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Review

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

Wolfgang Löscher *

*Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Hannover, Germany*
*Center for Systems Neuroscience, Hannover, Germany*

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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,

* Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany. Tel.: +49 511 856 8721; fax: +49 511 953 8581.
*E-mail address:* wolfgang.loescher@tiho-hannover.de.

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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).

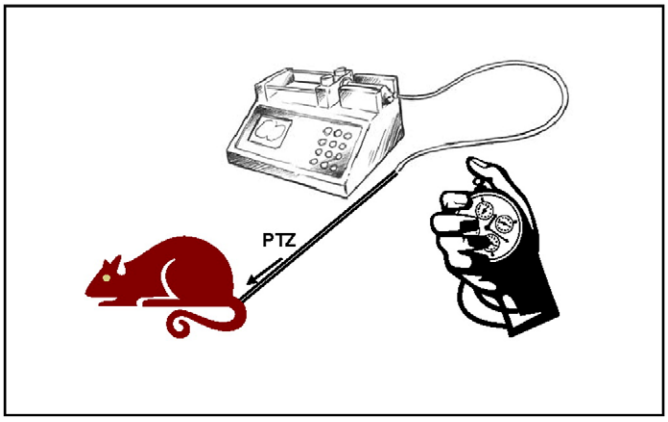


**Fig. 1.** Schematic illustration of timed i.v. PTZ infusion in an unrestrained mouse or rat. PTZ is continuously infused via a thin, flexible plastic catheter of about 30 cm or longer, connected by means of the sharp cut off end of an injection needle to a tail vein as described by Hint and Richter (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

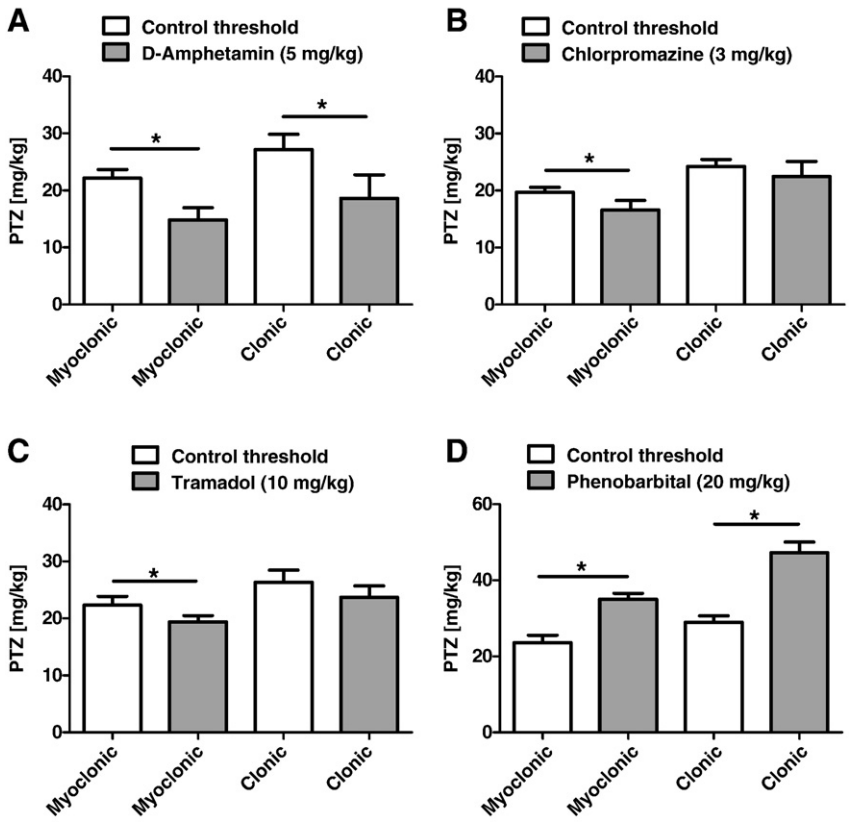


**Fig. 2.** Examples of pro- and anticonvulsant drug effects in the timed i.v. PTZ infusion seizure test in male Wistar rats. The seizure threshold is shown in mg/kg PTZ infused to the first myoclonic and clonic seizure. Data are means ± SEM of 5–8 rats per experiment. PTZ was infused at a concentration of 0.8% at 1 ml/min via the tail vein in unrestricted rats (see Fig. 1), resulting in a PTZ infusion rate of 8 mg/min. Based on the report of Pollack and Shen (1985), the seizure threshold was determined twice in each rat at an interval of 2–3 days, so that each rat served as its own control. The CNS stimulant d-amphetamine (5 mg/kg), the neuroleptic drug chlorpromazine (3 mg/kg), the atypical opioid analgesic tramadol (10 mg/kg) and the antiepileptic drug phenobarbital (20 mg/kg) were administered i.p. 30 min before onset of PTZ infusion. Significant drug effects are indicated by asterisk ($P<0.05$). Amphetamine and chlorpromazine decreased the seizure threshold for myoclonic or clonic seizures, i.e., exerted a proconvulsant effect, while phenobarbital induced an anticonvulsant effect. Tramadol, which was administered at a dose in the analgesic range of this drug in rats, induced a small (14%) but statistically significant decrease in the seizure threshold for myoclonic seizures. Data are from unpublished experiments by M. Bankstahl and W. Löscher.

seizure severity or duration. However, the variability of both seizure latency and incidence with i.p. or s.c. administration of fixed doses of PTZ in groups of rodents makes it difficult to use in evaluating the effects of treatment on seizure susceptibility (Nutt et al., 1986). In contrast, the infusion method of determining seizure threshold produces accurate measures of doses of PTZ required to elicit a seizure with little variability about the mean (Pollack and Shen, 1985; Green and Murray, 1989). As a consequence, compared to i.p. or s.c. PTZ administration, which has to be performed in relatively large groups of animals per dose level of a test drug, the saving in animals is considerable with individual seizure threshold determination by timed i.v. PTZ infusion. Furthermore, rapid termination of the infusion at the time of first myoclonic or clonic seizure prevents the onset of tonic seizures and respiratory arrest, which is not possible with administration of fixed, suprathreshold doses of PTZ (Pollack and Shen, 1985; Green and Murray, 1989).

## 3. Preclinical vs. clinical data on drugs that may lower seizure threshold

Various clinically approved drugs have been examined in the timed i.v. PTZ infusion seizure test, often after induction of seizures had been associated with such drugs in humans (Table 1).

### *3.1. CNS stimulants*

Considering that the CNS stimulant PTZ itself is a proconvulsant and convulsant drug, it is not surprising that administration of other CNS stimulants, including amphetamine, methylphenidate and theophylline, before onset of i.v. infusion of PTZ typically lowers the PTZ threshold in mice or rats (Table 1; Fig. 2), although also anticonvulsant effects of amphetamine were reported in this test (Kilian and Frey, 1973). Proconvulsant effects of the relatively weak CNS stimulant caffeine are more difficult to demonstrate in animal models. High doses of caffeine may induce seizures in both humans and experimental animals (Nehlig et al., 1992; Haller et al., 2005), and lower doses have been shown to increase seizure length during electroconvulsive therapy in patients with major depression, substantiating a proconvulsant potential of this methylxanthine in humans (Stern et al., 1999; Datto et al., 2002). In rats, caffeine significantly prolongs electroconvulsions at doses of 25 mg/kg and above (Francis and Fochtmann, 1994). However, at 20 mg/kg caffeine, a dose that produces convulsions in humans (Nehlig et al., 1992), no significant seizure threshold alteration was determined in the i.v. PTZ infusion test in rats (Table 1). When the threshold for clonic convulsions was determined in mice after s.c. injection of increasing doses of PTZ, caffeine significantly decreased the threshold at 90 mg/kg, whereas lower doses were ineffective (Luszczki et al., 2006). These data may indicate that electroconvulsions are more sensitive to proconvulsant effects of caffeine than PTZ-induced seizures.

However, what is the clinical relevance of proconvulsant activity of CNS stimulants administered at high doses in seizure models? Clinically, amphetamine has been widely used in epilepsy patients for counteracting the sedative effect of first generation antiepileptic drugs, such as phenobarbital or phenytoin, without adverse effect on the antiepileptic efficacy of the medication (Scholl, 1962). At doses

**Table 1**
Effects of drugs in the i.v. PTZ seizure threshold test that are considered (pro)convulsant in humans.

| Drug category | Drug | Effect in i.v. PTZ seizure threshold test | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Species | Seizure endpoint in PTZ threshold test | Dose range tested (mg/kg) | Route of administration | Effect in PTZ threshold test | Comments | References |
| Psychostimulants | Amphetamine | Mice | Clonic | 5 | i.p. | Proconvulsant | | Mannino and Wolf (1974) |
| | Amphetamine | Mice | Clonic | 1–4 | p.o. | Anticonvulsant | | Kilian and Frey (1973) |
| | Amphetamine | Mice | Tonic | 1–4 | p.o. | None | | Kilian and Frey (1973) |
| | Methylphenidate | Rats | Clonic | 4 | p.o. | Proconvulsant | | Oyungu et al. (2009) |
| | Caffeine | Rats | Myoclonic twitch | 20 | p.o. | None | | Himmel (2008) |
| Antiasthmatics | Theophylline | Rats | Myoclonic twitch | 15–30 | i.p. | Proconvulsant | | Murray et al. (1985) |
| | Theophylline | Rats | Myoclonic twitch and clonic | 100–200 | p.o. | Proconvulsant at 200 mg/kg | | Ault et al. (1987) |
| Neuroleptics | Haloperidol | Mice | Clonic | 0.1 | i.p. | None | | Kilian and Frey (1973) |
| | Haloperidol | Mice | Tonic | 0.1 | i.p. | None | | Kilian and Frey (1973) |
| | Droperidol | Mice | Clonic | 0.625 | s.c. | Proconvulsant | 1.25 and 2.5 mg/kg had no effect | Hashem and Frey (1988) |
| | Droperidol | Mice | Tonic | 0.625–2.5 | s.c. | Proconvulsant | | Hashem and Frey (1988) |
| Antidepressants | Venlafaxine | Rats | Clonic | 15–50 | i.p. | Tendency for increased seizure latency | Seizure latency and severity after i.p. PTZ (60 mg/kg). | Santos et al. (2002) |
| | Venlafaxine | Rats | Clonic–tonic | 75–150 | i.p. | Proconvulsant | Seizure latency and severity after i.p. PTZ (60 mg/kg). | Santos et al. (2002) |
| Local anesthetics | Lidocaine, tetracaine and others | Mice | Clonic | 5–50 (depending on drug) | s.c. | None | | Frey (1962) |
| | Lidocaine, tetracaine and others | Mice | Tonic | 5–50 (depending on drug) | s.c. | Anticonvulsant | | Frey (1962) |
| Antibiotics | Isoniazid | Rats | Myoclonic twitch | 120 | s.c. | Proconvulsant | | Himmel (2008) |
| Opioid analgesics | Morphine | Mice | Clonic | 15–100 | i.p. | Proconvulsant | Antagonism by naloxone | Mannino and Wolf (1974) |
| | Morphine | Rats | Not defined | 64 | s.c. | Anticonvulsant | | Adler et al. (1976) |
| | Morphine | Rats | Clonic | 10–50 | s.c. | Proconvulsant | Antagonism by naloxone | Foote and Gale (1984) |
| | Morphine | Mice | Clonic | 0.42–3.34 | s.c. | None | Analgesic dose range | Czuczwar and Frey (1986) |
| | | Mice | Tonic | 0.42–3.34 | s.c. | Anticonvulsant | Analgesic dose range | Czuczwar and Frey (1986) |
| | Morphine | Dogs | Myoclonic twitch | 1.0 | i.m. | Proconvulsant | | Frey et al. (1986) |
| | Morphine | Mice | Clonic or tonic–clonic | 0.5 | i.p. | Anticonvulsant | Antagonism by naloxone | Rocha Lauretti et al. (1994) |
| | Morphine | Mice | Clonic or tonic–clonic | 20 | i.p. | Proconvulsant | Antagonism by naloxone | Rocha Lauretti et al. (1994) |
| | Morphine | Rats | Myoclonic twitch | 10 | i.p. | Proconvulsant | | Himmel (2008) |
| | Hydromorphone | Mice | Clonic | 2–20 | i.p. | Proconvulsant | Antagonism by naloxone | Mannino and Wolf (1974) |
| | Levorphanol | Mice | Clonic | 2–20 | i.p. | Proconvulsant | Antagonism by naloxone | Mannino and Wolf (1974) |
| | (−)-Methadone | Mice | Clonic | 2–25 | i.p. | Proconvulsant | Antagonism by naloxone | Mannino and Wolf (1974) |
| | (+)-Methadone | Mice | Clonic | 15–25 | i.p. | None | | Mannino and Wolf (1974) |
| | Fentanyl | Mice | Clonic or tonic–clonic | 0.0075 | i.p. | Anticonvulsant | Antagonism by naloxone | Rocha Lauretti et al. (1994) |
| | Fentanyl | Mice | Clonic or tonic–clonic | 0.25 | i.p. | Proconvulsant | Antagonism by naloxone | Rocha Lauretti et al. (1994) |
| | Fentanyl | Mice | Clonic | 0.0125–0.2 | s.c. | None | Analgesic dose range | Czuczwar and Frey (1986) |
| | | Mice | Tonic | 0.0125–0.2 | s.c. | Anticonvulsant | Analgesic dose range | Czuczwar and Frey (1986) |
| | | Dogs | Myoclonic twitch | 0.01–0.03 | i.v. | Proconvulsant | | Frey et al. (1986) |
| | Pethidine | Mice | Clonic or tonic–clonic | 1.0 | i.p. | Anticonvulsant | Antagonism by naloxone | Rocha Lauretti et al. (1994) |
| | Pethidine | Mice | Clonic or tonic–clonic | 100 | i.p. | Proconvulsant | No antagonism by naloxone | Rocha Lauretti et al. (1994) |
| | Meperidine | Mice | Clonic | 2–16 | s.c. | None | Analgesic dose range | Czuczwar and Frey (1986) |
| | | Mice | Tonic | 2–16 | s.c. | Anticonvulsant | Analgesic dose range | Czuczwar and Frey (1986) |
| | | Dogs | Myoclonic twitch | 6 | i.m. | Proconvulsant | | Frey et al. (1986) |
| | Pentazocine | Mice | Clonic | 2–16 | s.c. | Anticonvulsant | Analgesic dose range | Czuczwar and Frey (1986) |
| | | Mice | Tonic | 2–16 | s.c. | Anticonvulsant | Analgesic dose range | Czuczwar and Frey (1986) |
| | | Dogs | Myoclonic twitch | 3.0 | i.m. | Proconvulsant | | Frey et al. (1986) |
| | Tramadol | Mice | Clonic | 10–68 | i.p. | None | | Friderichs (1987) |
| | Tramadol | Mice | Clonic | 100 | i.p. | Anticonvulsant | | Friderichs (1987) |
| | Tramadol | Mice | Tonic | 10–100 | i.p. | Anticonvulsant | | Friderichs (1987) |

below those exerting (pro)convulsant effects in animal models, amphetamine exerts anticonvulsant effects against PTZ-induced seizures in mice (Kilian and Frey, 1973) and absence seizures in a rat model with genetic absence epilepsy (Frey and Voits, 1991), which is in line with antiepileptic effects of amphetamine reported in children with absence seizures, i.e., petit mal epilepsy (Strauss, 1944; Livingston et al., 1948). In line with the effects of amphetamine on absence seizures, caffeine (5–15 mg/kg i.p.) significantly decreased absence-like spike-wave discharges in the tottering mutant mouse (Kostopoulos et al., 1987). Furthermore, long-term treatment of mice with caffeine via the drinking water in a dose commonly used in humans reduced the susceptibility to PTZ, possibly mediated by blocking adenosine $A_{2A}$ receptors (Johansson et al., 1996; El Yacoubi et al., 2008). The CNS stimulant modafinil (22.5–90 mg/kg i.p.) exerted anticonvulsant effects against PTZ and electroshock induced seizures in mice, but, at 180 mg/kg, exerted a proconvulsant effect in the PTZ model (Chen et al., 2007). Overall, these data indicate that different categories of CNS stimulants (amphetamines, methylxanthines, modafinil), which are often thought to be generally proconvulsant, exert proconvulsant effects only at high, therapeutically irrelevant doses, but may display anticonvulsant activity at lower doses. Yet, there is a great reluctance of clinicians to use CNS stimulants such as amphetamine or methylphenidate for treatment of attention deficit hyperactivity disorders in children with epilepsy, resulting in undertreatment of this disorder in epilepsy patients (Kanner, 2008). However, a review of the literature shows that CNS stimulants are generally safe for use in patients with epilepsy, including children with epilepsy and attention deficit hyperactivity disorders (Dunn et al., 2003; Dunn and Kronenberger, 2005; Kanner, 2008). Thus, even though CNS stimulants may exert (pro)convulsant activity at supratherapeutic doses, the general belief that therapeutic doses of such drugs may worsen seizures in patients with epilepsy is a misconception (Kanner, 2008).

### 3.2. CNS depressants

In contrast to CNS stimulants, for which proconvulsant and convulsant effects are biologically plausible, such effects are somewhat paradoxical and not fully understood for drugs that exert a depressant effect on the CNS. Drugs in this category include neuroleptics (antipsychotic drugs), some of which (e.g., chlorpromazine) are long known to induce (pro)convulsant effects in humans despite their sedative, CNS tranquillizing properties (Torta and Monaco, 2002), and some general anesthetics, e.g., etomidate, enflurane, ketamine and propofol, for which proconvulsant or convulsant effects have been reported in humans (Kofke et al., 1997; Datto et al., 2002). Furthermore, antidepressants are often believed to lower seizure threshold and exert (pro)convulsant effects in humans, particularly in patients with epilepsy or otherwise at risks for seizures, but this is a misconception for most antidepressant drugs (Edwards and Wheal, 1992; Alldredge, 1999; Torta and Monaco, 2002; Alper et al., 2007; Kanner, 2008).

#### 3.2.1. Neuroleptics

Neuroleptic (or antipsychotic) drugs can lower the seizure threshold and induce discharge patterns in the electroencephalogram (EEG) that are reminiscent of paroxysmal patterns associated with epileptic seizures (Markowitz and Brown, 1987; Pisani et al., 2002; Torta and Monaco, 2002). Thus, it is often thought that these drugs should be used with extreme caution, if at all, in epilepsy patients and in patients undergoing withdrawal from alcohol, barbiturates or benzodiazepines. However, psychiatric co-morbidity with epilepsy is common and often requires combined use of psychotropic and antiepileptic drugs (Alldredge, 1999; Kanner, 2008). A number of recent analyses have indicated that, while proconvulsant activity was a problem with first generation ("conventional") neuroleptics such as phenothiazines (e.g., chlorpromazine) and thioxanthenes, most newer neuroleptics, including atypical antipsychotics, can be used safely in epilepsy patients if moderate doses are attained gradually and if concomitant antiepileptic drug therapy is maintained (Alldredge, 1999; Alper et al., 2007; Kanner, 2008). An exception is the atypical neuroleptic clozapine, which can also induce paroxysmal EEG changes and seizures in nonepileptic patients and is considered the most potent (pro)convulsant antipsychotic drug (Torta and Monaco, 2002; Alper et al., 2007). Historically, the proconvulsant action of neuroleptics has been related to their antagonistic effect on dopamine receptors, particularly dopamine $D_2$ receptors, and, partially, to their activity on histamine receptors (Torta and Monaco, 2002). Numerous investigations have shown that dopamine agonists exert anticonvulsant effects in different animal models of seizures or epilepsy, including the i.v. PTZ seizure threshold test, and that this effect is mediated by both dopamine $D_1$ and $D_2$ receptors (Löscher and Czuczwar, 1986; Ogren and Pakh, 1993; Starr, 1996; Weinshenker and Szot, 2002). In line with its proconvulsant clinical activity, chlorpromazine lowers the PTZ threshold in rats (Fig. 2). However, other dopamine antagonists, including haloperidol, remoxipride and raclopride did not significantly modify seizures induced by PTZ (Kilian and Frey, 1973; Ogren and Pakh, 1993; Table 1), which is in line with the low seizure risk associated with these neuroleptics in humans (Kanner, 2008). Droperidol was reported to decrease the PTZ threshold (Hashem and Frey, 1988), although this antipsychotic drug is not associated with high seizure risk in patients, even under emergency conditions (Chase and Biros, 2002). In apparent contrast to its (pro)convulsant activity in humans, clozapine has been reported to delay the onset of seizures induced by PTZ in mice (George and Kulkarni, 1998).

#### 3.2.2. Antidepressants

Antidepressant drugs are often considered to be more (pro)convulsant than neuroleptics in humans, but this is obviously a misconception largely based on case reports involving tricyclic antidepressants at supratherapeutic doses (Markowitz and Brown, 1987; Edwards and Wheal, 1992; Dailey and Naritoku, 1996; Pisani et al., 2002; Torta and Monaco, 2002; Alper et al., 2007; Kanner, 2008). Whereas dopamine antagonism is a plausible explanation for the proconvulsant potential of some neuroleptics, this is not the case for the mechanism of action of antidepressants, i.e., reuptake inhibition of noradrenaline, 5-hydroxytryptamine (5-HT) or both, leading to increased synaptic concentrations of these monoamines. Instead, it is long known that increasing synaptic levels of noradrenaline or 5-HT increases seizure thresholds, including the i.v. PTZ seizure threshold, in mice and rats (Kilian and Frey, 1973; Peterson and Albertson, 1982; Przegalinski, 1985; Corcoran and Weiss, 1990; Weinshenker and Szot, 2002). At low, therapeutically relevant doses, antidepressant drugs thus exert anticonvulsant effects in seizure models, including the PTZ test (Lange et al., 1976; Yacobi and Burnham, 1991; Santos et al., 2002; Torta and Monaco, 2002; Ferrero et al., 2005; Uzbay et al., 2007). In contrast, proconvulsant activity may be seen at high doses in the PTZ test and other models (Lange et al., 1976; Escorihuela et al., 1989; Santos et al., 2002; Torta and Monaco, 2002), suggesting that antidepressants exert a biphasic (anti- and proconvulsant effect) on seizure threshold, depending on dosage, although this may differ among the various antidepressant drugs and the seizure models used (see data on venlafaxine as an example in Table 1). Clinically, many antidepressant drugs are thought to have the potential to provoke seizures, particularly in patients with a preexisting lowered seizure threshold (Markowitz and Brown, 1987; Edwards and Wheal, 1992; Pisani et al., 2002; Torta and Monaco, 2002). To a great extent, such concern is based on case reports involving high doses of tricyclic antidepressants (Dailey and Naritoku, 1996). Indeed, correlations between the incidence of antidepressant-induced seizures and doses or plasma concentrations strongly indicate that the apparent paradox

between preclinical and clinical findings is related to the fact that an increased risk for seizures in patients almost exclusively occurs after high doses or elevated plasma concentrations of antidepressant agents (Preskorn and Fast, 1992; Dailey and Naritoku, 1996; Alldredge, 1999). At lower doses, some antidepressants (e.g., fluoxetine and citalopram) have been shown to exert anticonvulsant effects in humans (Dailey and Naritoku, 1996; Torta and Monaco, 2002), which is in line with the dose-dependent biphasic (anti-/proconvulsant) activity of such drugs found in seizure models. Furthermore, in an analysis of Phase II and III clinical trials of antidepressants approved in the U.S. between 1985 and 2004, Alper et al. (2007) found that the incidence of seizures was significantly lower among patients assigned to antidepressants compared to placebo, indicating an anticonvulsant effect. The only exception was an immediate release preparation of bupropion, which increased seizure incidence in patients (Alper et al., 2007). This proconvulsant activity of bupropion was not identified by the PTZ model (Tutka et al., 2004). In summary, only a subset of psychotropic drugs, including clozapine, chlorpromazine and bupropion, is associated with a clinically relevant risk of inducing seizures at therapeutic doses (Alper et al., 2007; Kanner, 2008). Unfortunately, the misconception that antidepressants and other psychotropic drugs in general are proconvulsant at therapeutically relevant doses has resulted in the undertreatment of psychiatric comorbidities in patients with epilepsy (Kanner, 2008).

#### 3.2.3. General anesthetics

The idea that some general anesthetics may be proconvulsant in humans stems from several observations. With etomidate, myoclonic movements and seizure-like EEG activity are often observed during general anesthesia (Doenicke et al., 1999), and this drug augments seizure induction in electroconvulsive therapy, indicating a proconvulsant effect (Datto et al., 2002). Similarly, myoclonic movements and paroxysmal EEG activity have been observed during general anesthesia with propofol and enflurane (Ito et al., 1988; Sutherland and Burt, 1994). Methohexital has been reported to induce convulsions in patients with temporal lobe epilepsy (Modica et al., 1990) and sevoflurane may induce epileptiform EEG discharges during anesthesia (Jaaskelainen et al., 2003). Like etomidate, ketamine augments seizure induction in electroconvulsive therapy (Datto et al., 2002). Furthermore, ketamine activates epileptic discharges in the EEG of patients with partial epilepsy, substantiating its proconvulsant potential (Bacia et al., 1989). One possible factor to explain "proconvulsant" effects of anesthetics is inherent pharmacodynamic variability in the responsiveness of inhibitory and excitatory target tissues in the CNS (Modica et al., 1990). Depending on the brain concentration, centrally active drugs may produce differing effects on the CNS inhibitory and excitatory neurotransmitter systems. At lower doses, anesthetics may suppress inhibitory targets more effectively than excitatory targets, which also explains the preanesthetic excitation state that may occur before induction of surgical levels of anesthesia (Modica et al., 1990). However, much below the doses used for general anesthesia, several anesthetic drugs, including barbiturates, propofol and ketamine, are known to exert anticonvulsant effects in different models, including the PTZ test (Löscher et al., 1991a; Hasan et al., 1992; Hasan, 1997; Herink, 1997; Manocha et al., 2001). Furthermore, propofol and ketamine have been used to terminate benzodiazepine-resistant status epilepticus in humans (Abend and Dlugos, 2008). Thus, the term "proconvulsant" may be misleading for general anesthetics such as ketamine, propofol and others.

### 3.3. Local anesthetics and antibiotics

Similar to general anesthetics, local anesthetics such as lidocaine are known to exert both anti- and proconvulsant effects, depending on the administered dose (Modica et al., 1990). For instance, lidocaine has been shown to reduce mortality in mice treated with PTZ (Thompson and Aldrete, 1975). Furthermore, while lidocaine, tetracaine and other local anesthetics did not modify the PTZ threshold for clonic convulsions, they increased the threshold for tonic convulsions, indicating an anticonvulsant effect (Table 1).

In contrast to the typical biphasic anti- and proconvulsant effects of anesthetic drugs, pure proconvulsant activity occurs with some other pharmacological groups. For instance, several antibiotics, including isoniazid, penicillins, cephalosporins, carbapenems and fluoroquinolones can have proconvulsant activity and may precipitate seizures at therapeutic doses, even in patients who do not have epilepsy (Sander and Perucca, 2003). This proconvulsant activity of antibiotics can be demonstrated by the PTZ seizure threshold (Day et al., 1995; Himmel, 2008).

### 3.4. Opioids

The timed i.v. PTZ infusion seizure test has been widely used to characterize the proconvulsant effects of opioid analgesics, however, with mixed results (Table 1). High doses of morphine and other opioid analgesics can induce naloxone-insensitive electrographic epileptiform patterns and behavioral convulsions in a variety of species, including humans (Frenk, 1983; Reisine and Pasternak, 1996). Fentanyl and alfentanyl have been reported to enhance focal seizure activity in patients with temporal lobe epilepsy (Bowdle, 1998). In line with this clinical finding, small doses of fentanyl decreased the focal seizure threshold in the amygdala kindling model of temporal lobe epilepsy in rats (Schwark et al., 1986). Epilepsy may also increase the sensitivity to proconvulsant action of morphine; e.g., extremely low doses of morphine elicited seizures in genetically epilepsy-prone rats (Reigel et al., 1988), and amygdala kindled rats showed a heightened sensitivity to morphine's convulsive effects which could be blocked by naloxone (Mansour and Valenstein, 1984). Is this proconvulsant activity of morphine and other analgesic opioids reflected in the timed i.v. PTZ infusion seizure test? As shown in Table 1, both proconvulsant or anticonvulsant effects of morphine and other opioids have been reported. It is long known that administration of opioid analgesics at doses within the analgesic range induce naloxone-sensitive anticonvulsant effects in several animal models of seizures or epilepsy, including the kindling model, whereas higher doses may induce proconvulsant or convulsant effects (Frenk, 1983). This is, at least in part, also reflected by the timed i.v. PTZ infusion seizure test (Table 1). Thus, at low doses within the analgesic range, morphine and other opioid analgesics induce either no seizure threshold changes or increases in seizure threshold, whereas considerably higher doses decrease the threshold. This biphasic effect is most nicely illustrated in the experiments of Rocha Lauretti et al. (1994) in mice. However, both the seizure endpoint and the species may affect results obtained with opioids in the timed i.v. PTZ infusion seizure test (Table 1). For instance, analgesic doses of opioids did not affect the threshold for induction of clonic seizures, but increased the threshold for tonic seizures in mice (Czuczwar and Frey, 1986). The same doses of opioids that did not induce any proconvulsant activity in mice (Czuczwar and Frey, 1986) were proconvulsant in dogs (Frey et al., 1986). In addition to seizure endpoint, dose and species, brain alterations such as occurring during epilepsy alter the response to opioids, so that the use of healthy animals in the i.v. PTZ infusion seizure test does not reflect the enhanced susceptibility of epileptic individuals to such drugs. Thus, increased opioid receptor binding has been described in patients with temporal lobe epilepsy and different models of epilepsy, including amygdala kindling (Lee et al., 1986; Frost et al., 1988; Fisher and Frost, 1991; Engel and Rocha, 1992; Rocha et al., 1993), which may explain the increased susceptibility to proconvulsant effects of opioid analgesics.

The atypical opioid tramadol is one of the most widely used centrally acting analgesics worldwide (Grond and Sablotzki, 2004). Its

multi-modal effect results from a dual mode of action, i.e., weak agonistic acitivity at μ-opioid receptors and inhibition of monoamine (noradrenaline, 5-HT) reuptake, resulting in efficacy in both nociceptive and neuropathic pain (Lewis and Han, 1997; Grond and Sablotzki, 2004). Moreover, fewer instances of side effects such as constipation, respiratory depression, and sedation occur than with traditional opioids (Grond and Sablotzki, 2004). In contrast to traditional opioids, which induce respiratory depression and seizures (among other symptoms) at toxic doses, high-dose toxicity of tramadol in animals and in humans is characterized by seizures rather than respiratory depression, which may be one reason why tramadol is often considered more (pro)convulsant than typical opioids (Spiller et al., 1997; Shipton, 2000). However, in a recent study in which the convulsant potential of tramadol in mice was compared with that of various traditional opioids, tramadol did not differ from other opioid analgesics in that seizures were only determined at doses high above the analgesic dose range, thus excluding any unexceptional seizure potential of tramadol (Raffa and Stone, 2008). This conclusion is also supported by a large study of the California Poison Control System (Thundiyil et al., 2007) in patients in which seizures occurred in association with poisoning or drug intoxication. However, some reports described seizures in patients at recommended therapeutic doses of tramadol, although this seems to be an extremely rare event (Jick et al., 1998; Gasse et al., 2000). The risk is increased in patients with a history of alcohol abuse, stroke, head injury or epilepsy (Gardner et al., 2000). Furthermore, tramadol may increase the seizure risk in patients taking other medicinal products that lower the seizure threshold (Khan et al., 1997). In contrast to the often cited notion that tramadol may be particularly (pro)convulsant, this atypical opioid has been shown to exert anticonvulsant activity in the analgesic dose range in animal models, including electroshock-induced seizures in mice and kindled seizures in rats (Manocha et al., 1998, 2005; Potschka et al., 2000). The anticonvulsant effect of tramadol determined at i.p. doses of 10–50 mg/kg in mice was antagonized by naloxone (Manocha et al., 2005). Oral administration of tramadol at doses of 10–40 mg/kg did not affect convulsions induced by i.v. PTZ (70 mg/kg) in mice (Osterloh et al., 1978). In the timed i.v. PTZ infusion seizure test in mice (Table 1), tramadol did not exert any proconvulsant activity at doses ranging from 10–100 mg/kg i.p., but significantly increased latency to clonus at 100 mg/kg, and dose-dependently blocked tonic seizures (Friderichs, 1987). However, in another study, 50 mg/kg tramadol i.p. significantly decreased the latency to convulsions induced by i.p. administration of PTZ (80 mg/kg) in mice, which could be partially counteracted by naloxone (Rehni et al., 2008). Thus, the same i.p. dose of tramadol was anticonvulsant on electroshock seizures in mice (Manocha et al., 2005) but proconvulsant on PTZ-induced seizures in this species (Rehni et al., 2008).

Such apparently paradoxical data with anticonvulsant activity in one seizure test but proconvulsant activity in another test are also known from some antiepileptic drugs. Thus, the widely used antiepileptic drugs phenytoin, carbamazepine and lamotrigine exert anticonvulsant activity against electroshock-induced seizures and kindled seizures in mice or rats, but are ineffective or proconvulsant in the timed i.v. PTZ infusion seizure test, when myoclonic or clonic seizures are used as endpoint (Miller et al., 1986; Löscher et al., 1991a; White et al., 2008). However, nobody would consider these antiepileptic drugs proconvulsant. This illustrates that the timed i.v. PTZ infusion seizure test may yield false positive data on proconvulsant drug potential when relying solely on this test.

## 4. Limitations of the timed i.v. PTZ infusion seizure test in correctly predicting proconvulsant drug activity

The example of tramadol and antiepileptic drugs such as phenytoin, carbamazepine and lamotrigine illustrates that identification of proconvulsant (or anticonvulsant) drug activity should not rely on one test. In other words, the sole use of the timed i.v. PTZ infusion seizure test for identifying proconvulsant drug activity in safety pharmacology during drug development may lead to false negative (or positive) conclusions. Reasons for this include:

(1) The convulsant effect of PTZ is thought to be primarily due to its antagonistic action on $GABA_A$ receptors (Ramanjaneyulu and Ticku, 1984). Thus, the PTZ test is particularly sensitive to drugs that potentiate or antagonize GABA either directly or indirectly (Löscher and Schmidt, 1988; Green and Murray, 1989). However, a useful test for identifying proconvulsant activity should do so independently of the mechanism involved in the proconvulsant activity.

(2) The seizure endpoint (myoclonic, clonic, tonic, mortality) chosen in the PTZ test will affect results with drugs. Thus, as shown in Table 1 for opioid analgesics, the same drug may be proconvulsant when using clonic seizures as endpoint, but anticonvulsant when using tonic seizures. Traditionally, the PTZ test has been used for decades as a model of nonconvulsive seizures, which is based on the fact that antiepileptic drugs (such as ethosuximide) that are effective against absence or myoclonic seizures in patients block myoclonic or clonic seizures in the PTZ test (White et al., 2008). In contrast, antiepileptic drugs such as phenytoin or carbamazepine, which are not efficacious against absence or myoclonic seizures in patients, do not block these seizures (or even aggravate them) in PTZ models (Löscher et al., 1991a; White et al., 2008). However, there are also exceptions in that lamotrigine, which is effective against nonconvulsive seizures in patients, is ineffective or proconvulsant in the PTZ test, so that other models of nonconvulsive seizures have to be included during preclinical development of antiepileptic drugs (Perucca et al., 2007). In other words, the use of PTZ models in preclinical testing of antiepileptic drugs may yield false negative data.

(3) The species may affect results obtained when using the timed i.v. PTZ infusion seizure test for identifying proconvulsant drug activity, as exemplified for the differences in opioid effects in mice, rats and dogs shown in Table 1.

(4) Drugs are usually administered at single doses when being tested for proconvulsant activity. However, as shown for instance for antidepressant drugs (Escorihuela et al., 1989; Ferrero et al., 2005), chronic may differ from acute drug effects in this test, which, of course, is also true for other models used to identify proconvulsant activity (see below).

(5) Because PTZ has to penetrate from the blood to the brain during i.v. infusion, haemodynamic effects of investigational drugs may affect the test, e.g., a drug that enhances blood flow to the brain will reduce the PTZ threshold. Also, effects of drugs on respiration will affect the test (Woodbury, 1969).

(6) PTZ itself increases heart rate and blood pressure via effects on brain stem centers and, by this, may affect brain distribution of drugs (Herrmann and Coper, 1987). Furthermore, the blood–brain barrier is affected by PTZ-induced seizures, which may alter penetration of drugs into the brain (Scheld, 1989).

(7) Drug-induced alterations in body temperature affect the test in that hyperthermia is proconvulsant, while hypothermia is anticonvulsant (Woodbury, 1969). The proconvulsant activity of opioids determined in this test (Table 1) may therefore, at least in part, be due to their hyperthermic effect at high doses. This, however, may also form a bias in other tests used for identifying proconvulsant activity of drugs.

(8) Technical factors affect results obtained in the timed i.v. PTZ infusion seizure test. For instance, infusion rate and concentration of PTZ in the infusate may influence results obtained with anticonvulsant or proconvulsant drugs in this test (Fingl and McQuarrie, 1960; Nutt et al., 1986). Furthermore, restraint of the animals during PTZ infusion may confound the results of

the test, so that it is preferable to perform infusion in unrestrained animals, as described by several groups (e.g., Hint and Richter, 1958; Frey and Löscher, 1980; Vohland et al., 1981; Pollack and Shen, 1985; Löscher and Hönack, 1995) and illustrated in Fig. 1.

(9) Finally, as it applies to other tests used for safety pharmacology (Porsolt et al., 2002), the timed i.v. PTZ infusion seizure test is usually conducted in normal (healthy) animals. However, epilepsy and other brain disorders known to enhance the risk of proconvulsant drug effects may dramatically alter the adverse effect profile of drugs (Löscher and Hönack, 1991; Klitgaard et al., 2002). Thus, absence of any proconvulsant drug activity in the PTZ test does not preclude that a drug exhibits such activity in patients with epilepsy. As a consequence, alternative or additional tests are needed to investigate the potential proconvulsant effects of a substance.

## 5. Alternatives to the timed i.v. PTZ infusion seizure test for identifying proconvulsant drug activity

Electrically induced seizure models, such as the maximal electroshock seizure (MES) test in mice or rats, are the most frequently used model systems for the identification of anticonvulsant activity (White et al., 2008). Anticonvulsant effectiveness of a drug in the MES test is thought to predict clinical efficacy against generalized-tonic clonic seizures, and the predictive value of this test is high (Perucca et al., 2007). In the MES test, a current well above seizure threshold is applied by either corneal or ear electrodes to induce a tonic hindlimb seizure. A modification of this test can be used to determine the threshold for tonic seizures in groups of mice or rats by a "staircase" procedure (Löscher et al., 1991b). This MES threshold (MEST) test is more sensitive than the traditional suprathreshold test to identify new antiepileptic drugs (Löscher and Schmidt, 1988). The antiepileptic drug levetiracetam is an important example in this regard, because it was ineffective in the MES test but raised seizure threshold in the MEST test, which was predictive of its clinical efficacy (Löscher and Hönack, 1993). The electroconvulsive threshold, such as determined by the MEST test, is also useful to identify proconvulsant drug effects (Porsolt et al., 2002). The major advantage vs. the timed i.v. PTZ infusion seizure test is that the MEST is less mechanism-dependent, i.e. drugs with diverse mechanisms of anti- or proconvulsant activity are identified by this model (Löscher and Schmidt, 1988). Furthermore, as discussed above, electroconvulsions are also therapeutically used in humans (Taylor, 2008), so that drugs, such as caffeine, theophylline or aminophylline, that are known to exert proconvulsive effects on electroconvulsions in humans (Kelsey and Grossberg, 1995; Stern et al., 1999; Datto et al., 2002) can be used to validate the MEST test. Interestingly, in studies by Czuczwar et al. (1986, 1990) CNS stimulants such as caffeine or aminophylline did not decrease the MEST at subconvulsive doses in mice, but caffeine prolonged the duration of electroconvulsive seizures in rats (Francis and Fochtmann, 1994), indicating that both seizure threshold and seizure duration should be assessed for identifying proconvulsant drug effects. Furthermore, subconvulsive doses of caffeine and aminophylline have been shown to reduce the anticonvulsant activity of various antiepileptic drugs in the MES test in mice, possibly indicating that methylxanthines should be used with caution for antiasthmatic therapy in patients with epilepsy (Czuczwar et al., 1986, 1990). Using an increasing-current electroshock seizure threshold test in mice, Kitano et al. (1996) reported that subconvulsive doses of aminophylline, chlorpromazine and PTZ lowered seizure threshold, indicating that this test reliably identifies proconvulsant drug activities.

Another model with electrical induction of seizures is electrical kindling, in which the convulsive current is applied via a depth electrode to a limbic brain region such as the amygdala (Sato et al., 1990; Coulter et al., 2002). The term kindling refers to the phenomenon that periodic electrical stimulation of the amygdala or other limbic brain regions with initially subconvulsive stimuli results in the progressive development of focal and, later, secondary generalized seizures. Once the enhanced sensitivity to stimulation has developed, the animal is said to be fully kindled. The increased sensitivity to electrical stimulation is persistent and reflects permanent changes in brain function reminiscent of those occurring in epilepsy (Coulter et al., 2002). For instance, fully kindled rats have a decreased seizure threshold compared to normal rats. Thus, kindled rats are considered more sensitive to proconvulsant drug effects than nonkindled animals (Potschka et al., 2000). For instance, amygdala-kindled rats have been used for evaluating the (pro) convulsant effects of opioids, showing a heightened sensitivity to the convulsive activity associated with high doses of morphine and tramadol (Mansour and Valenstein, 1984; Potschka et al., 2000). Furthermore, clinically relevant doses of fentanyl, meperidine and pentazocine decreased the focal seizure threshold (afterdischarge threshold) in the amygdala kindling model (Schwark et al., 1986). However, analgesic doses of morphine and tramadol significantly increased seizure threshold in kindled rats (Schwark et al., 1986; Potschka et al., 2000). Thus, morphine and tramadol exerted a biphasic pattern in kindled rats with anticonvulsant activity at low doses but convulsant effects at higher doses (Mansour and Valenstein, 1984; Schwark et al., 1986; Potschka et al., 2000).

Another approach for identifying proconvulsant drug activity is the use of epileptic rodents, such as DBA/2 mice and genetically epilepsy-prone rats (GEPRs) with enhanced susceptibility to audiogenic seizure induction, or genetic absence epileptic rats of Strasbourg (GAERS) and WAG/Rij rats of Nijmegen with inborn absence epilepsy (Van Luijtelaar et al., 2002). The potential advantage of such rodent mutants for identifying proconvulsant drug activity is their increased seizure susceptibility, which simulates the increased risk of epilepsy patients to proconvulsant drugs. For instance, caffeine exerted proconvulsant activity in DBA/2 mice with an $ED_{50}$ of 0.04 mg/kg i.p. (De Sarro et al., 1999), i.e., much below the doses of caffeine that are proconvulsant in nonepileptic rodents (see above). In rats with spontaneous absence-like EEG paroxysms, low doses of opioids, including fentanyl (0.01–0.05 mg/kg), buprenorphine (0.1 and 0.2 mg/kg) and pentazocine (1–4 mg/kg), increased the number and duration of spike-wave discharges in the EEG, whereas d-amphetamine (0.5 mg/kg) and the tricyclic antidepressants imipramine (5 and 10 mg/kg) and amitryptiline (5 and 10 mg/kg) exerted an anticonvulsant effect (Frey and Voits, 1991). The anticonvulsant effect of imipramine in this absence epilepsy rat model is in line with clinical studies on antiepileptic activity of imipramine in patients with absence and myoclonic seizures (Fromm et al., 1978).

Proconvulsant or convulsant activity of a drug may be also assessed by studying the EEG effects of drugs in normal rodents or larger animals such as dogs (Zaczek et al., 1986; Porsolt et al., 2002; Dürmüller et al., 2007). Induction of paroxysmal ("epileptiform") EEG alteration in the absence of overt clinical seizure activity may be considered evidence of proconvulsant activity, while concomitant EEG and clinical seizure activity would indicate convulsant activity. The sensitivity may be enhanced by using kindled rats or epileptic rodents for such EEG studies. A simplified, widely used version of this approach is administration of a test compound at increasing doses to normal rats or mice and monitoring of clinical (behavioral) seizure activity without EEG, but this approach likely misses proconvulsant, subconvulsive activity of a substance.

Finally, proconvulsant or convulsant drug activity may be also studied in vitro. For instance, Saboory et al. (2007) used a preparation of mouse hippocampi in low magnesium, which induces seizure-like events (SLEs). Morphine suppressed these SLEs at low concentrations (10 μM) but enhanced them at high concentrations (100 μM). The proconvulsant effect could be blocked by naloxone. Thus, this in vitro preparation recapitulated the biphasic (anticonvulsant/proconvulsant) pattern typically found with morphine and some other opioids in in vivo models.

## 6. Conclusions

Timed i.v. infusion of PTZ is a simple, convenient seizure threshold test for identifying proconvulsant drug effects. As shown in this review, the chance that a potentially proconvulsant drug is identified by this test is relatively high, and for several drugs there is good correspondence to clinical experience. However, like all tests, the timed i.v. PTZ infusion seizure test has limitations. A major limitation is the specific mechanism of action of PTZ's convulsant effect, so that testing of drugs in this model may produce both false positive and false negative data. Only a battery of different tests can provide complete and more reliable conclusions about the proconvulsant potential of an investigational drug. This battery should include animals with lowered seizure threshold, e.g., kindled rats or epilepsy-prone rodents. Furthermore, the relation of doses producing (pro)convulsant effects to the therapeutic dose-range of a substance ("therapeutic index") should be considered. For instance, the label "proconvulsant" may be misleading if drug-induced convulsions only occur at doses high above the therapeutic range, but the drug is not proconvulsant or even exerts anticonvulsant effects at lower doses.

Indeed, as discussed in this review, most psychotropic and analgesic drugs bear the unwarranted stigma of being proconvulsant, leading to misconceptions regarding the safety of such drugs. To a great extent, this stigma is based on proconvulsant (or convulsant) activity at supratherapeutic doses of such drugs in animal models, including the PTZ seizure threshold test, and/or on clinical case reports on increased seizure risk associated with a subset of such drugs, often administered at overdose. At therapeutic doses, most psychotropic drugs are not associated with increased seizure risk, including patients with epilepsy or otherwise at risk for seizures (Alper et al., 2007). Misconceptions regarding proconvulsant effects of psychotropic drugs are particularly unfortunate with regard to epilepsy, in which psychiatric comorbidities tend to be undertreated despite markedly elevated rates of depression and suicide (Kanner, 2008). Limited studies of psychotropic drug use in patients with epilepsy demonstrate that these agents, including antidepressants, neuroleptics and CNS stimulants, usually have a positive effect on psychiatric comorbidities without a negative effect on seizure occurrence (Alldredge, 1999; Kanner and Gidal, 2008). As a consequence, stigmatization of drugs as proconvulsant should be replaced by a sound scientific assessment of the relevant proconvulsive potential, if any, at therapeutic doses. As shown in this review, a number of animal models, including the PTZ seizure threshold test, are useful in this regard, provided that the limitations of these models are taken into account.

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