Trazodone is a Potent Agonist at 5-HT$_{2C}$ Receptors Mediating Inhibition of the N-Methyl-D-Aspartate/Nitric Oxide/Cyclic GMP Pathway in Rat Cerebellum$^{1}$

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ABSTRACT

The effects of trazodone on the cyclic GMP elevation elicited by N-methyl-D-aspartate in rat cerebellar slices were analyzed. Trazodone inhibited in a concentration-dependent manner (EC$_{50}$ = 0.82 nM) the cyclic GMP response evoked by 0.1 μM N-methyl-D-aspartate. The inhibition was near complete at 10 nM trazodone. The effect of 10 nM trazodone was unaffected by 0.3 μM spiperone or rauvoscine, antagonists with selectivity for the 5-HT$_2$ serotonin receptor, or the 5-HT$_{2B}$ subtype, respectively, but it was totally prevented by 0.01 μM mesulergine, a 5-HT$_{2A}$/5-HT$_{2B}$/5-HT$_{2C}$ receptor antagonist. Trazodone was potent counteracted (IC$_{50}$ = 2.7 nM) by the selective 5-HT$_{2B}$/5-HT$_{2C}$ receptor antagonist N-(1-methyl-5-indolyl)-N-(3-pyridyl) urea HCl and, less potently (IC$_{50}$ = 95 nM), by ketanserin, a 5-HT$_{2A}$/5-HT$_{2C}$ receptor blocker. It is concluded that trazodone behaves as a potent full agonist at the 5-HT$_{2C}$ receptor mediating inhibition of the cerebellar N-methyl-D-aspartate/nitric oxide/cyclic GMP system.

Trazodone is an antidepressant drug marketed in several countries (see, for reviews, Brogden et al., 1981; Haria et al., 1994). Although the mechanisms by which the drug alleviates symptoms of depression are unknown, interactions of trazodone with 5-HT (serotonin) neurons and receptors have been proposed by several authors. Early papers reported activity of trazodone as an inhibitor of 5-HT uptake (Garattini et al., 1976; Stefanini et al., 1993); such an activity appears, however, too weak to account for the clinical efficacy of trazodone, particularly if compared with those of antidepressants that are selective serotonin uptake inhibitors. Based on a number of reports, trazodone is now generally thought of as a 5-HT receptor antagonist (Bryant and Ereshefsky, 1982; Wrighglesworth, 1983; Fuller et al., 1984; Clineschmidt et al., 1985; Jenck et al., 1993; Takeuchi et al., 1997). Trazodone appears to target preferentially receptors of the 5-HT$_2$ type; the few data available, in part based on behavioral studies, suggest that the drug may be a 5-HT$_{2C}$ (Jenck et al., 1993) and a 5-HT$_{2A}$ (Siegel et al., 1996; Takeuchi et al., 1997) receptor antagonist.

Functional models of native 5-HT$_2$ receptor subtypes are very scarce. It was recently shown that 5-HT$_{2C}$ receptors exist in the rat cerebellum; activation of these receptors potently inhibits the elevation of cyclic GMP levels elicited by NMDA (Marcoli et al., 1997). We now report that, in this model, trazodone mimics 5-HT and (-)-DOI, and may therefore behave as a full 5-HT$_{2C}$ receptor agonist.

Methods

Animals. Adult male rats (Sprague Dawley, 200–250 g) were housed at constant temperature (22 ± 1°C) and relative humidity (50%) under a regular light-dark schedule (light, 07.00–19.00 hr). Food and water were freely available. The animals were sacrificed by decapitation. The cerebellum was removed rapidly and placed in ice-cold medium.

Cyclic GMP production in cerebellar slices. The isolated cerebellum was chopped with a McIlwain tissue chopper in a sagittal plane into 400-μm slices weighing 12.0 ± 1.0 mg (mean ± S.E.M.; n = 10). Slices were preincubated for 90 min in a physiological medium having the following composition (millimolar): NaCl, 125; KCl, 3; MgSO$_4$, 1.2; CaCl$_2$, 1.2; NaH$_2$PO$_4$, 1; NaHCO$_3$, 22; glucose, 10 (aeration with 95% O$_2$–5% CO$_2$ at 37°C); pH 7.2 to 7.4, with changes of the medium every 30 min. After preincubation, slices were placed in plastic center wells (one slice per center well; approximately 0.90 mg of protein), transferred into tubes containing standard medium or medium added with serotonergic agonists and/or antagonists and incubated in a shaking water bath at 37°C for 15 min. The center wells were then transferred into tubes containing standard medium or medium added with NMDA (final concentration: 0.1 μM), with or without serotonergic agonists and/or antagonists and incubated for 3 min.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); NMDA, N-methyl-D-aspartate; DOI, (–)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; SB200646A, N-(1-methyl-5-indolyl)-N-(3-pyridyl) urea HCl; NO, nitric oxide; mCPP, m-(chlorophenyl)piperazine.
Incubation was terminated by transferring each center well into 1 ml of a solution containing 50 mM Tris-HCl and 4 mM EDTA (pH 7.5), and heating at 100°C. After 10-min incubation at 100°C, the slices were homogenized by sonication and, after centrifugation for 5 min at 5000 g, the cyclic GMP content of the supernatant (100-μl aliquots) was determined using a commercially available radioimmunoassay kit (Amersham Radiochemical Centre, Buckinghamshire, U.K.).

The sensitivity of the assay was approximately 0.04 pmol. Protein determinations were performed as described by Bradford (1976), using bovine serum albumin as standard. The levels of cyclic GMP were expressed as pico moles per milligram of protein. The cyclic GMP responses were calculated by subtracting the cyclic GMP present in the controls from that present in the samples containing the drugs tested. Drug effects are expressed as percent variation with respect to controls.

Calculation and statistics. EC50 or IC50 values (half-maximum effective concentrations) of agonists or antagonists were determined from the concentration-response curves obtained using a four-parameter logistic function fitting routine (Sigma Plot software). Mean ± S.E.M. values of the number of experiments (n) are indicated throughout. Student’s t test was used for analyzing the significance of the difference between two means.

Drugs. The following drugs were purchased: spiperone from RBI (Natick, MA) and ketanserin, (±)-DOI and NMDA from Tocris Cookson (Bristol, U.K.). The following drugs were gifts: trazodone from Istituto Ricerche Francesco Angelini (Pomezia, Roma, Italy); SB200646A from SmithKline Beecham Pharmaceuticals (West Sussex, U.K.); rauwolscine from Organon Scientific Development Group (Oss, the Netherlands) and mesulergine from Sandoz (Basel, Switzerland).

Results

It had previously been shown that NMDA, added to cerebellar slices from mature adult (2–3 months old) rats, stimulated the formation of cyclic GMP. The NMDA-evoked cyclic GMP response, which involves activation of NO synthase, could be inhibited potently by 5-HT and by the selective 5-HT2 receptor agonist (±)-DOI (Maura et al., 1995).

As shown in figure 1, the cyclic GMP response evoked by 0.1 μM NMDA was concentration-dependently inhibited by trazodone. The EC50 value (concentration causing half-maximal inhibition of the cyclic GMP response) was 0.82 nM; in a set of parallel experiments with trazodone, (±)-DOI inhibited the NMDA-evoked cyclic GMP production with an EC50 of 1.74 nM. Trazodone and (±)-DOI produced near-complete inhibition of the NMDA effect at approximately 10 nM. At the concentrations used, trazodone did not affect, on its own, the basal levels of cyclic GMP (not shown).

Table 1 shows that the inhibition by trazodone (0.01 μM) of the NMDA (0.1 μM)-evoked cyclic GMP response was prevented completely by 0.01 μM mesulergine, a potent 5-HT2A/5-HT2B/5-HT2C receptor antagonist. In contrast, neither spiperone (0.3 μM) nor rauwolscine (0.3 μM), antagonists with selectivity for the 5-HT2A or the 5-HT2B subtype, respectively, could significantly counteract the trazodone inhibition. None of the antagonists, at the concentrations tested, affected the basal or the NMDA-evoked cyclic GMP levels (not shown).

As illustrated in figure 2, the effect of 0.01 μM trazodone could be reversed in a concentration-dependent manner and with high potency (IC50 = 2.7 nM) by SB200646A, a selective 5-HT2B/5-HT2C receptor antagonist (Forbes et al., 1993). The effect of trazodone could also be counteracted by ketanserin (IC50 = 95 nM), a potent 5-HT2A receptor antagonist endowed with reasonable potency also toward the 5-HT2C subtype (Baxter et al., 1995).

Discussion

It was previously found that the NMDA receptor/NO/cyclic GMP pathway in adult rat cerebellar slices can be inhibited by 5-HT acting at receptors of the 5-HT2 type (Maura et al., 1995). In a subsequent study with the 5-HT2A/5-HT2B/5-HT2C agonist (±)-DOI and various antagonists, we have subclassified these receptors as 5-HT2C (Marcoli et al., 1997). The present work shows that trazodone can prevent the cyclic GMP response evoked by NMDA in a way quite similar to that of 5-HT and (±)-DOI. The elevation of cyclic GMP caused by NMDA was prevented completely by trazodone (fig. 1), as it was by 5-HT or (±)-DOI (Maura et al., 1995; and present data). Moreover, the three compounds displayed very similar potencies (table 2). These data raised the possibility that trazodone is an agonist at the 5-HT2C receptor mediating inhibition of the NMDA receptor/NO/cyclic GMP pathway in rat cerebellum.

To shed light on this unexpected behavior of trazodone, the effect of the drug was analyzed by using a number of antagonists endowed with relative selectivity for the 5-HT2A, 5-HT2B or 5-HT2C subtype of the 5-HT2 receptor (see, for reviews, Boess and Martin, 1994; Hoyer et al., 1994; Baxter et al., 1995).

Spiperone is a drug that displays high affinity (pKᵢ, 9.0–9.5) at native and cloned rat 5-HT2A receptors, but low affin-

![Fig. 1. Inhibition by trazodone (■) and (±)-DOI (▲) of the NMDA-evoked cyclic GMP response in rat cerebellar slices. Agonists were added concomitantly with 0.1 μM NMDA. Basal cyclic GMP levels amounted to 7.4 ± 0.50 (n = 14) pmol/mg of protein/3 min. The effect of 0.1 μM NMDA (81 ± 5.2% cyclic GMP increase, n = 14) was taken as 100%. Other experimental details as in "Methods." Results are expressed as percentages of the response to NMDA. Mean ± S.E.M. values of three to eight different experiments in triplicate are presented.](image-url)
The cerebellum. Whether trazodone possesses intrinsic inhibition of the NMDA receptor/NO/cyclic GMP pathway in receptors of the 5-HT2C subtype (SB200646A or ketanserin. Mesulergine is a potent 5-HT2 receptor antagonist (Forbes et al., 1993, 1994); SB200646A or ketanserin. Mesulergine is a potent 5-HT2 receptor antagonist endowed with some selectivity for the 5-HT2B subtype (Baxter et al., 1995). To conclude, the present results show that trazodone can inhibit the K+-evoked release of glutamate, similar to 5-HT and (±)-DOI (G. Maura and M. Raiteri, unpublished results). To conclude, the effect observed here is likely to reflect binding of authentic trazodone acting as a full agonist at 5-HT2C receptors. Of note, in rats trained to discriminate m-CPP from saline, trazodone substituted fully for m-CPP, like other direct 5-HT2C receptor agonists (Callahan and Cunningham, 1994). Based on the present findings, the idea that trazodone can inhibit the cerebral NMDA receptor/NO/cyclic GMP system through activation of receptors of the 5-HT2 subtype, this view is strongly supported by the blockade of trazodone effect caused by mesulergine, SB200646A or ketanserin. Mesulergine is a potent 5-HT2 receptor agonist endowed with some selectivity for the 5-HT2C subtype and often used as a 5-HT2C receptor ligand (Hoyer et al., 1993); SB200646A is a selective 5-HT2C/5-HT2C receptor antagonist (Forbes et al., 1993) and ketanserin, besides displaying particularly high affinity for the 5-HT2A receptor (pKᵢ = 8.9), is a moderately potent antagonist at receptors of the 5-HT2C subtype (pKᵢ = 7.0; Baxter et al., 1995). To conclude, the present results show that trazodone can behave as a full agonist at the 5-HT2C receptor mediating inhibition of the NMDA receptor/NO/cyclic GMP pathway in rat cerebellum. Whether trazodone possesses intrinsic activity also at 5-HT2C receptors located elsewhere in the central nervous system (Abramowski et al., 1995) remains to be determined. It should be added that, considering the complex binding profile of trazodone (Haria et al., 1994), the drug is likely to target multiple sites in the brain, including 5-HT receptors different from the 5-HT2 subtype and where trazodone could antagonize serotonin (for instance, 5-HT2A receptors; Takeuchi et al., 1997).

It was shown previously that the NMDA receptor/NO/cyclic GMP system in rat cerebellar slices can be inhibited potently also through the activation of postsynaptic receptors of the 5-HT1A subtype (Maura and Raiteri, 1996). Trazodone displays good affinity for these receptors (Jenck et al., 1993). However, two observations tend to exclude involvement of 5-HT1A receptors in the action of the drug: 1) spiperone, a potent 5-HT1A receptor antagonist, could not prevent significantly the effect of trazodone and 2) this effect was totally counteracted by SB200646A, a compound with negligible affinity for the 5-HT1A receptor (Forbes et al., 1993).

Under the in vitro conditions used in the present work, the effects observed would be more easily attributed to trazodone itself than to its metabolites. It has to be noted, however, that one major metabolite of trazodone, m-CPP (Caccia et al., 1981), is a 5-HT2C receptor agonist (Boess and Martin, 1994; Hoyer et al., 1994; Baxter et al., 1995). Thus, a contribution of m-CPP to the effect observed with trazodone cannot be ruled out entirely. On the other hand, according to Sanders-Bush and Breeding (1990), m-CPP is a partial agonist at 5-HT2C receptors. Moreover, the concentrations of m-CPP are significantly lower than those of trazodone in plasma and brain of rats administered the antidepressant (Smith and Suckow, 1985). Under these conditions, and assuming trazodone to behave as a 5-HT2C receptor agonist (Jenck et al., 1993), it would be quite difficult to explain how trazodone could display the same efficacy as 5-HT and (±)-DOI in cerebellar slices. In support of a genuine agonist action of trazodone, experiments with cerebellar mossy fiber synaptosomes superfused in conditions minimizing metabolism show that trazodone can inhibit the K+-evoked release of glutamate, similar to 5-HT and (±)-DOI (G. Maura and M. Raiteri, unpublished results). To conclude, the effect observed here is likely to reflect binding of authentic trazodone acting as a full agonist at 5-HT2C receptors. Of note, in rats trained to discriminate m-CPP from saline, trazodone substituted fully for m-CPP, like other direct 5-HT2C receptor agonists (Callahan and Cunningham, 1994). Based on the present findings, the possibility exists that penile erection, a known 5-HT2C receptor-mediated activity of m-CPP (Berendsen et al., 1990; Millan et al., 1997), is produced directly by trazodone also. Priapism has long been known as a side effect of the antidepressant therapy with trazodone (Haria et al., 1994). Several reports indicate that, in the cerebellum, 5-HT is able to inhibit glutamatergic transmission potently through pharmacologically distinct pre- and postsynaptic receptors (Raiteri et al., 1986; Maura et al., 1991, 1995; Maura and Raiteri, 1996; Marcoli et al., 1997). 5-HT-glutamate interactions may play an important role in cerebellar ataxia, a complex syndrome for which no established therapy is available. Symptomatic improvements have been reported in ataxic patients treated with the 5-HT precursor 5-hydroxytryptophan (see Trouillas and Fuxe, 1993), although large doses of the amino acid are necessary. Significant, although not dramatic, improvements have also been observed in patients administered buspirone, an anxiolytic
drug acting at 5-HT$_{1A}$ receptors (Lou et al., 1995; Trouillas et al., 1995, 1996); activation of somatodendritic 5-HT$_{1A}$ autoreceptors by buspirone and consequent depletion of serotonergic neuronal firing may, however, weaken the effectiveness of the drug. Because activation of 5-HT$_{2C}$ receptors appears as effective as that of 5-HT$_{1A}$ receptors in controlling cerebellar glutamatergic transmission (Maura et al., 1995; present work), 5-HT$_{2C}$ receptor agonists, like trazodone, may be particularly useful to treat cerebellar ataxia. Of note, 5-HT$_{2C}$ receptors seem to be present in relatively high density in human cerebellum (Abramowski et al., 1995). Actually, this receptor subtype appears to be the most common 5-HT receptor in the brain (Pompeiano et al., 1994; Wright et al., 1995). The involvement of 5-HT$_{2C}$ receptors in the therapeutic activity of trazodone and of other serotonergic antidepressants remains to be established.

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