A Facilitation Action of Reserpine on the Central Nervous System. (21149)

GRAHAM CHEN, CHARLES R. ENSOR, AND BARBARA BOHNER.
From the Research Laboratories, Parke, Davis & Company, Detroit, Mich.

Preparations from *Rauwolfia serpentina* have been used for centuries in Indian medicine for insomnia, hypochondria, and insanity (1). A sedative effect of these preparations was ascribed to be the rationale for therapy (2,3). Reserpine, the alkaloid isolated from *R. serpentina* recently, has been shown to possess a sedative property(4). In our studies of the influence of various agents on convulsive patterns in mice, reserpine was found to be capable of facilitating the extensor tonic component of the convulsion. This action is contrary to that of other central nervous system depressants which generally suppress the tonic extensor phase(5). The facilitation action of reserpine on the central nervous system appears to be specific in that it antagonizes selectively the anticonvulsant effects of the different depressants. This property of reserpine may be of diagnostic and therapeutic significance when applied to mental disorders.

In this report data are presented to show how reserpine affects the convulsive patterns induced in mice by chemical and electrical stimulation. The results obtained from experiments by antagonism between reserpine and the various anticonvulsant agents are to be reported in another communication.

Experimental. The reserpine, isolated from *R. serpentina* Benth. by Drs. Fleming and Crooks of the Research Laboratories, Parke, Davis & Co., was finely suspended in a gum acacia solution for intraperitoneal injection. Male albino mice weighing 18-22 g were used. They were allowed free access to food and water. Metrazol-, caffeine-, and strychnine-induced convulsions were produced by the intravenous infusion technic of Orloff, Williams, and Pfeiffer(6). Electrically-induced seizures were brought about in 2 ways: (a) with a low frequency stimulus similar to that employed by Toman(7), and (b) with a high frequency stimulus for maximal tonic convulsions as devised by Toman, Swinyard, and Goodman(8).

Results and discussion. The data in Table I indicate the influence of reserpine on seizure patterns of Metrazol-, caffeine-, and strychnine-induced convulsions. As described by Orloff, Williams, and Pfeiffer, Metrazol produces 3 "signs" of reaction which follow each other as the concentration of Metrazol is gradually increased in the blood stream. The first is a sharp twitching of the animal's body; this is followed very shortly by a series of clonic movements. After a period of alternate clonic movements and resting phases, the third sign, "persistent convulsion," occurs. It consists of a tonic flexion followed immediately by a tonic extension of the limbs. In the results here presented, only 2 endpoints were chosen for the seizure patterns by Metrazol: *viz.* the first sign of clonic movements and the final tonic extension component of the convulsion. Caffeine-induced convulsions are predominantly tonic seizures. Some clonic movements occur just before the tonic extension of the fore limbs. This, in a brief interval, is followed by the extension of hind limbs and usually death. The appearance of clonic movements was taken as one endpoint in our experiments. The full tonic extension of the hind limbs is the endpoint of injection. With strychnine, the mouse undergoes merely a short flexor and long extensor seizure of the limbs without eliciting any other premonitory signs as in the case of Metrazol and caffeine.

The effect of reserpine on Metrazol-induced seizures is a more rapid onset of the tonic phase of the convulsion. The final maximal extension of the limbs follows immediately the first sign of clonic movements. In other words, under the influence of reserpine, Metrazol will produce a seizure pattern in mice similar to that produced by strychnine. This is indicated by the fact that the same quantity of Metrazol employed for inducing the first clonic movements in untreated mice will produce a tonic extension seizure in reserpine-treated animals.

Reserpine exerted a similar effect on mice

TABLE I. Influence of Reserpine on Seizure Patterns of Mice Receiving Intravenous Infusion of Metrazol, Caffeine or Strychnine at Rate of 0.05 cc/10 Sec. 10 mice in each group.

Treatment	Metrazol (0.5%)			Caffeine (1.0%)			Strychnine (0.005%)	
	Wt. g	F.C.,* cc	T.E.,* cc	Wt, g	F.C., cc	T.E., cc	Wt, g	T.E., cc
Control	18-22	.14±.01	.44±.02	18-22	.29±.03	.36±.02	19-22	.34±.02
Reserpine, 8 mg/kg IP, 4-6 hr	19-22	.15±.00	.16±.01	18-20		.18±.02	18-22	.33±.01

* F.C., first clonic movements; T.E., tonic extension of hind limbs; mean value of cc solution and stand. errors.

with caffeine-induced seizure patterns. Here the amount of caffeine required for a maximal tonic extensor seizure in reserpine-treated mice is considerably less than that required for the first sign of clonic movements in untreated animals.

On the other hand, reserpine did not alter the strychnine-induced seizure patterns nor the dosage required for maximal tonic extensor response. That reserpine is capable of facilitating the tonic extensor seizure response in mice to a convulsive dose of Metrazol or caffeine but not to that of strychnine suggests that its site of action may be somewhere at the regions or pathways where both Metrazol and caffeine exert their analeptic action on the central nervous system.

This central effect of reserpine is slow in onset and long in duration. The dose and effect of reserpine at different periods after administration in Metrazol-induced seizures are given in Table II. No significant facilitation effect was evident 2 hours after the intraperitoneal injection of 4 mg kg reserpine (section A). This is shown either by the quantity of Metrazol required for inducing tonic extensor seizures or by the number of mice in which tonic extensor response followed the first clonic movements immediately. Only half of the animals were affected at the third or fourth hour. An intraperitoneal dose of 8 mg/kg of reserpine for a period of 4 hours was found to produce a full effect in all animals (section B). This dose and the time interval were subsequently employed in other experiments for its maximal effect.

The prolonged effect of reserpine is indicated by the data in section C. A facilitated tonic extensor response was observed in mice as long as 6 days after 2 mg/kg of reserpine had been administered intraperitoneally.

In Table III are presented the results obtained from experiments in mice receiving electrical stimuli. With a current of 50-100 m.a., rectangular pulses of 6 per second frequency, and 1 msec. width, a "stunning" seizure response was induced in animals without medication. In this condition the mouse assumes an upright position without extension of the limbs. Under the influence of 8 mg/kg reserpine (4 hours after intraperitoneal injection), stimuli of equal magnitude produced a maximal tonic extension of the limbs in all animals. Half the reserpine-treated mice gave a maximal tonic extensor response to 24 milliamperes. For a tonic extensor response in untreated mice, either a higher frequency or a greater amperage of current was required. In this experiment, a current of 88 milliamperes and 9 per second frequency was found to produce a tonic extension of the hind limbs in 50% of the untreated animals.

In the case of maximal tonic extensor seizures induced in mice by a 60-cycle alternating current, the threshold stimulus was found to be markedly lowered by reserpine. The results in Table IV were obtained in an experiment in which the threshold stimuli were determined for mice 24 hours after various doses of reserpine were administered intraperitoneally. Each dose of the drug was given to 30 animals. They were divided into 3 groups of 10 mice each and shocked with 3 different current intensities which would produce a maximal tonic extensor response in from 10 to 90% of the test animals. The current threshold that would cause convulsions in 50% of the mice (CS_{50}) was estimated graphically by probit units and current intensities in logarithms(9). The data indicate that the threshold stimulus for mice was significantly lowered by 0.25 mg/kg of

TABLE II. Dose and Effect of Reserpine in Metrazol-Induced Seizures by Intravenous Infusion in Mice (0.5%, 0.05 cc/10 Sec). 10 mice in each group.

(A) Time of onset of reserpine effect, 4 mg/kg intraperitoneally

Time after inj. (hr)	Wt, g	Seizures F.C., cc	Seizures T.E., cc	Seizures (T.E. – F.C.) cc ± S.E.	Diff. in (T.E. – F.C.) after inj. cc	"t"	"P"	No. mice, F.C.→T.E.*
0	19-21	.15	.44	.30 ± .03				0
1	19-22	.16	.43	.27 ± .09	.03	1.4	>.1	1
2	19-22	.14	.38	.24 ± .04	.06	1.0	>.1	1
3	19-21	.15	.28	.13 ± .04	.16	3.1	<.01	5
4	18-20	.15	.26	.11 ± .04	.18	3.3	<.01	6

(B) Effect of reserpine at various dose levels, 4 hr after intraperitoneal inj. 10 mice in each group.

Dose, mg/kg	Wt, g	Seizures F.C., cc	Seizures T.E., cc	Seizures (T.E. – F.C.) cc ± S.E.	Diff. in (T.E. – F.C.) for dose given cc	"t"	"P"	No. mice, F.C.→T.E.*
0	18-21	.14	.44	.30 ± .03				0
1	19-22	.16	.48	.32 ± .02	.02	.5	>.1	0
2	19-22	.13	.37	.24 ± .05	.06	1.1	>.1	2
4	19-22	.15	.25	.10 ± .04	.20	3.7	<.01	5
6	19-22	.14	.23	.09 ± .04	.21	3.9	<.01	6
8	20-22	.15	.16	.01 ± .01	.29	8.2	<.01	10

(C) Duration of reserpine effect, 2 mg/kg intraperitoneally

Days after inj.	1	2	3	4	5	6	7	8	9	10
No. mice, F.C.→T.E.*	3/3	3/3	2/3	3/3	3/3	2/3	1/3	0/3	0/3	0/3

* F.C.→T.E. = Tonic extensor seizures followed first clonic movements immediately.

reserpine. Only above 1 mg/kg did the animals show the other symptomatic effects of reserpine, *viz.*, ptosis, depression, and diarrhea. The lowering of a maximal tonic extensor seizure threshold of current by reserpine had also been observed by Jenny at 40 mg/kg orally(10). In our experience reserpine is nearly 10 times more effective intraperitoneally than orally.

In view of the fact that Dilantin specifically suppresses the maximal tonic extensor seizures of mice under electroshock(5), the influence of reserpine on the anticonvulsant effect of Dilantin was investigated as follows: Thirty mice received 5 mg/kg of reserpine and 15 mg/kg of Dilantin intraperitoneally; another 30 animals as controls received the same amount of Dilantin only. The electroshock procedure was conducted as in the above experiment 6 hours after reserpine and 3 hours after Dilantin administration. The CS_{50} of the Dilantin and reserpine-treated mice and that of the Dilantin-treated controls were found to be 11.2 ± 1.2 and 51.5 ± 10.3 m.a., respectively, giving a significant difference of 30.3 ± 7.0 m.a.(11). Reserpine thus antagonizes the anticonvulsant effect of Dilantin. As will be discussed together for other anticonvulsant agents in another communication, the reaction between reserpine and Dilantin seems to be competitive in that the effect of one may be reversibly antagonized by that of the other.

The mode of this facilitation action of reserpine on the central nervous system awaits investigation. It may be that reserpine causes an increase in excitability or in conductivity of certain central nervous system structures. Some of its beneficial effects in mental patients may be due to this property. The evidence here presented points out that a precaution should be taken when using reserpine in epileptic patients to guard against possible precipitation of seizure attacks by the same mechanism. Reserpine may serve, on the other hand, as an activating agent to bring on

epileptic discharges electroencephalographically. Some caution should be taken also in reserpine-treated patients on convulsive therapy either by chemical or by electrical stimulation. The dosage of a convulsant such as Metrazol or the current intensity required will be less, while the tonic extensor phase of the convulsion is expected to be facilitated under the influence of reserpine.

Summary. 1. Data are presented to show a facilitation action of reserpine on the central nervous system. The drug hastens the onset of maximal tonic extensor seizures in mice receiving intravenous Metrazol or caffeine (but not strychnine). It lowers the convulsive seizure threshold of mice to electrical stimuli and antagonizes the anticonvulsant action of Dilantin. 2. Some possible mechanisms of action and certain clinical implications of this property of reserpine are suggested.

TABLE III. Influence of Reserpine on Seizure Patterns of Mice Induced by a Low Frequency Rectangular Pulse Current.

Treatment, mg/kg I.P.	Current Cycles /sec.	m.a.	"Stunning" response No. reacted / No. used	Tonic extension No. reacted / No. used
0	6	25	10/10	0/10
0	"	50	10/10	0/10
0	"	100	10/10	0/10
Reserpine, 8*	"	10	5/5	0/5
	"	15	5/5	0/5
	"	20	7/10	3/10
	"	25	4/10	6/10
	"	35	1/10	9/10
	"	50	—	10/10
	"	100	—	10/10
0	9	50	9/10	1/10
0	"	75	6/10	4/10
0	"	100	5/10	5/10
0	12	100	—	10/10

T.E.$_{50}$† reserpine = 24 ± 1.4 m.a., 6 ~
T.E.$_{50}$ untreated = 88 ± 7.9 m.a., 9 ~

* 4.5 hr after inj.
† Tonic extension seizures in 50% of animals.

TABLE IV. Effect of Reserpine on Maximal Tonic Extensor Seizures in Mice Induced by a 60 Cycle Alternating Current for 0.2 sec. Thirty mice in each group.

Dose of reserpine, mg/kg IP (24 hr)	CS_{50},* m.a. ± S.E.	Difference† in CS_{50}	Significance "t"	Significance "P"
0	5.09 ± .10			
.0125	4.90 ± .09	.19	1.40	>.1
.025	4.80 ± .09	.29	2.10	.05
.05	4.71 ± .11	.38	2.50	.02
.10	4.72 ± .11	.37	2.40	.02
.25	4.70 ± .07	.39	3.10	<.01
.50	4.53 ± .07	.56	4.50	"
1.00	4.26 ± .07	.83	5.90	"
2.00	3.55 ± .06	1.54	11.30	"
4.00	3.10 ± .05	1.99	16.00	"

* CS_{50}—Convulsive stimulus for 50% of mice.
† Difference in convulsive stimuli required for untreated controls and for reserpine treated animals.

1. Chopra, R. N., Gupta, J. C., and Mukherjee, B., *Ind. J. Med., Res.,* 1933, v21, 261.
2. Chopra, R. N., Gupta, J. C., Bose, B. C., and Chopra, I. C., *ibid.,* 1943, v31, 71.
3. Datta, S. C., *Ind. J. Pharm.,* 1949, v11, 105.
4. Müller, J. M., Schlittler, E., and Bein, H. J., *Experientia,* 1952, v8, 338.
5. Chen, G., and Ensor, C. R., *Arch. Neurol. and Psych.,* 1950, v63, 56.
6. Orloff, M. J., Williams, H. L., and Pfeiffer, C. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 254.
7. Toman, J. E. P., *Neurology,* 1951, v1, 444.
8. Toman, J. E. P., Swinyard, E. A., and Goodman, L. S., *J. Neurophysiol.,* 1946, v9, 231.
9. Miller, L. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 1261.
10. Jenny, E. H., *Fed. Proc.,* 1954, v13, 370.
11. Burn, J. H., *Biological Standardization,* p31, 1937, Oxford University Press.

Received May 26, 1954. P.S.E.B.M., 1954, v86.