Pharmacological evidence for lack of central effects of reserpine methonitrate: a novel quaternary analog of reserpine

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Background: Reserpine, an alkaloid from *Rauwolfia serpentina* was widely used for its antihypertensive action in the past. However, its use was reduced in later years due to its sedative and extrapyramidal symptoms. **Objective:** The present investigation aimed to synthesize reserpine methonitrate, a quaternary analog of reserpine and evaluate pharmacologically its central and peripheral actions in comparison to reserpine. **Methods:** The change in behavior of mice and rats after treatment with reserpine or reserpine methonitrate as assessed by their effects on barbiturate hypnosis, spontaneous motor activity, body temperature, avoidance of conditioned response and palpebral closure were considered to be central actions while their influence on the blood pressure of anesthetized rats was measured for their peripheral actions. **Results:** The results indicate that reserpine produced central depression dose-dependently as determined from the battery of tests on the behavior of mice and rats. Reserpine methonitrate at doses equal to and double the equimolar doses of reserpine did not produce any behavioral changes compared with control animals. **Conclusions:** Both reserpine and reserpine methonitrate were found to produce a dose-dependent reduction in the blood pressure of anesthetized rats, although with higher doses of reserpine methonitrate indicated that quaternization of reserpine not only attenuated the entry of the analog into the CNS, but also reduced to the target tissue in the periphery.

Keywords: behavior, blood pressure, mice, rats, reserpine, reserpine methonitrate

Reserpine has been used for decades for the treatment of hypertension and schizophrenia [1–8]. It is known to act centrally as well as peripherally by depletion of biogenic amines such as, noradrenaline, serotonin (5HT) and dopamine. Reserpine exerts its depleting effect by specifically inhibiting the adenosine triphosphate–Mg^{2+}-dependent incorporation of biogenic amines into their storage vesicles [9,10]. For the most part, reserpine's peripheral depletion of amines is responsible for its antihypertensive effect while its antipsychotic action is due to its central depletion of biogenic amines [11–15]. However, due to the fact that it acts centrally, it produces sedation and Parkinsonism when used for the management of hypertension for prolonged periods [16,17]. As a result, it has reduced usage for chronic treatment in hypertensive patients and its use is limited to selective patient populations only [18,19]. Therefore, it would be a worthwhile aim to modify the structure of reserpine to make it more acceptable therapeutically for the treatment of hypertension.

Plummer and colleagues [20] and Schneider [21] were amongst the earlier investigators demonstrating the sedative effects of reserpine in monkeys and on sham rage behavior in cats. Lemieux and colleagues [16] and Harris [17] reported sedation followed by inhibition of spontaneous motor activity upon reserpine administration. Studies by Shore [22] and Salmoiraghi and colleagues [23] in mice also indicated that reserpine produces sedation and potentiates the depressant effects of hexobarbital. Beim [24] and other groups [25,26] observed hypothermia in mice after reserpine administration and attributed it to the depression of central thermoregulatory mechanisms. Later studies also established the tranquilizing actions of reserpine by measurements of spontaneous motor activity, conditioned avoidance response, eyelid closure and rotarod behavior after reserpine treatment in rats [27–31].

Attempts were made in the past to synthesize derivatives of reserpine with possibly higher and/or modified activities or those with fewer side effects [32–35]. Compared with reserpine itself, a number of reserpine analogs were found to exert a stronger influence on the amine concentration in the periphery than in the brain [36,37].

Based on the poor ability of quaternary derivatives to penetrate the blood–brain barrier (BBB), a great deal of research has been devoted to the quaternization of existing drugs to achieve preferential peripheral action [38–40]. Earlier reports have demonstrated the synthesis of

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quaternary derivatives of reserpine and isoreserpine, however, their pharmacology has not been studied [41,42]. The aim of the present study was to compare the effects of reserpine methonitrate (RMN) – a quaternary analog of reserpine – and reserpine on behavioral parameters of mice and rats and on blood pressure of anesthetized rats.

Materials & methods

Chemistry

The synthesis of RMN was carried out as follows: To a solution of reserpine (2 g, 3.3 mmols) in dichloromethane (DCM, 20 ml) was added methyl iodide (11 ml, 176 mmols) and the resulting mixture was stored in the dark for 2 days. The solid was filtered and washed with a little cold DCM and dried under vacuum at 70°C for 2 h to yield reserpine methiodide (RMI) [41,42]. To a solution of RMI (0.25 g, 0.67 mmols) in a mixture of DCM (3 ml) and aqueous ethanol (90%, 2 ml) was added a solution of silver nitrate (56 mg, 0.67 mmols) in aqueous ethanol (90%, 2 ml). The reaction mixture was stirred overnight at room temperature. The solution was filtered and washed thoroughly with chloroform:methanol (1:1). The solid obtained after evaporation of the solvent was passed through a silica gel column and eluted with chloroform:methanol (80:20) to yield RMN (**Table 1**).

Drugs

Reserpine and thiopentone were generous gift samples from Novartis India Limited and Abbott Laboratories, Mumbai respectively. All other chemicals used were of analytical grade. The solutions of reserpine and RMN under study were prepared in dimethyl sulphoxide (DMSO) and the volume of each dose was adjusted to 0.1 ml/100 g body weight for behavioral studies and to 0.05 ml/100 g body weight for blood pressure experiments as suggested by Varma and colleagues [43]. The doses of RMN were calculated on an equimolar basis of reserpine. In all the experiments, control groups were administered with the equivalent volumes of DMSO.

Table 1. Chemical properties of RMN.

Melting point	292
IR (KBr) V_{max}	3164, 2937, 1730, 1708, 1589, 1465, 1381, 1337, 1306, 1274, 1230, 1126, 997/cm
Positive FAB mass	623 m/z (100%, M^+NO_3, $C_{34}H_{43}N_3O_{12}$), 391, 232, 207, 195

FAB: Fast atom bombardment; IR: Infrared; KBr: Potassium bromide.

Animal experiments

Albino mice and rats of Wistar strain of either sex weighing between 20 to 25 g and 200 to 250 g respectively (Chakaborty 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 a standard diet and water *ad libitum* with 12 h light and dark cycle. The animal experiments conducted in this research were approved by the Institutional Animal Ethics Committee and by the government regulatory body for animal research (Reg. No. 516/01/A/CPCSEA).

Effect on barbiturate hypnosis in mice

The general procedure of Kuhn and Van Maanen [44] as described by Turner [45] was employed. The effect of RMN on the duration of thiopentone-induced hypnosis was determined and compared with reserpine.

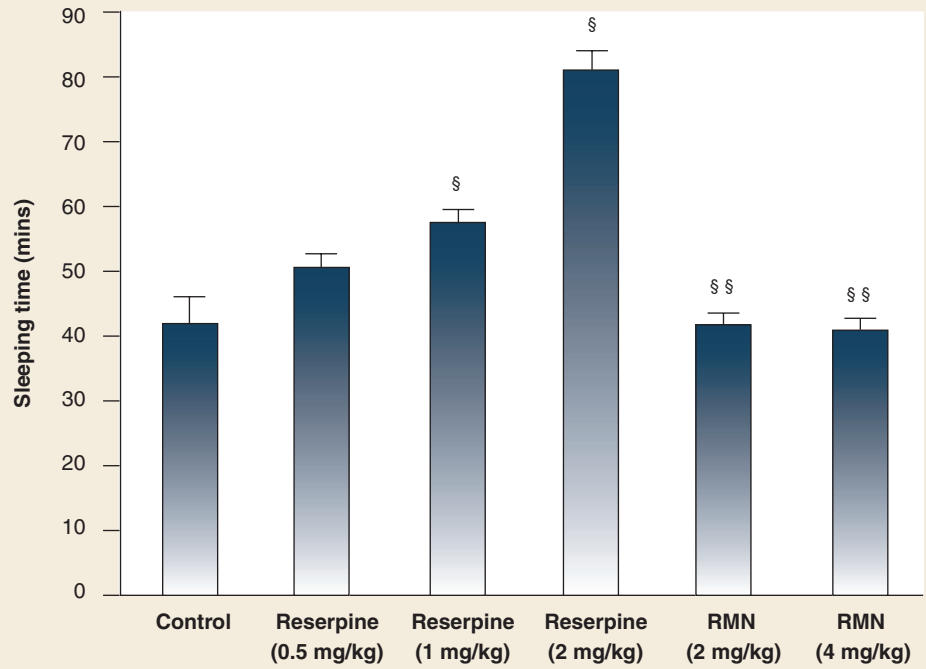
Groups of six of each mice strain were administered intraperitoneally (i.p.) with reserpine at doses of 0.5, 1 and 2 mg/kg body weight or RMN at doses of 2 and 4 mg/kg body weight while the control group was administered with DMSO at 0.1 ml/100 g body weight 1 h prior to the i.p. injection of thiopentone (40 mg/kg). The mice were turned on their backs after administration of thiopentone for 10 secs and then released. Loss of righting reflex was considered positive when mice remained on their backs for at least 1 min. Duration of sleep was assessed as the time that elapsed between loss and recovery of righting reflex.

Effect on spontaneous motor activity of mice

The method of Kuhn and van Maanen [44] was employed with slight modification. The effect of reserpine or RMN on the spontaneous motor activity of mice was registered with photoactometer (INCO, India).

Groups of six of each mice strain were administered intraperitoneally with reserpine at doses of 0.25, 0.50 and 1 mg/kg body weight or RMN at doses of 1 and 2 mg/kg body weight. All the animals were placed in the cage individually and the count was recorded for 5 mins before and after drug administration. Counts were recorded at 15 min intervals up to 2 h and then 4 h after drug administration. Control count for a group receiving only the control (DMSO, 0.1 ml/100 g) was also recorded.

Figure 1. The effect of reserpine and reserpine methonitrate on thiopentone-induced sleeping time of mice.



Each bar indicates the mean time elapsed between loss and recovery of righting reflex of six animals. Thiopentone (40 mg/kg, intraperitoneally [i.p.]) was injected 1 h prior to the i.p. administration of reserpine or reserpine methonitrate (RMN).
§:Significant difference from control group; $p < 0.001$; §§:Significant difference from reserpine (1 mg/kg) treated group; $p < 0.001.0$

Effect on body temperature of mice

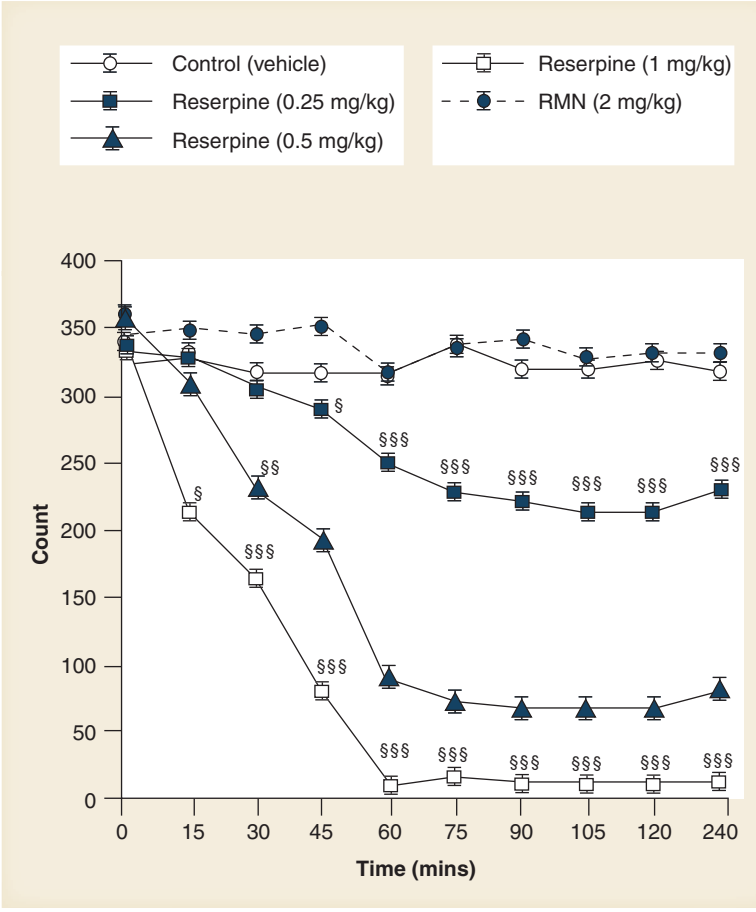
Mice from the groups of six of each strain were administered i.p. with reserpine at doses of 0.25, 0.5 and 1 mg/kg body weight or RMN at a dose of 2 mg/kg body weight. A record of rectal temperature of each animal was obtained by inserting the probe of a digital thermometer (CIE 305) about 1 cm into the rectum before and after administration of drugs [45]. The recordings were made at various intervals up to 24 h and the experiment was performed at room temperature (30 ± 1°C). A control group receiving the control (DMSO, 0.1 ml/100 g) was also studied.

Effect on conditioned avoidance response in rats

The method of Cook and Weidley was used [46]. In summary, the apparatus consists of a stainless steel grid floor in a wooden box which produces electrical shocks to rats who can escape the noxious stimulus by climbing a centrally located wooden pole. The stimulus is approximiately 0.1 mA of 40 V delivered for a period of 30 secs. The conditioning stimulus is a buzzer attached to the chamber.

Rats were trained to climb the pole by a shock of 30 sec duration. Jumping onto the pole functionally terminates the shock. They were later conditioned to do likewise at the sound of the buzzer of 15 sec duration. A 60 trial schedule with 1 min for each trial was followed. Satisfactorily pretrained rats that avoid over 90% of conditioned responses in a 60 trial schedule were selected in groups of six each. They were administered with reserpine at doses of 0.25, 0.5 and 1 mg/kg or RMN at a dose of 2 mg/kg body weight, while the control group received DMSO at 0.1 ml/100 g body weight i.p. The change in the avoidance of conditioned response compared with pretreatment response was recorded at 1, 2, 4, 6, 8 and 10 h after drug administration.

Figure 2. Influence of reserpine and reserpine methonitrate on spontaneous motor activity in mice.



Each point indicates the mean count for six animals during the 5 mins observation period. RMN: Reserpine methonitrate.
Significant difference from control group: $^{\S}p < 0.05$; $^{\S\S}p < 0.01$; $^{\S\S\S}p < 0.001$.

Palpebral test in mice

The purpose of this test was to measure the tendency of the animal to withdraw or to go into the resting state, such as that which precedes sleep. Thus, depression of the CNS and relaxation were measured [45]. Depression of the CNS generally produces some palpebral closure.

Groups of six of each strain of mice were administered with reserpine at doses of 0.25, 0.5 and 1 mg/kg or RMN at 1 and 2 mg/kg while the control group received DMSO at 0.1 ml/100 g body weight. The animals were housed in their respective cages and after 2 h of administration, were observed for palpebral closure without touching them. The scale of scoring for the animals increases with an increase in palpebral closure which evidenced from the degree of motor depression as follows:

- 1 for failure to observe any difference in motor activity
- 2 for a clear difference made unhesitatingly
- 3 if the difference can be owing to drug action

Effect on normal blood pressure of anesthetized rats

The procedure described by Noble was followed to evaluate the effect of RMN on normal blood pressure of anesthetized rats in comparison with reserpine [47]. Groups of six of each strain of rat were anesthetized with an i.p. injection of thiopentone (40 mg/kg body weight). The femoral vein was cannulated for administration of supplementary doses of anesthetic (if required) and drug solutions.

Hemodynamic set up was used to record the blood pressure of rats. The blood pressure of each animal was recorded from the left common carotid artery connected to a mercury manometer on kymograph paper. The normal blood pressure of rats was recorded after stabilization for 30 mins. The different doses of reserpine (0.25, 0.50, 1, 5, 10 and 15 µg/kg body weight) or RMN (10, 25 and 50 µg/kg body weight) were studied in separate groups (n = 6) to determine the change in blood pressure response. A control group receiving the control alone (DMSO, 0.05 ml/100 g body weight) was also studied.

Statistical analysis

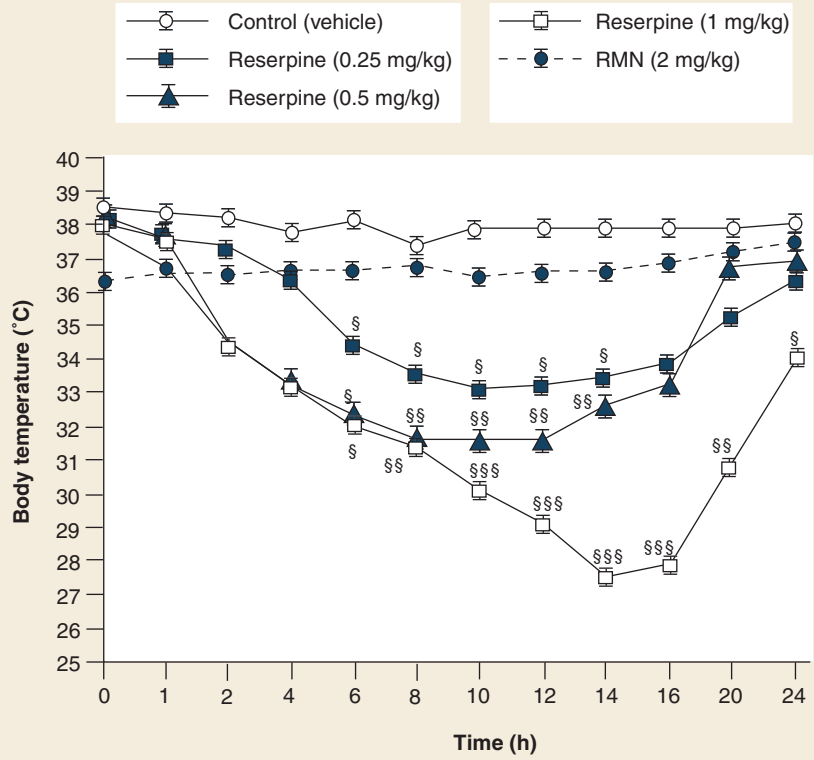
Data are expressed as mean ± standard error of means. Statistical analysis was carried out using one-way analysis of variance (ANOVA). *Post hoc* comparisons were performed using Dunnett's multiple comparisons test. In all the cases, $p < 0.05$ was considered statistically significant.

Results

Effect on barbiturate hypnosis in mice

The results produced by reserpine and RMN on the barbiturate sleeping time in mice are shown in **Figure 1**. Reserpine at doses of 0.5, 1 and 2 mg/kg body weight produced a tendency to increase the sleeping time to 50.67, 57.79 ($p < 0.001$) and 81.25 ($p < 0.001$) mins compared with control time of 42.50 mins. Reserpine at doses of 1 and 2 mg/kg body weight produced statistically significant increases in sleeping time compared with control or RMN-treated groups. No significant change in the sleeping time compared with control was observed in the RMN-treated group at dose levels of 2 and 4 mg/kg body weight.

Figure 3. Effect of reserpine and reserpine methonitrate on rectal temperature in mice at various time intervals.



Each point indicates the mean temperature of six animals. RMN: Reserpine methonitrate.
Significant difference from control group: §p < 0.05; §§p < 0.01; §§§p < 0.001.

Effect on spontaneous motor activity of mice

The effect of reserpine and RMN on the spontaneous locomotor activity of mice is shown in Figure 2. Dose-dependent inhibition of spontaneous motor activity compared with control was observed with reserpine at doses of 0.25, 0.50 and 1 mg/kg body weight. The reduction in locomotor activity by reserpine was of longer duration and continued with low activity for periods even after the experiment. RMN at doses of 1 and 2 mg/kg body weight did not affect the locomotor activity compared with control animals.

Effect on body temperature of mice

The effect of reserpine and RMN on the normal body temperature is shown in Figure 3. Reserpine, with all three doses studied, produced hypothermia in a dose-dependent manner. No such change in rectal temperature was observed with RMN at a dose of 2 mg/kg body weight.

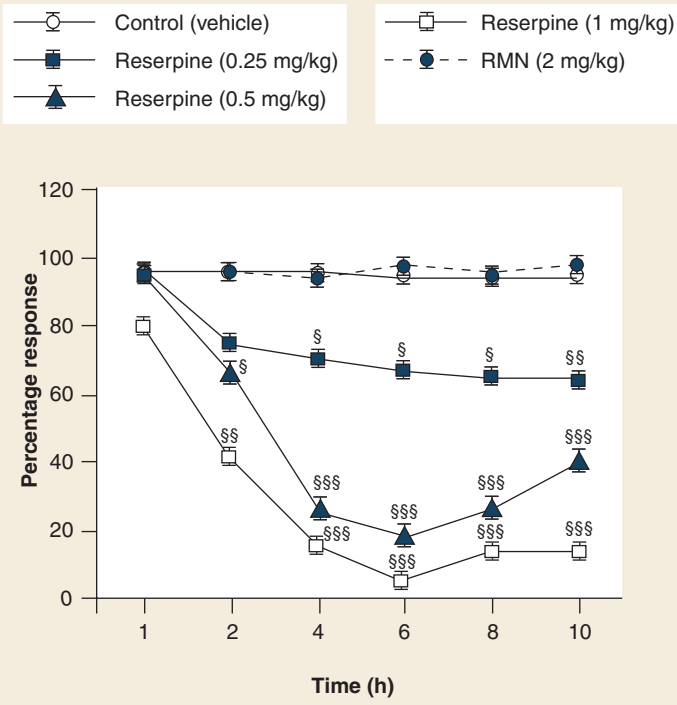
Effect on conditioned avoidance response in rats

The results produced by reserpine and RMN on the avoidance of conditioned response in rats is shown in Figure 4. Dose-dependent reduction in the avoidance of conditioned response was observed with reserpine at doses of 0.25, 0.5 and 1 mg/kg body weight. The reduced response was continued with all three doses even after the experiment. However, the analog RMN did not produce any change in the avoidance of conditioned responses compared with control animals.

Palpebral test in mice

The mean scores observed for palpebral movements in mice after i.p. administration of reserpine or RMN were plotted against the respective doses (Figure 5). Significant changes in the scores were observed with reserpine at doses of 0.25, 0.5 and 1 mg/kg body weight and the mean scores observed were 2.6 ($p < 0.001$), 2.8

Figure 4. Influence of reserpine and reserpine methonitrate on conditioned avoidance response in rats at various time intervals.



Each point represents the mean avoidance response of six animals calculated as percentage of predrug response. Rats were pretrained to avoid over 90% of conditioned responses in a 60-trial schedule. RMN: Reserpine methonitrate. Significant difference from control group: §p < 0.05; §§p < 0.01; §§§p < 0.001.

(p < 0.001) and 3.0 (p < 0.001), respectively. No significant difference in palpebral movements was observed with RMN at doses of 1 and 2 mg/kg body weight and the means scores observed were identical (mean score, 1.0) at both dose levels and the behavior of animals was normal compared with control (means score, 1.0). A clear nonhorizontal slope was observed with reserpine-indicated central depression in contrast to the horizontal slope observed with RMN.

Effect on normal blood pressure of anesthetized rats

The effect of reserpine and RMN on the normal blood pressure of anesthetized rats was shown in Table 2. Dose-dependent hypotension was observed with reserpine as well as with RMN. However, the control (DMSO) also produced hypotension which was approximately 15 mmHg from basal level. This fall in blood pressure was subtracted from the blood pressure recorded after the administration of the drug in the vehicle to obtain the actual effect of the drug.

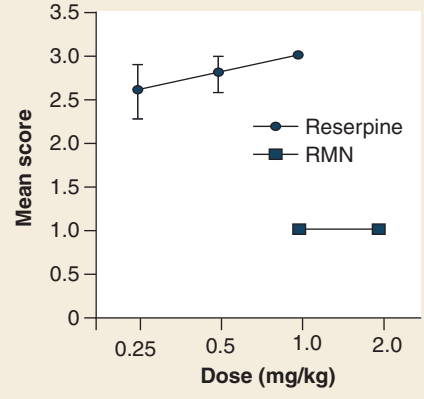
Reserpine at doses of 0.5, 1, 5, 10 and 15 µg/kg produced a significant (p < 0.01) reduction in blood pressure and the recovery time was found to increase significantly compared with control (p < 0.01) with increased doses. RMN was also found to produce a significant (p < 0.01) decrease in blood pressure at doses of 10, 25 and 50 µg/kg body weight and increased the recovery time (p < 0.01) with increases in dose compared with control

Expert opinion

The localization of drug effects to the peripheral or CNS has been of great interest to achieve drug activity at the desired site. From the considerable literature available, it is clear that quaternization reduces the diffusion of drugs through the BBB thereby confining their effects to the periphery only [38–40,48,49]. CNS depressants and tranquilizers are known to potentiate the hypnosis produced by barbiturates, reduce motor activity, reduce body temperature, avoid conditioned responses and increase palpebral closure through their central actions. The results of the present investigation on the central effects of reserpine correlate well with the observations of previous investigators [21,22].

It has been well established that the antihypertensive and tranquilizing actions of reserpine are

Figure 5. Effect of reserpine and reserpine methonitrate on palpebral movements in mice.



Each point represents the mean scores of a group treated either with reserpine or reserpine methonitrate (RMN).

Table 2. Effects of reserpine and reserpine methonitrate on the mean arterial pressure of anesthetized rats.

Drug	Dose (µg/kg)	Mean arterial pressure (mmHg, n = 6)				Recovery time (min)	Recovery due to drug
		Before drug	*After drug*	*Mean reduction*	*Reduction due to drug*		
Vehicle (DMSO)	0.05	126.2 ± 2.8	110.0 ± 4.1	16.2 ± 1.4	—	0.96 ± 0.1	—
Reserpine	0.25	138.3 ± 4.6	119.5 ± 4.2	18.8 ± 1.4	2.6	1.2 ± 0.2	0.2
	0.50	134.1 ± 5.5	91.2 ± 5.0	42.9 ± 0.9§	26.5	3.1 ± 0.4§	2.1
	1	135.2 ± 4.7	80.5 ± 3.8	54.8 ± 1.6§	38.6	4.9 ± 0.4§	3.9
	5	130.5 ± 5.1	69.0 ± 3.5	61.5 ± 2.8§	45.3	7.1 ± 0.3§	6.1
	10	130.0 ± 4.2	58.7 ± 3.9	71.2 ± 1.3§	55.0	10.2 ± 0.3§	9.2
	15	131.5 ± 5.1	41.1 ± 2.9	90.4 ± 3.1§	74.2	13.0 ± 0.3§	12.0
Reserpine methonitrate equivalent to reserpine	10	128.8 ± 6.4	90.0 ± 9.1	38.8 ± 3.6§	22.6	4.5 ± 0.3§	3.5
	25	135.0 ± 8.5	73.0 ± 7.4	62.0 ± 2.8§	45.8	10.5 ± 0.5§	9.3
	50	136.8 ± 5.3	42.5 ± 3.7	93.0 ± 8.0§	76.8	16.3 ± 1.0§	15.3

Significant difference from DMSO-treated group: §$p < 0.01$. DMSO: Dimethyl sulfoxide.

mediated through the depletion of biogenic amines in the body [15,50]. The peripheral depletion of amines is responsible for its antihypertensive effect [14,51] while their central depletion plays a role in sedation and depression of reserpine [27]. Reserpine exerts its depleting effect by specifically inhibiting the adenosine triphosphate–Mg^{2+}-dependent incorporation of biogenic amines into their storage vesicles [9,10]. Reserpine, being capable of central entry depletes monoamines including 5HT and such action is responsible for its central sedation and tranquilizing activities [15,22,52].

RMN at doses of twice the highest dose of reserpine (on equimolar basis) did not produce any effect on barbiturate sleeping time, spontaneous motor activity, body temperature, conditioned responses and palpebral movements. The possible explanation for the inability of RMN to elicit the above effects could be due to its nonpenetration through BBB to enter into the CNS. The large body of evidence on the quaternary methoderivatives of centrally active drugs available today also lends support to our observations with RMN [38–40,48,49].

In order to evaluate whether the quaternary analog of reserpine (RMN) still retains the peripheral blood pressure-lowering activity, further experiments were carried out on the blood pressure of anesthetized rats. Thus far, the results of RMN on the blood pressure response of anesthetized rats confirm that the peripheral actions of the reserpine molecule are not affected by quaternization. However, in the present study, the control (DMSO) also produced minor hypotensive effects on the blood pressure of rats when administered alone with the dose used for the administration of the drugs. Earlier workers also reported hypotension with DMSO supporting the present observations [43]. Reserpine produced a dose-dependent reduction in blood pressure as well as an increase in recovery time as demonstrated by previous investigators [53,54]. The intravenous doses required to produce hypotension were very small and the central effects were reported to be absent with such doses [24,55]. As indicated by earlier reports, the hypotensive effect of reserpine observed in rats is due to the depletion of catecholamines from the peripheral stores [10,14,56,57].

The effect of equimolar doses of RMN also indicated hypotension, however, with higher doses compared with reserpine. It is further indicated that quaternization of reserpine not only restricted the entry of RMN to the CNS but also reduced entry to target tissue in the periphery. Hence, relatively higher doses were required to produce a reserpine-like effect. Mechanistically, the hypotensive actions of RMN could also be due to peripheral depletion of catecholamines.

Finally, the present study indicated that quaternization of reserpine had not abolished the hypotensive response but that only higher doses were required. The added advantage that was seen with RMN was its nonsedative nature as observed from its inability to exert the central actions of reserpine.

Highlights

- Reserpine methonitrate (RMN), a quaternary analog of reserpine, was synthesized and evaluated for its central and peripheral actions in comparison to reserpine.
- RMN at doses equal to and double the equimolar doses of reserpine did not produce any central effects.
- RMN produced a dose-dependent reduction in blood pressure of anesthetized rats, although with higher doses compared with reserpine and increased the recovery time with an increase in dose.
- The hypotensive actions of RMN could also be due to its peripheral depletion of catecholamines.
- The studies indicated that quaternization of reserpine not only attenuated the entry of RMN into the CNS but also reduced to the target tissue in the periphery.

Furthermore, observations of the blood pressure-lowering response of RMN at low dose levels compared with conventional to investigate further with a view to advancing clinically availability.

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