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Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in human neocortex slices: involvement of 5-HT_{2C} and 5-HT_{1A} receptors

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1 The NMDA receptor/nitric oxide (NO)/cyclic GMP pathway and its modulation by 5-hydroxytryptamine (5-HT) was studied in slices of neocortical samples obtained from patients undergoing neurosurgery.

2 The cyclic GMP elevation produced by 100 μM NMDA was blocked by 100 μM of the NO synthase inhibitor N^G-nitro-L-arginine (L-NOARG) or by 10 μM of the soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-α] quinoxaline-1-one (ODQ).

3 The NMDA effect was prevented by 5-HT or by the 5-HT_2 agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ((±)-DOI; EC_{50} = 22 nM). The (±)-DOI inhibition was insensitive to the 5-HT_{2A} receptor antagonist MDL 100907 or the 5-HT_{2B} antagonist rauwolscine; it was largely prevented by 1 μM of the non-selective 5-HT_{2C} antagonists mesulergine (5-$HT_{2A,B,C}$), ketanserin (5-$HT_{2A,C}$) or SB 200646A (5-$HT_{2B,C}$); it was completely abolished by 0.1 μM of the selective 5-HT_{2C} receptor antagonist SB 242084.

4 The NMDA-induced cyclic GMP elevation also was potently inhibited by the selective 5-HT_{2C} agonist RO 60-0175 and by the antidepressant trazodone, both added at 1 μM, in an SB 242084-sensitive manner.

5 Finally, the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT; 1 μM) inhibited the NMDA-evoked cyclic GMP response, an effect blocked by the selective 5-HT_{1A} receptor antagonist WAY 100635.

6 In conclusion, the NMDA receptor/NO/cyclic GMP pathway in human neocortex slices can be potently inhibited by activation of 5-HT_{2C} or 5-HT_{1A} receptors.
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Keywords: human neocortex; 5-hydroxytryptamine-glutamate interaction; 5-HT_{2C} receptor; 5-HT_{1A} receptor; NMDA receptor; cyclic GMP; nitric oxide

Abbreviations: (±)-DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; L-NOARG, N^G-nitro-L-arginine; MDL100907, R-(+)-α-(2,3-dimethoxyphenyl)-1-(2-(4-fluorophenyl)ethyl)-4-piperidine-methanol; ODQ, 1H-[1,2,4]oxadiazolo[4,3,-α] quinoxaline-1-one; RO 60-0175, (s)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine; SB 200646A, N-(1-methyl-5-indoyl)-N-(3-pyridyl) urea HCl; SB 242084, 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl-carbamoyl]indoline; WAY 100635, [(3)H]N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridyl)cyclohexanecarboxamide 3HCl

Introduction

Glutamate, the major excitatory neurotransmitter in the mammalian CNS, plays important roles not only in physiological processes, but also in various pathological conditions generally linked to abnormally elevated glutamatergic transmission. Activation of ionotropic glutamate receptors, particularly of the NMDA type, causes influx of Ca^{2+}, activation of nitric oxide synthase (NOS) and formation of NO, resulting in increases in cyclic GMP (Garthwaite, 1982). The glutamate receptor/NOS/guanylate cyclase pathway has been studied by measuring cyclic GMP formation in slices of rat cerebellum (Garthwaite & Balasz, 1978; Bredt & Snyder, 1989; Raiteri *et al*., 1991) and hippocampus (East & Garthwaite, 1991; Wood *et al*., 1992) and by monitoring extracellular cyclic GMP during *in vivo* microdialysis in the cerebellum and hippocampus of awake, freely moving rats (Vallebuona & Raiteri, 1994; Fedele & Raiteri, 1999).

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The glutamate receptor/NO/cyclic GMP pathway has so far not been investigated in experiments of functional neurochemistry with fresh human brain tissue. This would certainly represent an excellent model in which to test agents able to curb excessive glutamatergic transmission.

Previously it was found that the release of glutamate from rat cerebellar synaptosomes (Davies & Leighton, 1984; Raiteri *et al*., 1986) and the cyclic GMP responses to ionotropic glutamate receptor agonists in slices of rat cerebellum (Dickie *et al*., 1990; Maura *et al*., 1995; Maura & Raiteri, 1996; Marcoli *et al*., 1997) can be inhibited by serotonin (5-HT) through the activation of multiple receptors indicating that 5-HT can exert a potent and sophisticated inhibitory control of the cerebellar glutamatergic transmission.

Recent experiments with human cerebrocortex synaptosomes suggest that 5-HT may be an important modulator of glutamate transmission also in telencephalic areas of the human brain. The release of endogenous glutamate evoked by depolarization from human neocortex synaptosomes could be

inhibited by 5-HT ($EC_{50}=2.9$ nM) acting at presynaptic heteroreceptors of the h5-HT_{1D} subtype (Maura *et al*., 1998), pharmacologically quite distinct from the h5-HT_{1B} autoreceptors regulating serotonin release (Marcoli *et al*., 1999).

It was important to investigate on the possibility that, in human cerebral cortex, 5-HT also modulates events that follow glutamate release and are triggered by postsynaptic glutamate receptor activation, in particular the NMDA receptor/NO/cyclic GMP pathway. We here find that the pathway can be very effectively inhibited by 5-HT acting at receptors that belong to the 5-HT_{1A} and 5-HT_{2C} subtypes.

Methods

Characteristics of human brain specimens

Fresh samples of human cerebral cortex were obtained from patients undergoing neurosurgery. The tissues used had to be removed by the surgeon to reach deeply located tumours or to treat epilepsy resistant to antiepileptic drugs. Since no significant differences were found between the results obtained from the two groups of patients, all data have been pooled. The samples removed represented parts of frontal (7), temporal (13), and parietal (5) lobes and were obtained from 11 male and 14 female patients (aged 36–73 years). Each sample was obtained and processed separately on a different day. Immediately after removal, the tissue was placed in a physiological salt solution (see below) kept at 0–4°C; slices (400 μm) perpendicular to the surface were prepared by use of a Mcllwain tissue chopper after cubic fragments had been obtained from the specimens.

Cyclic GMP production in cerebral cortex slices

Slices were equilibrated for 60 min at 37°C in a physiological medium having the following composition (mM): NaCl 125, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 22 and glucose 10 (gassed with 95% O_2 and 5% CO_2 at 37°C), pH 7.2–7.4, with changes of the medium every 20 min. After equilibration, slices were placed in glass vials (one slice per vial) containing 5 ml of standard medium or medium containing the antagonists and preincubated in a shaking water bath at 37°C for 15 min. Then preincubation medium was removed and replaced with standard medium or medium containing NMDA (final concentration 100 μM), with or without antagonists and/or serotonergic agonists, and incubated for 3 min. Incubation was terminated by transferring the slices into tubes containing 1 ml of Tris-HCl (50 mM; pH 7.5 containing 4 mM EDTA, at 100°C) and heating at 100°C for 10 min. The slices were then homogenized by sonication and centrifuged for 5 min at $5000\times g$. Supernatants were collected, lyophilized and resuspended in 100 μl buffer. Finally, their cyclic GMP content was determined using a commercially available radioimmunoassay kit (Amersham dual range, Amersham Radiochemical Centre, Buckinghamshire, U.K.); cyclic GMP was measured by the acetylation protocol which gives a sensitivity of 2 fmol 100 μl^{-1} (standard curve range 128–2 fmol 100 μl^{-1}). Protein determinations were carried out as described by Bradford (1976) using bovine serum albumin as standard.

Calculation and statistics

The levels of cyclic GMP were expressed as fmol mg^{-1} protein. The cyclic GMP response was calculated by subtraction of the cyclic GMP present in the controls from that present in the samples containing the drugs under study. The effects of drugs were expressed as per cent variation with respect to the appropriate controls. The EC_{50} (half-maximum effective concentration) value of (±)-DOI was determined from curves obtained using a function fitting routine (software Sigma Plot). Means ± s.e.mean of the given numbers of experiments (n) are indicated throughout. Student's t-test was used for analysing the significance of the difference between two means.

Materials

The following drugs were purchased: (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ((±)-DOI) and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) from RBI (Natick, MA, U.S.A.); 5-hydroxytryptamine creatinine sulphate (5-HT) from Calbiochem (Los Angeles, CA, U.S.A.); NMDA, ketanserin, N^G-nitro-L-arginine (L-NOARG) and 1H-[1,2,4]oxadiazolo[4,3,-α] quinoxaline-1-one (ODQ) from Tocris Cookson (Bristol, U.K.). The following drugs were gifts from the companies indicated: mesulergine (Sandoz, Basel, Switzerland); R-(+)-α-(2,3-dimethoxyphenyl)-1-(2-(4-fluorophenyl)ethyl)-4-piperidine-methanol (MDL 100907; Hoechst Marin Russel, Cincinnati, OH, U.S.A.); N-(1-methyl-5-indoyl)-N-(3-pyridyl) urea HCl (SB 200646A) and 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl-carbamoyl]indoline (SB 242084) (SmithKline Beecham Pharmaceuticals, West Sussex, U.K.); rauwolscine (Organon Scientific Development Group, Oss, The Netherlands); trazodone (Istituto Ricerche Francesco Angelini, Pomezia, Roma, Italy); (s)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine (RO 60-0175, Dr Jenck, F. Hoffman-La Roche Ltd, Basel, Switzerland) and [(3)H]N-(2-(4-(2-methoxyphenyl)- 1-piperazinyl)ethyl)- N-(2-pyridyl)cyclohexanecarboxamide 3HCl (WAY 100635, Wyeth Research, Berkshire, U.K.). Drugs were dissolved in distilled water or in physiological medium with the following exceptions: SB 200646A, SB 242084, ODQ (dimethylsulfoxide, DMSO) or ketanserin, mesulergine, MDL 100907 (acetic acid). Final bath concentrations of DMSO (up to 0.1%) or acetic acid (up to 0.001%) did not affect basal or NMDA-evoked cyclic GMP production.

Results

In human cerebral cortex slices the mean cyclic GMP basal level (3 min) was 47.9 ± 5.35 fmol mg^{-1} protein ($n=21$). NMDA stimulated formation of cyclic GMP: the cyclic GMP response to 100 μM NMDA amounted to 80.7 ± 5.82% ($n=21$) increase with respect to the basal level. When the cerebral cortex slices were exposed to the NO synthase inhibitor L-NOARG (100 μM) or to the soluble guanylate cyclase inhibitor ODQ (10 μM), the cyclic GMP response elicited by 100 μM NMDA was abolished (Figure 1).

The cyclic GMP response evoked by 100 μM NMDA was inhibited by 1 μM serotonin or by the 5-HT_2 receptor agonist (±)-DOI (Figure 2) indicating the involvement of serotonergic receptors of the 5-HT_2 type. As shown in Figure 3, the effect of (±)-DOI on the cyclic GMP response evoked by 100 μM NMDA was concentration-dependent: the EC_{50} value amounted to 22 nM. 5-HT or (±)-DOI did not affect the basal cyclic GMP level (data not shown).

Figure 4 shows that the effect of 1 μM (±)-DOI was counteracted by 1 μM mesulergine, a 5-HT_{2A}/5-HT_{2B}/5-HT_{2C} receptor antagonist (Hoyer *et al*., 1994). In contrast 0.1 μM MDL 100907, a potent and selective 5-HT_{2A} antagonist

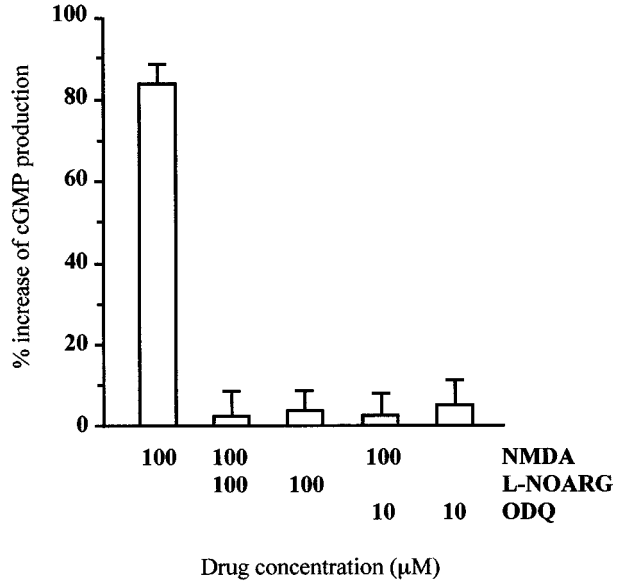

Figure 1 Inhibition by L-NOARG or by ODQ of the NMDA-evoked cyclic GMP production in human cerebral cortex slices. Slices were preincubated for 15 min with standard medium with or without enzyme inhibitors and then incubated for 3 min in the absence (controls) or in the presence of NMDA. Bars represent percentage increase of cyclic GMP levels as compared to the control values. Means±s.e.mean of three to four different experiments in duplicate are presented.

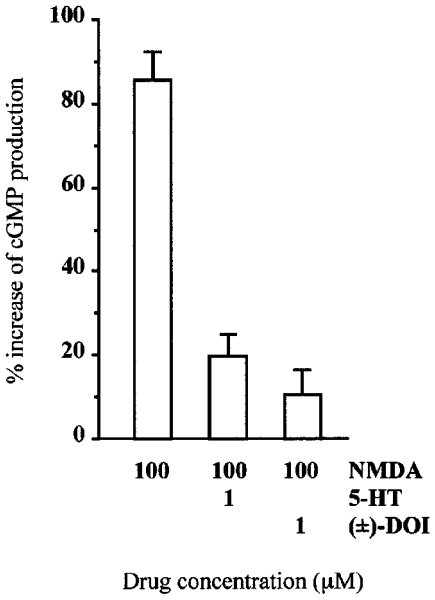

Figure 2 Inhibition by 5-HT or (±)-DOI of the NMDA-evoked cGMP production in human cerebral cortex slices. 5-HT or (±)-DOI were added concomitantly with NMDA. Bars represent percentage increase of cGMP levels as compared to the control values. Means±s.e.mean of three different experiments in duplicate are presented.

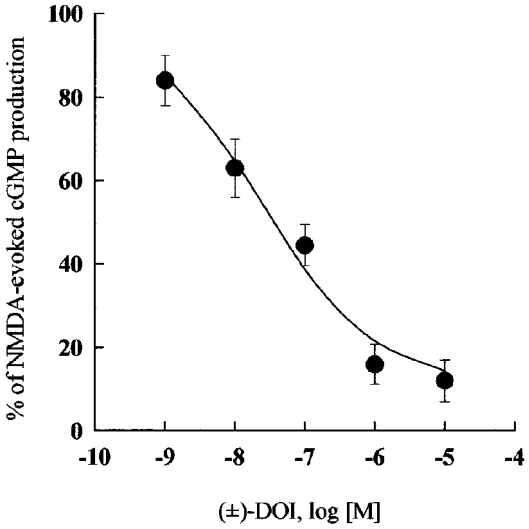

Figure 3 Inhibition by (±)-DOI of the NMDA-evoked cGMP production in human cerebral cortex slices. The effect of 100 μM NMDA was taken as 100%. The NMDA-evoked cyclic GMP production in the presence of (±)-DOI is expressed as percentage of the NMDA-evoked cyclic GMP production in the absence of drug. (±)-DOI was added concomitantly with NMDA. Means±s.e.mean of three to four different experiments in duplicate are presented.

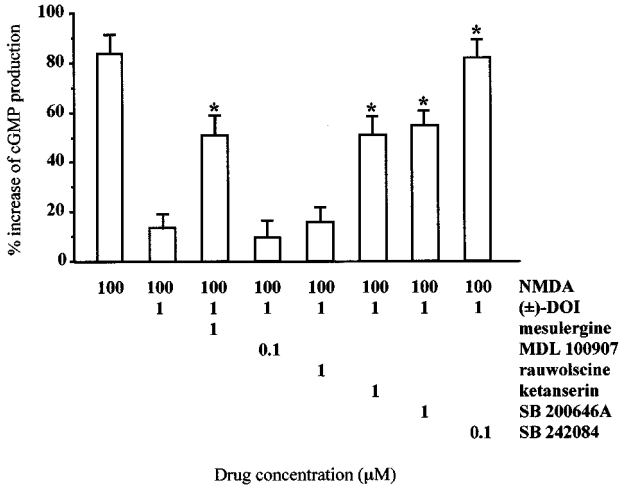

Figure 4 Effects of 5-HT_2 receptor antagonists with various receptor subtype selectivity on the (±)-DOI inhibition of the NMDA-evoked cGMP production in human cerebral cortex slices. (±)-DOI was added concomitantly with NMDA, antagonists 15 min before. The mean control values of each separate experiment did not differ significantly from each other and the values were therefore pooled for convenience. Bars represent percentage increase of cyclic GMP production with respect to the control value. Means±s.e.mean of three to thirteen different experiments in duplicate are presented. *$P<0.05$ when compared to (±)-DOI alone.

(Baxter *et al*., 1995), or 1 μM rauwolscine, antagonist with a relatively high affinity for the 5-HT_{2B} subtype and much lower affinities for the 5-HT_{2A} and 5-HT_{2C} subtypes (Wainscott *et al*., 1996), could not prevent the inhibition by (±)-DOI of the NMDA-evoked cyclic GMP elevation. Figure 4 also shows that the effect of (±)-DOI was prevented by 1 μM SB 200646A, a selective 5-HT_{2B}/5-HT_{2C} receptor antagonist (Forbes *et al*., 1993) and by 1 μM ketanserin, a potent 5-HT_{2A} receptor antagonist with activity also at the 5-HT_{2C} subtype (Baxter *et al*., 1995). Finally, the compound SB 242084, a potent and selective antagonist at the cloned human 5-HT_{2C} receptor subtype (Kennett *et al*., 1997), completely abolished the (±)-DOI inhibition of the NMDA-evoked cyclic GMP production, when added at 0.1 μM. None of the antagonists, at the concentration used, affected the basal or the NMDA-evoked cyclic GMP levels (data not shown).

We then tested if the novel compound RO 60-0175, found to be a selective 5-HT_{2C} receptor ligand in binding assays with cell lines expressing various human 5-HT receptors and to display agonistic properties in these cells and in the rat choroid

plexus (Martin *et al*., 1998), could affect the cyclic GMP elevation provoked by NMDA in human neocortex slices. Figure 5 shows that 1 μM of RO 60-0175 completely abolished the cyclic GMP response produced by 100 μM NMDA. The effect of RO 60-0175 was significantly prevented by 0.1 μM of the selective 5-HT_{2C} receptor antagonist SB 242084. Interestingly, the known antidepressant trazodone (1 μM) also abolished the NMDA-evoked cyclic GMP elevation in human tissue; the effect of trazodone was completely antagonized by 0.1 μM SB 242084 (Figure 5).

Finally, the cyclic GMP production caused by 100 μM NMDA in human neocortex slices was almost totally inhibited by the 5-HT_{1A} receptor agonist 8-OH-DPAT, added at 1 μM. The effect of 8-OH-DPAT could be prevented by 1 μM of the selective 5-HT_{1A} receptor antagonist WAY 100635 (Figure 6).

Discussion

The first result of this work is that the NMDA receptor/NO/cyclic GMP pathway can be studied in slices of human neocortex prepared from fresh specimens removed during neurosurgery. The cyclic GMP levels rise significantly upon addition of NMDA and this effect can be prevented by inhibiting NO synthase or soluble guanylate cyclase. An excellent model is therefore available to study functionally in human brain tissue a major glutamatergic pathway and its various steps including NMDA receptor activation and allosteric modulation, NO and cyclic GMP synthesis and phosphodiesterase activity.

The major finding of the paper concerns the important inhibitory control that 5-HT can exert on the glutamate/NO synthase/guanylate cyclase pathway in the human cerebrocortex through the activation of two subtypes of receptor, namely the 5-HT_{2C} and the 5-HT_{1A}. The involvement of 5-HT_{2C} receptors is indicated by (a) the differential sensitivity to a number of 5-HT_2 receptor antagonists of the inhibition of the NMDA-evoked cyclic GMP response produced by the 5-HT_2 agonist (±)-DOI; (b) the block of the (±)-DOI effect by the selective 5-HT_{2C} receptor antagonist SB 242084. The cyclic GMP elevation also was decreased by 8-OH-DPAT, a 5-HT_{1A} agonist with some affinity also at 5-HT_7 receptor (see Stowe & Barnes, 1998). 8-OH-DPAT effect was prevented by the selective 5-HT_{1A} antagonist WAY 100635 (Fletcher *et al*., 1996; Hirst *et al*., 1997), suggesting the involvement of receptors of the 5-HT_{1A} subtype.

The question arises as to whether the effects caused by 5-HT receptor activation result from inhibition of glutamate release (possibly induced by exogenous NMDA added to non-depolarized slices) onto receptors linked to cGMP generation or if 5-HT acts through mechanisms other than glutamate release inhibition. It was previously shown that the Ca^{2+}-dependent release of endogenous glutamate from human neocortex synaptosomes can be potently inhibited by 5-HT; however, this effect was totally insensitive to spiperone or the selective 5-HT_{1A} receptor antagonist WAY 100135 (Maura *et al*., 1998), which tends to exclude the existence on glutamatergic terminals of 5-HT_2 or 5-HT_{1A} receptors mediating inhibition of glutamate release. One possibility that should be considered is that inhibition of the NMDA-evoked cGMP response by 5-HT_{2C} receptor activation involves stimulation of the release of an inhibitory transmitter. It was recently reposed that (±)-DOI can increase extracellular GABA levels in the rat cortex during *in vivo* microdialysis (Abi Saab *et al*., 1999). One could therefore hypothesize that 5-HT_{2C} receptors located on GABAergic interneurons in the human neocortex mediate release of GABA onto receptors co-localized with NMDA receptors on NO synthase-containing cells, leading to inhibition of the NMDA-evoked cGMP elevation. As to the inhibitory 5-HT_{1A} receptors, they could be co-localized with NMDA receptors on the NO synthase-containing cells. Using intracellular recordings in slices of

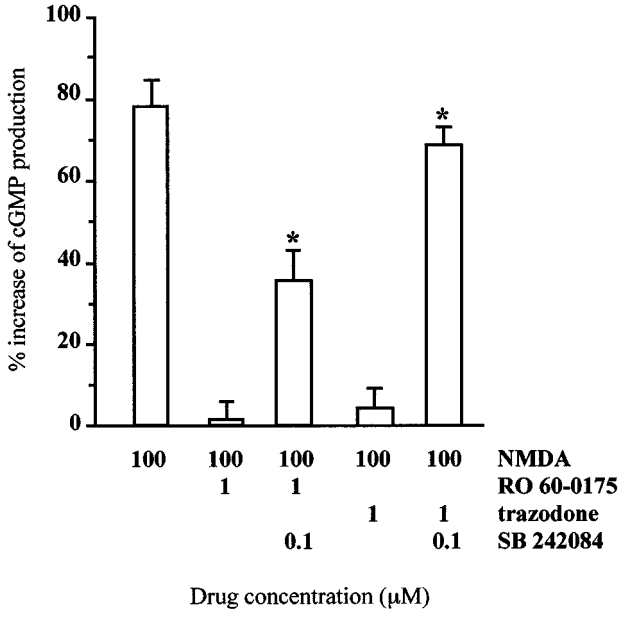

Figure 5 Inhibition of the NMDA-evoked cGMP production in human cerebral cortex slices by RO 60-0175 or trazodone. Agonists were added concomitantly with NMDA, the antagonist SB 242084 15 min before. Bars represent percentage increase of cGMP production with respect to the control value. Means ± s.e.mean of three to four different experiments in duplicate are presented. *$P<0.05$ when compared to the serotonergic agonist alone.

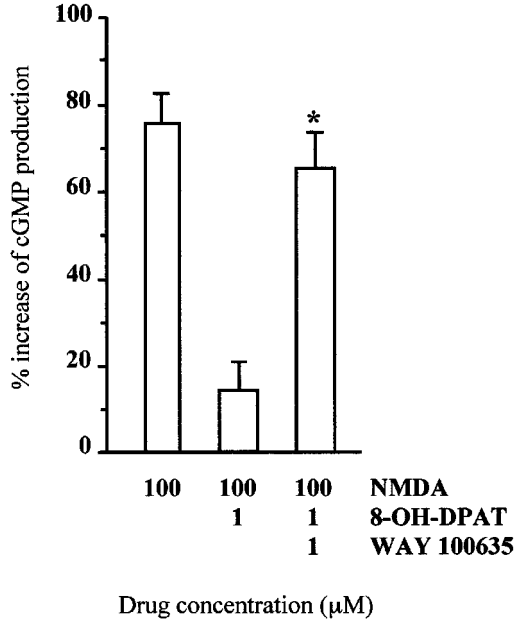

Figure 6 Inhibition of the NMDA-evoked cyclic GMP elevation in human neocortex slices by 8-OH-DPAT. The agonist was added concomitantly with NMDA, the antagonist WAY 100635 was present from 15 min before. Bars represent percentage increase of cyclic GMP production with respect to the control value. Means ± s.e.mean of three different experiments in duplicate are presented. *$P<0.05$ when compared to 8-OH-DPAT alone.

human neocortex, it was recently observed that neurons (apparently glutamatergic pyramidal neurons) can be hyperpolarized by serotonin via 5-HT_{1A} receptors (Newberry *et al*., 1999).

A third result of the present investigation is the ability of trazodone to inhibit the NMDA receptor/NO/cGMP pathway through the activation of 5-HT_{2C} receptors. Trazodone is an antidepressant drug marketed in several countries (see, for a review, Haria *et al*., 1994). Although it is unclear how the drug acts to alleviate symptoms of depression, interactions of trazodone with the 5-HT system have been proposed by several authors. The drug can inhibit 5-HT uptake (Garattini *et al*., 1976; Stefanini *et al*., 1976); such an activity appears, however, too weak to explain the clinical efficacy of trazodone, particularly if compared with those of antidepressants that are selective serotonin uptake inhibitors (Owens *et al*., 1997). Trazodone is thought of as a 5-HT receptor antagonist (Bryant & Ereshefsky, 1982; Fuller *et al*., 1984; Jenck *et al*., 1993; Cusack *et al*., 1994; Owens *et al*., 1997; Takeuchi *et al*., 1997). More precisely, trazodone appears to target preferentially receptors of the 5-HT_2 type and the few data available, in part based on behavioural 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. Our results with human neocortex slices appear to contrast with this view. In this model, trazodone mimics 5-HT and (±)-DOI, thus behaving as a 5-HT_2 receptor agonist. Moreover, the effect of trazodone is completely abolished by the selective 5-HT_{2C} receptor antagonist SB 242084. These results support the view that trazodone, at concentrations compatible with those reached during antidepressant treatment, can behave as a 5-HT_{2C} receptor agonist in the human cerebral cortex. Interestingly, a recent behavioural study in rats, mice and monkeys reports that the selective 5-HT_{2C} agonist RO 60-0175 exhibits a favourable therapeutic potential in depression (Martin *et al*., 1998); the compound was also reported to be sedative but lacking any anxiolytic or anxiogenic effects in rats (Kennett *et al*., 2000). In human neocortex slices RO 60-0175 inhibited the cGMP response similarly to trazodone (Figure 5). Our results suggest therefore that 5-HT_{2C} receptor activation could be relevant to the antidepressant activity of trazodone and, possibly, of selective serotonin reuptake inhibitors which also indirectly activate 5-HT_{2C} receptors. Receptors of the 5-HT_{2C} subtype can be found in high concentration in cortico-limbic regions suggesting that they may fulfil a major role in the control of mood (Pompeiano *et al*., 1994; Abramowski *et al*., 1995; Barnes & Sharp, 1999, for review). On the other hand, 5-HT_{1A} receptor agonists have been reported to exhibit effective antidepressant activity (see Lucki, 1991; Sussman, 1998), possibly through the activation of postsynaptic 5-HT_{1A} receptors located in limbic structures (Blier & de Montigny, 1994; Rueter & Blier, 1999). It should be added that antidepressants have been reported to produce adaptive changes, generally inhibitory, of NMDA receptor functions (Trullas & Skolnick, 1990; Cai & McCaslin, 1992; Kilts, 1994; Leonard, 1997; Nowak *et al*., 1998).

The present results, together with previous data on glutamate release from human neocortex synaptosomes, support the idea that 5-HT is a major inhibitory agent of glutamatergic transmission in the human cerebral cortex. Not only can serotonin inhibit the evoked release of glutamate from nerve terminals by acting at presynaptic h5-HT_{1D} receptors (Maura *et al*., 1998); it also can inhibit events triggered by glutamate release by acting at postsynaptic receptors of the 5-HT_{1A} and of the 5-HT_{2C} subtype. The finding that the cGMP production elicited by 100 μM NMDA can be almost totally abolished by either 1 μM (±)-DOI or 1 μM 8-OH-DPAT is intriguing as it seems that 5-HT_{1A} and 5-HT_{2C} receptors make up a dual control on the same pathway. Whatever the mechanisms, agonists at human 5-HT_{1D}, 5-HT_{2C} and 5-HT_{1A} receptors deserve attention as potentially useful drugs in neuropathologies with underlying excessive glutamatergic transmission.

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References

ABI-SAAB, W.M., BUBSER, M., ROTH, R.H. & DEUTCH, A.Y. (1999). 5-HT_2 receptor regulation of extracellular GABA levels in the prefrontal cortex. *Neuropsychopharmacol.,* **20,** 92–96.

ABRAMOWSKI, D., RIGO, M., DUC, D., HOYER, D. & STAUFENBIEL, M. (1995). Localization of the 5-hydroxytryptamine$_{2C}$ receptor protein in human and rat brain using specific antisera. *Neuropharmacology,* **34,** 1635–1645.

BARNES, N.M. & SHARP, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology,* **38,** 1083–1152.

BAXTER, G., KENNETT, G., BLANEY, F., BLACKBURN, T. (1995). 5-HT_2 receptor subtypes: a family re-united? *Trends Pharmacol. Sci.,* **16,** 105–110.

BLIER, P. & DE MONTIGY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.,* **15,** 220–226.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72,** 248–254.

BREDT, D.S. & SNYDER, S.H. (1989). Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proc. Natl. Acad. Sci. U.S.A.,* **86,** 9030–9033.

BRYANT, S.G. & ERESHEFSKY, L. (1982). Antidepressant properties of trazodone. *Clin. Pharm.,* **1,** 406–417.

CAI, Z. & MCCASLIN, P.P. (1992). Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainate- and N-methyl-D-aspartate-induced intracellular Ca^{2+} levels in neuronal culture. *Eur. J. Pharmacol.,* **219,** 53–57.

CUSACK, B., NELSON, A. & RICHELSON, E. (1994). Binding of antidepressants to human brain receptors: focus on newer generation compounds. *Psychopharmacology (Berl.),* **114,** 559–565.

DAVIES, J.A. & LEIGHTON, E. (1984). Effect of cinanserin on the release of glutamate from rat cerebellar slices. *Br. J. Pharmacol.,* **82,** 271P.

DICKIE, B.G., LEWIS, M.J. & DAVIES, J.A. (1990). Potassium-stimulated release of nitric oxide from cerebellar slices. *Br. J. Pharmacol.,* **101,** 8–9.

EAST, S.J. & GARTHWAITE, J. (1991). NMDA receptor activation in rat hippocampus induces cyclic GMP formation through the L-arginine-nitric oxide pathway. *Neurosci. Lett.,* **123,** 17–19.

FEDELE, E. & RAITERI, M. (1999). In vivo studies of the cerebral glutamate receptor/NO/cGMP pathway. *Prog. Neurobiol.,* **58,** 89–120.

FLETCHER, A., FORSTER, E.A., BILL, D.J., BROWN, G., CLIFFE, I.A., HARTLEY, J.E., JONES, D.E., McLENACHAN, A., STANHOPE, K.J., CRITCHLEY, D.J., CHILDS, K.J., MIDDLEFELL, V.C., LANFUMEY, L., CORRADETTI, R., LAPORTE, A.M., GOZLAN, H., HAMON, M. & DOURISH, C.T. (1996). Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. *Behav. Brain Res.,* **73,** 337–353.

FORBES, I.T., KENNETT, G.A., GADRE, A., HAM, P., HAYWARD, C.J., MARTIN, R.T., THOMPSON, M., WOOD, M.D., BAXTER, G.S., GLEN, A., MURPHY, O.E., STEWART, B.A. & BLACKBURN, P.T. (1993). N-(1-methyl-5-indolyl)-N′-(3-pyridyl)urea hydrochloride: the first selective 5-HT_{1C} receptor antagonist. *J. Med. Chem.,* **36,** 1104–1107.

FULLER, R.W., SNODDY, H.D. & COHEN, M.L. (1984). Interactions of trazodone with serotonin neurons and receptors. *Neuropharmacology,* **23,** 539–544.

GARATTINI, S., DEGAETANO, G., SAMANIN, R., BERNASCONI, S. & RONCAGLIONI, M.C. (1976). Effects of trazodone on serotonin in the brain and platelets of the rat. *Biochem. Pharmacol.,* **25,** 13–16.

GARTHWAITE, J. (1982). Excitatory amino acid receptors and guanosine 3′,5′-cyclic monophosphate in incubated slices of immature and adult rat cerebellum. *Neuroscience,* **7,** 2491–2497.

GARTHWAITE, J. & BALAZS, R. (1978). Supersensitivity to the cyclic GMP response to glutamate during cerebellar maturation. *Nature,* **275,** 328–329.

HARIA, M., FITTON, A. & McTAVISH, D. (1994). Trazodone. A review of its pharmacology, therapeutic use in depression and therapeutic potential in other disorders. *Drugs Aging,* **4,** 331–355.

HIRST, W.D., PRICE, G.W., RATTRAY, M. & WILKIN, G.P. (1997). Identification of 5-hydroxytryptamine receptors positive coupled to adenylyl cyclase in rat cultured astrocytes. *Br. J. Pharmacol.,* **120,** 509–515.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytrypamine (Serotonin). *Pharmacol. Rev.,* **46,** 157–203.

JENCK, F., MOREAU, J.L., MUTEL, V., MARTIN, J.R. & HAEFELY, W.E. (1993). Evidence for a role of 5-HT_{1C} receptors in the antiserotonergic properties of some antidepressant drugs. *Eur. J. Pharmacol.,* **231,** 223–229.

KENNETT, G., LIGHTOWLER, S., TRAIL, B., BRIGHT, F. & BROMIDGE, S. (2000). Effects of RO 60 0175, a 5-HT_{2C} receptor agonist, in three animal models of anxiety. *Eur. J. Pharmacol.,* **387,** 197–204.

KENNETT, G.A., WOOD, M.D., BRIGHT, F,. TRAIL, B., RILEY, G., HOLLAND, V., AVENELL, K.Y., STEAN, T., UPTON, N., BROMIDGE, S., FORBES, I.T., BROWN, A.M., MIDDLEMISS, D.N. & BLACKBURN, T.P. (1997). SB-242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. *Neuropharmacology,* **36,** 609–620.

KILTS, C.D. (1994). Recent pharmacologic advances in antidepressants therapy. *A. J. Med.,* **97,** 3S–12S.

LEONARD, B.E. (1997). The role of noradrenaline in depression: a review. *J. Psychopharmacol.,* **11,** S39–S47.

LUCKI, I. (1991). Behavioral studies of serotonin receptor agonists as antidepressant drugs. *J. Clin. Psychiatry,* **52,** 24–31.

MARCOLI, M., MAURA, G., MUNARI, C., RUELLE, A. & RAITERI, M. (1999). Pharmacological diversity between native human 5-HT_{1B} and 5-HT_{1D} receptors sited on different neurons and involved in different functions. *Br. J. Pharmacol.,* **126,** 607–612.

MARCOLI, M., MAURA, G., TORTAROLO, M. & RAITERI, M. (1997). Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in rat cerebellum: involvement of 5-hydroxytryptamine$_{2C}$ receptors. *J. Neurochem.,* **69,** 427–430.

MARTIN, J.R., BÖS, M., JENCK, F., MOREAU, J.-L., MUTEL, V., SLEIGHT, A.J., WICHMANN, J., ANDREWS, J.S., BERENDSEN, H.H.G., BROEKKAMP, C.L.E., RUIGT, G.S.F., KÖHLER, C. & VAN DELFT, A.M.L. (1998). 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. *J. Pharmacol. Exp. Ther.,* **286,** 913–924.

MAURA, G. & RAITERI, M. (1996). Serotonin 5-HT_{1D} 5-HT_{1A} receptors respectively mediate inhibition of glutamate release and inhibition of cyclic GMP production in rat cerebellum in vitro. *J. Neurochem.,* **66,** 203–209.

MAURA, G., GUADAGNIN, A. & RAITERI, M. (1995). Low nanomolar serotonin inhibits the glutamate receptor/nitric oxide/cyclic GMP pathway in slices from adult rat cerebellum. *Neuroscience,* **68,** 455–463.

MAURA, G., MARCOLI, M., TORTAROLO, M., ANDRIOLI, G.C. & RAITERI, M. (1998). Glutamate release in human cerebral cortex and its modulation by 5-hydroxytryptamine acting at h5-HT_{1D} receptors. *Br. J. Pharmacol.,* **123,** 45–50.

NEWBERRY, N.R., FOOTITT, D.R., PAPANASTASSIOU, V. & REYNOLDS, D.J. (1999). Actions of 5-HT on human neocortical neurones in vitro. *Brain Res.,* **833,** 93–100.

NOWAK, G., LEGUTKO, B., SKOLNICK, P. & POPIK, P. (1998). Adaptation of cortical NMDA receptors by chronic treatment with specific serotonin reuptake inhibitors. *Eur. J. Pharmacol.,* **342,** 367–370.

OWENS, M.G., MORGAN, W.N., PLOTT, S.J. & NEMEROFF, C.B. (1997). Neurotransmitter receptor and transporter binding profile of antidepressant and their metabolites. *J. Pharmacol. Exp. Ther.,* **283,** 1305–1322.

POMPEIANO, M., PALACIOS, J.M. & MENGOD, G. (1994). Distribution of the serotonin 5-HT_2 receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol. Brain Res.,* **23,** 163–178.

RAITERI, M., MAURA, G. & BARZIZZA, A. (1991). Activation of presynaptic 5-hydroxytryptamine$_1$-like receptors on glutamatergic terminals inhibits N-methyl-D-aspartate-induced cyclic GMP production in rat cerebellar slices. *J. Pharmacol. Exp. Ther.,* **257,** 1184–1188.

RAITERI, M., MAURA, G., BONANNO, G. & PITTALUGA, A. (1986). Differential pharmacology and function of two 5-HT_1 receptors modulating transmitter release in rat cerebellum. *J. Pharmacol. Exp. Ther.,* **237,** 644–648.

RUETER, L.E. & BLIER, P. (1999). Electrophysiological examination of the effects of sustained flibanserin administration on serotonin receptors in rat brain. *Br. J. Pharmacol.,* **126,** 627–638.

SIEGEL, B.W., FREEDMAN, J., VAAL, M.J. & BARON, B.M. (1996). Activities of novel aryloxyalkylimidazolines on rat 5-HT_{2A} and 5-HT_{2C} receptors. *Eur. J. Pharmacol.,* **296,** 307–318.

STEFANINI, E., FADDA, F., MEDDA, L. & GESSA, G.L. (1976). Selective inhibition of serotonin uptake by trazodone, a new antidepressant agent. *Life Sci.,* **18,** 1459–1465.

STOWE, R.L. & BARNES, N.M. (1998). Selective labelling of 5-HT_7 receptor recognition sites in rat brain using [^{3}H]5-carboxamidotryptamine. *Neuropharmacology,* **37,** 1611–1619.

SUSSMAN, N. (1998). Anxiolytic antidepressant augmentation. *J. Clin. Psychiatry,* **59,** 42–50.

TAKEUCHI, H., YATSUGI, S., HATANAKA, K., NAKATO, K., HATTORI, H., SONODA, R., KOSHIYA, K., FUJII, M. & YAMAGUCHI, T. (1997). Pharmacological studies on YM99, a novel antidepressant with selective serotonin re-uptake inhibitory and 5-HT_{2A} receptor antagonistic activity. *Eur. J. Pharmacol.,* **329,** 27–35.

TRULLAS, R. & SKOLNICK, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur. J. Pharmacol.,* **185,** 1–10.

VALLEBUONA, F. & RAITERI, M. (1994). Extracellular cGMP in the hippocampus of freely moving rats as an index of nitric oxide (NO) synthase activity. *J. Neurosci.,* **14,** 134–139.

WAINSCOTT, D.B., LUCAITES, V.L., KURSAR, J.D., BAEZ, M. & NELSON, D.L. (1996). Pharmacologic characterization of the human 5-hydroxytrypamine$_{2B}$ receptor: evidence for species differences. *J. Pharmacol. Exp. Ther.,* **276,** 720–727.

WOOD, P.L. RYAN, R. & LI, M. (1992). Excitatory amino acid signal transduction in the hippocampus: role of noradrenergic afferents and nitric oxide in cGMP increases in vivo. *Life. Sci.,* **51,** 601–606.

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