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Protection From Shock-Induced Seizures as a Measure of Hypnotic Potency of Drugs

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HALPERIN, J. M. AND L. C. IORIO. *Protection from shock-induced seizures as a measure of hypnotic potency of drugs.* PHARMAC. BIOCHEM. BEHAV. 13(2) 299–301, 1980.—Mice injected with drug or vehicle were administered a 13 mA shock via corneal electrodes. The number of mice in which the shock produced a tonic seizure was recorded. The dose that blocked seizures in 50% of mice (ED_{50}) was determined for each drug. The drugs evaluated consisted of 15 hypnotics, two antidepressants and two antihistamines. All hypnotics and antidepressants, and one antihistamine, caused a dose-dependent suppression of seizures. The ED_{50}'s were highly correlated with hypnotic potency in man. The advantages of this procedure, as compared to others currently used for the evaluation of hypnotic potential of novel compounds, are discussed.

Electroconvulsive shock Tonic seizures Hypnotics Antidepressants Antihistamines Mice

THE evaluation of hypnotic effects of drugs in infrahuman species is best performed using polygraphic recordings which directly assess the subject's sleep. This procedure, however, is both costly and time-consuming, making it impractical for the initial screening of novel compounds. Therefore, many researchers utilize a variety of simple tests which measure different aspects of behavioral depression prior to evaluation by polygraphic techniques. These tests include behavioral measures such as loss of righting reflex (LRR) [16], reduction of motor activity [4,10], potentiation of subhypnotic doses of other CNS depressants [6, 14, 15, 21], and reinduction of LRR in mice recovering from hexobarbital [7]. Other researchers have combined several of these tests along with subjective evaluation of drug-induced behavior to provide a pharmacological profile of compounds which might indicate hypnotic activity [9,12].

All of these procedures have the advantage over polygraphic evaluation of rapid evaluation and low cost. These advantages, however, are at the expense of predictive validity to potency in man (for review, see [7, 15, 16]). Besides their lack of usefulness as predictors of human clinical dose, many of these tests work only with certain classifications of hypnotics; benzodiazepines [16] and thalidomide [10], for example, do not appear active in LRR. Furthermore, thalidomide does not appear active in the hexobarbital reinduction test [7].

Change in seizure thresholds in rodents following electroconvulsive shock (ECS) has been employed to study anticonvulsant [18], as well as other neuropharmacologic properties of drugs [2]. This method, however, has not been systematically evaluated over a large range of sleep inducing drugs. We found that this technique can be used to yield data which is highly predictive of hypnotic potency in man in a variety of classes of hypnotic drug.

METHOD

Subjects

The subjects were CF-1 male albino mice (Charles River, Boston, MA) ranging in weight from 21–26 g. Prior to the onset of the experiment, they were housed in groups of 12 mice under 12/12 light/dark lighting conditions. Food and water were available ad lib.

Drugs Evaluated

Three classifications of drugs were evaluated: hypnotics, antidepressants, and antihistamines. Fifteen hypnotics were tested from a wide variety of dissimilar compounds. The hypnotics tested were four benzodiazepines (flurazepam, nitrazepam, triazolam, and quazepam), four barbiturates (phenobarbital, secobarbital, pentobarbital, and amobarbital), three glutarimide derivaties (glutethimide, methyprylon, and thalidomide), and four others (methaqualone, ethinamate, chloral hydrate and triclofos).

Two tricyclic antidepressants and two antihistamines were also evaluated. The antidepressants were amitriptyline, which has been shown to have strong sedative side effects [8,19], and imipramine, which is less sedating [8,11]. The antihistamines evaluated were diphenhydramine and chlorpheniramine; of these, diphenhydramine is more sedating [3].

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TABLE 1

EFFECT OF HYPNOTIC DRUGS ON ECS-INDUCED SEIZURES IN MICE AND THE RELATIONSHIP TO DOSES IN HUMANS

Drug	ED_{50} (95% C.L.)*, mg/kg, p.o.	Human hypnotic dose (mg)	
Triazolam	0.1 (0.06– 0.22)	0.25– 1.0	(17)
Nitrazepam	0.5 (0.35– 0.76)	2.5 – 10.0	(17)
Quazepam	0.9 (0.4 – 2.0)	15 – 20	(14)
Flurazepam	1.6 (1.1 – 2.3)	15 – 30	(12)
Phenobarbital	5.6 (2.9 – 11.1)	30 – 50	(12)
Glutethimide	15.9 (11.6 – 20.9)	250 – 500	(12)
Secobarbital	29.1 (20.8 – 40.9)	100	(12)
Pentobarbital	38.4 (25.5 – 62.1)	100	(12)
Methaqualone	45.9 (25.9 – 93.8)	150 – 300	(12)
Methyprylon	49.4 (30.0 – 91.7)	300	(12)
Chloral hydrate	83.8 (57.2 –121.0)	500	(12)
Thalidomide	97.7 (30.6 –685.3)	100 – 200	(1)
Amobarbital	126.1 (83.1 –230.1)	100 – 200	(12)
Ethinamate	150.8 (89.1 –252.8)	500 –1000	(12)
Triclofos	180.1 (133.3 –243.7)	1500	(12)

*Defined as that dose (95% confidence limits) that protects 50% of mice against ECS-induced seizures, evaluated according to the method of Finney [5].

Procedure

Groups of 10–20 mice were injected orally (0.02 ml/g) with test drugs suspended in 0.4% methylcellulose solution or vehicle alone. Each drug was tested in at least four groups of mice at logarithmically spaced doses.

One hour after treatment, response to ECS was evaluated in the following manner. A 13 mA, 60 Hz, AC shock was administered to each mouse via corneal electrodes for 0.2 sec. The number of mice in each group that had a tonic seizure, defined as an extension of the hindlimbs, was recorded. The ED_{50}, defined as that dose which blocked tonic seizures in 50% of the mice, was calculated using a probit analysis [5]. The relationship between the ED_{50} for each hypnotic and its relative potency in man was determined using a Pearson Product correlation coefficient.

RESULTS

ECS caused tonic seizures in >95% of the vehicle-treated mice. All hypnotics tested caused a dose-dependent suppression of tonic seizures. Table 1 indicates the ED_{50}'s and clinical doses of the hypnotics tested. The ED_{50}'s of the hypnotics significantly correlated ($r=.797$, $p<0.005$) with the midpoint of the human clinical dose range of these drugs.

The two tricyclic antidepressants both yielded dose-dependent protection from ECS. Amitriptyline, however, was about five times as potent as imipramine. Their respective ED_{50}'s were 31.2 mg/kg and 166.6 mg/kg.

Of the antihistamines tested, only diphenhydramine caused a dose-dependent suppression of tonic seizures following ECS. The ED_{50} for diphenhydramine was 16.7 mg/kg; chlorpheniramine was ineffective at doses as high as 160 mg/kg.

DISCUSSION

The data indicate that a compound's ED_{50} for protecting mice from tonic seizures following ECS is highly correlated with its hypnotic potency in man. This procedure is not only a more accurate predictor of human hypnotic potency than those previously utilized, but is also an objective measure that is valid for a broad range of hypnotics.

Furthermore, this procedure may yield data which is predictive of sedative side effect potential of drugs not normally used as sleep-inducers. Chlorpheniramine did not appear active in this test, whereas diphenhydramine, which is a more sedating antihistamine, was quite potent. Similarly, the more sedating antidepressant, amitriptyline, was about five times as potent as imipramine. Clearly, more drugs will have to be evaluated to test the predictive validity of this procedure with non-hypnotics.

This procedure is not meant to replace polygraphic evaluation for the assessment of hypnotic potential of novel compounds. It is likely to pick up non-sedating anticonvulsant compounds such as phenytoin and trimethadione. However, its effectiveness over such a wide variety of different compounds suggests that it is unlikely to miss any active hypnotic compounds.

In summary, we described and evaluated a procedure for the testing of hypnotic potential of novel compounds. This procedure has the following advantages over others currently being utilized: (a) low cost and rapid evaluation, (b) a simple, reasonably objective, dependent measure, (c) high predictive validity to human hypnotic potency, (d) yields predictive data over a large variety of classifications of hypnotic drugs, and (e) may be useful for predicting sedative side-effect potential in non-hypnotic drugs.

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