a methyl orange buffer solution which was prepared by saturating with methyl orange 1 l.

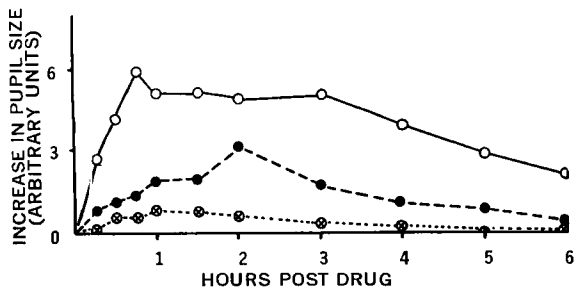

Figure 2—*Dose-time-action profiles of mydriatic response (average of eight mice) to 2 mg./kg. of isopropamide iodide administered orally. Key: ⊗. . ., solution alone; ●- - -, with 1 : 10 molar excess of trichloroacetate; ○—, with 1 : 50 molar excess of trichloroacetate.*

[1] Smith Kline and French, Philadelphia, Pa.
[2] Beckman Instruments, Inc., Fullerton, Calif.
[3] Cary model 15.

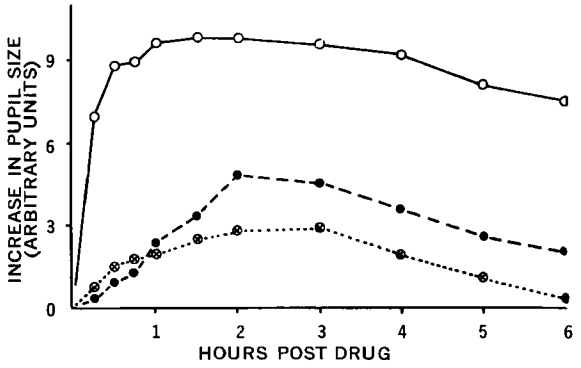

Figure 3—*Dose-time-action profiles of mydriatic response (average of 8 mice) to 4 mg./kg. of isopropamide iodide administered orally. Key: ⊗. . ., solution alone; ●- - -, with 1 : 10 molar excess of trichloroacetate; ○—, with 1 : 50 molar excess of trichoroacetate.*

of a solution containing 44 g. dipotassium hydrogen phosphate, anhydrous, and 21 g. sodium carbonate, anhydrous (pH 10.2). Approximately 2 g. of methyl orange was employed. The solution was extracted with three 50-ml. portions of chloroform and withdrawn through a chloroform-saturated cotton pledget into a 200-ml. volumetric flask. The color was developed with 20 ml. of 0.5 *N* HCl in absolute ethanol. The flask was brought to volume with chloroform and the absorbance was read on a spectrophotometer at 525 mμ. All readings were made against a reagent blank prepared in the same manner.

Absorbance readings were compared against a standard curve prepared with known quantities of isopropamide iodide.

Mydriasis Testing in Mice—Male albino mice (CF_1) weighing 18–22 g. were used. They were housed, 10 per cage, in a room maintained at 23° with food and water *ad libitum*. The mydriasis test described originally by Pulewka (6) was used. In this procedure the diameter of the pupil was measured, under constant illumination, by means of a micrometer in the ocular of a microscope.[4] Diameters were recorded as "ocular units." The right eye of each mouse was measured immediately before administration of the drug and again at 15, 30, 45, 60, 90, 120, 180, 240, and 360 min. postdrug. Therefore, each mouse served as its own control. Mydriatic activity was expressed as the average change in pupil diameter in each drug group.

Dose-Response, Time-Action Study—All drugs, prepared in distilled water, were administered by stomach tube in a dose volume of 10 ml./kg. at each dose level. Preparations studied were: (*a*) isopropamide iodide: 2, 4, 8, and 16 mg./kg. in distilled water; (*b*) isopropamide iodide: 2, 4, 8, and 16 mg./kg. in distilled water with a 50-fold molar excess of trichloroacetate ion; (*c*) isopropamide iodide: 2, 4, and 8 mg./kg. in distilled water with a 10-fold molar excess of trichloroacetate ion.

Doses of isopropamide iodide alone and isopropamide iodide plus TCA were administered to eight mice per dose-group. Time-action curves were drawn for each dose level by plotting the change in pupil diameter at the indicated time intervals postdrug. The significance

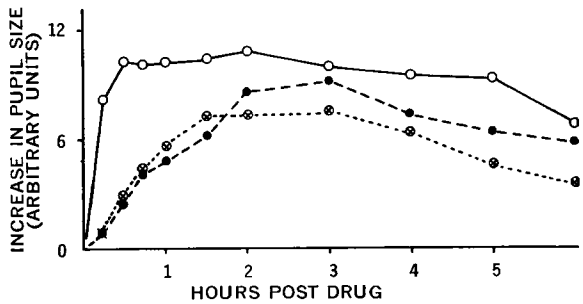

Figure 4—*Dose-time-action profiles of mydriatic response (average of eight mice) to 8 mg./kg. of isopropamide iodide administered orally. Key: ⊗. . ., in solution alone; ●- - -, with 1 : 10 molar excess of trichloroacetate; ○—, with 1 : 50 molar excess of trichloroacetate.*

[4] Stero-Zoom (R) model BVB 73, Bausch & Lomb, Rochester, N. Y.

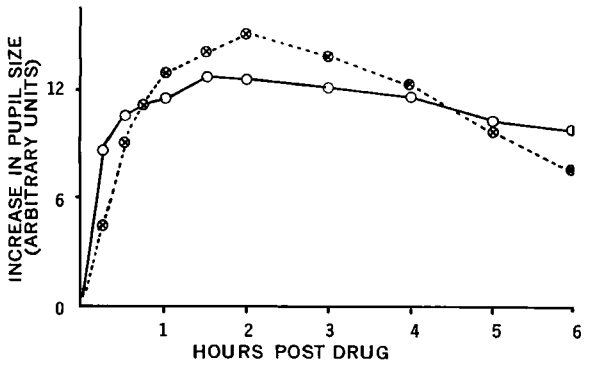

Figure 5—*Dose-time-action profiles of mydriatic response (average of eight mice) to 16 mg./kg of isopropamide iodide administered orally. Key: ⊗. . ., solution alone; O—, 1 : 50 molar excess of trichloroacetate.*

of the differences at these times between each isopropamide-TCA group and isopropamide alone were compared by the *t* test. Dose-response curves were plotted utilizing response at the time of peak activity as a function of dose. $ED_{\Delta 5}$ values (that dose which increased pupil diameter by five units) were also determined for 2-, 4-, and 8-mg. doses of each preparation at the time of peak activity.[5]

RESULTS AND DISCUSSION

The physical chemical characteristics of ion pairs have been reviewed by Kraus (7). Ion pairs are neutral species formed by electrostatic attraction between oppositely charged ions in solution, and they are often sufficiently lipophilic to dissolve in nonaqueous solvents. Isopropamide, a therapeutically useful anticholinergic drug, was chosen as a model quaternary ammonium compound for these studies because: (*a*) it exists as an ion over the entire range of physiologic pH; (*b*) a sensitive method of analysis for the drug is available; and (*c*) a simple quantitative pharmacologic test for the drug's activity is available. Trichloroacetic acid was chosen as the anion because: (*a*) it is a strong acid with pKa less than 1 and would be virtually completely ionized over the entire range of physiologic pH; (*b*) it is relatively nontoxic and has no pharmacologic activity; and (*c*) it is a fat-soluble acid which might be expected to form lipophilic ion pairs.

The effect of trichloroacetate on the partitioning of isopropamide between chloroform–water and *n*-octanol–water is illustrated in Fig. 1. The slopes of the two plots indicate that the isopropamide-TCA ion-pair has a greater affinity for *n*-octanol than for chloroform. The partition coefficient in absence of TCA, indicated by the intercept on the partition coefficient axis in Fig. 1, is the result of partitioning of isopropamide iodide ion pairs.

[5] An increase in pupil size of five units was a statistically significant mydriatic effect and was about midpoint of the range of activity obtained with doses up to 8 mg./kg.

Figures 2–5 show the effect of the addition of the anion TCA on the time-action curves for isopropamide at 2-, 4-, 8-, and 16-mg./kg. dosage levels. At 2, 4, and 8 mg./kg., isopropamide iodide plus TCA (1:10) was slightly more effective than isopropamide iodide alone, but less effective than isopropamide iodide plus TCA (1:50).

Figures 2–4 indicate that isopropamide iodide plus TCA (1:50) is more rapidly and efficiently absorbed than isopropamide iodide plus TCA (1:10) or isopropamide iodide alone. The apparently equivalent response indicated at a dosage level of 16 mg./kg. for isopropamide preparations both with and without TCA probably indicates that nearly maximum mydriasis is achieved at this dosage level of isopropamide.

Dose-response curves were plotted for these solutions and an $ED_{\Delta 5}$ (that dose which increased pupil diameter by five units) was determined for each preparation at the time of its peak activity. The $ED_{\Delta 5}$ for the 1:50 isopropamide-TCA preparation had to be estimated by extrapolation since the lowest dose administered with this concentration of TCA produced an increase greater than five units.

$ED_{\Delta 5}$ values (95% Fieller limits) were: isopropamide iodide, 5.3 (3.5–12.6) mg./kg.; isopropamide iodide plus TCA (1:10 molar ratio) 3.2 (2.2–5.0) mg./kg.; isopropamide iodide plus TCA (1:50 molar ratio) 1.4 mg./kg. estimated.

Trichloroacetic acid, at a dose equivalent to that amount which would be contained in a 20-mg./kg. dose of isopropamide iodide plus TCA (1:50), produced no mydriasis.

The results of the study indicate that the rate and efficiency of absorption of isopropamide are increased by trichloroacetate. These data suggest that it may be possible through selection of appropriate ion-pair formers to improve the efficiency and uniformity of absorption of quaternary ammonium compounds and other highly ionized drugs from the gastrointestinal tract.

Additional studies are in progress to test these assumptions and to further elucidate the observations presented in this communication.

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