# Pharmacological Studies with Pseudoreserpine and Raunescine, Two Alkaloids Isolated from *Rauwolfia canescens* Linn. (23365)

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A number of alkaloids have been isolated from *Rauwolfia canescens* Linn. Among these are deserpidine(1,2,3) raunescine and isoraunescine(4). Klohs *et al.* have isolated an alkaloid with chemical characteristics similar to raunescine and a new alkaloid, pseudoreserpine(5). Raunescine and pseudoreserpine were subjected to pharmacologic investigation for cardiovascular and sedative effects.

*Methods.* The sedative effect of the alkaloids in mice was determined by performance in roller cages(6). Raunescine and pseudoreserpine were administered intraperitoneally in varying doses to 189 and to 110 mice respectively, and the animals were tested 2 hours later. Dogs for cardiovascular testing were chosen at random and divided into groups of 5 animals regardless of sex or weight. Pseudoreserpine was given to one group at 0.5 mg/kg/day orally for 5 days. Raunescine was given orally to another group at 1 mg/kg/day for a like duration. The test methods have been described previously(7). The responses to epinephrine hydrochloride solution (1 and 3 $\mu$g/kg i.v.) were recorded in both control and test runs. Following the test run, the vagi were sectioned bilaterally and the responses to bilateral carotid occlusion and right central vagus faradization were recorded. Pseudoreserpine or raunescine was given intravenously at 0.3 or 1 mg/kg to 4 urethanized and vagotomized dogs. Responses to the following standard series of tests were observed: 3 and 6 $\mu$g/kg epinephrine, 1 $\mu$g/kg acetylcholine, 1 $\mu$g/kg isopropyl-norepinephrine, 1 $\mu$g/kg histamine, 1 minute tilting to 60°, bilateral carotid occlusion (30 sec.), and right central (2 volts, 15 sec.) and peripheral vagus stimulation (2 volts, to effect). The tests were repeated hourly for 5 hours. All drugs were administered intravenously; pressure from the femoral artery was recorded on an electrokymograph. Lack of material prevented additional testing in dogs.

*Results.* Both alkaloids produced visible "sedation" in mice and caused "fall-outs" from roller cages. The $ED_{50}$ (95% confidence limits) for pseudoreserpine was 11 (8-14) mg/kg and that for raunescine 7 (5-10) mg/kg. The slope functions were 2.7 (1.8-4.1) and 5.0 (2.5-9.7) respectively. There were no gross signs of ataxia. The corresponding figure for reserpine(8) was an $ED_{50}$ of 0.84 (0.62-1.10) mg/kg with a slope function of 2.1 (1.7-2.6).

After 5 days of oral medication the dogs exhibited sedation, diarrhea and prominence of the nictitating membrane, signs characteristic of treatment with a reserpine type alkaloid. The alkaloids reduced mean arterial pressure (MAP) and heart rate (HR) (Table I). After raunescine, MAP fell from 121 to 82 mm Hg, while HR decreased from 127 to 84 beats/min. Following pseudoreserpine MAP decreased from 117 to 89 mm Hg, with HR changing from 127 to 67 beats/min. No

TABLE I. Cardiovascular Effects in Dogs after 5 Days of Oral Medication. 5 dogs/series.

| Alkaloid and dose in mg/kg | Heart rate* (beats/min.) | | Mean art. pressure* (mm Hg) | | Response to i.v. (1 $\mu$g/kg) | | Epinephrine† (3 $\mu$g/kg) | |
|---|---|---|---|---|---|---|---|---|
| | Control | Test | Control | Test | Control | Test | Control | Test |
| Raunescine, 1.0 | 127 ± 22 | 84 ± 18 | 121 ± 22 | 82 ± 20 | 123 ± 26<br>+69 ± 16 | 88 ± 29<br>+114 ± 42 | 132 ± 28<br>+112 ± 17 | 86 ± 29<br>+144 ± 36 |
| Pseudoreserpine, .5 | 127 ± 22 | 67 ± 15 | 117 ± 12 | 89 ± 10 | 112 ± 24<br>+55 ± 36 | 94 ± 38<br>+77 ± 24 | 101 ± 21<br>+83 ± 44 | 89 ± 29<br>+128 ± 20 |

* Heart rate and mean arterial pressure measured under pentobarbital anesthesia and expressed as mean (± stand. dev.).

† Initial MAP (± stand. dev.) + rise in pressure (± stand. dev.).

TABLE II. Cardiovascular Responses in Dogs after Intravenous Administration in Acute Experiments (All Figures Refer to mm Hg).

| Drug and dose in mg/kg | Test | Control | ½ hr | 1½ hr | 2½ hr | 3½ hr | 4½ hr | 5½ hr | 8 hr |
|---|---|---|---|---|---|---|---|---|---|
| Raunescine, .3 | MAP in mm Hg | 106 | 106 | 110 | 121 | 126 | 120 | 122 | |
| | Epi* | +4§ | +24 | +36 | +55 | — | — | — | |
| | CO† | +22 | +11 | +20 | +49 | +36 | +42 | +48 | |
| | RCV‡ | +122 | +79 | +58 | +38 | +28 | +13 | +10 | |
| " , 1 | MAP in mm Hg | 96 | 70 | 62 | 74 | 88 | 80 | 77 | |
| | Epi | +4 | +20 | +28 | +46 | — | — | — | |
| | CO | +8 | −4 | +5 | +6 | +10 | +10 | +15 | |
| | RCV | +7 | +6 | +16 | +8 | +10 | +9 | +7 | |
| Pseudoreserpine, .3 | MAP in mm Hg | 111 | 109 | 114 | 118 | 100 | 92 | 81 | 60 |
| | Epi | +8 | +6 | +3 | +10 | +22 | +17 | +38 | +50 |
| | CO | +49 | +35 | +46 | +42 | +30 | +21 | +12 | +10 |
| | RCV | +26 | +38 | +39 | +21 | −9 | +13 | +8 | +3 |
| " , 1 | MAP in mm Hg | 105 | 100 | 93 | 91 | 92 | 80 | 75 | 68 |
| | Epi | +31§ | +30 | +35 | +67 | +63 | +74 | +64 | +68 |
| | CO | +46 | +16 | +39 | +16 | +21 | +23 | +18 | +15 |
| | RCV | +9 | −18 | −18 | −24 | −16 | −20 | −21 | −26 |

* Epinephrine, 3 μg/kg. † Carotid occlusion. ‡ Right central vagal stimulation.
§ Change in mm Hg.

definite changes were observed in the responses to bilateral carotid occlusion and right central vagus stimulation as compared to control studies(9). However, 1 and 3 μg kg of epinephrine evoked pressor responses which were generally greater, both in an absolute and relative sense than in tests before drug administration.

A delayed hypotensive response occurred 2½ to 3 hours following intravenous injection of both doses of pseudoreserpine and following the 1 mg/kg dose of raunescine (Table II). The 0.3 mg kg dose of raunescine evoked no fall in MAP.

In all dogs there occurred an increase in pressor response to epinephrine administered intravenously. The pressor response to bilateral carotid occlusion was greatly reduced in both pseudoreserpine-treated dogs, but not after raunescine. There was a reduction or reversal of the primary pressor response following right central vagal stimulation in all dogs except the dog on 1 mg/kg of raunescine. No significant changes were observed following acetylcholine, histamine, isopropylnorepinephrine, tilting or stimulation of the right peripheral vagus.

*Discussion.* Pseudoreserpine and raunescine differ from reserpine and deserpidine respectively by the presence of an hydroxyl rather than a methoxyl group at C-17 in ring E. The substitution of a hydroxyl group for the methoxyl group reduced the potency of the compound to one-tenth in causing mice "fall-outs" from roller cages (Table III). Concerning MAP and HR in dogs, alterations in the C-17 group resulted in potency changes similar to those observed in mice, namely reduction of the bradycardic and hypotensive effects. Thus, a change in the C-17 group resulted in parallel potency changes in dogs and mice. Such parallelism does not necessarily follow when other changes in the molecule are effected, as shown by the relative potency of reserpine and rescinnamine. If a methoxyl group is retained at C-17 and the trimethoxybenzoyl group of reserpine is replaced by a trimethoxycinnamyl group, thus forming rescinnamine, potency of the compound as measured by "fall-outs" was reduced to one-thirteenth (Table III). On the other hand, when potency was measured by

TABLE III. Comparison of Effect of Alkaloids on Mice "Fall-Outs" from Roller Cages.

| Alkaloid | $ED_{50}$* (mg/kg) | Slope function* |
|---|---|---|
| Raunescine | 7 (5 -10 ) | 5.0 (2.5-9.7)† |
| Pseudoreserpine | 11 (8 -14 ) | 2.7 (1.8-4.1) |
| Reserpine | 0.8 ( .62- 1.10) | 2.1 (1.7-2.6) |
| Deserpidine | 1.2 ( .8 - 1.8 ) | 2.2 (1.3-3.8) |
| Rescinnamine | 8.6 (7 -10 ) | 1.6 (1.3-2.0) |

* With 95% confidence limits.
† Not parallel to the other lines.

degree of hypotension and bradycardia produced in dogs, rescinnamine was twice as potent as reserpine.

The results indicate that both alkaloids are less potent than reserpine, rescinnamine or deserpidine. Shore and Brodie(10) recently found that both pseudoreserpine and raunescine reduced the serotonin content of rabbit brains but were both significantly less potent than reserpine.

*Summary and conclusions.* Two new alkaloids from *Rauwolfia canescens,* pseudoreserpine and raunescine have been studied for pharmacologic activity in mice and dogs. Both pseudoreserpine and raunescine have reserpine-like activity, but are less potent than reserpine. The reduction in potency has been attributed to the substitution of the methoxyl group on C-17 in ring E by a hydroxyl group.

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## Mode of Action of Aliphatic Amino Acids on Cortical Synaptic Activity. (23366)

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Electrophysiological and pharmacological evidence has disclosed that hyperpolarizing, inhibitory synapses, developing surface-positive cortical postsynaptic potentials (p.s.p.'s) occur abundantly on the superficial dendrites in the cerebral cortex, but not in the cerebellar(1,2). Recognition of this difference has facilitated analysis of the modes of action of various pharmacological agents on central synapses(1-5) that are otherwise obscured in the character of the overt response(6,7). Thus, central nervous system "stimulants" that produce convulsive activity by blockading inhibitory synapses (*e.g.* strychnine) may be distinguished(1,2) from those that activate excitatory synapses (*e.g.* Metrazol). The differential analysis is based on the absence of effect of topically applied strychnine-like agents on the cerebellar cortex, while topically applied activators of excitatory synapses produce convulsive activity in the cerebellar as well as the cerebral cortex. Differential analysis can also disclose the mode of action of "inhibitory" substances. If that overt effect results from selective blockade of excitatory synapses, topical application of the pharmacological agents to the cerebral cortex may be expected to produce inversion of the surface-negative dendritic p.s.p. to positivity as

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