A PHARMACOLOGICAL STUDY OF TWO BISBENZYLISOQUINOLINE ALKALOIDS, THALISTYLINE AND OBAMEGINE

JON W. BANNING, KADHIM N. SALMAN and POPAT N. PATIL

Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210

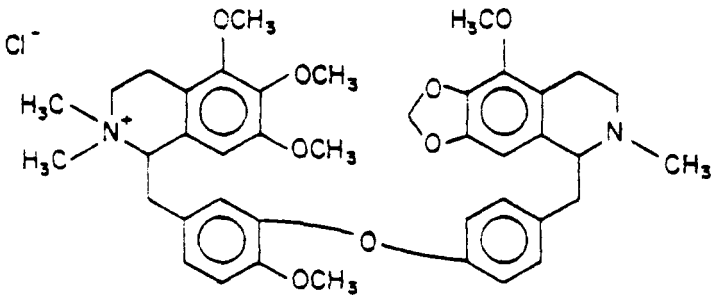
ABSTRACT.—Thalistyline, a monoquaternary bisbenzylisoquinoline alkaloid isolated from *Thalictrum* sp. inhibited respiration in anesthetized dogs. Thalistyline is about one-fourth as potent as d-tubocurarine in blocking neuromuscular transmission in the rat hemidiaphragm preparation. The pharmacological mechanism of action of the alkaloid is similar to that of d-tubocurarine. Obamegine did not exhibit curare-like activity. On the isolated rabbit aorta, contractions induced by an *alpha*-adreno-receptor agonist, phenylephrine, were antagonized by both alkaloids. Increasing concentrations of thalistyline produced parallel shifts to the right in the dose-response curves of phenylephrine. The pA_2 value for the competitive pharmacological antagonism was 6.33. Obamegine also antagonized the effects of phenylephrine on the aorta, but at the higher concentration blockade did not appear to be competitive. Thalistyline and obamegine lowered blood pressure in normotensive dogs. The effect was transient. Repeated injections of the alkaloids resulted in tachyphylaxis to blood pressure lowering effects. Although alkaloids exhibited *alpha* adrenergic blockade in the vascular preparation, the mechanism for the hypotensive effect remains to be established.

Alkaloids isolated from several species of plants in the genus *Thalictrum* have hypotensive, antimicrobial and antitumor activity (1). In 1976 we first reported the isolation of thalistyline, a monoquaternary bisbenzylisoquinoline alkaloid (fig. 1), from *Thalictrum longistylum* D.C. and *T. podocarpum* Humb. (2). Thalistyline as well as obamegine showed significant hypotensive activity in normotensive laboratory animals (3). Since these preliminary reports appeared, we have been able to isolate from *Thalictrum* species sufficient quantities of both obamegine and thalistyline to permit an additional pharmacological study. We shall present evidence that one of the major pharmacological actions of thalistyline is neuromuscular blockade. This neuromuscular blocking action of thalistyline will be compared with that of another well-known bisbenzylisoquinoline alkaloid, d-tubocurarine. Adrenoreceptor blocking and blood pressure lowering effects are also investigated for both compounds.

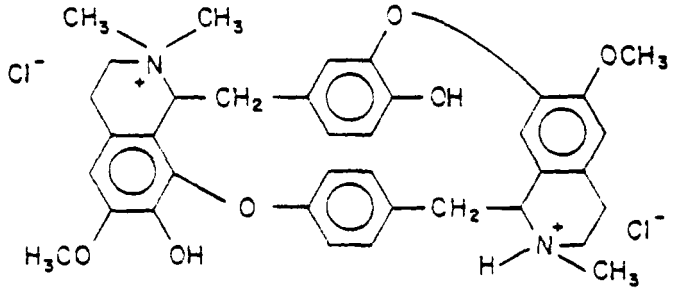
MATERIALS AND METHODS

I. CARDIOVASCULAR STUDIES.—Dogs of either sex with a weight of 7 to 12 kg were anesthetized with sodium pentobarbital (30 mg/kg iv). The right carotid artery was cannulated to record arterial blood pressure. Blood pressure responses were recorded on a Grass polygraph, and the results were expressed as changes in mean arterial pressure in mm Hg. Mean arterial pressure was calculated as the sum of the diastolic pressure plus one-third of the pulse pressure (4). Pulse pressure is the difference between the systolic and diastolic pressures. The right femoral vein was cannulated for intravenous injection. Heart rate was measured with a Grass tachograph which was triggered either by the ECG amplifier output or by the pulse pressure readout from the driver amplifier that was used to record arterial blood pressure. Bilateral vagotomy and tracheotomy were also performed. Drugs were injected in a volume of 0.1 to 1 ml followed by 2 ml of a saline flush and were prepared for intravenous injection as follows: obamegine was dissolved in a few drops of 0.1 M HCl and diluted to the proper volume with isotonic saline. Thalistyline Cl, dopamine HCl (Regis) and norepinephrine (Levophed bitartrate, Winthrop) were dissolved in isotonic saline with 0.1% ascorbic acid (Baker) added to prevent oxidation of these substances.

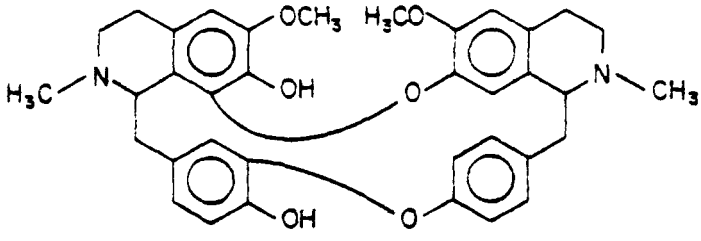
II. STUDIES ON RABBIT AORTIC STRIPS.—Rabbits of either sex with a weight of 2 to 3.4 kg were sacrificed by a sharp blow to the back of the head. Thoracic aortic strips (2 mm by 30 mm) were prepared as described by Furchgott and Bhadrakom (5). The strips were suspended in a 10 ml jacketed tissue bath containing modified Krebs' solution with the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 0.54, $NaHCO_3$ 24.9, glucose 11.1, and NaH_2PO_4 1. Ethylenediaminetetraacetic acid (10 mg liter) (G. Frederick Smith Chemical Co.) was added to the solution to prevent spontaneous oxidation of the catecholamines. The bath temperature was maintained at 37±0.5° and aerated with a mixture of 95% oxygen and 5% carbon dioxide. Responses of the aortic strips to drugs were recorded with an isotonic myograph on a physiograph. The isotonic lever exerted 4 g of tension. The strips were allowed to equilibrate for 2.5 hrs with frequent washing prior to the addition of drugs. Cocaine



THALISTYLINE Cl⁻



TUBOCURARINE Cl⁻



OBAMEGINE

FIG. 1. Comparison of chemical structures of bisbenzylisoquinoline alkaloids. Note the five methylated phenolic hydroxyl groups on the monoquaternary thalistyline structure. These structural features presumably contribute to the strong curare-like effect of thalistyline.

(Merck) (1 x 10^{-5}M) to prevent neuronal uptake of catecholamine and propranolol (Ayerst) (1 x 10^{-5}M) to prevent *beta*-adrenoceptor activation were added 20 and 25 min prior to the addition of phenylephrine (Gane's Chemical Works, Inc.). Cumulative dose-response curves were obtained by increasing the addition of phenylephrine by a factor of 3 according to the method of Van Rossum (6). The tissues were then washed several times until the base-line was reached. Aortic strips were incubated with a concentration of the alkaloid for 45 min. Cocaine and propranolol were again added at 20 and 25 min prior to the second dose-response curve to phenylephrine. One strip was always used as a control (not given antagonists) to detect changes in tissue sensitivity during the experiment. The dissociation constants (K_B) of the antagonists were calculated from the formula $K_B = (B)/DR-1$ where (B) is the concentration of the antagonist (7). The dose ratio, DR, is defined as the ED50 of the agonist in the presence of the antagonist (B) divided by the ED50 for the agonist alone. Where at least three different concentrations of antagonist produced apparently parallel shifts in the dose-response relationship, a Schild plot (8) was constructed to confirm that the antagonism

was of the competitive type. Negative log molar K_B is also referred to as pA_2 which can be obtained either from a Schild plot or from the above equation.

III. NEUROMUSCULAR STUDIES.—Male albino Sprague-Dawley rats (200 to 300 g) were sacrificed by a sharp blow to the head. The left hemidiaphragm with its attached phrenic nerve was removed as described by Bülbring (9) and suspended with 0.5 g tension in a 50 ml organ bath containing 30 ml Liley's solution of the composition (mM): NaCl 136.8, KCl 5.0, $CaCl_2 \cdot 2H_2O$ 2.0, $MgCl_2 \cdot 6H_2O$ 1.0, NaH_2PO 1.0, $NaHCO_3$ 12.0 and glucose 11. The bath temperature was maintained at 32±0.5° and aerated with 95% oxygen and 5% carbon dioxide. The phrenic nerve was passed across a pair of platinum electrodes and stimulated with 5 volt rectangular wave pulses of 0.4 msec duration at a frequency of 0.2 Hz. The hemidiaphragm was also directly stimulated with 50 volt rectangular wave pulses through an electrode in direct contact with the diaphragm muscle. All preparations were allowed to stabilize for 15 to 30 min before any treatments were initiated. Muscle contractions were recorded with a Grass isometric transducer (FTO3C) connected to a Grass polygraph. Logarithms of the molar concentrations at which contractile responses were 30, 50 and 70% of control were determined from the dose-response curve of each experiment by interpolation. The mean and confidence limits of the log values were calculated for each percentage level as described by Kaumann (10). Drugs used were: thalistyline Cl, obamegine, d-tubocurarine Cl (Squibb), eserine sulfate (Sigma) and α-bungarotoxin (Miami Serpentarium). The number of experiments was sufficient so that data could be analyzed statistically when required. The significance of the difference between the means was calculated by Students' t-test.

RESULTS

I. CARDIOVASCULAR STUDIES.

A. *Effects of Obamegine.*—Obamegine was very effective in lowering the blood pressure. In normotensive dogs (fig. 2) obamegine elicited dose-related decreases in mean arterial pressure (MAP). A high dose (4 mg/kg, iv) produced onset of hypotensive action at 5 sec after injection. Peak effect occurred at 1 min 30 sec and lasted for 1 min 45 sec. The total duration of hypotensive action was about 30 min. Obamegine caused a slight fall in heart rate of about 20 beats/min that was sustained for the duration of the hypotensive action. The alkaloid had no inhibitory effect on respiration, and it was not lethal for the dog from 0.5 mg/kg to 4 mg/kg dose range. Tachyphylaxis to the hypotensive effect of obamegine was observed. For example, in one experiment obamegine (2 mg/kg) lowered MAP from 100 to 39 mmHg, a 61 mmHg reduction. The duration of action for this dose was 22 min. About 2.5 hrs after injection of the first dose, a second dose (2 mg/kg) only reduced MAP by 11 mmHg from 108 (control) to 97 mmHg.

B. *Effects of Thalistyline.*—Thalistyline (0.1 to 0.4 mg/kg) produced decreases

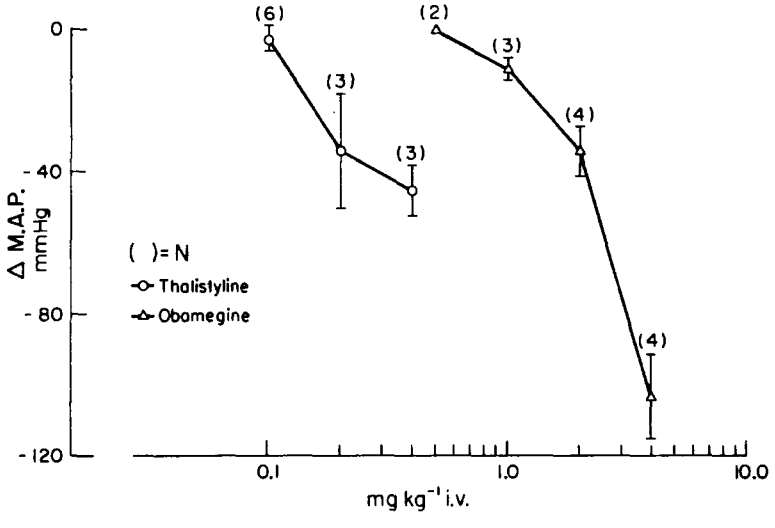


FIG 2. Comparison of blood pressure lowering effect of thalistyline and obamegine on mean arterial pressure in anesthetized dogs. Responses in mm Hg indicated here represent initial doses of each drug. Numbers in parentheses indicate the animals used at a given dose of the alkaloid.

in mean arterial pressure (fig. 2). A dose-effect relationship for the reduction of blood pressure exists only over the very narrow dose range indicated. Above 0.4 mg/kg thalistyline produced respiratory depression and death (fig. 3). The initial dose (0.1 mg/kg) generally caused a sustained rise in heart rate of 20 beats/min that persisted even though the MAP had returned to the control level. Subsequent doses of thalistyline produced both a rise and then a fall in heart rate of about 20 beats/min from the control level. The times for onset, peak effect and duration of action for the largest non-lethal dose of thalistyline (0.4 mg/kg) were generally about the same as reported for obamegine (4 mg/kg). Tachyphylaxis to the cardiovascular effects of thalistyline were also observed.

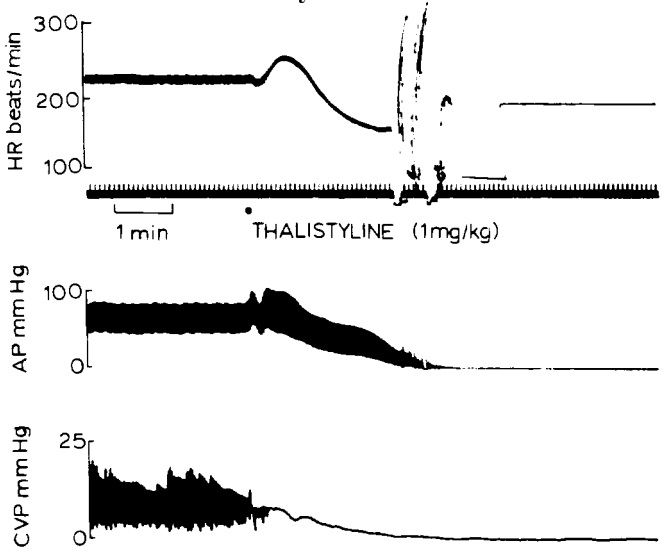


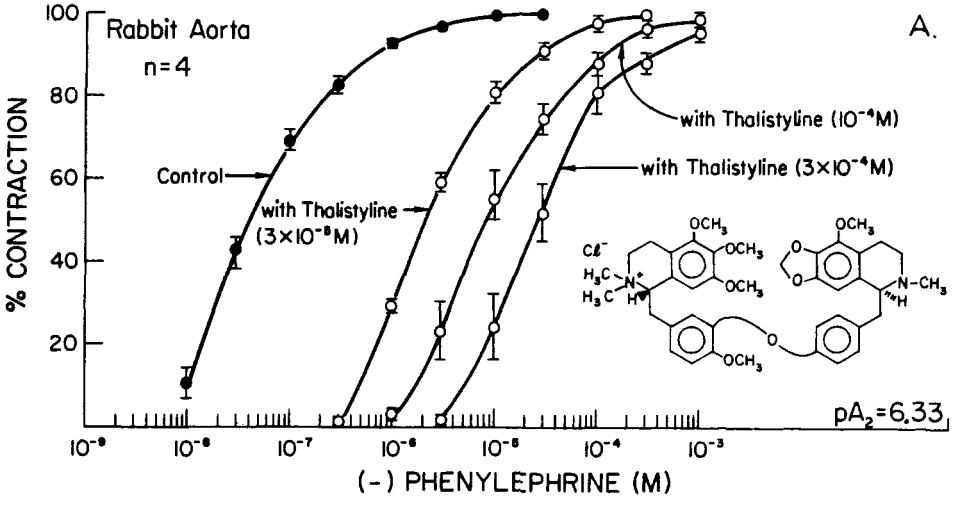
FIG. 3. Evidence of cardiovascular depression and death after a large dose of thalistyline. Dog was prepared as described in methods section to measure heart rate (HR), arterial pressure (AP) and central venous pressure (CVP). Dog received thalistyline (1 mg/kg) iv at the point in time indicated by the dot under the time line. Note that deflections of pen recording CVP fluctuations (via the brachial vein) are inhibited immediately following the injection of thalistyline. Cessation of respiratory activity (not shown) coincided with the inhibition of CVP fluctuations. Extremely irregular deflections of heart rate trace indicate the onset of cardiac arrhythmias as blood pressure reached minimal levels.

C. *Effects of Obamegine and Thalistyline on Cardiovascular Responses to Dopamine and Norepinephrine.*—In seven dogs, dopamine (50 to 100 μg/kg and norepinephrine (1 or 2 μg/kg) were injected (iv) before and during the hypotensive response elicited by obamegine (1 to 4 mg/kg) or thalistyline (0.2 to 0.4 mg/kg) to observe if these *Thalictrum* alkaloids would block blood pressure raising effects of these catecholamines. The blocking effect of thalistyline was inconsistent from animal to animal. Similar inconsistent results were obtained with obamegine: doses of more than 4 mg/kg could not be tested because the blood pressure was generally lowered to a range of 30 to 40 mmHg by this dose of obamegine.

II. ANTAGONISM OF PHENYLEPHRINE-INDUCED CONTRACTIONS OF RABBIT AORTIC STRIPS BY ALKALOIDS.

Since the mechanism of blood pressure lowering effects could not be assessed from the above experiments, we elected to test the antagonistic activity of our compounds on isolated tissues that contained *alpha*-adrenoreceptors. Thalistyline produced competitive antagonism of the contractile responses to phenylephrine (fig. 4A) as indicated by the parallel shifts to the right in the dose-response relation-

ship. A Schild plot of the data in fig. 4A produced a straight line with a slope of 1.1 and a pA_2 value of 6.33. Obamegine (3×10^{-5}M) caused a 10-fold shift to the right of the contractile responses to phenylephrine (fig. 4B). At the highest dose of obamegine (3×10^{-4}M), the increase in the shift to the right was accompanied by a 30% decrease in the maximal response to phenylephrine. This suggests an additional nonspecific inhibitory effect of obamegine at higher concentrations. Average negative log molar K_B calculated for two lower concentrations of obamegine was 5.30. Thus, obamegine has only 1/10 the blocking activity of thalistyline. The results showed that these alkaloids can block the *alpha*-adrenoreceptors.



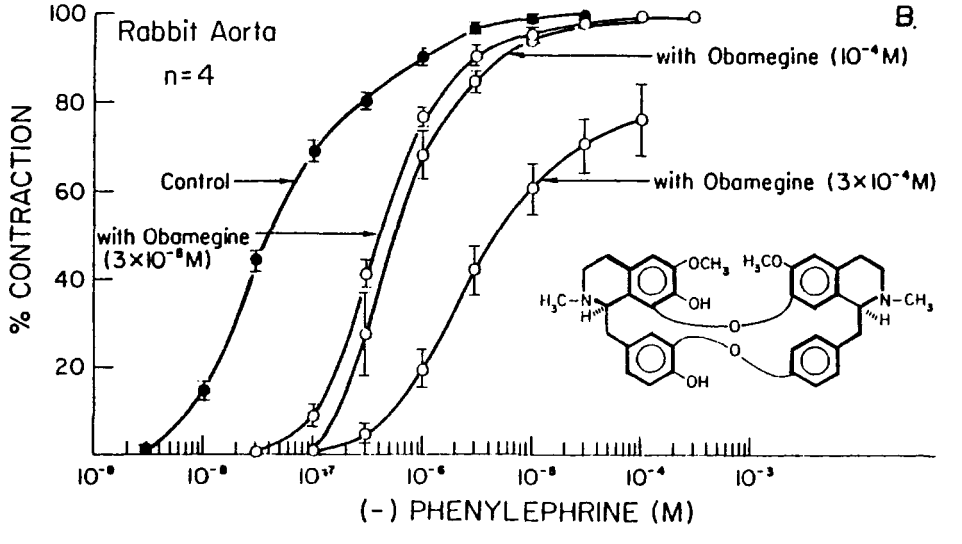


FIG. 4. Comparison of antagonistic activity of thalistyline (A) and obamegine (B) on phenylephrine-induced contractions of rabbit aortic strips. Increasing concentrations of thalistyline produced a greater blockade without a reduction in the maxima. pA_2 value is obtained from the Schild plot. Obamegine exhibited competitive blockade at 3×10^{-5} and 10^{-4}M concentrations. At higher concentration a reduction in maxima was observed.

III. Effects on neuromuscular transmission.

A. *Inhibition of Neuromuscular Transmission.*—Thalistyline (>0.4 mg/kg) produced respiratory arrest in dogs. Hence, the isolated left hemidiaphragm and phrenic nerve preparation from rats as described by Bülbring (9) was used to study the effects of the alkaloid on neuromuscular transmission. It inhibited the hemidiaphragm response to nerve stimulation as shown in fig. 5. If compared at the 50% level, which is equivalent to the IC_{50} (concentration causing 50% inhibition), the blocking effect of thalistyline was ¼ that of d-tubocurarine. The inhibitory effect of thalistyline on hemidiaphragm contractions was rapidly reversed upon washing the preparation. Usually one or two 30 ml washes were required to return the contractile responses to the control level. Responses returned to the control levels within 5 min after initial washout of thalistyline (1 x 10^{-5}M).

Obamegine, which had no effect on respiration in dogs, did not inhibit neuromuscular transmission at concentrations of 1 to 30 x 10^{-6}M.

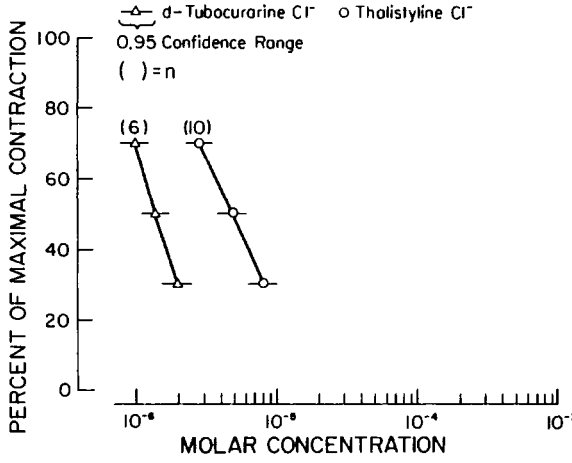


Fig. 5. Comparison of neuromuscular blocking activity in the isolated rat hemidiaphragm for thalistyline and d-tubocurarine. Inhibitory effects of these alkaloids on neuromuscular transmission are expressed as percentages of the maximal contractile response to electrical stimulation of the phrenic nerve. One-half maximal contractile response corresponds to the IC_{50} for these compounds. The IC_{50} and 0.95 confidence limits for thalistyline were 4.98 (4.0 to 5.9) x 10^{-6}M. The IC_{50} for d-tubocurarine was 1.42 (1.1 to 1.7) x 10^{-6}M.

B. *Reversal of Neuromuscular Blockage by Eserine.*—To explore the mechanism of neuromuscular blockade produced by thalistyline, eserine sulfate, a cholinesterase inhibitor was added to hemidiaphragm preparations during blockade with thalistyline (table 1). Eserine reversed the inhibitory effect of thalistyline on neuromuscular transmission. The contractile amplitude of thalistyline treated hemidiaphragms was increased an average of 2.5-fold by eserine.

C. *Effect of Thalistyline on Directly Stimulated Skeletal Muscle.*—In four hemidiaphragms the nerve-mediated stimulation of the muscle was blocked by incubation with d-tubocurarine (5 x 10^{-5}M) for 15 min. After blockade had fully developed, as indicated by a complete loss of contractile response, the electrode connections were switched to provide direct muscle stimulation via an electrode in direct contact with the muscle. Thalistyline was added cumulatively to the bath and the effect on directly stimulated muscle contraction was recorded. Results are shown in fig. 6. The reductions in contraction by thalistyline (1 to 10 x 10^{-5}M) were small and insignificant.

TABLE 1. Eserine reversal of inhibitory effect of thalistyline on neuromuscular transmission.

Exp. No.	Control[a] (g)	Thalistyline (10^{-5}M) (% of Control)[b]	
		Without Eserine	With Eserine (10^{-7}M)
1	3.1	45.1	77.4
2	9.0	11.1	50.0
3	4.2	19.0	57.1
4	3.4	23.5	70.5
Mean±S.E.		24.7±7.3	63.8±6.2

[a]Control responses of hemidiaphragms in grams of tension developed to electrically stimulated neuromuscular transmission are recorded in Column 2. [b]Results in Columns 3 and 4 are expressed as percentages of the control responses.

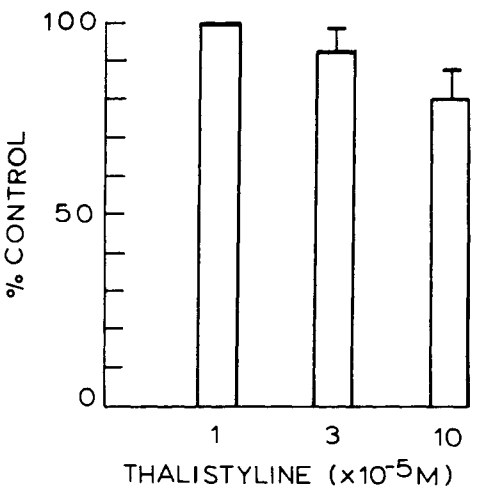


FIG.6. Effects of thalistyline on direct muscle stimulation in hemidiaphragms incubated with d-tubocurarine 5 x 10^{-5}M. At this concentration the contractions of the muscle to nerve stimulation were completely blocked. Responses expressed as a percentage of the control response to direct muscle stimulation. Each bar represents the mean of four experiments and standard error of the mean. Mean responses to direct muscle stimulation were slightly decreased by thalistyline at 3 and 10 x 10^{-5}M, but these decreases were not statistically significantly different from control with Students' t-test.

D. *Inhibition of α-Bungarotoxin Blockade of Nicotinic Cholinergic Receptors by Thalistyline.*—α-Bungarotoxin is a low molecular weight protein that binds selectively and irreversibly to the nicotinic cholinergic receptor of skeletal muscle. Treatment of nerve-stimulated muscle preparations with this protein results in a decline of muscle contractions which cannot be reversed even after repeated tissue washings (11). Drugs that interact at nicotinic cholinergic receptors either as agonists or as antagonists can compete with α-bungarotoxin in binding to the receptor (12).

Since the reduction of the toxin binding has been shown to occur in a dose-dependent manner (13), doses of thalistyline in excess of the functional IC_{50} for neuromuscular transmission were studied. The hemidiaphragms were incubated for 20 min with thalistyline (3×10^{-5}M) to produce complete neuromuscular block as shown by a total loss of contractile response to nerve stimulation. Without washing, α-bungarotoxin (1×10^{-6}g/ml) was added to the tissue baths. Two hrs after addition of toxin the hemidiaphragms were washed with 30 ml of buffer and every 5 to 10 min thereafter for 2 hrs or until the amplitude of muscle contractions became consistent. In preliminary experiments complete neuromuscular blockade by thalistyline (3×10^{-5}M) alone in contact with the hemidiaphragm for 2 hrs 20 min could be totally reversed within 50 to 70 min after initial washout of drug. In contrast, our results showed upon washout of the toxin that contractile responses returned, but mean response was only about two-thirds of the control responses (table 2).

TABLE 2. Thalistyline protection of rat hemidiaphragms from α-bungarotoxin blockade.[a]

	% Recovery of nerve-stimulated muscle contractions	
% Control	With α-Bungarotoxin 1×10^{-6} g/ml	With Thalistyline 3×10^{-5}M and α-Bungarotoxin 1×10^{-6} g/ml
(5)	(1)	(4)
100.....	0	65.8±14.2

[a]All hemidiaphragms were incubated with α-bungarotoxin for 2 hrs. Thalistyline (Column 3) was added 20 min prior to addition of toxin. Percent recovery of nerve-stimulated muscle contractions was established at 2 hrs after initial washout of toxin or sooner when amplitude of muscle contractions became consistent. Results are expressed as the mean ± standard error of mean. Number of experiments in parentheses.

Apparently, thalistyline is unable to completely protect the total population of functional receptors on the motor end plate and, instead, protects a fraction of the total population of nicotinic cholinergic receptors subject to irreversible blockade by α-bungarotoxin.

DISCUSSION

We have compared the pharmacology of two structurally closely-related bisbenzylisoquinolines, thalistyline and obamegine. We have found notable differences in the pharmacological activity for each compound. For example, obamegine did not affect respiration in dogs and did not block neuromuscular transmission in the rat diaphragm preparation. Thalistyline was a relatively potent neuromuscular blocker and the differences between the blocking effects of d-tubocurarine and thalistyline were small.

Our attempts to study the mechanism of action for thalistyline's effects revealed these facts: (a) A major portion of the neuromuscular blocking action can be attributed to a competitive type of blockade of the nicotinic receptors of the endplate. Eserine is known to reverse the blockade of neuromuscular transmission by non-depolarizing antagonists like d-tubocurarine Cl^- (14); in the present experiments eserine also reversed a substantial portion of the neuromuscular blockade by thalistyline. (b) Thalistyline did not inhibit responses to direct muscle stimulation at concentrations that produced complete blockade of nerve-mediated contractions. A small statistically insignificant magnitude of direct muscle block occurred above concentrations 20-fold greater than the IC_{50}

for neuromuscular blockade. (c) The slopes of the dose-response curves of d-tubocurarine and thalistyline (fig. 5) for the inhibition of neuromuscular transmission were parallel. Ariens (15) has pointed out that a similarity of dose-response curves suggests a similar mechanism of action. (d) The protective effects of an IC_{100} dose of thalistyline for neuromuscular transmission seemed to protect only about ⅔ of the functional response. These results at first glance would appear to contradict the evidence presented thus far favoring a curare-like mechanism of thalistyline on neuromuscular transmission. However, Miledi and Potter (13) found that d-tubocurarine at concentrations up to 100-fold greater than the apparent IC_{100} for neuromuscular inhibition was only able to maximally protect about 50% of ^{131}I-α-bungarotoxin labelled binding sites in frog sartorius muscle preparations. According to Miledi and Potter, the most "plausible explanation is that there are two different membrane molecules, both capable of reacting with Ach and α-bungarotoxin, but only one with a high affinity for d-tubocurarine" (13). Obviously, thalistyline behaved in a manner similar to d-tubocurarine in the receptor protection experiments. Thus, all lines of evidence presented here suggest to us that thalistyline is a curare-like alkaloid. Alternatively, unequal diffusion of substances at these sites could also explain these discrepancies in the receptor protection experiments.

Moreover, thalistyline possesses the structural requirements for a strong curare-like effect. Wintersteiner and Deutcher (16) experimentally increased the methylation of free phenolic hydroxyl groups on the quaternary bases, d-tubocurarine and d-chondrocurine, and the neuromuscular blocking potency increased 9-fold and 3-fold, respectively. In comparison, thalistyline contains a total of five methylated phenolic hydroxyl groups but d-tubocurarine has only two of these groups (fig. 1). The relatively potent curare-like effect of thalistyline may be attributed to the number of methylated phenolic groups on the molecule and its quaternary nitrogen atom.

In contrast, obamegine did not block neuromuscular transmission; its chemical structure (fig. 1) shows it to be devoid of a quaternary nitrogen and contains only two methoxy groups.

In addition to a curare-like action at the neuromuscular junction, thalistyline may also have curare-like side effects on the cardiovascular system such as histamine release (14). Histamine release can produce a transient hypotensive effect. Repeated doses of these *Thalictrum* alkaloids could deplete the readily releasable stores of histamine in the body and thereby cause tachyphylaxis to the hypotensive effects as we observed in dogs.

We have shown that thalistyline has an inhibitory effect on *alpha*-adrenoreceptors *in vitro*. The dissociation constant of thalistyline for the *alpha*-adrenoreceptors is 1/30 that for phentolamine under similar experimental conditions (7). Hence, phentolamine, a classical *alpha*-adrenergic receptor antagonist, has a 30-fold greater affinity for the receptor than does thalistyline. We can conclude that bisbenzylisoquinolines with the structures indicated can inhibit *alpha*-adrenergic neurotransmission. However, the mechanism may be either competitive as with thalistyline or noncompetitive as shown by obamegine. Since thalistyline is 30 times less potent than phentolamine, it is quite possible that the doses given to intact dogs were not sufficient to produce *alpha*-adrenoreceptor blockade, while concentrations of thalistyline in the studies *in vitro* were high enough to produce blockade.

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LITERATURE CITED

1. W.-N. Wu, J. L. Beal, R. Leu and R. W. Doskotch, *Lloydia*, **40**, 281 (1977).
2. W.-N. Wu, J. L. Beal and R. W. Doskotch, *Tetrahedron Lett.*, **41**, 3687 (1976).
3. W.-N. Wu, J. L. Beal, A. Mitscher, K. N. Salman and P. N. Patil, *Lloydia*, **39**, 204 (1976).
4. W. F. Ganong, *Review of Medical Physiology*, 8th edition, Lange Medical Publications, Los Altos, CA, 1977, p. 436.
5. R. F. Furchgott and S. Bhadrakom, *J. Pharmacol. Exp. Ther.*, **108**, 129 (1953).
6. J. M. Van Rossum, *Arch. Int. Pharmacodyn.*, **143**, 299 (1963).
7. R. F. Furchgott, The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: *Catecholamines*, ed. by H. Blaschko and E. Muscholl, Springer-Verlag, Berlin, 1972, pp. 283–335.
8. O. Arunlakshana and H. O. Schild, *Brit. J. Pharmacol.*, **14**, 48 (1959).
9. E. Bülbring, *Brit. J. Pharmacol.*, **1**, 38 (1946).
10. A. J. Kaumann, *J. Pharmacol. Exp. Ther.*, **173**, 383 (1970).
11. C. C. Chang and C. Y. Lee, *Arch. Int. Pharmacodyn. Ther.*, **144**, 241 (1963).
12. K. A. Skau and M. C. Gerald, *J. Pharmacol. Exp. Ther.*, **205**, 69 (1978).
13. R. Miledi and L. T. Potter, *Nature*, **233**, 599 (1971).
14. P. Taylor, Neuromuscular blocking agents. In: *The Pharmacological Basis of Therapeutics*, ed. by A. G. Gilman, L. S. Goodman and A. Gilman, MacMillan, New York, 1980, pp. 220–234.
15. E. J. Ariens, The mode of action of biologically active compounds. In: *Molecular Pharmacology*, ed. by E. J. Ariens, Academic Press, New York, 1964, Vol. I, pp. 136–156.
16. O. Wintersteiner and J. Dutcher, *Science*, **97**, 467 (1943).