MEDICAL RESEARCH AT DALHOUSIE

ALKALOIDS OF RAUWOLFIA SERPENTINA

ORIGINAL ARTICLE

A. K. Reynolds, B.A., M.Sc., Ph.D.[2]
Henry J. Presutti, B.Sc. '56[3]

Introduction: For the past few years, Rauwolfia serpentina—a drug used for centuries in the native medicine of India—has been studied intensively by chemists and pharmacologists. The several varieties of Rauwolfia have yielded a large number of alkaloids of which reserpine appears to be the most valuable therapeutically. Already this drug has found extensive use in psychiatry due to its rather unique sedative action. In addition, reserpine, as well as whole extracts of Rauwolfia and alkaloidal concentrates, have been found very useful in the management of hypertension. In milder cases, Rauwolfia preparations may be used alone. In more severe cases, they are used in conjunction with more powerful hypotensive agents such as hexamethonium and veratrum alkaloids. Its so-called tranquilizing properties make it particularly suited to the treatment of those cases of hypertension in which psychological factors are very prominent. (1)

A large number of alkaloids are present in the whole extracts and alkaloidal concentrates used clinically, and many of them are mutually antagonistic in some of their actions. It is therefore, important that the individual alkaloids be studied, not only for the purpose of establishing the presence or absence of therapeutically useful properties, but also to determine their possible influence on both the desirable and undesirable actions of reserpine. There is little doubt that the clinical usefulness of the whole extracts and alkaloidal concentrates is due to the presence of reserpine. None of the other alkaloids possess the unique central sedative properties, characteristic of this compound. It is logical to suppose, however, that the presence of the other alkaloids is not without influence. Several are known to exert a potent hypotensive action in experimental animals (2 - 7); some are central stimulants, (8 - 10); and nearly all exert some influence on smooth muscle structures (7 - 12). Pure reserpine has been reported to possess a therapeutic effectiveness ratio of about 1000to 1 compared with the whole root (13 - 15).

The mechanism by which reserpine produces its antihypertensive effect has not been completely established. It seems almost certain that it is intimately associated with its sedative action—an action which in itself is not well understood. The drug is not

1. These alkaloids were obtained through the generosity of Miss B. Rakshit, Bethune College, Government
2. Assistant Professor of Pharmacology.
3. Research Assistant.

a rapidly acting, potent antihypertensive compound; its effect on blood pressure are manifested rather slowly. The autonomic nervous system appears to be clearly involved, and the opinion has been expressed that reserpine acts primarily on the hypothalamus with a resulting inhibition of reflex sympathetic activity (16). It possesses no ganglionic blocking nor peripheral adrenolytic activity (14). Even before a significant fall in blood pressure occurs, reserpine suppresses those pressor responses mediated through the central nervous system, e.g. those due to carotid occlusion and central vagus stimulation (16).

Materials and Methods: The alkaloids that are being studied in this laboratory — chandrine, serpakrine, and raubrine — have been isolated from the Bengal variety of Rauwolfia serpentina. Their chemical structures have not yet been completely established. Freshly prepared aqueous solutions of hydrochloride salts have been employed throughout. To date, they have been examined for acute toxicity, and for their effects on blood pressure and gastrointestinal activity. In studies on **acute toxicity,** the compounds were administered intraperitoneally to mice and intravenously to rabbits, and the usual observations on behaviour, pupil size, respiration and so forth recorded. For **blood pressure** studies, cats and dogs were used exclusively. Cats were anaesthetized with either 100mg. per kg. of chloralose or 1 gram per kg. of urethane. Dogs were anaesthetized with 30 mg. per kg. of sodium pentobarbital given intravenously. A tracheal cannula was inserted, and either the left common carotid or femoral artery cannulated for recording arterial pressure with a mercury manometer. Drugs were administered via a cannula inserted in a femoral vein. For eliciting pressor responses, adrenaline (4 micrograms per kg.), or nicotine (15 micrograms per kg.) were injected intravenously. The carotid sinus reflex was elicited by 45 second occlusion of the carotid arteries caudad to the sinus. The actions on**gastrointestinal activity** were studied both **in vitro** and **in vivo.** Excised segments of rabbit jejunum and guinea pig ileum were suspended in Tyrode solution maintained at 37.0°C and aerated with a 95 percent 0_2 - 5 percent CO_2 mixture. Drugs were added to the 100 ml. bath to make the desired final concentration. Activity was recorded by means of a frontal lever writing on smoked paper. Acetylcholine and barium chloride were employed as neurotropic and musculotropic spasmogens respectively. For studying gastrointestinal activity **in vivo,** cats were anaesthetized with chloralose, and the intestine exposed by a midline incision. A 10 cm. length of jejunum was chosen and a ligature tied around the proximal end. At the other end, a T-piece was inserted and the loop of jejunum thus formed was filled with fresh corn oil. Care was taken to insure that the pressure (8 to 10 cms.) did not change throughout the experiment. The other end of the T-piece was connected to a tambour, and the movements recorded on smoked paper, using a 10:1 magnification. Drugs were injected into a cannulated femoral vein.

Results: Acute Toxicity. Neither chandrine nor serpakrine exert any profound action on the central nervous system in doses below 75 mg. per kg. In mice, doses in excess of 100 mg. per kg. cause increased irritability and convulsions from which the animals recover provided fixation of respiratory muscles does not occur during convulsive seizures. In surviving animals, recovery appears to be complete in 4 to 6 hours. In rabbits, doses of this magnitude produce serious respiratory embarassment. This can be effectively combatted by nalorphine. If adequate respiration can be maintained, animals recover completely. Raubrine is much more toxic, producing respiratory paralysis in doses about one-tenth those of serpakrine or chandrine. In the rabbit, all three alkaloids produce a transient miosis. A miotic effect is characteristic of resperine, but with that drug the effect is very prolonged.

Effects on Blood Pressure: All three alkaloids exert a hypotensive action in anesthetized cats and dogs. As shown in fig. 1, following the intravenous administration of 5 mg. per kg., the blood pressure falls sharply. This is followed by a gradual return to a level somewhat below the original pressure. The depressor action is seen even after bilateral cervical vagotomy and atropinization, and is not abolished by the previous administration of the antihistaminic compound, phenergan, as shown in fig. 2. The pressor response to injected adrenaline or nicotine is not altered, but due to carotid occlusion is markedly suppressed. Similar effects on blood pressure are produced by chandrine, serpakrine, and raubrine.

Gastrointestinal Actions: The action of chandrine on gastrointestinal activity are in distinct contrast to those of the other two alkaloids. On the excised guinea pig ileum, chandrine ($1:10^5$) augments both tonus and contractility and the action is atropine-resistant. It frequently initiates rhythmic movements in segments not exhibiting spontaneous activity. As shown in Fig. 3A, the application of a solution of raubrine ($1:10^5$) to the isolated rabbit jejunum results in a rapid and profound fall in tonus with a concomitant diminution in contractility. The actions of these alkaloids on isolated intestinal preparations is readily reversible. An antiacetylcholine action of raubrine on the guinea pig ileum is clearly shown in Fig. 3B. The first three contractions show the response to acetylcholine ($1:10^7$). The last two contractions show the response to the same concentration of acetylcholine, following 3 minute exposures to raubrine ($1:10^5$ and $1:5 \times 10^4$) respectively. The inhibitory action is non specific since the spasmogenic action of barium chloride is also suppressed. The action of serpakrine is similar to that of raubrine. This antiacetylcholine action has also been observed in intact animals. In rats given intraperitoneal injection of 5 mg. per kg. of serpakrine, the secretion of "bloody tears" (chromodacryorrhea) which follows the injection of the choline derivative, mecholyl, in untreated animals, is not seen.

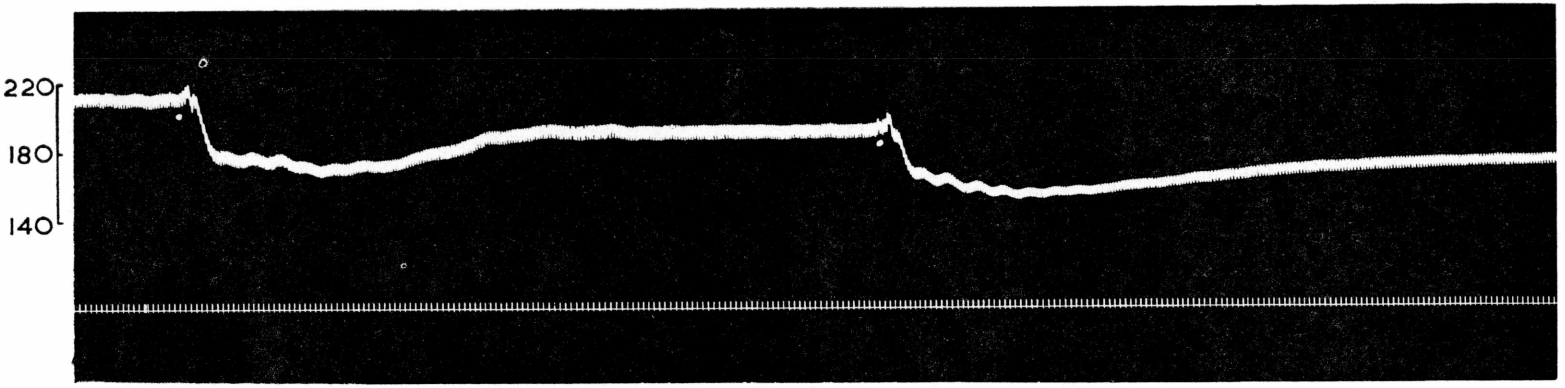

Fig. 1

The action of serpakrine on the blood pressure in the urethanized cat. At 1-histamine, 2-Phenergan, 3-histamine, 4-Serpakrine, 5-histamine. Time, 5 seconds.

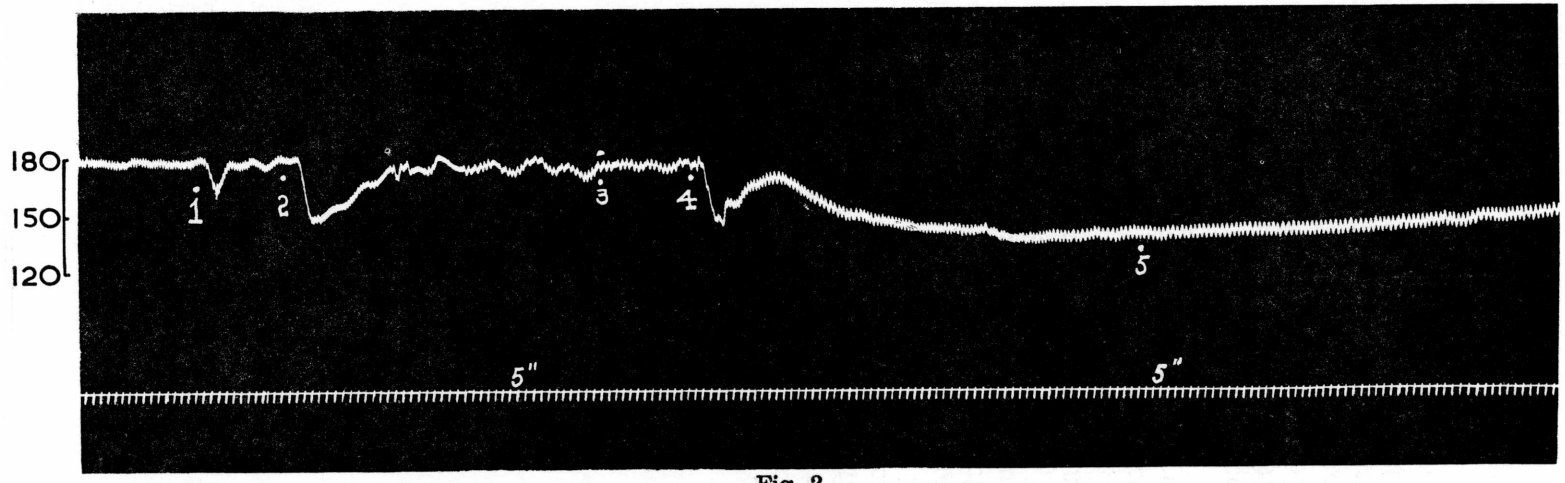

Fig. 2

The action of chandrine on the carotid blood pressure in the urethanized cat. At dots, Chandrine 5 mg. per kg. i.v. Time, 5 seconds.

Conclusion: None of these three bases exert a sedative action on the central nervous system. The unique type of sedation produced by reserpine appears to be peculiar to that alkaloid alone. It has not been reported for any of the other alkaloids of Rauwolfia studied thus far. Chandrine, serpakrine and raubrine all exert a depressor action in anaesthetized animals. The locus of this action has not yet been determined. However, it would appear to be mediated through a mechanism different to that by which reserpine brings about a fall in blood pressure, since the hypotensive action of that drug is intimately associated with its central sedative properties. These alkaloids do not exhibit ganglionic blocking or peripheral adrenolytic activity, nor does their depressor effect appear to result from histamine liberation. Studies in progress are designed to attempt to determine the site of action. Of the three alkaloids, sepakrine seems to be the most promising. It possesses both hypotensive and spasmolytic properties, both effects being produced by doses well below those required to elicit toxic signs. Chandrine is equally as potent in its depressor action, but tends to stimulate gastrointestinal motility. The action on the intestine **in vivo,** however, is neither marked or prolonged, and spasm has never been observed. The results of this study so far seem to emphasize the importance of investigating in detail the pharmacological actions of the individual alkalodial constituents of Rauwolfia serpentina. Diametrically opposite actions may be produced by the various bases, and there is wide variation in the toxicities.

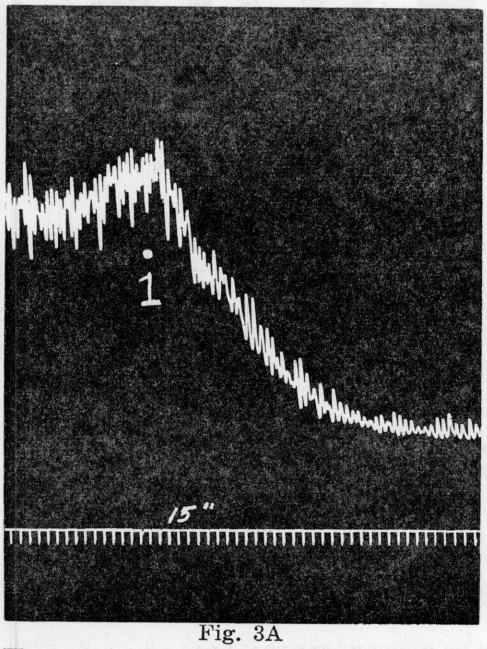

Fig. 3A

The action of raubrine on the isolated rabbitjejunum. At 1, raubrine 1:10^5. Time 15 seconds.

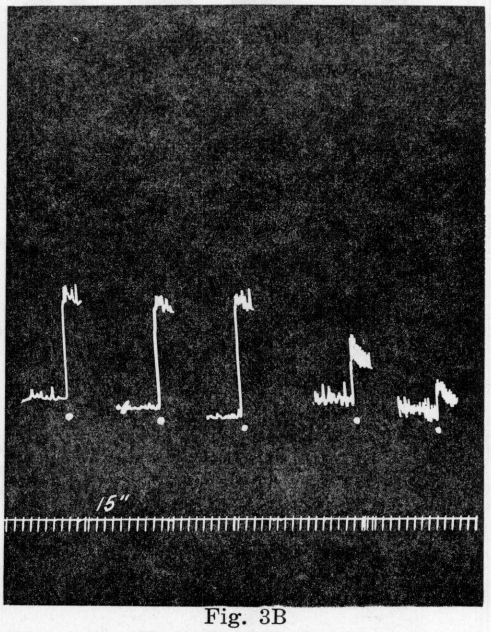

Fig. 3B

The antiacetylcholine action of raubrine on the isolated guinea pig ileum. At dots, acetylcholine 1:10^7. Between third and fourth contraction, exposure to raubrine 1:10^5. Between fourth and fifth contraction exposure to raubrine 1:5 x 10^4. Time, 15 seconds.

BIBLIOGRAPHY

1 Symposium: Ann. New York Acad. Sc., Ed. R. W. Miner, 59 Art 1 (1954) pp 1-140
2 Raymond-Hamet, M., Bull. Sci. Pharmacol. **43,** 364 (1936)
3 Raymond-Hamet, M., Compt. rend. **211,** 414, (1940)
4 Raymond-Hamet, M., Compt. rend. Soc. Biol. **134,** 96 (1940)
5 Raymond-Hamet, M., Compt. rend. Soc. Biol. **134,** 369 (1940)
6 Raymond-Hamet, M., Compt. rend. **223,** 927, (1946)
7 Gupta, J. C., and Kahali, B. S. Indian J. Med. Research **31,** 215 (1943)
8 Chopra, R. N., and Chakravarty, M. ibid **29,** 763(1941)
9 Chopra, R. N., Bose, B. C. and Gupta, J. C. ibid **30,** 319 (1942)
10 Chopra, R. N., Gupta, J. C., Bose, B. C., and Chopra, I. C. ibid **31,** 17, (1943)
11 Kapur, R. D., ibid **36,** 57, (1948)
12 Achelis, J. D., and Kroneberg, T., Naturwissenschaften **40,** 342, (1953)
13 Rubin, B., and Burke, J. C. Federation Proc. **13,** 400 (1954)
14 Wilkins, R. W., Ann. New York Acad. Sci. **59,** 36, (1954)
15 Wilkins, R. W., Judson, W. E., Stone, R. W., Hollander, W., Huckabee, W. E., and Friedman, I. H. New England J. Med. **250** 477, (1954)
16 Trapold, J. H., Plummer, A. J., and Yonkman, F. F., J. Pharmacol. **110,** 205, (1954)
17 Bein, H. J., Experientia **9,** 107, (1953)