The peak solubility value displayed during the dissolution of the anhydrous form of testosterone (Fig. 2) may correspond to a short-term steady-state situation involving equal rates of dissolution of the anhydrous form and crystallization of the stable hydrate. On the other hand, the peak solubility could also correspond to the solubility of the anhydrous form. However, the solubilities of both forms of testosterone appear to approach the same value with time (11).

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 19, 1971, from the *Department of Pharmacy, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication May 14, 1973.

* Present address: Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

▲ To whom inquiries should be directed.

Central Cholinomimetic Actions of 3,3-Dimethyl-1-butanol Carbamate

LYNN WECKER* and C. Y. CHIOU▲

Abstract □ The action of 3,3-dimethyl-1-butanol carbamate on the CNS was studied to determine the mechanism of its convulsant activity. Dimethylbutanol carbamate penetrated into the brain with maximum levels at 15 min. after intraperitoneal injection. The LD_{50} of dimethylbutanol carbamate in mice was 21 mg./kg., and atropine pretreatment protected animals against the toxic effects of dimethylbutanol carbamate, whereas physostigmine enhanced dimethylbutanol carbamate toxicity. Dimethylbutanol carbamate potentiated nicotine-induced convulsions in mice and had no effect on brain cholinesterase activity. Therefore, it appears that dimethylbutanol carbamate is a centrally active agent that produces convulsions *via* a mechanism similar to that postulated for nicotinic agents, although it produces antinicotinic actions peripherally.

Keyphrases □ 3,3-Dimethyl-1-butanol carbamate—mechanism of convulsant activity, central cholinomimetic actions, mice □ Cholinomimetic actions—3,3-dimethyl-1-butanol carbamate, mice □ Convulsant activity—3,3-dimethyl-1-butanol carbamate, mice

3,3-Dimethyl-1-butanol carbamate, a synthetic carbachol analog containing a carbon in place of the quaternary nitrogen atom, is an unusually interesting compound with a potent peripheral antinicotinic effect (1, 2) as well as a central convulsant action in mice, producing tonic-clonic convulsions (3, 4). The effects of this agent on cholinergic-induced drinking behavior in rats have been studied, and it was reported (5) that dimethylbutanol carbamate, when implanted into the lateral hypothalamic region of the rat brain, initiated a response similar to those of acetylcholine and carbachol. These results suggested a central cholinomimetic action of dimethylbutanol carbamate. It has been hypothesized that alterations in brain levels of acetylcholine may be correlated with behavioral changes and that there is a decrease in the acetylcholine content of the brain during convulsions (6–8). When an increase in cortical activity is evident, there is a release of acetylcholine from its storage sites in nerve terminals, resulting in an overall decrease in brain acetylcholine content (6–8).

Dimethylbutanol carbamate was found to cause a significant increase in the release of acetylcholine from the minced guinea pig cerebral cortex (1). Therefore, the present study investigated the possibility that the CNS actions of dimethylbutanol carbamate are due to a direct-releasing action of acetylcholine from storage sites inside nerve endings, a mechanism similar to that reported for the action of nicotinic agents (9). The unusual character of this compound could be used as a tool to elucidate cholinergic mechanisms.

EXPERIMENTAL

ICR Charles River mice, weighing 20–35 g., were used for all studies. To determine the extent of brain penetration of dimethylbutanol carbamate, mice were injected intraperitoneally with 20 mg./kg. ^{14}C-dimethylbutanol carbamate (0.7 μc./mg.). Dimethylbutanol carbamate was synthesized in this laboratory according to the method described previously (1). The animals were sacrificed by cervical dislocation at various times, and their brains were removed, weighed, homogenized (1 ml. water/1 g. tissue), and solubilized[1] at 50° for 12–15 hr. The samples were allowed to cool, and

[1] NCS solubilizer, Amersham Searle, Arlington Heights, Ill.

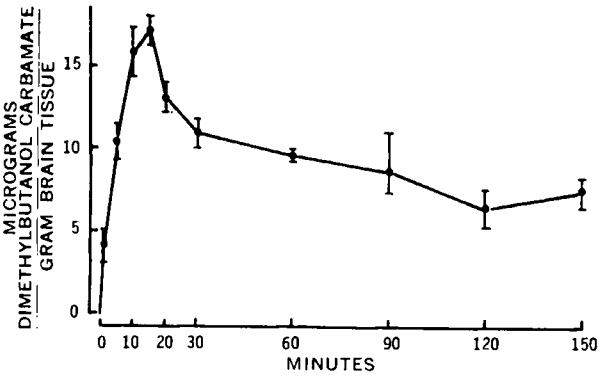

Figure 1—*Uptake of dimethylbutanol carbamate by mouse brain. Mice were injected with 20 mg./kg. ^{14}C-dimethylbutanol carbamate and sacrificed at time intervals shown on the abscissa. The brains were analyzed for radioactivity, and results are expressed as micrograms dimethylbutanol carbamate per gram brain tissue. Each point is a mean of three values, and the bars represent standard errors.*

then 20 ml. scintillation cocktail (6 g. 2,5-diphenyloxazole/l. toluene) was added. The samples were analyzed for radioactivity on a liquid scintillation system[2], with an error of ±1.0%.

The effects of atropine and physostigmine on the toxicity of dimethylbutanol carbamate were determined as follows. Mice were divided into three groups. The LD_{50} of dimethylbutanol carbamate was determined as the control using the first group. The second group of mice was pretreated with atropine (100 mg./kg.) 30 min. prior to dimethylbutanol carbamate administration. It was reported (10) that the effect of this atropine pretreatment is to decrease significantly brain levels of acetylcholine. The third group of mice was pretreated with 0.2 mg./kg. physostigmine 30 min. prior to dimethylbutanol carbamate injection. This physostigmine pretreatment has been found to produce significant behavioral alterations in mice. The LD_{50} and limits at the 95% confidence level for each group were determined by a log dose probit plot (11).

To determine the effects of dimethylbutanol carbamate on nicotine-induced convulsions, mice were divided into two groups. The first group was injected with nicotine intraperitoneally, and the number of animals that developed convulsions was noted. The second group was injected with a subconvulsant dose of dimethylbutanol carbamate (1 mg./kg.) intraperitoneally 30 min. prior to the nicotine injection, and the occurrence of convulsions was once again noted. The dose-response curves were constructed, and the ED_{50}'s to produce convulsant activity and potency ratios were calculated (11).

The effect of dimethylbutanol carbamate on intact brain cholinesterase was determined radioisotopically (12). Mice were sacrificed by cervical dislocation, and their brains were removed immediately, frozen, and weighed. The brains were homogenized for 30 sec. at 10,000 r.p.m.[3], in phosphate buffer (pH 8, 0.1 *M*), yielding a homogenate of 0.1 mg. brain/ml. phosphate buffer. A 0.1-ml. aliquot of the homogenate was added to centrifuge tubes containing phosphate buffer with or without dimethylbutanol carbamate (10^{-4} or 10^{-3} *M*). The tubes were preincubated for 15 min. at 37° before addition of ^{14}C-acetylcholine (1.2×10^{-5} *M*). The reaction mixtures were incubated for various periods, and 500 mg. cation-exchange resin[4] and 5 ml. absolute ethanol were added to stop the reaction. The mixture was shaken for 15 sec. and centrifuged at $1470 \times g$ for 5 min. Aliquots of the supernate (4.0 ml.) were sampled and added to 10 ml. scintillation cocktail (7 g. 2,5-diphenyloxazole and 100 g. naphthalene/l. dioxane). The samples were counted in a liquid scintillation system. Values for autohydrolysis of ^{14}C-acetylcholine were determined from incubation mixtures in the absence of homogenate. Initial rates of hydrolysis were determined during the first 30 min., and the rates were expressed as Δ c.p.m./min. Levels of statistical significance were determined with Student's *t* test at $p \leq 0.05$.

The drugs studied included 3,3-dimethyl-1-butanol carbamate, atropine sulfate, physostigmine sulfate, nicotine tartrate, and acetyl-1-^{14}C-choline iodine (4.53 mc./mmole)[5].

Table I—Effects of Atropine and Physostigmine on the Toxicity of Dimethylbutanol Carbamate in the Mouse

Treatment	Number of Animals	LD_{50} of Dimethylbutanol Carbamate, mg./kg.
Dimethylbutanol carbamate alone	150	21.0 (18.3–24.2)[a]
Atropine plus dimethylbutanol carbamate[b]	80	49.0 (29.7–80.9)[c]
Physostigmine plus dimethylbutanol carbamate[d]	80	17.0 (15.2–19.0)[c]

[a] Limits at the 95% confidence level determined by the method of Litchfield and Wilcoxon (11). [b] Treatment with atropine (100 mg./kg.) 30 min. prior to dimethylbutanol carbamate administration. [c] Significantly different from control at $p \leq 0.05$, determined by the method of Litchfield and Wilcoxon (11). [d] Treatment with physostigmine (0.2 mg./kg.) 30 min. prior to dimethylbutanol carbamate administration.

[2] Beckman liquid scintillation system, model 1650, Beckman Instruments, Fullerton, Calif.

RESULTS

To determine whether dimethylbutanol carbamate penetrated into the CNS, mice were injected intraperitoneally with ^{14}C-dimethylbutanol carbamate. Maximum brain levels were reached at approximately 15 min., and the concentration at that time was 17.1 mcg. dimethylbutanol carbamate/g. wet weight brain tissue (1.3×10^{-4} mole/kg.) (Fig. 1). After 15 min., the concentration of dimethylbutanol carbamate decreased steadily until a fairly constant level was apparent (8.5 mcg. dimethylbutanol carbamate/g. brain tissue) for the following 60 min.

When mice were injected with a lethal dose of dimethylbutanol carbamate, they exhibited initial ataxia followed by hyperactivity leading to tonic-clonic convulsions approximately 30–45 min. after injection. The duration of convulsions was approximately 5 min. before death occurred. At the time of death, the chest cavity of the animal was opened and heart function was inspected. Respiratory paralysis appeared to be the cause of death, but death was probably not due to a peripheral neuromuscular blockade since sciatic nerve gastrocnemius muscle blockade in rabbits was not achieved at doses that produced death. The LD_{50} for the mouse was 21 mg./kg. (Table I). When animals were pretreated with atropine, there was significant protection from dimethylbutanol carbamate toxicity and the LD_{50} was increased to 49 mg./kg. dimethylbutanol carbamate. Conversely, when mice were pretreated with physostigmine, dimethylbutanol carbamate toxicity was enhanced and the LD_{50} was lowered to 17 mg./kg.

The ED_{50} of nicotine to cause convulsion was lowered from 3.8 to 2.7 mg./kg. with dimethylbutanol carbamate pretreatment (Table II). This was a significant potentiation of convulsant activity, with a potency ratio of 1.4. The slopes of the dose-response curves indicate a parallelism with a slope ratio of 1.1.

The effect of dimethylbutanol carbamate on brain cholinesterase was studied to determine whether cholinesterase inhibition was involved with the central actions of dimethylbutanol carbamate. When mouse brain homogenates were incubated with dimethyl butanol carbamate (10^{-4} or 10^{-3} *M*), there was no effect on the rate

Table II—Effects of Dimethylbutanol Carbamate Pretreatment on Nicotine-Induced Convulsions in the Mouse

Treatment	Number of Animals	ED_{50} of Nicotine, mg./kg.	Slope
Nicotine alone	55	3.8 (3.4–4.2)[a]	1.3 (1.1–1.5)
Dimethylbutanol carbamate plus nicotine[b]	59	2.7 (2.3–3.1)[c]	1.4 (1.1–1.7)

[a] Limits at the 95% confidence level determined by the method of Litchfield and Wilcoxon (11). [b] Treatment with dimethylbutanol carbamate (1.0 mg./kg.) 30 min. prior to nicotine administration. [c] Significantly different from control at $p \leq 0.05$, determined by the method of Litchfield and Wilcoxon (11).

[3] Willems Polytron, Brinkmann Instruments, Westbury, N. Y.
[4] Amberlite CG120, Mallinckrodt Chemical Works, St. Louis, Mo.
[5] New England Nuclear, Boston, Mass.

Table III—Effects of Dimethylbutanol Carbamate on Rates of Acetyl-1-^{14}C-choline Hydrolysis by Mouse Brain Cholinesterases

Concentration of Dimethylbutanol Carbamate, *M*	Rate of Hydrolysis[a], Δ c.p.m./min.
Control[b]	96.1 ± 7.8
10^{-4}	105.3 ± 6.8
10^{-3}	108.0 ± 6.0

[a] Values expressed as the mean ± *SE* (n = 6). [b] Dimethyl sulfoxide (0.3% by volume) used as solvent control to prepare stock solutions of dimethylbutanol carbamate due to the limited solubility of dimethylbutanol carbamate in aqueous solutions.

of ^{14}C-acetylcholine hydrolysis (Table III), supporting a direct acetylcholine-releasing action by dimethylbutanol carbamate.

DISCUSSION

Dimethylbutanol carbamate has been shown to possess central cholinomimetic effects, although it has cholinolytic actions peripherally (1, 2). This agent has been shown to release acetylcholine from the minced guinea pig cerebral cortex (1) at concentrations that did not affect brain cholinestease activity, suggesting a direct acetylcholine-releasing action. From the studies on dimethylbutanol carbamate toxicity, it has been shown that atropine is able to protect mice from the lethal effects of dimethylbutanol carbamate, while physostigmine potentiates dimethylbutanol carbamate toxicity. Therefore, it appears that dimethylbutanol carbamate toxicity may be due to an increased transmitter release. This idea is supported by the observation that protection of cholinergic actions by atropine is able to decrease dimethylbutanol carbamate toxicity. Although precise action mechanisms of atropine in the CNS are not well established, it is clear that atropine reduces central acetylcholine levels (10), which are probably responsible for the reduction of central cholinergic actions of dimethylbutanol carbamate. Likewise, enhancement of toxicity has been shown to be evident in the presence of a cholinesterase inhibitor.

Dimethylbutanol carbamate was found to enhance nicotine toxicity. It has been shown that nicotine causes the release of acetylcholine in rat cerebral cortex (9), and nicotine-induced convulsions have been postulated to be due to an action on the CNS (13). Thus, it appears that the central actions of dimethylbutanol carbamate and nicotine may be quite similar. It was reported (14) that atropine was the most effective agent against nicotine-induced convulsions. It was also shown that atropine was very effective in protecting animals from dimethylbutanol carbamate toxicity, supporting a similar mechanism for the central actions of dimethylbutanol carbamate and nicotine.

In conclusion, it appears that dimethylbutanol carbamate is a potent central cholinomimetic agent whose action is due to the direct release of endogenous acetylcholine from nerve terminals. This may result in an overall decrease in brain acetylcholine to produce central convulsant activity in animals.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 4, 1973, from the *Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, FL 32601*

Accepted for publication May 14, 1973.

Supported in part by Research Grant NS-09302-02 from the National Institute of Neurological Diseases and Stroke.

* Supported in part by a teaching assistantship from the Florida State government. Present address: Department of Pharmacology, Vanderbilt University Medical School, Nashville, Tenn.

▲ To whom inquiries should be directed.