Utility of Two Convulsant Techniques as Indicators of CNS Excitability

By HAROLD H. WOLF and GERARD A. STOCK, JR.*

A study was undertaken to evaluate the relative usefulness of pentylenetetrazol (PTZ) *versus* hexafluorodiethyl ether (HFE) as chemoconvulsive agents which could be employed to quantitate CNS excitability in mice. The PTZ was given by constant i.v. infusion, whereas the HFE was administered by inhalation. Levels of excitability were altered with several doses of (+)-amphetamine and pentobarbital. The results indicate that the PTZ infusion procedure is considerably more sensitive and less variable than the HFE inhalation technique for the determination of drug-induced fluctuations in central activity.

THE OBSERVATION made by Krantz and his coworkers (1) that hexafluorodiethyl ether (HFE) caused convulsions when inhaled has led to the usage of this agent as a convulsant in psychiatric treatment. In recent years, a number of authors have commented on the utility of this procedure as a tool for the laboratory evaluation of central nervous system activity. For example, Davis and Webb (2) have employed the drug to quantitate a circadian rhythm of chemoconvulsive response and also to demonstrate that, in mice, susceptibilities to auditory and chemical convulsive stimuli are not necessarily correlated in a positive manner (3). Other workers have found the technique valuable for predicting the activity of anticonvulsants (4) and for demonstrating increased sensitivity of the central nervous system following cortical ablations (5).

Several of these studies (4, 5) have shown that the type of seizures produced by the inhalation of HFE are similar to those seen following the intravenous administration of pentylenetetrazol (PTZ). Moreover, the HFE technique has been shown to be considerably easier to employ. However, whereas much evidence is available concerning the sensitivity and reliability of the i.v. PTZ technique as a means of evaluating drug-induced fluctuations in central activity (6–9), comparatively little similar information is available for the inhalation of HFE. Therefore, a series of experiments were designed to compare the sensitivity and variability of the two techniques when used to quantitate changes in CNS activity induced by drugs.

EXPERIMENTAL

The experimental animals employed were 20 to 25 Gm., adult, male, albino mice of a random bred Swiss strain obtained from Maxfield Animal Supply. All experiments were conducted during the same time of day and room temperature was maintained between 23–26°.

PTZ was administered by timed intravenous infusion, employing a modification of a technique originally described by Orloff (10). In this procedure, the animal is briefly restrained while a 0.5% solution of the convulsant in saline is infused into a tail vein at a constant rate of 0.247 ml./min. The amount of time which elapses from onset of infusion until the animal displays 3 sec. of continuous clonic activity is recorded. From this information and the body weight of the mouse, the number of mg./Kg. of PTZ required to induce seizure can be calculated.

HFE[1] was administered as a 10% solution in 95% ethyl alcohol by means of a timed inhalation technique. The apparatus employed was a modified version of that described by Adler (5). With this procedure, the convulsant solution is infused at a constant rate of 0.136 ml./min. onto a gauze wick fixed near the top of a 300-ml. bell jar. The jar, whose open bottom edge had been coated with stopcock grease, is first placed over a mouse on a glass plate containing a filter paper mat, thus providing a relatively airtight container. A wire mesh screen dividing the chamber approximately in half effectively prevents the mouse from rising on its hind legs in an attempt to avoid the vapors of HFE which sink to the bottom of the jar. As with the PTZ technique, the amount of time which elapses from onset of infusion to 3 sec. of continuous clonic activity is recorded and the mg./Kg. of HFE required to induce seizure are calculated. Following each convulsive episode, the vapors are removed by means of a vacuum water pump which is connected to a side arm of the bell jar.

Employing these two techniques, several experiments were conducted to quantitate the fluctuations in central activity induced by pentobarbital and (+)-amphetamine. Aqueous solutions of soluble salts of these drugs, *i.e.*, sodium pentobarbital and (+)-amphetamine sulfate were administered intraperitoneally in a constant volume of 1 ml./100 Gm. body weight. Preliminary investigations, employing the chemoshock threshold procedures, revealed that the times of peak effect for pentobarbital and (+)-amphetamine were 10 and 15 min., respectively.

In order to compare the sensitivity and variability of the two convulsant techniques, groups of 14 mice each were given graded doses of pentobarbital, (+)-amphetamine, or requisite volumes of saline (controls) and evaluated for seizure susceptibility at the appropriate times of peak effect. From the accumulated data, a series of threshold ratios, *i.e.*, the mean mg./Kg. of convulsant required to produce clonic seizure in drug-treated animals divided by the mean mg./Kg. of convulsant required to reach the same end point in controls was computed for both PTZ and HFE. The 95% confidence intervals for the individual threshold ratios were calculated by a method described by Goldstein (11).

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* National Science Foundation Undergraduate Research Participant, 1965.

[1] Kindly supplied as Indoklon by Dr. A. H. Neeley, Ohio Chemical and Surgical Equipment Co., Murray Hill, N. J.

RESULTS AND DISCUSSION

The results obtained with five doses of pentobarbital, *i.e.*, 2, 4, 8, 16, and 32 mg./Kg., are shown in Fig. 1. The vertical bracketed lines represent 95% confidence intervals for the calculated ratios and any line which does not cross 1.0 indicates that the dose of pentobarbital under consideration had a significant ($p < .05$) effect on convulsive threshold.

It is obvious from the results presented that a decrease in CNS excitability, as expressed by an increase in threshold ratio, can be demonstrated for pentobarbital using either convulsant technique. However, it is also quite clear that the HFE procedure is considerably less sensitive than that employing PTZ. Thus, within the dose range examined, twice as much pentobarbital (16 *versus* 8 mg./Kg.) was required to demonstrate a significant elevation in threshold by the inhalation *versus* i.v. infusion technique. Moreover, in all instances the amount of CNS depression induced by equivalent doses of pentobarbital was more readily apparent by the PTZ procedure. The magnitude of this difference is statistically significant at doses of 8, 16, and 32 mg./Kg.

In this study the amount of data variability, as portrayed by the length of the vertical bracketed lines, was essentially the same for both convulsant procedures at four of the five doses of barbiturate tested. At the highest dose of pentobarbital the PTZ technique manifested more variability than inhaled HFE, but the former procedure was, as indicated, 1.6 times more sensitive.

The results of a similarly designed experiment employing (+)-amphetamine to alter level of CNS excitability are presented in Fig. 2. Here it can be seen that the PTZ technique is also considerably more sensitive to the effects of a central stimulant. In fact, with HFE, the authors were not able to demonstrate a significant decrease in threshold ratio with any dose of (+)-amphetamine tested. On the other hand, with PTZ, significant CNS excitability was evident at both 8 and 16 mg./Kg. of (+)-amphetamine.

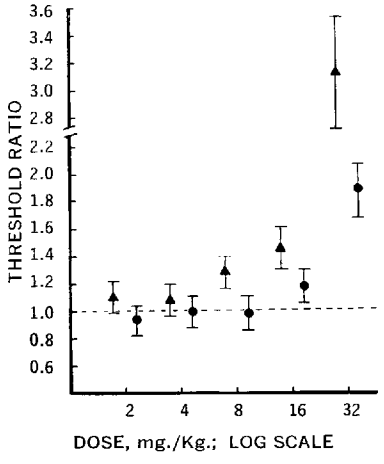

Fig. 1.—Alterations in CNS activity induced by pentobarbital. Key: ▲, PTZ technique; ●, HFE technique.

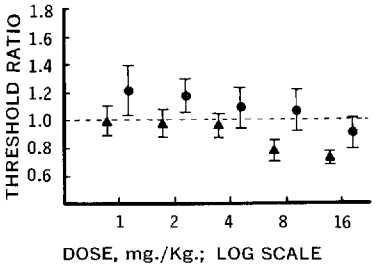

Fig. 2.—Alterations in CNS activity induced by (+)-amphetamine. Key: ▲, PTZ technique; ●, HFE technique.

It will be noted that small doses of (+)-amphetamine (1 and 2 mg./Kg.) induced a slight, but statistically significant, elevation in convulsive threshold as determined by the HFE technique. This seemingly paradoxical effect of (+)-amphetamine has been observed by other workers (12, 13) employing electroshock procedures and has been attributed to amphetamine's desynchronizing action in the frontal cortex (14) and its inhibitory action at the level of the reticular formation (15). The reasons for the inability of the PTZ technique to detect this phenomenon remain, as yet, unclear.

An examination of the 95% confidence intervals in Fig. 2 reveal that, in all instances, the inhalation of HFE provided more variable data than did the i.v. administration of PTZ. The maximum difference in variability between the two techniques amounted to 19% and was observed following the administration of 16 mg./Kg. of (+)-amphetamine.

The results obtained in this study are essentially in agreement with those reported by Truitt *et al.* (4), who observed that PTZ provided the most sensitive index of a change in convulsive threshold produced by phenobarbital. On the other hand, our results differ somewhat from those observed by Adler (5), who reported that, although an increased sensitivity to both PTZ and HFE results from ablations of either the frontal or posterior cortex, the data obtained with HFE manifest less variability. This apparent discrepancy in findings may result from species differences (mice *versus* rats) or from the methods employed to produce changes in level of central activity (drugs *versus* cortical ablations).

In summary, it would appear that although the HFE technique has the obvious advantage of simplicity of operation and lack of animal restraint, the utility of the procedure is hampered by a relative lack of sensitivity. Moreover, when the technique is employed to evaluate drug-induced changes in CNS excitability, the data collected manifest somewhat more variability than similar information obtained using timed intravenous infusion of PTZ.

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Determination of Carbonyl Compounds by Sodium Nitrite Titration of Excess 2,4-Dinitrophenylhydrazine in the Presence of Hydrazone

By LEON KURLANSIK and EDWARD F. SALIM

Numerous methods have been developed for the quantitative determination of carbonyl compounds with 2,4-dinitrophenylhydrazine. Generally, it is necessary to separate the prepared hydrazone from excess reagent before final measurement. A simplified method for the determination of aldehydes and ketones is presented in which excess 2,4-dinitrophenylhydrazine in the presence of hydrazone is titrated with standard sodium nitrite solution.

Since Mathewson (1) prepared hydrazones of water-soluble carbonyl compounds with 2,4-dinitrophenylhydrazine, this reagent has been used extensively for qualitative characterization and quantitative estimation of carbonyl compounds. Applications of gravimetric (2–4), spectrophotometric (5–7), and titrimetric procedures for quantitative determinations of aldehydes and ketones have been reported. Among the titrimetric methods developed are solution of the hydrazone in standard base and determination of excess sodium hydroxide (8), nonaqueous titration of hydrazone in pyridine with tetrabutylammonium hydroxide (9), determination of reduced nitro-groups of the hydrazone (10) or excess hydrazine (11), and direct amperometric titration of the carbonyl with 2,4-dinitrophenylhydrazine solution (12).

A common feature of most published methods is the separation of prepared hydrazone from excess reagent before the final measurement is conducted. The preponderance of procedures deals with the hydrazone and relatively few involve the determination of excess reagent. Isolation of hydrazones which are slightly soluble in the reaction media generally results in low recoveries.

Vulterin and Zyka (13) have described the potentiometric titration of 2,4-dinitrophenylhydrazine with 0.1 *M* sodium nitrite and have postulated the reaction to proceed by formation of the 2,4-dinitrophenylnitrosohydrazine. Since the β nitrogen of the hydrazone is substituted, corresponding nitroso addition presumably does not occur. Baldinus and Rothberg have reported the titration of hydrazones with sodium nitrite but only after vigorous treatment with sulfuric acid and tetrahydrofuran (14). Based on this information a simplified method for the determination of carbonyl compounds has been developed by an initial reaction with 2,4-dinitrophenylhydrazine and subsequent titration of excess reagent in the presence of prepared hydrazone using standard sodium nitrite.

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EXPERIMENTAL

All titrations were conducted potentiometrically using a Beckman Expandomatic pH meter equipped with a calomel and platinum electrode system.

Reagents.—*2,4-Dinitrophenylhydrazine Reagent Solution.*—Add 9 Gm. of finely powdered 2,4-dinitrophenylhydrazine (2,4-DNPH) to a stirred mixture of 100 ml. of 85% phosphoric acid, 100 ml. of ethanol, and 20 ml. of sulfuric acid. Stir for 30 min., add 100 ml. of ethanol, and continue mixing for an additional hour. Cool and filter prior to use. (The reagent is approximately 0.1 *M* and is stable for at least 3 weeks.)

Sodium Nitrite, 0.1 M.—Dissolve 7.5 Gm. of sodium nitrite in sufficient water to make 1000 ml. The solution was standardized against U.S.P. sulfanilamide reference standard, previously dried at 105° for 3 hr., by potentiometric titration using calomel *versus* platinum electrodes.

General Procedure.—Transfer about 1 meq. of test compound to a glass-stoppered, 125-ml. conical flask and dissolve or suspend in 20 ml. of ethanol. Add 25.0 ml. of 2,4-DNPH reagent, insert the stopper, and place the flask in a constant-temperature bath maintained at 50–55° for 1 hr. (*Note:* aldehydes are allowed to react at room temperature for the prescribed 1 hr.) Cool and transfer the contents of the flask to a 400-ml. beaker with the aid of 100 ml. of water. Add 10 ml. of hydrochloric acid, 5 Gm. of potassium bromide, and titrate slowly