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Neuropsychopharmacology (2003) 28, 244–252
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www.neuropsychopharmacology.org

Imaging Brain Phospholipase A_2 Activation in Awake Rats in Response to the 5-$HT_{2A/2C}$ Agonist (±)2,5-Dimethoxy-4-Iodophenyl-2-Aminopropane (DOI)

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Incorporation coefficients *k** of intravenously injected [^{3}H]arachidonic acid from blood into brain reflect the release from phospholipids of arachidonic acid by receptor-initiated activation of phospholipase A_2 (PLA_2). In unanesthetized adult rats, 2.5 mg/kg intraperitoneally (i.p.) (±)2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), which is a 5-$HT_{2A/2C}$ receptor agonist, has been reported to produce the behavioral changes of what is known as the 5-HT_2 syndrome, but only a few small regional decrements in brain glucose metabolism. In this study, 2.5 mg/kg i.p. DOI, when administered to unanesthetized rats, produced widespread and significant increases, of the order of 60%, in *k** for arachidonate, particularly in neocortical brain regions reported to have high densities of 5-HT_{2A} receptors. The increases could be entirely blocked by chronic pretreatment with mianserin, a 5-HT_2 receptor antagonist. The results suggest that the 5-HT_2 syndrome involves widespread brain activation of PLA_2 via 5-HT_{2A} receptors, leading to the release of the second messenger, arachidonic acid. Chronic mianserin, a 5-HT_2 antagonist, prevents this activation.
Neuropsychopharmacology (2003) **28,** 244–252. doi:10.1038/sj.npp.1300022

Keywords: serotonin (5-HT); phospholipase A_2; DOI; mianserin; arachidonic acid; imaging; brain

INTRODUCTION

Some atypical antipsychotic and other drugs effective in schizophrenia, depression, obsessive–compulsive disorder, and neurodegenerative disease are considered to act at serotonin (5-hydroxytryptamine) 5-HT_2 receptors (Barnes and Sharp, 1999; Breier, 1995; Ramasubbu *et al*, 2000; Rauser *et al*, 2001; Reynolds, 2001; Thase, 2002). In brain, 5-HT_2 receptors can be coupled via G-proteins to phospholipase C (PLC) activation, generating inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol as second messengers (Conn and Sanders-Bush, 1986b; Edagawa *et al*, 2000), or to phospholipase A_2 (PLA_2) activation, releasing arachidonic acid (AA) from phospholipids (Axelrod, 1995; Berg *et al*, 1998; Felder *et al*, 1990; Tournois *et al*, 1998). Both AA and its eicosanoid metabolites are important second messengers (Shimizu and Wolfe, 1990).

A *5-HT_2 syndrome* has been described in rats following administration of the 5-$HT_{2A/2C}$ receptor agonist (±)2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) (Glennon, 1986; Johnson *et al*, 1998; Pranzatelli, 1990; Wettstein *et al*, 1999). The syndrome is characterized by head and body shakes, ear scratching, skin jerks, and forepaw tapping. It is maximal in response to 3.0 mg/kg intraperitoneal (i.p.) DOI, and 2.5 mg/kg i.p. DOI has been used widely in behavioral and biochemical studies of the syndrome. Additionally, 2.5 mg/kg i.p. DOI in rats markedly stimulates the release of corticotropin (ACTH), corticosterone, oxytocin, renin, and prolactin, and activates hypothalamic corticotropin-releasing factor and oxytocin-expressing neurons (Van de Kar *et al*, 2001). DOI also induces hyperthermia in rats (Mazzola-Pomietto *et al*, 1997).

Despite the marked behavioral and neuroendocrine effects of 2.5 mg/kg DOI, the regional cerebral metabolic rate for glucose ($rCMR_{glc}$), a marker of neuronal activity measured with intravenous [^{14}C]2-deoxy-D-glucose, was minimally affected in unanesthetized rats given this dose of DOI (Freo *et al*, 1991). Of 75 brain regions examined using quantitative autoradiography, this dose of DOI reduced $rCMR_{glc}$ significantly in layer IV of the pyriform cortex, the ventral CA3 region of the hippocampus, the cortical nucleus of the amygdala, and the olfactory tubercle. The reductions were ascribed to inhibition by DOI of neuronal spike activity (Ashby *et al*, 1990; Bloom, 1985; Cooper *et al*, 1996), to which $rCMR_{glc}$ is said to be coupled (Sokoloff, 1999). In another study, adrenalectomy or pretreatment with metyrapone (an inhibitor of 11-β-hydroxylase, a rate-limiting enzyme in corticosterone syntheses) abolished $rCMR_{glc}$ declines in the dorsal CA1, CA2 and CA3 regions of the hippocampus in response to 10 mg/kg i.p. DOI, suggesting to the authors that hippocampal activity can be modu-

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Received 10 January 2002; revised 30 May 2002; accepted 3 June 2002
Online publication: 5 June 2002 at http://www.acnp.org/citations/Npp060502319

lated by the hypothalamic–pituitary–adrenal axis (Freo *et al*, 1992).

It is not evident why 2.5 mg/kg i.p. DOI produces the marked behavioral activation of the 5-HT_2 syndrome, while at the same time causing decrements in $rCMR_{glc}$. We thought that this discrepancy might be clarified if we could examine postsynaptic signal transduction *in vivo*, secondary to 5-HT_2 receptor occupancy by DOI. As noted above, such signaling can occur through activation of PLC or PLA_2. No method currently exists to image brain PLC activation *in vivo*, whereas PLA_2 activation can be imaged by using quantitative autoradiography to measure incorporation into brain of intravenously injected, radiolabeled AA (DeGeorge *et al*, 1991; Hayakawa *et al*, 2001; Nariai *et al*, 1991; Rapoport, 2001; Robinson *et al*, 1992). We thought that we would use this latter method. Tracer incorporation in response to an appropriate agonist reflects PLA_2-mediated hydrolysis of unlabeled AA from the stereospecifically numbered (*sn*)-2 position of synaptic brain phospholipids (DeGeorge *et al*, 1991; Fonlupt *et al*, 1994; Grange *et al*, 1998; Jones *et al*, 1996), independent of changes in regional cerebral blood flow (rCBF) (Chang *et al*, 1997; DeGeorge *et al*, 1991; Robinson *et al*, 1992; Robinson and Rapoport, 1986; Yamazaki *et al*, 1994). Receptors coupled to PLA_2 via membrane G-proteins include cholinergic muscarinic M_1 and M_3 receptors, dopaminergic D_2 receptors, and serotonergic 5-HT_2 receptors (Axelrod, 1995; Bayon *et al*, 1997; Cooper *et al*, 1996; DeGeorge *et al*, 1991; Felder *et al*, 1990; Hayakawa *et al*, 2001; Vial and Piomelli, 1995). PLA_2 can also be activated when Ca^{2+} enters cells by glutamate acting at *N*-methyl-D-aspartate (NMDA) receptors or by acetylcholine acting at nicotinic receptors (Brooks *et al*, 1989; Cooper *et al*, 1996; Vijayaraghavan *et al*, 1995).

In the present study, we injected tritiated AA ([^{3}H]AA) intravenously in unanesthetized rats and used quantitative autoradiography to determine regional brain incorporation coefficients k^* of the tracer in response to 2.5 mg/kg i.p. DOI. The racemic DOI commonly is used to study effects of *in vivo* 5-$HT_{2A/2C}$ receptor activation. Both stereoisomers bind with equivalent affinities to 5-$HT_{2A/2C}$ receptors, although ($-$)DOI is twice as potent as (+)DOI in inducing head twitches in mice (Glennon, 1986, 1987; PDSP Drug Database, 2000; Pranzatelli, 1990; Roth *et al*, 2000).

We also quantified k^* for [^{3}H]AA in response to chronically administered mianserin, an atypical tetracyclic antidepressant that has been used as a 5-HT_2-receptor antagonist in many animal studies, although having some adrenergic α_2-antagonist activity as well (Anji *et al*, 2000; Ashby *et al*, 1990; Blackshear and Sanders-Bush, 1982; Dijcks *et al*, 1991; Hoyer *et al*, 1995; PDSP, 2000; Pranzatelli, 1990; Rocha *et al*, 1994; Roth and Ciaranello, 1991; Roth *et al*, 2000; Sanders-Bush *et al*, 1987; Schreiber *et al*, 1995). Finally, we measured k^* in response to 2.5 mg/kg i.p. DOI 24 h after mianserin administration (Arvidsson *et al*, 1986; Berendsen and Broekkamp, 1991; Sanders-Bush *et al*, 1987), by which time mianserin is known to be largely washed out from the brain (Dijcks *et al*, 1991; Sanders-Bush *et al*, 1987).

An abstract of part of this work has been published (Qu *et al*, 2001).

MATERIALS AND METHODS

Chemicals

Radiolabeled [5,6,8,9,11,12,14,15-^{3}H]AA ([^{3}H]AA) at a specific activity of 200 Ci/mmol was purchased from Moravek Biochemicals (Brea, CA). Radiochemical purity by thin-layer chromatography always exceeded 96%. Mianserin and DOI were purchased from Sigma-Research Biochemicals International (Natick, MA). Pentobarbital sodium was purchased from Richmond Veterinary Supply Co. (Richmond, VA).

Animals

Male Fischer-344 rats (Charles River Laboratories, Wilmington, MA), 12 weeks old and weighing 290–320 g, were housed under standard laboratory conditions under a 12-h light/12-h dark cycle, with ready access to standard laboratory chow and water. The experimental protocol was approved by the National Institute of Child Health and Human Development Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication 86-23).

Arterial and Venous Catheterization

Rats were placed in four experimental groups of 10 animals each: (1) controls; (2) rats given 2.5 mg/kg i.p. DOI acutely; (3) rats administered 10 mg/kg i.p. mianserin daily for 14 days, then not given mianserin for 24 h; (4) rats administered 10 mg/kg i.p. mianserin daily for 14 days, then not given mianserin for 24 h, and then given 2.5 mg/kg i.p. DOI.

The *in vivo* fatty acid method has been described elsewhere (DeGeorge *et al*, 1991; Hayakawa *et al*, 2001). Briefly, rats in each of the four groups were anesthetized with halothane (1–3% v/v in O_2). PE 50 polyethylene catheters (Clay Adams, Lincolnshire, IL) filled with heparinized saline (100 IU/ml) were surgically implanted into a femoral artery and vein, after which the incision site was infiltrated with a local anesthetic (lidocaine) and closed with wound clips. The rats were wrapped loosely in a fast-setting plaster cast, secured to a wooden block with the upper body free, and allowed to recover from anesthesia in a temperature-controlled and sound-dampened box for 4 h. Body temperature was kept at 36–37°C by means of a rectal thermometer and a feedback heating device.

Drug Administration and Tracer Infusion

After the rat recovered from anesthesia for 4 h, 125 μl arterial blood was withdrawn to measure pH, pO_2, and pCO_2. Rats (8–10 per group) were administered either saline (control) or 2.5 mg/kg i.p. DOI. After 20 min, 1.75 mCi/kg [^{3}H]AA in 2 ml of 5 mM HEPES buffer, pH 7.4, containing 50 mg/ml fatty-acid-free bovine serum, was infused through the venous cannula with an infusion pump (Harvard Instrument Co., Holliston, MA) at a rate of 400 μl/min for 5 min.Timed 125-μl arterial blood samples were collected from the beginning of infusion to 20 min, when the rats were killed with 65 mg i.v. sodium pentobarbital. Brains were removed and frozen in 2-methylbutane at -50°C for subsequent autoradiography. Plasma was separated from

arterial blood by centrifugation, and lipids were extracted using the method of Folch *et al* (1957). Radioactivity in the organic fraction was measured by liquid scintillation spectroscopy.

Autoradiography and Calculations

Frozen brains were sectioned on a cryostat at -20°C. Sets of three adjacent 20-μm sections were collected and mounted on glass coverslips at 140-μm coronal intervals and dried. The sections were exposed together with [^{3}H]methylmethacrylate autoradiographic standards (Amersham, Arlington Heights, IL) to [^{3}H]hyperfilm (Amersham) for 15–18 weeks and then developed following the manufacturer's instructions. One of the three adjacent sections was collected and stained with cresyl violet to identify brain regions with reference to a rat-brain atlas (Paxinos and Watson, 1987).

Regional brain radioactivity was measured in sextuplicate by quantitative densitometry using the public domain image analysis program NIH Image (version 1.62) created by Wayne Rasband (National Institutes of Health, Bethesda, MD), installed on a Macintosh computer (Apple Computer, Cupertino, CA). Regional brain incorporation coefficients k^* were calculated as

$$k^* = \frac{c^*_{\text{brain}}(20\,\text{min})}{\int_0^{20} c^*_{\text{plasma}}\,dt}$$

where k^* is in units of ml/(s g); c^*_{brain} (20 min) is the brain radioactivity at 20 min in nCi/g, c^*_{plasma} is the plasma fatty acid radioactivity in nCi/ml, and t is time after onset of [^{3}H]AA infusion.

Data were compared using Prism software for the Macintosh (Abacus Concepts, Berkeley, CA) and are reported as means $\pm$SEM. A one-way ANOVA and Dunnett's (Dunnett, 1964) multiple comparison test were used to evaluate statistical significance between experimental and control means; $p<0.05$ was taken as statistically significant.

RESULTS

Table 1 summarizes mean physiological parameters in unanesthetized control rats and in rats treated chronically with mianserin. These values are similar to published values.

As illustrated in Figure 1, coronal autoradiographs showed widespread increments in k^* (brain radioactivity divided by integrated plasma radioactivity; Equation (1)) for [^{3}H]AA after 2.5 mg/kg i.p. DOI, compared with k^* from control rats. The largest increments were in motor and somatosensory cortical areas.

Mean regional [^{3}H]AA incorporation coefficients (k^*) in saline-treated control rats are presented in the first data column of Table 2. The values are comparable to previously published control values (DeGeorge *et al*, 1991; Hayakawa *et al*, 2001). Notable is the 6- to 10-fold greater k^* at the choroid plexus than in the brain parenchyma.

Compared with controls, 2.5 mg/kg i.p. DOI produced widespread and statistically significant increments in k^* for [^{3}H]AA, of the order of 60%, in many brain regions (second data column of Table 2), but particularly in the neocortex.

After 14 days of mianserin administration, and allowing 24 h for mianserin to be washed out from the brain (Dijcks *et al*, 1991; Sanders-Bush *et al*, 1987), there was no significant difference in mean k^* for [^{3}H]AA in any brain region compared with the respective k^* in control animals (third data column of Table 2). Furthermore, when DOI was administered after 2 weeks of mianserin after allowing for washout (fourth data column of Table 2), no statistically significant difference in mean k^* was found in any brain region or in the choroid plexus, compared with the respective control mean. Thus, chronic mianserin completely blocked all DOI-induced increments in [^{3}H]AA incorporation.

DISCUSSION

The 5-HT$_{2A/2C}$ receptor agonist DOI, at a dose of 2.5 mg/kg i.p., caused widespread and large (as high as 60%) increments in k^* for [^{3}H]AA in brains of unanesthetized adult rats. These increments are consistent with the reported marked behavioral (5-HT$_2$ syndrome) and neuroendocrine responses provoked by this dose (Johnson *et al*, 1998; Pranzatelli, 1990; Van de Kar *et al*, 2001; Wettstein *et al*, 1999). The increments in k^* could be completely blocked by chronic pretreatment with mianserin, a 5-HT$_2$ receptor agonist that has been reported to block the 5-HT$_2$ syndrome and the hyperthermia produced by DOI (Berendsen and Broekkamp, 1991; Mazzola-Pomietto *et al*, 1997).

The interpretation that k^* for [^{3}H]AA reflects regional PLA$_2$ activation derives from experimental observations that k^* is independent of rCBF, that incorporation of labeled AA from blood into brain phospholipids is very rapid, and that k^* reflects brain PLA$_2$ but not PLC activity (Rapoport, 2001; Rapoport *et al*, 2001; Robinson *et al*, 1992; Washizaki *et al*, 1991). That k^* is independent of rCBF is evident from

Table 1 Physiological Parameters of Rats after Surgery

	Control rat	Mianserin rat
Body temperature (°C)	35.6 ± 0.1	35.3 ± 0.2
Arterial blood pressure (mm Hg)	97. 7 ± 3.6/130.6 ± 4.8	95.2 ± 2.5/121.2 ± 2.4
Heart rate (beats/min)	425.0 ± 13.7	430.9 ± 13.6
Arterial pH	7.4 ± 0.008	7.4 ± 0.008
Arterial blood gas, pCO$_2$ (mmHg)	38.9 ± 1.6	41.1 ± 1.3
Arterial blood gas, pO$_2$ (mm Hg)	95.4 ± 2.2	93.8 ± 1.9

Mean ± S.E.M. ($n=11$–14). Chronic treatment with mianserin (i.p.) for 14 days, 1 day washout.

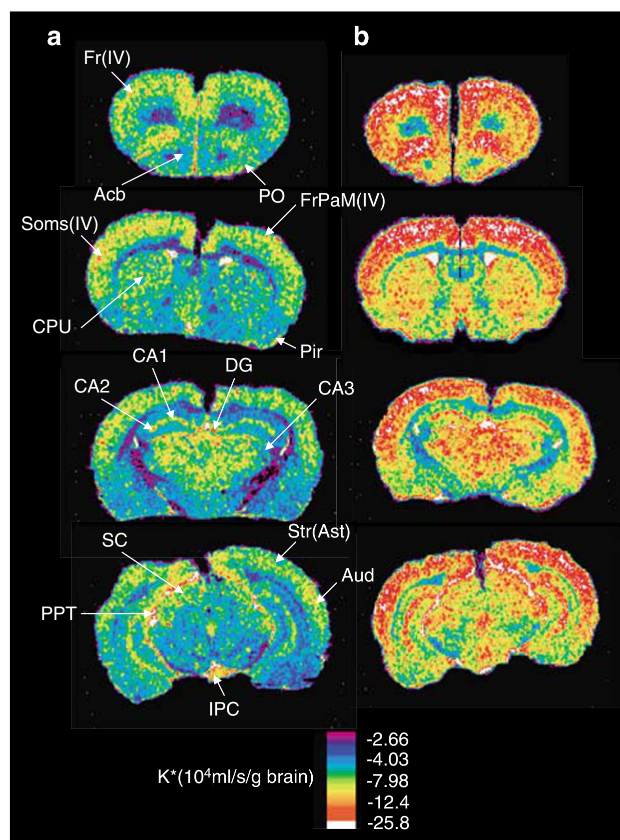

Figure 1 [^{3}H]arachidonate incorporation coefficients k^* in coronal sections from brain of (a) control rat and (b) rat given DOI (2.5 mg/kg i.p.); k^* is color-coded.

several observations. As shown in Table 2, k^* for [^{3}H]AA was markedly elevated in response to 2.5 mg/kg DOI (Table 2), despite evidence that $rCMR_{glc}$, to which rCBF is coupled (Reivich, 1974), declines or does not change with this dose (Freo *et al*, 1991, 1992). Likewise, administration to rats of arecoline, a cholinergic agonist that acts at muscarinic M_1 receptors coupled to PLA_2, increased $rCMR_{glc}$ and rCBF (as well as k^*) for labeled AA, without affecting k^* for labeled palmitic acid (DeGeorge *et al*, 1991; Jones *et al*, 1996; Maiese *et al*, 1994; Soncrant *et al*, 1985). Thus, fatty acid uptake by the brain is not increased by increased rCBF *per se*. Finally, values for k^* for both labeled palmitate and arachidonate were shown to be unaffected by two-fold increments in rCBF induced by hypercapnia in rats and monkeys (Chang *et al*, 1997; Yamazaki *et al*, 1994).

The independence of k^* from rCBF arises because circulating plasma albumin, to which fatty acid is highly bound but from which it can rapidly dissociate (Svenson *et al*, 1974), acts as an 'infinite source' of intravascular tracer for entry into brain (Robinson *et al*, 1992; Robinson and Rapoport, 1986; Washizaki *et al*, 1991). As blood passes through the brain, unesterified unbound labeled fatty acid is rapidly extracted and replaced by fatty acid released from albumin. About 5% of a plasma fatty acid is stripped from albumin as blood passes through the brain (Pardridge and Mietus, 1980).

Within 2 min after entering rat brain following its intravenous injection, 90% of radiolabeled AA has been incorporated into 'stable' brain lipids, largely into the *sn*-2 position of phospholipids. The remainder, found in the aqueous fraction, represents metabolites arising from comparatively slow β-oxidation (Osmundsen and Hovik, 1988). The rate of disappearance of labeled AA from brain phospholipids is only 10% per hour (DeGeorge *et al*, 1989; Rapoport, 2001; Rapoport *et al*, 2001; Washizaki *et al*, 1994), which means that we can image tracer incorporation at 20 min without worrying about loss from the phospholipids. Finally, inhibiting brain PLA_2 activity *in vivo* by drug produces a proportional reduction in k^* for [^{3}H]AA (Grange *et al*, 1998).

Chronically administered mianserin had no effect on baseline values of k^* for [^{3}H]AA, but prevented DOI-initiated increments in k^* (Table 2). The 5-HT_2 receptor-mediated activation of PLC by DOI, which increases phosphatidylinositol turnover and Ca^{2+} mobilization by IP_3, is also reported to be inhibited by chronic mianserin (Conn and Sanders-Bush, 1986b; Wolf and Schutz, 1997). Inhibition of signaling in both cases is probably due to mianserin-induced neuroplastic changes, rather than to physical blocking of 5-HT_2 receptors by mianserin, as the brain mianserin concentration falls to less than 0.1% of its peak concentration within 24 h after i.p. injection (Dijcks *et al*, 1991; Sanders-Bush *et al*, 1987). Chronic mianserin is reported not to alter extracellular serotonin levels in rat brain (Kreiss and Lucki, 1995), but is reported to reduce brain densities of 5-HT_{2A} receptors (Berendsen and Broekkamp, 1991; Blackshear and Sanders-Bush, 1982; Essom and Nemeroff, 1996; Frazer *et al*, 1988; Roth and Ciaranello, 1991) and 5-HT_{2C} receptors (Rocha *et al*, 1994). Phosphorylation and interaction of the receptors with membrane G-proteins are altered (Hartman and Northup, 1996; Ozawa *et al*, 1994; Westphal *et al*, 1995), and both receptor types are functionally hyposensitive (Mazzola-Pomietto *et al*, 1997). Chronic mianserin, on the other hand, produces a supersensitivity of adrenergic α_2 receptors (Pinder, 1985).

Head twitches of the 5-HT_2 syndrome appear to be related more to 5-HT_{2A} than to 5-HT_{2C} initiated signaling (Schreiber *et al*, 1995); thus, a selective 5-HT_{2C} antagonist (SB 200,646A) did not inhibit the twitches. Additionally, dopaminergic D_1 antagonists as well as agonists to α_1 and α_2 adrenoreceptors and to 5-HT_{1A} receptors reduced DOI-induced head twitches, suggesting a role for nonserotonergic mechanisms (Schreiber *et al*, 1995). A full 5-HT syndrome has been described in humans, with some components perhaps related to the 5-HT_2 syndrome in rodents. The clinical syndrome occurs with excess serotonergic therapy and can be exacerbated by coadministration of a monoamine oxidase inhibitor. Its features include an altered mental status, restlessness, myoclonus, hyperreflexia, diaphoresis, shivering, and tremor (Mills, 1997; Sternbach, 1991); it is treated by discontinuing serotonergic therapy.

The robust increments in k^* induced by 2.5 mg/kg i.p. DOI are accompanied by a few reductions in $rCMR_{glc}$ (Freo *et al*, 1991, 1992), which are ascribed to reduced neuronal spike activity (Ashby *et al*, 1990; Bloom, 1985; Cooper *et al*, 1996; Freo *et al*, 1991; Sokoloff, 1999). $rCMR_{glc}$ is a weighted average, reflecting energy consumption by many brain processes, and PLA_2-initiated AA release and reincorporation consume only a small fraction of net brain adenosine

Table 2 Regional [^{3}H]Arachidonate Incorporation Coefficients k^* (ml/(s g) brain × 10^4) in Rat Brain under Different Conditions

Brain regions	Abbreviation	Control	DOI	Mianserin	Mianserin +DOI
Cerebral cortex					
Prefrontal cortex IV	PFr	8.6 ± 0.4	14.0 ± 1.3***	9.1 ± 0.6	9.3 ± 0.9
Frontal cortex layer I	Fr I	7.2 ± 0.4	12.6 ± 1.5***	7.8 ± 0.8	9.5 ± 1.1
Frontal cortex layer IV	Fr IV	9.2 ± 0.5	15.5 ± 1.5***	9.9 ± 0.9	10.9 ± 1.0
Motor cortex layer I	FrPaM I	7.0 ± 0.5	11.8 ± 0.9***	7.8 ± 0.8	8.8 ± 1.1
Motor cortex layer II–III	FrPaM II–III	7.5 ± 0.5	13.7 ± 1.2***	8.4 ± 0.7	9.0 ± 0.9
Motor cortex layer IV	FrPaM IV	9.3 ± 0.5	16.6 ± 1.6***	10.4 ± 0.9	11.1 ± 1.1
Motor cortex layer V	FrPaM V	7.6 ± 0.5	13.3 ± 1.1***	8.5 ± 0.7	9.0 ± 0.9
Motor cortex layer VI	FrPaM VI	6.8 ± 0.4	11.7 ± 1.0***	7.4 ± 0.6	8.0 ± 0.8
Somatosensory cortex layer I	Soms I	7.4 ± 0.5	12.7 ± 1.0***	8.4 ± 0.8	8.9 ± 1.1
Somatosensory cortex layer II–III	Soms II–III	8.2 ± 0.6	14.1 ± 1.3***	8.6 ± 0.7	9.8 ± 1.0
Somatosensory cortex layer IV	Soms IV	9.43 ± 0 0.6	15.9 ± 1.5***	10.4 ± 0.9	11.2 ± 1.1
Somatosensory cortex layer V	Soms V	7.73 ± 0.5	12.3 ± 0.9***	8.1 ± 0 0.6	9.0 ± 1.0
Somatosensory cortex layer VI	Soms VI	6.2 ± 0.4	10.2 ± 0.9***	6.4 ± 0.6	7.1 ± 0.7
Anterior cingulate cortex	Acg	8.6 ± 0.5	13.1 ± 1.0***	8.6 ± 1.0	9.6 ± 1.1
Auditory cortex layer I	Aud I	6.7 ± 0.2	10.5 ± 1.0***	6.9 ± 0.7	8.3 ± 1.0
Auditory cortex layer IV	Aud IV	8.9 ± 0.4	12.1 ± 0.8*	8.4 ± 0.7	11.0 ± 1.0
Auditory cortex layer V	Aud V	7.2 ± 0.4	11.1 ± 1.1***	7.0 ± 0.7	8.2 ± 0.7
Visual cortex layer I	Str (Ast) I	6.3 ± 0.2	11.1 ± 1.1***	6.9 ± 0.7	7.6 ± 0 0.8
Visual cortex layer IV	Str (Ast) IV	8.2 ± 0.4	14.5 ± 1.3***	8.7 ± 0.8	10.1 ± 1.1
Visual cortex layer V	Str (Ast) V	6.8 ± 0.4	11.9 ± 1.1***	7.2 ± 0.7	8.0 ± 0 0.9
White matter					
Corpus callosum	cc	3.2 ± 0.3	5.4 ± 0.5***	3.5 ± 0.3	3.9 ± 0.5
Internal capsule	ic	3.1 ± 0.2	5.3 ± 0.4***	3.5 ± 0.4	3.5 ± 0.5
Olfactory system					
Olfactory cortex	PO	10.1 ± 0.6	15.5 ± 1.3***	10.6 ± 0.9	12.1 ± 1.2
Pyriform cortex	Pir	9.0 ± 0.7	13.8 ± 1.4***	9.7 ± 0.8	10.7 ± 1.1
Basal ganglia and related areas					
Nucleus accumbens	Acb	8.0 ± 0.4	12.5 ± 1.4***	11.1 ± 1.3	10.1 ± 1.0
Caudate-putamen dorsal	CPU	6.3 ± 0.3	10.1 ± 0.9***	6.6 ± 0.6	7.3 ± 0.8
Caudate-putamen ventral	CPU	6.4 ± 0.3	10.6 ± 1.0***	7.2 ± 0.6	7.7 ± 0.8
Caudate-putamen lateral	CPU	6.6 ± 0.3	10.5 ± 1.0***	6.9 ± 0.5	8.2 ± 0.7
Caudate-putamen medial	CPU	6.2 ± 0.3	10.2 ± 0.8***	6.8 ± 0.6	7.7 ± 0.7
Bed nucleus stria preoptic nucleus	LPO/MPO	5.5 ± 0.3	8.6 ± 0.8***	5.8 ± 0.5	6.3 ± 0.8
Bed nucleus stria suprachiasmatic nucleus	PSCH	6.3 ± 0.4	9.8 ± 0.7***	6.7 ± 0.4	7.2 ± 0.8
Bed nucleus stria terminalis	BSTPO	4.7 ± 0.2	7.2 ± 0.7***	5.1 ± 0.5	5.9 ± 0.7
Entopeduncular nucleus	EN	4.1 ± 0.2	6.9 ± 0.6***	4.6 ± 0.4	4.9 ± 0.6
Globus pallidus	GP	4.3 ± 0.3	7.0 ± 0.5***	4.9 ± 0.5	4.9 ± 0.6
Amygdala basolateral/basomedial nuclei	BL/BM	5.1 ± 0.2	8.0 ± 0.8***	5.6 ± 0.6	5.8 ± 0.7
Subthalamic nucleus	Sth	6.4 ± 0.4	9.9 ± 0.8***	6.5 ± 0.8	7.4 ± 0.8
Substantia nigra pars reticulata	SNR	5.3 ± 0.3	9.8 ± 1.0***	5.4 ± 0.4	6.6 ± 0.7
Substantia nigra pars compacta	SNC	5.6 ± 0.3	9.8 ± 1.0***	5.5 ± 0.5	6.8 ± 0.8
Lateral septal nucleus	LSI	5.0 ± 0.3	8.2 ± 0.7***	5.4 ± 0.6	6.2 ± 0.6
Medial septal nucleus	MS	5.9 ± 0.3	9.2 ± 0.8***	6.5 ± 0.5	7.3 ± 0.8
Dorsal diagonal band	VDBD	6.5 ± 0.4	9.8 ± 0.8***	7.0 ± 0.6	7.8 ± 0.9
Ventral diagonal band	VDBV	5.9 ± 0.4	8.8 ± 0.6***	6.3 ± 0.7	7.0 ± 0.9
Hippocampal formation					
Ammon's horn CA1	Hip CA1	7.8 ± 0.4	12.0 ± 0.9***	8.1 ± 0.6	8.8 ± 1.0
Ammon's horn CA2	Hip CA2	7.4 ± 0.4	11.8 ± 1.1***	7.7 ± 1.2	8.7 ± 0.9
Ammon's horn CA3	Hip CA3	6.8 ± 0.3	10.7 ± 0.8***	7.2 ± 0.6	8.2 ± 0.8
Dentate gyrus	DG	12.2 ± 0.8	18.9 ± 2.3*	14.5 ± 1.5	14.5 ± 1.7
Dorsal lateral geniculate nucleus	DLG	6.9 ± 0.3	10.9 ± 0.9***	7.6 ± 0.7	8.1 ± 0.8
Parafascicular nucleus	PF	6.6 ± 0.3	10.3 ± 0.9***	6.8 ± 0.6	7.3 ± 0.8
Thalamus					
Paratenial nuclei	PT	6.4 ± 0.3	1.0 ± 0.8***	7.2 ± 0.6	7.8 ± 0.8
Anteroventral nuclei	AV	9.4 ± 0.6	14.3 ± 1.5***	10.5 ± 1.0	11.2 ± 1.1
Anteromedial nuclei	AM	6.7 ± 0.3	10.1 ± 0.7***	7.4 ± 0.6	7.8 ± 0.8
Reticular nuclei	Rt	6.4 ± 0.3	10.4 ± 0.9***	7.5 ± 0.6	7.7 ± 0.8
Paraventricular nuclei	PVA	6.5 ± 0.2	10.2 ± 1.1***	7.2 ± 0.6	7.5 ± 0.9
Ventroposterior medial thalamus	VPL	6.5 ± 0.5	9.5 ± 0.8***	6.7 ± 0.5	7.1 ± 0.8
Ventroposterior lateral thalamus	VPM	6.8 ± 0.7	9.9 ± 0.9***	6.9 ± 0.8	7.7 ± 0.8
Lateral habenular nucleus	LHb	8.4 ± 0.6	12.1 ± 1.2*	8.1 ± 0.6	9.3 ± 1.1

Table 2 *Continued*

Brain regions	Abbreviation	Control	DOI	Mianserin	Mianserin +DOI
Medial habenular nucleus	MHb	9.1 ± 0.6	13.1 ± 1.2*	9.2 ± 0.8	9.8 ± 1.2
Medial geniculate nucleus	MG	6.6 ± 0.3	10.7 ± 1.1***	6.7 ± 0.5	7.8 ± 0.8
Hypothalamus					
Supraoptic nuclei	SO	14.5 ± 1.2	19.3 ± 3.1*	14.7 ± 1.2	13.1 ± 1.8
Subfornical organ	SFO	9.6 ± 0.5	19.5 ± 2.2***	12.1 ± 1.2	14.6 ± 1.6
Lateral nuclei	LH	4.8 ± 0.3	7.4 ± 0.5***	5.0 ± 0.5	5.6 ± 0.7
Anterior nuclei	Ahy	5.2 ± 0.3	7.9 ± 0.5***	5.5 ± 0.5	5.8 ± 0.7
Periventricular nuclei	Pe	7.8 ± 1.3	9.8 ± 0.8	8.1 ± 1.2	7.9 ± 1.1
Arcuate nuclei	Arc	5.5 ± 0.4	8.6 ± 0.5***	5.8 ± 0.7	6.8 ± 0.8
Ventromedial nuclei	VMH	5.1 ± 0.2	8.1 ± 0.5***	5.3 ± 0.5	6.1 ± 0.6
Posterior nuclei	PH	6.6 ± 0.3	10.1 ± 0.8***	6.8 ± 0.5	7.5 ± 0.8
Mammillary nucleus	MM	5.9 ± 0.4	8.5 ± 0.7*	6.0 ± 0.7	6.2 ± 0.9
Medial forebrain bundle	mfb	5.1 ± 0.3	7.9 ± 0.5***	5.3 ± 0.5	5.6 ± 0.7
Median eminence	ME	12.7 ± 1.6	16.0 ± 2.5	12.3 ± 2.5	12.5 ± 1.9
Brainstem and spinal cord					
Raphe magnus nuclei	RMg	6.1 ± 0.5	10.6 ± 1.4***	6.6 ± 0.7	6.6 ± 0 0.9
Raphe pallidus nuclei	Rpa	7.1 ± 0.8	10.7 ± 1.2*	6.2 ± 0.6	6.6 ± 1.0
Raphe median nuclei	MnR	6.1 ± 0.5	11.1 ± 1.2***	6.7 ± 0.9	7.2 ± 1.0
Raphe dorsal nuclei	DR	7.6 ± 0.6	12.8 ± 1.3***	7.9 ± 1.0	9.0 ± 1.2
Locus coeruleus	LC	8.9 ± 0.5	14.6 ± 1.6***	9.5 ± 0.9	9.7 ± 1.2
Cochlear nucleus	VCO,GrCo	10.1 ± 0.7	16.2 ± 1.5***	10.5 ± 1.2	11.2 ± 1.6
Vestibular nucleus (medial)	MVe	11.8 ± 0.7	19.0 ± 1.9***	12.1 ± 1.2	12.8 ± 1.8
Inferior colliculus	CICVI	10.3 ± 0.4	18.7 ± 2.2***	12.7 ± 1.3	12.7 ± 1.8
Superior colliculus	SuG	10.0 ± 0.9	16.9 ± 1.2***	11.2 ± 0.8	10.7 ± 1.0
Pretectal area	PPT	10.7 ± 1.2	18.0 ± 1.0***	12.1 ± 1.9	12.4 ± 1.6
Pedunculopontine nucleus	PPTGg	5.0 ± 0.6	9.0 ± 1.0***	5.5 ± 0.7	5.9 ± 0.9
Deep layers of superior colliculus	SC	10.0 ± 0.8	14.2 ± 1.3*	9.7 ± 0.7	11.7 ± 1.0
Flocculus	Fl	9.2 ± 0.5	15.40 ± 1.57***	10.47 ± 1.18	10.47 ± 1.62
Interpeduncular nucleus	IPC	9.1 ± 0.5	13.7 ± 1.7*	8.6 ± 0.8	10.9 ± 1.2
Spinal tract V nucleus	Sp5I	7.3 ± 0.6	10.9 ± 1.2	7.7 ± 0.7	7.2 ± 1.2
Choroid plexus	ChP	67.8 ± 3.2	92.6 ± 9.7*	76.7 ± 6.3	73.3 ± 7.9

k^* values are mean ± S.E.M ($n = 9$–10). Abbreviations according to Paxinos and Watson (1987). One-way ANOVA and Dunnett's multiple comparison test were used. Rats treated with saline (controls) were compared with each group. Differs significantly from control mean: *$p < 0.05$; ***$p < 0.001$.

triphosphate (ATP) consumption (Purdon and Rapoport, 1998; Rapoport, 2001). Large changes in [^{3}H]AA incorporation into the brain in contrast to small changes in $rCMR_{glc}$ have also been noted in rats administered the muscarinic agonist arecoline or the dopaminergic D_2 agonist quinpirol (Hayakawa *et al*, 2001; Nariai *et al*, 1991; Orzi *et al*, 1988; Wooten and Collins, 1981).

Recall that PLA_2 can be activated when a ligand binds to any of a number of receptor subtypes, including 5-HT_2 receptors (Axelrod, 1995; Cooper *et al*, 1996; Felder *et al*, 1990). 5-HT_2 receptors are widely distributed in rat brain (Appel *et al*, 1990; Morilak *et al*, 1993; Pazos and Palacios, 1985). High densities are reported in cerebral cortex, olfactory and pyriform cortex, nucleus accumbens, caudate-putamen body and tail, dentate gyrus of hippocampus, and medial mammillary nucleus of hypothalamus. In the neocortex, highest densities are in layer IV. Frontal and motor cortical regions have higher densities than other cortical regions, whereas densities are comparatively low in the caudate-putamen head, globus pallidus, red nuclei, septal nuclei, and most parts of the hippocampus (McKenna *et al*, 1989; McKenna and Peroutka, 1989), thalamus, hypothalamus, midbrain, brain stem, and spinal cord.

The most intense increments in k^* in response to DOI (Figure 1, Table 1) were seen in brain regions having high densities of 5-HT_{2A} compared with 5-HT_{2C} binding sites (Cooper *et al*, 1996; Li *et al*, 2001; Pazos and Palacios, 1985). High densities of 5-HT_{2A} binding sites are found in neocortical areas (layer IV), amygdala and midbrain (lateral amygdaloid nucleus, medial amygdaloid nucleus), and CA1 and CA3 regions of the hippocampus, with lesser densities in the caudate-putamen. There are fewer 5-HT_{2C} binding sites in rodent brain; they are found in the hypothalamus, amygdala and hippocampus, but minimally in the neocortex (except for the temporal horn). The correspondence between the brain distributions of PLA_2 activation by DOI and of 5-HT_{2A} receptors could be further examined using DOI and altanserin, a selective 5-HT_{2A} blocker (Hoyer *et al*, 1995; Leysen *et al*, 1988; PDSP Drug Database, 2000; Roth *et al*, 2000), or by studying DOI responses in a 5-HT_{2A}-receptor knockout mouse (Lira *et al*, 2001).

The choroid plexus has more than four-fold higher densities of 5-HT_{2C} binding sites than brain parenchymal regions as well as high levels of 5-HT_{2C} mRNA and 5-HT_{2A} binding sites (Kaufman *et al*, 1995; Li *et al*, 2001). The six- to 10-fold greater control value for k^* in the choroid plexus than in brain parenchymal regions, the marked increment in this k^* in response to DOI, and the ability of chronic mianserin to block this increment suggest that serotonin-related PLA_2 signaling plays an important role in choroid plexus function, particularly secretion of cerebrospinal fluid. On the other hand, serotonin is reported to decrease

cerebrospinal fluid secretion by increasing phosphorylation of Na^+, K^+-ATPase by protein kinase C following activation of PLC (Conn and Sanders-Bush, 1986a; Fisone *et al*, 1995, 1998), or through Ca^{2+}-dependent activation of PLA_2 (Kaufman *et al*, 1995). The uptake mechanism of [^{3}H]AA into the choroid plexus may differ from that into brain, as the plexus, unlike the brain parenchyma, has a leaky vasculature that can allow protein-bound [^{3}H]AA to access directly the choroid epithelium (Rapoport, 1976).

The DOI dose chosen in the present study may have been too large to identify PLA_2 signaling solely at 5-HT_2 receptors, because of downstream activation of dopaminergic D_2 and other receptors also coupled to PLA_2 (see the Introduction). A smaller dose may help in this regard. Although the affinities of both the (+) and (−) stereoisomers of DOI are reported to be equivalent at 5-HT_2 receptors (Glennon, 1986, 1987; PDSP Drug Database, 2000; Pranzatelli, 1990; Roth *et al*, 2000), as far as we know the affinities of the two stereoisomers at other receptors coupled to PLA_2 have not been examined. DOI can increase amphetamine-induced dopamine release in the brain (Ichikawa and Meltzer, 1995), brain extracellular dopamine and noradrenaline concentrations (Gobert and Millan, 1999), and dopamine turnover (Gaggi *et al*, 1997), and DOI will activate local γ-aminobutyric acid (GABA) inputs to serotonergic neurons in the dorsal raphe nucleus (Liu *et al*, 2000). 5-HT_2 receptor activation can also inhibit glutamate release from rat cerebellar mossy fibers (Marcoli *et al*, 2001) and the release of acetylcholine in the hippocampus and neocortex (Feuerstein *et al*, 1996), which may explain DOI's inhibition of $rCMR_{glc}$ (Freo *et al*, 1991, 1992).

In summary, our results suggest that labeled AA can be used to examine *in vivo* brain PLA_2 signaling initiated by serotonergic drugs. Increments in k^* for [^{3}H]AA in response to DOI largely correspond to the distribution of 5-HT_{2A} binding sites in the brain, although downstream receptors coupled to PLA_2 are probably activated as well. Chronic mianserin, a 5-HT_2 agonist known to inhibit the 5-HT_2 syndrome, blocks [^{3}H]AA incorporation completely in response to 2.5 mg/kg i.p. DOI. Imaging information gathered using labeled AA is clearly distinct from that using labeled 2-deoxy-D-glucose, and specific to PLA_2 activation rather than to general brain functional activity. As a result of this, it might be worthwhile to extend the fatty acid method to examine PLA_2 signaling in the human brain by means of positron emission tomography (Chang *et al*, 1997; Giovacchini *et al*, 2001).

ACKNOWLEDGEMENTS

This work was supported by the National Alliance for Research on Schizophrenia and Depression (NARSAD) under a Distinguished Investigator Award to S. Rapoport.

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