Inhibitory Presynaptic 5-Hydroxytryptamin_{2A} Receptors Regulate Evoked Glutamate Release from Rat Cerebellar Mossy Fibers

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ABSTRACT
We studied the pharmacological characterization of the 5-hydroxytryptamine_{2A} (5-HT_{2A}) heteroreceptor located on glutamatergic cerebellar mossy fiber nerve terminals. Depolarization-evoked overflow of endogenous glutamate from rat cerebellar “giant” mossy fiber synaptosomes was inhibited by 5-HT or (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ([+]-DOI), exhibiting pD_{2} (−log EC_{50}) values of 7.37 and 7.29, respectively. Trazodone inhibited the depolarization-evoked glutamate overflow, exhibiting lower potency (pD_{2} = 6.42) and lower efficacy with respect to 5-HT or (+)-DOI (maximal inhibition, 54%, compared with 70% for either 5-HT or (+)-DOI). Ketanserin, a 5-HT_{1A}/5-HT_{2C} receptor antagonist, counteracted the inhibitory effect of (+)-DOI or trazodone. Inhibition of glutamate overflow by 5-HT, (+)-DOI, or trazodone was prevented by the selective 5-HT_{2A} receptor antagonist R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenoxy)ethyl]-4-piperidine-methanol (MDL 100907), while the potent and selective 5-HT_{2C} receptor antagonist 6-chloro-5-methyl-1-[6-(methyl)pyridin-3-yl)pyridin-3-yl carbamoyl] indoline (SB 242084) was ineffective. In cerebellar slices, MDL 100907 increased on its own the K^{+}-evoked release of glutamate. It is concluded that the evoked release of glutamate from cerebellar mossy fibers can be controlled by inhibitory presynaptic 5-HT_{2A} heteroreceptors, the receptors can be activated by endogenously released 5-HT, and trazodone behaves as a partial agonist at these receptors.

The function of serotonin (5-hydroxytryptamine; 5-HT) as a modulator of neuronal activity and, in particular, of transmitter release in the mammalian central nervous system is well established. 5-HT receptors located on soma/dendrites and on terminals of 5-HT neurons can function as autoreceptors (Barnes and Sharp, 1999; Pinero and Blier, 1999). On the other hand, 5-HT_{1B,D}, 5-HT_{2A} or 5-HT_{3} heteroreceptors located on nonserotonergic neuronal terminals can regulate release of diverse neurotransmitters, such as acetylcholine, noradrenaline, dopamine, γ-aminobutyric acid, glutamate or cholecystokinin (Barnes and Sharp, 1999; Sarhan and Filion, 1999).

We previously found that 5-HT can potently inhibit the release of endogenous glutamate from adult rat cerebellar slices by acting at 5-HT_{1} and 5-HT_{2} receptors (Maura et al., 1988). Experiments with synaptosomes isolated from the cerebellum by a standard procedure indicated that the 5-HT_{1} receptors, belonging to the 5-HT_{1D} subtype, are sited on glutamate-releasing terminals of parallel/climbing fibers (Raiteri et al., 1986; Maura and Raiteri, 1996); surprisingly, release-regulating 5-HT_{1} receptors appeared to be lost during the preparation of synaptosomes.

Immunohistochemical, electrophysiological, and pharmacological evidence indicates that, in addition to parallel/climbing fibers, cerebellar mossy fibers use glutamate as their major transmitter (Beitz et al., 1986; Somogyi et al., 1986). Terminals of mossy fibers are relatively large; therefore, during homogenization of cerebellar tissue, two types of synaptosomes are produced: “standard” synaptosomes (the smaller synaptosomes that concentrate in the crude mitochondrial fraction) and giant synaptosomes (larger synaptosomes sediment in the nuclear fraction). According to Israel and Whittaker (1965), giant synaptosomes originate from the large terminals of mossy fibers. Because of the different sedimentation properties, giant synaptosomes are not present in standard synaptosomal preparations. However, they can be obtained from the nuclear fraction of the cerebellar homogenates. We previously prepared giant synaptosomes from rat cerebellum (Maura et al., 1991). Their morphology appeared well preserved and similar to that originally described by Israel and Whittaker (1965). In par-
cular, giant synaptosomes released endogenous glutamate upon K\(^+\)-depolarization in a calcium-dependent manner, indicating good viability. Furthermore, the evoked release of glutamate was inhibited by 5-HT or by (±)-DOI, suggesting that the 5-HT\(_2\) receptors originally identified in cerebellar slices could be heteroreceptors located on mossy fiber endings lost during standard synaptosomal preparation. Accordingly, 5-HT\(_1\) agonists displayed no activity in the giant synaptosome preparation indicating little or no contamination by standard glutamatergic synaptosomes carrying 5-HT\(_{1D}\) receptors (Maura et al., 1991).

Receptors of the 5-HT\(_2\) type have been shown to exist in at least three structurally different subtypes termed 5-HT\(_{2A}\), 5-HT\(_{2B}\), and 5-HT\(_{2C}\); moreover, ligands endowed with selectivity for these subtypes have been developed (for review, see Barnes and Sharp, 1999). It was therefore possible to approach a more detailed pharmacological characterization of the release-regulating 5-HT\(_2\) receptor presumably present on rat cerebellar mossy fibers. This work was carried out mainly with preparations of giant synaptosomes. 5-HT and (±)-DOI, which display similar affinities for the 5-HT\(_{2A}\), 5-HT\(_{2B}\), or 5-HT\(_{2C}\) subtype (Hoyer et al., 1994; Baxter et al., 1995) were used as agonists; the results originally obtained with the two drugs (Maura et al., 1991) have been completed by constructing full concentration-response curves in this study. The known antidepressant trazodone, generally thought of as a 5-HT receptor antagonist (Haria et al., 1994; Takeuchi et al., 1997), was studied because during preliminary experiments, carried out previously in our laboratory, it had exhibited some activity at the 5-HT\(_2\) receptors on giant cerebellar synaptosomes (see also Garrone et al., 2000). Various 5-HT\(_2\) receptor antagonists were used to discriminate between receptor subtypes. In a set of experiments the role of endogenous 5-HT acting at the 5-HT\(_2\) receptors present on mossy fiber terminals in regulating glutamate release was investigated using cerebellar slices.

**Materials and Methods**

**Animals.** Adult Sprague-Dawley male rats weighing 200–250 g were housed at constant temperature (22 ± 1°C) and relative humidity (50%) under a regular light/dark schedule (lights on 7:00 AM to 7:00 PM). Food and water were freely available. The animals were killed by decapitation. The cerebellum was rapidly removed and placed in ice-cold medium. Experimental procedures were approved by the Ethical Committee of the Pharmacology and Toxicology Section, Department of Experimental Medicine in accordance with the European legislation (European Communities Council Directive of 24 November 1986, 86/609/EEC).

**Experiments with Giant Synaptosomes.** Giant synaptosomes were prepared according to Israel and Whittaker (1965) with some modifications. The cerebellum was removed and homogenized in 40 volumes of 0.32 M sucrose buffered with phosphate at pH 7.4. The homogenate was centrifuged at 1000 g for 5 min. The pellet (P\(_1\); crude nuclear fraction) was resuspended in an equal volume of sucrose 0.32 M, pH 7.4, filtered through a double gauze layer, and then centrifuged at 1000 g for 5 min. The pellet containing the giant synaptosomes was resuspended in a physiological salt solution of the following composition: 125 mM NaCl, 3 mM KCl, 1.2 mM MgSO\(_4\), 1.2 mM CaCl\(_2\), 1 mM NaH$_2$PO\(_4\), 22 mM NaHCO\(_3\), and 11 mM glucose (aerated with 95% O\(_2\) and 5% CO\(_2\) at 37°C, pH 7.2 to 7.4). Giant synaptosomes were incubated 15 min at 37°C in standard medium. After incubation, identical aliquots of the synaptosomal suspension were distributed at the bottom of a set of parallel superfusion chambers maintained at 37°C (Raiteri et al., 1974). Superfusion was started at a rate of 0.6 ml/min with standard medium aerated continuously with 5% CO\(_2\) in O\(_2\). Glutamate release was evoked by exposure to 15 mM KCl (replacing an equimolar concentration of NaCl) for 120 s, after 38 min of superfusion. Two 3-min samples (basal outflow) were collected before and after the 6-min sample containing the glutamate released by the depolarization pulse. Superfusate fractions were collected in plastic vials and rapidly frozen. Agonists were added concomitantly with high K\(^+\) and antagonists 8 min before depolarization. Glutamate released in the collected fractions was expressed as picomoles per milligram synaptosomal protein or as percentage of the glutamate tissue content at the beginning of the fraction. Protein determination was carried out according to Bradford (1976). The K\(^+\)-evoked overflow was calculated by subtraction of glutamate content in the two 3-min fractions (pre- and poststimulation basal outflow) from the total content in the 6-min fraction corresponding to high K\(^+\) stimulation and was measured as percentage of increase with respect to the prestimulation basal outflow. Depolarization-evoked overflow in the presence of drugs was calculated as percentage of variation with respect to the control.

**Experiments with Slices.** The isolated cerebellum was chopped into 250-µm slices using a McIlwain tissue chopper. After 10 min of incubation at 37°C in standard medium, slices were transferred at the bottom of a set of parallel superfusion chambers (3 slices per chamber) at 37°C. Superfusion was started at a rate of 0.6 ml/min with standard medium aerated with 5% CO\(_2\) in O\(_2\). Two 120-s depolarizations (35 mM KCl replacing an equimolar NaCl concentration) were applied 35 and 78 min after start of superfusion (S\(_1\) and S\(_2\), respectively). Two 4-min samples (basal outflow) were collected before and after 8-min samples containing glutamate released by depolarization pulses; superfusate fractions were collected in plastic vials and rapidly frozen. Glutamate released in the collected fractions was expressed as picomoles per milligram protein or as percentage of the glutamate tissue content at beginning of the fraction. The K\(^+\)-evoked overflow during S\(_1\) or S\(_2\) was calculated by subtraction of glutamate content (picomoles or percentage of the glutamate tissue content) in the pre- and poststimulation basal fractions from the total content in the 8-min fractions corresponding to K\(^+\) stimulation. The antagonists were added 8 min before S\(_2\); their effects on K\(^+\)-evoked overflow were measured as percentage of variation of the S\(_2\)/S\(_1\) ratio with respect to the control value.

**Glutamate Determination.** The amount of endogenous glutamate released in the fractions collected (or remaining in synaptosomes or slices) was measured by high-performance liquid chromatography. Glutamate content of synaptosomes or slices was measured in the supernatant obtained after homogenization (Ultra Turrax, M. Cella, Milan, Italy; maximum speed, 20 s) in ice-cold distilled water and centrifugation at 20,000 g for 10 min. The analytical method involved automatic precolumn derivatization (Waters 715 ultra wisp; Waters, Milford, MA) with o-phthaldialdehyde followed by separation on C\(_{18}\) reverse phase chromatography column (Chrompack International, Middleburg, The Netherlands; 10 cm × 4.6 mm, 3 µm) and fluorometric detection. Buffers and gradients program were as follows: solvent A, 0.1 M sodium acetate (pH 5.8)/methanol, 80:20; solvent B, 0.1 M sodium acetate (pH 5.8)/methanol, 20:80; solvent C, 0.1 M sodium acetate (pH 6.0)/methanol, 80:20; gradient program, 100% solvent C for 4 min; 90% solvent A and 10% solvent B in 1 min; isocratic step for 2 min; 78% solvent A and 22% solvent B in 2 min; isocratic step for 6.5 min; 66% solvent A and 34% solvent B in 1.1 min; isocratic step for 1.5 min; 42% solvent A and 58% solvent B in 1.1 min; isocratic step for 3.5 min; flow rate 0.9 ml/min (Waters 600 MS gradient system). Homoserine was used as internal standard. The detection limit was 100 fmol/µl.

**Calculation.** EC\(_{50}\) values for agonists were determined from curves obtained using a four-parameter logistic function fitting routine (Sigma Plot software). pD\(_2\) (= log EC\(_{50}\)) values were taken as measure of the agonist potency. Means ± S.E. of n number of experiments are presented throughout. Significance of the difference was
analyzed by analysis of variance followed by post hoc Scheffé multiple comparison or Student’s t test. Level of significance was set at \( p < 0.05 \).

**Drugs.** The following drugs were purchased: \((\pm)-1-(2,5$-$dime-thoxy-4$-$iodophenyl)$-2$-$aminopropanoic acid \((\pm)$-DOI) from Sigma/RBI (Natick, MA); ketanserin from Torcim Cookson (Bristol, UK); 5-HT creatinine sulfate from Calbiochem (Los Angeles, CA). The following drugs were donated: \(R(-)+\alpha$-$[2,3$-$dimethoxyphenyl]$-1$-(2$-$fluorophenyl)ethyl$]-4$-piperidinemethanol \((MDL100907)\) from Hoechst Marin Russel (Cincinnati, OH); 6$-$chloro$-$5$-$methyl$-1$-$[6$-$methylpyridin$-$3$-$yl$]$-pyridin$-$3$-$yl$-carbamoyl indoline \((SB242084)\) from SmithKline Beecham Pharmaceuticals (West Sussex, UK); trazodone from Istituto Ricerche Francesco Angelini (Pomezia, Roma, Italy).

**Results**

**Release from Giant Synaptosomes.** The overflow of endogenous glutamate \((6$-$min samples) evoked during depolarization with \(15\) mM KCl \((120\) s) of superfused rat cerebellar giant synaptosomes amounted to \(470 \pm 52.4\) pmol/mg of protein \((n = 14)\); the basal outflow in the 3$-$min samples collected before and after K$^+$ depolarization amounted to \(134 \pm 11.8\) pmol/mg of protein \((n = 14)\) and \(119 \pm 9.5\) pmol/mg of protein \((n = 14)\), respectively. Expressed as percentage of the total synaptosomal glutamate content, the basal release in the 3$-$min sample collected before and after K$^+$ stimulation amounted to \(60 \pm 0.05\) pmol/min \((n = 4)\) and \(51 \pm 0.04\) pmol/min \((n = 4)\), respectively, whereas the K$^+$-evoked overflow \((6$-$min sample) was \(9.35 \pm 0.90\%\) \((n = 4)\). Previous experiments \((Maura et al., 1991)\) had shown that the overflow of endogenous glutamate evoked by K$^+$ depolarization of rat cerebellar giant synaptosomes in superfusion was almost entirely Ca$^{2+}$-dependent.

Figure 1 shows concentration$-$dependent inhibition of endogenous glutamate release by 5-HT, \((\pm)$-DOI, or trazodone \((5$-$HT, \(pD_2 = 7.37); \((\pm)$-DOI, \(pD_2 = 7.29); \) trazodone, \(pD_2 = 6.42\)\. Maximum glutamate overflow inhibition by the compounds tested amounted to \(70\%\) for 5-HT or \((\pm)$-DOI but only to \(54\%\) for trazodone.

We previously reported that ketanserin, a potent 5$-$HT$_{2A}$ receptor antagonist with moderate affinity for the 5$-$HT$_{2C}$ receptor subtype \((Baxter et al., 1995)\), counteracted the 5-HT inhibition of glutamate overflow from giant synaptosomes \((Maura et al., 1991)\). In Figs. 3 and 4 ketanserin is shown to antagonize \((\pm)$-DOI or trazodone effects. The compound SB 242084, a potent and selective antagonist at the recombinant human 5$-$HT$_{2C}$ receptor \((Kennett et al., 1997)\), was ineffective against 5-HT or \((\pm)$-DOI in our system \((Figs. 2 and 3)\). On the other hand, the 5$-$HT$_{2A}$ receptor antagonist MDL 100907 \((Baxter et al., 1995)\) prevented the inhibition of glutamate release from giant synaptosomes produced by 5-HT \((Fig. 2)\), \((\pm)$-DOI \((Fig. 3)\) or trazodone \((Fig. 4)\). None of the antagonists at the concentrations used affected on its own the basal release or the depolarization-evoked overflow of glutamate \((data not shown)\).

**Release from Cerebellar Slices.** The fractional basal overflow of glutamate in the 4$-$min fraction collected before S1 \((30.79 \pm 2.62\) pmol/mg of protein/min; mean \(\pm\) S.E., \(n = 6)\) was \(1.013 \pm 0.0145\%\)/min. The glutamate overflow evoked by K$^+$ \((35\) mM) depolarization during S1 \((758.7 \pm 63.6\) pmol/mg of protein; mean \(\pm\) S.E., \(n = 6)\) amounted to \(4.02 \pm 0.32\%\). The mean control S$_2$/S$_1$ ratio \((0.99 \pm 0.07;\ mean \(\pm\) S.E., \(n = 6)\) was significantly increased to \(1.35 \pm 0.10 (\pm 37\%);\ mean \(\pm\) S.E., \(n = 6)\) when MDL 100907 \((1\) \(\mu\)M) was added 8 min before S$_2$. The S$_2$/S$_1$ ratio in the presence of the 5$-$HT$_{2A}$ antagonist did not significantly differ from the value obtained in parallel superfusion chambers in the presence of ketanserin \((1\) \(\mu\)M): \(1.32 \pm 0.08 (\pm\) S.E., \(n = 6)\).
observed in situ and purified from cerebellar homogenates (Israel and Whittaker, 1965; Maura et al., 1991). In addition, these preparations release glutamate upon depolarization in a Ca\(^{2+}\)-dependent manner (Maura et al., 1991), and they are insensitive to agonists at 5-HT\(_{1}\) receptors known to be localized on parallel fiber/climbing fiber axon terminals (Raiteri et al., 1986; Maura and Raiteri, 1996). Furthermore, the system of synaptosomes superfused (a very thin layer of synaptosomes up-down superfused; for technical details, see Raiteri and Raiteri, 2000) can prevent indirect effects. Any compound released is rapidly removed by the superfusion fluid before it can act on the releasing terminal or on neighboring particles; therefore, the release modulations provoked by a drug added to the superfusion fluid can be assumed to be due to direct actions on the releasing particle. In our case, the release inhibition observed most likely represents a genuine effect of trazodone at presynaptic 5-HT\(_{2A}\) receptors. When this work was almost completed, a paper was published proposing that trazodone inhibition of glutamate release from cerebellar mossy fiber synaptosomes could be related to “its effects on a determinant common to \(\sigma\) compounds” and simultaneous action “as a partial 5-HT receptor agonist” (Garrone et al., 2000). Our data are in line with the idea that trazodone is a partial agonist at 5-HT receptors regulating glutamate release from cerebellar mossy fiber synaptosomes. In addition, we classified pharmacologically the receptor involved as 5-HT\(_{2A}\) subtype. The possibility that 5-HT\(_{2A}\) and \(\sigma\) receptors cooperate in regulating glutamate release cannot be ruled out and certainly a better understanding of such a possible receptor-receptor interaction would merit attention. No doubt all these new results add further complexity to the pharmacological profile of trazodone, an antidepressant drug formerly considered a 5-HT\(_{2}\) receptor antagonist (see Haria et al., 1994).

In the cerebellar cortex no morphological evidence for presynaptic 5-HT\(_{2A}\) receptors has been provided; receptors of this subtype were found to be expressed in the somatodendritic region of Purkinje cells (Maeshima et al., 1998). Nevertheless, it is of interest that mRNA encoding for the 5-HT\(_{2A}\) receptor or 5-HT\(_{2A}\) receptor-like protein was detected in reticular and pontine nuclei (Palacios et al., 1993; Pompeiano et al., 1994), from which mossy fibers project to the cerebellar cortex (see Palacios et al., 1993). The cerebellum has usually been considered an area of the central nervous system extremely poor in 5-HT receptors, based on binding or morphological studies. However, types and subtypes of the 5-HT receptor have been identified and characterized in this area as release regulating presynaptic auto- and heteroreceptors in functional studies (Raiteri et al., 1986, 1991; Maura et al., 1991; Maura and Raiteri, 1996; see below).

Inhibition of neurotransmitter release by presynaptic receptors coupled to phosphoinositide hydrolysis, including the 5-HT\(_{2A}\) subtype (see Lucaites et al., 1996; Grotewiel and Sanders-Bush, 1999), is not surprising. Indeed, either potentiation or inhibition of glutamate release was observed following activation of phosphoinositide-coupled metabotropic

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**Fig. 3.** Antagonism by ketanserin or MDL 100907 but not by SB 242084 of the (±)-DOI inhibition of the K\(^{+}\)-evoked glutamate overflow from rat giant synaptosomes. Data are expressed as percent inhibition of the K\(^{+}\)-evoked overflow of glutamate. (±)-DOI was added concomitantly with high K\(^{+}\); antagonists 8 min before. Data are means ± S.E. of three to four independent experiments in triplicate. *, significant difference \((p < 0.05)\) versus (±)-DOI alone.

**Fig. 4.** Antagonism by ketanserin or MDL 100907 of the trazodone inhibition of the K\(^{+}\)-evoked glutamate overflow from rat giant synaptosomes. Data are expressed as percent inhibition of the K\(^{+}\)-evoked overflow of glutamate. The agonist was added concomitantly with high K\(^{+}\); antagonists 8 min before. Data are means ± S.E. of three to four independent experiments in triplicate. *, significant difference \((p < 0.05)\) versus trazodone alone.

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The inability of the potent and selective 5-HT\(_{2C}\) receptor antagonist SB 242084 (Kennett et al., 1997) to block the effect of 5-HT tends to exclude the involvement of the 5-HT\(_{2C}\) receptor subtype. The finding that the selective 5-HT\(_{2A}\) receptor antagonist MDL 100907 (Baxter et al., 1995) could prevent the effect of 5-HT or (±)-DOI allows us to classify the serotoninergic receptor located on the glutamatergic giant nerve terminals of the rat cerebellar cortex as 5-HT\(_{2A}\) subtype.

Trazodone could inhibit the K\(^{+}\)-evoked glutamate release from giant synaptosomes, although with potency and efficacy lower than those of 5-HT or (±)-DOI. These findings, together with the antagonism by ketanserin or MDL 100907 of the effect of trazodone, suggest that the compound can behave as a partial agonist at the 5-HT\(_{2}\) presynaptic receptors sited on mossy fibers. Based on the technical arguments discussed above it can be assumed that trazodone acts directly on glutamate-releasing mossy fiber terminals by activating the 5-HT\(_{2A}\) receptors located on these terminals. It should be added that the superfusion fluid should immediately remove trazodone metabolites possibly formed during superfusion before they can act on nerve terminals. Thus the release inhibition observed most likely represents a genuine effect of trazodone at presynaptic 5-HT\(_{2A}\) receptors. When this work was almost completed, a paper was published proposing that trazodone inhibition of glutamate release from cerebellar mossy fiber synaptosomes could be related to “its effects on a determinant common to \(\sigma\) compounds” and simultaneous action “as a partial 5-HT receptor agonist” (Garrone et al., 2000). Our data are in line with the idea that trazodone is a partial agonist at 5-HT receptors regulating glutamate release from cerebellar mossy fiber synaptosomes. In addition, we classified pharmacologically the receptor involved as 5-HT\(_{2A}\) subtype. The possibility that 5-HT\(_{2A}\) and \(\sigma\) receptors cooperate in regulating glutamate release cannot be ruled out and certainly a better understanding of such a possible receptor-receptor interaction would merit attention. No doubt all these new results add further complexity to the pharmacological profile of trazodone, an antidepressant drug formerly considered a 5-HT\(_{2}\) receptor antagonist (see Haria et al., 1994).

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Inhibition of neurotransmitter release by presynaptic receptors coupled to phosphoinositide hydrolysis, including the 5-HT\(_{2A}\) subtype (see Lucaites et al., 1996; Grotewiel and Sanders-Bush, 1999), is not surprising. Indeed, either potentiation or inhibition of glutamate release was observed following activation of phosphoinositide-coupled metabotropic
glutamate receptors; in this case, a “switch” from potentiation to inhibition of release would depend on nerve terminal activity (Herrero et al., 1998). As to 5-HT2A receptors, Ca2+-dependent opening of hyperpolarizing K+ channels was observed after 5-HT2A receptor activation in C6 glioma cells (Bartrup and Newberry, 1994). Clearly, the precise mechanism by which 5-HT2A receptors mediate inhibition of the evoked glutamate release in cerebellar mossy fiber terminals remains to be determined.

A possible physiological role for the presynaptic 5-HT2A receptors characterized in this study is suggested by the effect of MDL 100907 in slices. The ability of the selective 5-HT2A antagonist to increase glutamate release during depolarization of cerebellar slices indicates that, at least under some conditions of stimulation, endogenously released 5-HT can reach 5-HT2A receptors able to modulate glutamate release onto granule cells.

Serotonergic projections originating from raphe nuclei or other nuclei in the reticular formation terminate with fine and diffuse varicosities in the three layers of the cerebellar cortex (Hökfelt and Fuxe, 1969; Chan Palay 1975; Bishop and Ho, 1985) and seem to exert a very sophisticated inhibitory control of glutamatergic transmission. Electrophysiological studies have shown that 5-HT can affect the firing of rat cerebellar neurons and modulate excitatory amino acid effects (see Strahle and Hubbard, 1983; Lee et al., 1985). Based on our studies with isolated nerve terminals and cerebellar slices (Raiteri et al., 1986, 1991; Maura et al., 1988, 1991, 1995; Maura and Raiteri, 1996; Marcoli et al., 1997, 1998), multiple types, and subtypes of 5-HT receptors appear involved in the control of cerebellar glutamate transmission in the adult rat. The mechanisms identified include glutamate release regulation by 5-HT1D and 5-HT2A presynaptic receptors and inhibition by postsynaptic 5-HT1A and 5-HT2C receptors of the nitric oxide/cyclic GMP pathway coupled to ionotropic glutamate receptor activation (Raiteri et al., 1986, 1991; Maura et al., 1995; Maura and Raiteri, 1996; Marcoli et al., 1997, 1998).

A better knowledge of the serotonergic control of cerebellar glutamate transmission is of potential interest for a number of pathological conditions including cerebellar ataxia. In fact, ionotropic glutamate receptor overactivation and excitotoxic degeneration of Purkinje or granule cells have been proposed to be involved in cerebellar ataxia (Baxter et al., 1995; Blaney et al., 1995; Blackburn et al., 1995; Maura et al., 1995; Maura and Raiteri, 1996; Marcoli et al., 1997, 1998). Trazodone, potentially able to activate postsynaptic 5-HT2A receptors characterized in this study is suggested by the effect of MDL 100907 in slices. The ability of the selective 5-HT2A antagonist to increase glutamate release during depolarization of cerebellar slices indicates that, at least under some conditions of stimulation, endogenously released 5-HT can reach 5-HT2A receptors able to modulate glutamate release onto granule cells.

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