European Journal of Pharmacology 610 (2009) 1–11

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Preclinical assessment of proconvulsant drug activity and its relevance for predicting adverse events in humans

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ARTICLE INFO

Article history:
Received 27 January 2009
Received in revised form 19 February 2009
Accepted 3 March 2009
Available online 16 March 2009

Keywords:
Safety pharmacology
Convulsion
Seizure threshold
CNS stimulant
Neuroleptic
Opioid
Antidepressant

ABSTRACT

Safety pharmacology studies, which are performed before first studies with investigational drugs in humans, often include experiments on proconvulsant drug activity, because such drugs are thought to promote seizures by decreasing seizure threshold. A commonly used model for the assessment of proconvulsant activity of investigational or marketed drugs is the timed intravenous pentylenetetrazole (PTZ) infusion seizure test, in which the latency to myoclonic or clonic seizures is determined by PTZ infusion in mice or rats. This test provides an extremely sensitive parametric method for assessing seizure threshold and allows detecting both anticonvulsant and proconvulsant drug effects. The aim of this review is to critically review the concept of "proconvulsant" drug activity and discuss data obtained by the PTZ and other seizure threshold tests as well as the various factors that may affect seizure threshold determinations. Furthermore, preclinical and clinical data on proconvulsant drug activity are compared. It is concluded that a battery of different tests is needed to provide the most reliable conclusions about the proconvulsant profile, if any, of drugs. Furthermore, misconceptions regarding proconvulsant drug effects, which can result in the undertreatment of brain diseases, are discussed.

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1. Introduction

Safety pharmacology studies in rodents, which are performed before first studies with investigational drugs in humans, often include experiments on seizure threshold for detection of proconvulsant drug activity (Porsolt et al., 2002; Gad, 2003; Kumar et al., 2007). Preclinical testing for proconvulsant activity is considered important,

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doi:10.1016/j.ejphar.2009.03.025

because such drugs may promote convulsions, e.g., in patients with epilepsy or in combination with other potentially proconvulsant drugs (Porsolt et al., 2002; Gad, 2003). It is thus imperative that any preclinical model that is used for identifying proconvulsant drug activity correctly predicts such activity and does not produce too many false negative results.

Clinically, the terms "proconvulsant" and "convulsant" are often mixed or even used synonymously, because it is difficult to identify a drug-induced decrease in seizure threshold in humans, whereas induction of seizures, particularly in association with drug intoxication, is an easily recognizable event. However, a drug that induces seizures at high, toxic doses is not necessarily proconvulsant at lower doses. Proconvulsant drugs are typically central nervous system (CNS) stimulants, such as pentylenetetrazole (PTZ), strychnine, theophylline, cocaine or amphetamine, which may lower seizure threshold at subconvulsive doses, but cause or produce convulsions at higher, convulsant doses (Herrmann and Coper, 1987; Smith and McBride, 1999). However, a convulsant or proconvulsant effect may also occur at high doses of drugs that are anticonvulsant at lower doses. Examples are local anesthetics, such as lidocaine, some antiepileptic drugs, e.g., carbamazepine and phenytoin, and the general anesthetic ketamine, all of which exert anticonvulsant effects in humans (Perucca et al., 1998; Walker and Slovis, 1997; Abend and Dlugos, 2008). Furthermore, convulsions are unspecific adverse symptoms of many drugs, particularly at high (toxic) doses.

Whether a drug with potential (pro)convulsant activity induces seizures in humans depends on a number of factors, including dose (risk usually increases at high, toxic doses), duration of treatment, comedication with other potentially (pro)convulsant drugs or use of illicit drugs with (pro)convulsant potential, specific diseases of the patient, e.g., epilepsy, brain insults, alcohol abuse, age (risk increases at high age) and genetic factors. Thus, in order to protect humans from the risk associated with proconvulsant drug effects, the potential of a drug to induce such effects needs to be determined preclinically. In view of the widespread use of the timed i.v. PTZ infusion seizure test for this purpose (White et al., 2008), the aim of this review is to critically review data obtained by this test, compare the PTZ test with other models used for assessing proconvulsant drug activity, and discuss the various factors that may affect seizure threshold and lead to false positive or negative results on proconvulsant potential of a drug candidate. Furthermore, preclinical and clinical data on proconvulsant drug activity are compared. Finally, misconceptions regarding proconvulsant drug effects will be discussed.

2. The timed intravenous pentylenetetrazole (PTZ) infusion seizure test

PTZ, also known as pentetrazol and metrazol, is a CNS stimulant that is widely used experimentally to study seizure phenomena and to identify pharmaceuticals that may alter seizure susceptibility (Löscher and Schmidt, 1988; Löscher, 1999; White et al., 2008). PTZ is also a prototypical anxiogenic drug and may exert positive effects on cognition (Jung et al., 2002; Rueda et al., 2008). PTZ acts predominantly by antagonizing GABAergic inhibition via an effect at the picrotoxin site of the chloride ionophore of the $GABA_A$ receptor (Ramanjaneyulu and Ticku, 1984). Because of its stimulatory effects on the brain stem, PTZ has clinically been used as a circulatory and respiratory stimulant and, before the invention of electroconvulsive therapy, for convulsive therapy in patients with major depression (Fink, 1972, 1984; Herrmann and Coper, 1987).

For assessing proconvulsant drug activity, the timed intravenous PTZ infusion seizure test has become a standard model (Fig. 1). This test can be used to assess the ability of a drug to modify seizure threshold by administering the drug before onset of PTZ infusion. PTZ is continuously infused intravenously (i.v.) with a constant flow rate in mice or rats until the appearance of seizures (Orloff et al., 1949; Hint and Richter, 1958; Fingl and McQuarrie, 1960; Kilian and Frey, 1973; Frey and Löscher, 1980; Pollack and Shen, 1985; Nutt et al., 1986; Löscher and Schmidt, 1988; Green and Murray, 1989; Löscher et al., 1991a; Mandhane et al., 2007). The threshold dose of PTZ (in mg/kg) is calculated from the time needed to produce convulsions, the body weight of the animal, and the rate of infusion and concentration of the convulsant in the infusate (Fig. 1). This timed intravenous PTZ infusion seizure test provides an extremely sensitive parametric method for assessing seizure threshold in individual animals (White et al., 2008). A quantifiable endpoint can be obtained with a minimal number of animals. Anticonvulsant drugs will delay the appearance of seizures (i.e., increase time of infusion to seizures), while proconvulsant drugs exert the opposite effect (Fig. 2). Different seizure types can be chosen as endpoint in this test. Usually, the first seizure occurring during PTZ infusion in rodents and other species is a myoclonic twitch, followed by clonic and, later, tonic seizures (Löscher and Schmidt, 1988).

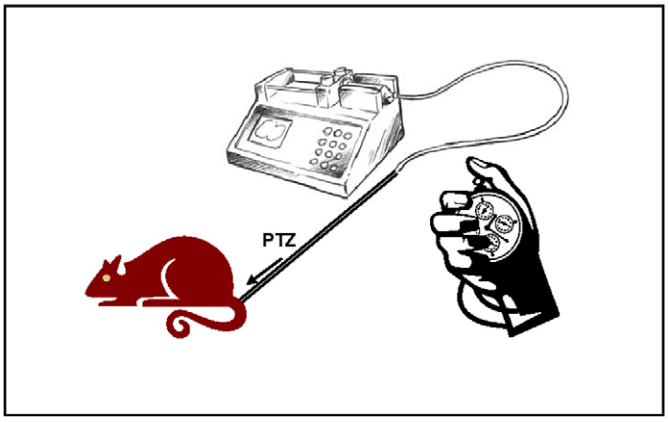

Fig. 1. Schematic illustration of timed i.v. PTZ infusion in an unrestrained mouse or rat. PTZ is continuously infused via a thin, flexible plastic catheter of about 30 cm or longer, connected by means of the sharp cut off end of an injection needle to a tail vein as described by Hint and Richter (1958). Some groups have infused PTZ into the jugular vein of rats (e.g., Pollack and Shen, 1985; Löscher and Hönack, 1995), but this necessitates surgery with implantation of a cannula into the vein under anesthesia at least one day before the PTZ seizure threshold test. During PTZ infusion, the animal is allowed to move freely, e.g. in a plastic cage or glass container, which is preferable to any restraint of the animal that may affect the seizure threshold. The catheter is attached to a syringe containing PTZ in aqueous solution. PTZ is infused at constant rate by a motor-driven infusion pump and the latencies to different seizure types occurring during infusion are recorded with a stop watch or other mechanism. The threshold dose of PTZ (in mg/kg bodyweight) is calculated from the infusion rate, the body weight of the animal and the time necessary to produce a specific seizure type, e.g., the first myoclonic twitch (which occurs with the first paroxysmal EEG activity) or the first clonic seizure. Usually, infusion of PTZ is terminated immediately following the onset of this seizure, but one may also continue the infusion to record latencies to all seizure types (myoclonic, clonic, tonic) occurring during PTZ infusion. Typical infusion rates are 4–8 mg PTZ per min in rats and ~3 mg PTZ per min in mice, resulting in seizure thresholds of about 30–40 mg/kg PTZ when using the first clonic seizure as an endpoint (Kilian and Frey, 1973; Frey and Löscher, 1980; Vohland et al., 1981; Pollack and Shen, 1985; Nutt et al., 1986; Löscher et al., 1991a). Test drugs are administered at a fixed time (e.g., 15 or 30 min) before onset of PTZ infusion to determine pro- or anticonvulsant effects on the seizure threshold. The potency of drugs to increase seizure threshold can be determined (and compared) by calculating the doses required to increase threshold by 50% (TI50 or TID_{50}) in rats or mice, testing a range of doses in groups of rodents (Green and Murray, 1989; Löscher and Nolting, 1991). For proconvulsant drugs, potency may be assessed as proconvulsant threshold dose, i.e., the lowest dose of a drug that significantly decreases the PTZ seizure threshold.

As an alternative to i.v. infusion of PTZ, which requires some technical expertise and equipment (Fig. 1), PTZ is often administered by the intraperitoneal (i.p.) or subcutaneous (s.c.) routes at convulsant doses for assessment of proconvulsant drug activity in groups of mice or rats (Lange et al., 1976; Urca and Frenk, 1980; Ogren and Pakh, 1993; Santos et al., 2002; Zienowicz et al., 2005; Yilmaz et al., 2007; Rehni et al., 2008). Seizure latency, seizure severity and seizure duration can be used as endpoints after i.p. or s.c. administration of PTZ. A proconvulsant drug may decrease seizure latency and increase