THE BIOLOGICAL ASSAY OF *RAUWOLFIA SCHUELI*

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The biological effects of *Rauwolfia schueli* are essentially the same as those of the pure alkaloid reserpine and the crude compound *R. serpentina* in the rat. No difference in relative potency between the small and large roots of large trees of the *R. schueli* species was apparent when either reserpine or *R. serpentina* was employed as the standard. *R. schueli* was estimated to be about 1/630 as active as reserpine. In terms of reserpine-like activity, this species appeared to be more potent than *R. serpentina* and by comparison with the results of others about as potent as *R. canescens* but more potent than *R. heterophylla*. Within the range of doses selected, blood pressure did not appear to be an adequate index for measuring reserpine-like activity in the rat.

NUMEROUS studies have shown that *Rauwolfia serpentina* is effective in the treatment of hypertension and certain mental disorders. Hypotensive and sedative properties also have been ascribed to *R. canescens* and *R. heterophylla*[1] and to *R. vomitoria*[2]. The preparations tested were either the powdered whole root or some extract thereof. It is the purpose of this study to compare the activity of *R. schueli*, a species peculiar to the northern part of Argentina and Bolivia, with the activity of the pure alkaloid reserpine and the crude compound *R. serpentina*.

MATERIALS AND METHODS

Test Preparations

The test preparations used were *R. schueli* YR-1, a blended batch of small roots of large trees, and *R. schueli* YC-1, a blended batch of large roots of large trees. Samples of these preparations were supplied by E. R. Squibb and Sons. The assumed relative potencies with reserpine as the standard were as follows: YR-1, 1·28 g. reserpine per kg.; YC-1, 1·30 g. reserpine per kg. Each sample was ground to a particle size that could pass a 200 mesh screen.

Standard Preparations

The crystalline alkaloid reserpine was dissolved in glacial acetic acid and enough distilled water added to yield a stock solution of 10 mg. alkaloid per ml. of 10 per cent acetic acid. This solution was stored at 7° and used within 2 weeks. A sample of the crude compound *R. serpentina* also was supplied by E. R. Squibb and Sons. It had an assumed relative potency of 1 g./kg. and was powdered to the same degree of fineness as the test samples, YR-1 and YC-1.

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Experimental Animals

The experimental animals were male albino rats, from 120 to 200 g., taken from the animal stock of the Department of Physiology at the University of Mendoza in Argentina. Each rat was fasted for 18 to 24 hours before dosing.

Methods of Dosing

The rats were divided into 4 groups. The first group received YR-1 or reserpine; the second, YR-1 or *R. serpentina*; the third, YC-1 or reserpine; and the fourth, YC-1 or *R. serpentina*. All preparations were administered at 3 dose levels corresponding in effect to 1, 2 and 4 mg. of reserpine per 100 g. of rat as judged from the assumed relative potencies. This amounted to 781, 1,562 and 3,124 mg./100 g. of rat for YR-1; 769, 1,538 and 3,176 mg./100 g. of rat for YC-1; and 1,000, 2,000 and 4,000 mg./100 g. of rat for *R. serpentina*. There were 16 rats at each dose level for the first group of rats treated (YR-1 and reserpine) and 20 rats at each dose level for the 3 remaining groups.

Because of the high dose volumes required of the 2 test preparations and *R. serpentina*, each dose was divided equally into 2 or 3 portions administered 45 minutes apart. Each preparation was fed by gastric tube after being suspended in a 0·25 per cent agar solution.

Measured Activity

Two types of activity were measured, ptotic activity and decrease in blood pressure. Ptotic activity was scored in whole numbers ranging from 0 to 4 according to a scale devised by Rubin and others[3,4]. Each eye was scored separately and the scores for both eyes totalled. Observations were made 8, 24 and 48 hours after dosing. Blood pressure readings were obtained on the tail by the plethysmographic method of Williams and others[5]. Measurements were taken before dosing, during, and immediately after the 24-hour period subsequent to dosing.

RESULTS

For all preparations, doses and animals tested, the peak ptotic effect was observed 24 hours after dosing. Average 24-hour readings are shown in Table I. At the middle and highest dose, effects still were present after 48 hours for YR-1 and YC-1 and to some extent *R. serpentina*.

TABLE I

AVERAGE 24-HOUR PTOTIC ACTIVITY IN RATS

Group	Preparations	Dose			No. of animals each dose
		1	2	3	
I	YR-1	2·50	4·35	5·92	14
	Reserpine	2·00	4·14	5·07	14
II	YR-1	2·47	4·42	5·23	19
	R. serpentina	2·15	4·10	5·00	19
III	YC-1	3·05	4·33	6·22	18
	Reserpine	2·50	3·94	5·88	18
IV	YC-1	3·05	4·15	5·95	20
	R. serpentina	2·50	3·85	5·65	20

During the first 24 hours, there was a decrease in average blood pressure at each dose for each preparation tested (Table II). But, in no instance was there a consistent decrease in average blood pressure with increasing dose. Sedation, diarrhoea and decrease in body temperature were common to all animals during the 24-hour period subsequent to dosing. During this same period of time, convulsions were observed at the highest dose in 3 rats on YR-1 and in 2 rats on YC-1. In addition, 2 animals died at the highest dose for YR-1 and 3 animals at the highest dose for YC-1.

TABLE II

MEAN BLOOD PRESSURE IN MM. OF HG. BEFORE AND AFTER A 24-HOUR PERIOD SUBSEQUENT TO DOSING

Preparation	Dose 1		Dose 2		Dose 3	
	Before	After	Before	After	Before	After
YR-1	99·7 (36)*	76·6 (34)	98·4 (36)	83·9 (32)	97·9 (36)	89·4 (32)
YC-1	95·6 (40)	70·1 (37)	98·6 (40)	81·0 (35)	101·0 (40)	70·4 (34)
Reserpine	99·6 (36)	81·2 (27)	97·1 (36)	73·6 (30)	99·9 (36)	79·5 (33)
R. serpentina	99·6 (40)	69·7 (40)	91·5 (40)	71·0 (40)	101·2 (40)	71·8 (39)

* Number of rats.

Relative potencies were estimated by the method of parallel line assays described in Finney[6]. Results are summarized in Table III for 24-hour ptotic activity only. Estimates of relative potency were not obtained from blood pressure readings, because the dosage-response curves were either flat or increasing within the range of doses selected for this study.

TABLE III

RELATIVE POTENCY DETERMINATIONS FOR *Rauwolfia schueli* YR-1 AND YC-1

Test preparation	Standard	Relative potency in g. of reserpine per kg. of test prep.*	95 per cent confidence limits
YR-1	Reserpine	1·603	1·196, 2·142
YR-1	*R. serpentina*	1·533	1·322, 1·787
YC-1	Reserpine	1·557	1·290, 1·868
YC-1	*R. serpentina*	1·550	1·396, 1·720

* 1 kg. of *R. serpentina* is assumed to be equivalent in effect to 1 g. of reserpine.

Although the relative potencies of YR-1 and YC-1 were higher than they initially were assumed to be, both preparations exhibited the same degree of reserpine-like activity. This is shown in Table III. Inasmuch as the relative potencies of YR-1 and YC-1 did not appear to depend on whether the standard was reserpine or *R. serpentina* administered in equivalent doses, the assumption on which equivalent doses were ascertained, that is, 1 kg. of *R. serpentina* is equivalent in effect to 1 g. of reserpine, was indirectly verified. These findings also suggested that the reserpine-like activity of *R. schueli* is greater than that of *R. serpentina.*

Discussion

Various criteria have been used as a basis for estimating the relative potency of rauwolfia compounds. Reputedly, the problem is a difficult one because the onset of action of reserpine is gradual and the dosage-response curve for most of its activity in mammals is notably flat[7]. In the present study, ptotic activity did provide for good estimates of relative potency. However, peak effects were not observed until about 24 hours after dosing.

Earl[7] suggests a unique biological assay of reserpine based on an all-or-none response. He uses a pigeon emesis test and obtains a relatively steep dosage-response curve. There is a possibility that his method will give estimates of relative potency which are subject to less error than those obtained through an evaluation of ptotic activity.

Convulsions and death are effects which previously have not been ascribed to the pure alkaloid reserpine, although in this study, they were observed at the highest dose for YR-1 and YC-1. Presumably, they also could occur at doses higher than 4 mg./100 g. of rat for reserpine considering that the biological equivalence of the *R. schueli* roots was underestimated in selecting the doses. Furthermore, ptotic activity was more sustained at higher doses for YR-1 and YC-1 than it was for reserpine. Of course, convulsions and death also could have been due to the presence of toxic substances in the whole root.

Rubin and others[1] studied the activity of *R. serpentina*, *R. heterophylla* and *R. canescens*. Based on measures of ptotic activity in the rat, they obtained the following reserpine equivalence ratios: *R. serpentina*, 1:401; *R. heterophylla*, 1:387; and *R. canescens*, 1:258. Inasmuch as it has been shown indirectly that *R. serpentina* is about 1/1,000 as active as reserpine, it would appear that the findings of Rubin and his colleagues are not directly comparable to those of the present study. Conceivably, this could be due to a difference in assay techniques. If the equivalence ratios for *R. heterophylla* and *R. canescens* are multiplied by a factor of 1/2·5 in order to adjust for this difference, the following results are obtained: *R. heterophylla*, 1:968 and *R. canescens*, 1:645. In the present study, the roots of *R. schueli* were about 1/630 as active as reserpine. Thus, it would appear through these adjustments that in terms of reserpine-like activity, *R. schueli* is about as potent as *R. canescens* but more potent than *R. heterophylla*.

Since all of the active principles of *R. schueli* have not been identified, it cannot be stated that the activity of this species is due simply to its reserpine content. For example, Rubin and others[1] used an isotope dilution method for ascertaining the reserpine content of the three species of rauwolfia root which they studied. They compared these results with those obtained from a biological assay in rats and in each instance they found that the activity of the roots was several times greater than that which could be predicted on the basis of their reserpine content alone. Studies on dogs and monkeys by Cronheim and others[8] and Kohli and Mukerji[9] also suggest that reserpine does not account for all of the

activity found in *R. serpentina* or any of its extracts. La Barre[2] found that reserpine is not the only active principle in *R. vomitoria.* He was able to show that the non-reserpine extract of this species had hypotensive effects in dogs, rabbits and rats and tranquillising effects in dogs which were not unlike those of reserpine.

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