A NOTE ON THE PHARMACOLOGY OF RESERPINE

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The introduction of extracts and concentrates of *Rauwolfia serpentina* Benth. into medicine[1] was followed by the isolation and chemical characterisation of reserpine[2]. A number of careful pharmacological studies on the properties and mode of action of reserpine have now been made[3]. These indicate that the drug produces its characteristic effects primarily by a direct action upon the brain, but that it also influences other tissues and organs, especially those containing contractile elements. In order to throw some light upon these effects and to confirm and extend the observations of others[4–11], we have investigated some of the actions of reserpine upon isolated tissues and organs.

Preparation of Reserpine Solutions

Reserpine was dissolved in an aqueous solution of 10 per cent. ascorbic acid to give a concentration of 2 mg. per ml. The pH of this solution was about 2·5. Immediately before use, the pH was raised to about 4·5 by the addition of small amounts of 5 per cent. sodium bicarbonate solution. The mixture was then diluted with the physiological saline being used to give a final reserpine concentration of 1 mg. per ml. Reserpine precipitated rapidly from the final solution; hence it was necessary to carry out the addition of sodium bicarbonate immediately before use. The control solution was prepared by treating the 10 per cent. ascorbic acid solution in exactly the same fashion. In some experiments, 0·2 per cent. citric acid solution was used as a solvent. These instances are mentioned specifically in the text.

Methods and Results

All drug concentrations, unless otherwise stated, refer to final bath concentrations, expressed as weight of drug per millilitre of bath fluid.

Skeletal Muscle

Frog Rectus Abdominis Muscle. Frogs of either sex were decapitated and pithed. The rectus abdominis muscle was dissected out and set up in an organ bath containing 10 ml. of frog Ringer's fluid (NaCl, 0·65; KCl, 0·014; $CaCl_2$, 0·012; $NaHCO_3$, 0·02; glucose, 0·2 per cent.). The bath was oxygenated and allowed to remain at room temperature. The muscle was stimulated by the addition of submaximal doses of acetylcholine bromide (ACh) or, using potassium-free frog Ringer's solution, potassium chloride solution (KCl) was added to the bath so as to cause a reproducible contractile response. Addition of reserpine solution did not modify the responses to ACh (0·1 μg.) or KCl (2 mg.) in experiments

carried out in August. When similar experiments were carried out in January, different results were obtained. Reserpine (200 μg.) now reduced the responses to 0·1 μg. ACh and itself caused a slow contraction (Fig. 1). The control solution caused a smaller and much slower contraction (Fig. 1). In some experiments, the control solution had no apparent effect, although reserpine still caused a contraction. When reserpine was dissolved in citric acid, it produced a contraction, but it did not influence the response to ACh.

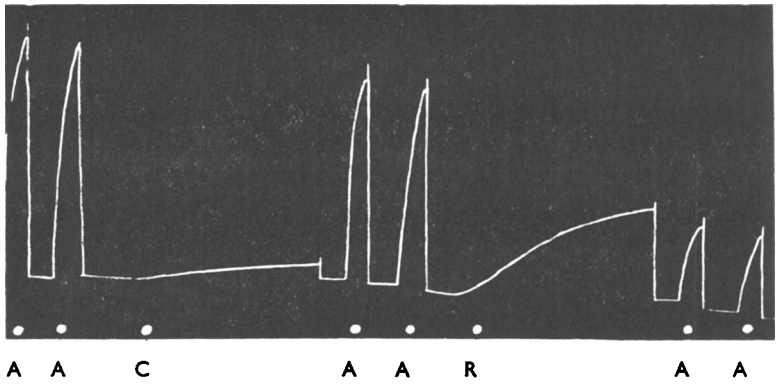

FIG. 1. Direct effect of reserpine on frog rectus abdominis muscle and its influence on ACh induced contractions. At A, 0·1 μg. ACh added. At C, 0·2 ml. ascorbic acid control added. At R, 10 μg. reserpine added.

Cardiac Muscle

Isolated, Perfused Kitten and Rabbit Hearts. The hearts were rapidly dissected out, freed from extraneous tissues and washed in heparinised Locke's solution (NaCl, 0·9; KCl, 0·042; $CaCl_2$, 0·024; glucose, 0·2; $NaHCO_3$, 0·05 per cent.) containing double the usual amount of glucose. They were perfused through the aorta by Langendorff's method[12]. The temperature was maintained at 37° C. The perfusion fluid was well oxygenated. Outflow was measured by a modification of Thorp's impulse counter. Reserpine in a concentration of 0·1 or 1·0 μg. per ml. increased the outflow, in some cases by as much as 100 per cent., but in other experiments, the increase was of the order of 10 per cent. (Fig. 2). There was a slight reduction in heart rate. The amplitude usually underwent a gradual reduction. In most instances, the effects upon amplitude and outflow were partially reversible when reserpine infusion ceased. After perfusion with respine, the heart muscle often showed a loss of tone. The control solution had qualitatively similar, but quantitatively weaker, effects. Reserpine had no significant influence upon the cardio-accelerator action of (−)-adrenaline hydrochloride (5 ng.), (−)-noradrenaline bitartrate* (1 ng.), histamine acid phosphate (Hm) (10 μg.) or 5-hydroxytryptamine creatinine sulphate (5-HT) (10 μg.), but appeared to reduce the duration of their action. Reserpine had no demonstrable effects

* (−)-adrenaline and (−)-noradrenaline were used throughout the work.

upon the characteristic actions of adrenaline, noradrenaline, Hm or 5-HT on cardiac amplitude or outflow. The reduction in outflow after barium chloride and pitressin was antagonised by reserpine. This confirmed the observations of Tripod and Meier[11]. Reserpine did not influence the effect of pitressin and barium chloride upon the heart rate or amplitude.

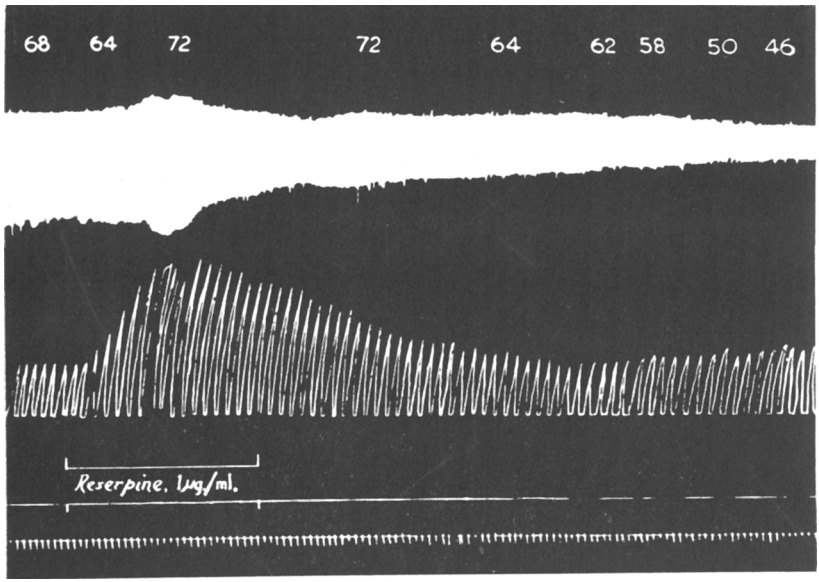

FIG. 2. Influence of 1 µg. per ml. reserpine on amplitude, rate and outflow of perfused kitten heart. Upper record, amplitude (beats per minute); middle record, outflow; lowest record, time = 60 seconds.

The Isolated Guinea-pig and Rabbit Auricles. The auricles were set up in a 25 ml. bath containing well oxygenated Locke's solution at 29° C. Reserpine, 1 µg. or 10 µg., reduced the rate and amplitude of the spontaneous contractions (Fig. 3). The reduction in amplitude was occasionally very marked and occurred soon after addition of the drug, but in general it was gradual and less dramatic. The increases in rate and amplitude produced by Hm (0·5 µg.), adrenaline (0·25 µg.) and noradrenaline bitartrate (0·1 µg.) were reduced, usually reversibly, by 1 and 10 µg. of reserpine added 5 minutes beforehand. The responses to noradrenaline were more actively antagonised than those to adrenaline (Fig. 3). Return to control levels of response was slow (up to 2 hours). A delayed reduction in amplitude was seen after washing out the bath (Fig. 3).

Smooth Muscle

The Isolated Guinea-pig Terminal Ileum. A 3-cm. length of the terminal ileum was removed, washed in Tyrode's solution (NaCl, 0·8; KCl, 0·02; $NaHCO_3$, 0·1; $CaCl_2$, 0·02; $MgCl_2$, 0·001; NaH_2PO_4, 0·005; and glucose, 0·1 per cent.) and set up in a bath containing 2 ml. oxygenated

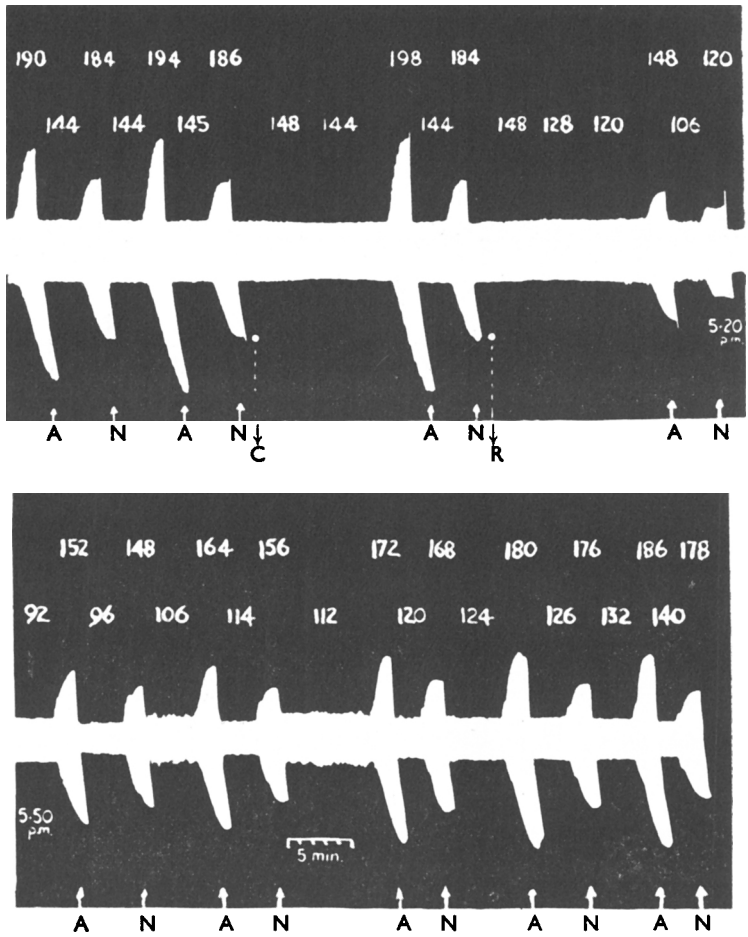

FIG. 3. Influence of reserpine on rate, amplitude and response to adrenaline and noradrenaline of isolated guinea-pig auricles. Figures refer to number of beats per minute; upper row—after drugs; lower row—normal beat (before drugs). At A, 0·25 μg. (−)-adrenaline added. At N, 0·1 μg. (−)-noradrenaline added. At C, 0·1 ml. control solution added. At R, 1 μg. reserpine added. Time = 60 seconds.

Tyrode's solution at 37° C. The responses to submaximal doses of ACh (0·5 μg.), Hm (0·5 μg.), 5-HT (20 ng.) and barium chloride (0·5 mg.) were reduced by doses of from 5 μg. to 30 μg. of reserpine (Fig. 4). The degree of inhibition was related to some extent to the dose of reserpine. Larger doses of reserpine exerted a more prolonged effect than smaller ones, recovery in some cases taking as long as an hour. Maximum inhibition of the response was usually observed after the second or third addition of the spasmogen (Fig. 4). Reserpine itself appeared to have no direct effect upon the ileum. The control solution had no effect. When these experiments were repeated using citric acid solution

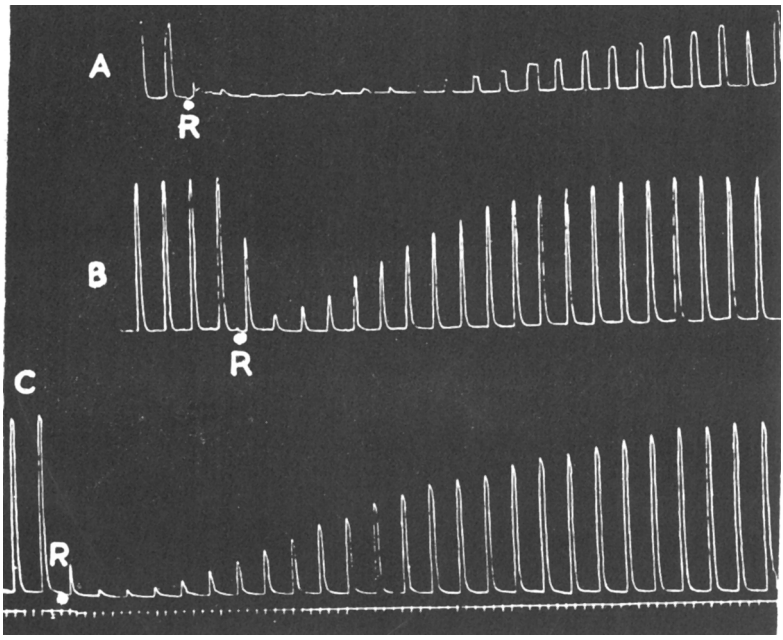

FIG. 4. Influence of reserpine on contractions of guinea-pig ileum induced by Hm, barium chloride and 5-HT. A, All contractions induced by 20 ng. 5-HT. B, All contractions induced by 0·5 μg. Hm. C, All contractions induced by 0·5 mg. barium chloride. At R, 30 μg. reserpine added to bath. Time = 60 seconds.

as solvent, the inhibition of responses of the ileum to ACh and Hm were reduced. Recovery to control levels of response was quicker (Fig. 5). It was decided to see whether citric acid would prevent reserpine from producing its usual inhibitory effects. When citric acid (200 to 800 μg.) was added to the bath with the reserpine, the inhibitory effect of 30 μg. reserpine was markedly reduced (Fig. 6). Doses of citric acid up to

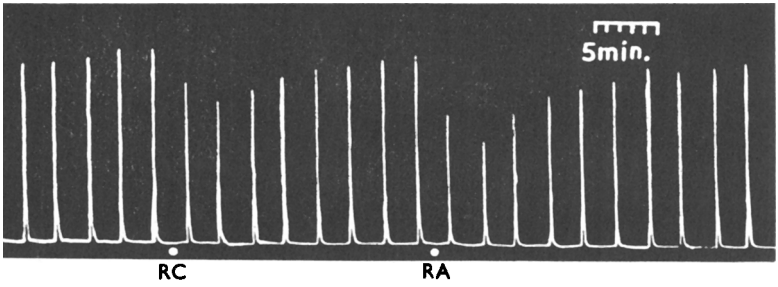

FIG. 5. Influence of reserpine in citric acid solution and ascorbic acid solution on ACh induced contractions of guinea-pig ileum. All contractions induced by 0·5 μg. ACh. At RC, 10 μg. reserpine in citric acid added. At RA, 10 μg. reserpine in ascorbic acid added. Time = 60 seconds.

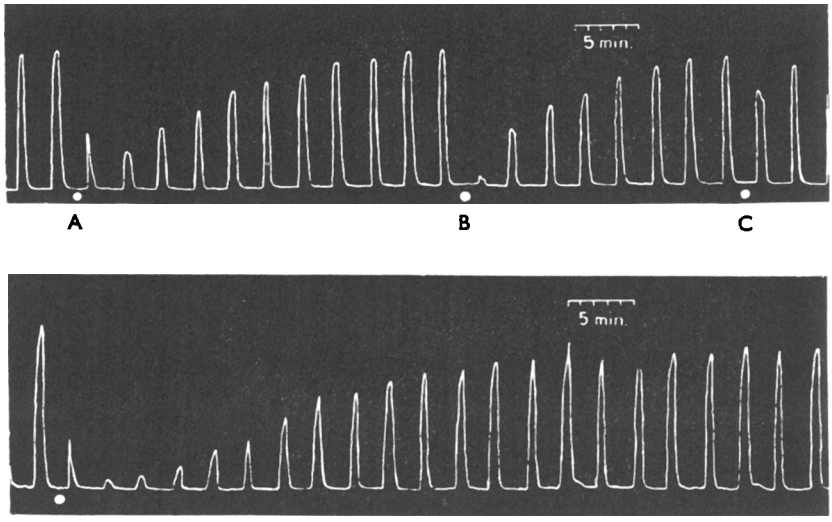

FIG. 6. The influence of added citric acid on reserpine inhibition of ACh induced contractions of the guinea-pig ileum. All contractions induced by 0·5 μg. ACh.
Upper tracing. At A, 400 μg. citric acid with 30 μg. reserpine added. At B, 800 μg. citric acid with 30 μg. reserpine added. At C, 800 μg. citric acid added.
Lower tracing. At R, 30 μg. reserpine added.
Time = 60 seconds.

1·6 mg., however, did not completely prevent the reserpine-induced inhibition. Sodium citrate in doses giving the same bath concentration of citrate ion did not have any inhibitory effect.

The Isolated Rabbit and Kitten Duodenum. About 3 cm. of duodenum were removed, taking a portion which began about 5 cm. distal to the pyloric sphincter. This was set up in a bath containing 5 ml. of oxygenated Locke's solution at 37° C. Reserpine (4 to 30 μg.) inhibited the spontaneous activity of the duodenum (Fig. 7). At the same dose levels,

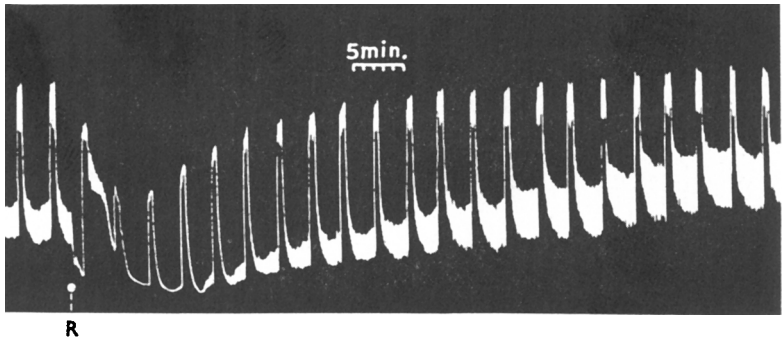

FIG. 7. Influence of reserpine on spontaneous activity and ACh induced contractions of rabbit duodenum. All contractions induced by 0·1 μg. ACh. At R, 30 μg. reserpine added. Time = 60 seconds.

the alkaloid antagonised the spasmogenic action of 0·1 μg. ACh. Reduction in tone caused by 0·5 μg. adrenaline bitartrate was not influenced. The control solution itself showed slight, rather variable, effects upon tone.

Isolated Rat Uterus. Virgin female rats weighing between 120 and 180 g. were brought into œstrus by subcutaneous injections of 0·1 mg. per 100 g. of body weight of stilbœstrol in arachis oil, given 24 hours

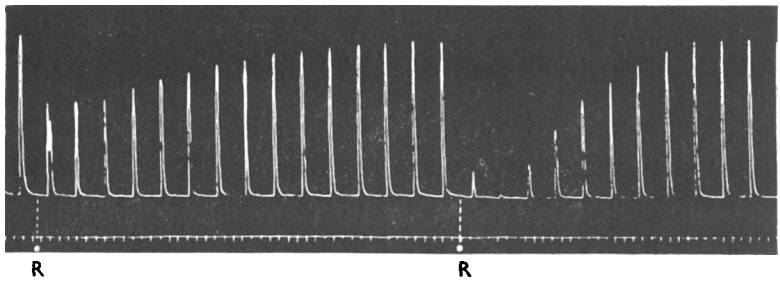

FIG. 8. Influence of reserpine on ACh induced contractions of rat uterus. All contractions induced by 0·25 μg. ACh. At R, 30 μg. reserpine added. Time = 60 seconds.

before use. One horn of the uterus was suspended in a bath containing 2 ml. of oxygenated de Jalon's solution (NaCl, 0·9; KCl, 0·42; $CaCl_2$, 0·006; glucose, 0·05; and $NaHCO_3$, 0·05 per cent.) at 29° C. No direct action was seen at dose levels of from 2 to 100 μg. of reserpine. The initial dose of reserpine reduced (usually reversibly) the responses to KCl (2 mg.), ACh (0·25 μg.) and 5-HT (20 ng.). Similar doses of reserpine, given later, again reduced the amplitude of the response but the effect was now easily reversible (Fig. 8).

The Spinal Cat. Cats of either sex were given atropine (1 mg. per kg.) by intraperitoneal injection 15 minutes before induction of anæsthesia

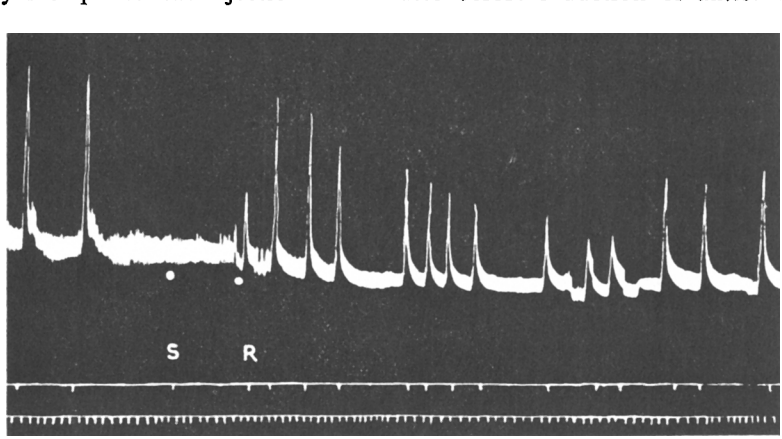

FIG. 9. Influence of reserpine on the pressor response to adrenaline in the spinal cat. All unmarked responses due to 5 μg. adrenaline + 3 ml. saline. At S, 3 ml. saline. At R, reserpine 1 mg. per kg. Time = 60 seconds.

with ether. The carotid arteries were tied and the spinal cord was transected at the level of the second cervical vertebra. The brain was destroyed by a blunt probe. Blood pressure was recorded from the carotid artery and drugs were given by a cannula inserted into the femoral vein. In young cats* a progressive, gradual reduction in blood pressure was noted with 1 mg. per kg. of reserpine. The responses to 5 μg. adrenaline and 1 μg. noradrenaline bitartrate were also progressively reduced (Fig. 9). The effect was not reversible during experiments of 6 to 8 hours. In older animals*, this effect was less marked. Older cats did not show any fall in blood pressure to reserpine. At the point of maximal sustained reserpine-hypotension, Hm (5 to 10 μg.) produced no further fall in blood pressure. Reserpine did not inhibit the pressor response to posterior pituitary extract. In some experiments, however, the latter appeared to reverse the reserpine-induced reduction of the adrenaline response.

Discussion

At the moment, we can offer no explanation for the varying effects of reserpine on the frog rectus abdominis muscle.

The effects of reserpine on the heart—decrease in amplitude with increase in outflow—may indicate a reduction in extravascular support for the coronary bed, that is, a reduced tone of cardiac muscle.

On the smooth muscle of the ileum and uterus, no decrease in tone is evident, but spontaneous activity is inhibited and tone is reduced in rabbit and kitten duodenum. There is a non-specific antagonism shown to the effects of ACh, Hm, HT and barium chloride. This is a prolonged effect and in most instances the maximum effect is delayed in onset.

In the spinal cat, where any vascular tone remained, this was progressively reduced by reserpine to a point where further administration of Hm caused no depressor response. There was antagonism to adrenaline in younger animals but not in older ones. In general, reserpine reduces the tone of cardiac and smooth muscle. Preliminary experiments using the isolated, perfused rabbit ear and rabbit hind quarters indicate that drug-induced vasoconstriction is reduced by low concentrations of reserpine, which themselves have no direct observable effect.

The results obtained indicate that as far as the preparations used are concerned, reserpine does not act upon specific receptors as does, for example, atropine. It may be acting at some point in the metabolic processes underlying contraction—drug-induced or myogenic. This suggestion is supported by the observation that reserpine inhibition is reduced by citric acid. This is now being investigated, as the possibility of an effect upon tissue calcium cannot yet be excluded.

The delay in reaching maximum inhibition may be explained in a number of ways, although experimental proof is lacking. Reserpine may undergo a chemical change to a more active form, or the delay may be related to its low solubility. It is also possible that reserpine is acting as an anti-metabolite.

* Cats of 8 years or over were considered to be "old".

SUMMARY

1. The actions of reserpine have been investigated using a number of tissues containing contractile elements.

2. Both the direct effects of reserpine and its actions upon drug-induced responses have been investigated.

3. Reserpine has been found to antagonise some of the effects of acetylcholine, (−)-adrenaline, (−)-noradrenaline, 5-hydroxytryptamine, pitressin, barium chloride and potassium chloride on such preparations as the frog rectus abdominis muscle, the isolated guinea-pig ileum, and auricles, the isolated perfused heart and the spinal cat.

4. It is suggested that reserpine may affect the metabolic processes which underlie muscular contraction.

We wish to thank Dr. C. Dale Falconer of Ciba Laboratories Ltd., for assisting us in these investigations with gifts of reserpine. We are also grateful to Messrs. Upjohn Ltd., and Messrs. Abbott Laboratories Ltd., for gifts of 5-hydroxytryptamine creatinine sulphate, and we thank Miss I. Topping and Miss J. Hall for technical assistance.

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