

CONVULSANT ACTION OF Ro-4-1778 AND ITS MODIFICATION BY BARBITURATES AND TRANQUILLISERS

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The convulsant action of the analgesic Ro-4-1778 was studied in rats at three different dose levels. Pentobarbitone protected the rats against convulsions but the mortality of rats increased. Reserpine and chlordiazepoxide protected the rats at some dose levels; protection is attributed to amine depletion in the case of the former and known anticonvulsant action in the case of the latter.

This compound (Ro-4-1778) which is chemically (1-(p-chlorophenethyl)—6-7 dimethoxy-2-methyl 1, 2, 3, 4 tetrahydro isoquinoline) was synthesised by Brossi et al. (1960). Its analgesic activity in rats has been reported by Gupta and Gaitonde (1964). An observation was made that the drug produced analgesia in a dose range 10-50 mg/Kg. but in a few animals receiving a dose of 50 mg/Kg., there was an evidence of hyperexcitability and some animals even developing convulsions. With higher doses of Ro-4-1778, the convulsant action became more marked.

It was thought worthwhile to study the convulsant action of the drug and effect of pentobarbitone, reserpine, and chlordiazepoxide in modifying the convulsions to help in elucidating the probable site and mechanism of action.

MATERIAL AND METHODS

Albino rats weighing between 65-220 Gms. were employed. These were distributed randomly so that each group consisted of more or less similar number of large and small rats.

Four groups of 10 rats each were taken and were given Ro-4-1778 in 3 different doses and were observed as to the onset of convulsions and ultimate result in survival or death.

In another group of 10 rats, pentobarbitone sodium 30 mg/Kg. was given to find out the onset and duration of loss of righting reflex.

Study of the modification of effects of Ro-4-1778 by pentobarbitone, reserpine and chlordiazepoxide was made as follows. Pentobarbitone 30 mg/Kg. was immediately followed by Ro-4-1778 in three different doses—in three groups of rats; other groups of rats were treated with reserpine 2.5 mg/Kg. on two consecutive days—24 and 48 hours before administration of Ro-4-1778 in three different doses. Chlordiazepoxide—10 mg/Kg. was soon followed by Ro-4-1778 in three different doses. All injections were given intraperitoneally. The doses of Ro-4-1778 in

control rats as well as in the drug treated groups were 50,60 and 80 mg/Kg. respectively.

RESULTS

Ro-4-1778 : The effect of Ro-4-1778 alone is shown in Table I. Out of the 10 rats given a dose of 50 mg/Kg. Ro-4-1778, seven developed convulsions and one amongst them died. The convulsions developed after a mean interval of 15.3 minutes (S. E. \pm 1. 2.). To start with the animals developed hyperexcitability, twitches became more frequent and clonic convulsions developed. With 60 mg/Kg. 9 out of 10 developed convulsions which is again clonic in type. The convulsions developed after a mean interval of 10.9 minutes (S. E. \pm 0.86) and all the animals developing convulsions died. Essentially similar observations were made with dose of 80 mg/Kg. (Table I). Here of course all the 10 rats died of convulsions. Results with higher doses of Ro-4-1778 were chosen to assess the protective actions, if any, of pentobarbitone, reserpine and chlordiazepoxide.

Pentobarbitone : A dose of 30 mg/Kg. pentobarbitone produced a loss of righting reflex within a mean period of 6.1 minutes (S.E. \pm 0.86) and the mean duration of the loss of righting reflex was 47.6 minutes. (S. E. \pm 6.6).

In three groups of rats, pentobarbitone was immediately followed by Ro-4-1778. The results are shown in Table II. With a dose of 50 mg/Kg. Ro-4-1778 administered immediately after pentobarbitone the righting reflex was lost within a mean period of 7.1 minutes (S.E. \pm 1.8). None of the rats developed convulsions but all died. With a dose of 60 mg and 80 mg/Kg. Ro-4-1778, the righting reflex was lost after mean interval of 5.5 minutes (S.E. \pm 0.64) and in 6.7 minutes (S.E. \pm 0.92) respectively. Again none of the rats survived.

Results show that though animals are protected from convulsions, the toxicity is increased resulting in more number of deaths in animals. There is statistically no difference as regards the onset of the loss of righting reflex in the three groups given different doses of Ro-4-1778.

Reserpine : A dose of 5 mg/Kg. reserpine given in two doses is known to produce depletion of 5HT and catecholamines from the central nervous system (Pletscher et al., 1956, Paasonen and Vogt, 1956 and Holzbauer and Vogt, 1956). It was thought that if reserpine modifies the convulsant action it might elucidate the mechanism of action of Ro-4-1778. The results are shown in Table I. It is seen that reserpine treated animals when given Ro-4-1778, 50 mg/Kg., out of 10 rats 6 did not develop convulsions, rest developed within a mean period of 45.0 minutes (S.E. \pm 3.2); 2 rats died of convulsions. With a dose of 60 mg/Kg. of Ro-4-1778 convulsions developed in 9 rats out of 10 within a mean period of 21.0 minutes (S.E. \pm 2.5); 4 rats died of convulsions. With a dose of 80 mg/Kg. of Ro-4-1778 convulsions developed in 8 rats out of 10 within a mean period of 13.5 minutes (S.E. \pm 3.9) and all the rats developing convulsions died.

TABLE I.

Effect of Ro-4-1778 alone and in combination with fixed doses of Pentobarbitone, Reserpine and Chlordiazepoxide

Dose of Ro-4-1778 in mg/Kg.		Ro-4-1778	Ro-4-1778 and Pentobarbitone 30 mg/Kg.	Ro-4-1778 and Reserpine 5 mg/Kg. in two divided doses	Ro-4-1778 and Chlordiazepoxide 10 mg/Kg.
50	No. of rats having convulsions ...	7 (10)	0 (10)	4 (10)	5 (10)
	P value ...	—	<0.01*	>0.05*	>0.05*
	Onset of convulsions in minutes ...	15.3±1.2†	—	45.0±3.2†	16.0±1.0†
	No. of rats dead ...	1 (10)	10 (10)	2 (10)	1 (10)
	P value ...	—	<0.01*	>0.05*	—
60	No. of rats having convulsions ...	9 (10)	0 (10)	9 (10)	6 (10)
	P value ...	—	<0.01*	—	>0.05†
	Onset of convulsions in minutes ...	10.9±0.86†	—	21.0±2.5†	14.5±0.9†
	No. of rats dead ...	9 (10)	10 (10)	4 (10)	4 (10)
	P value ...	—	>0.05*	<0.05*	<0.05*
80	No. of rats having convulsions ...	10 (10)	0 (10)	8 (10)	9 (10)
	P value ...	—	<0.01*	>0.05*	>0.05*
	Onset of convulsions in minutes ...	9.5±1.04*	—	13.5±3.9†	14.0±0.9†
	No. of rats dead ...	10 (10)	10 (10)	8 (10)	3 (10)
	P value ...	—	—	>0.05*	<0.01*

Figures in parantheses denote the number of rats used in each group.

* versus Ro-4-1778.

P value is calculated by chi square test.

† The values are mean ± standard error.

TABLE II.

Effect of Pentobarbitone alone (30mg/Kg.) and in combination with different doses of Ro-4-1778 in rats

	Pentobarbitone 30 mg/Kg.	Doses of Ro-4-1778 in mg/Kg. with Pentobarbitone 30/mg/Kg.		
		50	60	80
Onset of loss of righting reflex in minutes ...	6.1 ± 0.86*	7.1 ± 1.8*	5.5 ± 0.64*	6.7 ± 0.92*
Duration of loss of righting reflex in minutes ...	47.6 ± 6.6*	†	†	†
No of animals dead ...	0 (10)	10 (10)	10 (10)	10 (10)

*The values are mean of 10 observations ± Standard error.

Figures in parantheses denote the number of rats used in each group.

†Animals never recovered and ultimately died.

Results with reserpine show that it protects animals against the convulsant action of the drug both as regards the onset of convulsions which developed after a longer interval and as regards the number of animals developing convulsion.

Chlordiazepoxide: Chlordiazepoxide a nonphenothiazine tranquilliser was administered in a dose of 10 mg/Kg. intraperitoneally and was soon followed by Ro-4-1778. Chlordiazepoxide alone in this dose failed to show any effect. However, since it has been reported earlier (Gupta and Gaitonde, 1964) that it potentiates the analgesic action of morphine and Ro-4-1778, it was felt that it might as well modify the convulsant action of Ro-4-1778. With a dose of 50 mg/Kg. Ro-4-1778 administered immediately after chlordiazepoxide 5 rats out of 10 developed clonic convulsions within a mean period of 16.0 minutes (S.E. \pm 1.0). Before developing convulsions all the rats assumed a peculiar posture with hind paws wide apart possibly due to skeletal muscle relaxation. All the animals except one completely recovered; one rat only in this series died. With a dose of 60 mg/Kg. Ro-4-1778 administered immediately after chlordiazepoxide 6 rats out of 10 developed clonic convulsions. Convulsions developed within a mean period of 14.5 minutes (S.E. \pm 0.9); 4 rats out of 10 died. With a dose of 80 mg/Kg. Ro-4-1778 administered after chlordiazepoxide, 9 rats out of 10 developed clonic convulsions within a mean period of 14.0 minutes (S.E. \pm 0.9); 3 rats out of 10 died of convulsions.

Results with chlordiazepoxide show that it has a protective action against convulsant action of Ro-4-1778.

An observation was made during the study that the severity of the convulsion was more in the smaller rats.

DISCUSSION

The analgesic and the central excitatory action of Ro-4-1778 has been reported earlier (loc cit). It is not unusual for a compound to have multiplicity of actions. An analgesic of the type of morphine has excitatory action on the spinal cord.

The drug Ro-4-1778 produced a very well marked clonic convulsion the onset of which was a matter of dose as seen in the Table I, with higher doses the convulsions developed much more quickly, though the difference is not always statistically significant. Since the convulsions were of clonic type, it must have had its origin at supraspinal level. An observation was made that the severity of convulsion was more in rats weighing less than 100 Gm. though the ultimate outcome was not different. This may be because of some biochemical difference between the larger and smaller rats (Ferrini and Glasser, 1963).

To elucidate the mechanism or mode of convulsant action of Ro-4-1778, pentobarbitone, reserpine and chlordiazepoxide were used.

Drugs which elicit convulsions usually counteract the depressant effect of barbiturates without being specific competitive antagonists, but exerting a functional

type of antagonism (Joseph, 1961). It was thought that barbiturates would protect the animals against convulsions and reduce the mortality. It was found that all the rats given barbiturates were protected against convulsions but the barbiturate loss of righting reflex time was very much prolonged and mortality too was increased. This could be explained by assuming each of the two drugs to have at least two loci of action—at one they are synergistic and at the other antagonistic. Another inference possibly could be drawn that death of animals after Ro-4-1778 is not per se attributable to the convulsant action, since even when the convulsions are prevented, the toxicity is not only not prevented but very much aggravated and results are highly significant ($P < 0.01$) at all dose levels.

Reserpine is known to affect the convulsant and anticonvulsant actions of some drugs. Thus it is known that reserpine exacerbates some aspects of the convulsions produced in mice by injection of leptazol and caffeine and lowers the threshold for electroshock seizures. On strychnine, ammonium acetate or picrotoxin induced maximal tonic extensor seizures in mice, however, reserpine has a suppressive effect (Chen et al., 1954, Chen and Ensor, 1954, Chen and Bohner, 1956). Reserpine also antagonises the anti-convulsant activity of phenytoin, various barbiturates and myaensin. (Chen and Bohner, loc cit).

The effect of Ro-4-1778 in reserpine pretreated rats shows that the convulsions develop after a longer latent period which is more marked when the challenging dose of Ro-4-1778 is less. As regards the effect of reserpine on convulsions and death, though there is an apparent protective action it is not statistically significant in most of the cases. It is a conjecture from the study that the amine depletion may be concerned in the protective action of reserpine.

Chlordiazepoxide protected the rats against the lethal effects of Ro-4-1778 and not so much against the convulsant effects. There was slight delay in the development of convulsions also. This may be attributable to the anticonvulsant action of chlordiazepoxide as reported by Randall et al. (1960).

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