Some Physical Factors Affecting the Enhanced Blepharoptotic Activity of Orally Administered Reserpine-Cholanic Acid Coprecipitates

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Abstract □ In order to elucidate the mechanism by which orally administered cholanic acid derivative-reserpine coprecipitates potentiate the pharmacological activity of reserpine in mice, a study of the surface tension and dissolution properties of the various systems was undertaken. Deoxycholic acid, cholic acid, lithocholic acid, and 3,12,24-trihydroxycholane were found to significantly lower surface tension. These same compounds, as coprecipitates, enhanced the ptotic activity of reserpine. A fifth compound, 5β-cholanic acid, showed only minor surface tension lowering properties. No indication of micelle formation was observed with these five compounds in the pH 6.4 buffer system used. *In vitro* dissolution studies of the coprecipitates of reserpine with these compounds in ethyl acetate at 37° were also undertaken. An increase in the dissolution rate of the coprecipitates over that for pure reserpine was noted, due most probably to a reduction in the particle size of reserpine during the formation of the coprecipitates. The rate of dissolution of reserpine from the coprecipitates in a 1:16 molar ratio showed a rank order correlation with *in vivo* potency.

Keyphrases □ Reserpine-cholanic acid coprecipitate—blepharoptotic activity □ Blepharoptotic activity, reserpine—cholanic acid coprecipitation effect □ Surface tension lowering—reserpine activity effect □ Dissolution—reserpine-cholanic acid coprecipitates

Malone *et al.* (1) have shown that the onset of action and degree of blepharoptotic activity of reserpine is enhanced two to three fold when administered orally to mice as a reserpine-deoxycholic acid coprecipitate. Further studies have demonstrated that this enhancement is not specific to deoxycholic acid, but is obtained with combinations of reserpine and cholic acid, lithocholic acid, and 3,12,24-trihydroxycholane (2). No attempt was made by these investigators to ascertain the nature of the observed potentiation of blepharoptotic activity.

A consideration of the literature indicates that the observed pharmacological potentiation may arise from certain physicochemical and/or physiological effects. Insofar as the former are concerned, the rate of dissolution is often the rate-determining step in the absorption process of poorly soluble drugs such as reserpine (3–5). The factors involved in the dissolution rate of pharmaceuticals are embodied in the Noyes-Whitney equation (6) or the various modifications thereof (7–10). Accordingly, the *in vivo* dissolution of the various coprecipitates prepared by Malone *et al.* (1, 2) could be influenced by such physicochemical factors as micellar solubilization (11–13), and reduction of interfacial tension (14–16), and particle size (17, 18).

Certain of the physiological effects associated with bile acids may also alter the absorption or activity of reserpine. These would include changes in gastric motility and emptying (19), protein binding (20, 21), changes in membrane permeability (22), and involvement of the entero-hepatic circulatory system (23).

With these factors in mind, and with as yet no acceptable solution as to the mechanism of the enhanced blepharoptotic activity, an investigation was undertaken to study (*a*) the surface-active properties of the various cholanic acid derivatives used in the coprecipitate systems, and (*b*) the dissolution rates of reserpine from the various reserpine-cholanic acid coprecipitates. The present paper reports these findings.

EXPERIMENTAL

Materials—Cholic acid,[1] deoxycholic acid,[1] lithocholic acid,[1] reserpine,[1] and 5β-cholanic acid[2] were dried under vacuum for at least 24 hr. prior to use. 3,12,24-Trihydroxycholane was synthesized by means of $LiAlH_4$ reduction of deoxycholic acid in anhydrous tetrahydrofuran (24). The purity of the product was confirmed by IR and elemental analysis. All other chemicals were of reagent grade and used as received.

Preparation of Coprecipitates—All cholanic acid derivative-reserpine coprecipitates used in these studies were prepared in the same manner as those used by DeCato *et al.* (2) in the *in vivo* tests. Often the same samples of coprecipitate were employed for both the *in vivo* and *in vitro* experiments. Following its preparation, each sample material was screened and the fraction that passed through a 20-mesh sieve and was retained on a 60-mesh sieve was used for the dissolution studies.

Surface Tension Studies—An excess quantity of each of the pure cholanic acid derivatives was equilibrated at 37° for at least 72 hr. in a pH 6.4 McIlvaine buffer (25) in which the KCl was removed and the sodium ion concentration adjusted to a physiological level of 0.15 *M* (26). The concentrations of these saturated, filtered (Millipore, HAWP, 0.45 μ pore size) solutions were determined spectrophotometrically (see *Assay Procedure*) with the exception of 5β-cholanic acid which was determined by adding known increments of solid to the buffer solution until a saturated solution was obtained. The solutions were then progressively diluted with the pH 6.4 buffer and surface tension measurements made at 37° using a Cenco-DuNouy ring tensiometer equipped with a constant-temperature attachment. Dial readings were converted to dynes $cm.^{-1}$ by means of the tables prepared by Harkins and Jordon (27).

Dissolution Studies—The dissolution studies were carried out in 300 ml. of ethyl acetate contained in a three-necked round-bottom flask immersed in a constant-temperature bath maintained at 37°. The stirring speed of the Servodyne[3] constant speed stirring apparatus was 30 r.p.m. A polyethylene three-blade propeller, 3.8 cm. in diameter, was employed at a depth of immersion of 23 mm. The amount of coprecipitate used per dissolution run was such that each sample contained 150 mg. of reserpine.

Five-milliliter samples were removed at known time intervals and replaced with an equal quantity of solvent until at least 80% of the reserpine was in solution. All samples were filtered through a Whatman No. 2 filter paper and 1 ml. of the filtrate diluted with nine parts of absolute methanol prior to being assayed for reserpine at 268 mμ using a Beckman DB-G spectrophotometer. No significant absorbance was found for the various cholanic acid derivatives over the wavelength range used for the reserpine analysis.

Assay Procedures—The solutions of deoxycholic acid, cholic acid, and lithocholic acid were assayed according to the procedure of Eriksson and Sjovall (28), which involves heating the derivative in 65 % sulfuric acid at 60° for a specified period of time. The assay

[1] Obtained from Nutritional Biochemical Corp., Cleveland, Ohio.
[2] Obtained from Mann Research Laboratories, New York, N. Y.
[3] Cole-Parmer Instruments Co., Chicago, Ill., 60648

Table I—Comparison of Surface Tension-Lowering Effect of Cholanic Acid Derivatives with *In Vivo* Ptotic Activity of Respective Coprecipitates with Reserpine

Cholanic Acid Derivative[a]	Saturation Concentration, moles/l.	Surface Tension of Saturated Solution, dynes/cm.	Relative Ptotic Activity of 1:16 Coprecipitate[b]
Lithocholic acid	2.2×10^{-5}	54.6	2.93
Deoxycholic acid	5.5×10^{-4}	44.8	2.16
Cholic acid	3.7×10^{-3}	44.8	1.93
Trihydroxycholane	1.8×10^{-5}	45.1	1.87
5β-Cholanic acid	1×10^{-5}	61.2	1.41

[a] Ranked in decreasing order of *in vivo* ptotic activity for each respective 1:16 coprecipitate. [b] Ptotic activity = potency observed for 1:16 coprecipitate/potency observed for pure reserpine, 4 hr. after oral dosing with drug. Data obtained from *Reference* 2.

for 3,12,24-trihydroxycholane involved heating the substance at 60° for 45 min. in 96 % sulfuric acid.

RESULTS AND DISCUSSION

Surface Tension Studies—The ability of the various cholanic acid derivatives to lower the surface tension of an aqueous buffer solution at 37° is apparent from Table I which shows the surface tension of the buffer solution at the saturation concentration of each derivative studied. Saturated solutions of the various coprecipitates with reserpine produced reductions in surface tension similar to those exhibited by the cholanic acid derivatives alone. Reserpine alone lowered the surface tension of the buffer solution from 67.7 to 63.5 dynes $cm.^{-1}$

The cholanic acid derivatives are listed in Table I in decreasing order of their ptotic activity when administered as 1:16 coprecipitates with reserpine (2). The data for the 1:16 ratio was chosen here since it produced the greatest enhancement in ptotic activity (1). The rank order of potency shown in Table I was maintained over the period of 4–10 hr. after dosing; the values at 4 hr. have been included to demonstrate the relative magnitude of the observed increase in ptotic activity.

It is apparent that the surface tension-lowering ability of the various cholanic acid derivatives does not completely parallel the rank order of ptotic activity found for their respective coprecipitates with reserpine. However, there does appear to be a qualitative effect, in that those cholanic acid derivatives that reduce the surface tension of the buffer by at least 12 dynes $cm.^{-1}$ are also those which, as coprecipitates, enhance the ptotic activity of reserpine. A significant reduction in the surface tension of a solution does imply an increased ability of that solution to wet a dispersed solid particle. With a poorly soluble drug this can lead to an increase in dissolution rate, as has been shown in several studies using surfactants below their CMC (14–16). Accordingly, the relative abilities of the cholanic acid derivatives to lower surface tension could be a factor in enhancing ptotic activity *in vivo* by facilitating the wetting, and hence the dissolution rate, of reserpine.

It is also conceivable that the presence of micelles could, through solubilization, affect the dissolution rate of reserpine by increasing its saturation concentration (6). However, the surface tension data for all the cholanic acid derivatives studied at pH 6.4 precludes the presence of micelles at this pH, since no break in the surface tension *versus* concentration curve, characteristic of micelle formation, was observed prior to saturation. Surface tension determinations were then repeated on deoxycholic acid and trihydroxycholane at pH 7.4. A sharp break was observed in the case of deoxycholic acid, indicating the formation of micelles. The change in pH from 6.4 to 7.4 had no effect, however, on the surface tension curve of the trihydroxycholane, indicating the inability of the unionizable compound to form micelles. In view of the fact that none of the cholanic acid derivatives were capable of forming micelles at pH 6.4, which is close to the pH existing in the lumen of the small intestine, it appears that micelle formation is not a factor in increasing the *in vivo* ptotic activity of reserpine administered as a coprecipitate with a cholanic acid derivative.

Dissolution Studies—As shown in Fig. 1, the rate of dissolution of reserpine from the coprecipitates varied according to the cholanic acid component used to form the coprecipitates. Thus, the rank order of dissolution was lithocholic acid > cholic acid ≅ deoxycholic acid > 5β-cholanic acid > reserpine precipitate > 3,12,24-trihydroxycholane. The reserpine used as a standard in the dissolution rate studies was a sample recrystallized from methanol in the same manner as the coprecipitates, only in the absence of a cholanic acid derivative. Since the dissolution rate of the precipitated reserpine in Fig. 1 is different from that found for the coprecipitates, it may be assumed that the enhanced dissolution rate of reserpine as a coprecipitate is not due to recrystallization, *per se*. Also, a comparison of the dissolution rate of reserpine alone and from a 1:16 physical mixture with deoxycholic acid containing the same particle size drug shows that the rates are comparable (Table II). This fact tends to preclude the possibility that the cholanic acid derivatives in the coprecipitates function to increase the bulk solubility of the drug, thereby increasing its dissolution and absorption characteristics.

The correlation between dissolution rate and *in vivo* ptotic activity for each of the coprecipitates is illustrated in Table II. On the assumption that the activity of reserpine is related to dissolution rate, the low dissolution rate for the trihydroxycholane coprecipitate (approximately one-half that exhibited by reserpine alone) is anomalous. This behavior is probably inherent to this particular system, due to the low solubility of trihydroxycholane in ethyl acetate. In fact, in the synthesis of trihydroxycholane from a deoxycholic acid precurser, ethyl acetate is used to recrystallize the product from the reaction mixture (24). It can also be seen from Fig. 1 that reserpine from the coprecipitates goes into solution significantly faster than the precipitated reserpine alone. Since the same particle size fraction (20/60 mesh) was used for these systems, it is reasonable to suppose that the reserpine in the coprecipitate is available to the solvent in smaller particle form. Studies have

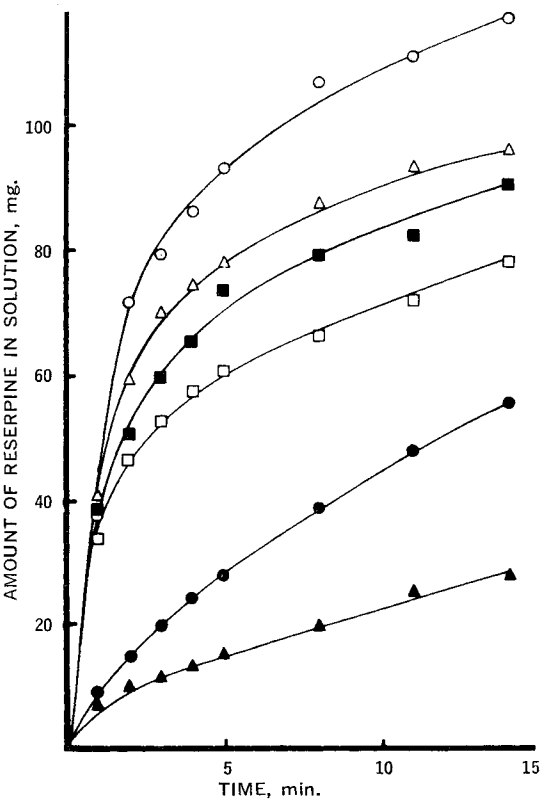

Figure 1—In vitro *dissolution rates of various cholanic acid derivative-reserpine coprecipitates in ethyl acetate at 37°. Key:* ○, *lithocholic acid;* △, *cholic acid;* ■, *deoxycholic acid;* □, *5β-cholanic acid;* ▲, *3,12,24-trihydroxycholane;* ●, *precipitated reserpine only.*

Table II—Comparison of *In Vitro* Dissolution Rate of Cholanic Acid Derivatives-Reserpine Coprecipitates with *In Vivo* Ptotic Activity

Coprecipitate with Reserpine[a]	Amount of Reserpine in Solution after 8 min., mg.	Dissolution Ratio[b]	*In Vivo* Activity[c]
Lithocholic acid	106.8	2.76	2.93 (2.63–3.64)[d]
Deoxycholic acid	79.2	2.04	2.16 (1.82–2.58)[d]
Cholic acid	87.7	2.26	1.93 (1.66–2.25)[d]
Trihydroxycholane	19.4	0.50	1.87 (1.51–2.32)[d]
5β-Cholanic acid	66.4	1.72	1.41 (1.05–1.88)[d]
Deoxycholic acid (physical mixture with reserpine)	36.6	0.95	—
Reserpine precipitated	38.7	1.00	1.00

[a] See Table I, *Footnote*[a]. [b] Dissolution ratio = amount of reserpine in solution for 1:16 coprecipitate/amount of reserpine in solution for precipitated reserpine, after 8 min. [c] See Table I, *Footnote*[b]. [d] Calculated 95% confidence limits for the listed potencies.

established that the smaller the particle size, the faster the rate of dissolution of pure reserpine in an ethyl acetate system (29). It seems likely that the observed *in vivo* ptotic activities are due to differences in dissolution rates, and hence the availability of reserpine from the various coprecipitates. It is probable that the several rates of dissolution observed are dependent on the particle size of the reserpine in the coprecipitates, although the wetting effect of the cholanic acid derivatives in the microenvironmental area around the dissolving drug particles must also be considered an effect, at least *in vivo*.

Gibaldi *et al.* (30) in a study of the dissolution properties of reserpine from various molar ratios of reserpine:deoxycholic acid coprecipitates showed that the dissolution rate increased with increasing molar ratio, which was in general agreement with the *in vivo* ptotic activity of reserpine (1). These investigators, likewise, attributed the observed potentiation in activity to differences in the particle size of reserpine produced during the preparation of the coprecipitates.

Since the various cholanic acid derivatives used in these studies have several physiological effects, it is not possible to attribute completely the observed *in vivo* response (1, 2) of the different coprecipitates to one single physical property of the materials used. Thus, orally administered bile salts can cause an increase in gastric volume which, in turn, can cause a decrease in the rate of gastric emptying (19), possibly increasing the residence time of the drug at the intestinal absorption site. This would result in increased drug absorption. Also, gastric and intestinal motility have been found to increase and decrease depending on the dose of bile salts administered (31, 32) and this could effect the absorption of reserpine administered as a coprecipitate with bile acids. It has also been reported that reserpine and bile acids and salts bind to plasma protein (20, 21). If the bile acids displaced reserpine from the binding sites, an increased pharmacological response to reserpine would be observed. Finally, drugs eliminated or involved in an entero-hepatic circulation mechanism may be reabsorbed after elimination in the bile (23). If the exogenous bile acids increase, either directly or indirectly, the reabsorption or decrease in the elimination through the bile of reserpine, an enhanced pharmacological response would be observed.

These same physiological factors should be operable both in the case of a physical mixture of the drug with a cholanic acid derivative and a coprecipitate. However, it is significant that the ptotic activity of reserpine in a 1:16 reserpine-deoxycholic acid coprecipitate was almost twice that in a physical mixture of similar composition (1), At best, therefore, the above-cited physiological factors could only play a minor role in the observed enhancement in reserpine induced ptotic activity. This indicates that the physical process of dissolution is probably the prime factor responsible for the enhanced activity observed.

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