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Convulsant and Anticonvulsant Cyclopentanones and Cyclohexanones

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SUMMARY

The convulsant and/or anticonvulsant activity of unsubstituted and mono-alkyl-substituted cyclopentanones and cyclohexanones were examined by testing the ability of these compounds to produce seizures or to inhibit seizures induced by pentylenetetrazol and maximal electroshock in CF-1 mice. In addition, these compounds were tested for their ability to bind to the picrotoxin receptor. The unsubstituted compounds, cyclopentanone and cyclohexanone, prevented both pentylenetetrazol- and maximal electroshock-induced seizures. Cyclopentanones and cyclohexanones with small (<3 carbon atoms) alkyl substituents in the 2-position were also anticonvulsant; all of these compounds, except 2-*t*-ethylcyclohexanone, blocked both pentylenetrazol- and maximal electroshock-induced seizures. 2-Ethylcyclohexanone was very effective against pentylenetetrazol seizures but did not prevent maximal electroshock seizures. Cyclohexanones with larger alkyl substituents in the 2-position, 2-propylcyclohexanone and 2-*t*-butylcyclohexanone, caused clonic seizures following injection into mice. Of the cyclopentanones and cyclohexanones with alkyl substitutions in the 3-position that were studied, one was an anticonvulsant (3-methylcyclopentanone), one was a mixed convulsant/anticonvulsant (3-ethylcyclohexanone), and the other two (3-ethylcyclopentanone and 3-*t*-butylcyclohexanone) were convulsants. Finally, two cyclohexanones with alkyl substituents in the 4-position were studied. Both 4-ethylcyclohexanone and 4-*t*-butylcyclohexanone produced convulsions when injected into mice. All the neuroactive cyclopentanones and cyclohexanones competitively displaced [^{35}S]*t*-butylbicyclophosphorothionate, a ligand specific for the picrotoxin receptor, from rat brain membranes. The convulsant compounds were generally more potent than the anticonvulsants. The cyclohexanones were more potent than their corresponding cyclopentanones and the binding potency of both increased as the size of the alkyl substituent increased. These results suggest that cyclopentanone, cyclohexanone, and their alkyl-substituted derivatives act at the picrotoxin receptor to increase or decrease neuronal activity. Thus, they appear to have sites and mechanisms of action similar to those of the neuroactive γ-butyrolactones and γ-thiobutyrolactones.

Both GBL and TBL are known to be highly neuroactive compounds. It has been reported that animals treated with GBL exhibit behavioral abnormalities that are similar to absence seizures in humans (1) and TBL has been reported to cause convulsions in experimental animals (2). When GBL and TBL are alkylated in the β-position, the resulting compounds are highly convulsant and produce seizures that resemble those caused by picrotoxinin (3, 4). In contrast, when GBL and TBL are substituted in the α-position and the β-position remains unsubstituted, the resulting compounds are usually anticonvulsant (4–6). Receptor binding studies indicate that both convulsant and anticonvulsant GBLs and TBLs bind to the picrotoxin receptor (7–9). In addition, electrophysiological experiments have shown that alkyl-substituted GBLs and TBLs alter GABA-mediated currents in cultured neurons. Convulsant GBLs and TBLs diminish GABA responses, whereas an anticonvulsant TBL, α-EMTBL, augmented GABA responses and an anticonvulsant GBL, α-EMGBL, had no direct effect but blocked the effects of β-EMGBL (a convulsant GBL), picrotoxinin, and α-EMTBL (9–11). α-EMGBL did not interfere with benzodiazepine- or barbiturate-induced potentiation of GABA responses.[1] These results are all consistent with the hypothesis that alkyl-substituted GBLs and TBLs act at the picrotoxin receptor as either agonists, inverse agonists, or antagonists.

Previous studies of alkyl-substituted GBLs and TBLs have concentrated on the size and location of the alkyl substituent (12). Also, some information about the importance of the heteroatom has been obtained by comparing the potencies of

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[1] J. A. Ferrendelli, unpublished observations.

ABBREVIATIONS: GBL, γ-butyrolactone; TBL, γ-thiobutyrolactone; α-EMTBL, α-ethyl-α-methyl-γ-thiobutyrolactone; α-EMGBL, α-ethyl-α-methyl-γ-butyrolactone; β-EMGBL, β-ethyl-β-methyl-γ-butyrolactone; CP, cyclopentanone; CH, cyclohexanone; TBPS, *t*-butylbicyclophosphorothionate; PTZ, pentylenetetrazol; PEG, polyethylene glycol; ED_{50}, half-maximal effective dose; TD_{50}, half-maximal toxic dose; CD_{50}, half-maximal convulsant dose; IC_{50}, half-maximal inhibitory concentration; K_D, equilibrium dissociation constant; B_{max}, total concentration of ligand binding sites; TMSM, tetramethylsuccinimide; β-EMTBL, β-ethyl-β-methyl-γ-thiobutyrolactone; GABA, γ-aminobutyric acid.

alkyl-substituted GBLs and TBLs (which represent a sulfur-for-oxygen substitution in the ring). These comparisons have shown that both α- and β-substituted TBLs are substantially more potent than the correspondingly substituted GBLs (4). This indicates that the identity of the heteroatom in the ring can effect the potency of these compounds. Very little is known about the neurological activity of nonheterocyclic compounds; therefore, we have examined the convulsant and anticonvulsant activities of CP (a five-membered cyclic ketone), CH (a six-membered cyclic ketone), and some of their alkylated derivatives (Fig. 1). To determine whether these compounds share a common mechanism of action with alkyl-substituted GBLs and TBLs, the CPs and CHs were also tested for their ability to displace [^{35}S]TBPS from the picrotoxin receptor in rat brain membranes.

Experimental Procedures

Materials. CP, PTZ, picrotoxinin, and PEG were purchased from Sigma Chemical Co. (St. Louis, MO); CH, 2-ethyl-CH, 4-ethyl-CH, and 2-methyl-CH were obtained from Wiley Organics (New York, NY); 2-*t*-butyl-CH and 4-*t*-butyl-CH were purchased from Aldrich Chemical Co. (Milwaukee, WI); 2-ethyl-CP and 3-ethyl-CP were purchased from K & K Labs (Plainview, NY); 2-methyl-CP, 3-methyl-CP, and 2-propyl-CH were purchased from Pfaultz & Bauer (Waterbury, CT); and both unlabeled and ^{35}*S*-labeled TBPS were purchased from New England Nuclear (Boston, MA). 3-Ethyl-CH and 3-*t*-butyl-CH were synthesized according to previously described methods (13).

Behavioral effects. Female CF-1 strain mice (Harlan; 6–8 weeks old) were maintained on a 12-12 light-dark cycle with food and water available *ad libitum*. Drug screening was accomplished by methods based on those of Swinyard and Woodhead (14). Test compounds were dissolved in 30% PEG and given by intraperitoneal injections (5–30 mice/dose) in a volume of 0.01 ml/g of body weight. Following the injection of the test compound, the mice were observed for 30 min and any seizure activity was noted. Compounds that did not induce clonic seizures were tested for anticonvulsant activity by examining their ability to block seizures caused by PTZ or maximal electroshock.

Maximal electroshock seizures were induced by a 60-Hz alternating current of 50 mA delivered via corneal electrodes for 0.2 sec using an electroshock stimulator (Wahlquist Instrument Co., Salt Lake City, UT). Sodium chloride (0.9%) drops were placed on the animals' eyes before application of the corneal electrodes. Seizure protection was defined as the failure to demonstrate tonic hindlimb extension past 90°. PTZ (85 mg/kg) was administered as a 0.85% solution in 0.9% NaCl and the mice were observed for 30 min for seizure activity. Protection was defined as the absence of clonic seizures.

Neurotoxic effects were assessed using the rotorod toxicity test (15). In this test, the mouse was placed on a 1-inch diameter rod rotating at 6 rpm. The animal was considered toxic if it fell from the rotating rod twice during the 10-min testing period.

The ED_{50}, TD_{50}, and CD_{50} values were determined by $\log_{10}$ probit analysis (16) of the dose-response data. The CD_{50} was determined from the number of animals that had clonic seizures following the administration of the test compound.

[^{35}S]TBPS binding. [^{35}S]TBPS binding was performed according to previously described methods (8, 17). The cerebral cortex from female Sprague-Dawley rats (250–300 g) was removed over ice immediately following decapitation. The brains were homogenized in 20 ml of ice-cold 0.32 M sucrose/g of tissue and were centrifuged at 1000 × *g* for 10 min. The supernatant was carefully decanted and centrifuged at 150,000 × *g* for 30 min. The resulting pellet was resuspended in 20 volumes of ice-cold deionized water and centrifuged at 150,000 × *g* for 30 min. The pellet was resuspended in 20 volumes of 50 mM Tris-citrate buffer (pH 7.5) and was centrifuged at 50,000 × *g* for 30 min. The resulting pellet was resuspended in 20 volumes of 50 mM Tris-citrate buffer. The membrane suspensions were stored at −70° and were thawed and resuspended in 50 mM Tris-citrate buffer immediately before use. The protein concentration was determined according to the method of Lowry *et al.* (18).

For binding assays, 100 μl of rat brain membranes were added to a solution containing 50 μl of [^{35}S]TBPS (specific activity, 60–85 Ci/mmol) in 1 M NaBr and 50 μl of the test compound dissolved in 50 mM Tris-citrate buffer. For the inhibition experiments, the final [^{35}S]TBPS concentration was 2 nM and for saturation studies the [^{35}S]TBPS concentration ranged from 1 to 300 nM. The samples were incubated in triplicate for 90 min at 25°, diluted with 3 ml of 0.9% NaCl, rapidly filtered through Whatman GF/B filters, and washed twice with 3 ml of 0.9% NaCl. Filter-bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined as that observed in the presence of 10 μM unlabeled TBPS or 100 μM unlabeled picrotoxinin.

The determination of the drug concentration that displaced 50% of specifically bound [^{35}S]TBPS and curve fitting of sigmoidal concentration-displacement curves were accomplished by $\log_{10}$ probit analysis using at least five different drug concentrations (16). Binding constants were determined by nonlinear regression analysis of the saturation data (19).

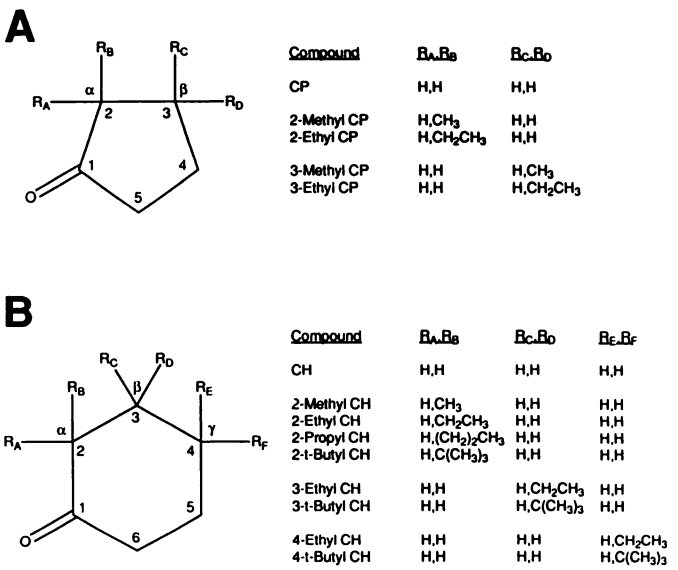

Fig. 1. Chemical structures of some alkyl-substituted CPs (A) and CHs (B).

Results

CPs have two distinct carbon atoms, the 2- and 3-carbons, that are subject to alkyl substitution. These correspond to the α- and β-positions of GBLs and TBLs. CHs have three distinct sites for alkyl substitution. These are the 2- and 3-carbons, which are equivalent to the α- and β-carbon atoms of the GBLs and TBLs, and the 4-carbon, which has no equivalently positioned carbon in the GBLs and TBLs.

Behavioral effects. All mice given intraperitoneal injections of 30% PEG in a volume of 0.01 ml/g of body weight were unprotected against PTZ- and maximal electroshock-induced seizures. In addition, these animals displayed no rotorod toxicity.

The unsubstituted compounds, CP and CH, prevented both PTZ and maximal electroshock seizures, although CP was only marginally protective against maximal electroshock. Both compounds were relatively nontoxic as well.

The 2-substituted derivatives of CP examined (2-methyl-CP

and 2-ethyl-CP) were able to block PTZ- and maximal electroshock-induced seizures at nontoxic doses (Table 1). 2-Ethyl-CP was more effective against PTZ (with a median effective dose of 205 mg/kg) than was 2-methyl-CP. Against maximal electroshock-induced seizures the converse was true, inasmuch as 2-methyl-CP was more potent than 2-ethyl-CP. At doses up to 562 mg/kg, none of the mice injected with CP or 2-methyl-CP became acutely neurotoxic. 2-Ethyl-CP was also relatively nontoxic; only 2 of the 20 animals injected with 316 mg/kg and only 6 of 25 injected with doses between 400 and 600 mg/kg were toxic as determined by the rotorod neurotoxicity test (see Experimental Procedures).

CHs with small (<3 carbon atoms) alkyl substituents in the 2-position (2-methyl-CH and 2-ethyl-CH) were more potent in preventing PTZ-induced seizures than their correspondingly substituted CPs (Table 1). As with the CPs, the 2-ethyl-substituted CH was more potent than the 2-methyl derivative in blocking PTZ seizures. Conversely, 2-methyl-CH was more potent than 2-ethyl-CH against maximal electroshock-induced tonic hindlimb extension. In fact, 2-ethyl-CH failed to prevent maximal electroshock seizures even when given in doses up to 562 mg/kg. Unlike the 2-substituted CPs, the corresponding CH derivatives produced severe neurotoxic effects within the range of doses studied.

Elongation of the 2-substituent on the CH ring produced profound differences in the biological activity of these compounds. Administration of 2-propyl-CH or 2-*t*-butyl-CH induced seizures that were characterized by myoclonic twitches and clonic convulsions. These seizures often lasted in excess of 30 min. Tonic seizures were not observed following the injection of either 2-propyl-CH or 2-*t*-butyl-CH even at the highest dose tested (562 mg/kg).

The 3-substituted CP 3-methyl-CP blocked both PTZ- and maximal electroshock-induced seizures (Table 1). However, unlike the other anticonvulsant CPs, animals injected with high doses of 3-methyl-CP (>237 mg/kg) became neurotoxic. When high doses (>237 mg/kg) of the other 3-substituted CP, 3-ethyl-CP, were administered, myoclonic twitches and, at higher doses, clonic convulsions were observed. The median convulsive dose for clonic seizures was 403 mg/kg. Tonic seizures were not observed even at the highest dose tested (562 mg/kg).

CHs substituted in the 3-position were also examined. At high doses, 3-ethyl-CH caused brief (<5 sec) clonic seizures within 1 min of intraperitoneal administration. The mice then became lethargic and did not exhibit any other evidence of seizure activity. Mice treated with subconvulsant doses of 3-ethyl-CH were protected when challenged with PTZ or maximal electroshock (Table 1). In contrast, 3-*t*-butyl-CH was highly convulsant. Injections of 3-*t*-butyl-CH produced myoclonic twitches, clonic seizures, and, at sufficiently high doses, tonic seizures. The CD_{50} for tonic seizures was 144 mg/kg.

Finally, 4-substituted CHs were tested for convulsant and/or anticonvulsant properties. We observed that intraperitoneal injections of 4-ethyl-CH caused seizures characterized by myoclonic twitches and clonic convulsions (Table 1). None of the 50 animals (including the five mice tested at 562 mg/kg) given 4-ethyl-CH had tonic convulsions. 4-*t*-Butyl-CH was 3–4-fold more potent than 4-ethyl-CH in producing clonic convulsions. In addition, 4-*t*-butyl-CH induced tonic seizures (CD_{50} of 125 mg/kg).

[^{35}S]TBPS binding. Initial binding experiments indicated that [^{35}S]TBPS bound to a single site in rat brain membranes with an apparent K_D of 39.3 ± 1.9 nM and a B_{max} of 1.34 ± 0.04 pmol/mg of protein. Picrotoxinin (IC_{50} = 300 nM) and PTZ (IC_{50} = 620 μM) both displaced specific [^{35}S]TBPS binding competitively. These results agree with those reported by other laboratories (8, 17, 20–22).

All of the CPs and CHs tested produced concentration-dependent inhibition of specific [^{35}S]TBPS binding from rat brain membranes. The IC_{50} values ranged from 20 μM for 4-*t*-butyl-CH to 29.8 mM for CP (Table 2). The CHs were more potent than similarly substituted CPs. Also, binding potency increased as the size of the alkyl substituent increased. Finally, as the distance between the carbonyl carbon and the alkyl substituent increased, the compounds became more effective displacers of [^{35}S]TBPS. For example, 4-ethyl-CH was more potent than 3-ethyl-CH and 3-ethyl-CH was more potent than 2-ethyl-CH (Fig. 2A).

Scatchard plots in the presence or absence of 1.2 mM 2-ethyl-CH, 200 μM 3-ethyl-CH, and 140 μM 4-ethyl-CH show that these compounds displace TBPS in a competitive manner (Fig. 2B). All of the CPs and CHs tested were competitive inhibitors of [^{35}S]TBPS binding. That is, these compounds approximately doubled the apparent K_D of TBPS for its receptor without significantly changing the number of TBPS binding sites in the tissue (Table 2).

TABLE 1

Convulsant activity or anticonvulsant activity and neurotoxicity of intraperitoneally administered CPs and CHs

The CD_{50} reported is for clonic seizures. Numbers in parentheses are 95% fiducial limits. MES, maximal electroshock.

Compound	Convulsant activity, CD_{50}	Anticonvulsant activity, ED_{50}		Toxicity, TD_{50}
		PTZ	MES	
	mg/kg	*mg/kg*		*mg/kg*
CP	None	473 (382–882)	362 (310–440)	>562
CH	None	238 (203–275)	397 (327–532)	473 (431–536)
2-Methyl-CP	None	268 (230–324)	366 (324–416)	>562
2-Ethyl-CP	None	205 (148–258)	316 (275–366)	>562
2-Methyl-CH	None	219 (186–255)	216 (170–263)	243 (218–270)
2-Ethyl-CH	None	140 (119–163)	>562	245 (210–293)
2-Propyl-CH	452 (321)[a]	None	None	NA[b]
2-*t*-Butyl-CH	151 (75–197)	None	None	NA
3-Methyl-CP	None	365 (285–468)	263 (201–331)	408 (323–723)
3-Ethyl-CP	396 (301–464)	None	None	NA
3-Ethyl-CH	613 (402)[a]	156 (108–190)	122 (98–155)	184 (148–219)
3-*t*-Butyl-CH	121 (73–184)	None	None	NA
4-Ethyl-CH	183 (43–253)	None	None	NA
4-*t*-Butyl-CH	53 (25–100)	None	None	NA

[a] Seizures in <50% of the mice at highest dose tested; no upper fiducial limit.
[b] NA, not assessed.

TABLE 2

Potencies of CPs and CHs as inhibitors of [^{35}S]TBPS binding and their effect on [^{35}S]TBPS binding constants

Data are expressed as mean ± standard error; the number of experiments is given in parentheses.

Compound	[^{35}S]TBPS IC_{50}	[^{35}S]TBPS Binding Constants:	
		K_D	B_{max}
	nM	nM	pmol/mg of protein
Control (10)		39.3 ± 1.9	1.34 ± 0.04
CP (3)	29.8 ± 2.5	74.0 ± 5.1*	1.23 ± 0.05
CH (3)	9.6 ± 1.5	84.8 ± 6.7*	1.29 ± 0.09
2-Methyl-CP (4)	2.8 ± 0.1	83.2 ± 2.2*	1.16 ± 0.05
2-Ethyl-CP (3)	2.4 ± 0.1	90.8 ± 1.9*	1.11 ± 0.13
2-Methyl-CH (3)	2.5 ± 0.4	72.1 ± 3.8*	1.24 ± 0.05
2-Ethyl-CH (3)	1.2 ± 0.1	87.1 ± 8.4*	1.15 ± 0.22
2-Propyl-CH (3)	0.70 ± 0.11	65.7 ± 3.9*	1.31 ± 0.04
2-*t*-Butyl-CH (3)	0.59 ± 0.03	83.5 ± 2.0*	1.38 ± 0.17
3-Methyl-CP (3)	3.5 ± 0.1	76.1 ± 4.5*	1.37 ± 0.05
3-Ethyl-CP (3)	0.63 ± 0.06	85.9 ± 3.7*	1.32 ± 0.06
3-Ethyl-CH (4)	0.20 ± 0.02	78.7 ± 8.2*	1.42 ± 0.10
3-*t*-Butyl-CH (3)	0.021 ± 0.001	81.8 ± 2.5*	1.44 ± 0.08
4-Ethyl-CH (3)	0.14 ± 0.01	90.5 ± 6.4*	1.31 ± 0.06
4-*t*-Butyl-CH (3)	0.020 ± 0.001	84.3 ± 5.1*	1.21 ± 0.09

*$p < 0.01$ as compared with control; *t* test with the Bonferroni correction.

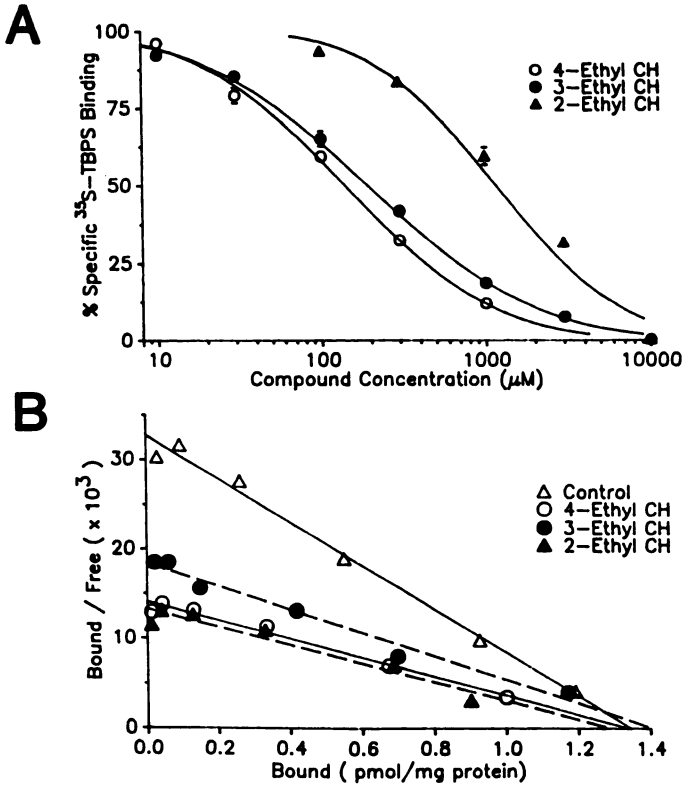

Fig. 2. A, Concentration-dependent displacement of specifically bound [^{35}S]TBPS from rat brain membranes by 2-ethyl-CH (▲), 3-ethyl-CH (●), and 4-ethyl-CH (○). Data are presented as mean ± 1 SE. B, Typical Scatchard plots of [^{35}S]TBPS binding data, control (△) and in the presence of 1.2 mM 2-ethyl-CH (▲), 200 μM 3-ethyl-CH (●), or 140 μM 4-ethyl-CH (○). The K_D and B_{max} values were obtained from nonlinear regression analysis of the binding data and are summarized in Table 2.

Discussion

By comparing the activity of the α- and β-substituted GBLs, succinimides, and related compounds, Klunk *et al.* (5, 6) proposed that (a) a carbonyl oxygen adjacent to the heteroatom (with two free electron pairs, such as oxygen, sulfur, or nitrogen with an ionizable hydrogen) of a heterocyclic ring and (b) suitable alkyl substituents on the carbon atom α- and/or γ- but not β- to the carbonyl were absolute requirements for the anticonvulsant activity of these cyclic compounds. The presence of alkyl substituents in the β-position produced compounds that were convulsants regardless of other substituents (3, 6). Compounds that lacked alkyl groups have been reported to be convulsant (1). Here we found that CP, CH, and their 2-, 3-, and 4-substituted derivatives all bind to a common receptor site (the picrotoxin receptor) and are effective as either anticonvulsants, convulsants, or both. These findings suggest that neither the presence of a ring heteroatom nor the presence of α- or γ-alkyl substituents are absolute requirements for anticonvulsant activity of small cyclic compounds.

The earlier conclusion that an oxygen, sulfur, or unsubstituted imide nitrogen in the ring was required for anticonvulsant activity had been based on three observations (6). First, *N*-methylation of TMSM, a potent convulsant, produces pentamethylsuccinimide (Fig. 3). This replaces the ionizable hydrogen in TMSM with a methyl group and results in greatly reduced convulsant activity. Second, β-ethyl-β-methyl-γ-butyrolactam is similar to β-EMGBL except that the ring oxygen is replaced by a nitrogen atom that is not significantly ionizable at physiological pH. However, β-ethyl-β-methyl-γ-butyrolactam is much less convulsive than β-EMGBL. Third, 5,5-dimethyl-1,3-cyclohexanedione (a six-membered cyclic di-ketone) is inactive, whereas β,β-dimethylglutarimide, the corresponding compound with the —CH_2— replaced by a —NH—, was a convulsant. In all three cases the activity of the analogous

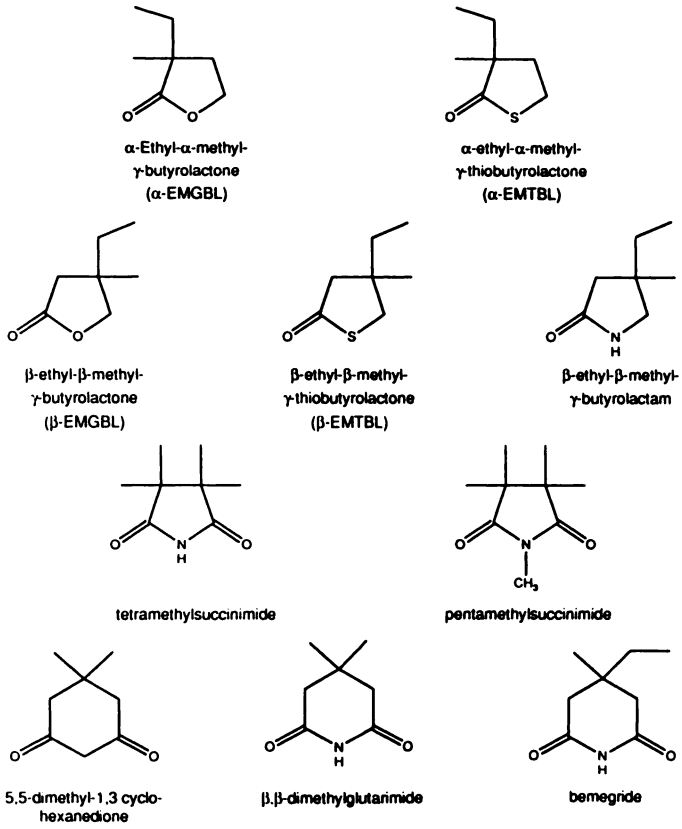

Fig. 3. Chemical structures of selected γ-butyrolactones, γ-thiobutyrolactones, γ-butyrolactams, succinimides, cyclohexanediones, and glutarimides.

α-substituted derivative was not assessed. Our results show that the presence of a heteroatom is not required for anticonvulsant activity because both unsubstituted and α-substituted CPs and CHs are at least as potent as GBLs and TBLs with similar alkyl substituents (12).

By contrast, the activity of 3-substituted (i.e., β-substituted) compounds is affected by the absence of a heteroatom. The β-methyl-substituted CP 3-methyl-CP is anticonvulsant, whereas the β-ethyl derivative, 3-ethyl-CP, is only a weak convulsant. In the CH series, the β-ethyl-substituted compound 3-ethyl-CH is both an anticonvulsant and a weak convulsant. The 3-ethyl-substituted cyclic ketones examined in the report are at least 10-fold weaker convulsants than the heterocyclic convulsant β-EMGBL (3) and 50- to 100-fold less potent than β-EMTBL (4). In addition, β-EMGBL and β-EMTBL have been reported to produce tonic convulsions at doses below 50 mg/kg, whereas we report that neither 3-ethyl-CH nor 3-ethyl-CP produced tonic seizures, even when administered in doses as high as 562 mg/kg. The only β-substituted cyclic ketones with substantial convulsant activity, 3-*t*-butyl-CH, is still 5-fold less effective than β-EMGBL and 30-fold less effective than β-EMTBL in producing both clonic and tonic seizures. The smaller size of the β-alkyl substituents may contribute to the lower convulsant potency of the 3-substituted CPs and CHs. However, heteroatom effects on convulsant potency are also seen in β-ethyl-β-methyl-substituted heterocyclic rings. β-Ethyl-β-methyl-γ-butyrolactam is weaker than β-EMGBL (CD_{50}, 20 mg/kg), which in turn is weaker than β-EMTBL (CD_{50}, 3 mg/kg) (3, 4). Thus, it appears that the presence of a heteroatom in the ring may be more important for the convulsant action of the β-substituted compounds than for the anticonvulsant activity of their α-substituted analogs.

Electrophysiological studies of alkyl-substituted GBLs and TBLs indicated the heteroatom was a major factor in determining the action of these compounds at the picrotoxin receptor (9). α-Alkyl-substituted GBLs were antagonists at this receptor site, whereas α-substituted TBLs acted as inverse agonists. In addition, β-GBLs were full agonists, whereas β-TBLs acted only as partial agonists. The sulfur-for-oxygen substitution in the ring significantly decreased the efficacy of β-substituted TBLs. A similar decrease in agonist efficacy might result from a carbon-for-oxygen substitution. This would explain why 3-ethyl-CH has both convulsant and anticonvulsant activity. 3-Ethyl-CH may only be a weak partial agonist at the picrotoxin receptor and as a result a weak convulsant. However, 3-ethyl-CH could prevent PTZ [a full agonist at the picrotoxin receptor (23–25)] from binding to this receptor site, and as a result this compound would also act as an anticonvulsant at least against PTZ-induced seizures.

The results obtained for the CPs and CHs confirm the observation that cyclic compounds with small α- (but not β-) alkyl substituents are anticonvulsants (5, 12). Our results suggest that unsubstituted compounds can be either convulsant (TBL), inactive (GBL), or anticonvulsant (CP and CH). α-Alkylation (one or two carbon atoms) increases both the anti-PTZ potency and affinity of the CPs and CHs for the picrotoxin receptor. However, α-alkylation seemed to have little effect on the ability of these compounds to block maximal electroshock-induced seizures. Like the GBLs and TBLs, the potency of CPs and CHs as displacers of specifically bound [^{35}S]TBPS was highly correlated with their ability to prevent PTZ seizures ($r = 0.86$, $p < 0.001$, $n=8$). This suggests that these compounds share a common mechanism of action with the heterocyclic compounds.

Like the GBLs and TBLs, these cyclic ketones appear to interact with the picrotoxin receptor with low affinity. Although we have not measured the brain concentrations of CPs and CHs required to produce neurological effects, this has been examined with α-EMGBL, α-EMTBL, and β-EMGBL. The effective brain concentrations of these agents is consistent with their low affinity interaction with the picrotoxin receptor. For example, the brain concentration of α-EMGBL when injected into mice at the ED_{50} against PTZ-induced seizures is 1.2 mM, whereas its IC_{50} for displacement of specifically bound [^{35}S]TBPS binding is 2.3 mM (8, 9).[2] Nevertheless, because of the relatively high concentrations of GBLs, TBLs, CPs, and CHs required for clinical effects we cannot, at this time, exclude definitively the possibility that cellular or neurochemical factors other than those related to the picrotoxin receptor contribute to the actions of these agents.

The effect of varying the size of alkyl substituents has been studied extensively with α-alkyl-substituted GBLs and TBLs (12). The results of that study suggest that GBLs with α-alkyl groups between two and five carbon atoms produce optimal anticonvulsant activity. Those GBLs and TBLs with larger alkyl substituents (six carbons) were either inactive or produced convulsions. A similar phenomenon is observed for the CHs; CHs with small alkyl substituents (one or two carbons) are anticonvulsant whereas those with alkyl groups larger than two carbon atoms act as convulsants. As with the α-position, the size of the substituent at the β-position appears to be an important determinant of activity. Larger alkyl β-substituents produce compounds with convulsant activity (e.g., 3-ethyl-CP and 3-*t*-butyl-CH), whereas compounds with smaller alkyl groups in the β-position have some anticonvulsant or mixed anticonvulsant/convulsant activity (3-methyl-CP and 3-ethyl-CH).

The interacton of six-membered ring systems with the picrotoxin receptor has not been systematically examined. Our results show that CHs exhibit convulsant or anticonvulsant activity depending on the location and size of alkyl substituents. In addition, glutarimides (six-membered cyclic imides) also possess convulsant or anticonvulsant activity (26, 27). The α-substituted compounds 2-methyl-CH, 2-ethyl-CH, and glutethimide (α-ethyl-α-phenylglutarimide) are anticonvulsant, whereas some of those with substituents in the β-position, 3-*t*-butyl-CH, bemegride (β-ethyl-β-methylglutarimide), and β,β-dimethylglutarimide are convulsant. Squires *et al.* (25), Klunk *et al.* (28), and Ticku (29) have suggested that bemegride acts at the picrotoxin receptor and we have shown that both anticonvulsant and convulsant CHs competitively displace [^{35}S]TBPS from sites on rat brain membranes. Our results show that certain five- and six-membered ring systems bind to the picrotoxin receptor and act as either convulsant or anticonvulsant agents.

In conclusion, we have found that CPs and CHs are neuroactive compounds that possess either anticonvulsant or convulsant activity depending upon the pattern and size of alkyl substitution. Like GBLs and TBLs, those cyclic ketones with small alkyl substituents in the α-position are anticonvulsant.

[2] D. Canney and D. Covey, unpublished observations.

The convulsant activity resulting from alkyl substitution at the β-position is reduced without a heteroatom. β-Substituted CPs and CHs are either anticonvulsants or weak convulsants or both. In addition, unlike TBL, which is a convulsant, the unsubstituted cyclic ketones examined in this report (CP and CH) are anticonvulsants. Thus, it appears that α-alkyl substituents are not necessary for anticonvulsant activity although they may increase the anti-PTZ potency of these agents. The activity of these cyclic ketones suggests that the presence of a heteroatom in the ring is not an absolute requirement for neurological activity of small cyclic compounds.

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