Studies on the Metabolism of Reserpine: Part I—Identification of Metabolites

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(*Manuscript received* 27 *June* 1955)

Metabolic studies with reserpine in rats have revealed, besides the drug, the presence of two metabolites, reserpic acid methyl ester and reserpic acid. These have been detected and identified in tissues, blood, intestinal contents and excreta with the aid of paper partition chromatography. *In vitro* experiments have established that degradation to reserpic acid methyl ester can occur in the small intestines, while both the metabolites can be formed in the liver.

ALTHOUGH reserpine has attained considerable importance as a therapeutic agent in hypertension and psychoneurotic conditions, its mode of action is not fully understood and the delayed action of the drug, even after intravenous administration, still remains unexplained. In the hope of shedding some light on this problem, metabolism studies of this drug in rats were undertaken. This paper records the detection of reserpine and identification of its metabolites in the biological materials. Further, with the aid of *in vitro* experiments, the possible sites of formation of these metabolites have been indicated.

Experimental procedure

Paper chromatography — The R_f values for reserpine, reserpic acid methyl ester and reserpic acid in various solvent systems on Whatman No. 1 circular discs are indicated in Table 1. Of these systems, the first four were used for the identification of the alkaloid and its metabolites in the extracts. The others are listed to illustrate the nature of partitioning in various solvent systems.

The alkaloids were spotted on the air-dried paper chromatograms by spraying them with 0·5 per cent methanolic hydrochloric acid and examining the re-dried papers in ultraviolet light when bright green fluorescent zones indicated their positions.

The circular chromatograms were found to be satisfactory for the final identification of the metabolites which were taken to be pure when after elution they ran to a constant R_f value in two solvent systems. For preliminary chromatograms, however, the extracts from the animal were run as streaks in a descending system on Whatman No. 1 sheets. The R_f values for the pure materials in the descending system are not dissimilar to those given for the circular discs.

Administration of the drug to the rats — C.D.R.I. inbred rats of both sexes weighing 150-200 g. were given 2·5 mg. of reserpine per 100 g. of body weight in propylene glycol-alcohol-water (1 : 1 : 2). The animals were placed in metabolism cages for separate collection of the dejecta. The orally fed animals were sacrificed 4 hr. after drug administration while those given the drug

TABLE 1 — Rf VALUES OF RESERPINE, RESERPIC ACID METHYL ESTER AND RESERPIC ACID IN DIFFERENT SOLVENT SYSTEMS

Sl. No.	Solvent system	Reserpine	Reserpic acid methyl ester	Reserpic acid
1.	Hexane (40)-dioxan (20)-1% NH_4OH (40)	0·44	0·01	nil
2.	Ethyl acetate (50)-N-acetic acid (50)	0·90	0·37	0·10
3.	*n*-Butanol (50)-N-hydrochloric acid (50)	0·98	0·85	0·60
4.	*n*-Butanol (50)-1% NH_4OH (50)	1·00	1·00	0·44
5.	Pet. ether (45°-65°) (40)-dioxan (20)-1% NH_4OH (40)	0·73	0·10	nil
6.	Benzene (50)-1% NH_4OH (50)	0·60	0·10	nil
7.	Hexane (50)-dioxan (50)-10% trichloracetic acid (50)	0·80	0·60	nil
8.	Chloroform (30)-methanol (10)-1% NH_4OH (20)	1·00	0·90	0·15
9.	Chloroform (30)-methanol (10)-N-hydrochloric acid (20)	1·00	0·90	0·45
10.	Chloroform (30)-methanol (10)-1% trichloracetic acid (20)	1·00	0·90	0·40
11.	Chloroform (30)-methanol (10)-5% trichloracetic acid (20)	1·00	1·00	1·00
12.	Chloroform (50)-N-acetic acid (50)	0·35	0·05	nil

by the intravenous and intraperitoneal route, 3 hr. after drug administration. Blood was collected by heart puncture under ether and the tissues removed thereafter.

Extraction and identification of reserpine and its metabolites from tissues and blood — (1) The tissues (liver, kidneys and brain) were homogenized separately in a Waring blendor in ethanol (20 cc.), transferred to centrifuge tubes, more alcohol (30 cc.) added and allowed to stand for 45 min. with occasional stirring as was the oxalated blood in ethanol (50 cc.). The tubes were centrifuged, the solid materials extracted again with ethanol (40 cc.) and the supernatants evaporated to dryness under reduced pressure. The residues were then taken up in ligroin, extracted twice with 2 per cent trichloracetic acid and these extracts in turn extracted with chloroform-methanol (3 : 1). The chloroform-methanol extracts were dried over anhydrous sodium sulphate, neutralized with alcoholic ammonia and evaporated to dryness. The residue was then chromatographed on a column of neutral activated alumina (7·5 g.) in benzene. The second fraction (15 cc.) contained reserpine, the next (chloroform-4 per cent ethanol, 50 cc.) contained some reserpine, the reserpic acid methyl ester and some reserpic acid, the rest of which came through with ethanol. With each new solvent, the flask containing the residues was re-extracted.

(2) The tissues were homogenized separately in a Waring blendor in water (20 cc.) and treated with 10 per cent trichloracetic acid (20 cc.) after transferring to suitable centrifuge tubes, as was the oxalated blood. After standing at room temperature for 15 min. with occasional stirring, the tubes were centrifuged and the precipitated proteins re-extracted with 5 per cent trichloracetic acid (30 cc.). The combined supernatants were then extracted with chloroform-methanol (3 : 1) twice and these solutions dried over anhydrous sodium sulphate. The presence of reserpic acid and reserpic acid methyl ester could be established in these solutions by neutralizing them with alcoholic ammonia, evaporating them to a small volume (*c.* 0·2 cc.) under reduced pressure and applying them first to descending chromatograms as streaks. The detection of reserpine in these extracts was not possible because of the large amount of interfering material present. The chloroform-methanol extracts were, therefore, treated with silver carbonate (1 g.) and allowed to stand overnight. After filtering off the precipitated silver trichloracetate, the solution was evaporated to dryness and the residues chromatographed on neutral alumina as in the previous method. Reserpine and reserpic acid methyl ester could always be detected and sometimes reserpic acid as well.

Extraction and identification of reserpine and its metabolites in small and large intestines — The contents of the small intestines and those of the large intestines and the faeces were washed through and extracted with ethanol (total volume 50 cc.), centrifuged, the supernatants evaporated to *c.* 1 cc. under reduced pressure and a few drops of alcohol added to take all into solution. All the three, reserpine, reserpic acid methyl ester and reserpic acid, could be detected in these solutions.

Identification of metabolites in the urine — Because of the diarrhoea caused by reserpine, the urine excreted between the time of drug administration and animal sacrifice was contaminated with faeces. A drop of pure urine was, therefore, taken by pressing the bladder just prior to sacrificing the animal. Reserpic acid and reserpic acid methyl ester could always be detected in this drop by applying it directly to the paper chromatogram. Reserpine, however, showed up only occasionally in the absence of some interfering material in the urine. When not detectable, the urine was treated with 2 per cent trichloracetic acid (2 cc.) extracted out with chloroform-methanol (3 : 1) and chromatographed on neutral alumina as in the case of the organs.

In vitro degradation of reserpine by rat liver slices — Reserpine (2 mg.) was taken up in citric acid (4 cc.) and 36 cc. of N/10 Na_2HPO_4 added. This solution (*p*H 7·3) was made up to 100 cc. with oxygenated Ringer solution and divided into four vessels. Rat liver was removed quickly after stunning the animal. Free hand sections of the liver were cut and approximately equal lots of the slices were added to each vessel. The flasks were then incubated at 37°C. with constant shaking for 3 hr. The contents were then filtered and the combined filtrates were made ammoniacal and extracted with chloroform twice and then with *n*-butanol. The presence of considerable amounts of reserpic acid methyl ester and of reserpic acid in these extracts was established by paper chromatography.

In vitro degradation of reserpine by the small intestines — Intestinal loops, 1½-2 in. long, were removed from overnight-starved rats from the proximal part of intestines. Reserpine solution (1 mg. per cc. in propylene glycol, alcohol and water) (1:1:8) was introduced into these loops which were tied up from both ends. These loops were then suspended in an organ bath maintained at 37° ±1°C. in Ringer Locke solution with constant oxygen bubbling. After 3-4 hr. incubation in the bath, the contents of these loops were washed and worked up as in the previous experiments. Considerable amount of reserpic acid methyl ester could be detected.

Results and discussion

The results of these investigations can be summarized as follows:

(1) It has been possible to detect and identify reserpine and its two metabolites, reserpic acid methyl ester and reserpic acid, in all the biological materials examined, viz. blood, liver, brain, kidneys, washings of the small intestines, faeces and washings of the large intestines and urine, irrespective of the mode of administration of the drug.

(2) Degradation of reserpine to both reserpic acid methyl ester and reserpic acid has been shown to take place in the liver by *in vitro* incubation of reserpine with liver slices.

(3) Degradation of reserpine to reserpic acid methyl ester alone has been shown to take place in the small intestines by *in vitro* experiments.

It will be apparent that the first finding is corroborated by the next two. Reserpine administered by any of the three methods must reach the liver and the degradation to both reserpic acid methyl ester and reserpic acid in this organ would explain the presence of these metabolites in all the tissues examined. Although the experiments reported are of a purely qualitative nature, obviously larger amounts of reserpic acid methyl ester were present in the fractions from animals administered the drug orally. This is to be expected since the small intestines appear to be efficient in degrading reserpine to reserpic acid methyl ester.

As reserpic acid methyl ester has been found to be a metabolite of reserpine, we administered this substance intravenously to rabbits and orally to monkeys but found it to be inactive. Huebner *et al.*[1] have also reported the inactivity of reserpic acid methyl ester. In view of the inactive nature of this compound the other metabolite, reserpic acid, a further degradation product, would also be expected to be devoid of physiological activity. It is, therefore, possible that the reserpine molecule is itself responsible for the pharmacological action.

While our investigation was in progress, we came across a report on the metabolic studies with reserpine by Glazko *et al.*[2]. These authors also detected reserpic acid methyl ester as a metabolite and showed with the aid of *in vitro* experiments that it was formed by the enzymatic hydrolysis of reserpine by the intestinal mucosa. Surprisingly, however, only insignificant amounts of this metabolite could be detected in animals given the drug by routes other than the oral one and no mention of the second metabolite, reserpic acid, has been made.

Acknowledgement

The authors are grateful to Drs. B. Mukerji, N. N. De and M. L. Dhar for their interest in this work and to Messrs Ciba Ltd., Basle, for a generous gift of pure reserpine.

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