Ital. J. Neurol. Sci. 16:73-77, 1995

The early identification of anticonvulsant activity: role of the maximal electroshock and subcutaneous pentylenetetrazol seizure models

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A number of widely different animal seizure models have been employed in the search for new and novel anticonvulsant drugs useful for the tratment of human epilepsy. At present, no single laboratory test will, in itself, establish the presence or absence of anticonvulsant activity or fully predict the clinical potential of a test substance. Of the many available animal models, the maximal electroshock (MES) and subcutaneous pentylenetetrazol (scPTZ) tests still represent the most commonly employed models for the routine screening and identification of new anticonvulsant drugs. This chapter will briefly describe how these two tests are conducted, their limitations and how they have contributed in the past and to the present day anticonvulsant drug discovery process.

Key Words: maximal electroshock — pentilenetetrazol — anticonvulsant drugs — animal models of epilepsy.

Historical Perspective

The MES test has been employed extensively in the search for new anticonvulsant substances ever since Putnam and Merritt (1937) successfully identified phenytoin in a systematic screening program (see [5] for historical discussion and references). Their discovery of phenytoin and its subsequent success in epileptic patients for whom the barbiturates or bromides were largely ineffective demonstrated that it was possible to discover new clinically effective drugs using an experimental animal model. Subsequently, Everett and Richards [1] demonstrated that pentylenetetrazol-induced threshold seizures could be blocked by trimethadione and phenobarbital but not by phenytoin. Goodman and colleagues [3] later demonstrated that phenytoin and phenobarbital but not trimethadione could modify MES seizures. This observation, coupled with those of Everett and Richards [1] suggested that the two seizures tests (i.e., MES and pentylenetetrazol) could be used selectively to identify drugs effective against maximal versus threshold seizures. A critical link between experimental seizure models and human seizure disorders was provided by Lennox [7] when he demonstrated that trimethadione was effective in decreasing or preventing petit mal attacks in 50 patients and was ineffective or worsened grand mal attacks in 10 patients. Thus, the

correlation between anticonvulsant efficacy in human seizure disorders and maximal and threshold seizures in animal models was established.
For the last 20 years, the Anticonvulsant Drug Development Program of the National Institute of Neurological Disorders and Stroke has routinely employed these two tests in its early identification procedures. As discussed below, this is due in part to their somewhat predictive nature.

The Tests

The MES and scPTZ tests are routinely conducted with either mice or rats. There are advantages and disadvantages associated with both species that are beyond the scope of this chapter and which have been described elsewhere [9, 10, 15, 16].
For the MES test, individual animals receive an electrical stimulus that is delivered through either corneal or pinneal electrodes for 0.2 sec duration and is of sufficient intensity to induce a maximal or tonic extension seizure of the hindlimbs (e.g., 50 mA in mice and 150 mA in rats). Typically, this stimulus is 5 to 10 times higher than the threshold current necessary to evoke a tonic extension seizure. This test measures the ability of a drug to abolish the tonic extensor component of the seizure and is routinely conducted at the predetermined time of peak effect following oral or intraperitoneal administration of a test substance [17].
Pentylenetetrazol administered subcutaneously, is routinely employed to produce a minimal clonic seizure of the vibrissae and/or forelimbs which persists for at least five seconds. Typically, a dose of PTZ sufficient to induce seizure activity in 97% of the animals challenged (CD97) is administered subcutaneously to mice (85 mg/kg) or rats (70 mg/kg) at the time of peak effect of the investigational agent (see [10] and [17] for specific details). Animals are then observed over the next thirty minutes for the presence or absence of clonic seizure activity. Thus, this test measures the ability of a drug to prevent a threshold clonic seizure.

Resultant Seizures and Pharmacological Profiles

The induction of a MES seizure with a supramaximal current delivered via corneal electrodes results in a behavioral seizure in rats that is characterized by tonic extension of both forelimbs and hindlimbs. Tonic extension is followed by brief episodes of clonic activity of the forelimbs and hindlimbs which is followed by a prolonged postictal period lasting several minutes. Stimulation of the brain via corneal electrodes also results in a higly characteristic electrographic seizure which is generalized from the anterior to the posterior surface of the brain. The electroencephalograph displays high frequency, high amplitude spiking activity which correlates with the behavioral seizure.
One disadvantage associated with the use of rats, as opposed to mice, in the MES test is that not all rats will display a full hindlimb tonic extension seizure in response to a supramaximal stimulation. The incidence of nonresponders in Sprague-Dawley derived, albino rats is approximately 15% but can increase markedly with increasing age and seasonal variations [17]. Thus, it is necessary to prescreen all rats the day before a drug trial to identify and eliminate the nonresponders from the experimental group.
Drugs are said to be effective in the MES model when they are found to block the hindlimb tonic extensor component of the seizure. Thus, it is not necessary or even expected that they will block the secondary clonic activity which follows tonic extension.
Drugs can modify the MES seizure pattern by several different mechanisms. For example, they can stabilize the neuronal membrane or decrease the tendency for neurons to discharge repetitively by altering the threshold for focal seizure discharge or they can decrease the spread of a seizure discharge from the focus [20]. Because the stimulus employed in the MES test is several times greater (supramaximal) than that required to produce a tonic extension seizure, a drug which acts primarily by raising seizure threshold is not likely to prevent tonic extension in this model. Thus, the MES test is primarily thought to detect drugs which act by preventing seizure spread. Numerous technical, biological, and pharmacokinetic factors have been identified which can "*qualitatively*" affect the results obtained in a drug-testing trial and these have been described elsewhere [9] and [17]. However, it is noteworthy that while these factors are not likely to contribute to "missing" a MES-active drug, they may certainly contribute to erroneous conclusions regarding potency and duration of action of an active drug.
The behavioral seizure which results from the subcutaneous administration of PTZ is markedly different from that of a MES seizure. Depending on the dose administered, PTZ can produce myoclonic jerks, repeated clonic seizures of the vibrissae, forelimbs and hindlimbs without loss of righting reflex, clonic seizures of the limbs with loss of righting reflex, and loss of righting reflex followed by tonic extension of the forelimbs and hindlimbs [10]. This is particularly important be-

cause these different endpoints have been shown to be associated with different pharmacological profiles [10, 13]. For example, phenytoin and ethosuximide, two drugs with markedly different clinical profiles, are both effective against tonic extension seizures induced by scPTZ [13]. Ethosuximide is effective because it raises the threshold for a tonic extension seizure and phenytoin because it prevents seizure spread. Drugs screened by the Anticonvulsant Drug Development program, are said to be effective in the scPTZ test if they block only the clonic seizure which typically follows myoclonic jerks. This is an important point because, by choosing an inappropriate endopoint, a rather discriminating threshold model can be converted into a nondiscriminating seizure test [13].

The EEG of a PTZ treated animal is characterized by 6-7 Hz spike and wave discharges which correlate with the behavioral seizure. With minor exceptions, drugs which are effective against generalized absence seizures in humans are also effective against scPTZ seizures in animals. Thus, ethosuximide, trimethadione, and valproate are effective in this model; whereas phenytoin and carbamazepine are ineffective against pure clonic seizures [13] and [17].

Discussion

When conducted properly the MES and scPTZ tests are probably the best validated of all the experimental models available for evaluating anticonvulsant drugs and provide the most useful information concerning the potential clinical utility of an investigational agent. Unlike animal seizure models developed in later years, the MES and PTZ tests have both been validated in human studies. In these studies, the EEG and resultant behavioral seizure following electroconvulsive shock therapy and PTZ administration were demonstrated to be remarkably similar to that recorded in animals (see [14], [18] and [19] for discussion). Likewise, phenytoin was shown to block selectively electroconvulsive shock-induced tonic extension [18] but not PTZ-induced seizures [2]. In a similar manner, drugs like trimethadione were found ineffective against electroconvulsive shock-induced tonic extension [18]. This early demonstration in man led to the ultimate inclusion of these two tests into the screening protocol of the Anticonvulsant Drug Development Program [6].

Results obtained over the last five decades of anticonvulsant drug testing have demonstrated that, with minor exceptions, substances which obtund the tonic extension component of MES seizures (e.g., phenytoin) have been found to be clinically useful for the management of generalized tonic-clonic seizures; whereas substances which prevent clonic seizures induced by pentylenetetrazol (e.g., ethosuximide) have been found to be useful for the treatment of generalized absence seizures. Likewise, compounds which are effective against both seizure types (e.g., valproate) have been found to be effective against both human seizure disorders.

The majority of currently available anticonvulsant drugs are thought to exert their anticonvulsant effect by: (1) delaying recovery of sodium channel activation; (2) reducing low-threshold (T-type) calcium currents; or (3) enhancing $GABA_A$ receptor-mediated inhibition [11]. In general, most of the prototype anticonvulsant drugs with demonstrated clinically efficacy against generalized tonic-clonic seizures (phenytoin, carbamazepine, and valproate) have been found to reduce sustained high-frequency repetitive firing of action potentials through an effect on sodium channels. Drugs that are effective clinically against generalized absence seizures (ethosuximide, trimethadione and valproate) have been shown to reduce low-threshold calcium currents of the T-type. Drugs most effective against myoclonic seizures (barbiturates and benzodiazepines) enhance $GABA_A$-mediated inhibition (for further discussion, see [11]. From this discussion, one might reasonably expect "pure" MES-active compounds to reduce sodium channel activity and "pure" scPTZ-active compounds to reduce T-type calcium channel currents. Furthermore, those drugs with a broad anticonvulsant profile in animal models might be expected to possess multiple mechanisms of action.

Based on our current understanding, one would not expect a drug that is thought to reduce T-type calcium currents to block MES-induced seizures nor would one expect a sodium channel blocker to be active against scPTZ seizures. Beyond this, little can be said about the molecular mechanism of action of drugs which prevent seizures induced by MES or pentylenetetrazol. For this reason, the MES and scPTZ tests are generally thought to be useful for identifying compounds which act by limiting seizure spread and raising seizure threshold, respectively [17].

Clearly, an ideal model of epilepsy would be able to predict the clinical utility of a newly identified anticonvulsant drug. However, none of the present models can predict with absolute certainty whether a drug will be successful in human studies. Only after a drug has demonstrated efficacy in the clinic will one begin to appreciate its overall anticonvulsant potential. This is not to say that animal models are unimportant to the discovery process. However, it does emphasize the wisdom of demonstrating that the models employed

TABLE I. *Anticonvulsant profile and proposed mechanism of action of newly released and investigational anticonvulsants.*

	Experimental Model		
	MES	scPTZ	Mechanism of Action
CPPene	+	−	NMDA antagonist
Felbamate	+	+	↓ SRF[a]; modulate SI-Glycine receptor[b]
Gabapentin	+	−	↓ L-amino acid transport
Lamotrigine	+	−	↓ Na ↓ EAA[c] release
Loreclezole	+	+	↑ GABA?
Losigamone	+	+	↑ GABA
Vigabatrin	+	+/−	↑ GABA by blocking metabolism
Remacemide	+	−	NMDA antagonist?
Topiramate	+	−	↓ SRF; ↑ GABA
Tiagabine	−	+	↑ GABA by ↓ uptake
Zonisamide	+	−	? Na^+

(+) active; (−) inactive
[a] SRF - sustained repetitive firing
[b] SI - strychnine insensitive
[c] EAA - excitatory amino acid

in an anticonvulsant discovery program are not missing mechanistically novel anticonvulsant drugs. A number of investigational anticonvulsants currently in clinical development appear to possess novel mechanisms of action (Table 1) and some have shown significant promise in epileptic patients. Moreover, all of these agents have been found to possess activity against MES-and/or scPTZ induced seizures. Albeit, the GABA transaminase inhibitor gamma vinyl GABA was originally found to be inactive against both MES and scPTZ. However, it was found to be active in both tests when appropriate consideration was given to its proposed mechanism of action. For example, the time of peak effect of gamma vinyl GABA in most models is between 24 and 48 hours. This long time to peak effect reflects the slow yet irreversible inhibition of GABA transaminase and subsequent increase in brain GABA concentrations.

It is important to note that there are a number of factors unrelated to the inherent mechanism of action of a drug that can contribute to whether it will be effective in a particular seizure model. Besides time of peak effect, other factors might include inadequate absorption following oral administration, inability to cross the blood-brain-barrier, and species-dependent differences in drug metabolism (for further discussion, see [8]. These and other factors can all contribute to "missing" an active drug in a primary screen and every attempt should be made to control for their influence.

In summary, the MES and scPTZ tests have survived the test of time. The resultant seizures from maximal electroshock stimulation and subcutaneously administered PTZ are higly reproducible and have well-defined endpoints. The MES test possesses a pharmacological profile in laboratory animal and human studies which is consistent with human generalized tonic-clonic seizures, while the scPTZ test correlates well with generalized absence seizures.

When appropriate consideration is given to certain pharmacokinetic parameters, all of the investigational anticonvulsants under development have been found active in one or both of these models. Thus, it is possible to identify mechanistically novel anticonvulsants with these two tests. Because of this, the MES and scPTZ tests represent two highly appropriate first screens for any anticonvulsant discovery program. Such an approach would not be expected to limit further studies in other seizure models.

Sommario

Numerosi modelli animali di crisi epilettiche sono stati impiegati per ricercare nuovi farmaci antiepilettici utili per il trattamento delle epilessie umane. Attualmente nessuno dei tests di laboratorio è in grado, da solo, di stabilire il valore entiepilettico di una determinata sostanza o di predirne l'utilità clinica. Tra i molti modelli animali disponibili quelli più comunemente impiegati per selezionare nuovi farmaci antiepilettici sono il test di risposta all'elettroshock massimale (MES) e alla somministrazione

subcutanea di pentilenetetrazolo (scPTZ). Il presente capitolo passa in rassegna la metodologia dei due tests, le loro limitazioni e il contributo che essi hanno fornito in passato e tutt'ora forniscono alla scoperta di nuovi farmaci antiepilettici.

References

[1] EVERETT G.M., RICHARDS R.K.: *Comparative anticonvulsive action of 3,5,5-trimethyloxazolidine-2,4-dione (Tridione), Dilantin and phenobarbital.* J. Pharmacol. Exp. Ther., 81: 402-407, 1944.

[2] GOLDSTEIN H.H., WEINBERG J.: *Experimental evidence of anticonvulsant properties of sodium diphenyl hydantoinate (Dilantin Sodium N-N.R.).* Arch. Neurol. Psychiat., 43: 453-455, 1940.

[3] GOODMAN L.S., SWINYARD E.A., TOMAN J.E.P.: *Laboratory technics for the identification and evaluation of potentially antiepileptic drugs.* Proc. Am. Fed. Clin. Res. 2: 100-101, 1945.

[4] KAUFMAN I.C., MARSHALL C., WALTER A.E.: *Metrazol activated electroencephalography.* Res. Publ. Ass. Nerv. Ment. Dis. 21: 476-486, 1947.

[5] KRALL R.L., PENRY J.K., KUPFERBERG H.J., SWINYARD E.A.: *Antiepileptic drug development: I. History and a program for progress.* Epilepsia 19: 393-408, 1978.

[6] KRALL R.L., PENRY J.K., WHITE B.G., KUPFERBERG H.J., SWINYARD E.A.: *Antiepileptic drug development: II. Anticonvulsant drug screening.* Epilepsia 19: 409-428, 1978.

[7] LENNOX W.G.: *The petit mal epilepsies. Their treatment with Tridione.* J.A.M.A. 129: 1069-1074, 1945.

[8] LOSCHER W., SCHMIDT D.: *Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations.* Epilepsy Res. 2: 145-181, 1988.

[9] LOSCHER W., FASSBENDER C.P., NOLTING B.: *The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models.* Epilepsy Res. 8: 79-94, 1991.

[10] LOSCHER W., HONACK D., FASSBENDER C.P., NOLTING B.: *The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazol seizure models.* Epilepsy Res. 8: 171-189, 1991.

[11] MACDONALD R.L., KELLY K.M.: *Mechanisms of action of currently prescribed and newly developed antiepileptic drugs.* Epilepsia 35 (S4): S41-S50, 1994.

[12] ORLOF M.J., WILLIAMS H.L., PFEIFFER C.C.: *Times intravenous infusion of Metraxol and strychnine for testing anticonvulsant drugs.* Proc. Soc. Exp. Biol. Med. 70: 254-257, 1949.

[13] PIREDDA S.G., WOODHEAD J.H., SWINYARD E.A.: *Effect of stimulus intensity on the profile of anticonvulsant activity of phenytoin, ethosuximide and valproate.* J. Pharmacol. Exp. Ther 232: 741-745, 1985.

[14] PUTNAM T.J., MERRITT H.H.: *Experimental determination of the anticonvulsant properties of some phenyl derivatives.* Science 85: 525-526, 1937.

[15] SWINYARD E.A.: *Laboratory evaluation of antiepileptic drugs.* Epilepsia, 10:107-119, 1969.

[16] SWINYARD E.A., BROWN W.C., GOODMAN L.S.: *Comparative assays of antiepileptic drugs in mice and rats. 1952.* J. Pharmacol. Exp. Ther. 106: 319-330, 1952.

[17] SWINYARD E.A., WOODHEAD J.H., WHITE H.S., FRANKLIN M.R.: *General principles. Experimental selection, quantification, and evaluation of anticonvulsants*, in: Levy, R. Mattson, R. Meldrum, B. Penry, J.K. and Dreifuss F.E. (eds.), Antiepileptic Drugs, Third Edition, Raven Press, Ltd., New York, pp. 85-102, 1989.

[18] TOMAN J.E.P., LOEWE S., GOODMAN L.S.: *Physiology and therapy of convulsive disorders. I. Effect of anticonvulsant drugs on electroshock seizures in man.* Arch. of Neurol. Psychiat. 58: 312-324, 1947.

[19] WOODBURY D.M.: *Applications to drug evaluations.* In Purpura D.P., Penry J.K., Tower D., Woodbury D.M. and Walter R. (eds). Experimental Models of Epilepsy-A Manual for the Laboratory Worker, Raven Press, Ltd., New York, pp. 557-583, 1972.

[20] WOODBURY D.M., ESPLIN D.W.: *Neuropharmacology and neurochemistry of anticonvulsant drugs.* Proceedings of the Assoc. for Research in Nervous Mental Disease 37: 24-56, 1959.

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