Comparative Studies of Protoveratrine A and Protoveratrine B Intravenously in Hypertensive Man

By Bertram M. Winer, M.D.

Veratrum alkaloids are powerful hypotensive agents with many desirable characteristics, but the usefulness of the available preparations has been limited by a narrow dosage range between hypotensive and emetic effects. After the physiochemical separation of some of the alkaloids had been accomplished, a search for an alkaloid with a wider spread between hypotensive and emetic doses was undertaken. Parenteral studies in dogs demonstrated that the hypotensive activity of protoveratrine A was slightly greater than that of protoveratrine B, but did not show differing degrees of dissociation of hypotensive from emetic activity. A comparison between protoveratrine A and protoveratrine B in man, however, revealed marked differences when the alkaloids were administered orally: protoveratrine A had strong hypotensive activity but a narrow therapeutic dosage range, whereas protoveratrine B required much larger doses to elicit hypotensive effects but had a wider range between hypotensive and emetic effects. These differences prompted a comparative study of the responses of hypertensive patients to intravenous administration of the 2 alkaloids.

Material and Methods

Comparative bioassays of protoveratrine A and protoveratrine B were obtained from 13 patients who were known to have essential or renal hypertension, sustained despite the oral or intravenous administration of placebos. At least 4 different hypotensive doses of each alkaloid were given intravenously to each subject. The largest dose given to each patient was determined by the production of a fall in blood pressure to or less than normal or by nausea or vomiting. Protoveratrine A was given on 81 occasions, protoveratrine B on 109. No more than 1 study was conducted in a given patient on the same day. Each alkaloid was administered in a measured period of 2 minutes. With the patient in the recumbent position, a technician determined blood pressure and pulse rate at intervals of a few minutes during periods of at least 20 minutes before and several hours after administration. Blood pressure was measured by the auscultatory technique according to the criteria of the American Heart Association. Mean blood pressure was calculated as the diastolic pressure plus one third of the difference between systolic and diastolic pressures. The mean value of the lowest 2 mean blood pressures obtained within 25 minutes after administration of an alkaloid was taken as the maximal response to the given dose of the alkaloid. These responses were used to construct logarithmic dose-response curves. The occurrence and severity of paresthesias, nausea, and vomiting were noted. Electrocardiograms were taken during studies of the larger doses and whenever bradycardia was marked.

Observations of the effects of protoveratrine B intravenously were made an additional 48 times in 21 other adult hypertensive patients. This information was used with the above to estimate the dose of protoveratrine B required to produce a fall in blood pressure to 150/100 mm. Hg or less.

Results

Qualitative Similarities

Each alkaloid had strong hypotensive and bradycrotic activity, and each was capable of inducing nausea or vomiting. The occurrence and magnitude of these effects varied with the amount of alkaloid given (table 1), but the time of onset and duration of the effects were similar for the 2 alkaloids.

The first effect was the occurrence within several minutes of paresthesias, sensations of warmth or burning, which were noted sequentially in the throat, chest, perineum, and extremities. Brief sneezing was common. The paresthesias regressed within 30 to 60 minutes.

Systolic and diastolic blood pressure began to fall a few minutes after administration of the drugs with maximal response within
12 to 25 minutes. Blood pressure was maintained at the lower leve's for 45 to 90 minutes (fig. 1). Return to control values occurred in approximately 3 hours.

Pulse rate usually decreased slightly during the hypotensive action of both alkaloids. Marked sinus bradycardia, however, of less than 48 beats per minute occurred in 5 patients after each alkaloid. At rates of approximately 40 beats per minute, the site of impulse formation varied between the sinoatrial and atrioventricular nodes in the course of consecutive beats or groups of beats for periods of minutes. During the height of the bradycardia ventricular or supraventricular premature beats, at times in bigeminal fashion, were occasionally seen. The T waves in leads V₄, V₅, and V₆ were transiently inverted in several patients. All of these effects, the sinus bradycardia, atrioventricular rhythm, premature beats, and T-wave inversions, were immediately reversible by administration of 0.4 to 1.0 mg. of atropine sulfate intravenously, usually without affecting the blood pressure response (fig. 2).

At the height of the paresthesias, approximately 10 minutes after drug administration, a tight sensation in the chest and epigastrium was occasionally noted after the larger doses of either alkaloid. This pain forewarned nausea with or without vomiting and hiccoughs. Emetic responses lasted less than a half hour.

Quantitative Differences

For a considerable portion of the dose ranges given to 10 subjects, there was a linear relation between hypotensive response and the log dose in each case. These data were used for the estimation of the relative hypotensive potencies of the 2 substances (table 2, columns 1 and 2). On a weight basis protoveratrine B was slightly less potent (82 per cent) than protoveratrine A.

A dose producing nausea was reached in 10 patients with protoveratrine A (table 2, column 3). Vomiting occurred in 7 of these patients. Nausea was produced in 6 patients after protoveratrine B, vomiting in 3. The emetic doses of protoveratrine B were .24,
.25, and .38 mg., equivalent to 2.9, 4.4, and 4.6 µg per Kg. The vomiting induced by protoveratrine B occurred after doses that produced subnormal blood pressures. Following normotensive doses of protoveratrine A, small increases in dose induced nausea and vomiting, and a diminished blood pressure response (fig. 3). In contrast, in the same patients subnormal blood pressure could be achieved by protoveratrine B without an emetic response. The remarkable reproducibility of the response of an individual to a given dose of intravenous veratrum is also evident in this figure.

Protoveratrine B had less emetic activity than protoveratrine A at equipotent hypotensive doses. A statistical comparison of the hypotensive and emetic activities of the 2 alkaloids (table 2) was obtained by determination of tolerance ratios in which the largest doses of protoveratrine B given without nausea were compared with the doses of protoveratrine A producing nausea. These ratios were adjusted for the differences in hypotensive potency of the alkaloids. Protoveratrine B was better tolerated than protoveratrine A (p < .001).

Optimal Dose of Protoveratrine B

The doses of protoveratrine B that lowered the blood pressure of 34 hypertensive patients to 150/100 mm. Hg or less are indicated in

Figure 1

Time course of blood pressure response to varying doses of protoveratrine A and protoveratrine B.
Table 2
Relative Hypotensive and Emetic Potencies of Protoveratrine A and Protoveratrine B

<table>
<thead>
<tr>
<th>Hypotensive potency ratio</th>
<th>Prototveratrine B/prototveratrine A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
</tr>
<tr>
<td>Patient</td>
<td>(1)</td>
</tr>
<tr>
<td>DG</td>
<td>74%</td>
</tr>
<tr>
<td>DP</td>
<td>102%</td>
</tr>
<tr>
<td>JC</td>
<td>71%</td>
</tr>
<tr>
<td>FK</td>
<td>76%</td>
</tr>
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<td>SG</td>
<td>74%</td>
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<td>65%</td>
</tr>
<tr>
<td>MB</td>
<td>95%</td>
</tr>
<tr>
<td>AD</td>
<td>79%</td>
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<tr>
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<td>95%</td>
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<tr>
<td>CW</td>
<td>—</td>
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<tr>
<td>BK</td>
<td>—</td>
</tr>
<tr>
<td>IF</td>
<td>—</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>82%</td>
</tr>
</tbody>
</table>

95% Confidence limits 73-98 150-173 124-152

Figure 4. Total single doses of .15 to .20 mg. (1.8 to 3.0 μg./Kg.) were effective in 28 patients. It is likely that some of these patients required less, but received doses in this range after recognition that such doses were both strongly hypotensive and well tolerated. Interestingly, the patient who required the smallest dose per kilogram was a strikingly obese woman, and the subject requiring the largest dose was a poorly nourished man.

Discussion

Protoveratrine A and protoveratrine B are tetraesters of the alkaline, protoverine. They differ by a single hydroxyl group in one of the acids4 (table 3).

The hypotensive effect is largely a reflex action, which originates in the heart (Bezold-Jarisch effect), carotid sinus, and lungs.9-15 The receptors transmit impulses to the vasomotor center by the vagi and carotid sinus nerves. Vasodilatation is the result of changes in the transmission of vasoconstrictor nerve impulses.16,17 Efferent vagal activity is increased, largely reflexly. Cardiac output is variably affected.18,19 Cerebrovascular20 and renal resistances21 decrease.

The emetic action appears to be a reflex effect that originates in or near the nodose ganglion.22 The veratrum alkaloids do not directly stimulate the vomiting center nor the chemoreceptor trigger zone.23

The dissociation between the hypotensive and emetic activities of the tetraesters of veratrum may be ascribed to differences in the accessibility of the receptors or to intrinsic differences in the receptors themselves at the different sites of hypotensive and emetic action. The degree of the dissociation of activities appears to be influenced by the chemical configurations of the alkaloids.

Protoveratrine B had less emetic activity than protoveratrine A at doses causing equivalent hypotensive effects in hypertensive man.
Protoveratrine B-induced sinus bradycardia, intermittent A-V nodal rhythm (third strip), and T-wave inversions of the sinus beats in lead V4. Atropine reversed the bradycardia and T-wave changes, although the hypotensive effect continued.

Figure 2

Table 3

<table>
<thead>
<tr>
<th>Hydrolysis of Protoveratrine A and Protoveratrine B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoveratrine A</td>
</tr>
<tr>
<td>C₆H₆O₇N</td>
</tr>
<tr>
<td>Hydrolysis</td>
</tr>
<tr>
<td>Protoverine</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>2-methyl butyric acid</td>
</tr>
<tr>
<td>2-hydroxy-2 methyl butyric acid</td>
</tr>
</tbody>
</table>

(p <.001). In contrast to protoveratrine A, protoveratrine B had little emetic activity when given intravenously in doses producing normotension. Protoveratrine B appears to have properties that warrant its preferential use for parenteral administration.

The data in this bioassay demonstrated considerable precision. The 95-per cent confidence limits of both the hypotensive potency ratios and the tolerance ratios were relatively narrow. For example, the ratio of the upper
95 per cent limit to the lower 95 per cent limit of the hypotensive potency ratios was less than 150 per cent in 9 of 10 cases.

Protoveratrine B has qualities that favor its clinical use intravenously in hypertensive emergencies. Its potent vasodepressor and cardiodecelerator effects, as well as its development of maximal action within minutes, are particularly valuable in acute left ventricular failure and in eclampsia, acute glomerulonephritis, or malignant hypertension with hypertensive encephalopathy. Derivatives of Rauwolfia, administered intramuscularly, have been exceedingly useful in the management of urgent hypertensive problems, but in instances requiring a quick and strong hypotensive effect protoveratrine B may be preferable. Indeed, the two have been used together, protoveratrine B intravenously to achieve control within 10 to 15 minutes, and alkaloids of Rauwolfia intramuscularly to maintain it.

Orally protoveratrine A is the active alkaloid in mixtures of protoveratrine A and B. Its long-term use is difficult because of a narrow range between hypotensive and emetic doses. Protoveratrine B can be an effective hypotensive agent when administered orally, but large doses have been required due to incomplete absorption or inactivation by the gastrointestinal tract or liver. The hypotensive effect of protoveratrine B orally tends to be prolonged; an emetic response has been rare. Mild neuromuscular effects, muscle tightness and muscle weakness, may be induced.
Figure 4
The total dose of protoveratrine B and the dose in micrograms per kilogram that reduces the blood pressure of 34 hypertensive patients to or below 150/100 mm. Hg, are plotted against the number of patients yielding the response.

The clinical use of tetraesters of veratrum appears to be safe. No deaths or vascular catastrophes have been ascribed to their use. An oral overdose of 75 times the recommended dose of a mixture of protoveratrinines A and B was tolerated. Excessive hypotension after parenteral administration is rare and can be overcome by the injection of 25 mg. of ephedrine sulfate intramuscularly or by other vasoconstrictor agents. Intravenous administration of atropine sulfate in doses of 0.4 to 1.0 mg. abolishes undesired vagal effects on heart rate and rhythm, usually without loss of the hypotensive response.

The transient T-wave inversions induced by veratrum in the left precordial leads resemble those observed in anxious patients during hyperventilation. In both cases the T-wave changes can be reversed by vagolytic agents.

Although biologic assay techniques are uncommonly used in man, fixed hypertension lends itself to their application. The virtues of such techniques in human pharmacology have been demonstrated in studies of digitalis glycosides and diuretics. Comparative bioassays in stable clinical disease states foster the perception of clinically significant differences among drugs that are chemically closely related.

Summary
Comparative studies of intravenously administered protoveratrine A and protoveratrine B in hypertensive man indicate that the alkaloids have qualitatively similar hypotensive, bradycrotic, and emetic actions, but quantitatively different hypotensive and emetic potency. The relative hypotensive potencies of the two substances were determined by analyses of logarithmic dose-response curves in the same patients. On a weight basis protoveratrine B was slightly less potent than protoveratrine A.

A favorable dissociation between hypotensive and emetic activity was found. Tolerance ratios were determined in which the largest doses of protoveratrine B given without nausea were compared with the doses of protoveratrine A producing nausea. These ratios were adjusted for the differences in hypotensive potency of the alkaloids. Protoveratrine B was better tolerated than protoveratrine A (p < .001). Indeed, protoveratrine B had little emetic activity when given intravenously in doses producing normotension. Protoveratrine B appears to have properties that warrant its preferential selection for parenteral administration. Its effective dosage range has been studied.

The strong hypotensive activity of intravenously administered protoveratrine B, its development of maximal action within minutes, and its pulse-slowing effect favor its use in those hypertensive states in which almost immediate lowering of the blood pressure is indicated.

Fixed hypertension lends itself to the application of biological assay techniques. The use of such techniques in human pharmacology fosters the perception of clinically significant differences among drugs that are chemically closely related.

Acknowledgment
I am indebted to Miss Margaret McDonough for technical assistance, to Dr. Mindel C. Sheps of the Department of Public Health, Harvard Medical School, for the helpful analyses, to Dr. Otto Krayfer for his statistical criticism, and to Dr. Phillip Boyer of the Pitman-Moore Company for generous supplies of the alkaloids.
PROTOVERATRINE A AND B

Summario in Interlingua

Studies comparative de protoveratrina A e de protoveratrina B, administrate per via intravenose in humanos hypertensive, indica que le alcaloids ha qualitativa e simile effectos hypotensive, brady-erotic, e emetic, sed que lor potencias hypotensive e emetic es quantitativa e differentemente. Le relative potencias hypotensive del duo substancias esseva determine per analises del curvas logarithmic de dose e responsa in le mesme patientes. Super le base de pesos equal, protoveratrina B esseva levemente minus potente que protoveratrina A.

Un dissociation favorable inter le activitate hypotensive e le activitate emetic esseva constatate. Proportiones de tolerancia esseva determinate in que le doses maximal de protoveratrina administrate sin subsequent nausea esseva comparete con le doses de protoveratrina A que produciva tal nausea. Iste proportiones esseva adjustate pro le differentias in le potentia hypotensive del duo alcaloids. Protoveratrina B esseva melo tolerate que protoveratrina A (p minus que 0,001). De facto, protoveratrina B habeva paue activitate emetic quando illo esseva administrate intravenosemente in doses producente normotension. Protoveratrina B pare haber proprietates que justifica su selection preferential pro administrations parenteral. Su gamma de dosage efficient esseva studiate.

Le forte activitate hypotensive de protoveratrina B in administration intravenose, le facto que illo disveloppa su effecto maximal intra alium minutus, e su effecto rellentator super le pulso es rationes pro recommendar su uso in le statos de hypertension in le quales un reduction quasi immediato del tension de sanguine es indicate.

Hypertension fixe se presta al application de technicas de essayage biologic. Le uso de tal technicas in le pharmacologia human promove le perception de clinicamente significative differentias inter drogas que es chimicamente auffin.

References

A teacher I had in college, Professor Edwin B. Holt, once pointed out to me a fact of considerable bearing when one is dealing with abstract and collective nouns. "A word," he said, "that has many connotations can denote nothing; conversely, a word that denotes but one thing has no connotations." The word water, for example, has numerous connotations—"weak as water," "As the hart panteth after the water books," "as wet as water," etc.—but H2O denotes but one thing and so has no connotations.

Now, it seems to me that part of the task of the poet or the essayist is to use the connotative words that exactly convey his feeling, whereas a part of the task of the scientist is to employ denotative words with comparable logical skill and verifiable precision—Alan Gregg, M.D. Challenges to Contemporary Medicine. New York, Columbia University Press, 1956, p. 100.
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