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Indirect biochemical evidence that reserpine methiodide produces selective depletion of peripheral biogenic amines in rats

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Received February 5, 2004, accepted June 23, 2004

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Pharmazie 60: 294–297 (2005)

Reserpine, an alkaloid from *Rauwolfia serpentina* was widely used for its antihypertensive action in the past. In the present investigation, reserpine methiodide (RMI), a quaternary analogue of reserpine was synthesised and evaluated biochemically for its central and peripheral amine depleting actions in rats and compared with reserpine. The 24 h urinary excretion of vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), the respective metabolites of noradrenaline, serotonin and dopamine were estimated and considered as indirect biochemical indices for the amine depleting action of reserpine and RMI. The results indicate that RMI at doses of equal to and double the equimolar doses of reserpine was found to deplete the peripheral amines without affecting the central stores of the amines. The results further suggest that the quaternization of reserpine might restrict its transfer across the blood-brain barrier and could be the reason for its selective peripheral action.

1. Introduction

Reserpine, an alkaloid isolated from *Rauwolfia* species, was introduced for the treatment of hypertension and schizophrenia in 1950's but was replaced by more effective drugs by the end of 1970's (Vakil 1949; Mueller et al. 1952; Wilkins and Judson 1953; Vakil 1954; Wilkins 1954; Bleuler and Stoll 1955). Reserpine is known to act centrally as well as peripherally by depletion of biogenic amines *viz.,* noradrenaline, serotonin and dopamine. Mostly, its peripheral depletion of amines is responsible for its antihypertensive effect while the central depletion of amines is responsible for its antipsychotic action (Wolley and Shaw 1954; Pletscher et al. 1955; Bertler et al. 1956; Holzbauer and Vogt 1956; Muscholl and Vogt 1958; Brodie et al. 1960; Bertler 1961). However, because of its central action it produces sedation and Parkinsonism when used for the management of hypertension for prolonged periods (Dustan et al. 1954; Achor et al. 1955; Lemieux et al. 1956; Harris 1957). As a result it has only limited value for chronic treatment of hypertensive patients (Noce et al. 1954; Hughes et al. 1955). Hence there is a need for structural modification of the drug to make it more acceptable for the treatment of hypertension.

Attempts were made in the past to synthesize derivatives of reserpine with possibly higher and/or modified activities or with fewer side effects (Garatfini et al. 1959; Protiva et al. 1960; Agbalyam 1961; Karim et al. 1961). Compared to reserpine itself, a number of reserpine analogues were found to influence the amine concentration in the periphery stronger than in brain (Trcka et al. 1963; Trcka and Clarsson 1965; Trcka and Clarsson 1967).

Based on the poor ability of quaternary derivatives to penetrate the blood-brain barrier, a great deal of research has been devoted towards quaternization of existing drugs to achieve preferentially peripheral action (De la Lande et al.1955; Khromov-Borisov and Yanovitskaya 1959; Lapin 1970; Goldberg et al. 1979; Brewster et al. 1996; Janowsky 2002). Earlier reports have demonstrated the synthesis of quaternary derivatives of reserpine and isoreserpine, however their pharmacology was not studied (Schlittler 1965; Gaskell and Joule 1967). In the present investigation, a quaternary analogue of reserpine, namely reserpine methiodide which was synthesized and evaluated in rats for its amine depleting action compared to reserpine. Urinary levels of vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), which are the respective metabolites of noradrenaline, serotonin and dopamine were estimated after reserpine or RMI treatment in rats.

2. Investigations and results

The main aim of this study was to determine whether RMI was able to deplete the central and peripheral biogenic amines to the same extent as produced by reserpine. Reserpine at a dose of 5 mg/kg body weight produced significant ($p < 0.05$) increase in the urinary excretion profile of VMA compared to control animals. The analogue at doses equimolar to reserpine of 5 and 10 mg/kg body weight produced a more significant ($p < 0.05$) increase in VMA excretion compared to controls and to that observed with reserpine (Fig. 1). However, the higher dose (10 mg/

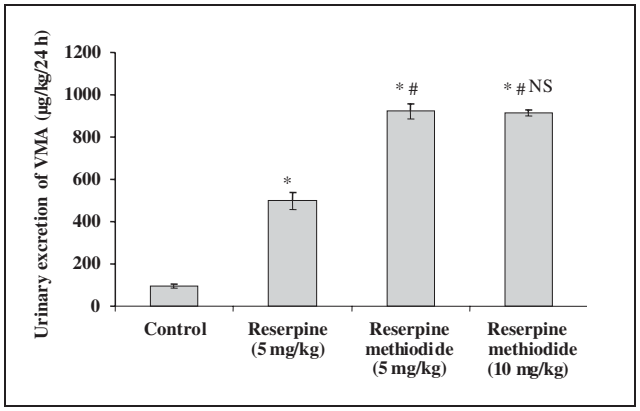

Fig. 1: Diagram illustrating the effect of reserpine and reserpine methiodide on the 24 h urinary excretion of VMA in rats. Each bar indicates the mean excretion of six animals. Significant difference from control group: * #$p < 0.05$. Significant difference from reserpine treated group: $p < 0.05$ NS - No significant difference between 5 and 10 mg/kg treated groups of reserpine methiodide

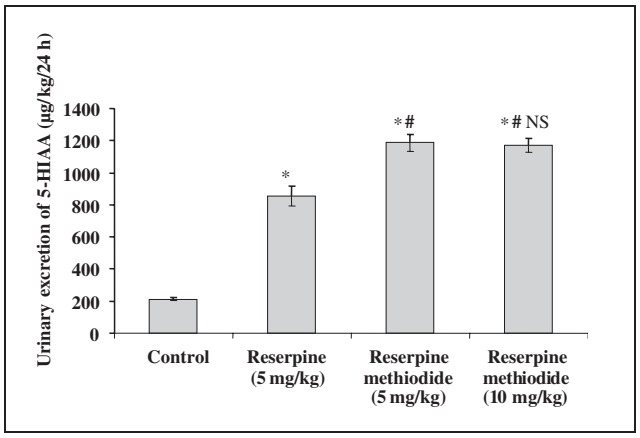

Fig. 2: Diagram illustrating the effect of reserpine and reserpine methiodide on the 24 h urinary excretion of 5-HIAA in rats. Each bar indicates the mean excretion of six animals. Significant difference from control group: * $p < 0.05$. Significant difference from reserpine treated group: #$p < 0.05$ NS - No significant difference between 5 and 10 mg/kg treated groups of reserpine methiodide

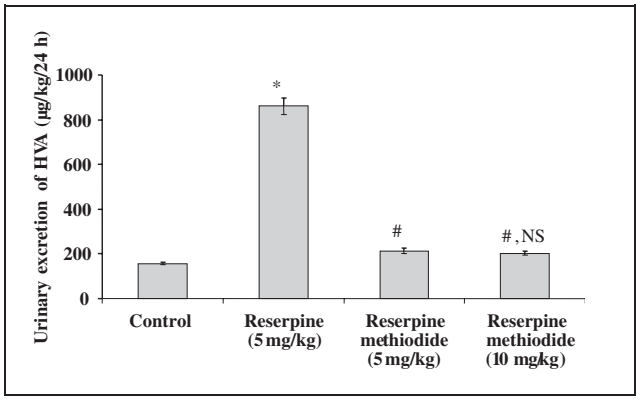

Fig. 3: Diagram illustrating the effect of reserpine and reserpine methiodide on the 24 h urinary excretion of HVA in rats. Each bar indicates the mean excretion of six animals. Significant difference from control group: * $p < 0.05$. Significant difference from reserpine treated group: #$p < 0.05$ NS - No significant difference between 5 and 10 mg/kg treated groups of reserpine methiodide

kg body weight) of RMI did not further enhance the excretion of VMA produced by 5 mg/kg body weight dose.

A significant ($p < 0.05$) increase in 5-HIAA excretion was observed with reserpine at a dose of 5 mg/kg body weight and with the equivalent dose of RMI (Fig. 2). A higher amount of 5-HIAA was found excreted in animals treated with the analogue/reserpine than in the control. However the effect was more pronounced with the analogue compared to reserpine. The enhancement in dose to 10 mg/kg body weight of RMI did not produce any further increase in 5-HIAA excretion.

A marked increase in HVA excretion was observed in animals treated with reserpine ($p < 0.05$) compared to controls while a minor change was observed in animals treated with RMI at doses of 5 and 10 mg/kg body weight compared to control (Fig. 3).

3. Discussion

The structural modification of existing drugs to achieve selective action is not uncommon in providing better biopharmaceutical properties of drugs. It has been well established that the antihypertensive and tranquilizing actions of reserpine are mediated through the depletion of biogenic amines in the body (Shore et al. 1957; Brodie et al. 1960; Bertler 1961). The peripheral depletion of amines is responsible for its antihypertensive effect (Muscholl and Vogt 1958; Trendelenburg 1963) while their central depletion plays a role in sedation and depression (Carlsson et al. 1957; Sheppard and Zimmerman 1960). Reserpine exerts its depleting effect by specifically inhibiting the adenosine triphosphate-Mg^{2+}-dependent incorporation of biogenic amines into their storage vesicles (Carlsson et al. 1962, 1963).

Since reserpine depletes noradrenaline, 5-HT and dopamine from their storage sites, this results in a consequent increase in their metabolite levels in urine. Previous investigators have demonstrated a marked increase in the urinary excretion of peripheral and central metabolites of biogenic amines in animals treated with reserpine (Brodie et al. 1955; Pletscher et al. 1955; Shore et al. 1955; Erspamer and Ciceri 1957).

In the present investigation, a non-invasive biochemical approach was followed to determine the 24 h urinary excretion of VMA, 5-HIAA and HVA in rats treated with reserpine or RMI. VMA is the peripheral metabolite of noradrenaline, 5-HIAA is considered to be the main metabolite of 5-HT while HVA is the predominant metabolite of dopamine. Noradrenaline exists both centrally and peripherally, serotonin exists mainly peripherally while the majority of dopamine exists centrally. Since 99% of the total body's content of serotonin is present in the periphery, it is considered that the major part of the excreted 5-HIAA is from the peripheral release (Aizentein et al. 1979; Some and Helander 2002). Similarly, high levels of dopamine are found in the centre rather than periphery, and any change in the HVA excretion in urine is considered to correspond with changes in dopamine levels at the central regions (Kott et al. 1971). These indices provide an indirect evidence for the peripheral and central monoamine depleting effects of reserpine and its analogue.

The results show that reserpine increased the urinary excretion of VMA, 5-HIAA and HVA indicating the depletion of peripheral as well as central biogenic amines. These results are in agreement with observations of previous investigators (Brodie et al. 1955; Pletscher et al. 1955; Shore et al. 1955; Erspamer and Ciceri 1957).

The increase in the urinary excretion of VMA and 5-HIAA with RMI is higher than with reserpine at an equi-

molar dose of 5 mg/kg body weight. This could be explained based on the results of HVA excretion in the analogue treated animals. The inability of the analogue to increase HVA excretion unlike reserpine could be due to its non-entry across the blood-brain barrier and into the central nervous system to deplete dopamine which is present predominantly in certain areas of the brain namely mesolimbic, nigrostriatal and tuberoinfendibular systems (Rang et al. 1995). The localized distribution of the analogue in the periphery could lead to a higher degree of peripheral noradrenaline and serotonin depletion and hence their metabolite levels were found to be increased much more.

The increased urinary levels of 5-HIAA observed with RMI could be due to the peripheral release of serotonin as it is found predominantly at the periphery in enterochromaffin cells. The higher dose (10 mg/kg) of RMI did not produce any further increase in VMA and 5-HIAA excretion. The possible reason for this could be that 5 mg/kg dose was sufficient to deplete the amines completely from their storage sites.

In conclusion, the present study indicated that the quaternization of the reserpine molecule prevents its access into the central nervous system thereby causing selective peripheral depletion of biogenic amines.

4. Experimental

4.1. Chemistry

Reserpine methiodide was prepared as previously described (Schlittler 1965; Gaskell and Joule 1967). Briefly, the compound was prepared by adding a solution of reserpine (2 g, 3.3 mmol) in dichloromethane (20 ml) to methyl iodide (11 ml, 176 mmol) and the resulting solution was kept for two days in the dark. The solid was filtered and washed with a little cold dichloromethane and dried under vacuum at 70 °C for 2 h to yield reserpine methiodide.

4.2. Chemicals used

Reserpine was a generous gift sample from Novartis India Limited, Mumbai. The standard samples of VMA, 5-HIAA, HVA and iso-VMA (internal standard) were purchased from Sigma-Aldrich, St.Louis, USA. All other chemicals used were of HPLC or analytical grade as appropriate.

The solutions of reserpine and RMI under study were prepared in DMSO and the volume of each dose was adjusted to 0.1 ml/100 gm body weight as suggested by Varma et al. (1987). The doses of RMI were calculated on an equimolar basis of reserpine.

4.3. Animal experiments

Albino rats of either sex weighing between 100–150 g (Charkaborty Enterprise, Kolkata) were used in the study. They were acclimatized to the laboratory conditions for at least 10 days prior to the experiment and were provided with standard diet and water *ad libitum* with a 12 h light and dark cycle.

Animals were divided into 4 groups of six each and were housed individually in metabolic cages. Funnels of suitable size were arranged at the bottom of the metabolic cages for collection of urine. Perforated plastic discs were arranged in the funnels to retain fecal matter. The animals were maintained at room temperature and acclimatized to metabolic cages for few days prior to drug administration.

The treatment given to the groups of animals was as follows:

Group 1: Control animals treated with DMSO intraperitoneally at a dose of 0.1 ml/100 gm body weight.

Group 2: Animals administered intraperitoneally with reserpine at a dose of 5 mg/kg body weight.

Group 3: Animals administered intraperitoneally with reserpine methiodide at a dose equivalent to 5 mg/kg body weight of reserpine.

Group 4: Animals administered intraperitoneally with reserpine methiodide at a dose equivalent to 10 mg/kg body weight of reserpine.

In each group, animals were placed individually in metabolic cages after drug administration and were allowed access to water. The 24 h urine samples from the point of drug administration was collected for each animal in a beaker containing 5 ml of 6 M HCl arranged at the bottom of the funnel. The volumes of the 24 h urine samples collected in the beakers were noted individually and about 2 ml of urine (mixture) from each animal was taken into sample tubes and centrifuged at 3000 rpm for 10 min. The supernatants were transferred into another set of clean and dry tubes and stored at −20 °C until analysis by HPLC.

4.4. Simultaneous HPLC determination of VMA, 5-HIAA and HVA in urine

The procedure described by Wako-chem. Co., was used for the simultaneous determination of the above metabolites. The urine samples were thawed before analysis. To 0.2 ml of each sample, 0.1 ml of internal standard (1000 ng) and 0.7 ml of mobile phase were added. The solutions were mixed well and filtered through a 0.4 μm membrane filter. The filtrate (20 μL) was injected into the column (RP C-18, 250 mm × 4.6 mm I.D; particle size 5 μm; YMC Inc., USA). The mobile phase (filtered through a 0.4 μm membrane filter) comprised of 10 : 90 v/v of acetonitrile and 0.1 M KH_2PO_4 and the flow rate of the mobile phase was maintained at 0.8 ml/min, which yields a column back pressure of 220–230 kgf/cm^2. Detection was done by UV absorption at 230 nm. The range of the detector was set at 0.001 a.u.f.s. The peak area ratios of VMA, 5-HIAA and HVA to that of internal standard were calculated and substituted in the respective regression equations to estimate the amount of the metabolite present in the sample.

4.5. Statistical analysis

Data are expressed as mean ± standard error of means. Statistical analysis was done using one-way analysis of variance (ANOVA). Post-hoc comparisons were done by using Dunnet's t–test. In all the cases, $p < 0.05$ was considered statistically significant.

Acknowledgements: The authors are indebted to M/s Laila Impex Group, Vijayawada for their co-operation in the synthesis of RMI. The financial support by the Council of Scientific and Industrial Research (CSIR), New Delhi to S Nammi is gratefully acknowledged.

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