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BIOCHEMORPHOLOGICAL EFFECTS OF N-ALLYL SUBSTITUTION ON BARBITURATE COMPOUNDS

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Interest in the study of structural alteration of the substituents of barbiturate nitrogen was engendered in two ways. The substitution of an allyl group on the nitrogen of morphine gives rise to allyl-normorphine, a compound virtually devoid of pharmacological similarity to morphine but which exhibits a rather specific antagonism for the respiratory depressive action of morphine and its derivatives (Unna, 1943; Hart, 1943; Huggins, *et al.*, 1950; and Eckenhoff, *et al.*, 1952).

It was felt that the investigation of the analogous compounds in the barbiturate series should be carried out. There were indications in the literature that the substitution of larger radicals on the nitrogen atom may decrease the hypnotic properties of barbiturates (Shonle and Doran, 1936; Buck, *et al.*, 1938; and Henze and Spurlock, 1941). The results of preliminary investigation in this direction showed that the proposed analogy between N-allyl barbiturates and N-allyl normorphine did not hold true.

It was also reasoned that these compounds might be studied with a view toward ascertaining the effect of a barbiturate antagonist, Metrazol, on the action of the altered compounds. Such studies would throw light on whether or not the structural change had conceivably altered the anticonvulsive properties of the molecule. Consequently, this work was undertaken to compare the anticonvulsant (antimetrazol) properties of Barbital (5,5 - diethylbarbituric acid), Phenobarbital (5,5 - ethylphenylbarbituric acid), and their nitrogen substituted derivatives.

Methods and Materials

The procedure employed was the timed metrazol infusion technique developed by Orloff, *et al.* (1949). For discussion of other techniques (electrical and pharmacological stimulation methods) the reader is referred to Toman and Goodman (1947, 1948), Toman (1951), Swinyard, *et al.* (1952), and Woodbury and Davenport (1952). In general it can be stated that the information obtained using one type of experimental technique is rather specific. It is commonly observed that favorable results with one technique cannot be reproduced by a different method. With these limitations in mind our work has been confined to antimetrazol studies.

Animals used were Webster strain albino mice of both sexes, weighing 14-20 gms. They were fed Purina chow diet *ad libitum*. Food was withdrawn from the mice 16 to 20 hrs. before each experiment but leaving free access to water.

Barbituric acid derivatives (acid form) were dissolved in excess of

0.1 N NaOH and neutralized with 0.1 N HCl. In preparing solutions care was taken to limit the volume to be injected. Solutions used were: Barbital, Na U.S.P. 440 mgm. %; Metrazol, 500 mgm. %; Phenobarbital, Na U.S.P. 440 mgm. %; N-Allyl Barbital, 400 mgm. % and 800 mgm. %; N-Allyl Phenobarbital, 400 mgm. % and 800 mgm. %; and N-Phenacyl Phenobarbital, 500 mgm. % and 800 mgm. %.

Compounds 1 and 2 were prepared by C. D. Proctor. Compound 3 was prepared according to Henze and Spurlock (1941). Elemental analysis was performed by Dr. L. I. Diuguid, St. Louis, Mo., using micro Kjeldahl procedure.

Syringes for injections were the tuberculin type, graduated at 0.01 ml. Needles used were 27-gauge for both intravenous and intraperitoneal injections. A cone-shaped plastic cylinder was used for holding and for observing the animals during intravenous injections.

Animals were divided into weight groups (15-17 gm. and 18-20 gm.). The anticonvulsant agent was administered intraperitoneally at doses of 50-100 mgm./kg. based on the acid weight. Metrazol solution (500 mgm. %) was infused through the caudal vein at the rate of 0.05 cc./10 sec. at periods of 5, 60, and 120 minutes after administration of the anticonvulsant. The reaction of the mouse to this carefully controlled intravenous infusion of Metrazol showed the definite pattern described by Orloff, *et al.* (1949). The signs of reaction followed each other in a strict sequence with increasing concentration of Metrazol in the blood. The first of these symptoms was the "first twitch"—a sharp single twitch of the entire body. This was followed very shortly by "pseudoconvulsions" — a series of clonic movements accompanied by an audible squeak by the animal and ventral flexion of the head. Later, the animal showed clonic movements alternating with resting phases. The third sign — "persistent convulsions" — appeared when the maximal Metrazol concentration of the blood was reached; it was the final point of injection. These lethal convulsions were characterized by tonic flexion and were followed by extension of the hind legs. The operator only observed the volume of Metrazol injected at the appearance of "first twitch" and "persistent convulsions" because the "pseudoconvulsions" followed very closely the "first twitch". The efficacy of the anticonvulsant was denoted as:

Protective ratio: *threshold after treatment* (in cc. of 0.5% Metrozol) *over threshold of the corresponding weight group which was untreated.*

A protective ratio equal to 1.00 is the control value obtained from untreated mice. A value above 1.00 indicates the action of the compound antagonistic to the effects of Metrazol, while any value less than 1.00 shows synergistic action to the convulsant. The ability of the compound to modify the convulsive pattern of Metrazol was also considered.

Results and Discussion

N-Allyl Barbital and Barbital.— Results with N-Allyl Barbital indicated that this compound had different anticonvulsive properties than its parent compound, Barbital. Its activity, 5 minutes after the ad-

TABLE 1.—Physical and Chemical Data on the Compounds Synthesized in our Laboratory.

Compound	Molecular wt.	Formula	Data of chemical analysis		Observed melting point °C.	H_2O solubility
			found N%	calculated N%		
N-Allyl Barbital (1)	226_6	$C_{11}O_3N_2H_{16}$	11.89	12.50	77-78	poor
N-Allyl Phenobarbital (2)	273_6	$C_{15}O_3N_2H_{16}$	10.11	10.27	68-69	poor
N-Phenacyl Phenobarbital (3)	351_8	$C_{20}O_4N_2H_{18}$	8.03	8.00	156-158	poor

ministration of the same dose (50 mgm./kg.) as Barbital, showed noteworthy activity both in raising the protective ratios and in modifying the pattern of convulsions. The "first twitch" and "pseudoconvulsions" were changed to the extent that they were difficult to observe. The other finding was the delay of death after appearance of "persistent convulsions" (these were of weaker intensity and not continuous). The delay lasted up to 30 minutes and was seen in about 50% of the animals. The lethal convulsions were tonic with flexor and extensor components as in control animals, but the latter animals always died immediately upon the appearance of "persistent convulsions". Barbital animals at the corresponding time interval (5 min.) showed smaller protective ratios than N-Allyl Barbital animals. The pattern of convulsions was essentially unmodified and no delayed deaths were observed. At one hour postadministration, the same dose (50 mgm/kg.) of N-Allyl Barbital showed a markedly decreased anticonvulsive activity, whereas the activity of an equal dose of Barbital rose in respect to the protective ratios and ability to modify the seizure pattern, although no delay of death was seen. A double dose of N-Allyl Barbital (100 mgm./kg.) proved to be more effective, but the protection afforded was below that observed at the 5-minute interval, and animals showed a more or less typical seizure pattern. At two hours postadministration, the activity of 100 mgm./kg. of N-Allyl Barbital showed a progressively decreasing protection, while the protection afforded by 50 mgm./kg. of Barbital remained on the same level as at the 1-hour interval.

The description of results in Table 2 points out that N-Allyl substitution increased the immediate antimetrazol activity of the parent compound but simultaneously decreased the duration of action. The first property is in agreement with Toman and Goodman (1948) who stated that N-alkylation may increase antimetrazol activity. It would appear that the immediate metabolic product of N-Allyl Barbital is not Barbital, as was reported

TABLE 2.—The Anticonvulsive Potency of N-Allyl Barbital in Comparison with Barbital.

Anticonvulsive	Mg./kg. intra-peritoneal	Convulsive threshold in cc. of 0.5% Metrazol		Protective ratio	
		1st. twitch	persistent convulsions	1st. twitch	persistent convulsions
Control, 15-17 gms.	0	0.13 (24)[1]	0.42 (24)[1]	1.00	1.00
Control, 18-20 gms.	0	0.16 (24)	0.52 (22)	1.00	1.00
Barbital— 5 min.	50	0.20 (20)	0.58 (20)	1.61	1.36
— 60 min.	50	0.27 (19)	0.69 (18)	2.07	1.64
—120 min.	50	0.19 (22)	0.65 (22)	1.46	1.55
N-Allyl-Barbital					
— 5 min.	50	0.47 (23)	0.97 (31)	2.87	1.87
— 60 min.	50	0.26 (18)	0.66 (20)	1.62	1.27
— 60 min.	100	0.27 (19)	0.60 (19)	2.08	1.43
—120 min.	100	0.17 (21)	0.46 (23)	1.30	1.09

[1] Figures in parentheses indicate the number of observations.

in the case of N-Methyl Barbital which was converted to Barbital *in vivo* (Butler and Bush, 1939).

N-Allyl Phenobarbital and Phenobarbital.—N-Allyl Phenobarbital, at the same dose (50 mgm./kg.) as Phenobarbital at 5 minutes postadministration, showed inferior activity with respect to protective ratios of the persistent convulsions, but it was more effective in raising the threshold of both "first twitch" and "pseudoconvulsions". Both compounds were able to make these two signs less defined. The modification of "persistent convulsions" by Phenobarbital was much more pronounced than by N-Allyl Phenobarbital. Phenobarbital abolished the typical tonic convulsive death by changing it to the clonic type death occurring after some delay. N-Allyl Phenobarbital, at a dose of 100 mgm./kg. at the 5-minute interval, caused very profound modifications of the "first twitch" and "pseudoconvulsions"; only 2 of 11 animals showed these symptoms in any recognizable form. In a majority of cases the persistent convulsions showed a clonic pattern which was especially well defined in animals dying after some delay. The protective ratios at the higher dose (100 mgm./kg.) were superior to those of the parent compound at the 50 mgm./kg. dose. At one hour postadministration, N-Allyl Phenobarbital (100 mgm./kg.) showed a marked decrease in the protective ratios and in ability to modify the character of persistent convulsions. There was delayed death in 3 of 21 animals. The Phenobarbital protection at one hour was increased in comparison to its immediate activity in all aspects. At two hours postadministration, N-Allyl Phenobarbital showed a more decreased potency, affording only a very slight protection as evidenced by a poor protection ratio. Just the opposite picture

TABLE 3.—The Anticonvulsive Potency of N-Substituted Phenobarbital in Comparison with Phenobarbital.[1]

Anticonvulsive	Mg./kg. intraperitoneal	Convulsion threshold in cc. of 0.5% Metrazol		Protective ratio	
		1st. twitch	persistent convulsions	1st. twitch	persistent convulsions
Phenobarbital— 5 min..	50	0.22 (13)[2]	0.75 (18)[2]	1.38	1.44
— 60 min..	50	0.30 (14)	0.67 (17)	2.30	1.60
—120 min..	50	0.26 (17)	0.77 (19)	2.00	1.83
N-Allyl Phenobarbital					
— 5 min.....	50	0.23 (19)	0.50 (22)	1.77	1.19
— 5 min.....	100	0.31 (2)	0.77 (11)	2.38	1.86
— 60 min.....	100	0.25 (16)	0.66 (21)	1.84	1.57
—120 min.....	100	0.16 (19)	0.46 (19)	1.23	1.10
N-Phenacyl Phenobarbital— 5 min.....	62	0.20 (15)	0.57 (17)	1.54	1.38
— 5 min.....	100	0.18 (19)	0.72 (21)	1.12	1.40
— 60 min.....	100	0.14 (17)	0.44 (18)	1.07	1.05
—120 min.....	100	0.12 (21)	0.39 (21)	0.92	0.93

[1] For control values, see Table 2.
[2] Figures in parentheses indicate the number of observations.

was seen with the Phenobarbital animals in which protection reached a maximum at two hours after administration. Delayed death, characterized by clonic convulsions, was observed in 58% of the animals.

From these results one may conclude that N-Allyl Phenobarbital had a considerably shorter action than the parent compound, as was observed in the case of N-Allyl Barbital and Barbital. Such results infer that metabolic degradation products of N-Allyl Phenobarbital were different than Phenobarbital which showed a continuously increasing activity. The compound giving Phenobarbital as a metabolic product is N-Methyl Phenobarbital (Butler, 1952). A difference between the two allyl derivatives can be noted in that the N-allyl substitution of Phenobarbital did not increase the anticonvulsive properties of the parent compound as did the change from Barbital to N-Allyl Barbital. The latter derivative showed a markedly increased initial activity over the parent compound but lacked sustained action.

N-Phenacyl Phenobarbital and Phenobarbital. — The results with N-Phenacyl Phenobarbital (62 mgm./kg., 5 minutes postadministration) indicated a moderate protective action and poor ability to modify the convulsive pattern. With a greater dose (100 mgm./kg.) the protective ratios did not become higher, but a very pronounced ability to change the tonic convulsions to the clonic type was observed (17 delayed deaths in 21 animals). This delay sometimes reached 30 minutes,

and several seizures were observed before the outcome was lethal. At 60 minutes postadministration, there was no significant elevation of protective ratios over the control values and the pattern of the convulsions was not changed. At 120 minutes, there was some indication of synergism between this drug and Metrazol, because the protective ratios at the persistent convulsion level were below unity.

The findings at 120 minutes were in agreement with those obtained by Henze and Spurlock (1941) who, using an older Metrazol technique, could not detect any anticonvulsive properties of this compound. The results obtained with the compound under study indicate limited protective properties at shorter waiting periods, with pronounced ability to modify convulsions at five minutes. The significance of this property was stressed by Toman, Swinyard and Goodman (1946). They pointed out that the efficacy of a given antiepileptic compound may sometimes be more related to a reduction in the ability of the brain to support self-sustaining discharges than to a simple increase in the chemical or electrical threshold for initiating discharges.

The hypnotic properties of the N-subsituted compounds studied were characterized by an anesthetic power greater than that of the parent compounds (on a weight basis), by immediate onset of anesthesia, by a shorter duration of action, and by a shorter postanesthetic depression (Gylys and Proctor, unpubl. data). These properties are distinctly different from those of the parent compounds. The short anesthetic action is analogous to the short duration of the anticonvulsive effect. The fact that these compounds had a stronger hypnotic effect than the parent compounds would indicate that the hypnotic properties do not necessarily develop parallel to the anticonvulsive ones. This is similar to the findings of Everett and Richards (1945).

Summary

Studies were undertaken to evaluate the pharmacological effects of N-alkyl substitution on barbiturate molecules using the intravenous Metrazol infusion test. Changes in both convulsive threshold and pattern of convulsions were taken into consideration. It was ascertained that the allyl substitution on Barbital produced a quantitative increase in the intensity of the anticonvulsant activity. Such substitution decreased the latent period for manifestation of the peak of the anticonvulsant activity of Barbital, but it also decreased the duration of such activity. N-Allyl substitution of Phenobarbital yielded a compound with somewhat reduced anticonvulsive potency; this substitution also decreased the latent period of the peak of action and duration of such activity. N-phenacyl substitution yielded a compound with reduced anticonvulsive effect and, late in the course of its action, possible synergistic action with Metrazol.

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