Med.exp. (Basel) 10, 327-344 (1964). LSD 1221

Med. exp. *10:* 327–344 (1964)

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Pharmacologic Properties of a New Tranquillizing Agent, 2-Methyl-2-propyltrimethylene butylcarbamate carbamate (Tybamate)*

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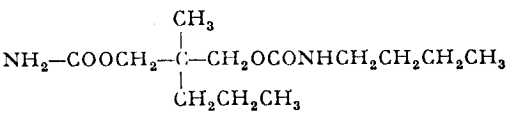
The widely recognized clinical usefulness of meprobamate in the management of anxiety and tension states has prompted a search among related chemical structures for a new substance which would retain the older drug's tranquilizing properties, yet display other properties of potential utility in the management of as yet intractable behavioral disorders.

Tybamate (2-methyl-2-propyltrimethylene butylcarbamate carbamate) was found to possess a spectrum of pharmacological properties which merited close study. Tybamate exhibited neuropharmacological, relaxant, and taming properties generally similar to those of meprobamate, but differed from meprobamate in several significant respects. The most important differences are shown in its ability to suppress the electroencephalographic effects induced by the hallucinogen LSD-25 in rabbits, its ability to counteract certain effects of serotonin, and by its absence of a stimulatory effect on the microsomal enzymes of the liver.

Physical and chemical properties. Tybamate was first synthesized at Wallace Laboratories in 1957 *(Berger and Ludwig,* 1960). Chemically tybamate is 2-methyl-2-propyltrimethylene butylcarbamate carbamate, with the following structural formula:



A bitter, white, crystalline substance, tybamate dissolves readily in common organic solvents. It melts at 48-50°C and has limited

* Tybamate will be distributed by Wallace Laboratories under the trade name "Solacen". Tybamate is the generic name of the drug.

Received: February 12, 1964.

solubility in water (0.05 g/100 ml at 25°C and 0.1 g/100 ml at 37°C). At 37°C it is not broken down by dilute acid or alkali solutions or gastric and intestinal juices.

The partition ratios of tybamate and meprobamate between a number of organic solvents and water at room temperature are given in Table I. The striking differences between the 2 compounds are due to the higher lipid solubility of tybamate.

TABLE I

Partition ratios of tybamate and meprobamate between organic solvents and water at 25°C

Solvent	Partition Ratio solvent/water	
	Tybamate	Meprobamate
Benzene	43	0.22
Carbon tetrachloride	10.1	0.03
Chloroform	> 100	3.5
Cottonseed oil	28	0.3

Methods

Because of its poor water solubility, tybamate was suspended in 5% aqueous gum acacia when given by intraperitoneal, subcutaneous or oral routes to rats or mice. Ten to 15 animals were used at each dose level. A warm supersaturated 1% aqueous solution was used for intravenous administration to mice. Cats and dogs received tybamate as a 10% solution in propylene glycol. The response to the vehicle alone was determined in every experiment. *Miller and Tainter*'s (1944) method was used to calculate mean responses and their error, and Student's t test to evaluate the significance of differences between the means (*Burn* et al., 1950).

For neurophysiological studies cats were prepared under ether anesthesia. All wound margins and pressure points were infiltrated with lidocaine and the animals were immobilized with succinylcholine or gallamine. The respiratory pump was connected to the tracheal cannula. At least 1 hour was allowed for the elimination of ether before any studies were begun. Electroencephalographic recordings were taken from bipolar concentric electrodes placed stereotaxically in the dorsal hippocampus, fornix, thalamus, and mesencephalic reticular formation, and from needle electrodes inserted into the cranium over chosen areas of the cerebral cortex. Activation of the cerebral cortex was elicited either by a 5 second train of square waves applied to the reticular formation (150 cps, 0.5 ms pulses), by 60 cps stimulation of the sciatic nerve through a shielded electrode, or by sensory stimulation (acoustic or tactile). Limbic seizures were produced by excitation of the fornix (150 cps, 1.0 ms pulse width) and recorded from the hippocampus. Recruiting was elicited by stimulation of one of the nuclei of the diffuse thalamic system (N. centralis

lateralis or N. ventralis anterior) with 9-10 cps., 1.0 ms pulses. All drugs were given intravenously and at the end of each experiment the cat was sacrificed and the brain was removed and preserved in 10% formalin for histological verification of electrode locations.

Rabbits were given subcutaneous injections of lidocaine in and around the scalp to provide local anesthesia. A skin incision was made and needle electrodes were inserted into burr holes in frontal, parietal and occipital regions of the skull. The rabbit, usually blindfolded, was kept in a special box which required minimum restraint. EEG activation was produced by acoustic (handclap) or tactile (puff of air to the back) stimuli and recorded on a Grass electroencephalograph.

Muscle relaxant properties were investigated in cats decerebrated at an intercollicular level (*Berger* et al., 1959). Spinal reflexes were studied in cats as described by *Berger* (1949). Anticonvulsant action was studied in mice receiving electroshock (*Berger,* 1952), strychnine 2.5 mg/kg or pentylenetetrazol 120 mg/kg intraperitoneally 30 minutes after the administration of graded doses of tybamate or meprobamate, using 10 mice at each dose level.

Barbiturate synergism was studied in groups of 10 mice receiving jointly a dose of the drug under test and hexobarbital sodium 100 mg/kg intraperitoneally. The period of time for which the righting reflex was lost ("sleeping time") was compared with the sleeping time of the control animals that received hexobarbital only. The experiments with the control and test groups were carried out simultaneously.

The effect of drugs on the metabolizing enzymes was studied in male rats according to the technique of *Conney* et al. (1960). The animals were pretreated with the drug under study twice daily for 4 days. All drugs were given intraperitoneally with the exception of phenobarbital which was given orally. On the fifth day the animals received hexobarbital sodium 95 mg/kg intraperitoneally and the time during which the righting reflex was lost was measured and compared with that observed in control animals which were pretreated with gum acacia solution. To minimize the effect of the day to day variations in the duration of the hexobarbital "sleeping time", experiments with control and test groups were always carried out simultaneously.

Pyrexia in rabbits with LSD-25 was produced according to the method of *Horita and Dille* (1954). LSD-25 5 μg/kg was given intravenously to male albino rabbits weighing 2 to 3 kg and the temperature was measured by a telethermometer. All drugs were administered intravenously.

The effects of vagus stimulation or of the administration of acetylcholine, epinephrine or serotonin on the blood pressure were studied in mongrel dogs anesthetized with pentobarbital sodium 35 mg/kg. The antiserotonin activity was evaluated according to the method of *Schneider and Rinehart* (1956). Intravenous serotonin creatinine sulfate 100 μg/kg consistently elicited a characteristic pressure response when injected at 15-minute intervals. Drugs were given at a slow rate intravenously over a 4-minute period, 5 minutes prior to the serotonin injection. The carotid pressor reflex was elicited by bilateral clamping of the common carotid arteries for 30 seconds. The central stump of the vagus nerve was stimulated through bipolar shielded electrodes for 10 seconds using a Harvard inductorium with the secondary coil at 3.6 cm.

For peripheral vagus stimulation an AEL 751 electronic stimulator (2 to 5 V, 120 stimuli per second for 5 seconds) was used. Electrocardiograms were made with a Sanborn polygraph employing standard Lead II.

Ganglionic blocking was evaluated in cats anesthetized with sodium pentobarbital 35 mg/kg intraperitoneally. The preganglionic nerve trunk of the left superior cervical ganglion was severed and stimulated through shielded bipolar electrodes utilizing an AEL 751 stimulator (120 stimuli per second at 2 to 6 V for 5 seconds). The contractions of the nictitating membrane were recorded on a kymograph.

The antispasmodic effect of drugs was determined on the guinea pig ileum using acetylcholine chloride 0.2 μg/ml, serotonin creatinine sulfate 2.0 μg/ml, and histamine diphosphate 0.2 μg/ml, as spasmogens. The drugs, administered in aqueous solution, were added 2 minutes before the spasmogen.

The concentration of tybamate in body fluids was determined according to the method of *Hoffman and Ludwig* (1959) employing this compound as a standard. Protein binding of tybamate in plasma was studied by the equilibrium dialysis technique described by *Anton* (1960).

Results

Gross Behavior. Tybamate given to mice or rats in suitable doses produced profound muscular relaxation, which with increasing dosage led to the loss of the righting reflex and flaccid paralysis. The appearance and condition of the animals was similar to that observed after administration of meprobamate. Tybamate, like meprobamate, relaxed only the voluntary skeletal muscles and did not materially affect respiration and other vital functions, even when given in large dosage that completely paralyzed the animals. Regardless of the route of administration, tybamate produced loss of the righting reflex at lower doses than meprobamate. This greater potency was not accompanied by a comparable increase in toxicity; tybamate had a greater safety ratio than meprobamate (Table II).

When given orally to dogs in doses of 100 mg/kg a slight hind limb ataxia appeared after about an hour. However, this was noticeable only when the animals made sharp turns during walking. This locomotor disturbance lasted for approximately 1 hour. Marked ataxia appeared after 200 mg/kg. After 300 mg/kg orally, ataxia was followed by flaccid paralysis and prostration which developed over a period of 3 hours. The dogs appeared to be asleep (moderate ptosis with downturned eyes, but with little relaxation of the nictitating membrane), but remained responsive to auditory stimulation. When called, they promptly opened their eyes and

TABLE II

Acute toxicity and paralyzing action of tybamate and meprobamate in mice and rats after different routes of administration

Species and route of administration	PD_{50} (mg/kg)		LD_{50} (mg/kg)		Safety Ratio LD_{50}/PD_{50}	
	Tybamate	Meprobamate	Tybamate	Meprobamate	Tybamate	Meprobamate
Mice i.p.	96 ± 2 *	235 ± 7	540 ± 13	800 ± 15	5.6	3.4
Mice i.v.	68 ± 4	175 ± 12	254 ± 13	482 ± 33	3.7	2.7
Mice oral	235 ± 15	300 ± 12	830 ± 65	1000 ± 44	3.5	3.3
Rats i.p.	126 ± 8	300 ± 16	465 ± 39	545 ± 50	3.7	1.8
Rats oral	450 ± 41	1140 ± 78	1040 ± 94	1600 ± 163	2.3	1.4

* Standard error.

seemed alert. The ease with which these animals could be aroused even at a time when gross locomotor impairment was manifest was characteristic of the action of the drug.

The action of tybamate was also investigated in aggressive cats. When these animals were received they were so hostile and wild that they could not be handled without special precautions. The cats reacted with displeasure and belligerence to any person approaching their cages. With backs arched, hair raised, and claws and teeth bared, they greeted any attempt to open the cage door with hissing and spitting. Often savage strikes were made at the handler even before the door was opened. The cats could be removed from the cage only with the use of animal snares. When caught, they continued to struggle violently, biting and scratching; only when confined in a burlap bag could they be controlled sufficiently for the injection of drugs.

Thirty minutes after receiving 50 to 100 mg/kg of tybamate intraperitoneally, the cats became calm and tractable, almost friendly. They were alert, tolerated handling, and some even responded to stroking and petting by purring. These animals returned to their full pre-drug belligerence 4 to 5 hours after administration of the drug. In crossover studies in these same animals, 50 to 100 mg/kg of meprobamate intraperitoneally, had similar taming action as tybamate, but of somewhat shorter duration.

Electroencephalographic studies. In 15 unanesthetized, immobilized cats, 10 mg/kg of tybamate injected intravenously, had no effect on the spontaneous electrical activity of the cerebral cortex.

After 20 mg/kg, some animals showed a decrease of the high frequency (30-40 cps) components and a moderate increase in the amplitude of the slower waves. Occasionally spindles appeared. These effects became more prominent with larger doses of tybamate, such as 40 mg/kg.

In studies carried out in 13 cats the duration of hippocampal seizures induced by electrical stimulation of the fornix was sharply reduced from an average of 76 ± 5 seconds to 11 ± 3 seconds. This 86% reduction in seizure duration following the intravenous administration of tybamate (20-40 mg/kg) was statistically significant at the 0.001 level. These same doses produced a slight depression of cortical activation elicited by stimulation of the mesencephalic reticular formation and elevated the threshold for such activation. In 8 cats recruiting elicited by low-frequency stimulation of the diffuse thalamic system was hardly affected after 20 mg/kg, but there was a slight depression after a 40 mg/kg dose of the drug. Table III summarizes the effects of tybamate on neurophysiological phenomena and compares the findings with the previously published data obtained with meprobamate and carisoprodol (*Berger* et al., 1959).

TABLE III

Effects of drugs on electrophysiological phenomena in cats (20 mg/kg, i.v.)

Compound	Spontaneous cortical activity	Cortical and hippocampal activation	Limbic seizures	Recruiting
Tybamate	Slight depression	Slight depression	Depressed	Unchanged
Meprobamate	Unaffected	Unaffected	Depressed	Depressed
Carisoprodol	Depressed	Depressed	Unaffected	Enhanced

We have observed that LSD-25, 10 μg/kg, administered intravenously to 14 unanesthetized, minimally restrained rabbits, produces a long-lasting state of EEG activation. This state was counteracted by tybamate or chlorpromazine but was not affected by meprobamate. Thus, tybamate in doses of 5 to 10 mg/kg, which by themselves had no effect on the EEG, caused complete abolition of the LSD-25-induced activation pattern and produced the appearance of a resting type of EEG. Meprobamate, on the other hand, even in very large doses of 60 to 80 mg/kg, had no effect on EEG activation by LSD-25 (Figure 1). Chlorpromazine 5 mg/kg,

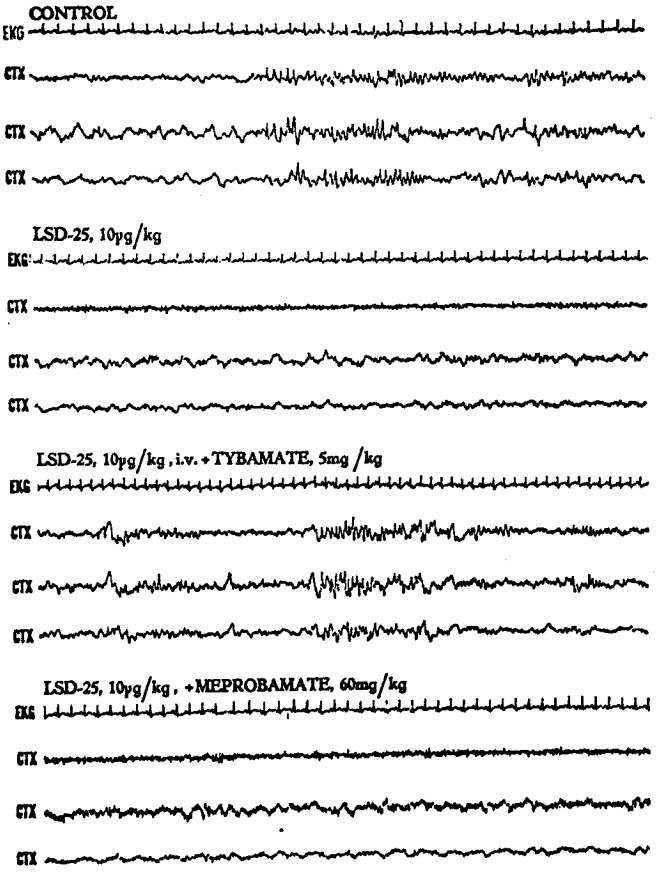


Fig. 1. Activation of the EEG by LSD-25 i.v. in unanesthetized rabbits and the effect of tybamate and meprobamate on the LSD-25 activation. Lead I EKG, Lead II, III and IV frontal, parietal and occipital bipolar cortical tracings.

intravenously, however, like tybamate, reversed the effect of LSD-25 on the EEG.

Muscle relaxant action. The muscle relaxant action of tybamate was evaluated by studying its effect on decerebrate rigidity and spinal reflexes. Recordings were made from the extensor muscles of the hind limb of 9 decerebrate cats. The electromyogram showed the characteristic high-voltage, high-frequency potentials. These could be completely abolished and a normal electromyogram produced by the administration of tybamate 10 mg/kg. In order for meprobamate and carisoprodol to produce similar re-

laxation of decerebrate rigidity, 24 mg/kg and 3 mg/kg, respectively, were required.

The action of tybamate on spinal reflexes was studied in 4 chloralosed cats. Tybamate, 20 mg/kg, i.v., blocked or diminished by at least 80 per cent the flexor reflex in the hind limb. Onset of action was rapid, and the effect persisted for 1 to 3 hours. Tybamate administration did not affect the patellar reflex. Its effect on spinal reflexes appeared to be similar to that of meprobamate.

Anticonvulsant action. Tybamate in suitable doses eliminated the occurrence of the tonic extensor phase of maximal electroshock seizures in mice. The mean effective dose on oral administration was 198 ± 15 mg/kg, which is of the same order of magnitude as the value obtained with meprobamate (*Berger,* 1952). Tybamate was inferior to meprobamate as a pentylenetetrazol antagonist. The mean doses required to prevent pentylenetetrazol convulsions or deaths in mice were 120 ± 10 mg/kg and 106 ± 12 mg/kg, respectively. Meprobamate prevented death at 31 ± 2 mg/kg. Tybamate also provided poor protection against strychnine convulsions and deaths (Table IV).

TABLE IV

The antagonistic effects of tybamate and meprobamate in mice receiving strychnine sulfate 2.5 mg/kg i.p. or pentylenetetrazol 120 mg/kg i.p. 30 minutes after the drug

	Mean Protective Doses ± Standard Error in mg/kg Against			
	Strychnine		Pentylenetetrazol	
	Convulsions	Deaths	Convulsions	Deaths
Tybamate	388 ± 29	> 420	120 ± 12	106 ± 12
Meprobamate	470 ± 23	210 ± 28	67 ± 3.4	31 ± 2.5
Carisoprodol	> 980	> 980	145 ± 12.8	86 ± 6.2

Barbiturate synergism. Prolongation of the duration of hexobarbital anesthesia was examined in mice. Tybamate 100 mg/kg, i.p., significantly prolonged sleeping time, but half this dose had no marked effect. Meprobamate in similar doses produced comparable effects. These results are illustrated in Table V.

Stimulatory effect on barbiturate metabolism. Conney et al. (1960) have shown that pretreatment of rats with phenobarbital, phenylbutazone, and many other drugs increases enzyme activity in the liver and that the resulting accelerated drug metabolism

TABLE V

Effects of tybamate and meprobamate on hexobarbital sleeping time in mice

Compound	Dose mg/kg i.p.	Number of mice	Mean sleeping time minutes ± s.e.	T	P
Tybamate	100	10	129 ± 13	5.6	< 0.001
	50	10	53 ± 4	0.8	0.4
Meprobamate	100	10	122 ± 13	5.4	< 0.001
	50	10	65 ± 8	1.8	0.1
Controls	—	20	47 ± 4		

could be detected in the intact animal by the shortened duration of hexobarbital anesthesia. Tybamate pretreatment at various dose levels did not shorten the duration of hexobarbital anesthesia. Meprobamate pretreatment had a similar effect at most dose levels. After repeated administration of large doses of meprobamate, however, a moderate decrease in the duration of anesthesia became apparent. Phenobarbital pretreatment, used as the control substance, invariably produced a striking reduction in the duration of hexobarbital anesthesia. These results are illustrated in Table VI.

Effect on LSD-25 pyrexia. Horita and Dille (1954) have shown that LSD-25 will produce fever when administered to rabbits and that this effect can be antagonized by chlorpromazine or pentobarbital. We have found that tybamate evaluated in 12 rabbits, in

TABLE VI

Effect of drug administration repeated 8 times during 4 days on hexobarbital sleeping time administered on the 5th day

Drug	Dose mg/kg	Number of rats used	Mean sleeping time after hexobarbital 95 mg/kg, i.p. Minutes ± standard error	T	P
Tybamate	200	15	26.7 ± 1.6	0.24	> 0.8
	100	15	40.2 ± 2.6	4.06	< 0.001
	50	15	25.3 ± 2.3	0.21	> 0.8
Meprobamate	200	15	18.3 ± 1.3	2.8	< 0.01
	100	15	30.3 ± 1.9	1.39	> 0.1
	50	15	24.6 ± 2.3	0.41	> 0.6
Phenobarbital	50	15	9.1 ± 1.2	6.26	< 0.001
	25	15	8.9 ± 0.7	6.84	< 0.001
Control	—	30	26.0 ± 2.4		

doses up to 50 mg/kg, i.v., did not significantly alter the course of LSD-25-induced fever. Chlorpromazine, 5 mg/kg, i.v., on the other hand, prevented the appearance of LSD-25-induced fever in rabbits. These results are illustrated in Figure 2.

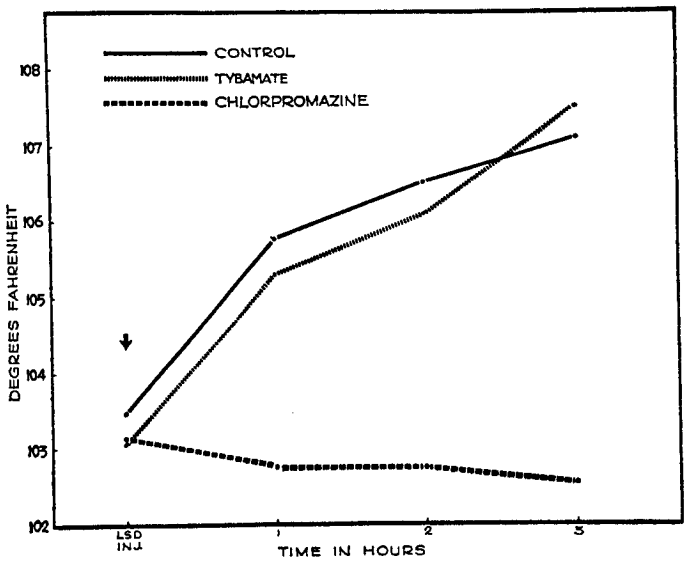


Fig. 2. Effect of tybamate 50 mg/kg i.v. and of chlorpromazine 5 mg/kg i.v. on fever produced in rabbits by administration of LSD-25 5 μg/kg i.v., 6 rabbits per point.

Cardiovascular effects. Rapid injection of 20 mg/kg, i.v., of tybamate in anesthetized dogs produced a transient drop in arterial pressure with full recovery after 20 to 30 minutes. This was associated with a transient reduction in femoral venous pressure and flow. No significant hypotensive action could be shown in unanesthetized rabbits. Estimates of peripheral resistance computed according to the method of Wiggers (1950) indicated no significant changes. In an experiment carried out in 10 dogs, the carotid sinus pressor response induced by occlusion of the common carotid arteries was diminished by 60 to 70 per cent after administration of tybamate 20 mg/kg. A similar dose of the drug also inhibited the pressor effect of central vagal stimulation in 10 dogs. Cardiac arrest elicited by electrical stimulation of the peripheral end of the vagus was prevented in 5 out of 10 dogs by tybamate 20 mg/kg. The pressor response to electrical stimulation of hypo-

thalamic, mesencephalic or medullary vasomotor centers in unanesthetized, curarized cats was sharply curtailed by 30 mg/kg, i.v.

In 5 dogs, tybamate, 20 mg/kg, i.v., did not significantly alter the arterial pressor response to epinephrine 2 μg/kg. The pressure before drug was 67 ± 11 mm Hg and 55 ± 8 mm Hg after tybamate. There was no significant effect on the blood pressure drop induced by the injection of acetylcholine 20 μg/kg. Tybamate, evaluated in 22 dogs, had a marked effect on the blood pressure elevation produced by serotonin 100 μg/kg. These responses were quantitatively compared with those obtained after chlorpromazine and meprobamate. The results of the experiments summarized in Table VII indicate that chlorpromazine was about twice as potent as tybamate in counteracting the pressor response produced by serotonin. Meprobamate was inactive in this respect.

TABLE VII

The ability of drugs to prevent blood pressure rise produced in dogs by administration of serotonin 100 μg/kg

Drug	Dose mg/kg	Number of dogs	Blood Pressure Rise		Median effective dose mg/kg
			Control mm Hg	After drug mm Hg	
Tybamate	20	14	54 ± 6.6	18 ± 4.5	
	10	4	55 ± 15.4	28 ± 4.3	7.8
	5	4	58 ± 9.3	47 ± 8.7	
Chlorpromazine	10	3	45 ± 14.4	0	
	5	3	63 ± 7.3	17 ± 2.1	3.5
	1.25	4	28 ± 8.1	26 ± 7.5	
Meprobamate	40	4	50 ± 11.6	40 ± 9.5	No activity
	20	4	48 ± 8.7	48 ± 9.8	

Other pharmacological actions. In cats, contractions of the nictitating membrane produced by preganglionic stimulation of the isolated superior cervical ganglion were reduced by 25% after administration of tybamate 20 mg/kg. Tybamate did not possess marked antispasmodic action when evaluated on the isolated guinea pig ileum. Contractions of the ileum produced by acetylcholine or histamine were not antagonized by the drug. To prevent serotonin-induced contractions, tybamate in a concentration of 17 μg/ml was required. Tybamate did not possess analgesic action when evaluated in mice on the hot plate and did not protect dogs from apomorphine-induced emesis.

Blood levels. Orally administered tybamate was readily absorbed from the gastrointestinal tract and reached peak blood concentrations in laboratory animals and in humans in about 1 hour. Significant blood levels were maintained for 4 to 6 hours. The estimated half-life of the drug, that is the time when one-half of the peak concentration of the drug remained in the blood, was about 4 hours in the dog. Twenty-four hours after administration, no traces of the drug were present in the blood.

Distribution in the animal body. In these studies tybamate labeled with C^{14} in the unsubstituted carbamate carbonyl position was used. No selective organ specificity for the drug was discovered. Twenty-four hours after intraperitoneal administration to rats, 94.5% of the administered dose of labeled tybamate was recovered. Of this amount, 91.6% was present in the urine and the remainder in the feces. All tissues and organs, as well as the blood and exhaled carbon dioxide did not contain any significant radioactivity.

Metabolic fate. Most of the drug was present in the urine in the form of an hydroxylated carbamate derivative, the precise structure of which has not yet been ascertained. The urine also contained small amounts of the unchanged drug as well as other as yet unidentified metabolites.

Protein binding. The binding of plasma proteins with tybamate was quite high. The percentage of tybamate bound in human, rabbit, and mouse plasma was 77%, 81%, and 65% respectively. Meprobamate, investigated under similar conditions, showed little or no binding with proteins.

Chronic toxicity. Groups of 20 male and 20 female newly weaned Charles River rats received tybamate in concentrations of 2%, 1%, and 0.5%. The drug was mixed with Wayne Rodent Diet. An untreated group of animals served as controls. Body weights and food consumption were determined at weekly intervals for 18 months. At termination, neurological examination and careful gross observation of the animals failed to disclose any significant changes, except for some lowering of body weight gains in the females on a 2% drug diet. Locomotion was normal as was the response to touch and noxious or auditory stimuli. Examination of the blood showed normal values for hemoglobin, erythrocyte, leukocyte and leukocyte differential values. At autopsy no signs of any drug-induced alterations were found upon gross examination of the vital organs, except for some liver enlargement at the highest

(2%) tybamate level. However, the liver grossly appeared normal in color and texture, and the absence of abnormal findings upon histological examination suggests that this enlargement of the organ may reflect the role of the liver in tybamate detoxification.

Four groups of 4 mongrel dogs (2 males and 2 females) were given tybamate orally twice daily, 5 days a week, half of the total daily dose being given at each feeding. The dose levels given were: 150 mg/kg, 250 mg/kg, 350 mg/kg, 550 mg/kg. After 4 months on the drug, no gross or histological evidence of drug-induced pathological changes were found. Efficient drug absorption was evidenced by muscle relaxation and ataxia. Some noticeable decrease in weight gain was observed in dogs at the highest dose. Hematological values were normal. Urine analyses indicated normal kidney function.

A second chronic toxicity study (18 months) in dogs with 4 males and 4 females in each group was carried out. In addition to a control group which received no drug, 3 additional groups received 50, 150, and 300 mg/kg/day. The lowest level (50 mg/kg) was given in a single dose and the 150 mg/kg and 300 mg/kg/day doses in 2 equally divided amounts. The drug was well tolerated at 50 mg/kg/day and 150 mg/kg/day as evidenced by no significant changes in the body weights. Hematological determination and urine analyses indicated normal values for all groups. Liver function values (bromsulphalein, blood urea nitrogen, serum transaminase, and alkaline phosphatase) approximated normal values for the controls in the 50 mg/kg and 150 mg/kg/day groups. Some dogs in the 150 mg/kg group showed small increases in alkaline phosphatase values. At the highest dose of 300 mg/kg/day there was severe loss of weight; bromsulphalein and blood urea nitrogen were normal but serum transaminase and alkaline phosphatase were elevated. Histopathological examination of the livers of these high dose animals indicated that these changes reflected the inability of these animals to take sufficient nourishment for they were seriously depressed and ataxic for the greater part of the day. Upon gross and microscopic examination of the vital organs of all the control and drug-treated dogs pathological alterations that could be firmly assigned to the administration of the drug were not found at either 50, 150, or 300 mg/kg of drug per day.

Suspensions of tybamate were administered orally to adult Rhesus monkeys 5 times a week for 26 consecutive weeks in dosages

of 450 mg/kg, 150 mg/kg, or 50 mg/kg/day; 2 males and 2 females received each drug level. An additional group was given meprobamate at 150 mg/kg/day as a reference standard, and a control group was given the vehicle only. Although acute drug-induced effects, such as muscle relaxation, were consistently induced at the 450 mg/kg and 150 mg/kg dosage levels, body weight changes were similar in all groups. Urine and blood examinations did not disclose any abnormal findings. Gross and histological examination of organs and tissues failed to demonstrate any evidence of pathology attributable to the administration of the drug. The absence of signs of toxicity that could be attributed to the drug in this species conforms to the observation of the very low toxicity with this drug after prolonged administration to rats and dogs.

Teratogenic studies. Female rats that had been on a diet containing tybamate in concentrations of 2.0%, 1.0%, and 0.5% for 14 weeks were mated. Careful examination of the rats born to these females failed to show any evidence of teratogenic changes.

Teratogenic studies were also carried out in rabbits using a procedure similar to that described by *Sommers* (1962). Tybamate, in a concentration 0.25% body weight, was included in the diet during the first week of pregnancy and maintained until parturition. There were no teratogenic abnormalities in the litters. Thalidomide tested concurrently under these same conditions produced obvious limb and skull malformations.

Discussion

Tybamate possesses a number of pharmacological properties that are characteristic of tranquilizing agents of the meprobamate type (*Berger*, 1960; *Kletzkin*, 1962). In low doses that do not affect spontaneous cortical or subcortical electrical activity, the drug shortens the duration or completely eliminates the appearance of afterdischarges set up by electrical stimulation of the fornix or other parts of the limbic system (*Kletzkin and Berger*, 1959). Another neurophysiological property which tybamate and meprobamate share and which differentiates them from the barbiturates and many other central nervous system depressants, is the absence of a direct action on the midbrain reticular formation and the presence of a blocking action on the interneurons. Tybamate, also, like meprobamate, reduces the belligerent behavior of naturally

aggressive cats and produces sedation in animals without impairment of their capacity to respond to environmental stimuli. These experimental findings suggested that tybamate may be useful as a tranquilizer, and this has been demonstrated by controlled clinical trials in humans.

Tybamate, however, possesses several interesting pharmacological properties which are not shared by meprobamate. The most intriguing of these is the ability of tybamate to counteract the pronounced and persistent EEG activation produced by the hallucinogen LSD-25. Low doses of tybamate (5 to 10 mg/kg) change the activated EEG to one characteristic of the resting state. This effect cannot be brought about even with very large doses of meprobamate (60 to 80 mg/kg). Tybamate, however, cannot counteract all the effects of LSD-25. Thus, for instance, it does not affect the pyrexia produced by administration of LSD-25.

Tybamate also differs from meprobamate in being able to counteract the pressor response produced by serotonin. This antiserotonin activity of tybamate is of interest particularly because it is a property also displayed by LSD-25. Thus, according to the response measured, tybamate may share one activity with LSD-25 or antagonize another.

The high plasma binding observed with tybamate but not with meprobamate is also of interest, particularly if it is recalled that tybamate disappears from the blood somewhat more rapidly than the other drug. The meaning of this observation is not clear, but it shows that protein binding and the rate at which a drug is eliminated from the circulation need not be related.

Tybamate, like meprobamate (*Berger*, 1954) depresses polysynaptic reflexes in the chloralosed cat at doses which leave monosynaptic reflexes unimpaired. Although it is a more potent centrally acting muscle relaxant, as determined by its effects on decerebrate rigidity, it is a weaker anticonvulsant than meprobamate. Carisoprodol (*Berger* et al., 1959) on the other hand, is a more potent muscle relaxant and a poorer anticonvulsant than either tybamate or meprobamate. It appears, therefore, that in these 3 chemically related compounds, muscle relaxant properties and anticonvulsant properties are dissociated and independent from each other. Carisoprodol, moreover, does not attenuate limbic seizures even in doses greatly exceeding those which produce profound muscular relaxation (*Kletzkin*, 1960) and differs in this re-

spect from both tybamate and meprobamate. In addition, carisoprodol has a pharmacologically demonstrable analgesic action (*Berger* et al., 1959; *Margolin*, 1960) which neither meprobamate nor tybamate possess. From a chemical standpoint the rather sharp differences in properties observed in comparisons of carisoprodol and tybamate, as well as other members of the propanediol dicarbamate series, stress the uncertainty encountered in endeavoring to establish structure-activity relationships. It appears that the most reliable predictions of tranquilizing activity at present derive from careful electrophysiological analysis of drug action on the brain.

Despite the antiserotonin action exhibited by tybamate, its autonomic effects are insignificant. Only when given intravenously in large doses does it alter the vasomotor reflexes of the carotid bodies, or affect the slowing of the heart after peripheral vagus stimulation or inhibit the pressor response to electronic stimulation of the central portion of the divided vagues nerve. In pharmacodynamic studies with cats and dogs, only transient hypotensive effects could be demonstrated. The pharmacological responses *in vitro* or *in vivo* to histamine or acetylcholine were not altered by tybamate.

Repeated administration of tybamate to rats failed to yield data suggestive of the development of tolerance. This finding, as well as observations made during chronic administration in dogs and monkeys, suggests that little, if any, tolerance to the drug is likely to occur.

Summary

Tybamate (2-methyl-2-propyltrimethylene butylcarbamate carbamate) shares many pharmacological properties with meprobamate. Their common properties include the ability to tame wild and aggressive cats, the ability to shorten limbic seizures in doses that do not affect spontaneous cortical potentials, the selective depressant effect on interneurons, and a centrally mediated muscle relaxant action. Tybamate differs from meprobamate in that it reverses the electroencephalographic effect of the hallucinogen LSD-25 in rabbits, antagonizes the serotonin-produced blood pressure rise in dogs, and is extensively bound to plasma protein. The drug is well tolerated over long periods of time by rats, dogs and monkeys, and tolerance to its effects does not readily develop.

Zusammenfassung

Tybamat (2-Methyl-2-propyltrimethylen-butylcarbamat-carbamat) ähnelt in vielen pharmakologischen Eigenschaften dem Meprobamat. Mit Meprobamat

gemeinsam sind: die Fähigkeit, wilde und aggressive Katzen zu zähmen, die Fähigkeit, durch Reizung des Fornix ausgelöste hippocampale Anfälle in ihrer Dauer zu verkürzen, und zwar in Dosen, welche die corticalen Spontanpotentiale nicht verändern, ferner die selektiv depressive Wirkung auf Interneuronen und eine zentrale Muskel-relaxierende Wirkung. Tybamat unterscheidet sich von Meprobamat in den folgenden Reaktionen: es kehrt die durch LSD-25 ausgelöste Aktivierung im Elektroencephalogramm um, es antagonisiert den durch Serotonin beim Hund hervorgerufenen Blutdruckanstieg; ferner besitzt Tybamat im Gegensatz zu Meprobamat ein starkes Bindungsvermögen an die Plasmaproteine. Die Substanz wird von Ratten, Hunden und Affen, über eine lange Zeit verabreicht, gut vertragen. Eine Gewöhnung entwickelt sich, wenn überhaupt, nur sehr langsam.

Résumé

Le Tybamate (2-méthyl 2-propyltriméthylène butylcarbamate) possède plusieurs des propriétés pharmacologiques du méprobamate. Parmi leurs propriétés communes se trouvent la capacité de calmer les chats sauvages et agressifs, de raccourcir les décharges convulsives (limbiques) à des doses qui n'aggissent pas sur les potentiels corticaux spontanés ; ils ont un effet dépressif sélectif sur les neurones intermédiaires et une action centrale de relaxation musculaire.

Le Tybamate est différent du méprobamate par le fait qu'il inverse l'effet EEG du LSD-25 chez les lapins ; il antagonise l'hypertension sérotoninique chez les chiens et il est lié aux protéines plasmatiques. La drogue est bien tolérée lors d'une administration prolongée chez les rats, chiens et singes. Les phénomènes d'accoutumance sont plus rares.

Acknowledgement

The authors are indebted to Dr. B. J. Ludwig for supplies of tybamate, Dr. W. Diamantis, Dr. E. Fedor and Dr. O. Plekss for the autonomic data, Dr. R. Vinegar for the hematological data and to Drs. B. J. Ludwig and J. D. Douglas for the preliminary biochemical data. The authors are also indebted to J. Elenewich, M. Hall, N. Ivins, R. Loetzer, Z. Reinhardt, E. Ruhman and G. Sweeton for valuable technical assistance. Supplies of LSD-25 were available through the courtesy of Sandoz Pharmaceuticals, Hanover, New Jersey.

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