Bile Acid Enhancement of Reserpine-Induced Blepharoptosis

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Abstract □ A more rapid onset of blepharoptotic activity in mice as well as a significantly increased oral potency relative to reserpine base was noted for reserpine coprecipitates with deoxycholic, cholic, and lithocholic acids. Some enhancement of potency was seen for a reserpine–trihydroxycholane combination, while a reserpine–cholanic acid coprecipitate was about equivalent to reserpine base. The use of water-soluble reserpine acetate increases both the apparent relative potency and the duration of activity, while the relatively water-insoluble reserpine hydrochloride only increases the duration of reserpine-like activity.

Keyphrases □ Reserpine—blepharoptosis □ Bile acid effect—reserpine blepharoptosis □ Solubility effect—reserpine activity

The ability of aqueous solutions of bile salts to solubilize highly nonpolar molecules *in vitro* has been recognized for many years. The increase in effectiveness of medicinal agents such as vitamin K (1) and phenolphthalein (2) upon administration with bile salts and acids has been attributed to interfacial effects and inclusion compound formation.

Previous work with mice in this laboratory (3) has shown that various molar coprecipitates of deoxycholic acid (desoxycholic acid) with reserpine base have the ability to increase the apparent oral blepharoptotic potency of reserpine, as well as to produce a more rapid onset of action. The administration of a series of reserpine-deoxycholic acid coprecipitates with a molar ratio from 1:2 to 1:16 showed a progressive increase in the ptotic potency as compared to reserpine base alone.

The fact that deoxycholic acid is not a normal bile acid for mice (4), suggested that this phenomenon with reserpine might be peculiar only for this acid and have little biological significance and practical application. In the present study, the effects of various orally administered reserpine–bile acid coprecipitates upon blepharoptotic activity were used as parameters of reserpine absorption. The authors' series of test coprecipitates contains one bile acid indigenous to mice (cholic acid) and others not present in mice. The substitution of various hydroxyl groups on the basic cholanic acid molecule and the reduction of deoxycholic acid to trihydroxycholane were studied for their relative effect upon reserpine absorption.

EXPERIMENTAL

Reserpine–bile acid coprecipitates (all 1:16 molar combinations) were prepared according to the technique of Malone *et al.* (3), Method *A*, although absolute methanol was substituted for absolute ethanol to prevent formation of any bile acid–alcohol inclusion compounds.

The mice[1] were removed from food 18 hr. before oral dosing and placed back on food after the +6-hr. ptotic readings had been completed. Doses of 3, 6, and 12 mg./kg. were used for the reserpine standard and reserpine as the hydrochloride, while doses of 1.5, 3, and 6 mg./kg. were used for the reserpine of the 1:16 *M* reserpine–bile acid combinations and doses of 2, 4, and 8 mg./kg. used for reserpine as the acetate. All drugs and test combinations were suspended by trituration to the point of uniform opalescence in the 0.25% aqueous agar-dosing vehicle (30 ml./kg.). In all other respects, the blepharoptotic assays were carried out exactly as described by Malone *et al.* (3).

Table I—Apparent Oral Potencies of Reserpine in Various Bile Acid Coprecipitates as Compared to Reserpine Alone[a]

Identification	Hours After Dosage 2	4	6	10	24
Reserpine–deoxycholic acid	3.19[b] (2.38–4.28)	2.16[b] (1.82–2.58)	2.25[b] (1.83–2.77)	1.93[b] (1.61–2.31)	2.28[c] (1.84–2.83)
Reserpine–cholic acid	3.15[b] (2.47–4.02)	1.93[b] (1.66–2.25)	2.16[b] (1.69–2.75)	1.70[b] (1.39–2.09)	1.94[c] (1.63–2.31)
Reserpine–lithocholic acid	2.88[b] (2.20–3.78)	2.93 (2.36–3.64)	3.50 (2.68–4.59)	2.63 (2.14–3.23)	2.40[c] (2.00–2.88)
Reserpine–trihydroxycholane	1.79[c] (1.33–2.43)	1.87[c] (1.51–2.32)	1.99[c] (1.57–2.51)	2.11[c] (1.77–2.52)	2.04[c] (1.60–2.61)
Reserpine–cholanic acid	1.28[c] (0.94–1.76)	1.41 (1.05–1.88)	1.43 (1.08–1.88)	1.29 (0.95–1.76)	<1.44[d]

[a] The range of figures within parentheses indicates the calculated 95% confidence limits for the listed potency. [b] Significant departure from parallelism ($p < 0.05$), with the coprecipitate dose-response curve being the steeper. [c] Calculated as a 2 × 2 assay; the others calculated as 3 × 3 assays. [d] Only a graphical estimate of potency was possible.

[1] Albino mice (15–30 g.) were donated from their stock (bred from HAM-ICR strain, caesarean delivered, Charles River mice) by Atlas Chemical Industries, Inc., of Wilmington, Del., and maintained in this laboratory on Purina laboratory chow and water *ad libitum* for at least 1 week prior to testing.

Table II—Apparent Oral Potencies of Reserpine Salts as Compared to Reserpine Base[a]

	Hours After Dosage				
	2	4	6	10	24
Reserpine acetate	1.95 (1.38–2.75)	2.15[b] (1.62–2.84)	2.22[b] (1.67–2.94)	1.99[b] (1.44–2.73)	≥2.70[c]
Reserpine hydrochloride	0.52[d] (0.28–0.98)	0.90 (0.64–1.25)	1.20 (0.85–1.68)	1.79 (1.24–2.57)	≥1.62[c]

[a] The range of figures within parentheses indicates the calculated 95% confidence limits for the listed potency. [b] Significant departure from parallelism ($p < 0.05$), with the salt dose-response curve being the steeper. [c] Only a graphical estimate of potency was possible. [d] Calculated as a 2 × 2 assay; the others calculated as 3 × 3 assays.

RESULTS AND DISCUSSION

Seven balanced log dose-response ptotic assays were conducted as shown in Tables I and II. In each case the reserpine–bile acid coprecipitate or reserpine salt was assayed against reserpine base as a standard. The statistical evaluation of potency with its 95% confidence limits was done according to the techniques of Bliss and Calhoun (5). The statistical treatment for the 3 × 3 (108–120 mice, 18–20 animals/dosage level) and 2 × 2 (72–80 mice) assays involved analysis of variance and factorial analysis. When the slow

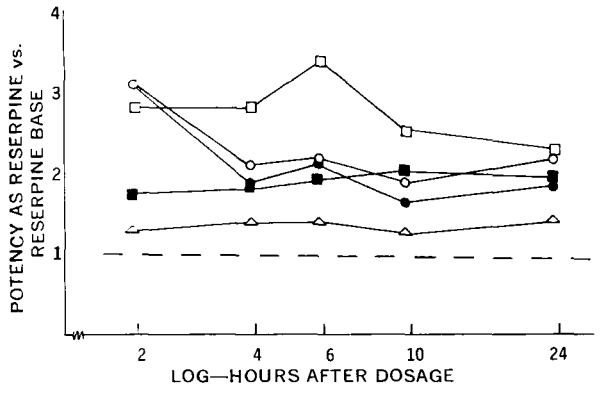

Figure 1—*Variation with time of the oral potencies of various 1:16 molar reserpine–bile acid coprecipitates as compared to reserpine base as a standard. Key: ○, reserpine–deoxycholic acid; ●, reserpine–cholic acid; □, reserpine–lithocholic acid; ■, reserpine-reduced deoxycholic acid(trihydroxycholane); △, reserpine–cholanic acid.*

onset or waning of the reserpine-like activity did not allow 3 × 3 calculations (lack of ptotic activity at the lowest dosage level), a 2 × 2 assay was calculated attempting to match two doses of the standard with two doses of the test material in the same linear portion of the dose-response curves. An average λ value (s/b) of 0.28 was obtained which agrees well with the assay characteristics of 0.27 and 0.29 reported earlier for this assay (3, 6).

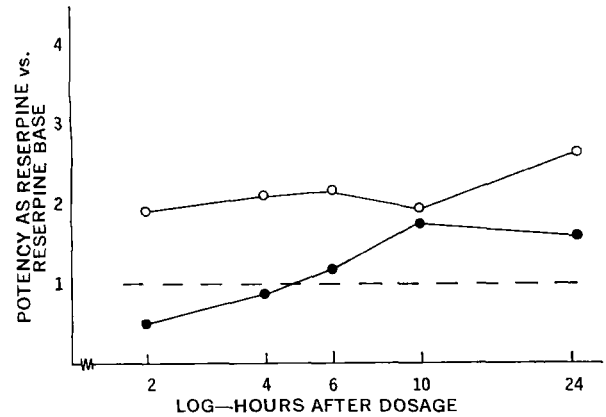

Figure 2—*Variation with time of the oral potencies of reserpine salts as compared to reserpine base as a standard. Key: ○, reserpine acetate; ●, reserpine hydrochloride.*

As summarized in Table I and illustrated in Fig. 1, the oral potency curve of the deoxycholic acid coprecipitate in relation to time after administration was similar qualitatively to that reported earlier (3). The apparent potency was greatest at the +2-hr. ptotic reading indicating a possible enhancement of alkaloidal absorption relative to the reserpine base alone. The potency figures for the cholic acid coprecipitate closely follow those for the deoxycholic acid combination. While the magnitude of the lithocholic acid-induced +2-hr. enhancement of reserpine-like activity was quite comparable to that calculated for both the deoxycholic and cholic acid combinations, the lithocholic acid coprecipitate appeared qualitatively different since its potency was well sustained with time and the maximum enhancement was noted with the +6-hr. readings.

The potency of the reserpine–cholanic acid coprecipitate was about equivalent to that of reserpine alone, while that of the reserpine–trihydroxycholane combination was only modestly enhanced beyond that of reserpine base. Neither of these two coprecipitates showed an accentuated +2-hr. ptotic potency.

In appropriate control studies, deoxycholic, cholic, lithocholic, and cholanic acids and trihydroxycholane were without ptotic activity when administered alone to mice.

Table II outlines the effects of reserpine salt formation on ptotic activity. Reserpine acetate (totally soluble in the vehicle at the concentrations used) produced a statistically significant increase in the apparent potency at all the measured time intervals. The general enhancement and the peaking of this effect at +24 hr. (Fig. 2) indicate both a hastened absorption and a longer duration of reserpine-like activity. Reserpine hydrochloride (insoluble) was essentially equipotent with the reserpine base at the +2, 4, and 6-hr. readings, indicating no significant facilitation of absorption. Only after +10 hr. did reserpine hydrochloride statistically increase its apparent potency over the reserpine standard, thus indicating some potential for prolonging the duration of action of reserpine.

The preceding data should dispell any doubts that the enhancement of reserpine's blepharoptotic activity might be peculiar only for deoxycholic acid. Both cholic acid (indigenous to mice) and lithocholic acid (not indigenous) evidenced ability to exhibit this property. The mechanism of action for bile acid enhancement of reserpine-induced blepharospasm has not been delineated here; however, there are several possible correlates between the physical and biological properties of these compounds and the noted *in vivo* phenomena (7). Moreover, it is of interest to note that the oral presentation of reserpine in a solubilized form also enhances its apparent potency. While reserpine was the only test drug used in this study, it is felt that the results obtained may be applicable to other poorly soluble and inefficiently absorbed medicinals.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 14, 1968, from the *Divisions of Pharmacology and Medicinal Chemistry, Pharmacy Research Institute, University of Connecticut, Storrs, CT 06268*

Accepted for publication October 25, 1968

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

This work was supported in part by University of Connecticut Research Foundation grant 400-5-178.

Effect of Polyethylene Glycol 300 on the Viability of Bacterial Spores

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Abstract □ In an earlier study polyethylene glycol 300 (PEG 300) reduced the dry-heat sterilization time for the spores of two species of aerobic bacilli. Further investigation indicates that PEG 300 also affects the viability of the spores without heat.

Keyphrases □ Polyethylene glycol 300 (PEG 300) effect—bacterial spores □ Bacterial spore germination—PEG 300 effect □ Oil, bacterial growth—PEG 300 effect

The authors recently reported that polyethylene glycol 300 (PEG 300) significantly reduced the dry-heat sterilization time for the spores of two species of aerobic bacilli (1). Since this was unexpected and contrary to an earlier report that polyethylene glycol (PEG) did not affect sterilization time (2), additional studies were conducted to confirm the sterilizing activity of PEG 300 with heat and to determine its effect without heat. The results of experiments conducted without heat follow.

Several low-molecular weight glycols are effective in low concentrations as air sterilizers (3, 4). Glycerol and various polyols inhibited bacteria through an osmotic effect (5). A specific inhibitory effect has been suggested for propylene glycol (6).

Although PEG compounds have been used in pharmaceutical preparations for many years, only recently has any antibacterial activity been attributed to these compounds. Cox (7) reported in 1965 that PEG affected the survival of bacteria as aerosols. Subsequently (8) he reported the toxic effect of solutions of various PEG compounds on vegetative bacterial cells. Sachs and Alderton (9) described a procedure for separating spores from vegetative cells using a two-phase aqueous polymer system containing PEG 4000 and potassium phosphate. They found that spores recovered in this manner showed no reduction in thermal resistance or viable count. The authors have not found any reports that any of the PEG compounds adversely affect the viability of bacterial spores.

EXPERIMENTAL

The growth of the spores of *Bacillus subtilis* ATCC 9372 and *Bacillus stearothermophilus* and the preparation of the spore test disks have been reported (1). The results reported here were obtained concurrently with those of the previous study, the test interval being one in which the spores were highly heat resistant in sesame oil and in air. The procedures and materials used were the same as those used in the heat study, the only difference being that the spore disks were held in the sealed oil or PEG 300 ampuls at room temperature for periods up to 3 weeks before transfer to the culture medium.

Analysis of PEG 300—The PEG 300 used in this study was analyzed to determine if some minor component other than the PEG 300 might be responsible for the results. Table I suggests that PEG 300 alone was involved.

RESULTS

B. subtilis—When PEG 300 was used as a control in the oil-sterilization study (1), the time from first exposure of the spores to PEG 300 until inoculation into the culture medium rarely exceeded 24 hr. Under those conditions PEG 300 did not affect the germination time of the unheated controls. When the *B. subtilis* spore disks were stored in PEG 300 for longer periods at room temperature, a different result was observed as shown in Table II. *B. subtilis* spores did not survive after storage in PEG 300 for 1 week, whereas those stored in the unpreserved sesame oil and in the phenol and benzyl alcohol-preserved sesame oil survived up to 2 weeks. No viable counts were made, thus it is impossible to determine what the viability loss rate was or the time required to kill all cells. Although these tests were much more limited than those in which heat was used, there is good correlation between the data obtained under the two conditions, *i.e.*, decreased survival times in the presence of PEG 300 compared to those in sesame oil.

B. stearothermophilus—Control disks of *B. stearothermophilus* produced visible growth within 12 hr. after transfer to the culture medium. Germination time was not altered by storage in either the

Table I—Analysis of PEG 300

Moisture[a]	0.12%
Ethylene glycol[b]	0.18%
Diethylene glycol[b]	0.24%
Aldehyde[c]	14 p.p.m.
Ethylene oxide[d]	Negative

[a] Karl Fischer method. [b] GLC, flame detector. [c] Aldehyde (as formaldehyde) spectrophotometric determination. [d] Spectrophotometric method.