A NOTE ON THE PHARMACOLOGY OF THE ALKALOIDS OF *RAUWOLFIA SERPENTINA* BENTH.

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THE sedative and hypotensive properties of extracts of the root of *Rauwolfia serpentina* have been known in India for some time[1,2], but have only relatively recently aroused interest in western clinical medicine[3,4,5]. The recent isolation of reserpine[6] (an alkaloid possessing these specific sedative and hypotensive properties) has clarified a somewhat confused picture of the nature and identity of the active principles.

The precise mode of action of these active rauwolfia extracts and alkaloids is not yet known, and there is some confusion in the literature. The work of Plummer, Barrett, Wagle and Yonkman[7] indicates that, in the dog, the alkaloids have cholinergic properties. Bein[8] has pointed out that in hypotensive doses, the drug did not act as a ganglionic blocking agent or as a sympatholytic. On the other hand, it potentiated the pressor actions of sympathomimetic amines. The hypotensive effects were not affected by atropine or vagotomy. It was suggested that there was a depression of the central portions of the sympathetic nervous system. Trapold, Osborne and Yonkman[9] divided the effects of reserpine into immediate and delayed actions. With respect to the latter, they confirmed much of the work of Bein; but they found that during the period of maximal hypotensive activity, there was potentiation of the effects of acetylcholine or vagal stimulation, and administration of adrenaline or noradrenaline produced the usual pressor response, coupled with bradycardia. Bradycardia and miosis caused by reserpine were antagonised by atropine. Gourzis, Sonnenschein and Barden[10], working with a mixture containing the hypotensive and sedative principles of rauwolfia, point out that in the anæsthetised dog, there was potentiation of the pressor response to adrenaline; the effects of afferent vagal stimulation were abolished; but the hypotensive responses to acetylcholine, histamine and efferent vagal stimulation were not affected. There is a relatively long latent period before the sedative and hypotensive actions become apparent, even after intravenous injection[11]. The reason for this is not clear.

The genera Aspidosperma and Rauwolfia are closely related botanically and our interest and observations on the former[12,13,14,15] led us to investigate some of the pharmacological properties of the alkaloids of the latter. We feel that our results may throw a little light on the mode of action of the Rauwolfia alkaloids.

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MATERIALS

The alseroxylon fraction of the *Rauwolfia serpentina* alkaloids, which contains the specific bradycardiac, hypotensive and sedative principles of *Rauwolfia serpentina*, was used in these experiments. A 0·5 per cent. solution of the drug in the appropriate saline solution was used throughout and further dilutions made from this where necessary.

RESULTS

On the frog rectus-abdominis muscle, the stimulant actions of 10 μg. of acetylcholine chloride were inhibited by doses of 0·075 mg. of the alkaloids. (Fig. 1). The alkaloids were kept in contact with the tissues for 90 seconds. Acetylcholine chloride (10 μg.) was then added and an inhibition noted. The bath was then emptied, and after refilling in the usual way, acetylcholine chloride (10 μg.) was again added, when a greater inhibition was observed. Additions of acetylcholine chloride (10 μg.) were made at the stated intervals until the response returned to normal. (Fig. 1). In one experiment, a solution containing 0·025 mg. of the alkaloid was added to a solution containing 10 μg. of acetylcholine chloride and the mixture allowed to stand out of contact with the muscle for 90 seconds. An appropriate volume of this mixture was then added to the bath. Slight antagonism to the stimulant actions of acetylcholine chloride was shown. There appeared to be no chemical antagonism. After washing out, acetylcholine chloride (10 μg.) was again added and a marked antagonism shown.

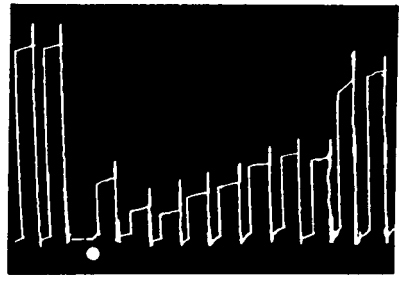

FIG. 1. The antagonism to acetylcholine by the "alseroxylon" fraction of *Rauwolfia serpentina* alkaloids on the frog rectus abdominis preparation in a 10-ml. bath. All contractions are due to the addition of 10 μg. of acetylcholine which was preceded 90 seconds earlier at A by 0·075 mg. of the Rauwolfia alkaloids. Note the progressive inhibition after washing out.

On the rabbit duodenum a very similar effect was noted, especially at higher doses of the alkaloids (Fig. 2). When compared with atropine sulphate, the anticholinergic actions (first inhibition) on rabbit duodenum were of about one thirtieth the strength. The maximum inhibition showed the drug to have about one tenth of the potency of atropine sulphate. In the guinea-pig ileum, this latent period before anticholinergic activity was not seen. In this preparation, the alkaloids were about one fortieth the strength of atropine sulphate. There was, in addition, inhibition of the spasmogenic actions of histamine acid phosphate (1 μg.) and barium chloride (4 mg.). In the isolated rabbit auricles, the inhibiting actions of 50 μg. of acetylcholine chloride were antagonised by 1·5 mg. of the alkaloids. Once again latency was shown and in this case, as in those

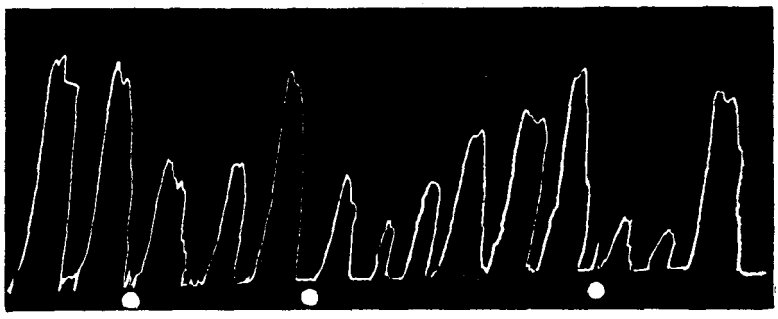

FIG. 2. Antagonism to acetylcholine by the Rauwolfia alkaloids on the isolated duodenum of the rabbit in a 50-ml. bath containing Tyrode's solution at 36·5° to 37° C. All contractions due to the addition of 1 μg. acetylcholine which was preceded 75 seconds earlier at A by 0·25 mg. at B by 0·30 mg., and at C by 0·50 mg. of the Rauwolfia alkaloids.

mentioned above, a considerable period of time elasped before the effects of the drug disappeared completely. Repeated washings were necessary. (Fig. 3). Antagonism to the vasodilator actions of 20 μg. of acetyl-

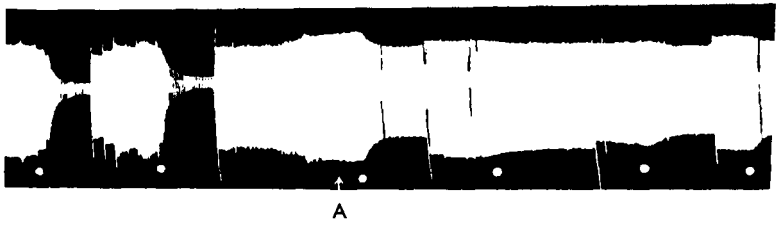

FIG. 3. Antagonism to acetylcholine on the isolated rabbit's auricles in a 50-ml. bath containing oxygenated Ringer Locke's solution at 29° C. 50 μg. acetylcholine was added to the bath at the points. The tissue was washed after each addition of acetylcholine which was preceded 1 minute earlier at A by 1·5 mg. of the Rauwolfia alkaloids.

choline chloride on the rat's hindquarters preparation was shown by 1·5 mg. of the alkaloids. There was no apparent latency. The drugs appeared to have no direct vasodilator or vasoconstrictor actions at this dose level. No significant local anæsthetic activity could be demonstrated by the method of Sollmann[16] as modified by Bulbring and Wajda[17], but in mice 2 mg. of alkaloids caused a marked fall in body temperature. It will be seen that there was a delay of about 90 minutes before the full effect became apparent. (Fig. 4).

DISCUSSION

The alseroxylon fraction of the alkaloids of *Rauwolfia serpentina* appears to possess anticholinergic properties on skeletal, smooth and cardiac muscle. These effects are persistent, and in some tissues, there is a latent period before the maximal effect is shown. It is well known that there is a relatively long latent period before sedation and hypotension

become apparent when rauwolfia preparations are used, and that these effects persist. The parallelism of the effects we have shown on isolated tissues is therefore interesting.

This latency of action on isolated tissues could be explained in a number of ways. For example, the initial stage, when anti-acetylcholine activity is small, may be due to the adsorption of the drug on to the muscle cell receptors in a relatively inactive form which, when in contact with fresh and alkaloid-free saline, is in some way altered chemically or physically, the change depending upon the presence of the muscle cells. There is evidence that the alkaloids are firmly attached to the tissues. The altered compound might stimulate some step in the production of cholinesterase (yet specific anti-acetylcholine activity is not apparent), or it might even be used by the cells as a metabolite. A decreased permeability to acetylcholine cannot of course be excluded nor can an increased permeability to sodium and potassium ions.

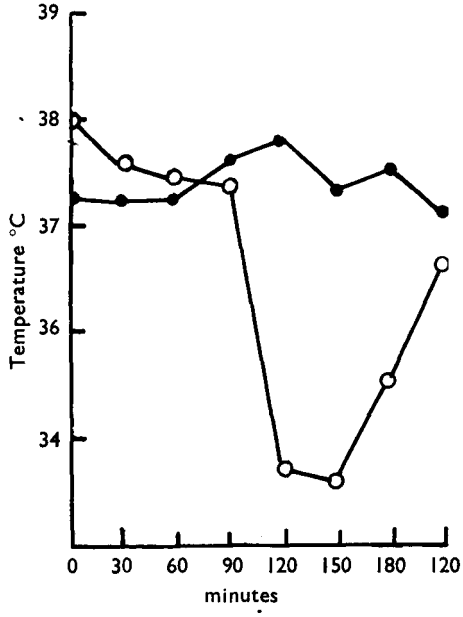

FIG. 4. The effect on temperature of mice after the injection of 2 mg. doses of Rauwolfia alkaloids (alseroxylon fraction). There is a delay of 90 minutes before the onset. Each point represents a mean of 5 observations.
○—○ Rauwolfia alkaloids.
●—● Controls (0·2 ml. of saline solution).

The latent period which is seen before there is full hypotension or sedation in certain intact animals or in man may be due to partial inhibition of the production of some essential metabolite so that it is not until the existing supplies of this substance have been used up by the organism, that the full effects of the drug are seen. On the other hand, production or activity of indigenous acetylcholine may be curtailed, possibly due to stimulation of the production of cholinesterase. If this is so, an anticholinesterase, such as eserine, should reverse the characteristic actions of the drug.

SUMMARY

1. The alseroxylon fraction of the alkaloids of *Rauwolfia serpentina* possesses both delayed and immediate anticholinergic actions on skeletal, smooth and cardiac muscle.

2. Possible modes of action are discussed.

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