Convulsions Induced by 2-*N*-Di-*n*-Butylaminoethanol[1]

ROLF HARTUNG, LESTER B. PITTLE, AND HERBERT H. CORNISH

The University of Michigan School of Public Health,
Department of Industrial Health, Ann Arbor, Michigan 48104

Convulsions Induced by 2-*N*-Di-*n*-Butylaminoethanol. HARTUNG, ROLF, PITTLE, LESTER B., and CORNISH, HERBERT, H. (1970). *Toxicol. Appl. Pharmacol.* **17**, 337–343. Dibutylaminoethanol (Bu$_2$AE) produces convulsions in rats leading to respiratory arrest regardless of route of administration. The lethality of 5 × LD50 doses of Bu$_2$AE can be readily overcome by artificial respiration for 2–4 hr. *In vivo* phrenic nerve–diaphragm preparations indicate that the respiratory arrest is due to a neuromuscular blockade. Spontaneous activity of the phrenic nerve increases during convulsions and persists during blockade. Pretreatment with mephenesin or diphenylhydantoin, but not pentobarbital, was effective in counteracting convulsions and respiratory arrest. Bu$_2$AE has also been shown to be a moderate inhibitor of acetylcholinesterase. It is proposed that the increased nerve activity during convulsions with its simultaneous increase in acetylcholine release, acts in concert with the moderate level of cholinesterase inhibition, producing a neuromuscular blockade due to excessive acetylcholine accumulation at the junction.

Dibutylaminoethanol (Bu$_2$AE) has found use as a conditioning agent for cellulose acetate filaments to facilitate textile manufacture, a catalyst in polyurethane foam manufacture, an anticorrosion additive for lubricants and hydraulic fluids, in the manufacture of emulsifying and dispersing agents, and in the curing of silicone resins.

Bu$_2$AE has been investigated by us because it is one of the more toxic in a long series of alkyl-substituted aminoethanols. The oral LD50 of this compound has been reported as 1.07 g/kg by Smyth *et al.* (1954). Cornish *et al.* (1969) found that Bu$_2$AE in drinking water at 0.2 and 0.4 g/kg per day in rats resulted in reduced body weight and slight increases in kidney weight without accompanying pathology. Inhalation studies at 70 ppm for 6 hr daily for 5 days, resulted in weight losses, chromodacryorrhea, tremors, and some convulsions. At that dose level there was 20% mortality.

Characteristically Bu$_2$AE acts by inducing strong clonic-tonic convulsions which closely resemble a Grand Mal seizure. These convulsions were noted after oral and intraperitoneal administrations as well as inhalation of Bu$_2$AE. All observed mortalities due to Bu$_2$AE were preceded by convulsions. Because of this close relationship, they were studied in greater detail.

METHODS

There appeared to be no qualitative difference in the convulsions associated with acute or subchronic exposures or with variations in route of administration. Therefore

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the following series of experiments were standardized using ip injections of Bu_2AE. Male Sprague-Dawley rats (Spartan strain) were used throughout.

Like most alkyl-substituted aminoethanols, Bu_2AE is normally quite alkaline with a pK_a of 9.67 (Gero, 1963). Prior to use, commercial Bu_2AE (Eastman Organic Chemicals) was further purified by fractional vacuum distillation. It was then neutralized with HCl to avoid the direct effects of alkalinity. Doses are reported in terms of Bu_2AE base. Statistical analyses of the ED50 and LD50 data were done using the logistic analysis of Berkson (1944), which we have programmed for computer use. Electroencephalograms were taken under pentobarbital anesthesia between right frontal and right parietal areas with the electrodes directly attached to the skull. *In vivo* nerve-diaphragm preparations were prepared as illustrated in Fig. 1. Mathematical analysis of nerve activity was done by computer-assisted polynomial regression.
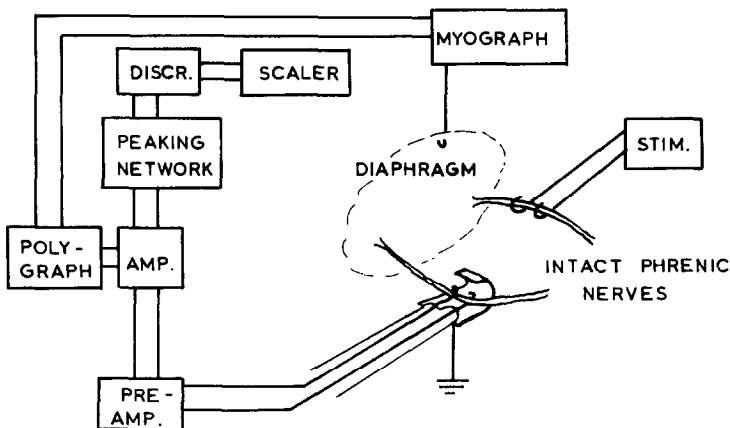

FIG. 1. Experimental configuration for measuring spontaneous phrenic nerve activity and for simultaneous determination of threshold voltage for stimulation of contraction of the rat diaphragm.

RESULTS

After ip injection the median effective dose for the production of convulsions was 50.6 mg/kg (95% fiducial limits: 31.6–80.9), and the LD50 was found to be 89.1 mg/kg (95% fiducial limits: 40.6–195.6). There is a significant overlap between convulsive dose and lethal dose. Many of the rats which reached the convulsive stage also died.

Shortly after Bu_2AE injection, there appears to be an increase in rhythmic electrical activity of the EEG which becomes very pronounced during the ensuing convulsions (Fig. 2). In this case, these convulsions were reduced to very heavy tremors under the influence of the barbiturate anesthesia. During light pentobarbital anesthesia, the rat will frequently wake up after Bu_2AE and shortly go into frank convulsions. The convulsions then lead to a respiratory arrest and a flattening of the EEG due to anoxia. The convulsions arose centrally as indicated by the EEG and substantiated by the fact that convulsions disappeared distally upon spinal section.

The immediate cause of death after Bu_2AE appears to be the respiratory arrest which follows immediately after the convulsions. We attempted to counteract the respiratory

arrest phase by artificial respiration after doses up to 500 mg/kg ip Bu₂AE. This repre-
sents approximately 5–6 LD50. The rats were placed under light pentobarbital anes-
thesia and respired via a tracheal cannula using a Physiograph respirator. After an
initial period of moderate convulsions and tremors, the experimental animals stopped
breathing spontaneously, as tested by briefly interrupting the artificial respiration. The
animals did not develop cyanosis at any time during this treatment. The artificial respira-
tion counteracted the respiratory failure, but not the convulsions.

After the convulsive phase, the animals appeared to be in a generalized state of
flaccid paralysis for 2–4 hr. After these 2–4 hr of artificial respiration, all animals were
able to resume breathing on their own and were able to walk a short time after that.
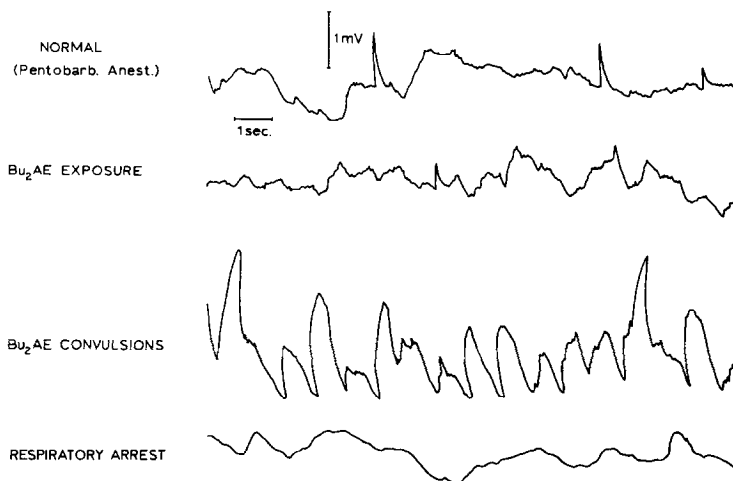When function was regained, the signs of tremors and convulsions did not reappear.

Fig. 2. EEG—right frontal to right parietal; electrodes placed on the surface of the skull in a rat after
250 mg/kg ip Bu₂AE.

Since artificial respiration was effective in counteracting the lethality of Bu₂AE due
to respiratory arrest without appreciably affecting the convulsions, we investigated the
nature of that respiratory arrest. For this investigation, we utilized the *in vivo* nerve-
diaphragm preparation as illustrated in Fig. 1. We were thus able to measure the spon-
taneous activity of the intact phrenic nerve, and also were able to study the effect of
stimulating the phrenic nerve on the contractility of the rat diaphragm. Stimulating the
phrenic nerve indicated that a neuromuscular blockade developed whose onset was dose
related (Fig. 3). For individual rats within these groups, the onset of the blockade was
typically abrupt. There also was a general indication that the severity of the blockade
was dose related.

The recordings of spontaneously occurring nerve activity exhibited a great deal more
variability. The basic method of measurement, using a scaler and counting all impulses
above a certain threshold voltage, plainly suffered from lack of sophistication and
equipment. Nevertheless, the data in Fig. 4 demonstrate that some activity in the intact
phrenic nerve persists even during the neuromuscular blockade, and that there is a
trend toward higher nerve activity for higher dose levels of Bu₂AE. Much of that increase

occurs during the convulsive stage and is probably due to the increased central stimulation after Bu_2AE.
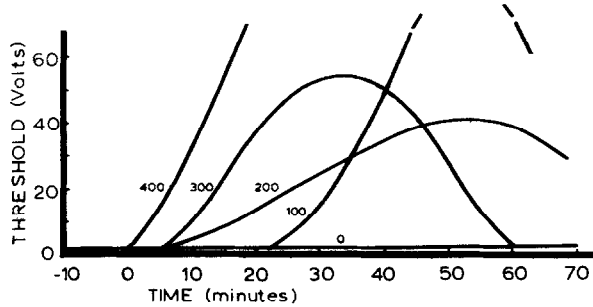

FIG. 3. Polynomial regressions (5th degree) of threshold voltages for phrenic nerve stimulation eliciting contraction of the diaphragm. Time of injection is at 0 min; dosages are indicated as mg/kg ip of Bu_2AE. 400 mg/kg: No. of rats (NR) = 5, No. of determinations (ND) = 299, F value = 72.40; 300 mg/kg: NR = 5, ND = 332, F = 23.50; 200 mg/kg: NR = 8, ND = 500, F = 27.15; 100 mg/kg: NR = 2, ND = 137, F = 205.53; 0 mg/kg: NR = 2, ND = 137, F = —.
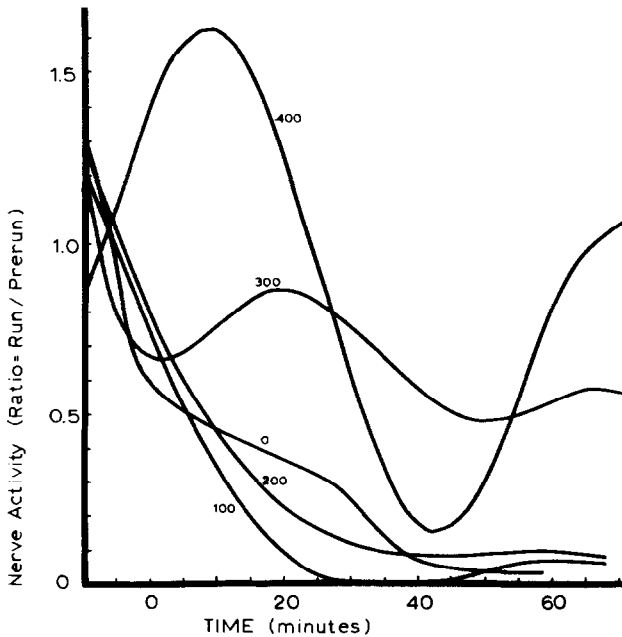

FIG. 4. Polynomial regressions (5th degree) of relative spontaneous phrenic nerve activity. Time of injection is at 0 min, dosages are indicated as mg/kg ip of Bu_2AE. 400 mg/kg: NR = 5, ND = 391, F = 7.01; 300 mg/kg: NR = 5, ND = 332, F = 0.59; 200 mg/kg: NR = 8, ND = 500, F = 21.99; 100 mg/kg: NR = 2, ND = 139, F = 38.62; 0 mg/kg: NR = 2, ND = 78, F = 6.98.

Many of the preceding data of course had to be gathered during artificial respiration. Without artificial respiration or other treatment, the time course of these events is normally quite short. At 250 mg/kg, which represents approximately a 3 × LD50 dose, time to first convulsion was typically 2.9 ± 0.3 (mean ± SE) min and time to death

TABLE 1

EFFECT OF MEPHENESIN ON Bu$_2$AE CONVULSIONS IN RATS

Pretreatment Mephenesin (mg/rat)	Bu$_2$AE (mg/kg)	Interval between treatments (min)	Time to 1st convulsion, t_c (min)	Time to death, t_d (min)	Survival
20 iv	400	2.0	—	—	Survived
20	320	3.0	—	—	Survived
20	400	3.0	2.0	4.0	
20	400	3.0	37.0	—	Survived
20	400	3.0	15.0	37.0	
40 po	250	90	—	—	Survived
40	250	90	1.8	6.2	
40	250	90	—	—	Survived
40	250	90	10.0	—	Survived
40	250	90	1.0	5.5	
40	250	90	1.8	6.0	
80 po	250	90	4.0	8.0	
80	250	90	—	—	Survived
80	250	90	1.8	5.5	
80	250	90	1.8	8.6	
80	250	90	—	—	Survived
80	250	90	2.2	7.8	
80	250	45	11.5	—	Survived
80	250	45	—	—	Survived
80	250	45	6.5	—	Survived
80	250	45	—	—	Survived
80	250	45	2.5	15.8	
80	250	30	—	—	Survived
80	250	30	4.2	9.0	
80	250	30	4.2	—	Survived
80	250	31	—	—	Survived
80	250	30	3.2	9.0	Survived

TABLE 2

EFFECT OF DIPHENYLHYDANTOIN ON Bu$_2$AE CONVULSIONS IN RATS

Pretreatment diphenyl-hydantion (iv) (mg/kg)	Bu$_2$AE (mg/kg)	Interval between treatments (min)	Time to 1st convulsion, t_c (min)	Time to death t_d (min)	Survival
5	250	2.0	—	—	Survived
5	250	2.0	2.0	7.2	
5	250	2.0	—	—	Survived
5	250	1.8	—	—	Survived
5	250	1.8	—	—	Survived
5	250	2.0	—	—	Survived
5	250	2.0	—	—	Survived
					(85.7%)

7.8 ± 1.0 min. Under these conditions, the mortality was 100% in 10 experiments. The same dose was used in most of the subsequent experiments which tested various Bu_2AE drug interrelations.

We had previously demonstrated that Bu_2AE was a moderately active inhibitor of acetylcholinesterase (Hartung and Cornish, 1968). Therefore, we investigated the effect of pretreatment with atropine on Bu_2AE-induced convulsions. The Bu_2AE was injected when atropinization had been achieved, as judged by pupil size. Dosages of atropine ranging from 0.02 to 0.2 mg/kg had no significant effect on the time course or the outcome of convulsions and respiratory arrest induced by Bu_2AE doses as low as 125 mg/kg. Since atropine is mainly effective in counteracting the parasympathomimetic effects of anticholinesterases, but not neuromuscular effects, these results were not unexpected.

The possibility still remained that this was a curarelike neuromuscular blockade. Therefore we employed 0.85 mg/kg iv edrophonium, which is a valuable antagonist against some curariform neuromuscular blockers. It too had no effect on the time course or outcome.

Since none of the tested peripherally acting antagonists to neuromuscular blocking agents had been effective in counteracting Bu_2AE toxicity, we decided to test some of the centrally acting anticonvulsants.

Throughout our work with anesthetized rats we had noted that barbiturates in moderate doses were unable to control Bu_2AE-induced convulsions. Therefore we investigated a series of other anticonvulsants.

Magnesium sulfate (1 mg/kg iv) given as pretreatment resulted in 1 survival in 6. The significance of that effect may be questionable. Higher dose levels may be more effective.

Mephenesin was far more effective as an anticonvulsant against Bu_2AE (Table 1). In these tests, a curious effect became evident. Animals which did not develop convulsions, survived without developing any indication of respiratory arrest. When the onset of convulsions could be delayed to 6 min, the rats usually survived. In those cases the convulsions were much weaker and alternated with heavy tremors for as long as 1.5 hr without producing a respiratory arrest.

Diphenylhydantoin (5 mg/kg iv) was the most effective drug for preventing Bu_2AE-induced convulsions (Table 2). It almost completely prevented convulsions and mortalities after normally lethal doses of Bu_2AE.

On the other hand, trimethadione was ineffective in controlling Bu_2AE-induced convulsions even at doses (150 mg/kg ip) which had been found effective in counteracting a lethal dose of the convulsant pentylenetetrazol.

DISCUSSION

Bu_2AE has been shown to produce convulsions and neuromuscular blockade resulting in respiratory arrest. None of the peripherially acting drugs selected for possible antagonistic action to the neuromuscular blockade produced by Bu_2AE had any noticeable effect. However, two centrally acting anticonvulsants were effective in counteracting both the convulsive seizures and the respiratory arrest produced by Bu_2AE. Thus for Bu_2AE there appears to be a relationship between the primary mechanism

causing convulsions and the subsequent neuromuscular blockade which results in death due to respiratory arrest.

Bu$_2$AE appears to possess a duality in its mode of action. First, it produces a greatly increased CNS activity which results in strong tonic-clonic convulsions. Respiratory arrest due to the neuromuscular blockade develops during this period of elevated CNS activity. Since Bu$_2$AE is also a moderate acetylcholinesterase inhibitor, this increased nerve activity with its simultaneous increase in release of acetylcholine at the junction, may result in a neuromuscular blockade due to excess acetylcholine at that site.

ACKNOWLEDGMENTS

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