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Effects of convulsant barbiturates on vascular smooth muscle

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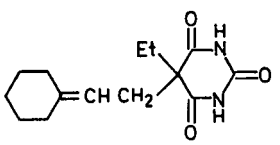
The convulsant barbiturate, 5-(2-cyclohexylidene-ethyl)-5-ethyl barbituric acid (CHEB), produces contraction in rabbit aortic strips. Contractions effected by either CHEB or tyramine were preceded by a lag time and both agents induced tachyphylaxis; however, cross-tachyphylaxis could not be demonstrated. Phenoxybenzamine and atropine failed to affect CHEB-induced responses, whereas pentobarbitone selectively blocked and also reversed CHEB contractions. Prevention, but not reversal, of tachyphylaxis was also accomplished with pentobarbitone. These results suggest that CHEB does not act through the release of noradrenaline or acetylcholine; nor does it exert an effect on the receptors for these amines or on those for histamine. Pentobarbitone, however, appears to compete with CHEB for common receptors. Another convulsant barbiturate, 5-ethyl-5-(dimethylbutyl)barbituric acid (DMBB), and its optical isomers were also examined. The racemic mixture had no contractile activity, but the (+)-isomer elicited CHEB-like effects. The (−)-isomer, on the other hand, was like pentobarbitone in that it antagonized both CHEB- and (+)-DMBB-induced contractions. These studies illustrate that convulsant barbiturates are able to stimulate vascular smooth muscle; therefore, it is suggested that the rabbit aortic strip may serve as an *in vitro* working model for study of the mechanism of action of these drugs in the central nervous system.

Convulsant barbiturates have usually been examined for their ability to produce central nervous system (CNS) excitation in contrast to the depression characteristically associated with hypnotic barbiturates. While studying the CNS activity of 5-(2-cyclohexylidene-ethyl)-5-ethyl barbituric acid (CHEB; I), a convulsant barbiturate, Downes, H. & Williams, J. K. (personal communication) observed that the drug produced a large increase in blood pressure in unanaesthetized spinal cats. We found it to induce a contraction on the rabbit aortic strip. This report describes the results of experiments designed to study some characteristic effects of CHEB on this preparation. In addition, a comparison of some of the actions of CHEB was made with another convulsant barbiturate, 5-ethyl-5-(dimethylbutyl)barbituric acid (DMBB; II), and its two optical isomers.



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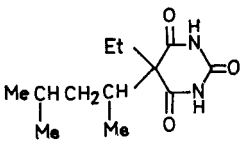

II

Recently, Perry, Downes & Karler (1969) reported that the convulsant activity associated with the racemic mixture of DMBB resides in the (+)-isomer which is a potent convulsant, whereas the (−)-isomer has primarily depressant activity. Like CHEB, the (+)-isomer of DMBB produces a contraction of the aortic strip. It is suggested that this smooth muscle preparation may serve as a working model for the study of the mechanism of action of the convulsant barbiturates on the CNS.

EXPERIMENTAL

Materials and methods

Aortic strips were obtained from 2·0–3·5 kg rabbits killed by rapid injection of air into an ear vein. Spiral strips, 3–4 mm in width and 20–30 mm in length, were prepared according to Furchgott (1960). The aortic segments were mounted vertically in jacketed 30 ml tissue baths maintained at 37·5°, and the tissues were bathed in Krebs bicarbonate solution gassed with 5% carbon dioxide in oxygen. The fluid in the bath was exchanged by overflow. Inactivation of catecholamines was retarded by the presence of the sodium salt of ethylenediamine tetra-acetic acid ($1{\cdot}0 \times 10^{-5}$M) in the Krebs bicarbonate solution.

A Grass stain gauge transducer (FT03C) and model 5 Polygraph were used to measure isometric contractions. Drug effects were recorded as mm of pen deflection at a sensitivity of 0·2 mV/cm and a chart speed of 0·25 mm/s.

The drugs used were: noradrenaline bitartrate (Sterling-Winthrop, New York, N.Y.); histamine dihydrochloride (Eastman Organic Chemicals, Rochester, N.Y.); acetylcholine bromide (Eastman Organic Chemicals, Rochester, N.Y.); tyramine hydrochloride (Mann Research Laboratories, New York, N.Y.); atropine sulphate (Mallinckrodt Chemicals, St. Louis, Mo.); sodium pentobarbitone (Robinson Laboratory Inc., San Francisco, Calif.); sodium phenobarbitone (Merck & Co.,

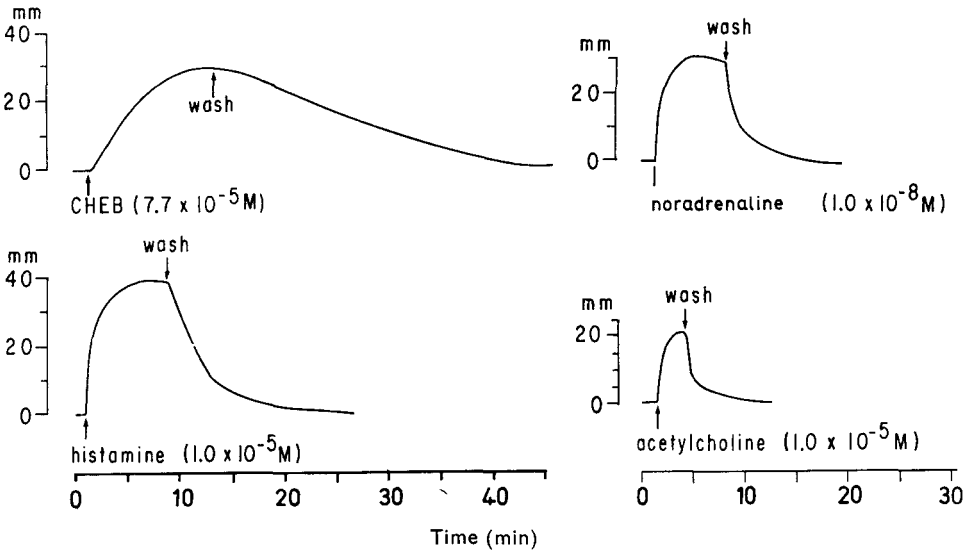

FIG. 1. Response of the aortic strip to CHEB and other agonists. Tissue bathed in a 37·5° Krebs bicarbonate solution. The responses are copies of original recordings with a compressed time scale. The bathing solution containing drug was replaced at the peak of contraction, as indicated by the term *wash* in each schematic response.

Rahway, N.J.); phenoxybenzamine hydrochloride (Smith Kline & French, Philadelphia, Pa.); the sodium salts of CHEB and DMBB, and of the two optical isomers of DMBB (all prepared in our laboratory). Phenoxybenzamine hydrochloride was first dissolved in a small amount of propylene glycol and final concentrations were made by diluting with isotonic NaCl solution. All other drugs were dissolved in isotonic NaCl solution; the sympathomimetic amines were similarly prepared and, in addition, were in 0·01M HCl. Drug solutions were added to the tissue bath in a volume of 0·5 ml or less.

RESULTS

Effect of CHEB on rabbit aortic strips. The addition of CHEB to the muscle bath caused a contraction of the aortic strip (Fig. 1). The onset of the effect and the recovery after wash out are slower than those of noradrenaline, histamine or acetylcholine. The (+)-isomer of DMBB displayed effects similar to those of CHEB; but the (−)-isomer did not produce contraction.

Effect of repeated exposure to CHEB. Repeated exposure of the aortic muscle preparation to CHEB produced tachyphylaxis (Fig. 2A). The (+)-isomer of DMBB caused a similar effect (Fig. 2B).

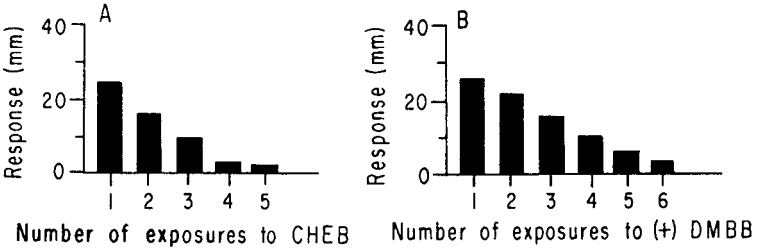

FIG. 2. (A) Effect of repeated exposures to the same concentration ($7{\cdot}7 \times 10^{-5}$M) of CHEB. The figure illustrates data from a typical experiment. The bars of the histogram represent the magnitude of the peak responses.

(B) Effect of repeated exposures to the same concentration ($1{\cdot}5 \times 10^{-4}$M) of the (+)-isomer of DMBB. The figure illustrates data from a typical experiment. The bars of the histogram represent the magnitude of the peak responses. In both A and B, each exposure was followed by a wash procedure.

Effect of pentobarbitone and phenobarbitone on the CHEB response. The prior addition of pentobarbitone ($5{\cdot}5 \times 10^{-5}$M) to the tissue bath blocked the contraction produced by CHEB ($7{\cdot}7 \times 10^{-5}$M) (Table 1); after washing pentobarbitone from the bath, a normal CHEB response could be elicited. Phenobarbitone was also able to block the CHEB-induced contraction, but in a concentration approximately ten times

Table 1. *Effect of pentobarbitone on the CHEB response*

Treatment (in order of exposure)	Concentration (M)	Response (mm)
Noradrenaline	$1{\cdot}0 \times 10^{-7}$	31
Pentobarbitone + noradrenaline	$5{\cdot}5 \times 10^{-5}$ + $1{\cdot}0 \times 10^{-7}$	32
Pentobarbitone + CHEB	$5{\cdot}5 \times 10^{-5}$ + $7{\cdot}7 \times 10^{-5}$	0
CHEB	$7{\cdot}7 \times 10^{-5}$	32
Noradrenaline	$1{\cdot}0 \times 10^{-7}$	32

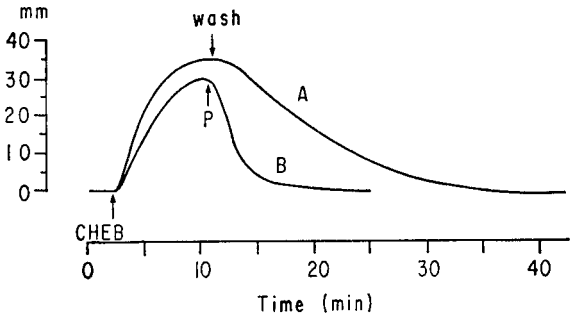

FIG. 3. Effect of (A) the wash and of (B) the addition of a blocking concentration of pentobarbitone ($5{\cdot}5 \times 10^{-5}$M) on the rate of relaxation of a CHEB-induced contraction (P = pentobarbitone). The different treatments were applied at the peak of response.

that of pentobarbitone. The blocking concentrations of both drugs did not influence the control response to noradrenaline.

The interaction between pentobarbitone and CHEB was observed in two other types of experiments. Fig. 3 shows the influence of pentobarbitone on the relaxation rate of CHEB-induced contraction. When pentobarbitone was added to the bath at the peak of a CHEB contraction, the muscle relaxed more rapidly than it did after washing out CHEB. The data in Fig. 4 illustrate another interaction between CHEB and pentobarbitone in which pentobarbitone not only blocked the CHEB contraction, but also protected the muscle against the effect of repeated exposures to CHEB. Fig. 4A shows that a CHEB response was unchanged even after several exposures of the muscle to CHEB in the presence of pentobarbitone; therefore, pentobarbitone blocked the development of tachyphylaxis. The results shown in Fig. 4B demonstrate that pentobarbitone could not reverse an existing CHEB-induced tachyphylaxis.

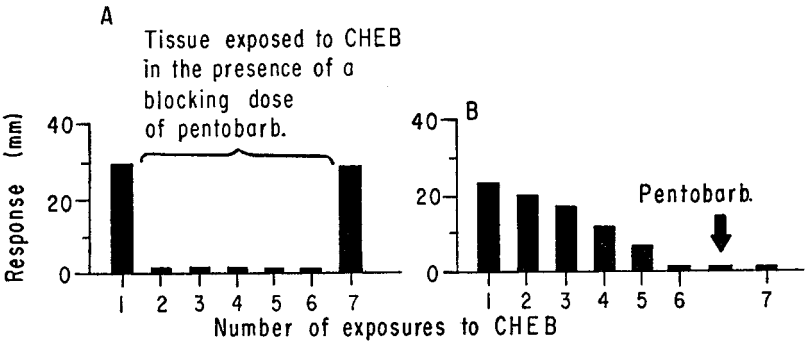

FIG. 4. The influence of pentobarbitone on the tissue response to CHEB. In (A), the initial and final responses were produced by CHEB alone in a concentration of $7{\cdot}7 \times 10^{-5}$. The intervening exposures to the same concentration of CHEB were made in the presence of a blocking dose of pentobarbitone ($5{\cdot}5 \times 10^{-5}$M). In (B), tachyphylaxis was produced by repeated exposures to CHEB. The arrow indicates the exposure of the muscle to pentobarbitone ($5{\cdot}5 \times 10^{-5}$M) for a 10-min period. In both (A) and (B), each exposure was followed by a wash procedure.

Pentobarbitone, previously placed in the tissue bath, also blocked the contraction produced by the (+)-isomer of DMBB; but doses reversing the CHEB contraction (Fig. 3) did not reverse the (+)-DMBB response.

Effect of noradrenaline depletion on the CHEB response. Tachyphylaxis to certain drugs has been explained on the basis of a depletion of transmitter substance. For example, the depletion of noradrenaline stores in tissue has been invoked as an explanation of tyramine-induced tachyphylaxis. To test the possibility that CHEB acts indirectly by stimulating the release of noradrenaline, the influence of tyramine-induced tachyphylaxis on the CHEB response was studied. No significant difference was found between the CHEB response after tyramine-induced tachyphylaxis and the control CHEB contraction at CHEB concentrations of $2{\cdot}0 \times 10^{-4}$–$5{\cdot}0 \times 10^{-5}$M. Furthermore, tissue rendered insensitive to CHEB responded normally to tyramine. Cross-tachyphylaxis was therefore absent.

Table 2. *Effect of atropine on the CHEB response*

Treatment	Concentration (M)	Control response (mm)	Response after atropine (1×10^{-5}M) (mm)
Acetylcholine	$1{\cdot}0 \times 10^{-5}$	24	0
Noradrenaline	$5{\cdot}0 \times 10^{-8}$	41	39
CHEB	$1{\cdot}0 \times 10^{-4}$	39	26

Effect of atropine on the CHEB response. The ability of atropine to block CHEB-induced contractions was examined in four experiments. Atropine was placed in the bath 5 min before an agonist and the results in Table 2 show that atropine, in a concentration that blocked an acetylcholine response, was ineffective against a contraction produced by either noradrenaline or CHEB. Although atropine appears to reduce the CHEB response in this experiment, the diminished second contraction can be explained by the occurrence of tachyphylaxis. Experiments of the type depicted in Fig. 2A demonstrated that a 10–40% reduction in the contractions produced by CHEB occurs with the second exposure of the tissue to the drug. Atropine did not produce a greater reduction in the CHEB responses than could be accounted for by tachyphylaxis; therefore, it did not exert any antagonism to CHEB.

Effect of phenoxybenzamine on the CHEB response. In three experiments, the influence of phenoxybenzamine on the contraction produced by CHEB and other agonists was studied. Phenoxybenzamine was placed in the muscle bath 45 min before the agonists. Table 3 shows that the α-receptor blocking drug blocked the noradrenaline contraction and greatly reduced the histamine response. On the other hand, it had little influence on an acetylcholine-induced contraction, and the CHEB response was not diminished more than expected from the occurrence of tachyphylaxis.

Table 3. *Effect of phenoxybenzamine on the CHEB response*

Treatment	Concentration (M)	Control response (mm)	Response after phenoxybenzamine ($1{\cdot}0 \times 10^{-5}$M) (mm)
Noradrenaline	$1{\cdot}0 \times 10^{-6}$	23	0
Acetylcholine	$1{\cdot}0 \times 10^{-4}$	6	5
Histamine	$1{\cdot}0 \times 10^{-5}$	28	10
CHEB	$7{\cdot}7 \times 10^{-5}$	12	10

DISCUSSION

CHEB initiated a contraction in the rabbit aortic strip after a brief lag time (30–60 s). This lag time is characteristic; it did not occur with the direct-acting agonists, noradrenaline, acetylcholine or histamine. However, tyramine, an indirect-acting agent, displayed a lag time comparable to that of CHEB. The similarity suggests that CHEB might exert its action on smooth muscle in a manner analogous to that of tyramine. The onset of contraction induced by the (+)-isomer of DMBB was also preceded by a lag time.

Burn (1959) reported that the vasoconstriction caused by thiopentone in normal rabbits could be prevented if the tissue stores of noradrenaline were first depleted by pretreatment with reserpine. It appears that the release of noradrenaline accounts for the vasoconstrictor activity of thiopentone. Furchgott (1963) has shown that tachyphylaxis to tyramine in rabbit aortic strips results from the depletion of releasable noradrenaline. Both CHEB and the (+)-isomer of DMBB produced tachyphylaxis, suggesting that they may also act through the release of a biologically active substance. Further study has since demonstrated that cross-tachyphylaxis exists between CHEB and the (+)-isomer of DMBB. The existence of cross-tachyphylaxis implies that these agents possess a similar mode of action, perhaps release of an active substance from a common pool. Tyramine also caused tachyphylaxis; however, cross-tachyphylaxis between CHEB and tyramine did not exist. Its absence indicates that CHEB and tyramine either do not release the same substance or, if the same substance is released, it must be from a different pool.

Furchgott (1954) observed that the blockade of α-adrenergic receptors also reduced the response to histamine and 5-hydroxytryptamine; therefore, response to an agent acting through the release of any of these substances or at their receptor level should be markedly reduced by phenoxybenzamine. In the present experiments, phenoxybenzamine did not exert any influence on the CHEB-induced contraction. On the basis of these findings, it appears that CHEB does not act through a release of noradrenaline, histamine or 5-hydroxytryptamine; nor does it act directly on the receptors for these biogenic amines. These data support the conclusion that CHEB and tyramine do not act by release of the same substance.

Because atropine failed to influence the CHEB effect, it is unlikely that the contractile effect is mediated either indirectly through the release of acetylcholine or directly at cholinergic sites. It was found that the acetylcholine-induced contraction can be selectively blocked by atropine without influencing the activity of either noradrenaline or CHEB.

Pentobarbitone appears to exert a specific antagonism towards the contractile activity of CHEB. In a concentration similar to that of CHEB, it has no effect on the response to noradrenaline, but blocked the response to CHEB. When this same dose of pentobarbitone was given at the peak of a CHEB-induced contraction, the muscle rapidly relaxed. These data suggest that pentobarbitone does not antagonize the action of CHEB by a physiological depression but possibly by a competition for common receptors.

Phenobarbitone can also block a CHEB-induced contraction, but the concentration required is ten times that of pentobarbitone. The difference in the blocking concentration may be explained on the basis of differences in lipid solubility; that is, in a methylene chloride:aqueous system, the partition coefficient of pentobarbitone is about ten times that of phenobarbitone.

When tissue was exposed to CHEB several times, tachyphylaxis developed. However, if the tissue was repeatedly exposed to CHEB in the presence of pentobarbitone and then to CHEB alone, the final CHEB response was equal in magnitude to the initial response. This prevention of tachyphylaxis lends further support to the conclusion that CHEB and pentobarbitone compete for a common receptor and that pentobarbitone is capable of antagonizing the interaction between CHEB and its receptor. Failure of pentobarbitone to reverse CHEB-induced tachyphylaxis indicates that CHEB probably produces contraction in smooth muscle through a chain of events, one of which is slowly reversible and responsible for the development of tachyphylaxis. Responsiveness to CHEB reappears only after time is allowed for this process to return to its original state.

Racemic DMBB, like CHEB, was reported to be convulsant in mice (Perry & others, 1969); however, unlike CHEB, it has no contractile effect on vascular smooth muscle. The *in vivo* studies established that the (+)-isomer of DMBB possesses the convulsant activity and that the (−)-isomer is depressant to the CNS. Our studies demonstrate that the (+)-isomer of DMBB exerted a CHEB-like effect on the aortic strips and the the (−)-isomer, like pentobarbitone, blocked contractions induced by both the (+)-isomer of DMBB and CHEB.

The close correlation between convulsant activity and the ability to induce vascular smooth muscle contraction suggests that the *in vitro* muscle preparation may serve as a working model for study of the mechanism of action of the convulsant barbiturates on the CNS.

Acknowledgements

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