Review

5-HT₃ receptor antagonists and anxiety; a preclinical and clinical review

Berend Olivierᵃᵇᶜ, Ineke van Wijngaardenᵈ, Willem Soudijnᵈ

ᵃDepartment of Psychopharmacology, Faculty of Pharmacy, Rudolf Magnus Institute for Neurosciences, University of Utrecht, Utrecht, The Netherlands
ᵇPsychoGenics Inc., 4 Skyline Drive, Hawthorn, NY 10532, USA
ᶜDepartment of Psychiatry, Yale University School of Medicine, New Haven, CT, USA
dLeiden/Amsterdam, Center for Drug Research, Leiden, The Netherlands

Received 25 May 1999; received in revised form 25 September 1999; accepted 19 October 1999

Abstract

The present paper reviews the evidence for anxiolytic activity of 5-HT₃ receptor antagonists in animal models of anxiety and in clinical trials in humans. Compared to the established anxiolytics (benzodiazepine receptor agonists and, to a lesser extent, 5-HT₁₅ receptor agonists) 5-HT₃ receptor antagonists display a different anxiolytic profile. They are anxiolytic in a limited number of animal anxiety models. If active, they often are very potent and display bell-shaped dose response curves, whereas the ratio between therapeutic activity and side effects appears remarkably large. 5-HT₃ receptor antagonists remain active after chronic dosing and no indications for tolerance, dependence or rebound effects were found, which seems to make these drugs an attractive alternative to the benzodiazepines. However, the large body of animal data indicating a complete lack of psychotropic activity of 5-HT₃ receptor antagonists weakens the prediction of anxiolytic activity in these drugs. Human data are also controversial; some investigators have reported positive effects in anxiety disorders (panic disorder, GAD), others did not. It can be concluded that 5-HT₃ receptor antagonists do not represent a breakthrough in the treatment of various anxiety disorders, as initially suggested.

Keywords: 5-HT₃ receptor; 5-HT₁₅ receptor; 5-HT₃ receptor antagonists; Benzodiazepines; Anxiety; Animal models; Conflict; Potentiated startle; Periaquaductal gray stimulation; Conditioned place preference; Defensive burying; Ultrasonic pup vocalization; Adult ultrasonic vocalization; Defeat-induced analgesia; Elevated plus-maze; Social interaction; Light–dark exploration; Stress-induced hyperthermia; Drug discrimination; Generalized anxiety disorder; Panic disorder

1. Introduction

Traditionally, pharmacological research in the area of anxiety and stress treatment is very much influenced by the availability of anxiolytic drugs. Throughout recorded history ethanol was, and is the standard drug for (self)treatment of feelings of discomfort, tension, anxiety and stress. Mankind has sought purposefully for substances that modify these feelings and in the last century compounds such as bromide salts, paraldehyde and chloral hydrate were used in medical practice for such symptoms.

In the early 1900s the barbiturates were introduced, which were by far the dominant anxiolytic agents throughout the first half of the twentieth century. But considerable concern arose about their safety, leading to the search for better alternatives. This search resulted in development of compounds like meprobamate, but these compounds, which were still considered ‘risky’, were rapidly abandoned when the benzodiazepines were discovered and introduced to the market in the late fifties and early sixties. The benzodiazepines (BDZ) have been, and still are, the drugs of choice for the treatment of anxiety and stress over the last three decades, because of their effectiveness and relative safety. As with most other drugs, continued use revealed side effects, the most serious of them being tolerance and both psychological and physical dependence.

This once again has led to the search for new and better anxiolytics without the side effects of the BDZ. Serotonergic agents seem to be promising in this regard. The accidental finding of buspirone, a 5-HT₁₅ receptor agonist having anxiolytic effects (Goldberg and Finnerty, 1979), focused on the role of serotonin in anxiety processes,
leading to the development of various 5-HT$_{1A}$-anxiolytics, like ipsapirone, gepirone, flesinoxan and others (Yocca, 1990; Glennon et al., 1991a).

Halfway through the eighties, 5-HT$_3$ receptors were identified and this discovery was soon followed by the synthesis of 5-HT$_3$ receptor antagonists. In 1987 the first reports appeared describing the anxiolytic effects of the 5-HT$_3$ receptor antagonist ondansetron in different animal species and models (Costall et al., 1987a), followed by numerous investigations on this and other 5-HT$_3$ receptor antagonists, like tropisetron (ICS 205,930), bemesetron (MDL 72222), granisetron (BRL 43694) and others (Kilpatrick et al., 1990). Although several positive studies were reported, negative studies also appeared quite frequently. The explanation for this perhaps can be found in the fact that the anxiolytic (and other) properties of 5-HT$_3$ receptor antagonists have some unique characteristics. Firstly, anxiolytic effects reported occur at extremely low doses (often in the nano or microgram/kg range). Secondly, often an inverted U-shaped dose-response curve is found and third, the extremely large safety margin between effective dose and side effects (if any) is very remarkable.

Besides the effects of 5-HT$_3$ receptor antagonists on anxiety states (Barnes et al., 1992), these compounds are also claimed to be active in animal models of psychosis (Kilpatrick et al., 1990; Costall et al., 1991), memory and learning (Barnes et al., 1990b), and withdrawal/drug abuse (Costall et al., 1990a,b).

In the present contribution the available animal behavioural data on anxiolytic effects of 5-HT$_3$ receptor antagonists and potential human anxiolytic effects are critically reviewed. Before dealing with the behavioural studies a short overview will be given of the history of the 5-HT$_3$ receptor, the available 5-HT$_3$ receptor antagonists, their localization in the brain and some receptor neurobiology, including (electro)physiology and functional correlates.

1.1. 5-HT$_3$ receptors

The name ‘5-HT$_3$ receptor’ was first proposed in 1986 (Bradley et al., 1986), and immediately gained worldwide acceptance. In fact this was a mere renaming of the ‘serotonin-M’ receptor, as described by Gaddum and Picarelli (1957). The first reference to this receptor however, dates from 1953, when Rocha e Silva and coworkers reported on the antagonistic activity of cocaine against serotonin-induced contractions of guinea pig ileum. It was not until the late seventies that studies by Fozard et al. (1978, 1979) using metoclopramide and cocaine renewed interest in what are now called 5-HT$_3$ receptors. The subsequent discovery of extremely potent and selective 5-HT$_3$ receptor antagonists ignited an explosion of research on 5-HT$_3$ receptor pharmacology.

1.2. 5-HT$_3$ receptor antagonists

Since the first published 5-HT$_3$ (M) receptor antagonist bemesetron (MDL 72222) in 1983, many potent and selective 5-HT$_3$ receptor antagonists, belonging to different chemical classes, have been reported (for reviews see Gaster and King (1997), Gozlan (1997), King (1994) and Kilpatrick et al. (1990).

The best known compounds are tropisetron, granisetron and ondansetron (for reviews see Lee et al. (1993), Yarker and McTavish (1994), Wilde and Markham (1996), respectively). These compounds display nanomolar affinities for 5-HT$_3$ receptors, whereas the affinities for other transmitter receptors tested are at least two orders of magnitude less.

1.3. Structure of the 5-HT$_3$ receptor

The 5-HT$_3$ receptor, a member of the superfamily of ligand gated ion channels (Orteils and Lunt, 1995), is cation selective. Boess et al. (1995) demonstrated that the 5-HT$_3$ receptor isolated from mouse neuroblastoma × rat glioma hybrid cells (NG 108-15) is a pentameric homooligomer that when modelled as a cylinder has a length of 11 nm, a diameter of 8 nm and a central cavity 3 nm in diameter.

In analogy to other members of the superfamily the subunits consist of a large extracellular NH$_2$-terminal with a highly conserved 15 residues containing a Cys–Cys loop, four putative transmembrane segments M$_1$–M$_4$, a large cytoplasmic loop connecting M$_3$ and M$_4$ and a short -COOH terminal (Mariq et al., 1991). In the large cytoplasmic loop there are two potential phosphorylation sites.

Due to the spacing of polar and negatively charged amino acids in the M$_2$ region the M$_2$ helix seems a likely candidate for the lining of the ion channel as was postulated for the structurally closely related neuronal nicotinic acetylcholine receptor (see e.g. Leonard et al., 1988; Unwin, 1989).

The concept that the binding site(s) of 5-HT$_3$ receptor agonists are situated at the extracellular N-terminal is strengthened by the results published, using a receptor chimera whereby the N-terminal of the 5-HT$_3$ receptor subunit is exchanged for (parts of) the N-terminal of the nicotinic α7 receptor subunit. Functional expression of one of the chimaeric constructs was induced by acetylcholine but not by 5-HT and inhibited by nicotinic antagonists.

Cloning of the 5-HT$_3$ receptor subunits from different animal species including man resulted in either a long (5-HT$_3$-A$_k$) or a short (5-HT$_3$-A$_s$) splice variant. In the short variant a string of 6 amino acids (GSDDLGP) is deleted from the large internal loop between M$_1$ and M$_4$ (see Belelli et al., 1995; Miyake et al., 1995 and references therein). The functional properties of the homo-oligomeric
complexes of both variants expressed in different cell types are comparable though not identical with those of the wild type 5-HT$_3$ receptor.

1.4. Localization of central 5-HT$_3$ receptors

Central 5-HT$_3$ receptors have been labelled using various radioligands such as $[^3$H]-GR65630; $[^3$H]-tropisetron; $[^3$H]-Ly 278584; $[^3$H]-(S)-zacopride; $[^125$I]-(S)-zacopride (for review see Gozlan, 1997). Quantitative autoradiographic studies in several species, including man show that the highest concentration of 5-HT$_3$ receptors is present in four areas of the medulla oblongata: nucleus tractus solitarius, dorsal motor nucleus of the vagus nerve, nucleus of the spinal tract of the trigeminal nerve and the area postrema. Lower levels are detectable in cortical areas (the piriform and entorhinal cortex), limbic areas (hippocampus, amygdala, septum), subcortical areas (nucleus accumbens, hypothalamus) and spinal cord (dorsal horn) (for review see Laporte et al., 1992). In situ hybridization experiments confirm the presence of 5-HT$_3$ receptor mRNA in cortical, limbic, subcortical areas and the spinal cord (dorsal horn). In addition, mRNA is also detectable in striatum, olfactory tubercle and ventral horn of the spinal cord (Tecott et al., 1993; Johnson and Heinemann, 1993). No mRNA is found in the nucleus tractus solitarius, the dorsal motor nucleus of the vagus and the area postrema, areas with a high level of 5-HT$_3$ receptors. Tecott et al. (1993) suggest that the lack of mRNA in these areas might be due to a presynaptic localization of 5-HT$_3$ receptors on peripheral afferents to the dorsal vagal complex. This suggestion is in agreement with lesion studies (Tecott et al., 1993) and with ‘in vitro’ and ‘in vivo’ release experiments (vide infra).

The localization of 5-HT$_3$ receptors in the dorsal vagal complex is in good agreement with the antiemetic effect of 5-HT$_3$ receptor antagonists in cancer chemotherapy (for review see Sorbe, 1996). The presence of 5-HT$_3$ receptors in cortical and limbic region is supportive for a possible role of 5-HT$_3$ receptors in ‘emotional’ behaviour (this review).

1.5. Functional correlates of central 5-HT$_3$ receptors

Central 5-HT$_3$ receptors are involved in the release of various neurotransmitters in the brain (for review see Saito et al., 1996). Activation of 5-HT$_3$ receptors facilitates the release of 5-HT from guinea-pig hypothalamic, frontal cortical and hippocampal slices in vitro and rat hippocampus in vivo. This response is blocked by 5-HT$_3$ receptor antagonists, providing support for the anxiolytic effect of these compounds.

Stimulation of 5-HT$_3$ receptors decreases the release of noradrenaline from rat hypothalamic slices and in vivo from rat hippocampus. This result is consistent with the antidepressant effect of 5-HT$_3$ receptor antagonists in the learned helplessness paradigm.

5-HT$_3$ receptor activation increases the release of dopamine in vitro (rat striatal and nigral slices) and in vivo (rat nucleus accumbens). This effect is probably due to a direct or indirect effect on the dopamine transporter or may involve cholecystokinin, which is released by activation of 5-HT$_3$ receptors (Raiteri et al., 1993).

In in vitro experiments using cortical slices or human cortical synaptosomes, acetylcholine release is inhibited by stimulation of 5-HT$_3$ receptors. In vivo, 5-HT has a dual effect on the release of acetylcholine: inhibition in the guinea pig cerebral cortex and stimulation in the rat hippocampus.

2. Animal models of anxiety

There are basically two types of animal behaviour models used to detect anxiolytic drugs. Models can be based on conditioned behaviour and involve responses controlled by operant conditioning procedures. The other type of models involves unconditioned behaviour which rely on natural behavioural reactions and do not require specific training. Often the latter models rely on species-specific responses (e.g. social interaction, ultrasonic vocalization) and are sometimes referred to as ‘ethologically-based’ models (Lister, 1990). Table 1 summarizes these

<table>
<thead>
<tr>
<th>Conditioned models</th>
<th>Unconditioned models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conflict procedures</td>
<td>Light–Dark exploration</td>
</tr>
<tr>
<td>Fear-potentiated startle</td>
<td>Elevated Plus Maze</td>
</tr>
<tr>
<td>Periaqueductal Gray Stimulation</td>
<td>Defensive Burying</td>
</tr>
<tr>
<td>Drug Discrimination</td>
<td>Ultrasonic Vocalization</td>
</tr>
<tr>
<td>Conditioned Tasted Aversion</td>
<td>Social Interaction</td>
</tr>
<tr>
<td>Conditioned Place Preference or Aversion</td>
<td>Defeat-induced Analgesia</td>
</tr>
<tr>
<td>Conditioned ultrasonic vocalization</td>
<td>Stress-induced Hyperthermia</td>
</tr>
</tbody>
</table>
models currently in use to study the anxiolytic effects of drugs.

In the following sections the effects of 5-HT$_3$ receptor antagonists in conditioned and unconditioned anxiolytic models will be described. Each model will be shortly described and its specific character outlined; when possible a short comparison with effects of benzodiazepine and 5-HT$_{1A}$ anxiolytics will be given.

2.1. Conflict models

In conflict models ongoing behaviour is suppressed due to the presentation of aversive consequences for the behaviour. Punished consequences suppress either lever-pressing that is reinforced by food in hungry rats (Geller and Seifter, 1960), key-pecking reinforced by food intake in hungry pigeons (Barrett et al., 1986), water-licking in thirsty rats (Vogel et al., 1971) or exploration of mice of a new environment as in the four-plate test (Aron et al., 1971; Boissier et al., 1968). Signalled or unsignalled delivery of electric shock pulses may be used as aversive consequence. The release of suppressed behaviour without affecting the levels of unpunished responding following pharmacological intervention is predictive of an ‘anxiolytic’ effect. In rodents, pigeons and primates benzodiazepines (BDZ) are consistently found to be effective in these models (Barrett and Gleeson, 1991; Barrett, 1991).

In rat conflict models non-benzodiazepine anxiolytics such as buspirone have no or only weak anxiolytic effects (Barrett and Gleeson, 1991). Increases in punished responding in rats after 5-HT$_{1A}$ receptor agonists are more likely seen with the Vogel-type procedure than with the Geller conflict procedure (Barrett and Gleeson, 1991). In contrast, Gleeson et al. (1989) found anxiolytic activity not only of 5-HT$_{1A}$ receptor agonists in pigeons but to a lesser extent also of mixed 5-HT$_1$ receptor agonists and 5-HT$_2$ receptor antagonists (Barrett and Gleeson, 1991).

5-HT$_3$ receptor antagonists have been studied over a wide range of doses. In rats, Jones et al. (1988) examined ondansetron in a dose range of 0.05–1.6 mg/kg IP in the Vogel water lick test, whereas Piper et al. (1988) examined ondansetron (0.0005–5 mg/kg PO) and BRL 43694 (0.0005–50 mg/kg PO) both in food-reinforced and water-reinforced conflict tests. Dunn et al. (1991) studied ondansetron (0.01–0.1 mg/kg IP), zacopride (0.1–1 mg/kg IP), tropisetron (0.5–1 mg/kg IP) and bemesetron (MDL 72222) (10–20 mg/kg IP) in a modified food–reinforced conflict procedure. Higgins et al. (1991) studied ondansetron (10–1000 ng) and tropisetron (1–1000 ng) in the water-lick conflict test following microinjections in the amygdala. Borsini et al. (1993) studied several 5-HT$_3$ receptor antagonists in a conflict test. Cervo and Samanin (1995) studied ondansetron and tropisetron in punished responding in rats. None of these studies reported any anxiolytic activity in conflict tests. However, Filip et al. (1992) found anxiolytic activity in the Vogel-lick test for some (ondansetron, granisetron, zacopride, tropisetron) but not all (bemesetron, DAU 6215) 5-HT$_3$ receptor antagonists. In a modified Geller-Seifter procedure, extinction of conflict behaviour (Ketelaars et al., 1988), which is sensitive for benzodiazepines and 5-HT$_{1A}$ receptor agonists, tropisetron was also inactive (Ketelaars and Bruinvels, 1991). In the four plate test (Mos et al., 1989) no anxiolytic effects of ondansetron were found. In pigeons, Gleeson et al. (1989) studied ondansetron (0.001–1 mg/kg IM), tropisetron (0.001–0.3 mg/kg IM) and bemesetron (0.01–3 mg/kg IM). Ondansetron exhibited no anxiolytic activity at any dose, but tropisetron showed an anxiolytic effect at two doses (0.003 and 0.01 mg/kg) and bemesetron at one dose (0.03 mg/kg). Since increases in punished responding were variable within as well as across animals, and often were not replicated when the effects of the relevant doses were redetermined, the authors conclude that tropisetron and bemesetron were at best only marginally active in the conflict test, which was confirmed by Colpaert and Koek (1991). Artaiz et al. (1995) studied the 5-HT$_3$ receptor antagonists ondansetron, granisetron, tropisetron and VA 21B7 (3-[2-(4-piperonylpiperazinyl)-phenyl]propionic acid) in a Vogel test in rats. Ondansetron, granisetron and tropisetron were all anxiolytic in a narrow dose range showing a bell-shaped dose-response curve, whereas VA 21B7 was anxiolytic (both IP and PO) over the whole dose range tested.

Overall, 5-HT$_3$ receptor antagonists do not show up as consistent anxiolytics in conflict tests. If active, very narrow dose-ranges are general found. Compared to benzodiazepines, 5-HT$_3$ receptor antagonists can be considered as very weakly anxiolytic in these procedures, which are considered as very reliable anxiolytic paradigms.

2.2. Fear-potentiated startle response

A model consisting of a mixture of conditioned and unconditioned behaviour is the fear-potentiated startle response (Davis, 1979, 1986).

The startle response of a rat to a loud tone can be augmented by prior pavlovian fear conditioning. During the fear conditioning phase a light stimulus, serving as the conditioned stimulus, may signal the presentation of the light will augment the startle response (Davis, 1979, 1986). The startle response of a rat to a loud tone can be increased by prior presentation of the light stimulus, serving as the conditioned stimulus signals the presence of a shock (unconditioned stimulus). During the startle response measurement, presentation of the light will augment the startle amplitude. Both the anxiolytic effect of benzodiazepines and the anxiogenic effect of yohimbine and DMCM can be detected in this model (Davis, 1979, 1986; Davis et al., 1979; Glenn and Green, 1989; Hijzen et al., 1995). The partial 5-HT$_{1A}$ receptor agonists buspirone, gepirone (Davis et al., 1988; Kehne et al., 1988) and the full 5-HT$_{1A}$ receptor agonists desipramine and 8-OH-DPAT (Joordens et al., 1996, 1998) are also effective in this model. However, Davis et al. (1988) found that the full agonist 8-OH-DPAT and the partial agonist ipsapirone are not effective and the effect of buspirone could not be antagonised by 5-HT.
receptor antagonists or lesioning of the 5-HT system (Davis et al., 1988). In line with this, Joordens et al. (1998) also were not able to antagonize the anxiolytic effects of 8-OH-DPAT and fleisinoxan by 5-HT\textsubscript{1A} receptor antagonists. The latter findings suggest that 5-HT\textsubscript{1A} receptor stimulation is not involved in the anxiolytic profile of 5-HT\textsubscript{1A} receptor agonists in this model. Glenn and Green (1989) showed, in a somewhat modified procedure, that ondansetron reduced the potentiated startle amplitude in this model, suggesting anxiolytic activity comparable to the benzodiazepines. Nevins and Anthony (1994) studied the effects of ondansetron, (R)-zacopride and granisetron in the fear-potentiated startle paradigm using two training protocols, one similar to those used in most laboratories to test for anxiolytic anxiety and one using a less intensive aversive stimulation. Their hypothesis was that 5-HT\textsubscript{3} receptor antagonists are more effective under low aversive than under high aversive conditions. Under high aversive conditions, the control substances diazepam and buspirone effectively reduced the potentiated startle, but neither of the 5-HT\textsubscript{3} receptor antagonists did so, whereas under low aversive conditions both diazepam/buspirone and the 5-HT\textsubscript{3} receptor antagonists were able to reduce it. Bill et al. (1992) showed that the 5-HT\textsubscript{3} receptor antagonist WAY 100289 was active in the rat fear potentiated startle paradigm, showing a bell-shaped dose-response curve like ondansetron in the Glenn and Green (1989) study. Nevins and Anthony (1994) suggest that decreasing the shock intensity as a tool to manipulate anxiety, may either produce qualitatively different types of conditioned fear or alternatively may activate different brain mechanisms involved in the generation of different anxiety-like states. In any case, these data suggest that 5-HT\textsubscript{3} receptor antagonists may display anxiolytic activity only under mild aversive conditions.

2.3. Periaquaductal gray stimulation

Electrical stimulation of specific sites in the midbrain central gray (PAG) of rats induces aversive effects. Animals can readily be trained to switch off the current to escape the aversive stimulation.

This paradigm has been used by Graeff and coworkers (Graeff, 1984; Graeff et al., 1988) who reported anti-aversive effects of benzodiazepines, a finding replicated by Bovier et al. (1982). This suggests that aversive PAG stimulation may be of interest for the study of mechanisms possibly underlying fear and anxiety. In contrast to what would be expected if this were the case, the 5-HT\textsubscript{1A} receptor agonist 8-OH-DPAT had anxiogenic properties (Jenck et al., 1989b), whereas the 5-HT\textsubscript{2c} receptor agonist ketanserin had anxiolytic effects in this model, an effect shared with 5-HT\textsubscript{2c} receptor agonists (Jenck et al., 1990), of which mCPP has been reported to be clinically anxiogenic in sensitive patients. Thus, it can be stated that the observed effects in this model are equivocal with regard to potential anxiolytic effects. Using this paradigm Jenck et al. (1989a) have studied the effects of 5-HT\textsubscript{3} receptor antagonists. Tropisetron, bemesetron and ondansetron had no effects, neither anti-aversive nor pro-aversive. The predictive validity of this model as an anxiolytic test is not established yet, but regarding the effects of the benzodiazepines and the serotonergic drugs, the absence of any effect of 5-HT\textsubscript{3} receptor antagonists points to their weak or absent psychotrophic activities.

2.4. Defensive burying

Defensive burying, i.e. pushing and spraying of bedding material with alternating thrusting movements of forepaws and shovelling movements of the snout directed towards a discrete source of unfamiliar, aversive or noxious stimuli, has been demonstrated in rats (Pinel and Treit, 1978), mice (Broekkamp et al., 1986), gerbils (Davis et al., 1982) and ground squirrels (Heynen et al., 1989). The behaviour is interpreted as an unconditioned, species-specific response toward certain olfactory (Jackson et al., 1984; Pinel et al., 1981), tactile (Broekkamp et al., 1986) and visual stimuli (Pinel et al., 1980), which will elicit avoidance behaviour under appropriate conditions. Together with flight, freezing and certain forms of agonistic behaviour it constitutes the defensive behavioural repertoire in these species.

Defensive burying is also easily evoked as a conditioned response toward originally neutral stimuli with acquired aversive properties, e.g. by one-trial experience with electric shock through a wire-wrapped dowel (Hudson, 1950; Pinel and Treit, 1978). Defensive burying, both as a conditioned and an unconditioned response, has been proposed as a useful paradigm for screening of novel anxiolytic drugs (Treit et al., 1981; Treit, 1985; Broekkamp et al., 1986).

Anxiolytics belonging to two different pharmacological classes (benzodiazepines, 5-HT\textsubscript{1A} receptor agonists) have been found to suppress defensive burying both in rats and mice (De Boer et al., 1991), though the behavioural selectivity of the effects of 5-HT\textsubscript{1A} receptor agonists has been questioned in mice (Broekkamp et al., 1989). Broekkamp and Jenck (1989) and Njung‘e and Handley (1991) did find a selective blockade by a number of 5-HT uptake-blocking drugs. Both groups also studied the effects of ondansetron and tropisetron in a wide dose range. Both 5-HT\textsubscript{3} receptor antagonists were found ineffective. Njung‘e and Handley (1991) found however, that tropisetron in the very high dose of 10 mg/kg (IP) facilitated the inhibition induced by zimelidine (10 mg/kg IP). It should be noticed that various other 5-HT receptor antagonists had a similar potentiating effect, thereby questioning the specificity of 5-HT\textsubscript{3} receptors in this case.

2.5. Ultrasonic pup vocalisation

In the rat, removal of the pup from its nest, mother and
littermates elicits ultrasonic calling. These ultrasounds are in the frequency range of 30 to 50 kHz. The function of calling is probably to alert the mother and to stimulate her to search and retrieve pups (Allin and Banks, 1972). Ultrasonic calling shows a characteristic developmental pattern. Hård et al. (1982) have shown that sounds increase gradually to peak levels at about 9 to 11 days. Thereafter it gradually decreases and disappears around 16 to 18 days of age.

Several authors have reported that ultrasonic calling is sensitive to treatment with clinically proven anxiolytics as well as with putative anxiolytic compounds (Winslow and Insel, 1991). Gardner and Budram (1987) and many others (cf. Olivier et al. 1998a for a review) have reported that benzodiazepines reduce ultrasounds in rats, and similar results have been found by Nastiti et al. (1991) in mice. Conversely, benzodiazepine inverse receptor agonists increase ultrasounds suggestive of anxiogenic effects. 5-HT\textsubscript{1A} anxiolytics like buspirone, fluoxetine and ipsapirone reduce pup sounds too (Mos and Olivier, 1989; Winslow and Insel, 1991; Olivier et al., 1998b). These authors also established the fact that different 5-HT receptor agonists have differential effects on pup calls. Reuptake blockers of serotonin also reduce ultrasounds (Winslow and Insel, 1991; Mos and Olivier, 1989; Olivier et al., 1998b), but noradrenaline reuptake inhibitors like desipramine increase calling. Although this paradigm, like any other anxiety test, has its drawbacks and limitations, all authors so far have agreed upon the effects of benzodiazepines and 5-HT\textsubscript{1A} anxiolytics as reducing ultrasounds.

Not many studies have been reported on the effects of 5-HT\textsubscript{1A} receptor antagonists on rat pup ultrasounds. Mos et al. (1989), Mos and Olivier (1989) and Olivier et al. (1998b), did not observe any significant change in response to treatment with ondansetron over a wide dose range (0.001–1.0 mg/kg IP) both in a stressful (18°C) as well as in a less stressful (37°C) condition. Kehne et al. (1991) also reported the lack of effects on rat pup calls after MDL 73,147EF (1.25–10 mg/kg). Olivier et al. (1998b) tested the 5-HT\textsubscript{1A} receptor agonist phenylbiguanide (1–10 mg/kg IP) under high (18°C) and low (37°C) stress conditions and found neither effects on ultrasounds, nor on the negative geotaxis, a measure for side effects. In a distress vocalization paradigm in guinea pig pups (sonic vocalizations), ondansetron (0.001–0.1 mg/kg IP), in contrast to benzodiazepines and 5-HT\textsubscript{1A} receptor agonists, was not able to change the vocalizations (Molewijk et al., 1996).

No other publications have dealt with 5-HT\textsubscript{3} receptor ligands in such paradigms. Since in this test the results of most other compounds is quite comparable between different laboratories, it must be assumed that minor modifications of experimental procedures by other investigators will not lead to positive results with 5-HT\textsubscript{3} receptor antagonists.

It is as yet not clear why 5-HT\textsubscript{3} receptor antagonists have no effects whatsoever in the pup ultrasonic test. One possibility is that 5-HT\textsubscript{3} receptors in the CNS are not properly functioning at this age.

### 2.6. Vocalizations in aversive situations

Adult rats emit ultrasonic vocalizations (in approximately the 20–28 kHz range) under various aversive conditions such as, for example, in the presence of a predator (Blanchard et al., 1991), an aggressive male opponent (Tornatzky and Miczek, 1995; Vivian and Miczek, 1993), after exposure to a painful shock (Molewijk et al., 1995, Van der Poel et al., 1989), a loud acoustic (Kaltwasser, 1991; Miczek and Vivian, 1993) or tactile startle stimulus (Barros and Miczek, 1996).

Interestingly, rats can also produce ultrasonic vocalizations in association with a prior aversive event without the actual physical presence of threat. This latter feature may have face validity with regard to situational panic attacks, when environmental stimuli may acquire aversive properties and become triggers for panic attacks (Molewijk et al., 1995). Ultrasonic vocalizations in adult rats may represent affective expressions by rats exposed to threatening, startling or painful stimuli (cf. Miczek et al., 1995).

Socially experienced rats emit ultrasonic vocalizations at a higher rate after being withdrawn from chronic treatment with opioid drugs in a time- and dose-dependent manner (Vivian and Miczek, 1993). Similarly, when rats are startled 24 h after the last of 10 diazepam injections, they emit high rates of ultrasonic vocalizations (Miczek and Vivian, 1993). These ultrasonic vocalizations during withdrawal from diazepam can be attenuated with the 5-HT\textsubscript{1A} partial receptor agonist gepirone or with diazepam (Vivian and Miczek, 1993). Withdrawal from cocaine, either via long-term access via the drinking fluid or via intravenous self-administration in ‘binges’, produces large increases in ultrasounds in rats, mimicking the clinically observed ‘crash’ phase (Barros and Miczek, 1996).

Anxiolytic targets at 5-HT\textsubscript{1A} and benzodiazepine-GABA\textsubscript{A} receptors decrease these ultrasounds, when rats are exposed to acoustic startle stimuli (Kaltwasser, 1991) or to footshock, but not to tail shock (Cuomo et al., 1988; De Vrij et al., 1993; Sanchez, 1993), and also when they are anticipating a confrontation with an aggressive opponent (Tornatzky and Miczek, 1995).

Under a conditioning procedure, when rats were exposed to the environment where they have received electric foot shock previously, they emitted fewer ultrasounds after treatment with 5-HT\textsubscript{1A} receptor agonists, but not with benzodiazepines, except for alprazolam (Molewijk et al., 1995). This pattern of results suggests that this experimental procedure may serve as model for detecting anti-panic activity. In this procedure, ondansetron (0.001–0.1 mg/kg IP) had no activity whatsoever. Schreiber et al. (1998) found that ondansetron (0.1 mg/kg IP) was not able to...
antagonize the decrease in ultrasonic vocalizations induced by paroxetine, a selective serotonin reuptake inhibitor.

2.7. Defeat-induced analgesia in mice

A characteristic feature of conspecific defence in mice is the reduction in pain sensitivity that follows the experience of defeat by a conspecific. Mice exposed to a resident in his territory show two different forms of analgesia upon defeat. In one, an opioid substrate is involved. The other is a non-opioid form, and behavioural and pharmacological evidence indicates that this is a consequence of anxiety provoked by ecologically relevant aspects of the stimulus situation (Rodgers and Randall, 1986; 1986b). Rodgers and coworkers (Rodgers and Randall, 1987, 1987a, 1988) have shown that benzodiazepines block the non-opioid short-lasting analgesic effects of defeat, i.e. mice show no longer the decrease in pain sensitivity. However, non-neuronal benzodiazepine agonists also reduced defeat-induced analgesia, whereas for example chlordiazepoxide and midazolam were without effect. Thus the precise role of the benzodiazepine receptor is not yet definitively established.

5-HT<sub>1A</sub> receptor agonists like buspirone, ipsapirone and gepirone also blocked the non-opioid defeat-induced analgesia, although bell-shaped dose-response curves were observed for buspirone and ipsapirone (Rodgers and Randall, 1988). The effects of benzodiazepines and 5-HT<sub>1A</sub> receptor agonists, although far from fully understood, point to a role of anticipatory anxiety in the non-opioid defeat-induced or defensive analgesia (Rodgers, 1991).

The first report on 5-HT<sub>3</sub> receptor antagonists (Rodgers et al., 1990) showed that ondansetron suppressed the defeat-induced analgesia. Similar, but less strong reactions were also observed for tropisetron, MDL 72222 and MDL 73147 (Rodgers and Shepherd, 1990). It is therefore suggested that 5-HT<sub>3</sub> receptor antagonists might act as anxiolytics in this paradigm, although a peripheral mechanism of action cannot be excluded.

2.8. Elevated plus-maze

The elevated plus maze as used by Handley and Mithani (1984) is a procedure that relies on exploratory behaviour by rats that are placed in an elevated maze, consisting of two (opposite) open and two walled alleys. The animals will explore the different alleys (total number of entries). The open arms are more aversive to the rats than the closed ones, as revealed by a preference of the animals to explore the closed alleys. Anxiolytic drugs will help to overcome the fear-induced inhibition of open alley exploration. The ratio of open- versus total arm entries gives a sensitive measure of the anxiolytic effect of a drug and this measure is not very sensitive for general effects on exploration. Recently, modifications of the plus-maze have been developed, like the zero-maze (Shepherd and Rodgers, 1990).

In these paradigms the anxiolytic activity of benzodiazepines can reliably be demonstrated by the increase in percentage of open arm entries and time spent in the open arms. Drugs stimulating the 5-HT<sub>1A</sub> receptor, such as 8-OH-DPAT, buspirone and ipsapirone are difficult to detect as anxiolytic or have either been reported to be ineffective or even to show an opposite effect, i.e. decreasing the percentage of entries or time spent in the open arms (File et al., 1987; Pellow et al., 1987; Critchley and Handley, 1987; Moser, 1989; Griebel, 1995). This latter effect may be due to some anxiogenic effects of these drugs, but it may also represent a ‘false negative’ in this test. However, using ethologically derived measures in this paradigm (risk assessment and exploration) 5-HT<sub>1A</sub> receptor agonists have anxiolytic potential (Griebel et al., 1997).

Similar results have been reported for 5-HT<sub>2</sub> receptor antagonists. Thus the 5-HT<sub>3</sub> receptor antagonist ritanserin has been reported to show both anxiolytic and anxiogenic activity in this model (Pellow et al., 1987; Critchley and Handley, 1987).

Costall et al. (1989a, 1989b) reported an anxiolytic effect of the 5-HT<sub>3</sub> receptor antagonists ondansetron, zacopride, tropisetron, bemesetron, granisetron and RS-42358-197 (Costall et al., 1993) as revealed by the increase in time spent exploring the open arms of an elevated plus-maze. Similar results were found by Dunn et al. (1991) and Silvestre et al. (1996) for granisetron. File and Johnston (1989), Piper et al. (1988), Kshama et al. (1990) and Rodgers et al. (1995) failed to detect any effect of various 5-HT<sub>3</sub> receptor antagonists in the same test.

Griebel et al. (1997) tested (R,S)-zacopride, ondansetron, tropisetron and bemesetron in a rat elevated plus-maze test using spatiotemporal (open arm time/entry) and ethologically derived measures (risk-assessment/exploration). Over quite a dose-range (0.01–1 mg/kg SC) no effect on spatiotemporal parameters were found, whereas only zacopride and ondansetron reduced the number of aborted attempts to enter the open arms. It is unclear why different 5-HT<sub>3</sub> receptor antagonists display differential effects.

Andrews and File (1993) showed that (R, S) Zocapride had an anxiolytic effect in this model only in handling-naive rats, but not in handling-habituated rats. (R, S) Zocapride is considered a partial receptor agonist, which, according to Andrews and File (1993) may behave as a weak agonist in conditions of low 5-HT functioning, but as a full antagonist under conditions of high 5-HT functioning.

Rodgers et al. (1997b) administered ondansetron (0.1–100 µg/kg) subchronically (21 days) to mice, and found no evidence for anxiolytic activity using an ethologically derived elevated-plus maze scoring system in mice, in contrast to chlordiazepoxide (Rodgers et al., 1997a).
The lack of effect and the inconsistency of effects of new putative anxiolytic drugs such as 5-HT\textsubscript{1A} receptor agonists and 5-HT\textsubscript{3} receptor antagonists is at variance with the effects of classic anxiolytics in the elevated plus-maze (Moser, 1989).

2.9. Social interaction test

In this test, first described by File (1980), either two rats or two mice unfamiliar to each other are placed in an arena. This arena may either be familiar or unfamiliar to the animals, and may be brightly lit or not. The most aversive situation is obviously a brightly lit, unfamiliar environment in which low levels of social interaction occur. The time spent by the animals in social interaction is measured as well as locomotor activity. Benzodiazepines increase the time spent in social interaction. A similar effect has been reported for the 5-HT\textsubscript{1A} receptor agonists buspirone and ipsapirone (Gardner, 1985; Schuurman et al., 1987), though File (1985) could not confirm the anxiolytic potential of buspirone in this model.

The 5-HT\textsubscript{3} receptor antagonist ondansetron increased social interaction (Jones et al., 1988; Piper et al., 1988; Dunn et al., 1991), a result which could not be confirmed by File and Johnston (1989). Controversies exist over zacopride (active according to Dunn et al., 1991 and Costall et al., 1988a,b,c; not active according to File and Johnston, 1989) and granisetron (active according to Piper et al., 1988 and Kennett et al., 1990; inactive according to File and Johnston, 1989). Dunn et al. (1991) found the anxiolytic activity of bemesetron in this paradigm at relatively high doses concomitantly producing sedation. RS-42358-197 appeared a very potent 5-HT\textsubscript{3} receptor antagonist in this test (Costall et al., 1993). Cutler (1990) reported anxiolytic effects of granisetron and tropisetron in a social interaction test in gerbils.

Because 5-HT\textsubscript{1A} receptor antagonists may display anxiolytic effects, 5-HT\textsubscript{3} receptor agonists may exert anxiogenic effects. Mitchell et al. (1991) tested metachlorophenylbiguanide (mCPBG; intraventricularly, because they suggest that the compound does not penetrate the blood-brain barrier) in the social interaction test in rats. mCPBG reduced social interaction under low light/familiar conditions, but not under high light/unfamiliar condition, which is considered the most anxiogenic condition. Apparently, when anxiety levels are not high, a 5-HT\textsubscript{3} receptor agonist may have anxiogenic effects.

Thus some groups report an anxiolytic profile of the new drug types (5-HT\textsubscript{1A} receptor agonists and 5-HT\textsubscript{3} receptor antagonists) whereas others fail to find such an effect. The recent findings with the optical isomers of zacopride add further confusion to the understanding of possible anxiolytic activity of 5-HT\textsubscript{3} receptor antagonists. It was reported that the least active R(+) isomer of zacopride (defined biochemically; Pinkus et al., 1990; Waebber et al., 1990) increased social interaction, whereas the more active S(-)-zacopride was devoid of activity (Barnes et al., 1990a). The finding that S(-)-zacopride exerted 5-HT\textsubscript{3} receptor agonistic activity in the ferret, whereas the R(+) isomer behaved as an antagonist (Middlefell et al., 1990) may (partly) explain the confusing effects of racemic zacopride.

2.10. Light-dark exploration

This model was originally described by Crawley and Goodwin (1980) and is based on the natural aversion of mice and rats for brightly lit places. In a two compartment box, one dark and one brightly lit, the total activity, the time spent in the light compartment and the crossings between the light and dark compartment provides information about the preference of the animal for the dark compartment. As anxiolytics should reduce the natural aversion to light, the essential feature of this model is that anxiolytic drugs increase the number of crossings and/or the time spent in the light compartment. The latter parameter is generally considered to be the most relevant one.

Benzodiazepines are reliably detected in this paradigm using mice (Crawley, 1981; Jones et al., 1988; Kilfoil et al., 1989; Costall et al., 1989c). Also the 5-HT\textsubscript{1A} receptor agonist buspirone has been reported to show an anxiolytic profile in this test (Costall et al., 1988b; Carli et al., 1989; Kilfoil et al., 1989). The 5-HT\textsubscript{3} receptor antagonists ondansetron, bemesetron, tropisetron, zacopride, itasetron (DAU 6215), WAY 100289, RS-42385-197 and WAY-SEC-579 have been reported to produce similar effects as the benzodiazepines (Costall et al., 1987a, 1993; Kilfoil et al., 1989; Onavi and Martin, 1989; Bill et al., 1992; Borsini et al., 1993; Middlefell et al., 1996a,b). Morinan (1989) did not find anxiolytic activity of ondansetron and BRL 24924 in a rat black/white test box, but Sanchez (1996) found ondansetron active in a similar set-up. Also, Kshama et al. (1990) using mice were not able to detect anxiolytic activity in a number of 5-HT\textsubscript{3} receptor antagonists. The anxiolytic activity of the two isomers of zacopride does not parallel their 5-HT\textsubscript{3} receptor blocking activity (Pinkus et al., 1990; Waebber et al., 1990). The most active antagonist has been found to be less active than the weaker R(+) isomer of zacopride, which is highly active (Barnes et al., 1990a; Young and Johnson, 1991). On the other hand, Bill et al. (1991) could not find differences in the anxiolytic potential of R(+), S(-) and racemic zacopride in the mouse light/dark box using female mice. Moreover, they found a good correlation between the minimally effective doses in the light/dark box and the minimum doses required to block the von Bezold–Jarisch reflex in mice. This may point to species differences. Both isomers of zacopride possess long acting anxiolytic-like effects (>16 h) (Young and Johnson, 1991).

Costall and Naylor (1991) tested ondansetron for 14
days (10 µg/kg IP b.i.d.) and showed that it retained its anxiolytic activity in a white/dark box in mice and in a social interaction test in rats. Moreover, no evidence for tolerance or sensitization occurred. After stopping treatment, the anxiolytic effects slowly waned until control levels were reached after about 96 h. No rebound anxiety was observed. A similar pattern was observed with buspirone (1.0 mg/kg IP b.i.d.), but not with diazepam, which, upon cessation of treatment, led to anxiogenic effects. When diazepam (7 days treatment, 10 mg/kg IP b.i.d.) was withdrawn, a clear anxiogenic effect was noted when mice were tested 8, 24, 48 and 96 h after withdrawal in the black-white exploration test (Costall and Naylor, 1991). This withdrawal anxiety could not be antagonized by buspirone, but ondansetron (10 µg/kg IP b.i.d.) was able not only to restore control levels but indeed gain the anxiolytic level previously obtained by diazepam. A similar result was found in the social interaction test in rats. RS-42358-197 (Costall et al., 1993) appeared very effective in reducing withdrawal anxiety after stopping treatment with alcohol, nicotine, cocaine or diazepam. WAY-SEC-579 was also able to antagonize the anxiolytic-like profile after diazepam-withdrawal in the light/dark box (Middlefell et al., 1996a,b). Repeated co-administration of WAY-SEC-579 with diazepam prevented the development of diazepam withdrawal-induced anxiety (Middlefell et al., 1996a,b). WAY-SEC-579 itself did not lead to withdrawal anxiety. However, when rats were made completely tolerant to the anxiolytic effects of oxazepam in a modified light-dark box (21 days, 5 mg/kg/day IP), ondansetron (0.1 mg/kg IP) did not have anxiolytic activity, whereas in partially tolerant rats (7 or 14 days oxazepam), ondansetron was active. Apparently, ondansetron displays different activities during stages of full tolerance or withdrawal (Nowakowska et al., 1998).

The most unequivocal results with the new types of anxiolytic drugs are obtained in this model. The results obtained with the isomers of zacopride, however, suggest the involvement of another (subtype?) receptor, at least in rats.

2.11. Stress-induced hyperthermia

The arousing effect of handling can induce hyperthermia in rats (Poole and Stephanson, 1977; Eikelboom, 1986). Using this phenomenon of stress- or handling-induced hyperthermia in mice, Borsini et al. (1989) suggested that the higher rectal temperature of mice removed last from their home cage compared to those removed first from the same cage can be regarded as a form of anticipatory anxiety.

This increase in rectal temperature was prevented in a dose dependent way by prior treatment with benzodiazepines and the 5-HT$_{1A}$ receptor agonists 8-OH-DPAT and buspirone, and was not affected by non-anxiolytic compounds (e.g. imipramine, haloperidol) (Lecci et al., 1990a,b). The same authors showed that ondansetron did not affect the stress-induced hyperthermia, which was confirmed by Zethof et al. (1991, 1994, 1995). Borsini et al. (1993) could also find no evidence for anxiolytic effects of 5-HT$_3$ receptor antagonists in the stress-induced hyperthermia test.

2.12. Drug discrimination

Drug discrimination has not been developed as a model for anxiety and cannot simply be fitted into a categorization of animal models as homologous, physiological or empirical (Slangen, 1991). It is suggested that the drug-discrimination procedure in animals is the closest available experimental model for assessing the subjective effects of drugs. Moreover, drug discrimination studies may provide additional information about receptor mechanisms involved in the action of diverse anxiolytic drugs. Drug discrimination procedures can reveal very subtle differences in the mechanism of action of different drugs, as e.g. evidenced in the 5-HT system (Stolerman et al., 1987). Drug discrimination seems remarkably sensitive for in vivo measurement of a selective drug effect, especially with regard to the mechanism of action or the involvement of various neurochemical systems. In this sense it is worthwhile to consider whether it is feasible that manipulation of a central 5-HT$_3$ receptor leads to a discriminative stimulus (cue) for an animal. The available evidence is very meager in contrast to the evidence on 5-HT$_{1A}$ receptor agonists and benzodiazepines (Barrett and Gleeson, 1991). Glennon et al. (1991b, 1992) describe that the 5-HT$_3$ receptor agonists 2-methyl-5-HT (5 mg/kg) and metachlorophenylbiguanide (mCPBG) could be discriminated from saline. The 2-methyl 5-HT stimulus (ED$_{50}$=2.6 mg/kg) generalized to mCPBG (ED$_{50}$=1.7 mg/kg) and was antagonized by zacopride and tropisetron, but never completely by the quaternary salt of tropisetron, suggesting that the cue was centrally mediated. Till now no reports of 5-HT$_3$ receptor antagonists as training stimuli have appeared, although Tricklebank (1989) suggests that tropisetron has weak stimulus properties. 5-HT$_3$ receptor antagonists have been used as tools in order to study whether substitution or antagonism of a certain drug cue occurs. 5-HT$_3$ receptor antagonists were neither able to antagonize 5-HT$_{1A}$ receptor mediated cues (Arnt, 1989; Nader et al., 1989; Ybema et al., 1991), nor a mixed 5-HT$_{1A/1B}$ receptor mediated cue (eltoprazine; Ybema et al., 1992). The 5-HT$_3$ receptor antagonists tropisetron and bemesetron, but not zacopride were able to antagonize the ethanol cue in pigeons (Grant and Barrett, 1991) and in rats (Grant and Colombo, 1993). Although structural/chemical features may underlie the differential effects of tropisetron and bemesetron on one side and zacopride on the other side in antagonizing the ethanol cue in pigeons, it is also possible that the 5-HT$_4$ receptor agonistic activity of
zacopride (Bockaert et al., 1990) interferes with the 5-HT3 receptor antagonistic activity.

Stefanski et al. (1996) trained rats on ethanol (1 g/kg) versus saline in a two lever operant conditioning paradigm. None of a very broad range of doses (0.001–10 mg/kg) of the two 5-HT3 receptor antagonists tropisetron or ondansetron was able to antagonize the discriminative stimulus properties of ethanol. The 5-HT3 receptor agonist mCPBG, centrally or intraperitoneally administered, did not substitute for the ethanol cue. In an alternative to operant drug discrimination, the cross-familiarization conditioned taste aversion (CTA) procedure, pre-exposure to centrally administered mCPBG was also not able to alter the ethanol-induced CTA, whereas ethanol itself was (Bienkowski et al., 1998). These data suggest that 5-HT3 receptors are not primarily involved in ethanol’s stimulus properties. Stefanski et al. (1996) discuss several possible reasons why their findings deviate from those found by Grant and Barrett (1991) and Grant and Colombo (1993), e.g. dose, route of administration, and training dose of ethanol.

Similarly, 5-HT3 receptor antagonists were not able to antagonize the discriminative stimulus properties of other major drugs of abuse, such as morphine (Joharchi et al., 1993), cocaine (Paris and Cunningham, 1991; Lane et al., 1992; Schechter, 1993; Johanson and Barrett, 1995; De La Garza et al., 1996) or d-amphetamine (Moser, 1992). Cocaine has affinity for the 5-HT3 receptor (Kilpatrick et al., 1987) and has structural similarity to some 5-HT3 receptor antagonists, e.g. tropisetron and bemesetron (Richardson et al., 1985). In cocaine-trained (10 mg/kg) rats (Paris and Cunningham, 1991), tropisetron (2–24 mg/kg) and bemesetron (2–16 mg/kg) were not able to either substitute for or antagonize the cocaine-cue. This was somewhat unexpected because 5-HT3 receptor blockade can interfere with the behavioural effects of several drugs of abuse like cocaine or amphetamine (Costall et al., 1987b; Van der Heek and Cooper, 1990). Apparently, stimulation of 5-HT3 receptors does not play an important role in the discriminative stimulus and rewarding properties of stimulants (d-amphetamine, cocaine), although they may do in the case of alcohol (Grant and Barrett, 1991). However, the 5-HT3 receptor agonist mCPBG showed partial substitution (36%) for the cocaine stimulus in rats trained to discriminate 10 mg/kg cocaine from saline. Moreover, pretreatment with a 5.6 mg/kg dose of mCPBG shifted the dose-response curve for lower doses of cocaine to the left, but not for higher doses (Koetzner et al., 1995). These data indicate only a very limited role for 5-HT3 receptors in cocaine’s discriminative stimulus, which was further supported by a study where animals were either trained on cocaine or on mCPBG (De La Garza et al., 1996). mCPBG only very marginally (max. 50%) substituted for cocaine, whereas coadministration of mCPBG and cocaine did not change generalization. Neither ondansetron, nor bemesetron were able to antagonize cocaine’s cue. In this study, also mCPBG (15 mg/kg) could be trained and the 5-HT precursor 5-HTP fully substituted (dose-dependently) for mCPBG. The 5-HT3 receptor agonist zacopride only partially antagonized the cue and the rate-suppressant effects of mCPBG, suggesting a partial 5-HT3 receptor mediated cue. Cocaine did only partially (50%) mimic the mCPBG-cue in substitution tests. Glennon et al. (1992) were able to train 4 out of 6 rats to discriminate 2-methyl-5-HT (5 mg/kg IP) from saline (ED50 = 2.6 mg/kg). The cue generalized to the 5-HT3 receptor agonist mCPBG (ED50 = 1.6 mg/kg), but not to the 5-HT2 receptor agonist DOM or the 5-HT1/2 receptor agonist 5-OMe-DMT. The stimulus of 2-methyl-5-HT could be almost completely antagonized by tropisetron (0.1–10 µg/kg), but not by a quaternary amine analog of tropisetron (even not at doses 10,000 times the dose of tropisetron), strongly suggesting that 2-methyl-5-HT’s cue is mediated via the 5-HT3 receptor and is centrally mediated. Further support (Young and Glennon, 1992) for a 5-HT3 receptor mediated cue of 2-methyl-5-HT came from its antagonism by (±) zacopride and its isomers in a stereo selective way consistent with their 5-HT3 receptor binding profile (S(−) > RS(±) > R+). These data suggest that the cue of mCPBG is serotonergic in nature and only partially mediated by 5-HT3 receptors, whereas 2-methyl-5-HT seems to engender a full 5-HT3 receptor-mediated cue.

However, in an unpublished study, we (Slangen, 1991) tested two 5-HT3 receptor agonists, 2 methyl-5-HT and 3,4-dichlorophenylbiguanide in a two lever drug-discrimination paradigm in rats. 2-methyl-5-HT, in a dose range of 0.1 to 4.0 mg/kg PO could not be discriminated from vehicle in more than 80 training sessions in contrast to what was found by Glennon et al. (1991b, 1992; Young and Glennon, 1992). However, the same animals thereafter rapidly learned to discriminate chlordiazepoxide. Furthermore it was demonstrated that 2-methyl-5-HT, which very weakly binds to 5-HT1A receptors, does not substitute for flesinoxan in flesinoxan-trained animals. In a completely parallel way, ondansetron (0.1 to 4.0 mg/kg) was also neither able to function as a discriminable cue, nor to replace the chlordiazepoxide-cue.

The effects of the 5-HT3 receptor agonist 3,4-dichlorophenylbiguanide (3,4DCPG) were rather complex. The results suggest that, although 3,4DCPG may be able to induce discriminable stimulus effects in rats, these effects are obscured because food reward by bar pressing is disrupted at higher doses (8 mg/kg). It may be suggested that other drug discrimination tests, not involving food rewards, are more suited for training rats to discriminate between 3,4DCPG and saline.

In conclusion: Activation or blocking of centrally located 5-HT3 receptors does not generate clear discriminative stimulus effects. Whether certain stimuli (e.g. alcohol) can be blocked by 5-HT3 receptor antagonists (but not by others) is still a matter of controversy. The data so far are not pointing to clearly detectable (discriminable) stimulus
effects of 5-HT \textsubscript{3} receptor antagonists, in contrast to what has been demonstrated for compounds acting at 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, 5-HT\textsubscript{2} and GABA\_A-benzodiazepine receptors.

### 2.13. Miscellaneous

When marmosets or cynomolgous monkeys are confronted with a human observer, they retreat from the cage front to the rear and show characteristic postures indicating fear (Costall et al., 1988). Treatment with diazepam or ondansetron makes animals less fearful, i.e. they spend more time in the front of the cage and show less fearlike behaviour (Jones et al., 1988). In subsequent experiments other 5-HT\textsubscript{3} receptor antagonists were also found effective (Tyers et al., 1987; Piper et al., 1988; Borsini et al., 1993; Costall et al., 1993).

Cutler (1991) studied the effects of buspirone, granisetron and tropisetron (Cutler and Dixon, 1989) in an approach–avoidance situation, where female mice in a neutral cage were confronted with an unfamiliar male. Both buspirone and granisetron (given for 5–10 days in the drinking water) decreased flight of the females and enhanced active social investigation, which was taken as indicative of an anxiolytic action of the drugs. Such an effect was not found in male mice. Moreover no anti-aggressive effects of granisetron were found, which is in line with similar findings using ondansetron (Mos et al., 1989; Sanchez et al., 1993) and zacopride (White et al., 1991) in mice and ondansetron in rats (Mos and Olivier, 1990; Sanchez, 1993). Cutler and Piper (1990) found enhanced social investigation after administration of granisetron to male gerbils. When granisetron, BRL 46470 and diazepam were given to intruder mice in a resident-intruder situation (Cutler and Piper, 1991), all three compounds exerted behavioural changes indicative of anxiolytic activity.

Placing a hungry rat into an unfamiliar environment with food, suppresses feeding behaviour compared to a familiar environment. Benzodiazepines (Cooper, 1980) and 5-HT\textsubscript{1A} receptor agonists (Dourish et al., 1986) reduce this hyponeophagia. Fletcher and Davis (1990) confirmed these effects for chlordiazepoxide, 8-OH-DPAT and buspirone. Tropisetron (0.01–1 mg/kg) however had an opposite effect, although only at the lowest dose tested.

Shepherd and Rodgers (1990) used an elegant experiment to determine whether feeding behaviour in male mice was differentially affected under basal (free-feeding) or social conflict conditions. They reasoned that elicitation of feeding under conflict situations may point to a specific direct effect of the drug on mechanisms controlling food intake. 8-OH-DPAT enhanced feeding both under basal and conflict conditions. The 5-HT\textsubscript{3} receptor antagonist ondansetron (1.0–2.0 mg/kg) enhanced feeding only under basal conditions. Rodgers and Shepherd conclude that ondansetron does not show up as an anxiolytic agent in this conflict procedure. In the same set-up ondansetron had no anti-aggressive effects, in contrast to 8-OH-DPAT, which had an aggression-enhancing effect.

When mice were placed in a so called communication box for 16 h, in which non-shocked mice (responders) were placed adjacent to mice receiving shocks (senders), both types of mice developed gastric ulcers. The responder mice are thought to be psychologically stressed and developed more severe gastric ulcers than the physically stressed animals. 5-HT\textsubscript{3} receptor antagonists (ondansetron (ID\textsubscript{50}=0.1 mg/kg PO), tropisetron (ID\textsubscript{50}=0.2 mg/kg PO) and MDL 72222 (ID\textsubscript{50}=0.4 mg/kg PO) were able to prevent ulcer formation, whereas a peripherally acting 5-HT\textsubscript{1A} receptor antagonist (M-840) was not (Nomura et al., 1994), suggesting the involvement of centrally located 5-HT\textsubscript{3} receptors. In a procedure in female mice where animals displayed cataleptic-like immobility after repeated pinching at the scruff, ondansetron (0.1 and 1 mg/kg IP) decreased the frequency and shortened the duration of pinch-induced catalepsy at non-sedating doses (Fundaro, 1998). This immobility resembles behaviour seen in natural situations under high stress or fear conditions (inescapable stress).

### 2.14. General discussion animal data

In the present review the effects of 5-HT\textsubscript{3} receptor antagonists in various putative animal paradigms of anxiety are described and these effects are compared with those of the established anxiolytic benzodiazepines and 5-HT\textsubscript{1A} receptor agonists. Table 2 summarizes the overall conclusions drawn by us from the various studies performed and described.

In practically all paradigms used, benzodiazepine receptor agonists show up as potent anxiolytic agents. 5-HT\textsubscript{1A} receptor agonists have a different profile, being very

| Table 2 Summary of anxiolytic effects of 5-HT\textsubscript{3} receptor antagonists, benzodiazepine (BZ) receptor agonists and 5-HT\textsubscript{1A} receptor agonists in anxiety paradigms* |
|-------------------------------------------------|-----------------|-----------------|
| Conflict Models                                 | 5-HT\textsubscript{3} receptor agonists | BZ receptor agonists | 5-HT\textsubscript{1A} receptor agonists |
| Fear-potentiated startle                        | 0/+             | +/+             | 0/-             |
| Periaqueductal Gray stimulation                 | 0/+             | +/0             | 0/+             |
| Conditioned Ultrasonic vocalization            | 0/0+            | ++              | +/+             |
| Vocalizations in Aversive situations            | 0/+             | ++              | +/+             |
| Defensive Burying                               | 0/+             | +/0             | 0/-             |
| Ultrasonic Pupvocalisation                      | 0/+             | ++              | +/+             |
| Defensive Analgesia                            | 0/+             | ++              | +/+             |
| Elevated Plus-maze                              | +/0             | ++              | 0/-             |
| Social Interaction                              | +/0             | ++              | +/0             |
| Light-Dark Exploration                          | +/+             | ++              | +/0             |
| Stress-induced Hyperthermia                     | 0/+             | ++              | +/+             |
| Drug-discrimination (cue)                       | 0/+             | ++              | +/+             |

* = anxiogenic effect; 0 = no effect; +/++/+++++ = weak/moderate/strong (anxiolytic) effects.
active in some paradigms, and not (or even anxiogenic) in some others.

It is remarkable that the anxiolytic activity of 5-HT\textsubscript{1A} receptor agonists was not predicted from animal studies. Thus, buspirone, a 5-HT\textsubscript{1A} receptor agonist and dopamine D\textsubscript{2}-receptor antagonist, was tested clinically as an antipsychotic, but found inactive; concomitantly anxiolytic activity was shown, leading to its development as an agent in Generalized Anxiety Disorder (Goa and Ward, 1986). This finding triggered a new area of research and development of 5-HT\textsubscript{1A} (partial) receptor agonists, of which several are in advanced stages of development (ipsapirone, gepirone, flesinoxan). Although 5-HT was already earlier implied in the modulation of anxiety (Stein et al., 1973), the anxiolytic effects of 5-HT\textsubscript{1A} receptor agonists renewed the interest in the role of other serotonergic receptors in anxiety. The synthesis of specific 5-HT\textsubscript{3} receptor antagonist and (besides other effects like anti-emesis) the subsequent finding of their highly potent anxiolytic effects in some animal anxiety tests, led to an enormous effort in several pharmaceutical companies to synthesize and develop such specific agents.

Considering the activity of 5-HT\textsubscript{3} receptor antagonists in various anxiety tests (Table 2), it is clear that these agents deviate in their behavioural profile from both the benzodiazepines and the 5-HT\textsubscript{1A}-ligands. In several paradigms (conflict, periaquaductal gray stimulation, defensive burying, ultrasonic pupvocalization, stress-induced hyperthermia and drug-discrimination) they are completely inactive, whereas in other models contradicting results are described. Only in one test, the light/dark exploration in mice, 5-HT\textsubscript{3} receptor antagonists are always consistently found.

In some tests there are remarkable differences; in the social interaction test in rats, some groups find very potent and very consistent effects of all 5-HT\textsubscript{3} receptor antagonists (Costall, Glaxo), whereas other groups (File) essentially find no effects. A similar situation holds for the elevated plus-maze. It is unclear why such dramatic differences are found, but it is conceivable that subtle (but maybe also less subtle) differences in methodology (strains, handling, housing etc, etc) can account for such effects. Although positive reports in the elevated plus-maze, social interaction and light/dark exploration tests by far outnumber the negative reports, this may be partly due to a publication bias; negative results in a test are mostly not published in the understanding that more investment is needed to optimize the model in order to find the (dramatic) effects of 5-HT\textsubscript{3} receptor antagonists. Alternatively, a number of animal paradigms may be too sensitive and pick up properties of drugs which are not related to anxiety thus generating false positives. Although speculative, the social interaction test and to a lesser extent, the elevated plus maze, could be such tests. The predictive validity of animal models of anxiety has always to be considered cautiously. Mostly, false negatives are highly unwanted; clinical proof of anxiolytic activity of a drug not predicted by the animal models is rather frequent, e.g. in the case of 5-HT\textsubscript{1A} receptor agonists. The 5-HT\textsubscript{3} receptor antagonists may be proof of false positives, at least in some animal paradigms. This should be considered a serious explanation for the results described in this review.

Thus, some preclinical evidence for anxiolytic effects of 5-HT\textsubscript{3} receptor antagonists is present, though not as massive as one would hope for. Especially the data on chronic application look promising. The results obtained so far, though derived from a limited number of laboratories and in need of independent replication, show evidence for lack of tolerance and absence of withdrawal symptoms and rebound anxiety, thereby sharply contrasting the effects of the benzodiazepines. Such effects would strongly favour the development of this class of drugs for treatment of anxiety disorders, including generalized anxiety disorder, panic disorder, phobias and others. The finding that ondansetron antagonizes the rebound anxiety after benzodiazepine withdrawal (Costall et al., 1989a,b; Goudie and Leathley, 1990; Costall and Naylor, 1991) may be of great importance because of its implications for withdrawal anxiety upon cessation of drugs of abuse, like alcohol, cocaine or nicotine.

### 3. Clinical studies

The final proof for putative anxiolytic effects of a new class of drugs is testing in humans. Several 5-HT\textsubscript{3} receptor antagonists are presently in development or are marketed for various therapeutic areas (emesis, anxiety, cognitive dysfunctions, drug addiction and psychosis). Several 5-HT\textsubscript{3} receptor antagonists have been or are studied clinically as potential therapeutics in the treatment of anxiety disorders (Roca et al., 1995). However, there is still a paucity of fully published papers on the results of these studies. Most clinical work has been done on ondansetron. In human volunteers no sedation was observed, whereas constipation emerged as the main significant side-effect (Millson and Preston, 1991; Hall and Ceuppens, 1991). A similar profile was observed for granisetron (Leigh et al., 1991).

Lader (1991) summarized the preliminary findings of ondansetron in a multi center, double-blind, placebo-controlled, parallel group study with 58 general practitioners and a total of 402 patients, suffering from generalized anxiety disorder. Two doses of ondansetron (1 mg/kg t.i.d. and 4 mg/kg t.i.d.) were compared to diazepam (2 mg/kg t.i.d.) and placebo for a treatment period of 4 weeks, followed by a two-week withdrawal period. Notwithstanding a very high placebo response, some efficacy was shown, the 1 mg/kg dose being slightly superior to the 4 mg/kg dose, which could be interpreted as being in line with the bell-shaped dose-response curves emerging in
animal models. Diazepam also had a limited efficacy in this set-up. There was no rebound anxiety after ondansetron cessation, in contrast to diazepam, which confirms the animal data and also suggests no dependence liability. Mathew and Wilson (1991) measured the effects of diazepam (0.12 mg/kg), ondansetron (0.24 mg/kg) and saline on cerebral blood flow (CBF) and anxiety in GAD-patients. Diazepam, but not ondansetron or saline reduced CBF and anxiety.

Schweizer and Rickels (1991) are less optimistic about the anxiolytic properties of 5-HT<sub>3</sub> receptor antagonists in humans. They shortly describe two ondansetron (daily dosage range 8–48 mg) and one zacopride (daily dose range 0.04 µg to 400 µg) studies in GAD, but report no clear-cut significant treatment effects different from placebo. Negative results were also reported in a review article by Wilde and Markham (1996). In a randomized double blind trial 0.24 mg/kg ondansetron did not reduce anxiety in GAD patients whereas 0.12 mg/kg diazepam did. In the review by Greenshaw and Silverstone (1997) in a preliminary study, it was shown that the anxiolytic effect of ondansetron in GAD patients was similar to that of diazepam (57–59% vs. placebo 45%) when the Hamilton Anxiety Rating scores were used. Using the Montgomery Åsberg Depression Rating Scale the anxiolytic effect is a little higher than that of diazepam (54–59%) vs. 51% of placebo.

Lecrubier et al. (1993) observed in a double-blind, placebo-controlled evaluation of 0.5, 5, or 25 mg/day of tropisetron in 91 GAD patients a dose-dependent efficacy after 7 days of treatment (Hopkins Symptom Checklist total score, Global Impression Scale). However using the Hamilton Anxiety Scale statistical significance was not reached. Freeman et al. (1997) did a randomized, double-blinded, placebo-controlled study in Generalized anxiety disorder patients which was part of a parallel multicenter study with eight participating groups. Ondansetron (0.25 mg b.i.d., 0.5 mg b.i.d.), diazepam (5 mg b.i.d.) or placebo were given after a placebo run-in phase for 1–2 weeks. The study lasted eight weeks. Only 1 mg daily ondansetron significantly decreased anxiety as measured by the HAM-A and CGI severity scales. GAD was diagnosed in this study using DSM-III-R diagnostic (18) criteria, whereas in DSM-IV six core symptoms are used. Since ondansetron was found earlier ineffective in GAD, and suggested to be effective (absence of placebo group) in panic disorder (Schneier et al., 1996; Ballenger et al., in press), the Freeman et al. (1997) study could be confounded by mixing diagnostic criteria for GAD and panic disorder. Freeman et al. (1997) question that if ondansetron treats panic disorder but not GAD, there must be pharmacological evidence and pathophysiological implications for the 5-HT<sub>3</sub> receptor in panic disorder.

During a meeting of the American College of Neuropsychopharmacology (Puerto Rico, 1994) a paper was presented by Metz, Evoniuk and De Veauh-Geiss on the efficacy of ondansetron in panic disorder. In a multicentre double-blind, placebo-controlled clinical trial ondansetron reduced frequency and intensity of panic attacks. In another paper presented by Bell and De Veauh-Geiss the efficacy of ondansetron was also shown in social phobia (275 patients). Full papers of these studies however have not been published yet. In a small study, 14 patients with panic disorder or social phobia, were challenged with pentagastrin, a CCK-receptor agonist, which induces anxiety and panic in such patients (MCann et al., 1997). Pentagastrin induced anxiety, physical symptoms of panic attacks, pulse, plasma ACTH and cortisol, which could not be blocked by ondansetron, suggesting that pentagastrin’s effects (behavioural and physiological) are not 5-HT<sub>3</sub> receptor mediated. The efficacy of ondansetron as adjunctive medication in the discontinuation of chronic benzodiazepine treatment (alprazolam or lorazepam) was investigated in 108 patients (Romach et al., 1998). 97 patients completed a randomized double-blind discontinuation treatment program during which they received either ondansetron (2 mg twice daily) or placebo and flexibly tapered their benzodiazepine over a 6-week period. There were no significant effects of ondansetron on any outcome parameter and the authors (Romach et al., 1998) concluded that ondansetron had no significant effects on severity of withdrawal symptoms or levels of anxiety.

In a small study, 10 OCD patients which were on a stable treatment with the SSRI fluvoxamine (200–300 mg daily) were treated with ondansetron (8 mg/day) or placebo in a double-blind randomized controlled design for two weeks (Smeraldi et al., 1992). Ondansetron was not effective in further changing the antiobsessional effects of fluvoxamine.

Of course, much more studies over an extensive dose range are needed to decisively conclude whether 5-HT<sub>3</sub> receptor antagonists are the “anxiolytics” for the coming decades, but the data so far are far from promising.

**Acknowledgements**

We thank Marijke Mulder for secretarial assistance.

**References**


Cervo, L., Samanin, R., 1995. 5-HT1A receptor full and partial agonists and 5-HT1c (but not 5-HT1D receptor antagonists increase rates of punished responding in rats. Pharmacol. Biochem. Behav. 52, 671–676.


Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tyers, M.B., 1990b. Ondansetron inhibits a behavioural consequence of...


Cutler, M.G., Piper, D.C., 1990. Chronic administration of the 5-HT1 receptor antagonist BRL 43694; effects on reflex epilepsy and social behaviour of the Mongolian gerbil. Psychopharmacology 101, 244–249.


pharmacological properties and therapeutic efficacy as an anxiolytic. Drugs 32, 114–129.
King, F.D., 1994. 5-hydroxytryptamine-3-receptor antagonists. In: King, F.D., Jones, B.J., Sanger, G.J. (Eds), CRC: Boca Raton FLA, pp. 1-44.


