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COMMENTARY

ALKYL-SUBSTITUTED γ-BUTYROLACTONES AS POTENTIAL TOOLS IN THE STUDY AND TREATMENT OF EPILEPSY

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This commentary article will focus on the potential of alkyl-substituted γ-butyrolactones as agents for the treatment and study of epilepsy. Before considering these agents specifically, however, a brief discussion of epilepsy itself, its present treatment, and approaches used to study the actions of antiepileptic drugs will be given. This short discussion will provide a background for the remainder of the article in which we will discuss the history and pharmacology of the alkyl-substituted γ-butyrolactones, speculate on the mechanism of action of these drugs, and finally consider the future of these agents in both experimental research and clinical medicine.

Epilepsy is by no means a rare disease, and it is estimated that in the United States a minimum of four million persons suffer from some form of this disorder [1]. It is important to realize, however, that epilepsy is a broad term which refers to many types of recurrent seizures. A clinical seizure is a physical manifestation produced by paroxysmal, excessive, neuronal discharges in various susceptible parts of the brain. For the purposes of this article, seizures can be classified as either generalized or partial. Generalized seizures may be further subclassified as absence (petit mal) or tonic-clonic (grand mal).

Usual treatment of epilepsy is either by means of antiepileptic drugs or surgery. The latter is generally reserved for those patients who have frequent disabling seizures that cannot be controlled by any treatment regimen of antiepileptic drugs. Just as there are different classes of seizures, there are different classes of antiepileptic drugs used to treat them. For example, ethosuximide is effective only for petit mal absence seizures; phenytoin is ineffective for these seizures, but prevents generalized tonic-clonic convulsions. Barbiturates, benzodiazepines, and valproic acid may be effective in both. The present rate of complete control of seizures on the whole is about 60% [2]. Thus, while the presently available drugs have substantial benefits, there is still need for additions to the current therapeutic armamentarium.

The fact that our present therapy for epilepsy is productive does not imply that it is understood. On the contrary, while effective seizure therapy dates back to the use of bromide in 1857, the mechanisms of action of the sixteen currently used antiepileptic drugs are unknown with the possible exceptions of acetazolamide and the benzodiazepines. Understanding how all these drugs work is important for two reasons. First, it would provide important insights into the pathophysiology of seizure processes and, second, it would provide a basis for the rational design of more effective and safer antiepileptic drugs.

While several approaches to the study of antiepileptic drugs may be taken, the investigation of their effects on experimental seizure models has been one of the most widely employed. The rationale for this approach holds that a good antiepileptic drug should have little effect on the normal functions of the central nervous system and would exert its major effects in the prevention of an abnormal event (i.e. a seizure). Experimental seizures are usually produced either electrically or by convulsant drugs. These seizure models closely resemble human epilepsy in many respects. In addition, the effects of convulsant agents on the central nervous system are much less subtle than those of anticonvulsant agents. One can, therefore, more easily identify an important and specific effect on which to study the interactions of convulsant and anticonvulsant drugs. Thus, the study of drugs which induce seizures is closely tied to the study of the actions of drugs which prevent seizures.

The above strategy plays an integral role in our work which attempts to define the mechanism of action of anticonvulsant drugs and to use this knowledge in the design of new drugs. In this commentary, the study of a class of drugs called alkyl-substituted γ-butyrolactones will be described.

The history of alkyl-substituted γ-butyrolactones

The history of the alkyl-substituted γ-butyrolactones (GBLs) dates back to the discovery of the neuropharmacological effects of unsubstituted γ-butyrolactone in 1947 [3]. Much interest has been given to this drug, and its molecular and cellular actions have been reviewed recently [4]. It has been reported by several laboratories that systemic administration of γ-butyrolactone to experimental

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animals produces seizures. The behavioral and electroencephalographic characteristics of these seizures have been suggested to resemble petit mal absence seizures in humans [5–7].

One of the reasons that γ-butyrolactone-induced seizures were said to resemble petit mal absence seizures was that they both were prevented by drugs in the ethosuximide class but not by drugs in the phenytoin class. The antagonism between γ-butyrolactone and ethosuximide is very interesting in light of the structural similarities between the succinimide and γ-butyrolactone rings (Fig. 1). The fact that the alkyl groups of ethosuximide are necessary for activity prompted our interest in the neuropharmacological actions of alkyl-substituted γ-butyrolactones. Since the γ-butyrolactone molecule is not symmetrical like the succinimide molecule, it was necessary to study γ-butyrolactones with alkyl groups substituted on the carbon alpha to the carbonyl, α-ethyl-α-methyl γ-butyrolactone (α-EMGBL), as well as on the carbon beta to the carbonyl, β-ethyl-β-methyl-γ-butyrolactone (β-EMGBL) (Fig. 1).

Some work had been done previously on the β-substituted γ-butyrolactones. Prior to the study described below, β-EMGBL and several similar compounds were reported to produce seizures in rats, but the characteristics of the seizures were not described in any detail [8–11], and there is nothing known about their mechanism of action. γ-Butyrolactones substituted on the α-position with groups such as allyl plus α-benzimidazolyl, (*p*-methoxyphenyl)-allophanyl, or (*p*-bromophenyl)-allophanyl were studied by Sieroslawska *et al.* [12] in 1974 and were reported to have sedative-hypnotic, tranquilizer, anticonvulsant, and analgesic effects. However, α-EMGBL had never been investigated. Therefore, in order to directly compare alkyl-substituted γ-butyrolactones to ethosuximide, it was necessary to characterize the convulsant effects of β-EMGBL more fully, as well as to test α-EMGBL for neuropharmacological activity.

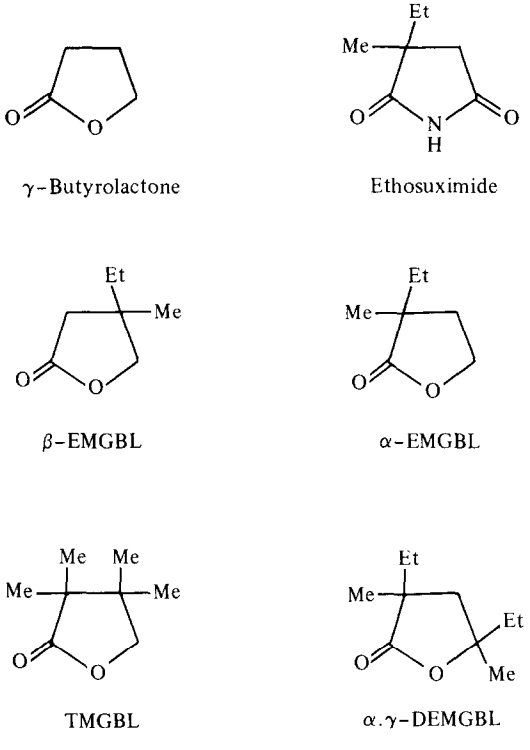

Fig. 1. Chemical structures of ethosuximide, γ-butyrolactone, and several alkyl-substituted γ-butyrolactones.

Epileptogenic properties of β-substituted γ-butyrolactones

The epileptogenic properties of β-EMGBL and several related compounds have been studied and compared to unsubstituted γ-butyrolactone [13]. The compounds were tested in mice and guinea pigs *in vivo* and in incubated guinea pig hippocampal brain slices. In all of these systems, β-EMGBL behaved much differently than GBL. In unrestrained mice, γ-butyrolactone produced nonconvulsive "absence-like" seizures. Given systemically, both γ-butyrolactone and its hydrolysis product, γ-hydroxybutyrate, were active. Previous studies [14, 15] indicate that γ-hydroxybutyrate is the active form, and γ-butyrolactone exerts an effect only after it is hydrolyzed by blood lactonase. In contrast, β-EMGBL produced convulsive seizures identical to those produced by pentylenetetrazol (a convulsant drug often used in experimental models of petit mal absence epilepsy). Also, the hydrolysis product of β-EMGBL was approximately one-tenth as potent as β-EMGBL itself.

In paralyzed-ventilated guinea pigs, γ-butyrolactone and β-EMGBL produced electroencephalographic changes which were markedly different. γ-Butyrolactone and γ-hydroxybutyrate produced hypersynchronous slow waves while β-EMGBL produced high frequency high voltage discharges. In addition, structurally rigid alkyl-substituted γ-hydroxybutyrate analogs with a *trans* configuration, which cannot relactonize, caused seizures similar to γ-butyrolactone while the corresponding *cis* analog, which can easily relactonize, caused seizures identical to β-EMGBL.

In incubated hippocampal slices the differences were even more pronounced. Both γ-butyrolactone and γ-hydroxybutyrate had no effect, β-EMGBL, however, had profound excitatory effects and induced spontaneous epileptiform discharges. The hydrolysis product of β-EMGBL had no effect on the activity of the slice.

Thus, it was concluded that the alkyl-substituted γ-butyrolactones and the unsubstituted compound are very different drugs and that the alkyl-substituted γ-butyrolactones probably have a site of action distinct from that of the unsubstituted compound. What the site of action of alkyl-substituted γ-butyrolactones may be will be suggested later in this article.

Anticonvulsant properties of α-substituted γ-butyrolactones

As was stated previously, the α-substituted compound, α-EMGBL, had not been tested prior to our study [16]. It was surprising, therefore, to find that a compound so chemically similar to the convulsant β-EMGBL (Fig. 1) would have the potent anticonvulsant activity which α-EMGBL was found to have.

Like β-EMGBL, α-EMGBL was tested in mice and guinea pigs *in vivo* and in incubated guinea pig hippocampal brain slices. In mice, α-EMGBL was tested alone and against the convulsant effects of β-EMGBL, pentylenetetrazol, picrotoxin, and maximal electroshock. In each case the effects of α-EMGBL were compared to ethosuximide and phenytoin. When given alone α-EMGBL had no effect on mice except at higher doses where it had a sedative effect. Ethosuximide and phenytoin had similar effects. Both ethosuximide and α-EMGBL pretreatment prevented seizures induced by pentylenetetrazol, picrotoxin and β-EMGBL but phenytoin pretreatment did not. Neither ethosuximide nor α-EMGBL could prevent maximal electroshock seizures but phenytoin was extremely effective. A quantitative comparison of the anti-pentylenetetrazol activity, anti-maximal electroshock activity and neurotoxicity of α-EMGBL, ethosuximide and phenytoin [17] showed α-EMGBL to be almost identical to ethosuximide in terms of spectrum of activity, potency and therapeutic index.

In paralyzed-ventilated guinea pigs α-EMGBL again caused no electroencephalographic changes when given alone. It was very effective in preventing a β-EMGBL-induced seizure or stopping a seizure already in progress. In fact, the most impressive effect of α-EMGBL was its ability to stop an ongoing seizure within 3 sec of being administered intravenously. Ethosuximide had similar effects except that its onset of action was slower. Phenytoin also did not prevent β-EMGBL-induced seizures in this system.

In hippocampal slices α-EMGBL caused a slight suppression of normal activity. However, when given in combination with β-EMGBL it blocked the epileptiform activity induced by β-EMGBL. The hydrolysis product of α-EMGBL had no activity alone and did not block β-EMGBL-induced excitation.

Thus, α-EMGBL appeared to be antagonistic to the actions of β-EMGBL in every system studied. This is a very intriguing finding in vew of their structural and chemical similarities. It suggests that they could act as agonist and antagonist at the same receptor site. This is supported by the inactivity of the hydrolysis products in both cases, suggesting that both α-EMGBL and β-EMGBL are active in the lactone form. This idea will be discussed more fully below.

Furthermore, α-EMGBL seems to fall into the same class of drugs as ethosuximide. Its similar potency and therapeutic index make it a promising candidate for clinical investigation.

Structure–activity relationships of alkyl-substituted γ-butyrolactones

The intriguing difference in activity between α-EMGBL and β-EMGBL prompted a more detailed study of the structure–activity relationships of alkyl-substituted γ-butyrolactones. While several analogs of both the γ-butyrolactone and succinimide class were included in the study [18], the major findings can be appreciated by examining only two of these compounds, α,α,β,β-tetramethyl-γ-butyrolactone (TMGBL) and α-γ-diethyl-α,γ-dimethyl-γ-butyrolactone (α,γ-DEMGBL) (Fig. 1).

TMGBL proved to be a convulsant [18]. It acted like β-EMGBL in every way and had a similar potency. In addition, TMGBL-induced seizures were blocked by the same anticonvulsants as were β-EMGBL-induced seizures, i.e. ethosuximide and α-EMGBL prevented the seizures but phenytoin did not.

α,γ-DEMGBL proved to be an anticonvulsant, almost identical in activity to α-EMGBL [16]. It had a somewhat lower potency than either ethosuximide or α-EMGBL and a slightly higher neurotoxicity, but its spectrum of activity against the various seizure models mentioned above was the same.

With the data from these and several other compounds the following model for the hypothetical binding site of γ-butyrolactone drugs was proposed [18]: (a) the compound with no alkyl groups has very little affinity for this site and is therefore inactive by our convulsant and anticonvulsant criteria, and (b) compounds with small alkyl groups on any or all of the α-, β- or γ-positions of the γ-butyrolactone ring will be active and will be convulsant if there are alkyl groups on the β-position (regardless of other substituents) or will be anticonvulsant if there are alkyl groups on the α- and/or γ-positions but not on the β-position. Thus, it is the β-position of alkyl-substituted γ-butyrolactones which is paramount in determining the activity of the compound.

During the development of this model, it became increasingly apparent that all of the structural requirements for alkyl-substituted γ-butyrolactones were fulfilled by picrotoxinin. Picrotoxinin is the more active component of picrotoxin, a convulsant drug which blocks the action of the major inhibitory neurotransmitter in mammalian brain, γ-aminobutyric acid (GABA). Picrotoxinin is believed to do this not by directly blocking GABA binding to its receptor but instead by blocking the GABA-induced increase in chloride flux which stabilizes the neuronal membrane near its resting potential [19]. Chemically, picrotoxinin is an alkyl-substituted γ-butyrolactone (Fig. 2A). In fact, it has a β-alkyl substitution (an

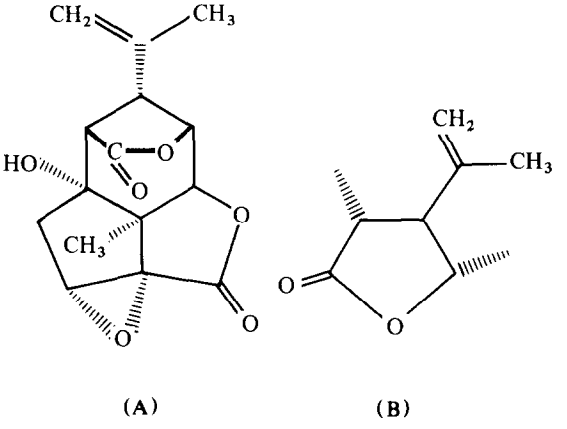

Fig. 2. Chemical structure of picrotoxinin. (A) Complete structure of picrotoxinin. (B) An isolated view of the essential β-substituted γ-butyrolactone ring oriented in the same way as those in Fig. 1.

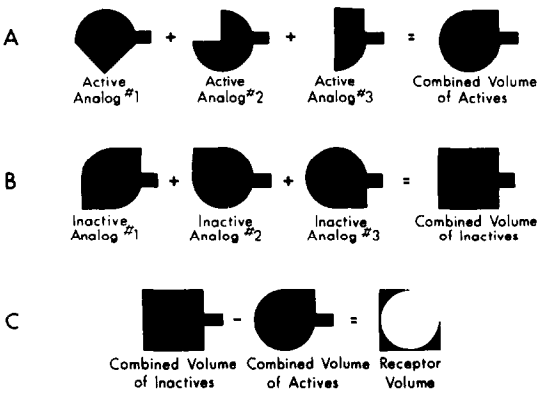

Fig. 3. A schematic of the methods used to generate computer models of binding sites. (A) Calculation of the combined active analog volume. (B) Calculation of the combined inactive analog volume. (C) Calculation of the volume of the receptor molecule which surrounds the binding site.

isopropenyl group) on a γ-butyrolactone ring (Fig. 2B), and both are known to be necessary for activity. In contrast, the remainder of the molecule has considerably less strict structural requirements [20].

Given that the picrotoxinin molecule fits the requirements for activity at the γ-butyrolactone site, might not the γ-butyrolactones fit the requirements for the picrotoxinin site and, in fact, might not these sites actually be the same?

To answer this question, a study was done with the aid of the computer-assisted molecular modelling system developed by Molnar *et al.* [21]. In this study [22], three-dimensional models were constructed for the alkyl-substituted γ-butyrolactones and for a series of picrotoxinin analogs. This method assumes that a structurally similar inactive molecule does not fit into the binding site of the receptor because it would need to occupy some volume which is already occupied by the receptor molecule itself.

Each model was developed independently and consisted of two elements. The first was a representation of the space available to active analogs (either convulsant or anticonvulsant). This was determined by computing the combined volume of all the active γ-butyrolactone or picrotoxinin derivatives (Fig. 3A). The second element was a representation of the portion of the receptor molecule which surrounds the binding site. This was determined by subtracting the combined active analog volume from the combined volume of the inactive analogs (Fig. 3C). It should be noted that the most important aspect of the receptor volume model thus derived is the contour of the binding site (depicted by the empty circular area in Fig. 3C). The overall shape of the outer limits of this portion of the receptor (depicted by the three corners of the square in Fig. 3C) has little importance.

When these two independently derived models were compared by testing whether the combined volume of the active γ-butyrolactone analogs would fit into the picrotoxinin receptor volume and vice versa, they were found to be extremely compatible. The models were then combined and shown to accommodate not only γ-butyrolactone and picrotoxinin derivatives, but also convulsant and anticonvulsant derivatives of succinimide, glutarimide and tetrazole.

Proposed mechanism of action of alkyl-substituted γ-butyrolactones

The structure–activity studies discussed above provided a link between alkyl-substituted γ-butyrolactones and picrotoxinin. This proved to be helpful in developing a hypothesis for the mechanism of action of the γ-butyrolactones. In addition, this association allowed the expansion of the present hypothesis of picrotoxinin action.

The first point in this proposed mechanism is that both the alkyl-substituted γ-butyrolactones and picrotoxinin work at the same site. This is supported not only by the structural studies but also by pharmacological studies which suggest α-EMGBL to be a competitive inhibitor of the actions of both picrotoxinin and β-EMGBL [16]. As was mentioned above, the site of action of picrotoxinin is believed to involve the GABA-regulated chloride channel.

The second point in the proposed mechanism is as follows. The convulsant compounds, β-substituted γ-butyrolactones and picrotoxinin, bind to a receptor and block GABA-induced chloride flux which results in the production of seizures. The anticonvulsant α-substituted γ-butyrolactones also bind this same receptor but do not cause a blockade of GABA-induced chloride flux or may even augment the flux. Therefore, these compounds have little activity when given alone but block the action of convulsant drugs of the β-EMGBL or picrotoxinin type.

The third point of our mechanism goes further to attempt to explain, on molecular terms, how all this might occur. Figure 4 graphically depicts this point. The figure contains combinations of two computer-generated elements. The first of these elements is the gray cross-hatched areas which represent the combined volume occupied by either the anticonvulsant α-substituted γ-butyrolactone molecules (Fig. 4A), the convulsant β-substituted γ-butyrolactone molecules (Fig. 4B), or picrotoxinin (Fig. 4C). These volumes were obtained by the method described in Fig. 3. The second of these elements is the two circles which represent the chloride channel. For a detailed discussion of how this model of the chloride channel was obtained, see Klunk *et al.* [22]. The larger circle represents the cross-sectional area of the channel itself as determined independently by Araki *et al.* [23] and Takeuchi *et al.* [24]. The inner circle represents the cross-sectional area of a hydrated chloride ion [23].

The structure–activity studies outlined above pointed out the importance of the substituent at the β-position of the γ-butyrolactone ring. Therefore, the channel was aligned with the model so that it fell over the β-carbon. The distance from the molecule was chosen to be that distance at which the anticonvulsant α-substituted compounds just failed to overlap the area occupied by the chloride ion (Fig. 4A). With this same alignment, both the convulsant β-substituted γ-butyrolactones (Fig. 4B) and picrotoxinin (Fig. 4C) overlap the area of the chlor-

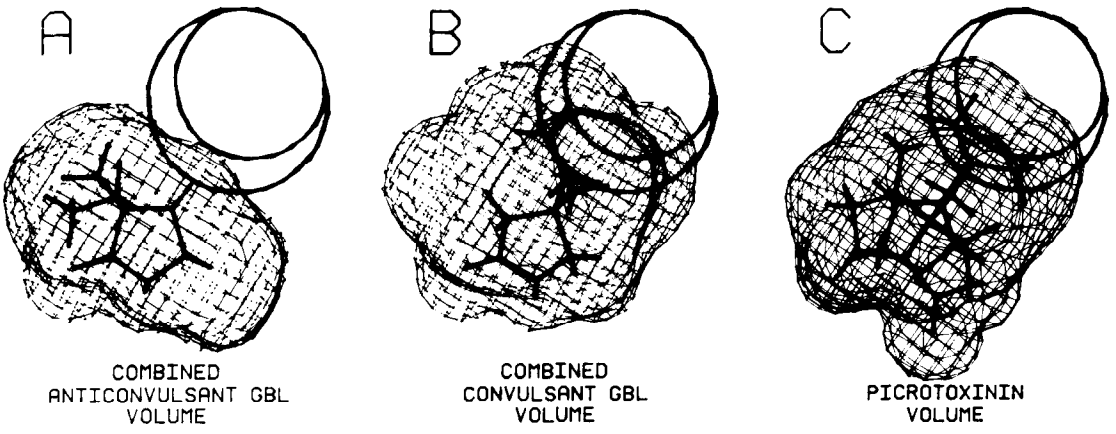

Fig. 4. Computer-generated models depicting the interaction of various molecular volume elements with the chloride channel. (A) The chloride channel model (larger circle) was placed at the β-position of the anticonvulsant γ-butyrolactones so that the molecular volume (grey cross-hatched area) just failed to overlap the chloride ion (inner circle). (B) When in exactly the same orientation as in A, the convulsant γ-butyrolactones overlap the chloride ion model. (C) Picrotoxinin in this same orientation shows a pattern of overlap very similar to that of the convulsant γ-butyrolactones.

ide ion, and this overlap is entirely accounted for by the β-substituents.

Our conclusion is that the physical dimensions of the chloride channel, chloride ion, and the alkyl-substituted γ-butyrolactones are such that it is possible to explain the mechanism of chloride flux blockade (or lack of it) on steric grounds alone. Thus, the anticonvulsant compounds, such as α-EMGBL would bind to the receptor but allow unobstructed passage of the chloride ion. The convulsant compounds such as β-EMGBL and picrotoxinin would bind to the same receptor and would directly impede the movement of chloride ions. This would destabilize the neuronal membrane and ultimately result in a seizure. This mechanism may also explain the actions of structurally analogous convulsant and anticonvulsant succinimides, glutarimides and tetrazoles.

A mechanism suggesting that a drug physically impedes an ionic channel is not new. It is generally accepted that tetrodotoxin blocks sodium channels in a similar manner [25]. However, the suggestion that the area overlapped by the β-substituents is the chloride channel itself remains highly speculative. Whether this speculation proves to be true or not does not discount the fact that, whatever this area may be, it is a very important area of space which can be used to predict the activity of γ-butyrolactone and picrotoxinin analogs. It is this predictive value that is the true importance of models such as this. This precise description of volume requirements of this site will allow the exploitation of these requirements in future drug design.

Future goals in the study of alkyl-substituted γ-butyrolactones

The investigation of this class of drugs has obviously only just begun. One of the first areas which requires attention is the demonstration of competitive binding by the alkyl-substituted γ-butyrolactones and picrotoxinin. While two assays exist for picrotoxinin binding, they have the problem of either extremely low specific binding [26] or a tedious purification of receptors which hampers reproducibility [27]. Both of these problems relate to the fact that the radioligand [^{3}H]-α-dihydropicrotoxinin has a low affinity ($K_D = 2\ \mu M$). Based on theoretical calculations, increasing this affinity only 5-fold to equal that of picrotoxinin itself ($K_D = 0.4\ \mu M$) would boost the specific binding into ranges which would be much more reliable. Thus, the first goal is the synthesis of a better radioligand for picrotoxinin binding.

The second area is that of chloride flux. The actual demonstration that the convulsant β-substituted γ-butyrolactones block GABA-induced chloride flux remains to be accomplished. Also, it would be necessary to clearly show that the α-substituted compounds have no blocking effect alone and prevent β-substituted γ-butyrolactone- and picrotoxinin-induced inhibition of chloride flux.

Potential for clinically useful antiepileptic drugs

In recent years, few new antiepileptic drugs have become available for use. Since the introduction of clonazepam in 1975 the only new drug has been valproic acid, introduced in 1978 [28]. However, as was pointed out at the beginning of this article, about 40% of epileptics still cannot achieve complete control of their seizures [2]. Thus, the need for newer and more effective drugs remains great.

The anticonvulsant α-substituted γ-butyrolactone, α-EMGBL, has passed only the initial phase of anticonvulsant drug screening. It will take much longer to see if it withstands further experimental trials of activity and toxicity before clinical trials can even be contemplated. However, its similarity to ethosuximide in terms of potency and therapeutic index in mice is a hopeful sign. Regardless, the alkyl-substituted γ-butyrolactones are likely to play

an important role in defining the mechanism of action of ethosuximide and similar antiepileptic drugs.

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