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Effects of Route of Administration on the Chronic Toxicity of Reserpine*

JOHN A. ROSECRANS

Departments of Pharmacology, University of Michigan, Ann Arbor, Michigan and University of Pittsburgh, Pittsburgh, Pennsylvania

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WESTERMANN (1962) initially observed that single doses of reserpine were more potent in lowering brain amine levels when given intramuscularly than when administered intraperitoneally. More recently BHAGAT (1964) has reported comparable differences when cardiac amine levels were also studied following various single doses of reserpine. His data clearly demonstrates that both the intramuscular and the subcutaneous routes were more potent than the intraperitoneal route of administration. It has further been demonstrated in these laboratories that the degree of toxicity from chronic reserpine treatment can also be influenced by route of administration.

Methods

HOLTZMAN (Wisconsin) male albino rats were used in this experiment. Reserpine (kindly supplied by CIBA, Inc., Summit, N.J.) was administered daily either subcutaneously or intraperitoneally in the following vehicle: 5% polyethylene glycol 300, 1% benzyl alcohol and 0.25% citric acid. Control rats received 1 ml/kg of this vehicle. Experimental groups were selected randomly and animals were housed two/cage for convenience in estimating food and water consumption. Food and water intake was measured by difference. Each group received 50 g/day of purina rat chow which was placed directly inside the cage. These animals were also presented 125 ml/day H_2O via standard water bottles.

Due to the high degree of physical deterioration and difficulty in handling chronic reserpinized animals, certain criteria for the duration of any single experiment were used. Drug administration to any experimental group was terminated when either an LD-50 was attained, or when the mean body weight of the group had decreased by more than 30%.

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Results

Effects of the Chronic Administration of Reserpine on the Growth Rate of Rats when Administered Intraperitoneally

In a previous investigation ROSECRANS and DEFEO (1965) observed that the chronic administration of reserpine to male albino rats did not induce severe cumulative effects when administered by the intraperitoneal route (1 mg/kg for 30 days). Since this observation was not anticipated, and was in conflict with our previous knowledge of reserpine's pharmacology, the particular experiment in question was repeated. The results were essentially the same (Table 1). Reserpine produced few toxic effects, and did not severely impair the feeding behavior of these animals. This was true for at least 42 daily doses of reserpine.

Table 1. *Effects of reserpine (1 mg/kg, i.p.) on the feeding behavior and growth rate of male albino rats (42 daily doses)*[1]

	Initial body weight g	Final body weight g	Mortality rate Dead/N	Mean daily food intake g	Mean daily water intake ml
Controls	148 ± 14	348 ± 34	0/12	19.7 ± 1.4	33.9 ± 2.9
Reserpine	158 ± 3	217 ± 9	4/24	15.7 ± 1.9	27.8 ± 2.2
% Control	104	63		84	83

[1] All values are means ± S.E. Also all data are expressed/animal.

The sedation produced by this regimen did not appear to be cumulative as indicated by the gross behavior of these animals. Maximal sedation developed within 8 hours, whereas minimal sedation was apparent with in 24 hours. Complete recovery to normal behavior was not observed, but the degree of sedation at both the 8th and the 24th hour was similar on each day of reserpine administration. An extremely interesting behavioral syndrome developed in most animals following 15 doses of reserpine. 24 hours following a previous dose reserpinized animals became extremely hyperexcitable and hypersensitive to handling and sound stimuli. In some cages an increase in fighting behavior was also observed. Unfortunately this behavior was evident until about the 30th day of administration and was greatly diminished thereafter.

Two other factors, although not studied extensively, may also be important in maintaining animals for prolonged periods on their daily reserpine regimen. Early in these studies it was recognized that animals paired with a minimal difference in body weight (not more than 15 g), exhibited less toxicity following daily reserpine treatment. Thus, it is recommended that partners demonstrating a great body weight variation, while under reserpine treatment, be reassigned new mates in order

to minimize the difference. This procedure was carried out routinely on each day of the experiment. A second factor which also appears to be important, is the weight of the animal at the initiation of an experiment. Animals weighing 280—300 g (90—120 days old) were found extremely sensitive to chronic reserpinization. 18 of 24 animals died following 14 doses of reserpine (1 mg/kg, i.p.) with a mean body weight loss of 100 g. As observed consistently throughout these studies, animals weighing 140—160 g (30—45 days old) tolerated reserpine best.

Effects of the Chronic Administration of Reserpine on the Growth Rate of Rats when Administered Subcutaneously

In contrast to the intraperitoneal administration of reserpine, 1 mg/kg when administered subcutaneously induced an LD-50 within 8 daily doses. To obtain a better picture of this initial observation, a second

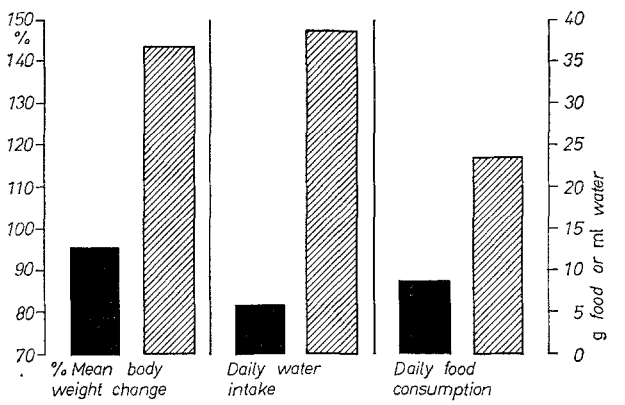

Fig. 1. Effects of 8 daily doses of reserpine (0.5 mg/kg) on the growth rate and daily mean food and water intake of male albino rats. At the initiation of this experiment, animals weighed 136—156 g (N = 48). The mortality rate of animals receiving reserpine subcutaneously was 50%, by the eighth day. ■ subcutaneous; ▨ intraperitoneal

experiment was conducted in which the dose of reserpine was reduced to 0.5 mg/kg (Fig. 1). At this dosage the subcutaneous route was certainly more toxic. A progressive depression of both the gross behavior and feeding behavior was also evident. On the other hand, there was no evidence of any cumulative effect when 0.5 mg/kg reserpine was administered i.p. The sedative effect was mild, and there was no apparent depression of either the growth rate or feeding behavior during the duration of the experiment.

To determine the best tolerated s.c. dose of reserpine, a third experiment was conducted (Table 2). In this last experiment, slightly larger

animals were used in order to obtain a better spread of the data. A positive growth rate was obtained with only the smallest dose of reserpine (0.125 mg/kg), whereas maximal toxic effects were again observed with the 0.5 mg/kg dose. In relation to the i.p. route, 0.125 mg/kg appeared equivalent to 0.5 mg/kg.

Table 2. *Effects of graded repeated subcutaneous doses of reserpine on the feeding behavior and growth rate of male albino rats*[1]

Dose mg/kg	n	Mean daily food intake g	Mean daily water intake ml	Mean % change of initial body weight %
0.125	6	23.9 ± 2.6	37.7 ± 5.7	+ 18.0 ± 11.4
0.250	6	15.4 ± 8.3	25.7 ± 7.3	− 1.7 ± 2.8
0.500	6	11.9 ± 11.7	20.3 ± 4.7	− 22.3 ± 5.3

[1] All values are means ± S.E. and all data are expressed/animal (25—27 determinations). This experiment was conducted for 8 days at which time 50% of the animals receiving 0.500 mg/kg died. Initial weight of these animals was 205 g.

Discussion

Classical pharmacology teaches us that, in general, the order of drug in relation to route of administration is as follows: intravenous > intraperitoneal > subcutaneous > intramuscular > oral route. However, in contrast to the anticipated effect, reserpine produced a much greater effect when administered subcutaneously, as compared to the intraperitoneal route. WESTERMAN (1962), in an extensive study of the influence of route of administration on the potency of certain drugs, discussed such variability. He concluded that, for a drug to be more potent when administered intramuscularly or subcutaneously, as compared to the intraperitoneal route, its rate of metabolism must be extremely rapid. In fact, for such a difference to occur, the rate of metabolism must be of the same order as, or greater than the rate of absorption. Therefore, it is suggested that the differences between routes in reserpine's toxic effects are the result of less of the drug being metabolized following a subcutaneous dosage.

The behavioral manifestations described here are certainly interesting, but are at present difficult to explain. In support of these observations, a recent study has also demonstrated that reserpine can greatly facilitate the hyperexcitability of animals receiving an acute dose of amphetamine (MORPURGO and THEOBOLD, 1966). In fact, the described social behavior of these animals, for the most part, is characteristic of that behavior experienced in this investigation following 15 daily doses of reserpine. Nevertheless, it is clear that reserpine can induce a variety of behavioral effects, and depending on the route of administration and dosage used, one may observe any number of them.

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JOHN A. ROSECRANS, Ph. D.
Department of Psychiatry
Yate University School of Medicine
333 Ceder Street
New Haven, Connecticut, U.S.A.

Erratum

The Effects of Posttrial Injections of Pentylenetetrazole, Strychnine and Mephenesin on Discrimination Learning

JARA KRIVANEK and EARL HUNT

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The legend to Fig. 1 on page 192 should be read: Decision speeds during the learning phase.