

THE MAXIMAL ELECTROSHOCK SEIZURE (MES) MODEL IN THE PRECLINICAL ASSESSMENT OF POTENTIAL NEW ANTIEPILEPTIC DRUGS

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SUMMARY

The choice of appropriate animal models for the initial in vivo testing of potential anticonvulsant compounds is one of the most important steps in the successful search for new antiepileptic drugs. The purpose of this paper is to describe the most important aspects to take into account when performing the maximal electroshock seizure (MES) test in the routine laboratory screening of new antiepileptics: the conventional and threshold MES test experimental procedures, the factors affecting experimental data (laboratory conditions, administration vehicles and drug formulations, time after drug administration, and stimulus duration and site of stimulation) and the assessment of anticonvulsant activity are discussed.

Key words: Antiepileptic drugs - In vivo testing - Acute seizure model - Electroshock

INTRODUCTION

In spite of the currently available therapeutic arsenal of old and new antiepileptic drugs (AEDs), almost one-third of epileptic patients continue to present seizures that appear to be refractory to all pharmacological schemes (1, 2). Subsequently, there is a substantial need to develop new and more effective AEDs, and numerous compounds are now in preclinical and clinical trials (2, 3).

The choice of appropriate animal models for the initial in vivo testing of a potential anticonvulsant drug is one of the most important steps in the successful search for new AEDs. At present, preclinical animal studies are indispensable in exploring the efficacy and safety of an investigational AED before its introduction in human volunteers (4-7).

Although modern cellular neurophysiological and biochemical approaches have made it possible to identify molecular targets of AEDs, in vitro testing is not likely to replace screening in animal models: on the one hand, in vitro systems cannot model the specific pharmacodynamic actions required for seizure protection since they do not assess the multidimensional parameter space, which includes not only the target molecules but also critical biomolecules that could cause side effects or interfere with the desired activity; on the other hand, in vitro testing does not assess bioavailability, brain accessibility and local delivery to the target. Therefore, only animal test systems can select compounds that are inherently anticonvulsant and are able to access the relevant brain targets (8).

If the purpose of the research is not to study the epileptic phenomenon itself but to screen new AEDs, the animal models fall into two

main categories: models of acute seizures (nonepileptic animals induced to have a seizure by an electrical or chemical stimulus) and models of chronic epilepsy (animals induced to have enhanced seizure susceptibility or spontaneous seizures) (8). For practical reasons, experimental research on new AEDs has mostly been carried out on normal mice and rats in which seizures were induced by chemical or electrical means (6, 7). Indeed, with respect to screening purposes, electrically or chemically induced seizures have advantages over most genetic models since seizure-susceptible animal species may lead to an exaggeration of the anticonvulsant potency of a new drug (9). Over the years, the maximal electroshock seizure (MES) model has remained one of the gold standards in early stages of testing (8).

In our opinion, despite the continuous search for new models closer to the human epilepsy phenomenon, the MES model will persist as the most useful tool at least at the anticonvulsant compound identification stage. Hence, we intend to describe in a single paper all the useful yet dispersed information on performing anticonvulsant screening by MES tests, drawing particular attention to experimental procedures and factors affecting the accuracy of experimental data.

THE MES TEST

The MES test, developed by Toman and collaborators more than 60 years ago (10), is probably the best-validated preclinical test that predicts drugs effective against generalized seizures of the tonic-clonic (grand mal) type (5, 9, 11-13). It permits evaluation of the

ability of a substance to prevent seizure spread through neural tissue (12, 14-16).

In the MES test, mice or rats receive an electrical stimulus of sufficient intensity to induce maximal seizures of their hind limbs, with tonic extension as the endpoint of the test (e.g., 14, 17-24).

The test is easily conducted, requires a minimal investment in equipment and technical expertise, and is well standardized. Additionally, the epileptic activity is no longer contaminated by the epileptogenic agent since it occurs only during application of the current. Unfortunately, the animals can be used only once (11). Several standard and newly developed AEDs are effective in the MES test, thus making it possible to quantify their anticonvulsant potency after both single and combined application (5, 24-26). The high correlation demonstrated between the ability of a drug to inhibit MES in rodents and its effectiveness in generalized tonic-clonic seizures in humans, in addition to the ease with which anticonvulsant activity can be detected in rodents, are probably the principal reasons for the popularity of the MES test (27).

Although it has been stated that the MES test restricts the testing of drugs acting on Na⁺ channels (e.g., carbamazepine, phenytoin) (28, 29), the majority of standard and newly developed anticonvulsant drugs are effective in the MES model, despite the fact that these drugs interact with other drug targets. In fact, the use of the MES model, not in its standard fashion with fixed supramaximal current application, but as a threshold test with determination of the individual seizure threshold for tonic seizures, extends its susceptibility in anticonvulsant drug testing, including drug categories such as γ -aminobutyric acid (GABA)-enhancing drugs (e.g., phenobarbital) or glutamate receptor antagonists (e.g., topiramate) (4, 8, 13, 16, 25, 26).

Conventional MES test experimental procedures

In the traditional MES test, rodents receive an electrical stimulus of sufficient intensity to induce maximal seizures of their hind limbs (a stimulus about 5-10 times higher than the individual electrical seizure threshold of the animals, in order to avoid bias in the induction of tonic seizures due to daily fluctuations in seizure threshold) (14-16).

MES stimulation can be applied through transcorneal or transauricular (ear-clip) electrodes from an electroshock apparatus at an intensity sufficient to elicit tonic hind limb extension (HLE) in 100% of the control animals. A seizure is generally considered to be *maximal* if increments in current intensity do not alter the pattern or the duration of its various components (30). The conventional MES test has standardized parameters such as a 50-mA (mice) or 150-mA (rats) fixed current, a 50-60-Hz pulse frequency, a 0.6-ms pulse width and a 0.2-s stimulus duration (9, 11, 16, 27, 31, 32). Corneal electrodes are mainly used. During stimulus application, the animal should be restrained only by hand and released at the moment of stimulation to permit observation of the seizure throughout its entire course (11, 33). If bipolar corneal electrodes are used, a drop of an electrolyte/local anesthetic should be applied into the eyes before placement of the electrodes (not only to ensure adequate electrode contact and anesthesia, but, in mice, also to reduce the incidence of

fatalities from maximal electroshock seizures almost to zero) (11, 15, 18, 31, 34) (Fig. 1A).

Briefly, following stimulus application (Fig. 1B) an immediate severe tonic seizure with maximal extension of the anterior and posterior legs occurs and the body becomes stiffened (Fig. 1C); at the end of this tonic phase, which usually lasts for 10-15 s, clonic seizures start, characterized by paddling movements of the hind limbs and shaking of the body; 20-30 s later, the animal is usually able to come back to an upright position and start moving around, apparently recovering its normal behavior (Fig. 1D) (35).

The test will be considered positive if the animal exhibits tonic extensor seizure with rearward HLE more than 90° from the body and sustained for more than 3 s following 10 s after stimulation (20, 27, 36). The tonic HLE finishes at the time of onset of generalized clonus (30). Only animals that consistently exhibit the tonic HLE component of MES in three trials on separate days, while unmedicated, should be used (the animals become their own controls) (18, 27, 34).

Threshold MES (MEST) test experimental procedures

The principal disadvantage of the MES test with supramaximal stimulation is that it does not detect the anticonvulsant drugs that increase the seizure threshold but are not potent enough to raise the threshold above 50 mA (mice) or 150 mA (rats), although such drugs (e.g., ethosuximide) could be of clinical value (14, 15). In order to minimize this, a parallel or alternative way to perform the MES test should be considered: the threshold for maximal electroshock seizures (MEST), in which doses of the drugs are fixed but current intensity is adjusted appropriately. In the MEST test it is possible to determine the effect of the drug on the seizure threshold of an individual animal (or group of animals) without ignoring individual differences of animals in terms of seizure susceptibility. It makes the maximal electroshock much more sensitive and extends its susceptibility in anticonvulsant drug testing, including drug categories such as GABA-enhancing drugs, which are inactive or only weakly active against the standard MES test with supramaximal stimulation (9, 16, 25, 37).

MEST in rodents can also be determined via corneal or ear electrodes by means of a stimulator which delivers either constant current or constant voltage at a frequency of 50-60/s for 0.2 s (9, 14, 33). In this test it is very important to ensure the application of the present current, so a powerful apparatus with selfadjusting stimulus voltage, according to the impedance of the test object (usually the external resistance of the animal is about 5 k), should be used (the serial resistance of the stimulator should be switched to 10 k for both mice and rats) (9). Each group of animals should be used for only one threshold determination (16). In contrast with the traditional MES test, in which no daily control groups are necessary for experiments, in the MEST control groups receiving vehicle should be tested together with drug-treated groups on each experimental day, since the control threshold in rodents varies as a result of age and daily (circadian) or hormonally induced rhythms (9, 16, 33).

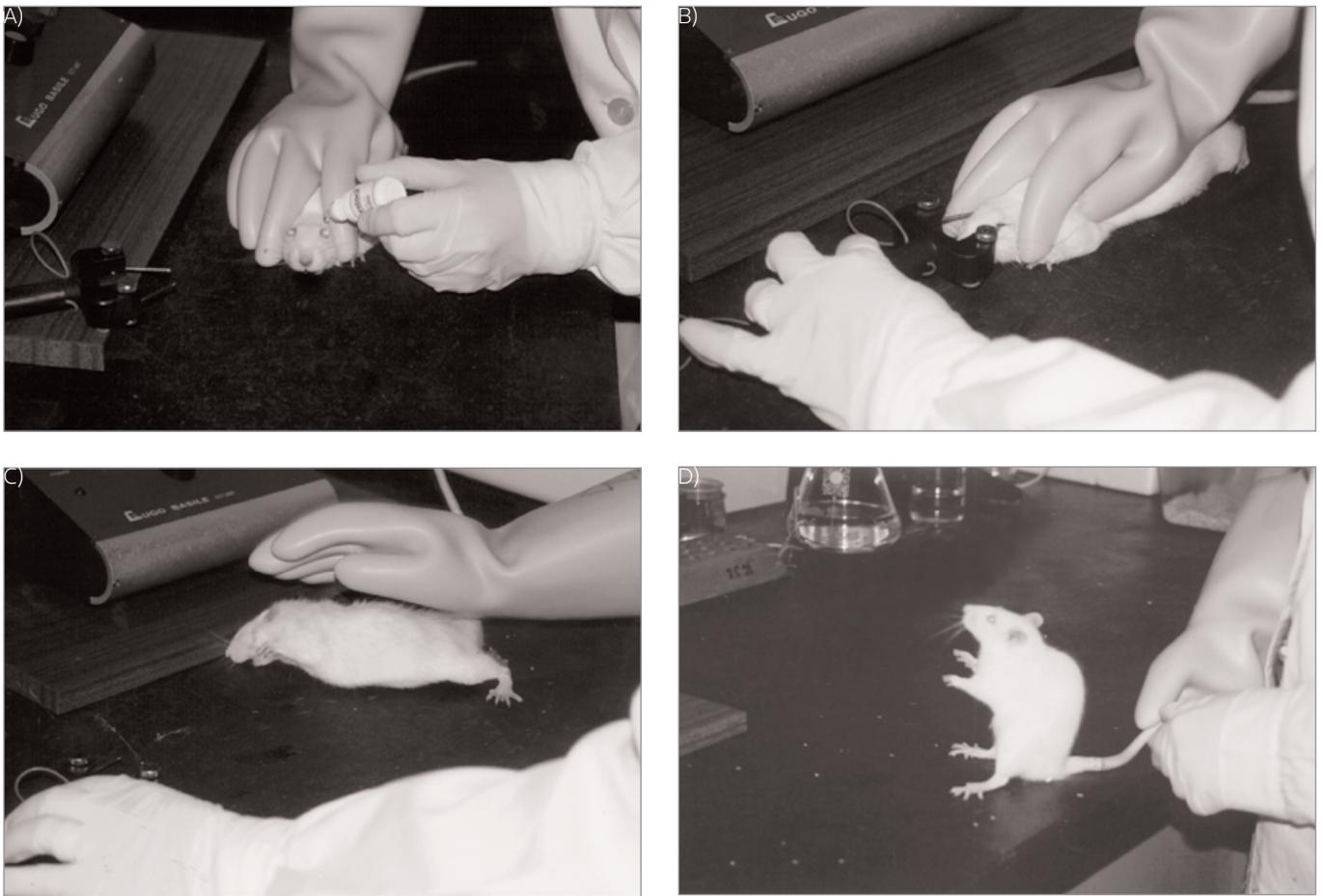


Figure 1. A. Local anesthetic application. B. Corneal stimulus application: 50-mA (mice) or 150-mA (rats) fixed current; 50-60-Hz pulse frequency; 0.6-ms pulse width; 0.2-s stimulus duration. C. Tonic phase. D. Clonic phase.

FACTORS AFFECTING EXPERIMENTAL DATA OBTAINED IN ELECTROSHOCK MODELS

The fact that different groups of researchers report different experimental data on the same compounds at comparable doses in the same model and species strongly suggests that there are technical, biological and/or pharmacological factors other than species, strain, sex or age which affect experimental data obtained by apparently standardized procedures (16). In this paper, the following factors affecting experimental data are discussed: laboratory conditions, administration vehicles and drug formulations, time after drug administration, and stimulus duration and site of stimulation.

Laboratory conditions

As usual, the animals used in anticonvulsant testing must be maintained under standard laboratory conditions, which include a temperature of 20-24 °C, relative humidity of about 50-60% and a 12-h light cycle beginning early in the morning (38). These conditions are very relevant. For instance, sudden changes in the ambient temperature may cause temporary changes in the seizure threshold in the animal (an increase in the temperature can elevate the seizure threshold) (31). The diet used should also be standardized.

Furthermore, the animals should be allowed free access to food and water except during the short time they are removed from their cages for testing. Previous work has shown that starvation increases the severity of MES, prolonging the tonic extensor component, and significantly reduces the seizure threshold (15, 34).

Administration vehicles and drug formulations

One of the initial problems in laboratory drug testing is the choice of an adequate vehicle and formulation, since administration vehicles and drug formulations could have consequences for drug bioavailability and drug pharmacodynamic response. Although clinically efficacious anticonvulsant drugs are usually administered orally, preclinical testing should be started with parenteral, usually intraperitoneal, administration, to ensure that the gastrointestinal tract is not interfering with the results. Only in the case of activity by this route should the efficacy of the compound after oral administration be studied (9). Bearing in mind that AEDs have to cross the blood-brain barrier to exercise their therapeutic effect in the central nervous system, most of them are lipophilic (39); consequently, water insolubility becomes a common problem in the laboratory evaluation of these drugs. To resolve this problem, it often becomes

necessary to resort to a suspension or a lipophilic vehicle to inject the drug into the laboratory animal. However, if this choice is incorrect, low or retarded absorption may occur or additional pharmacodynamic effects may appear, possibly leading to misinterpretation of study results. For this reason, the choice of an adequate vehicle and formulation becomes a critical factor in the development of this kind of laboratory testing (16, 40). Certain lipophilic vehicles (such as 30% glycofurol) potentiate the anticonvulsant action of drugs (e.g., primidone) in the MES test (by a seizure threshold-increasing action of this solvent), in spite of the administration of this vehicle alone not exerting measurable effects in this test. Nevertheless, 30% polyethylene glycol 400 does not increase seizure threshold or drug potencies, similar to lower concentrations of glycofurol (10%). Therefore, they can be used for preparation of drug solutions in case of poor aqueous solubility of drugs. Finally, when water-insoluble drugs are administered as aqueous suspensions, their anticonvulsant effects can be considerably lower when compared to injections of the same doses as solutions, which could be related to lower drug absorption in the case of intraperitoneal administration of drug suspensions (16, 41).

Time after drug administration

Another issue that should be discussed as early as possible in pre-clinical testing to avoid false conclusions is the time required after administration for peak activity to be exhibited. In fact, it is the time of peak anticonvulsant effect that should be used for quantification of the anticonvulsant potency. This procedure is especially important in the case of drugs with short half-lives, because late determination of activity would lead to underestimation of true anticonvulsant potency. Some care should also be given to the dose administered, since after higher doses more time might be needed to reach maximum concentrations in blood and brain. Finally, the time of peak effect determined by the MES test should be assumed for future anticonvulsant testing (9, 16, 34).

Stimulus duration and site of stimulation

In relation to technical factors, it seems relevant to consider stimulus duration and site of stimulation as determining factors. In relation to the duration of the stimulus, an inverse relationship can be observed between the stimulus duration and the threshold for tonic seizures, irrespective of the site of stimulation: stimuli of longer duration are more effective in activating paroxysmal discharge than are stimuli of shorter duration (27). Zablocka and Esplin (42) also demonstrated that increasing the stimulus duration in the MES test from 0.2 to 0.5 s could cause HLE in rats that are normally resistant to this response, whereas increasing the current intensity from 150 mA to 500 mA was largely ineffective in this regard. However, as referred to previously, 0.2 s is accepted as the usual duration of the stimulus.

The site of stimulation is also a determining factor in drug evaluation by electroshock seizures. Seizures produced with transcorneal electroshock in rats differ from those produced by transauricular electroshock in several important ways. This results from the fact that different electrode placement induces preferential activation of different anatomical substrates within the brain due to higher current densities reaching these structures (corneal stimulation produces a

preferential activation of the forebrain structures, while stimulation through ear-clip electrodes activates the brain stem). As the components of tonic seizures have been shown to depend on the brain stem, transauricular stimulation is more effective at eliciting tonic convulsions, which are more severe and present lower and less variable seizure thresholds. Consequently, MES elicited with ear stimulation would be more difficult to inhibit with anticonvulsant drugs than MES elicited with transcorneal electrodes. In other words, drugs preventing the spread of a seizure are possibly more potent against transcorneal-induced seizures – a point that should be kept in mind when comparing results from different laboratories (16, 27, 35).

EVALUATION OF ANTICONVULSANT ACTIVITY

Conventional MES test design

To evaluate the anticonvulsant potency of a certain drug in the traditional MES test at least three groups of animals, consisting of 8-10 animals per group, should be administered increasing doses of the drug. Then, at a predetermined time following drug administration each animal should be challenged with MES. The anticonvulsant activity of the drug is determined as a quantal endpoint, i.e., the presence or absence of HLE. The number of animals per group protected against MES is converted to a percentage, and a dose-response curve can be constructed. By the method described by Litchfield and Wilcoxon (43), the protective efficacy of the drug is evaluated as ED_{50} (with 95% confidence limits), defined as the dose (in mg/kg) required to protect 50% of the animals challenged with MES against the endpoint (9, 16, 18, 20, 37, 44).

MEST test design

The threshold is usually determined by an "up-and-down method". The principle of this method is that the stimulus intensity or voltage for each animal is determined by the response of the animal just tested: the current is lowered or raised by 0.06-log intervals (mice; 0.6 log rats) if the preceding animal did or did not exert HLE, respectively. Using the method originally described by Kimball et al. (45), the data thus generated in a single group of 15-20 animals is used to calculate the threshold current inducing HLE in 50% of the animals (convulsant current [CC_{50}] or convulsant voltage [CV_{50}] with confidence limits for 95% probability) (9, 16, 33, 37). For comparison of drug effects, the dose which elevates the threshold by 20% is calculated by plotting the doses of the respective drug against the percentage threshold increase on a semilogarithmic scale (9, 16).

DISCUSSION AND CONCLUSIONS

No single animal model mimics exactly the complex human epileptic phenomenon (46, 47). Although acute seizure models have proven useful for the identification of drugs with anticonvulsant activity, they are not closely related to human epilepsy, but represent models for induction of single epileptic seizures (models of seizure states) rather than models of epilepsy. Nonetheless, the obvious differences between the known features of the human condition and those of acute models (i.e., the general lack of previous pathology, the presence of a well-defined focus, and the absence of lasting

anatomic and physiological alterations) all have to be considered before wide-ranging conclusions are drawn from the use of acute models alone (9, 32). In fact, there is no guarantee that drugs effective in "neurologically normal" rodents will be equally effective in "neurologically abnormal" rodents (5, 7).

The predictive value of acute routine screening tests, including the MES test, has been questioned recently within the scientific community (48). Is the MES test, together with the pentylenetetrazol test (chemical induction), still one of the gold standards in the early stages of testing? Indeed, the MES test can fail to identify drugs that are clinically effective in treating partial seizures (e.g., vigabatrin, tiagabine and levetiracetam) (4), it does not distinguish between efficacy in the treatment of primarily and secondarily generalized tonic-clonic seizures, and it is not a good model for the identification of drugs for pharmacoresistant epilepsies (6). Are chronic epilepsy models, in which animals exhibit long-term enhanced seizure susceptibility and spontaneous seizures, preferred to acute models, in which normal animals are induced to have seizures? Some authors agree that chronic models can potentially identify AEDs that are not detected by the acute models (26). Indeed, of the multitude of tests that might be conducted, the chronic model of kindling is currently employed by most AED discovery programs (7).

In our opinion, taking into consideration that at present there are no validated models of refractory epilepsy (49), that chronic epilepsy models are technically difficult and not suited to routine screening, and that the mentioned acute models do not require extraordinary experimental logistics, are not time-consuming, are well validated with several AEDs, show good reproducibility between laboratories and are responsible for the initial identification of all the currently approved AEDs, besides having diverse and often distinctive clinical activities (7, 8, 26, 50), we consider that acute animal screening models are still essential tools in initial AED discovery. Furthermore, these models provide some insight into the central nervous system bioavailability of a particular investigational AED, they are nonselective with respect to mechanism of action, display clear and definable seizure endpoints and require minimal technical expertise. The lack of dependence on molecular mechanism also makes them ideally suited for screening large numbers of chemically diverse entities. In fact, they represent an ideal screening tool for the routine testing of potential AEDs (5, 7).

Preclinical assessment of potential AEDs will still be carried out by resorting to acute animal models, such as the MES model revisited here. This paper has described the most important aspects to take into account when performing the MES test in the routine laboratory screening of AEDs. Nevertheless, some attention should be paid to the results: they should be used as evidence supporting central nervous system activity against generalized seizures, but lack of activity should not be considered definitive evidence that a new AED is without clinical antiepileptic activity (5). The efficacy of AEDs in animal models of seizures is only partially predictive of their clinical profile (20, 50). In fact, there are now several examples wherein the pharmacology of AEDs can be affected by the disease state and, because these acute animal models are conducted in pathologically normal rodents, there is no guarantee that identified AEDs will be equally effective in pathologically abnormal rodents (7). It should be noted that the most important feature of acute preclinical tests in

the initial AED screening is the opportunity to determine which compounds should be developed, rather than predicting efficacy in the treatment of human epilepsy. In effect, the definitive test for evidence of anticonvulsant activity always requires the use of patients to validate the conclusions arising from animal models (50, 51).

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