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Effects of Some Anti-Epileptic, Neuroleptic and Gabaminergic Drugs on Convulsions Induced by D,L-Allylglycine

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ASHTON, D. AND A. WAUQUIER. *Effects of some anti-epileptic, neuroleptic and gabaminergic drugs on convulsions induced by D,L-allylglycine.* PHARMAC. BIOCHEM. BEHAV. 11(2) 221–226, 1979.—The antagonism of various seizure and time-related components of the convulsions resulting after IV injection of D,L-allylglycine into male Wistar rats were assessed in a standard test procedure. Trimethadione and ethosuximide did not antagonize the seizure components, whereas clonazepam, phenobarbital, diphenylhydantoin, primidone, valproate sodium, aminoxyacetic acid, etomidate, acetazolamide, flunarizine, pipamperone and baclofen did. The allylglycine test may thus represent a relatively specific method of differentiating between drugs effective against partial or generalized convulsive seizures from those effective against absence seizures. The neuroleptics haloperidol and pimozide were completely inactive in contrast to their reported antagonism of bicuculline seizures. The spectra of the active substances are discussed with respect to Principal Component and Cluster Analysis. Noteworthy are the similarities between baclofen and etomidate; between aminoxyacetic acid, phenobarbital and valproate sodium; and between diphenylhydantoin and flunarizine.

Anti-epileptic Neuroleptic Convulsions D,L-allylglycine Gabaminergic drugs

IN ANY neuronal aggregate when epileptic activity is displayed, the basic phenomenon is an excessively synchronous discharge of neurones [1]. Although cerebral grey matter displays immense variety in its pattern of cellular organization, a universal feature is the use of inhibitory interneurones activated by direct or recurrent collateral pathways, to prevent synchronous discharge [11]. With the exception of the spinal cord and lower brain stem, where glycine is the major transmitter producing postsynaptic inhibition [31], GABA is the principal inhibitory substance identified in the central nervous system [24]. The convulsant activity of allylglycine was first reported by Schneider *et al.* [27] and later was more extensively described in mice by McFarland and Walner [23]. Alberici *et al.* [2] showed an in vitro and in vivo inhibition of glutamine acid decarboxylase (GAD) in rats after allylglycine treatment, which led to a 40% decrease in the concentration of γ-aminobutyric acid in the cerebral cortex. The inhibition of GAD by allylglycine was not by way of the cofactor pyridoxal phosphate [2]. Horton [17] explained the long delay between injection of allylglycine and the first convulsion (1–3 hr) as being due to the metabolic conversion of allylglycine to 2-keto-4-pentenoic acid, which is a more potent GAD inhibitor than the parent compound. It is thus likely that compounds with Gabamimetic or γ-aminobutyrate aminotransferase (Gaba-T) inhibiting properties would be antagonists of allylglycine produced convulsions. This led us to investigate the action of some anti-epileptic drugs and various compounds described as possessing Gabaminergic properties on the convulsions produced by D,L-allylglycine.

METHOD

All experiments were carried out on 200 (± 10) g male Wistar rats of the laboratory strain. Prior to experimentation the animals were food deprived overnight but had free access to water. During the experiments animals were housed individually in transparent perspex observation cages, in which the floor was covered with sawdust. Manipulation of the animals and noise in the experimental room were kept to a minimum as earlier experiments had shown a marked sensitivity of allylglycine treated rats to these phenomena. Each animal was used once only.

All compounds were given orally except for etomidate which was given SC. For both routes drugs were injected at a volume of 1 ml per 100 g body weight. All compounds were given 2 hr prior to the allylglycine injection, except baclofen and clonazepam which were given 1 hr before and etomidate which was injected immediately prior to the allylglycine.

Diphenylhydantoin (10, 40, 160, 320 and 640 mg/kg), flunarizine (5, 20, 80 and 160 mg/kg), clonazepam (1.25, 5 and 10 mg/kg), primidone (5, 20 and 80 mg/kg), trimethadione (20, 160 and 320 mg/kg) and ethosuximide (320 mg/kg) were given as freshly ultrasonified aqueous suspensions in 1% Tween 80. Aminoxyacetic acid (5, 20, 80, 160 and 320 mg/kg), etomidate (0.63, 2.5, 10 and 40 mg/kg), pipamperone (0.63, 2.5, 10 and 40 mg/kg), phenobarbital (5, 20, 40 and 80 mg/kg), sodium valproate (160, 320 and 640 mg/kg), meprobamate (80 mg/kg) and amitryptyline (5 mg/kg) were dissolved in distilled water. Baclofen (0.63, 2.5, 10, 20 and 40

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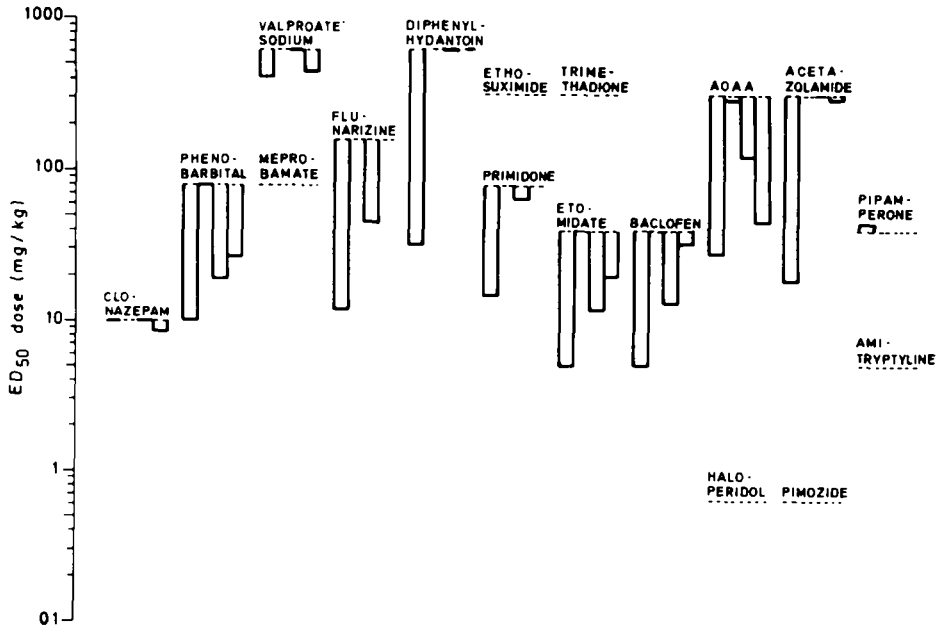

FIG. 1. Log plot of ED_{50} values (n=5) for absence of: 1st column clonic followed by tonic convulsions of fore and hind limbs (CBP/TBP). 2nd column: absence of clonic seizures (CLO). 3rd column: absence of tonic seizures (TON). 4th column: survival (SUR). All observations apply to the 4 hr after injection of allylglycine. Upper dotted line indicates highest dose administered. All compounds, except etomidate, given orally. AOAA=aminoxyacetic acid.

mg/kg), pimozide (0.04, 0.16 and 0.63 mg/kg) and haloperidol (0.01, 0.04, 0.16 and 0.63 mg/kg) were dissolved in distilled water and tartaric acid. Acetazolamide (20, 80 and 320 mg/kg) was dissolved in distilled water and a few drops of NaOH. Control rats received tap water orally at a volume of 1 ml/100 gram body weight.

D,L-allylglycine was injected in a tail vein at a dose of 160 mg/kg at a volume of 0.4 ml per 100 g body weight.

Animals were continuously observed for the 4 hr following the allylglycine injection. The rats were tested in groups of 6, composed of 5 drug treated animals and 1 tap water treated control. In any one session 2 groups of rats were observed by one experimenter.

On the basis of results from a pilot experiment the following seizure components were assessed. The time of onset of the first clonic or tonic movements (ONS). The presence or absence of a clonic seizure of both fore and hind limbs followed by a tonic seizure of both fore and hind limbs (CBP/TBP). The presence or absence of clonic seizures (CLO). The presence or absence of tonic seizures (TON). The time of death (MORT). Survival of any animal for 4 hr (SUR). The duration of the convulsion prior to death (DUR).

The 55 control animals all showed CLO and TON convulsions and died (MORT) within the 4 hr observation period. Ninety-two percent of the animals also had a CBP/TBP. Failure of a drug animal to show any one of these seizure components within 4 hr after allylglycine was counted as protection against that seizure component.

The range of the control population with respect to ONS, DUR and MORT was then calculated parametrically and nonparametrically. The parametric range was wider than the nonparametric range and thus the following parametric criteria were selected for the assessment of protection: ONS greater than 116 min after allylglycine; DUR greater than 82 min; and MORT exceeding 184 min after allylglycine. The calculation of drug effects on DUR is complicated by its relationship to ONS and the 4 hr observation period. Thus doses of drugs at which a significant survival at 4 hr and a significant delay of ONS occurred were not used for the calculation of ED_{50} values for DUR.

RESULTS

ED_{50} values for antagonism of the 4 seizure-components, i.e., absence of CBP/TBP, CLO, TON and SUR are represented in Fig. 1. ED_{50} values for increase in the time-related effects, i.e., ONS, DUR and MORT are represented in Fig. 2. All ED_{50} values were calculated according to Finney [13]. For convenience drug results will be given under the following headings: antiepileptic drugs, neuroleptic drugs, and miscellaneous compounds.

Anti-Epileptic Drugs

These compounds had various actions within the dose range tested. Clonazepam antagonized CBP/TBP, TON ($ED_{50} \geq 10$ mg/kg) and SUR in the seizure components; and increased ONS, DUR and MORT times. Phenobarbital antagonized CBP/TBP, TON, CLO ($ED_{50} \geq 80.0$ mg/kg), and SUR, in addition it increased the time of ONS, and MORT. Valproate sodium antagonized CBP/TBP, TON and SUR, and increased the time of ONS, and MORT. Flunarizine effected CBP/TBP and TON; and increased the time of ONS and DUR ($ED_{50} \geq 160$ mg/kg). Diphenylhydantoin effected CBP/TBP and TON ($ED_{50} \geq 640$ mg/kg); and increased ONS and MORT. Primidone antagonized CBP/TBP and TON; and increased ONS, DUR and MORT. Trimethadione increased

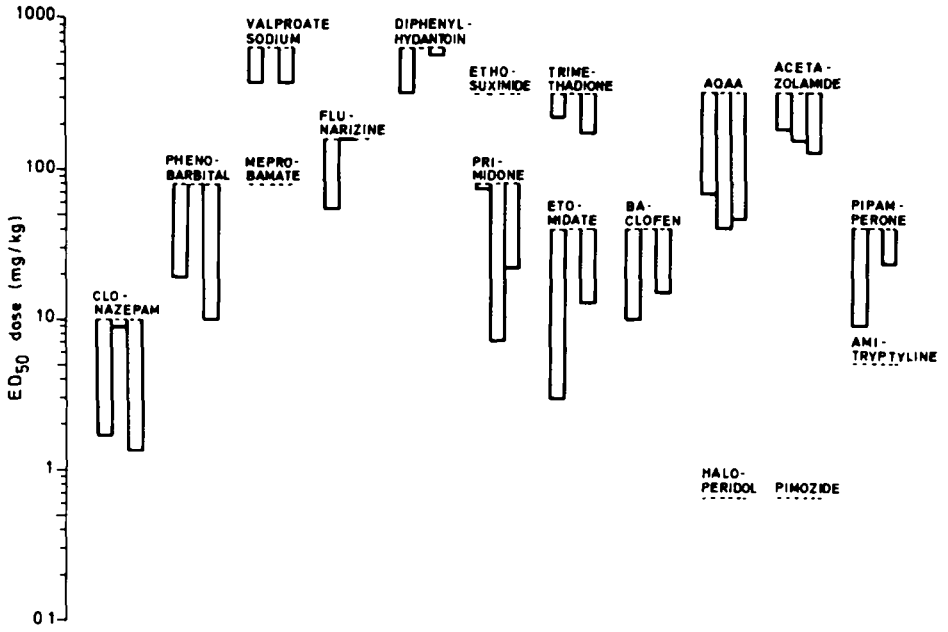

FIG. 2. Log plot of ED_{50} values (n=5) for: 1st column: onset of seizure (ONS) in excess of 116 min. 2nd column: duration (DUR) of convulsion before death greater than 82 min. 3rd column: time of mortality (MORT) in excess of 184 min. All times in minutes after administration of allylglycine. Upper dotted line is highest dose given. All compounds given orally except etomidate (SC).

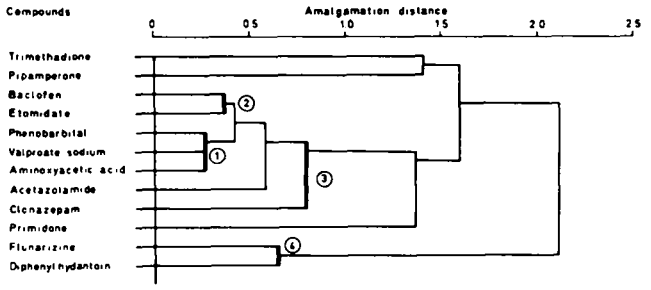

FIG. 3. Cluster analysis illustrating the inter-relationships of 12 active compounds in function of ED_{50} values against 5 variables: onset of seizures (ONS), mortality time (MORT), survival at 4 hr (SUR), absence of tonic (TON), and absence of clonic of fore and hind limbs followed by tonic of fore and hind limbs (CBP/TBP).

ONS and MORT. Acetazolamide effected CBP/TBP and SUR; and increased ONS, DUR and MORT. Ethosuximide and meprobamate did not affect either the seizure components or the time-related factors.

Neuroleptic Drugs

Haloperidol (0.01–0.63 mg/kg) and pimozide (0.04–0.63) did not affect either the seizure components or the time-related factors. In contrast, pipamperone antagonized CBP/TBP and increased ONS, and MORT.

Miscellaneous Compounds

Etomidate and baclofen antagonized CBP/TBP, TON and SUR; and increased ONS, and MORT. Etomidate also had some affect on CLO ($ED_{50} \geq 40.0$ mg/kg).

Aminoxyacetic acid antagonized all the seizure components and increased all the time related-factors. Amitryptyline was inactive.

Spectral Mapping; Cluster and Principal Component Analysis

In order to analyse the anti-allylglycine activity profiles of the drugs, the ED_{50} values for CBP/TBP, TON, SUR, ON and MORT (or in their absence 100× the highest dose tested; except in the case of $\geq$ or $\leq$ where the value remained the same) were transformed by the spectral mapping method (for detailed methodology see [20]) in order to eliminate absolute potency. Cluster analysis (Fig. 3) and Principal component analysis (Fig. 4) were performed on the resulting data [20].

DISCUSSION

Injection of 160 mg/kg D,L-allylglycine IV into 200 g male Wistar rats produced a convulsion in all control animals. Seven components of these convulsions proved stable enough to evaluate the effect of drugs thereon. Of the 17 compounds tested, amitryptyline, meprobamate, ethosuxi-

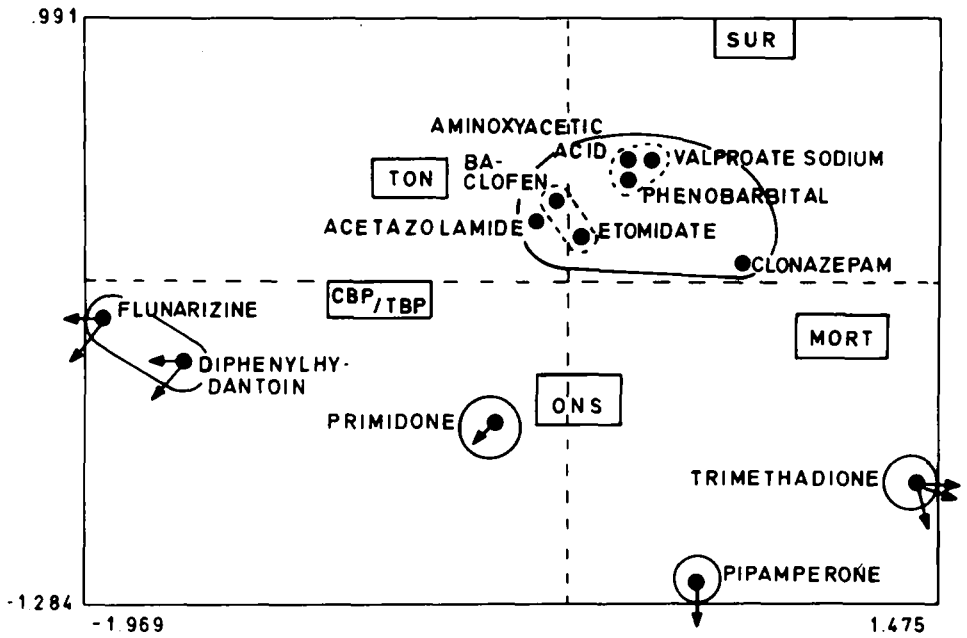

FIG. 4. Principal component mapping illustrating the spatial configuration of 5 variables and 12 compounds in a plane defined by the two components explaining 64.3% (abscissa) and 19.7% (ordinate) of the variance. Compounds grouped according an amalgamation distance of 0.37 (---) and 0.80 (——), respectively.

mide, haloperidol and pimozide had no affects; and trimethadione only affected the time related components.

Ethosuximide and trimethadione are considered to be the agents of choice for the treatment of simple absence seizures in humans [14]. In the present test system doses of these compounds of 320 mg/kg orally had no effect on the seizure components. In addition, ethosuximide had no affect on the time-related factors, whereas trimethadione delayed ONS and MORT. Thus the finding that phenobarbital, valproate sodium, primidone, clonazepam and diphenylhydantoin had affects on the seizure components induced by allylglycine whereas ethosuximide and trimethadione did not, suggests that the allylglycine test may represent a relatively specific method of differentiating between drugs effective against partial or generalized convulsive seizures from those effective against absence seizures.

At low doses pimozide and haloperidol block bicuculline convulsions in mice [32]. The convulsant effects of bicuculline is believed to be mediated via a blockade of central GABA receptors [7]. In the present experiments haloperidol (0.01–0.63 mg/kg) and pimozide (0.04–0.63 mg/kg) did not alter either the seizure or the time-related components of the allylglycine convulsion. This result is also at variance with those obtained by Meldrum *et al.* [24], who showed that haloperidol and pimozide slightly enhanced the photically induced seizures in *Papio papio* after administration of 180–200 mg/kg of allylglycine IV.

The spectra of the active compounds will be discussed within the framework of Principal component analysis (Fig. 4) and Cluster analysis (Fig. 3). These spectra are derived by transforming pharmacological data so as to remove the potency whilst leaving the activity profiles intact (method and rationale extensively described in [20]). Cluster analysis then illustrates the inter-relationships of the compounds but fails to indicate the spatial configuration of the cluster. Principal component analysis reveals general trends but yields no accurate information on the degree of resemblance of the compounds. This loss of information occurs through the transformation of a multidimensional space into a two-dimensional space, however the present figure contains 84% of the information contained in the original multidimensional space (64% abscissa, 20% ordinate). Multivariate statistics are best applied to large numbers of compounds which all show activity in 5–12 test systems. Two important restrictions were not met by the present data. Firstly 6 out of the 17 compounds tested were active against less than 2 of the 7 test parameters. Secondly, of the remaining compounds only 3 were active against the duration component. For these reasons CLO and DUR were not included in the analysis; and all compounds inactive against 4 or more components were disregarded in order to preserve the consistency of the data matrix. The resulting cluster and principal component analysis can be best used to serve as a mathematical confirmation of the similarity between profiles observed in Figs. 1 and 2.

At an amalgamation distance (i.e., Euclidean distance between drugs or amalgamated drugs) of 0.27 valproate sodium, phenobarbital, and aminoxyacetic acid (AOAA) appear as a single cluster. All three of these compounds have a wide activity in animal models of epilepsy. AOAA is known to inhibit γ-aminobutyrate aminotransferase (Gaba-T) and hence markedly elevate brain levels of GABA [4]. However, AOAA also binds to pyridoxal phosphate which results in a dysfunction of B-6 dependent enzymes, and thus of glutamate decarboxylase [24]. AOAA is thought to mainly inhibit GABA-T in vivo [6]; and this accords with the results of the present study where no evidence of a biphasic dose-response curve was obtained.

The mechanism of action of valproate sodium is also generally believed to involve an elevation of brain GABA con-

centrations, brought about through an inhibition of GABA-T [4]. Other authors have proposed an alternate mechanism of action for valproate sodium involving the inhibition of succinic semialdehyde dehydrogenase [26]. Valproate sodium did have activity in the present test system but in most cases the ED_{50} values were high in comparison with other chemically-induced seizure test systems; indicating that its Gabaminergic effects may be subsidiary to its major anticonvulsant action.

A major action of phenobarbital on the GABA system remains to be elucidated. Pentobarbital, but not phenobarbital, prolongs the response of cultured spinal cord neurons to exogenously applied GABA [21]. Similarly pentobarbital, but not phenobarbital inhibits the uptake of GABA by astrocytes [16].

At an amalgamation distance of 0.37 baclofen, a GABA derivative which unlike GABA itself can penetrate the blood brain barrier, and etomidate, a hypnotic compound, appear as a cluster. Baclofen has been shown to enhance ^{3}H-GABA release from rat globus pallidus in vitro [19] and is mainly used in the alleviation of spasticity [5]. Baclofen has activity in the maximal metrazol test in rats [10] where it inhibits tonic-hind paw extension (ED_{50} 11.0 mg/kg IP). Etomidate, like baclofen, has activity in the maximal metrazol test in rats and inhibits tonic-hind paw extension at a dose of 10 mg/kg [10]. A comparison between the antagonism by bicuculline and strychnine of the effects on GABA or etomidate on rat isolated superior cervical ganglia, frog isolated hemisected spinal cords and rat central neurones in vivo indicated that etomidate has either direct agonist action on GABA receptors or acts indirectly via release or potentiation of the effects of endogenous GABA [12]. In vitro etomidate does not compete with ^{3}H-muscimol in a GABA receptor binding assay (J. Leysen, Personal Communication); thus both etomidate and baclofen may increase release of GABA.

Etomidate was introduced as a short acting, non-barbiturate hypnotic to be given intravenously [18]. In 300 g rats hypnosis of duration 20 min is obtained after a rapid IV dose of 5.04 mg/kg [18]. After SC administration of etomidate the duration of hypnosis is longer but higher doses are required for induction (C. Niemegeers, Personal Communication). ED_{50} values of etomidate in the allylglycine test range from 2.99 mg/kg SC for onset at 118 min to $\geq$40.0 mg/kg for absence of clonic seizures at 240 min. It is thus unlikely that the effects of etomidate result solely from its hypnotic effect.

Clonazepam and acetazolamide cluster with the other antiepileptic compounds used in human for generalized convulsive seizures. GABA and gabamimetics increase the affinity of benzodiazepines for their post-synaptic receptors; in addition GABA-antagonists lower the affinity of benzodiazepines for their receptors [28]. Thus reducing GABA levels may lower the anti-convulsant activity of benzodiazepines. This could explain the relatively high doses of clonazepam that were required in order to antagonize the allylglycine convulsions. Alternatively if the anti-convulsant action of benzodiazepines results from modulation of the affinity of GABA receptors for GABA, then their anti-convulsant activity would also be expected to be attenuated in this model. It is presently difficult to explain the acetazolamide effects in terms of GABA because of the paucity of the literature in this respect. Acetazolamide (200 mg/kg orally) did not alter GABA levels [22], however other actions on the GABA system remain to be investigated.

At an amalgamation distance of 0.65 flunarizine and diphenylhydantoin appear as a cluster because of their pronounced effects on CBP/TBP. Flunarizine is a long acting difluoro derivative of cinnarizine [29], whose primary action is a selective antagonism of calcium-induced responses in peripheral vascular smooth muscle. However, flunarizine is also active against tonic-hind paw extension in the maximal metrazol test [10], clonic seizures in the kindled rat [3] and against tonic and clonic kindled seizures in the Beagle dog [30].

The similarity between flunarizine and diphenylhydantoin has been pointed out by De Smedt *et al.* [9] after comparing the profiles of the two compounds in the maximal electroshock in mice and maximal metrazol test in rats. In addition, flunarizine has been shown to be of benefit in therapy—resistant mentally retarded epileptic children [8].

Primidone, trimethadione and pipamperone did not cluster with any other compounds in the present series because of predominant effect on particular seizure or time-related components.

In conclusion, various anti-convulsants antagonized the seizures induced by D,L-allylglycine in rats. The inactivity of trimethadione and ethosuximide against the seizure components suggest that this test may represent a relatively specific method of differentiating between drugs effective against partial or generalized convulsive seizures from those effective against absence seizures. Principal component and cluster analysis of the spectra of the active compounds revealed a similarity between aminoxyacetic acid, valproate sodium and phenobarbital, between etomidate and baclofen; and between flunarizine and diphenylhydantoin.

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