Comparative Neurotoxicity and Antipentylenetetrazol Activity of Some Piperidinediones

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A new piperidinedione, DAPD, has been compared with four other piperidinediones—glutethimide, amino-glutethimide, methyprylon, and bemegride—and also phenobarbital. With two techniques that measured neurotoxicity and pentylenetetrazol seizure threshold, the onset, peak, and duration of activity of each drug was plotted. DAPD and glutethimide reached peak activity by 5 minutes and exhibited the same neurotoxicity. DAPD had the shortest duration of action and produced the least increase in seizure threshold of the piperidinediones studied. Amino-glutethimide resembled phenobarbital with a slow onset but a long duration of activity. Methyprylon and bemegride reached peak activity by 2.5 minutes; the former increased seizure threshold, whereas the latter was the only agent to decrease seizure threshold. Bemegride was the most toxic drug and amino-glutethimide the least toxic.

SEVERAL USEFUL therapeutic agents have been found in the piperidinedione series. Glutethimide[1] and methyprylon[2] are sedatives and hypnotics, amino-glutethimide[3] is an anticonvulsant, and bemegride[4] is an analeptic. Our interest in these compounds was prompted by the synthesis of a new piperidinedione (1), 3-(N,N-diethylamino)-3-phenyl-2,6-piperidinedione, hereafter called DAPD. To compare the pharmacology of DAPD with that of closely related agents, a comprehensive comparative pharmacologic study of several piperidinediones has been initiated in our laboratories. This report deals with the onset and duration of activity, the neurotoxicity, and the antipentylenetetrazol activity of DAPD, four piperidinediones, and one barbiturate. The structural formulas of the agents investigated are shown in Fig. 1.

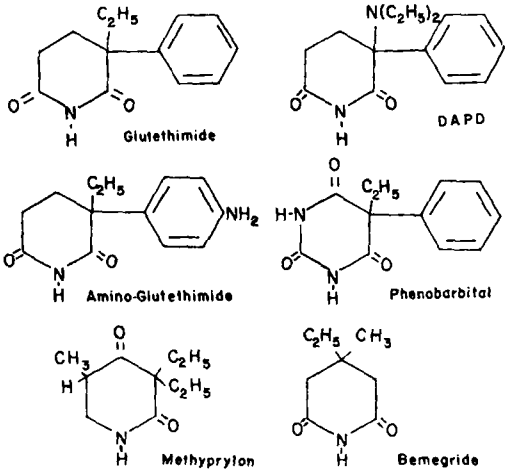

Fig. 1.—Structures of the compounds investigated. Glutethimide (3-ethyl-3-phenyl-2,6-piperidinedione), DAPD [3-(N,N-diethylamino)-3-phenyl-2,6-piperidinedione], amino-glutethimide (3-ethyl-3-(*p*-aminophenyl)-2,6-piperidinedione), phenobarbital (5-ethyl-5-phenyl-barbituric acid), methyprylon (3,3-diethyl-5-methyl-2,4-piperidinedione), and bemegride (4-ethyl-4-methyl-2,6-piperidinedione).

METHODS

The experimental animals were adult male albino mice (CF No. 1) obtained from the Carworth Farms. The drugs investigated (listed in Fig. 1) were given by the oral route as suspensions in 1% methylcellulose solution. Activity-duration curves were determined for each drug by two techniques that measured neurotoxicity and pentylenetetrazol seizure threshold. The neurotoxicity test was based on muscular incoordination; mice that could not remain for 1 minute on a horizontal rod (diameter, 2.5 cm.) rotating at 6 r.p.m. were considered toxic. The mice were tested for neurotoxicity at 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes and thereafter at hourly intervals until no mice were toxic. Groups of six to 12 mice were given various doses of the drugs until at least three points were established in the range between 0 and 100% neurotoxicity at the indicated time intervals. The dose of each drug required to produce toxicity in 50% of mice (TD_{50}) at the indicated times were determined and 95% confidence limits calculated by the method of Litchfield and Wilcoxon (2). The second technique measured seizure threshold. Pentylenetetrazol[5] was used as the convulsant stimulus; seizure threshold was measured by the intravenous infusion technique of Orloff, *et al.* (3), as modified by MacQuarrie and Fingl (4). The mice were randomly divided into groups of ten or more and given equivalent doses ($^1/_2$ of the TD_{50}) of the drugs to be tested, except

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[1] Marketed as Doriden by Ciba Pharmaceutical Products, Inc., Summit, N. J.
[2] Marketed as Noludar by Hoffmann-La Roche, Inc., Nutley, N. J.
[3] Marketed as Elipten by Ciba Pharmaceutical Products, Inc., Summit, N. J.
[4] Marketed as Megimide by Abbott Laboratories, North Chicago, Ill.
[5] Marketed as Metrazol by the Knoll Pharmaceutical Co., Orange, N. J.

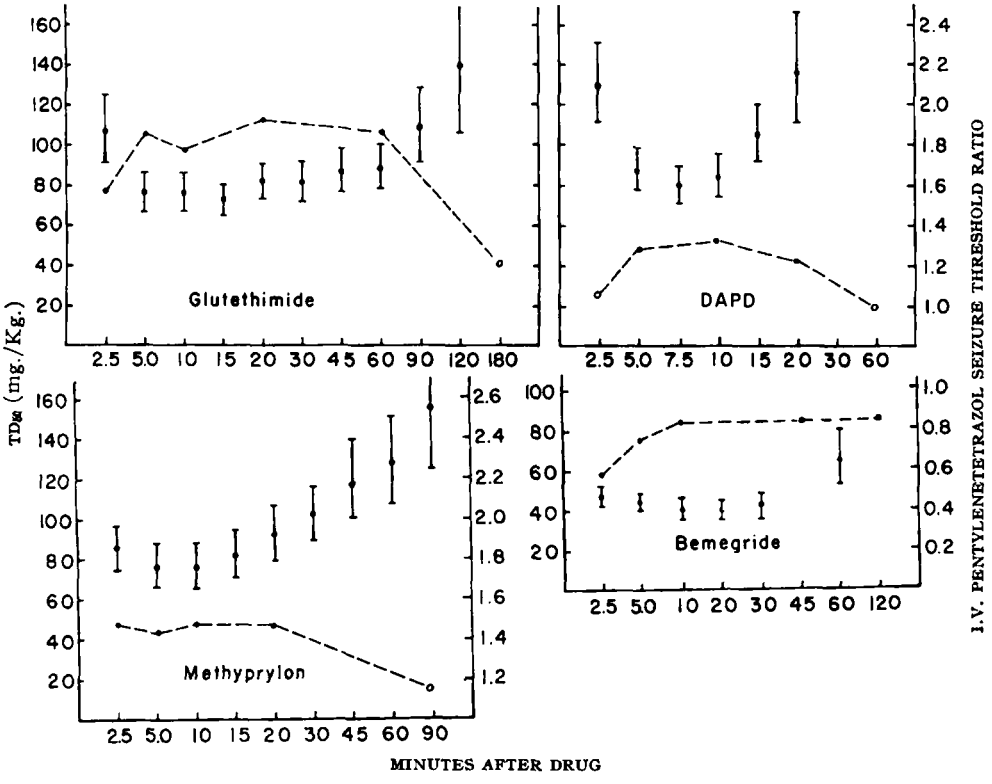

Fig. 2.—Neurotoxicity (TD_{50}) of some piperidinediones and their effect on intravenous pentylenetetrazol seizure threshold (PST) in mice. The vertical bracketed lines represent 95% confidence limits. Solid symbols at a PST ratio (mean threshold of drug-treated mice/mean threshold of control mice) indicate a significant change ($p < 0.05$) from 1.0, normal threshold. The PST ratios were determined at the indicated times after oral administration of 40 mg./Kg. of glutethimide, DAPD, and methylprylon, and after 20 mg./Kg. of bemegride.

that one of the groups was given the requisite volume of vehicle only and served as the control. At selected times after oral drug administration seizure thresholds were determined and the results expressed as threshold ratios (mean threshold of drug-treated group/mean threshold of concurrently tested control group).

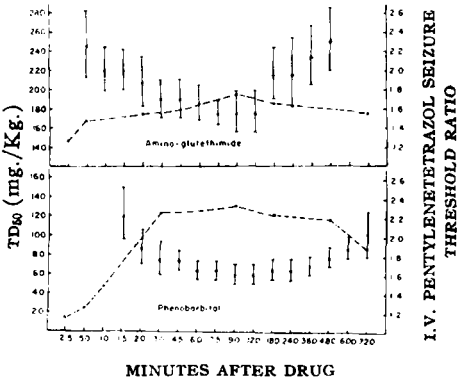

Fig. 3.—Neurotoxicity (TD_{50}) of amino-glutethimide and phenobarbital and their effect on intravenous pentylenetetrazol seizure threshold (PST) in mice. The vertical bracketed lines represent 95% confidence limits. Solid symbols at a PST ratio (mean threshold of drug-treated mice/mean threshold of control mice) indicate a significant change ($p < 0.05$) from 1.0, the normal threshold. The PST ratios were determined at the indicated times after oral administration of 90 mg./Kg. of amino-glutethimide and 30 mg./Kg. of phenobarbital.

RESULTS

The onset of action, time of peak activity, and duration of activity of the six drugs investigated are illustrated in Figs. 2 and 3. Glutethimide reached peak toxicity within 5 minutes, maintained this activity about 1 hour, then toxicity began to wane. The pentylenetetrazol seizure threshold (PST) ratio was 1.84 at 5 minutes, 1.88 at 60 minutes, but not significantly different from 1.0 at 3 hours. DAPD, in a similar manner, reached peak toxicity within 5 minutes, but toxicity was decreasing within 15 minutes. PST ratios were 1.05 at 2.5 minutes, 1.29 at 5 minutes, 1.31 at 10 minutes, 1.22 at 20 minutes, and 0.99 at 60 minutes. The peak activity of methyprylon, however, was reached by 2.5 minutes, but was waning by 30 minutes. PST ratios were 1.48 at 2.5 minutes, 1.47 at 20 minutes, but not significantly different from 1.0 at 90 minutes. Bemegride also reached peak activity by 2.5 minutes. This was the only compound which significantly lowered PST ratios to less than 1.0. It was difficult to delineate the complete toxicity curve of bemegride since its TD_{50} (40 mg./Kg.) approached the dose that was

lethal for 50% of the mice (80 mg./Kg.). Amino-glutethimide and phenobarbital took longer to reach peak activity—approximately 30 minutes. Both drugs exhibited antipentylenetetrazol activity before toxicity was evident. Although both drugs exhibited little neurotoxicity after 12 hours, they still exhibited significant PST ratios.

The peak TD_{50}s in mg./Kg. (with 95% confidence limits) of the six agents arranged in ascending order were: bemegride 40 (36–45), phenobarbital 60 (50–72), glutethimide 74 (67–81), methyprylon 77 (67–88), DAPD 80 (72–89), and amino-glutethimide 175 (164–187). The maximum PST ratios of the compounds in ascending order were: DAPD, 1.31; methyprylon, 1.49; amino-glutethimide, 1.75; glutethimide, 1.92; and phenobarbital, 2.31. Bemegride, a stimulant drug, lowered seizure threshold and exhibited PST ratios less than 1.0.

DISCUSSION

The minimal overt evidence of neurotoxicity (muscular incoordination) following the oral administration of five piperidinediones and one barbiturate to mice has been utilized to plot comparative activity-duration curves of these agents. In addition, intravenous pentylenetetrazol seizure threshold (PST) ratios were determined at selected times after drug administration and another set of activity-duration curves of the six drugs plotted. The PST ratios were determined at dose levels representing one-half of the TD_{50} of each compound; none of the mice at this dosage level of the six agents exhibited neurotoxicity. In general, the data obtained by the two techniques showed good correlation. Thus the time of onset and duration of activity, the TD_{50}, and the effect on PST was delineated for each compound.

Glutethimide, DAPD, and amino-glutethimide are closely related piperidinediones whose structural formulas resemble that of phenobarbital (Fig. 1). The only difference between glutethimide and phenobarbital is that a CH_2-CH_2 group in the piperidine ring of the former compound has replaced a NH-CO group in the pyrimidine ring of the latter. The substitution of a diethylamino group for an ethyl group of glutethimide produces DAPD and the addition of an amino group on phenyl ring of glutethimide produces amino-glutethimide. These four compounds, however, varied considerably in onset and duration of action and in their effects on PST.

It is probably best to compare DAPD with glutethimide. Although both drugs reached peak activity within 5 minutes, DAPD was active only a few minutes, while glutethimide was active more than 1 hour. DAPD appeared to have less depressant properties than glutethimide and appeared to excite the mice during the time of peak activity. Furthermore, DAPD had less antipentylenetetrazol activity and only raised seizure threshold 27% (based on an average of the PST ratios obtained during peak activity), whereas glutethimide raised seizure threshold 86%. Both compounds exhibited the same TD_{50}—about 80 mg./Kg.

Amino-glutethimide had a slower onset of action than glutethimide and DAPD but also had a much longer duration of action. In this respect it resembled phenobarbital more than the other piperidinediones. It was intermediate between DAPD and glutethimide in antipentylenetetrazol activity since it increased seizure threshold an average 66%. The high TD_{50}, 175 mg./Kg., exhibited by amino-glutethimide may indicate that this drug has less sedative properties than glutethimide. Clinical studies have confirmed its comparative lack of sedative-hypnotic effects (5–8).

Two other piperidinediones studied, methyprylon and bemegride, exhibited a very rapid onset of action after oral administration. Both compounds reached peak activity within 2.5 minutes by both techniques. Methyprylon, a sedative-hypnotic, increased seizure threshold; whereas bemegride, an analeptic agent, added to the convulsant effect of pentylenetetrazol and decreased the seizure threshold. Methyprylon exhibited the same TD_{50} as glutethimide and DAPD, 80 mg./Kg. Bemegride, with a TD_{50} of 40 mg./Kg., was the most toxic piperidinedione studied. Phenobarbital had a slower onset and a longer duration of activity and also greater antipentylenetetrazol activity than the five piperidinediones studied.

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