Yohimbine Potentiation of Reserpine Blepharoptosis

By MARVIN H. MALONE and ROBERT H. ROTH, Jr.†

Yohimbine, with an oral LD_{50} in mice of 36.5 mg./Kg. (S.E. = ±3.02), does not show any reserpine-like sedative-blepharoptotic activity in nonlethal dosage. Using factorially designed, graded response assays, yohimbine was shown to potentiate reserpine palpebral ptosis significantly for at least 5 hours when administered concurrently with and at the same oral dosage level as reserpine. Yohimbine administered in higher dosage extended the potentiation effect to a greater degree and for longer periods of time (≥24 hours). Since yohimbine is reported to be both an adrenolytic agent and an antimetabolite of serotonin, this study may indicate that the mechanism of action of reserpine sedation involves depletion of norepinephrine more than serotonin release as a neurohormone. Male mice were more susceptible to reserpine ptosis than females.

YOHIMBINE is a classic adrenolytic agent and recently has been shown to have central nervous system activity (1, 2). Yohimbine is also an antimetabolite of serotonin (3). At the present time there are several theories as to the mechanism of action of reserpine, but all theories implicate two compounds with possible neurohormonal activity in the central nervous system; these compounds are serotonin and norepinephrine (4–6). Since both reserpine and yohimbine have been reported to be natural constituents of *Rauwolfia serpentina* Benth (7, 8), it appeared to be of theoretical and practical interest to determine whether yohimbine would either potentiate or antagonize the action of reserpine. A persistant blepharoptosis is characteristic only of the reserpine-like ataraxics, since the eyelid closure of the phenothiazine ataraxics is easily reversed by environmental stimulation. Rubin, *et al.* (9, 10), have devised a graded response assay for reserpine-like activity utilizing the palpebral ptosis of mice recorded 5 hours after oral dosage. This ptosis is generally considered to be due to central depression and is seen concurrent with facilitation of caffeine and pentylenetetrazol induced extensor seizures; these phenomena are antagonized by prior or simultaneous iproniazid administration (11). Since localized lesions of the human hypothalamus, as in Horner's syndrome, produce reserpine-like symptoms: miosis, enophthalmus, hypothermia, lethargy, and palpebral ptosis (12), it was decided to utilize the mouse ptosis assay to determine the interactions of reserpine and yohimbine.

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This paper is dedicated to Dr. Harald G. O. Holck on the occasion of his retirement from the Institute for Cellular Research and the Department of Pharmacology of the University of Nebraska.

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EXPERIMENTAL

General.—White mice were obtained from Joseph E. Stocker, Ramsey, N. J., and were maintained in this laboratory on Purina Laboratory Chow and water *ad libitum* for at least 4 days prior to test. Only mice within a weight range of 15–25 Gm. were used for the ptotic assays. Animals were taken off food 3 to 4 hours prior to dosing and placed back on food 10 hours after dosing. Water was allowed throughout the entire test period. A dosage volume of 0.6 ml./20 Gm. of mouse was constant throughout this study. Animals were dosed utilizing precision grade syringes and cut-off, blunted, polished, 30-mm. long, 20 gauge hypodermic needles. Test solutions were coded using randomized numbers, so that the two dosing technicians did not know the actual dosage or identity of any of the test groups. For the ptotic assays, the animals were randomized in respect to the six test groups of the 3 × 3 assay format, and also randomized between the two sexes, and between the two dosing technicians. The mean time off food prior to dosing for each group was statistically constant. Only one technician read the ptosis values for all animals at all indicated times, and this technician did not know the dosage level or identity of any of the test groups. Each mouse was permanently and individually identifiable throughout the test period due to spot applications of a 0.5% picric acid solution in 70% ethanol. Ptosis for each eye was scored following the scale adopted by Rubin, *et al.* (10): 4 = complete, 3 = 3/4, 2 = 1/2, 1 = 1/4 closure of the eyelids from the normal aperture. A nonptotic response was scored as 0. The individual metameter was the sum of the ptotic scores for both eyes, so that the graded test response varied from 0–8 per mouse. Prior to ptotic scoring, each mouse was manually aroused, then placed on a screen and held by the tail facing the scorer at the scorer's eye level. Eye scores of a 4 rating were checked to insure that the lid was not encrusted shut and that the ptosis was an active phenomenon.

Yohimbine hydrochloride and reserpine alkaloid were obtained from California Corporation for Biochemical Research. Reserpine was solubilized by glacial acetic acid and sufficient double-distilled water was added to make a 10 mg./ml. solution in 10% acetic acid. All dosages of reserpine and yohimbine are expressed here in terms of the alkaloid rather than the alkaloidal salt. Where

TABLE I.—MEAN BLEPHAROPTOTIC SCORES AFTER ORAL DOSAGE OF RESERPINE AND RESERPINE-YOHIMBINE MIXTURES TO MICE

Test Code S *vs.* U	Standard (S) Dosage, mg./Kg., Reserpine[a]	Standard (S) Hr. After Dosage 3	5	10	24	*vs.* Unknown (U) Dosage, mg./Kg.[a] Reserpine	Yohimbine	Unknown (U) Hr. After Dosage 3	5	10	24
R *vs.* R[1]:Y[0]	2	2.26[b]	3.61	3.92	1.18	2	..	2.07	3.41	3.61	1.33
	4	3.59	4.94	5.04	3.11	4	..	3.76	4.94	4.83	2.17
	8	4.65	6.52	7.09	6.41	8	..	5.31	6.74	7.35	6.81
R *vs.* R[1]:Y[1]	2	2.06[c]	3.25	3.44	1.78	2	2	3.14	3.86	3.28	1.36
	4	3.47	4.83	4.83	2.61	4	4	5.30	5.61	5.14	2.11
	8	5.50	6.89	7.67	6.72	8	8	6.64	7.25	7.44	5.17
R *vs.* R[1]:Y[2]	2	2.53[c]	3.78	4.06	0.78	1	2	2.83	3.67	3.11	0.94
	4	4.17	5.42	5.28	2.75	2	4	4.08	4.56	4.64	1.64
	8	4.92	6.67	7.25	6.78	4	8	5.08	5.92	5.72	3.03
R *vs.* R[1]:Y[4]	2	1.92[c]	3.50	3.80	1.22	1	4	2.75	3.50	2.72	0.86
	4	3.39	4.58	4.69	2.56	2	8	4.25	5.19	5.61	2.22
	8	4.53	6.33	6.75	6.33	4	16	5.44	6.06	5.75	3.80
R[F] *vs.* R[M]	2	1.87[d]	2.98	3.20	0.92	2	..	2.46	4.04	4.33	1.59
	4	3.57	4.68	4.62	2.50	4	..	3.78	5.20	5.24	2.78
	8	5.11	6.61	7.00	6.20	8	..	4.85	6.65	7.44	7.02

[a] The constant dosage volume for each test dose was 30 ml./Kg. [b] 54 mice per dosage group, equally divided between sexes. [c] 36 mice per dosage group, equally divided between sexes. [d] 54 mice per dosage group. The "standard" group is all female; the "unknown" group is all male.

yohimbine and reserpine were administered concurrently, they were given together in the same vehicle.

RESULTS AND DISCUSSION

Preliminary Evaluation of Yohimbine.—As calculated by the Bartlett modification of the Miller and Tainter log-probit method (13), the oral LD_{50} for yohimbine was determined to be 36.5 mg./Kg. with a standard error of ±3.02. The dosage increment used was 1.414×, and the number of animals (N') contributing to effects between probits 3.5 and 6.5 was 37. The estimated LD_5/LD_{95} values were 20.4/65.0 mg./Kg. Acute death from yohimbine was preceded by the following major symptoms listed in order of appearance: respiratory stimulation, respiratory depression, hyperkinetic phenomena (increased motor activity, fine body tremors, Straub tail erection), transient minimum ptosis (maximum rating of 1 per eye), clonic convulsions, cyanosis, and death by respiratory arrest. No evidence of priapism was seen in the males. The transient ptosis was seen only at dosage levels of 45.2 mg./Kg. or greater and was always seen prior to convulsions and death. Considering only quantal incidence of detectable ptosis, reserpine has a PtD_{50} (ptotic dose for 50% of a population) graphically estimated at 0.5 mg./Kg.; in this respect, reserpine is $\geq$128× the ptotic potency of yohimbine. Considered as a graded response, reserpine was graphically determined to be $\geq$64× the ptotic potency of yohimbine. Previous work in this laboratory has indicated that the acute oral LD_{50} of reserpine (acetate) was $\geq$150 mg./Kg. Therefore, the LD_{50}/PtD_{50} ratio for reserpine is $\geq$300; while for yohimbine, this ratio only equals 0.6. Yohimbine qualitatively displays little to no reserpine-like, sedative-ptotic activity.

Yohimbine Potentiation of Reserpine-Induced Palpebral Ptosis.—Four balanced log dose-response assays were conducted, assaying various reserpine-yohimbine mixtures against reserpine alone as a standard. The yohimbine content of the mixtures ranged from 0–4× the reserpine content. The maximum dose of yohimbine given with reserpine was 16 mg./Kg. Ptosis was rated for all test groups at 3, 5, 10, and 24 hours after dosing. Maximal mean ptotic effects after reserpine alone were seen 10 hours after dosing, in conflict with previous reports indicating that maximal effects were seen 5 to 7 hours after dosing (10). Mean results are tabulated in the first sections of Table I. Not reported in Table I are the mean ptotic scores of the 2 control groups receiving 8 and 16 mg./Kg. of yohimbine orally, respectively. Each group consisted of 18 mice, the number divided equally between the sexes, dosed and scored concurrently with the assay test groups. Mice receiving 8 mg./Kg. of yohimbine did not have detectable ptosis at any time, while the group receiving 16 mg./Kg. was observed to have a mean ptotic score of 0.18 and 0.12 at the 3- and 5-hour readings, respectively, with complete absence of ptosis at the 10- and 24-hour readings.

The statistical treatment for the 3 × 3 and 2 × 2 assays involved analysis of variance, factorial analysis, and calculation of potency and confidence limits as per the techniques outlined by Bliss (14). The results are tabulated in the first sections of Table II, with graphical interpretations illustrated in Figs. 1 and 2. An average λ value (s/b) of 0.29 was obtained with the 16 3 × 3 assays calculated; standard error was determined to be ±0.015. With this λ value, a population of 210 mice per assay would have been needed to insure a standard error for potency of ±10% (14). The number of mice used in this study was either 216 (36 mice/dose) or 324 (54 mice/dose) per each 3 × 3 assay.

Peak potentiation was noted at the third hour for 1:1 and 1:2 reserpine-yohimbine mixtures, while peak effects were reached with the tenth-hour readings with the 1:4 mixture (Fig. 1), indicating that at these concentrations and at these times, the presence of yohimbine in an unknown preparation will maximally distort any ptotic assay attempting to document only reserpine activity. The control

TABLE II.—DETERMINATION OF APPARENT POTENCIES OF TEST UNKNOWNS IN RELATION TO RESERPINE ALONE OR TO RESERPINE IN FEMALE MICE

S *vs.* U Test Code from Table I	Hr. After Oral Dosage 3	5	10	24
R *vs.* R[1]:Y[0]	1.112[a] (0.933–1.326) 0.25–0.20[b]	1.003 (0.869–1.157) >0.50[b]	0.966[c] (0.847–1.102) >0.50[b]	0.967[cd] (0.865–1.081) >0.50[b]
R *vs.* R[1]:Y[1]	1.715 (1.418–2.075) <0.001[b]	1.259 (1.088–1.456) 0.005–0.001[b]	0.991 (0.874–1.124) >0.50[b]	0.820[e] (0.716–0.939) 0.005–0.001[b]
R *vs.* R[1]:Y[2]	2.161 (1.737–2.689)	1.467 (1.203–1.790)	1.219 (1.006–1.477)	1.265[e] (0.929–1.722)
R *vs.* R[1]:Y[4]	1.576 (1.245–1.994)	2.118 (1.716–2.613)	2.586[e] (2.161–3.095)	1.707[e] (1.129–2.580)
R(F) *vs.* R(M)	1.092 (0.917–1.301) 0.50–0.25[b]	1.269[f] (1.098–1.467) <0.001[b] 1.462[e] (1.188–1.800) <0.001[b]	1.340[c] (1.170–1.534) <0.001[b] 1.174[e] (1.036–1.330) 0.01–0.005[b]	1.164[c] (1.040–1.303) 0.01–0.005[b] 1.100[e] (0.991–1.221) 0.10–0.05[b]

[a] Figures in parentheses indicate the 95% confidence limits for the potency. [b] Observed *P* of test of S *vs.* U. [c] Significant curvature in 3 × 3 assay, *P* 0.05. [d] Significant opposite curvature in 3 × 3 assay, *P* 0.05. [e] Calculated as a 2 × 2 assay. [f] Significant departure from parallelism in 3 × 3 assay, *P* 0.05.

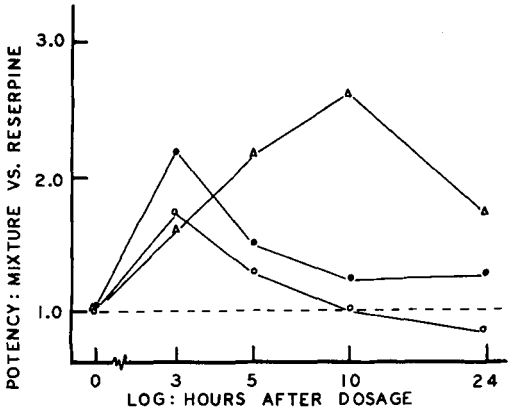

Fig. 1.—Variation with time of ptotic potency of various reserpine-yohimbine mixtures: O, 1:1 reserpine-yohimbine; ●, 1:2 reserpine-yohimbine; △, 1:4 reserpine-yohimbine.

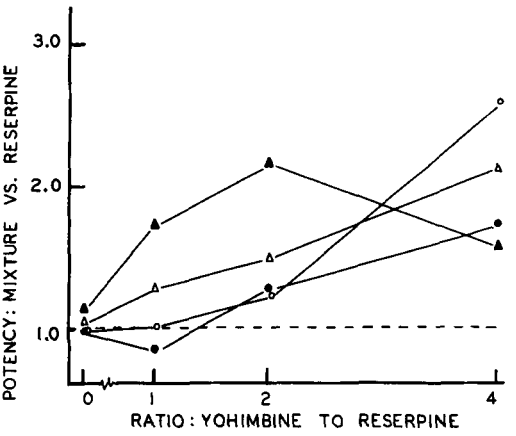

Fig. 2.—Variation of ptotic potency with ratio of yohimbine to reserpine in reserpine-yohimbine mixtures: ▲, 3-hour ptosis response; △, 5-hour; O, 10-hour; ●, 24-hour.

assay with no yohimbine in the unknown showed no potentiation at any of the time intervals.

Yohimbine in a 1:1 ratio in a reserpine-yohimbine mixture potentiated the 3- and 5-hour ptosis readings, had no effect on the 10th-hour ptosis, and may have been actually instrumental in reversing the residual 24-hour reserpine ptosis (Fig. 2). Yohimbine, in a ratio of 1:2 in reserpine-yohimbine mixtures, potentiated reserpine on the 3-, 5-, and 10-hour ptotic responses, but the effect on the 24-hour ptosis was not statistically significant. Yohimbine potentiated at all observed time intervals when in a 1:4 reserpine-yohimbine mixture indicating that in high levels, yohimbine is capable of exerting considerable sustained effects.

Sex Variation in Response to Reserpine.—In the preceding assays a consistent sex variation to the reserpine-induced blepharoptotic response was detected, so a factorially designed assay was performed to document this observation. The results are tabulated in the last part of Table I, and the statistical evaluation noted in the last lines of Table II. At 5, 10, and 24 hours after dosing with reserpine alone, the males were significantly more susceptible than the females, with the greatest sensitivity being apparent at the 5- and 10-hour ptotic readings.

CONCLUSIONS

Yohimbine, although lacking true ptotic activity, potentiated reserpine-induced blepharoptosis without altering slope, dose-response relationships of this phenomenon, thus indicating that the mechanism of action of reserpine may involve depletion of norepinephrine more than the release of serotonin as a neurohormone. In mice, the males are more susceptible to oral

reserpine than the females. The presence of yohimbine in crude plant material may distort results obtained by mouse blepharoptotic assays where the goal of the assay is the quantitation or the detection of only reserpine-like alkaloids.

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Synthesis and Pharmacological Effects of Bis-trialkylammonium Alkanol Carbonates

By LEO A. POHORYLES, L. WISLICKI, and SHALOM SAREL

The synthesis and pharmacological effects of a series of bis-trialkylammonium alkanol carbonates of the type I, are described. The compounds were tested in the cat for their effects on electrical excitability of striated muscle, respiration, and blood pressure, and in the frog rectus abdominis. While the ethyl derivative, (I-1), lowers blood pressure without affecting the muscle twitch, the propyl-(I-3) and, to a much higher extent, the butyl derivative, (I-6), depress neuromuscular transmission and also direct muscle excitability. Branching of the alkanol moiety (I-2), or replacement of the carbonate oxygen by sulfur (I-7), or insertion of ethyl for the methyl groups on the quaternary nitrogen (I-4 and I-5), weaken the effect. The block is similar to that caused by depolarizing agents.

IT IS WELL known that the curarizing activity of bis-quaternary ammonium compounds of the structure $R_3\overset{+}{N}—(CH_2)_x—\overset{+}{N}R_3$ is not substantially weakened by inserting in the linear polymethylene chain bivalent radicals like carbonyl and/or oxygen for the methylene groups. Introduction of one or more ester groupings into the chain linking the two ammonium groups provides powerful curare-like neuromuscular blocking agents; thus, choline esters of dicarboxylic acids, particularly that of succinic acid, proved to be valuable clinically. However, these substances were found to be not without drawbacks. These include, for the nondepolarizing drugs, their long action and side effects on heart rate or blood pressure. With suxamethonium, which *in vivo* is usually quickly decomposed by enzymatic hydrolysis, a high incidence of muscle pain (1, 2), the absence of an effective antagonist, and instability within a range of pH's (3) have been troublesome.

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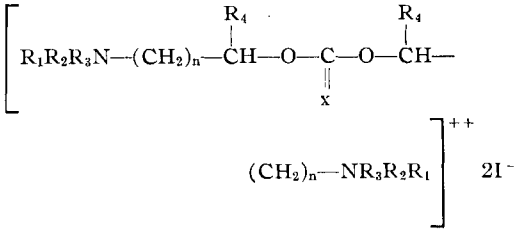
In view of the great stability shown by open chain carbonates toward hydrolysis both in acid and alkaline solutions (4), the synthesis of a series of bis-trialkylammonium alkanol carbonates of the structure I was undertaken.



I

I-1 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 1$; $x = O$.
I-2 $R_1 = R_2 = R_3 = R_4 = CH_3$; $n = 1$; $x = O$.
I-3 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 2$; $x = O$.
I-4 $R_1 = R_2 = CH_3$, $R_3 = C_2H_5$, $R_4 = H$, $n = 2$, $x = O$.
I-5 $R_1 = R_2 = C_2H_5$; $R_3 = CH_3$; $R_4 = H$; $n = 2$; $x = O$.
I-6 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 3$; $x = O$.
I-7 $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $n = 3$; $x = S$.

Differences in structure were designed with a