PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES OF α-METHYL-α-PHENYLGLUTARIMIDE

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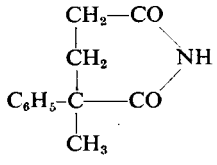
A NUMBER of derivatives of glutarimide have been reported to possess anticonvulsant activity, in particular α-methyl-α-phenylglutarimide and its *N*-methyl derivative[1].

Methylphenylglutarimide justified a more detailed investigation which is here reported. Included are studies of narcotic action since screening tests indicated that it was more active and had a longer duration of action than 3-methylpentyn-3-ol. The method of test previously described[1] was based on the maximal leptazol seizure pattern test of Goodman *et al*[2]. The method for the determination of narcotic activity allows an estimate to be made of the relative median narcotic doses (ND50) of methylphenylglutarimide and methylpentynol, and also compares the duration of action at different doses. Since the toxicity of the two compounds is not the same, the durations of action were compared at dose levels which bore the same relation to the respective estimates of LD50 and therefore allowed a similar margin of safety. This procedure has been used by others[3,4,5]. Finally, sub-acute toxicity tests employing mice, weanling rats and rabbits, were carried out.



α-methyl-α-phenylglutarimide

METHODS

General

The animals used throughout the investigations of acute toxicity, anticonvulsant activity, and narcotic action were female albino mice of Schofield strain, weighing approximately 20 g. Food was withdrawn overnight before use. The doses of all the compounds were administered in solution or suspension in 5 per cent. acacia solution, the volume being adjusted to 0·5 ml./20 g. of body weight. For the sub-acute toxicity experiments, the animals used and modes of administration of the compounds are stated in the appropriate section.

Acute Toxicity

Methylphenylglutarimide and methylpentynol were administered orally at dose levels which increased in geometrical progression by a factor of 1·1, 20 mice being used at each dose. The animals were then kept at approximately 18° C. for 5 days with free access to food (Diet 41) and

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water, and the LD50 estimates (and confidence limits, P = 0·95) were made from the number of deaths at the end of this period, using the method of Litchfield and Wilcoxon[6].

Anticonvulsant Activity

Varying doses of methylphenylglutarimide and aloxidone were administered orally to groups of 20 mice in doses which increased by a factor of 1·5. Two hours later, all the animals receiving this premedication were given an intravenous injection of 60 mg. of leptazol per kg. (0·2 ml. of a 0·6 per cent. solution per 20 g. of body weight), a dose known to induce convulsions in all but very resistant mice, and confirmed by observation in a control group of animals receiving no premedication. Only those animals showing the hind-leg tonic extensor component of the leptazol-induced seizure were considered to show positive response, and from these numbers in each group were calculated the percentage convulsing and hence the degree of protection. The PD50 and its confidence limits, (P = 0·95) was calculated according to the method of Litchfield and Wilcoxon[6].

Narcotic Activity

Varying doses of methylphenylglutarimide (0·23 to 0·77 g./kg.) and methylpentynol (0·35 to 0·77 g./kg.) were administered to groups of 30 marked animals, which were then kept in a ventilated cabinet at 29·5° C. for the next 6 hours. At 30 minute intervals the mice were placed on their backs, and if unable to right themselves within 30 seconds they were classed as showing a positive response. An estimate of the median narcotic dose (ND50) was derived by means of Kärber's formula[7] from the total number of mice at each dose level showing a positive response at any time during the period of observation.

As the animals were observed at fixed intervals, the time of onset of narcosis was recorded midway between the last time at which the righting reflex was present and the first time at which it was absent. The time of recovery was similarly estimated.

Duration of Narcosis

A fixed dose of methylphenylglutarimide and methylpentynol was administered to groups of marked mice. In the first experiment, using groups of 20 mice, the doses were respectively 0·46 and 0·56 g./kg. of body weight, corresponding to 70 per cent. of the respective LD50 estimates (see Experiment 1, Table I). In the second experiment, using groups of 40 mice, the doses were respectively 0·38 and 0·49 g./kg., i.e. 60 per cent. of the LD50 estimates (see combined results of Experiments 1 and 2, Table I). The animals were then kept in the ventilated cabinet at 29·5° C. for 7½ hours in the first experiment, and for 8 hours in the second. At 30 minute intervals, the mice were tested for abolition of the righting reflex. The time of onset of narcosis and the time of recovery were estimated as described above. The difference provides a measure of the duration of narcosis.

Subacute Toxicity

The animals used were female albino mice of Schofield strain weighing initially approximately 20 g.; weanling male rats of Tuck strain; and adult female rabbits of varying breeds.

(*a*) *Mice.* The mice were divided into groups of 20. Varying doses of methylphenylglutarimide and methylpentynol were administered orally, 5 days per week for 6 weeks, as suspensions or solutions in 0·5 ml. of 5 per cent. acacia per 20 g. of body weight. The actual doses were as follows:—methylphenylglutarimide, 380, 190 and 95 mg./kg.; methylpentynol, 490, 245 and 122 mg./kg. of body weight (corresponding to 60 per cent., 30 per cent., and 15 per cent. of the respective acute LD50 estimates). A control group received acacia solution alone. The animals were allowed free access to food (cube diet 41) and water, and their weights were recorded at the commencement of the experiment and at suitable intervals. Any deaths were also recorded, and after 6 weeks, the remaining animals were killed. Post-mortem examinations were made on several from each group.

(*b*) *Rats.* The rats were divided into groups of 10. Throughout the experiment they were fed on meal prepared according to diet 41, the amount being adjusted as they increased in weight, so that little wastage was found to occur. In the first group of animals, admixed with the meal was an amount of methylphenylglutarimide sufficient to provide each animal with an average daily intake of 150 mg./kg. (corresponding to 24 per cent. of the acute LD50 in mice). Similarly, those in the second group received an average daily intake of 50 mg./kg., i.e., 8 per cent. of the mouse LD50. The third group served as controls. The animals were weighed at frequent intervals for 7 weeks, and then killed. A histological examination was made of the liver, spleen and kidney from several animals of each group.

(*c*) *Rabbits.* The rabbits were fed daily with a mash of bran and oats, supplemented with hay, cabbage, carrots or turnips. Two animals served as controls and two received 30 mg. of methylphenylglutarimide per kg. of body weight (i.e., 5 per cent. of the mouse LD50) 5 days per week for 5½ weeks. The dose was administered by stomach tube in 5 ml. of 5 per cent. acacia solution/kg., the control animals receiving the acacia solution alone. Blood samples were taken twice weekly from the marginal ear veins of all four rabbits, and hæmoglobin estimations, erythrocyte counts, total leucocyte counts, and differential white cell counts were carried out. Body weights were also recorded on the same days.

Intravenous injection

Some preliminary experiments were made to find a suitable solvent for the intravenous administration of methylphenylglutarimide in rabbits. Five per cent. solutions in both propylene glycol and polyethylene glycol 400 were used, the control animals receiving the solvent alone.

RESULTS

Acute Toxicity

The results of two experiments to compare the acute toxicity in mice of methylphenylglutarimide and methylpentynol are given in Table I, and it can be seen that there is good agreement between them. If the two results are combined, the respective estimates are 0·63 g./kg. and 0·81 g./kg., thus methylphenylglutarimide is about 1·3 times as toxic as methylpentynol.

TABLE I

THE ACUTE ORAL TOXICITIES OF α-METHYL-α-PHENYLGLUTARIMIDE AND METHYLPENTYNOL IN FEMALE ALBINO MICE

Experiment	Compound	LD50 g./kg.	Confidence limits (P = 0·95) g./kg.
1.	Methylphenyl-glutarimide	0·63	0·59–0·67
	Methylpentynol	0·76	0·73–0·79
2.	Methylphenylglutarimide	0·63	0·59–0·67
	Methylpentynol	0·86	0·81–0·91

Anticonvulsant Activity

Table II contains the results of two experiments comparing the anticonvulsant activity of the new compound with that of aloxidone. These results are also in good agreement, and when the two sets are combined the values for the PD50 estimates are, methylphenylglutarimide, 62 mg./kg. and aloxidone, 195 mg./kg. Thus, the former appears to be about three times as active as the latter.

TABLE II

COMPARISON OF THE ANTICONVULSANT ACTIVITIES OF α-METHYL-α-PHENYLGLUTARIMIDE AND ALOXIDONE IN MICE INJECTED WITH LEPTAZOL

(60 mg./kg. body weight, intravenously)

Experiment	Compound	Median protective dose mg./kg.	Confidence limits (P = 0·95) mg./kg.
1.	Methylphenylglutarimide	65	55–77
	Aloxidone	215	175–264
2.	Methylphenylglutarimide	55	44–68
	Aloxidone	175	139–220

Narcotic Activity

The results of the experiment to compare the narcotic action of the compound and methylpentynol are summarised in Table III. For each dose level the table gives the number dead, the number of the remainder that were narcotised but subsequently recovered, the average time at which narcosis occurred and the average time at which narcosis ceased. The respective ND50 estimates are, methylphenylglutarimide, 0·34 g./kg. and methylpentynol, 0·43 g./kg. i.e., the former is about 1·3 times as active as the latter, and the results also suggest that of the two the new compound has a longer duration of action. Thus, at a dose level of 0·52 g./kg. for example, narcosis occurred about 30 minutes after the administration of methylpentynol and lasted for about 3 hours, but with methylphenylglutarimide, although the onset was slower, narcosis continued for more than 5¼ hours in most of the animals. Even at a dose level of 0·35 g./kg., it induced narcosis which lasted for more than 5¼ hours in 14 out of the 20 animals which lost the righting reflex. Thus it appears to have a longer duration of action than methylpentynol when the two substances are compared on a weight for weight basis and even

when doses in the ratio of 2:3 are compared. The latter finding is of considerable interest since the dose of methylphenylglutarimide is a smaller proportion of the LD50 than is the dose of methylpentynol.

The results of the experiments designed to compare the duration of narcosis produced by doses bearing a similar relation to the respective

TABLE III

COMPARISON OF THE NARCOTIC ACTIVITIES OF α-METHYL-α-PHENYLGLUTARIMIDE AND METHYLPENTYNOL IN FEMALE ALBINO MICE

Compound	Dose g./kg.	Number narcotised	Number failing to recover	Average time from injection (in hrs.) at which righting reflex was (*a*) lost	(*b*) regained
Methylphenylglutarimide ..	0·77	30/30	23/30	¼	>6*
	0·52	25/30	0/30	¼	>6
	0·35	20/30	0/30	¼	>6
	0·22	1/30	0/30	1¼	2¼
Methylpentynol	0·77	30/30	1/30	¼	>6
	0·52	26/30	0/30	¼	3¼
	0·35	3/30	0/30	¼	¾

* Where the sign ">" is used, it indicates that the righting reflex had not returned at the end of the 6 hour observation period in at least 70 per cent. of the animals at that dose level.

LD50 estimates confirm that methylphenylglutarimide is the longer-acting substance. In a preliminary experiment, in which a dose of 70 per cent. of the LD50 was given, methylpentynol produced narcosis in all 20 mice, and in two of them loss of the righting reflex occurred about 15 minutes after dosing and the narcosis lasted for more than 7¼ hours. In the other 18, however, the average induction time was about 30 minutes, and the duration of narcosis only about 4½ hours. With methylphenylglutarimide on the other hand, the righting reflex was still absent at the end of the observation period in 16 out of the 20 mice. As the average induction time for these animals was about 30 minutes, the duration of narcosis was evidently greater than 7 hours. Narcosis also occurred in the remaining 4 mice, the average times of onset and duration being about 30 minutes and 4½ hours respectively. When the doses were reduced to 60 per cent of the respective estimates of LD50, there were again marked differences between the two compounds. The administration of methylpentynol was followed by narcosis in 29 out of the 40 mice, the average induction time being about 30 minutes, and the average duration of narcosis being about 4 hours. Methylphenylglutarimide produced loss of the righting reflex in 35 out of the 40 mice, and in 20 of these the average time of onset was about 30 minutes and the average duration of narcosis about 5¾ hours. The average induction time for the remaining 15 was also about 30 minutes, but in these animals the righting reflex had not returned at the end of the observation period, i.e., the duration of narcosis was more than 7½ hours.

Sub-acute Toxicity

The effects of repeated administration of varying doses of the two substances on the growth rates of female albino mice are shown in Table IV. This records the average body weights of the different groups

of animals at intervals throughout the experimental period of 6½ weeks. No significant differences can be detected between the groups, i.e., the animals receiving the various doses of methylphenylglutarimide and

TABLE IV

EFFECT OF REPEATED ADMINISTRATION OF α-METHYL-α-PHENYLGLUTARIMIDE AND METHYLPENTYNOL ON GROWTH OF FEMALE ALBINO MICE

Compound	Dose (per cent. of LD50)	Average body weights (in g.) at stated times (in days) from beginning of experiment								Total number of deaths
		1	5	9	19	26	33	40	46	
—	—	27·7	21·2	23·0	27·2	26·3	27·4	26·6	27·0	2/20
Methylphenyl-glutarimide	60	22·3	21·4	23·7	25·6	27·4	27·7	28·0	29·7	6/20
	30	22·5	23·7	23·7	24·2	26·8	27·1	28·6	29·0	0/20
	15	22·7	23·9	24·7	27·2	27·5	28·5	29·2	30·9	2/20
Methylpentynol	60	22·1	20·7	22·7	25·7	26·5	27·5	27·6	28·6	3/20
	30	22·5	23·7	23·6	26·8	27·3	27·1	27·3	28·3	0/20
	15	22·3	23·5	24·7	27·5	27·8	28·6	28·8	30·0	1/20

methylpentynol all increased in weight at the same rate as the control mice. Also included in the table are the total mortalities which occurred in each group before the completion of the experiment. Those at the low dose of each compound may be attributed to external factors, being no greater than the number of control animals dying. At the high dose (380 mg./kg.) of methylphenylglutarimide, however, it will be seen that 30 per cent. of the animals eventually died, i.e., twice as many as in the group given the corresponding dose of methylpentynol. Post-mortem examination of the animals at the end of the experiment revealed no gross pathological changes in the animals in any of the groups. Thus, prolonged administration of the new compound does not appear to produce any marked deleterious effects on mice, except perhaps at a dose level corresponding to 60 per cent. of the acute LD50.

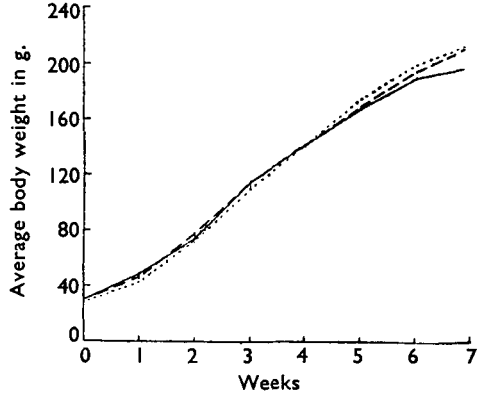

FIG. 1. Effect of continued administration of methylphenylglutarimide on the growth of weanling rats.

———— Treated group given 150 mg./kg./day.
. . . . . Treated group given 50 mg./kg./day.
– – – – – – Control group.

The average growth curves of weanling rats fed either on untreated meal or on meal containing different proportions of methylphenylglutarimide are compared in Figure 1. The curves for the three groups of animals are the same during the first 5 weeks, but the growth rate

of the animals given the high dose of the compound then appears to decrease, compared with that of those receiving the low dose and with that of the controls. Thus, although the average increases in weight of the controls and of the animals given this substance at a dose level of 150 mg./kg./day had previously been the same, during the final 13 days they were 42 g. and 29 g. respectively. However, these treated animals each received a total of approximately 7 g. of methylphenylglutarimide/kg. during the 7-week period of the experiment without the development of any other evident toxic effects. No significant differences between the control and the treated groups were revealed as a result of post-mortem examinations, including histological examination of the liver, kidney and spleen.

TABLE V

EFFECT OF REPEATED ADMINISTRATION OF α-METHYL-α-PHENYLGLUTARIMIDE ON MEAN BODY WEIGHTS, ERYTHROCYTE, TOTAL AND DIFFERENTIAL LEUCOCYTE COUNTS AND HÆMOGLOBIN VALUES OF FEMALE RABBITS

	Average body weights in kg.		Average RBC count (millions per cu. mm.)		Average WBC count (thousands per cu. mm.)		Average differential count (granulocytes per cent.)		Average Hb. per cent.	
Day	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	2·83	2·65	5·74	5·77	12·0	8·4	36	34	93	93
4	2·82	2·82	5·68	5·68	10·2	7·5	34	25	92	95
8	2·82	2·83	5·85	5·41	8·4	6·5	40	32	92	93
11	2·76	2·78	5·60	5·86	9·1	6·2	31	35	92	92
15	2·79	2·81	5·94	5·72	8·7	8·3	33	39	95	96
18	2·74	2·70	5·79	5·89	9·8	7·0	32	38	94	96
22	2·78	2·74	5·94	5·52	7·5	6·1	28	38	92	89
25	2·73	2·71	6·31	6·11	7·9	6·7	25	38	95	91
28	2·76	2·81	5·81	5·61	10·6	7·5	38	39	88	88
31	2·70	2·71	5·77	5·61	8·5	7·6	28	31	92	92
35	2·75	2·72	5·80	5·57	9·5	7·7	27	34	91	90
38	2·67	2·65	6·44	6·03	7·4	6·8	24	33	92	89

Table V records the changes in the average body weight, hæmoglobin content of the blood, and red, white and differential white blood cell counts of two control rabbits and of two rabbits treated with methylphenylglutarimide 5 days a week for nearly 6 weeks. Although the results of this preliminary experiment must be treated with caution, owing to the small number of animals used, prolonged administration does not appear to have had any adverse effect on the body weight or on the hæmopoietic system of rabbits. It will be noted, in particular, that there is no evidence of a significant decrease in the number of circulating granulocytes.

Intravenous administration in rabbits

Although methylphenylglutarimide was found to be nearly insoluble in most of the solvents normally used for intravenous injection, a 5 per cent. solution in propylene glycol could be prepared. In some cases an equal volume of water was added immediately before injection. Six rabbits were injected intravenously with doses of 50 to 100 mg./kg.; none was anæsthetised, but two died and the remainder showed local

inflammatory reactions. Four rabbits, injected with propylene glycol alone, also showed local inflammatory reactions. Higher doses of methylphenylglutarimide were subsequently given and it was found that a minimum dose of 150 mg./kg. was required to anæsthetise a rabbit for several hours. Since most of the toxic manifestations could have been attributable to the solvent, it was decided to try polyethylene glycol 400 as the solvent. No lesions were produced in rabbits injected with this solvent alone, but 1 animal out of 7 died during the injection, possibly owing to faulty technique. It was found that 150 mg./kg. of the new compound was sufficient to anæsthetise rabbits for 2 to 3 hours, and it was concluded that polyethylene glycol was a more suitable solvent for any further intravenous experiments than propylene glycol.

Discussion

The value of leptazol-induced seizures for the screening of new compounds for anticonvulsant activity has been established by various workers, including Everett[8], Swinyard[9], Toman *et al*[10], and Swinyard *et al*[11]. However, the usual method of test is based on the ability to afford complete protection against seizures induced by the injection of leptazol, and some drugs, e.g., diphenylhydantoin[12], which have clinical usefulness in epilepsy, are inactive by this test. Goodman *et al*[2]. accordingly described a method based on the ability of anticonvulsant drugs to modify the pattern of maximal seizures elicited by leptazol injections. Eight anti-epileptic drugs, including troxidone and diphenylhydantoin, were tested and all were found to be capable of modifying the maximal leptazol seizure pattern. It appears that this test provides a useful index of antiepileptic activity.

It is interesting to note, therefore, that methylphenylglutarimide appears to be at least three times as active as aloxidone (which itself is about 1·6 times as potent as troxidone[1]) by the maximal leptazol seizure-pattern test. It is, however, more toxic. Although the toxicities of methylphenylglutarimide and aloxidone have not been compared directly, it appears that the new compound is probably not more than three times as toxic as aloxidone since its LD50 is about 1·3 times that of methylpentynol, whilst methylpentynol has been found to be about twice as toxic as aloxidone in our experience. The therapeutic index (i.e., LD50/PD50) of methylphenylglutarimide therefore appears to be at least as great as that of aloxidone, i.e., it possesses a similar or even greater margin of safety. It will also be noted that there is a considerable margin between the anticonvulsant and the narcotic doses. Thus, the median protective dose against metrazol (60 mg./kg.) is less than one-fifth of the median narcotic dose (340 mg./kg.).

There is some reason to believe, therefore, that methylphenylglutarimide might be of use in the treatment of epilepsy, being considerably more potent than aloxidone and troxidone, and apparently being free from undesirable side-effects, e.g., sedation, at anticonvulsant doses. It is not possible, however, particularly on the basis of a single method of assay, to predict any specificity for the various seizure types. Swinyard

et al[10]. have emphasised that the ultimate value of an anticonvulsant drug can only be ascertained by clinical trial.

The narcotic properties of methylphenylglutarimide are also of some interest. Thus it appears to be more active than methylpentynol (1·3 : 1) when given orally, and it has a similar therapeutic index. The most important feature of the results, however, is the relative duration of narcosis. Whether the comparison is made on a weight for weight basis, at dose levels representing similar proportions of the LD50, or even at dose levels allowing a somewhat greater margin of safety, the average duration of narcosis appears to be at least 50 per cent. greater with it than with methylpentynol. This estimate of a 50 per cent. increase in duration of action is a very conservative one, however, for in most of the animals the action of the substance outlasted the period of observation and consequently the total duration of narcosis was not measured. There was, on the other hand, little difference between the two compounds in induction time.

In conclusion, it may be remarked that the results of the sub-acute toxicity experiments do not suggest that any undue hazards arise from the continued administration of the compound at various dose levels.

Summary

1. The anticonvulsant and narcotic activities of α-methyl-α-phenylglutarimide, a new central depressant, have been investigated. Some toxicological studies have also been made.

2. It is slightly more toxic than methylpentynol, the acute LD50 in mice being (*a*) 630 and 760 mg./kg. respectively (Experiment 1), (*b*) 630 and 860 mg./kg. respectively (Experiment 2).

3. It is at least three times as active as aloxidone as an antagonist of leptazol in mice, the respective median protective doses being (*a*) 65 and 215 mg./kg. (Experiment 1), (*b*) 55 and 175 mg./kg. (Experiment 2).

4. The narcotic activity is about 1·3 times that of methylpentynol, the median narcotic doses being 340 and 430 mg./kg. respectively.

5. The duration of narcosis induced by methylphenylglutarimide is at least 50 per cent. greater than that induced by methylpentynol when doses corresponding to similar proportions of the respective LD50 estimates are administered.

6. Anæsthesia lasting several hours has been produced in rabbits by the intravenous injection of the substance (150 mg./kg.) dissolved in polyethylene glycol 400.

7. It has been administered for prolonged periods to mice, rats and rabbits with no evidence of undue toxic effects at moderate dose levels.

8. The possible therapeutic uses of the compound have been discussed.

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