

Imaging Brain Phospholipase A₂ Activation in Awake Rats in Response to the 5-HT_{2A/2C} Agonist (±)2,5-Dimethoxy-4-Iodophenyl-2-Aminopropane (DOI)

Ying Qu^{*1}, Lisa Chang¹, Justin Klaff¹, Andrea Balbo¹ and Stanley I Rapoport¹

¹Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

Incorporation coefficients k^* of intravenously injected [³H]arachidonic acid from blood into brain reflect the release from phospholipids of arachidonic acid by receptor-initiated activation of phospholipase A₂ (PLA₂). 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-HT₂ 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-HT₂ receptor antagonist. The results suggest that the 5-HT₂ syndrome involves widespread brain activation of PLA₂ via 5-HT_{2A} receptors, leading to the release of the second messenger, arachidonic acid. Chronic mianserin, a 5-HT₂ antagonist, prevents this activation.

Neuropsychopharmacology (2003) **28**, 244–252. doi:10.1038/sj.npp.1300022

Keywords: serotonin (5-HT); phospholipase A₂; 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-HT₂ receptors (Barnes and Sharp, 1999; Breier, 1995; Ramasubbu *et al*, 2000; Rauser *et al*, 2001; Reynolds, 2001; Thase, 2002). In brain, 5-HT₂ receptors can be coupled via G-proteins to phospholipase C (PLC) activation, generating inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol as second messengers (Conn and Sanders-Bush, 1986b; Edagawa *et al*, 2000), or to phospholipase A₂ (PLA₂) 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-HT₂ 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 [¹⁴C]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-

*Correspondence: Ying Qu, Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Building 10, Room 6N202, Bethesda, MD 20892, USA, Tel: +1 301 594 3134, Fax: +1 301 402 0074, E-mail: quying@mail.nih.gov

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-HT₂ 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-HT₂ receptor occupancy by DOI. As noted above, such signaling can occur through activation of PLC or PLA₂. No method currently exists to image brain PLC activation *in vivo*, whereas PLA₂ 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 PLA₂-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 PLA₂ via membrane G-proteins include cholinergic muscarinic M₁ and M₃ receptors, dopaminergic D₂ receptors, and serotonergic 5-HT₂ 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). PLA₂ can also be activated when Ca²⁺ 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 ([³H]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 [³H]AA in response to chronically administered mianserin, an atypical tetracyclic antidepressant that has been used as a 5-HT₂-receptor antagonist in many animal studies, although having some adrenergic α₂-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 [5,6,8,9,11,12,14,15-³H]AA ([³H]AA) at a specific activity of 200 Ci/mmol was purchased from Moravsek 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 O₂). 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, pO₂, and pCO₂. 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 [³H]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 [^3H]methylmethacrylate autoradiographic standards (Amersham, Arlington Heights, IL) to [^3H]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 $\text{ml}/(\text{s g})$; $c_{\text{brain}}^*(20 \text{ min})$ 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 [^3H]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 [^3H]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 [^3H]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 [^3H]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 [^3H]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 [^3H]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 [^3H]AA in brains of unanesthetized adult rats. These increments are consistent with the reported marked behavioral (5-HT₂ 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-HT₂ receptor agonist that has been reported to block the 5-HT₂ syndrome and the hyperthermia produced by DOI (Barendsen and Broekkamp, 1991; Mazzola-Pomietto *et al*, 1997).

The interpretation that k^* for [^3H]AA reflects regional PLA₂ 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 PLA₂ 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 ($^{\circ}\text{C}$)	35.6 \pm 0.1	35.3 \pm 0.2
Arterial blood pressure (mm Hg)	97.7 \pm 3.6/130.6 \pm 4.8	95.2 \pm 2.5/121.2 \pm 2.4
Heart rate (beats/min)	425.0 \pm 13.7	430.9 \pm 13.6
Arterial pH	7.4 \pm 0.008	7.4 \pm 0.008
Arterial blood gas, pCO ₂ (mmHg)	38.9 \pm 1.6	41.1 \pm 1.3
Arterial blood gas, pO ₂ (mm Hg)	95.4 \pm 2.2	93.8 \pm 1.9

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

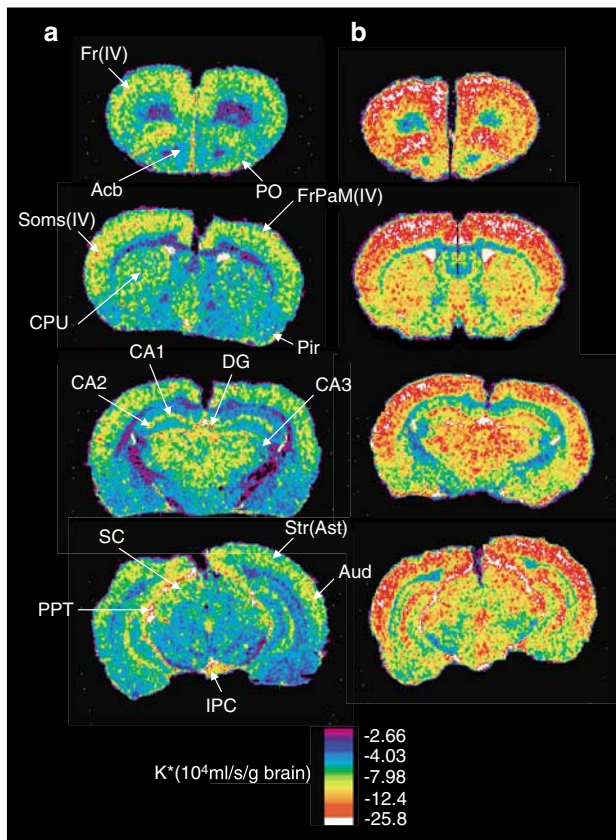


Figure 1 [^3H]arachidonate incorporation coefficients k^* in coronal sections from brain of (a) control rat and (b) rat given DOI (2.5 mg/kg i.p.); k^* is color-coded.

several observations. As shown in Table 2, k^* for [^3H]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 [^3H]AA (Grange *et al.*, 1998).

Chronically administered mianserin had no effect on baseline values of k^* for [^3H]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 [^3H]Arachidonate Incorporation Coefficients k^* (ml/(s g) brain $\times 10^4$) in Rat Brain under Different Conditions

Brain regions	Abbreviation	Control	DOI	Mianserin	Mianserin +DOI
<i>Cerebral cortex</i>					
Prefrontal cortex IV	PFr	8.6 ± 0.4	$14.0 \pm 1.3^{***}$	9.1 ± 0.6	9.3 ± 0.9
Frontal cortex layer I	Fr I	7.2 ± 0.4	$12.6 \pm 1.5^{***}$	7.8 ± 0.8	9.5 ± 1.1
Frontal cortex layer IV	Fr IV	9.2 ± 0.5	$15.5 \pm 1.5^{***}$	9.9 ± 0.9	10.9 ± 1.0
Motor cortex layer I	FrPaM I	7.0 ± 0.5	$11.8 \pm 0.9^{***}$	7.8 ± 0.8	8.8 ± 1.1
Motor cortex layer II–III	FrPaM II–III	7.5 ± 0.5	$13.7 \pm 1.2^{***}$	8.4 ± 0.7	9.0 ± 0.9
Motor cortex layer IV	FrPaM IV	9.3 ± 0.5	$16.6 \pm 1.6^{***}$	10.4 ± 0.9	11.1 ± 1.1
Motor cortex layer V	FrPaM V	7.6 ± 0.5	$13.3 \pm 1.1^{***}$	8.5 ± 0.7	9.0 ± 0.9
Motor cortex layer VI	FrPaM VI	6.8 ± 0.4	$11.7 \pm 1.0^{***}$	7.4 ± 0.6	8.0 ± 0.8
Somatosensory cortex layer I	Soms I	7.4 ± 0.5	$12.7 \pm 1.0^{***}$	8.4 ± 0.8	8.9 ± 1.1
Somatosensory cortex layer II–III	Soms II–III	8.2 ± 0.6	$14.1 \pm 1.3^{***}$	8.6 ± 0.7	9.8 ± 1.0
Somatosensory cortex layer IV	Soms IV	9.43 ± 0.06	$15.9 \pm 1.5^{***}$	10.4 ± 0.9	11.2 ± 1.1
Somatosensory cortex layer V	Soms V	7.73 ± 0.5	$12.3 \pm 0.9^{***}$	8.1 ± 0.06	9.0 ± 1.0
Somatosensory cortex layer VI	Soms VI	6.2 ± 0.4	$10.2 \pm 0.9^{***}$	6.4 ± 0.6	7.1 ± 0.7
Anterior cingulate cortex	Acg	8.6 ± 0.5	$13.1 \pm 1.0^{***}$	8.6 ± 1.0	9.6 ± 1.1
Auditory cortex layer I	Aud I	6.7 ± 0.2	$10.5 \pm 1.0^{***}$	6.9 ± 0.7	8.3 ± 1.0
Auditory cortex layer IV	Aud IV	8.9 ± 0.4	$12.1 \pm 0.8^*$	8.4 ± 0.7	11.0 ± 1.0
Auditory cortex layer V	Aud V	7.2 ± 0.4	$11.1 \pm 1.1^{***}$	7.0 ± 0.7	8.2 ± 0.7
Visual cortex layer I	Str (Ast) I	6.3 ± 0.2	$11.1 \pm 1.1^{***}$	6.9 ± 0.7	7.6 ± 0.08
Visual cortex layer IV	Str (Ast) IV	8.2 ± 0.4	$14.5 \pm 1.3^{***}$	8.7 ± 0.8	10.1 ± 1.1
Visual cortex layer V	Str (Ast) V	6.8 ± 0.4	$11.9 \pm 1.1^{***}$	7.2 ± 0.7	8.0 ± 0.09
<i>White matter</i>					
Corpus callosum	cc	3.2 ± 0.3	$5.4 \pm 0.5^{***}$	3.5 ± 0.3	3.9 ± 0.5
Internal capsule	ic	3.1 ± 0.2	$5.3 \pm 0.4^{***}$	3.5 ± 0.4	3.5 ± 0.5
<i>Olfactory system</i>					
Olfactory cortex	PO	10.1 ± 0.6	$15.5 \pm 1.3^{***}$	10.6 ± 0.9	12.1 ± 1.2
Pyriform cortex	Pir	9.0 ± 0.7	$13.8 \pm 1.4^{***}$	9.7 ± 0.8	10.7 ± 1.1
<i>Basal ganglia and related areas</i>					
Nucleus accumbens	Acb	8.0 ± 0.4	$12.5 \pm 1.4^{***}$	11.1 ± 1.3	10.1 ± 1.0
Caudate-putamen dorsal	CPU	6.3 ± 0.3	$10.1 \pm 0.9^{***}$	6.6 ± 0.6	7.3 ± 0.8
Caudate-putamen ventral	CPU	6.4 ± 0.3	$10.6 \pm 1.0^{***}$	7.2 ± 0.6	7.7 ± 0.8
Caudate-putamen lateral	CPU	6.6 ± 0.3	$10.5 \pm 1.0^{***}$	6.9 ± 0.5	8.2 ± 0.7
Caudate-putamen medial	CPU	6.2 ± 0.3	$10.2 \pm 0.8^{***}$	6.8 ± 0.6	7.7 ± 0.7
Bed nucleus stria preoptic nucleus	LPO/MPO	5.5 ± 0.3	$8.6 \pm 0.8^{***}$	5.8 ± 0.5	6.3 ± 0.8
Bed nucleus stria suprachiasmatic nucleus	PSCH	6.3 ± 0.4	$9.8 \pm 0.7^{***}$	6.7 ± 0.4	7.2 ± 0.8
Bed nucleus stria terminalis	BSTPO	4.7 ± 0.2	$7.2 \pm 0.7^{***}$	5.1 ± 0.5	5.9 ± 0.7
Entopeduncular nucleus	EN	4.1 ± 0.2	$6.9 \pm 0.6^{***}$	4.6 ± 0.4	4.9 ± 0.6
Globus pallidus	GP	4.3 ± 0.3	$7.0 \pm 0.5^{***}$	4.9 ± 0.5	4.9 ± 0.6
Amygdala basolateral/basomedial nuclei	BL/BM	5.1 ± 0.2	$8.0 \pm 0.8^{***}$	5.6 ± 0.6	5.8 ± 0.7
Subthalamic nucleus	Sth	6.4 ± 0.4	$9.9 \pm 0.8^{***}$	6.5 ± 0.8	7.4 ± 0.8
Substantia nigra pars reticulata	SNR	5.3 ± 0.3	$9.8 \pm 1.0^{***}$	5.4 ± 0.4	6.6 ± 0.7
Substantia nigra pars compacta	SNC	5.6 ± 0.3	$9.8 \pm 1.0^{***}$	5.5 ± 0.5	6.8 ± 0.8
Lateral septal nucleus	LSI	5.0 ± 0.3	$8.2 \pm 0.7^{***}$	5.4 ± 0.6	6.2 ± 0.6
Medial septal nucleus	MS	5.9 ± 0.3	$9.2 \pm 0.8^{***}$	6.5 ± 0.5	7.3 ± 0.8
Dorsal diagonal band	VDBD	6.5 ± 0.4	$9.8 \pm 0.8^{***}$	7.0 ± 0.6	7.8 ± 0.9
Ventral diagonal band	VDBV	5.9 ± 0.4	$8.8 \pm 0.6^{***}$	6.3 ± 0.7	7.0 ± 0.9
<i>Hippocampal formation</i>					
Ammon's horn CA1	Hip CA1	7.8 ± 0.4	$12.0 \pm 0.9^{***}$	8.1 ± 0.6	8.8 ± 1.0
Ammon's horn CA2	Hip CA2	7.4 ± 0.4	$11.8 \pm 1.1^{***}$	7.7 ± 1.2	8.7 ± 0.9
Ammon's horn CA3	Hip CA3	6.8 ± 0.3	$10.7 \pm 0.8^{***}$	7.2 ± 0.6	8.2 ± 0.8
Dentate gyrus	DG	12.2 ± 0.8	$18.9 \pm 2.3^*$	14.5 ± 1.5	14.5 ± 1.7
Dorsal lateral geniculate nucleus	DLG	6.9 ± 0.3	$10.9 \pm 0.9^{***}$	7.6 ± 0.7	8.1 ± 0.8
Parafascicular nucleus	PF	6.6 ± 0.3	$10.3 \pm 0.9^{***}$	6.8 ± 0.6	7.3 ± 0.8
<i>Thalamus</i>					
Paratenial nuclei	PT	6.4 ± 0.3	$1.0 \pm 0.8^{***}$	7.2 ± 0.6	7.8 ± 0.8
Anteroventral nuclei	AV	9.4 ± 0.6	$14.3 \pm 1.5^{***}$	10.5 ± 1.0	11.2 ± 1.1
Anteromedial nuclei	AM	6.7 ± 0.3	$10.1 \pm 0.7^{***}$	7.4 ± 0.6	7.8 ± 0.8
Reticular nuclei	Rt	6.4 ± 0.3	$10.4 \pm 0.9^{***}$	7.5 ± 0.6	7.7 ± 0.8
Paraventricular nuclei	PVA	6.5 ± 0.2	$10.2 \pm 1.1^{***}$	7.2 ± 0.6	7.5 ± 0.9
Ventroposterior medial thalamus	VPL	6.5 ± 0.5	$9.5 \pm 0.8^{***}$	6.7 ± 0.5	7.1 ± 0.8
Ventroposterior lateral thalamus	VPM	6.8 ± 0.7	$9.9 \pm 0.9^{***}$	6.9 ± 0.8	7.7 ± 0.8
Lateral habenular nucleus	LHb	8.4 ± 0.6	$12.1 \pm 1.2^*$	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
<i>Hypothalamus</i>					
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
<i>Brainstem and spinal cord</i>					
Raphe magnus nuclei	RMg	6.1 ± 0.5	10.6 ± 1.4***	6.6 ± 0.7	6.6 ± 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
Pretecal 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	FI	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 D₂ agonist quinpirol (Hayakawa *et al*, 2001; Nariai *et al*, 1991; Orzi *et al*, 1988; Wooten and Collins, 1981).

Recall that PLA₂ can be activated when a ligand binds to any of a number of receptor subtypes, including 5-HT₂ receptors (Axelrod, 1995; Cooper *et al*, 1996; Felder *et al*, 1990). 5-HT₂ 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 PLA₂ 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 PLA₂ 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 Ca²⁺-dependent activation of PLA₂ (Kaufman *et al*, 1995). The uptake mechanism of [³H]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 [³H]AA to access directly the choroid epithelium (Rapoport, 1976).

The DOI dose chosen in the present study may have been too large to identify PLA₂ signaling solely at 5-HT₂ receptors, because of downstream activation of dopaminergic D₂ and other receptors also coupled to PLA₂ (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-HT₂ 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 PLA₂ 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-HT₂ 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} (Freio *et al*, 1991, 1992).

In summary, our results suggest that labeled AA can be used to examine *in vivo* brain PLA₂ signaling initiated by serotonergic drugs. Increments in *k*^{*} for [³H]AA in response to DOI largely correspond to the distribution of 5-HT_{2A} binding sites in the brain, although downstream receptors coupled to PLA₂ are probably activated as well. Chronic mianserin, a 5-HT₂ agonist known to inhibit the 5-HT₂ syndrome, blocks [³H]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 PLA₂ 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 PLA₂ 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.

REFERENCES

- Anji A, Kumari M, Sullivan Hanley NR, Bryan GL, Hensler JG (2000). Regulation of 5-HT_{2A} receptor mRNA levels and binding sites in rat frontal cortex by the agonist DOI and the antagonist mianserin. *Neuropharmacology* 39: 1996–2005.
- Appel NM, Mitchell WM, Garlick RK, Glennon RA, Teitler M, De Souza EB (1990). Autoradiographic characterization of (±)-1-(2,5-dimethoxy-4-[¹²⁵I]iodophenyl)-2-aminopropane ([¹²⁵I]DOI) binding to 5-HT₂ and 5-HT_{1C} receptors in rat brain. *J Pharmacol Exp Ther* 255: 843–857.
- Arvidsson LE, Hacksell U, Glennon RA (1986). Recent advances in central 5-hydroxytryptamine receptor agonists and antagonists. *Prog Drug Res* 30: 365–471.
- Ashby Jr CR, Jiang LH, Kasser RJ, Wang RY (1990). Electrophysiological characterization of 5-hydroxytryptamine₂ receptors in the rat medial prefrontal cortex. *J Pharmacol Exp Ther* 252: 171–178.
- Axelrod J (1995). Phospholipase A₂ and G proteins. *Trends Neurosci* 18: 64–65.
- Barnes NM, Sharp T (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* 38: 1083–1152.
- Bayon Y, Hernández M, Alonso A, Nuñez L, García-Sancho J, Leslie C *et al* (1997). Cytosolic phospholipase A₂ is coupled to muscarinic receptors in the human astrocytoma cell line 1321N1: characterization of the transducing mechanism. *Biochem J* 323: 281–287.
- Berendsen HH, Broekkamp CL (1991). Attenuation of 5-HT_{1A} and 5-HT₂ but not 5-HT_{1C} receptor mediated behaviour in rats following chronic treatment with 5-HT receptor agonists, antagonists or anti-depressants. *Psychopharmacology (Berl)* 105: 219–224.
- Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clark WP (1998). Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol Pharmacol* 54: 94–104.
- Blackshear MA, Sanders-Bush E (1982). Serotonin receptor sensitivity after acute and chronic treatment with mianserin. *J Pharmacol Exp Ther* 221: 303–308.
- Bloom FE (1985). CNS plasticity: a survey of opportunities. In: Bignami A, Bolis CL, Bloom FE, Adey A (eds). *Central Nervous System Plasticity and Repair*. Raven Press: New York, pp 3–11.
- Breier A (1995). Serotonin, schizophrenia and antipsychotic drug action. *Schizophr Res* 14: 187–202.
- Brooks RC, McCarthy KD, Lapetina EG, Morell P (1989). Receptor stimulated phospholipase A₂ activation is coupled to influx of external calcium and not to mobilization of intracellular calcium in C62B glioma cells. *J Biol Chem* 264: 20147–20153.
- Chang MCJ, Arai T, Freed LM, Wakabayashi S, Channing MA, Dunn BB *et al* (1997). Brain incorporation of [1-¹¹C]-arachidonate in normocapnic and hypercapnic monkeys, measured with positron emission tomography. *Brain Res* 755: 74–83.
- Conn PJ, Sanders-Bush E (1986a). Agonist-induced phosphoinositide hydrolysis in choroid plexus. *J Neurochem* 47: 1754–1760.
- Conn PJ, Sanders-Bush E (1986b). Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5-HT₂ binding site. *J Neurosci* 6: 3669–3675.
- Cooper JR, Bloom FE, Roth RH (1996). *The Biochemical Basis of Neuropharmacology*. 7th edn. Oxford University Press: Oxford.
- DeGeorge JJ, Nariai T, Yamazaki S, Williams WM, Rapoport SI (1991). Arecoline-stimulated brain incorporation of intravenously administered fatty acids in unanesthetized rats. *J Neurochem* 56: 352–355.
- DeGeorge JJ, Noronha JG, Bell JM, Robinson P, Rapoport SI (1989). Intravenous injection of [1-¹⁴C]arachidonate to examine regional brain lipid metabolism in unanesthetized rats. *J Neurosci Res* 24: 413–423.
- Dijcks FA, Ruigt GS, de Graaf JS (1991). Antidepressants affect amine modulation of neurotransmission in the rat hippocampal slice: II. Acute effects. *Neuropharmacology* 30: 1151–1158.
- Dunnnett C (1964). New tables for multiple comparisons with a control. *Biometrics* 20: 484–491.
- Edagawa Y, Saito H, Abe K (2000). The serotonin 5-HT₂ receptor-phospholipase C system inhibits the induction of long-term potentiation in the rat visual cortex. *Eur J Neurosci* 12: 1391–1396.

- Essom CR, Nemeroff CB (1996). Treatment of depression in adulthood. In: Shulman KI, Tohen M, Kutcher SP (eds). *Mood Disorders across the Life Span*. John Wiley: New York, pp 251–264.
- Felder CC, Kanterman RY, Ma AL, Axelrod J (1990). Serotonin stimulates phospholipase A₂ and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. *Proc Natl Acad Sci USA* 87: 2187–2191.
- Feuerstein TJ, Gleichauf O, Landwehrmeyer GB (1996). Modulation of cortical acetylcholine release by serotonin: The role of substance P interneurons. *Naunyn Schmiedeberg's Arch Pharmacol* 354: 618–626.
- Fisone G, Snyder GL, Aperia A, Greengard P (1998). Na⁺,K⁺-ATPase phosphorylation in the choroid plexus: synergistic regulation by serotonin/protein kinase C and isoproterenol/cAMP-PK/PP-1 pathways. *Mol Med* 4: 258–265.
- Fisone G, Snyder GL, Fryckstedt J, Caplan MJ, Aperia A, Greengard P (1995). Na⁺K⁺-ATPase in the choroid plexus. Regulation by serotonin/protein kinase C pathway. *J Biol Chem* 270: 2427–2430.
- Folch J, Lees M, Sloane Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497–509.
- Fonlupt P, Croset M, Lagarde M (1994). Incorporation of arachidonic and docosahexaenoic acids into phospholipids of rat brain membranes. *Neurosci Lett* 171: 137–141.
- Frazer A, Offord SJ, Lucki I (1988). Regulation of serotonin receptors and responsiveness in the brain. In: Sanders-Bush E (ed). *The Serotonin Receptors*. Humana Press: New Jersey, pp 319–362.
- Freo U, Holloway HW, Kalogeras K, Rapoport SI, Soncrant TT (1992). Adrenalectomy or metyrapone-pretreatment abolishes cerebral metabolic responses to the serotonin agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) in the hippocampus. *Brain Res* 586: 256–264.
- Freo U, Soncrant TT, Holloway HW, Rapoport SI (1991). Dose- and time-dependent effects of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a serotonergic 5-HT₂ receptor agonist, on local cerebral glucose metabolism. *Brain Res* 541: 63–69.
- Gaggi R, Dall'Olio R, Roncada P (1997). Effect of the selective 5-HT receptor agonists 8-OHDPAT and DOI on behavior and brain biogenic amines of rats. *Gen Pharmacol* 28: 583–587.
- Giovacchini G, Chang MC, Bokde A, Connolly C, Channing MA, Herscovitch P et al (2001). Brain incorporation of [¹¹C]arachidonic acid (AA) and palmitic acid (PA). Human studies with PET (Abstract). *J Nucl Med* 42(Suppl): 65P.
- Glennon RA (1986). Discriminative stimulus properties of the serotonergic agent 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). *Life Sci* 39: 825–830.
- Glennon RA (1987). Central serotonin receptors as targets for drug research. *J Med Chem* 30: 1–12.
- Gobert A, Millan MJ (1999). Serotonin (5-HT)_{2A} receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely moving rats. *Neuropharmacology* 38: 315–317.
- Grange E, Rabin O, Bell J, Rapoport SI, Chang MCJ (1998). Manoalide, a phospholipase A₂ inhibitor, inhibits arachidonate incorporation and turnover in brain phospholipids of the awake rat. *Neurochem Res* 23: 1251–1257.
- Hartman IV JL, Northup JK (1996). Functional reconstitution *in situ* of 5-hydroxytryptamine_{2c} (5HT_{2c}) receptors with α_q and inverse agonism of 5HT_{2c} receptor agonists. *J Biol Chem* 271: 22591–22597.
- Hayakawa T, Chang CJ, Rapoport SI, Appel NM (2001). Selective dopamine receptor stimulation differentially affects [³H]arachidonic acid incorporation, a surrogate marker for phospholipase A₂-mediated signal transduction, in a rodent model of Parkinson's disease. *J Pharmacol Exp Ther* 296: 1074–1084.
- Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ et al (1995). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 46: 157–203.
- Ichikawa J, Meltzer HY (1995). DOI, a 5-HT_{2A/2C} receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. *Brain Res* 698: 204–208.
- Johnson RG, Stevens KE, Rose GM (1998). 5-Hydroxytryptamine-2 receptors modulate auditory filtering in the rat. *J Pharmacol Exp Ther* 285: 643–650.
- Jones CR, Arai T, Bell JM, Rapoport SI (1996). Preferential *in vivo* incorporation of [³H]arachidonic acid from blood into rat brain synaptosomal fractions before and after cholinergic stimulation. *J Neurochem* 67: 822–829.
- Kaufman MJ, Hartig PR, Hoffman BJ (1995). Serotonin 5-HT_{2C} receptor stimulates cyclic GMP formation in choroid plexus. *J Neurochem* 64: 199–205.
- Kreiss DS, Lucki I (1995). Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured *in vivo*. *J Pharmacol Exp Ther* 274: 866–876.
- Leysen JE, Gommeren W, Janssen PF, Van Gompel P, Janssen PA (1988). Receptor interactions of dopamine and serotonin antagonists: binding *in vitro* and *in vivo* and receptor regulation. *Psychopharmacol Ser* 5: 12–26.
- Li Q, Ma L, William K, Murphy DL (2001). 5-HT_{2A} and 5-HT_{2C} receptors are differently regulated in 5-HT transporter mice: Studies on the density and gene expression of the receptors. *Soc Neurosci Abstr* 26: 380.5.
- Lira A, Bradley-Moore M, Zhou M, Fairhurst S, Menzaghi F, Brunner D et al (2001). Cognitive performance in 5-HT_{2A} receptor knockout mice. *Soc Neurosci Abstr* 27: 3804.
- Liu R, Jolas T, Aghajanian G (2000). Serotonin 5-HT(2) receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Res* 87: 34–45.
- Maiese K, Holloway HH, Larson DM, Soncrant TT (1994). Effect of acute and chronic arecoline treatment on cerebral metabolism and blood flow in the conscious rat. *Brain Res* 28: 65–75.
- Marcoli M, Rosu C, Bonfanti A, Raiteri M, Maura G (2001). Inhibitory presynaptic 5-hydroxytryptamine(2A) receptors regulate evoked glutamate release from rat cerebellar mossy fibers. *J Pharmacol Exp Ther* 299: 1106–1111.
- Mazzola-Pomietto P, Aulakh CS, Tolliver T, Murphy DL (1997). Functional subsensitivity of 5-HT_{2A} and 5-HT_{2C} receptors mediating hyperthermia following acute and chronic treatment with 5-HT_{2A/2C} receptor antagonists. *Psychopharmacology (Berl)* 130: 144–151.
- McKenna DJ, Nazarali AJ, Hoffman AJ, Nichols DE, Mathis CA, Saavedra JM (1989). Common receptors for hallucinogens in rat brain: a comparative autoradiographic study using [¹²⁵I]LSD and [¹²⁵I]DOI, a new psychotomimetic radioligand. *Brain Res* 476: 45–56.
- McKenna DJ, Peroutka SJ (1989). Differentiation of 5-hydroxytryptamine-2 receptor subtypes using ¹²⁵I-R-(–)-2,5-dimethoxy-4-iodo-phenylisopropylamine and ³H-ketanserin. *J Neurosci* 9: 3482–3490.
- Mills KC (1997). Serotonin syndrome. A clinical update. *Crit Care Clin* 13: 763–783.
- Morilak DA, Garlow SJ, Ciaranello RD (1993). Immunocytochemical localization and description of neurons expressing serotonin-2 receptors in rat brain. *Neuroscience* 54: 701–717.
- Nariai T, DeGeorge JJ, Lamour Y, Rapoport SI (1991). *In vivo* brain incorporation of [¹⁻¹⁴C]arachidonate in awake rats, with or without cholinergic stimulation, following unilateral lesioning of nucleus basalis magnocellularis. *Brain Res* 559: 1–9.
- Orzi F, Diana G, Casamenti F, Palombo E, Fieschi C (1988). Local cerebral glucose utilization following unilateral and bilateral lesions of the nucleus basalis magnocellularis in the rat. *Brain Res* 462: 99–103.

- Osmundsen H, Hovik R (1988). β -Oxidation of polyunsaturated fatty acids. *Biochem Soc Trans* 16: 420–422.
- Ozawa H, Katamura Y, Hatta S, Amemiya N, Saito T, Ohshika H et al (1994). Antidepressants directly influence *in situ* binding of guanine nucleotide in synaptic membrane. *Life Sci* 54: 925–932.
- Pardridge WM, Mietus LJ (1980). Palmitate and cholesterol transport through the blood–brain barrier. *J Neurochem* 34: 463–466.
- Paxinos G, Watson C (1987). *The Rat Brain in Stereotaxic Coordinates*. 3rd edn. Academic Press: New York.
- Pazos A, Palacios JM (1985). Quantitative autoradiographic mapping of serotonin receptor in the rat brain: I. Serotonin-1 receptors. *Brain Res* 346: 205–230.
- PDSP Drug Database (2000). Psychoactive Drug Screening Program drug database (available at <http://pdsp.cwru.edu/pdsp.asp>) (see also description in Hot picks: Psychopharmacokinetics unbound. *Science* 287: 543c).
- Pinder RM (1985). Adrenoreceptor interactions of the enantiomers and metabolites of mianserin: are they responsible for the antidepressant effect? *Acta Psychiatr Scand Suppl* 320: 1–9.
- Pranzatelli MR (1990). Evidence for involvement of 5-HT₂ and 5-HT_{1C} receptors in the behavioral effects of the 5-HT agonist 1-(2,5-dimethoxy-4-iodophenyl aminopropane)-2 (DOI). *Neurosci Lett* 115: 74–80.
- Purdon AD, Rapoport SI (1998). Energy requirements for two aspects of phospholipid metabolism in mammalian brain. *Biochem J* 335: 313–318.
- Qu Y, Chang L, Klaff J, Balbo A, Rapoport SI (2001). Imaging phospholipase A₂ mediated signal transduction in response to a 5-HT₂ agonist in brain of awake rats (Abstract). *J Neurochem* 78(Suppl 1): 144.
- Ramasubbu R, Ravindran A, Lapierre Y (2000). Serotonin and dopamine antagonism in obsessive-compulsive disorder: effect of atypical antipsychotic drugs. *Pharmacopsychiatry* 33: 236–238.
- Rapoport SI (1976). *Blood–Brain Barrier in Physiology and Medicine*. Raven Press: New York.
- Rapoport SI (2001). *In vivo* fatty acid incorporation into brain phospholipids in relation to plasma availability, signal transduction and membrane remodeling. *J Mol Neurosci* 16: 243–261, 279–284.
- Rapoport SI, Chang MCJ, Spector AA (2001). Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. *J Lipid Res* 42: 678–685.
- Rausser L, Savage JE, Meltzer HY, Roth BL (2001). Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine(2C) receptor. *J Pharmacol Exp Ther* 299: 83–89.
- Reivich M (1974). Blood flow metabolism couple in brain. *Res Publ Assoc Res Nerv Ment Dis* 53: 125–140.
- Reynolds GP (2001). Antipsychotic drug use in neurodegenerative disease in the elderly: problems and potential from a pharmacological perspective. *Expert Opin Pharmacother* 2: 543–548.
- Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI (1992). A quantitative method for measuring regional *in vivo* fatty-acid incorporation into and turnover within brain phospholipids: review and critical analysis. *Brain Res Rev* 17: 187–214.
- Robinson PJ, Rapoport SI (1986). Kinetics of protein binding determine rates of uptake of drugs by brain. *Am J Physiol* 251: R1212–R1220.
- Rocha B, Rigo M, Di Scala G, Sandner G, Hoyer D (1994). Chronic mianserin or eltopazine treatment in rats: Effects on the elevated plus-maze test and on limbic 5-HT_{2C} receptor levels. *Eur J Pharmacol* 262: 125–131.
- Roth BL, Ciaranello RD (1991). Chronic mianserin treatment decreases 5-HT₂ binding without altering 5-HT₂ receptor mRNA. *Eur J Pharmacol* 207: 169–172.
- Roth BL, Kroeze WK, Patel S, Lopez E (2000). The multiplicity of serotonin receptors: uselessly diverse molecules or an embarrassment of riches? *Neuroscientist* 6: 252–262.
- Sanders-Bush E, Breeding M, Roznoski M (1987). 5-HT₂ binding sites after mianserin: comparison of loss of sites and brain levels of drug. *Eur J Pharmacol* 133: 199–204.
- Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan MJ (1995). (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: Modulation by novel 5-HT_{2A/2C} antagonists, D₁ antagonists and 5-HT_{1A} agonists. *J Pharmacol Exp Ther* 273: 101–112.
- Shimizu T, Wolfe LS (1990). Arachidonic acid cascade and signal transduction. *J Neurochem* 55: 1–15.
- Sokoloff L (1999). Energetics of functional activation in neural tissues. *Neurochem Res* 24: 321–329.
- Soncrant TT, Holloway HW, Rapoport SI (1985). Arecoline-induced elevations of regional cerebral metabolism in the conscious rat. *Brain Res* 347: 205–216.
- Sternbach H (1991). The serotonin syndrome. *Am J Psychiatry* 148: 705–713.
- Svensson A, Holmer E, Andersson LO (1974). A new method for the measurement of dissociation rates for complexes between small ligands and proteins as applied to the palmitate and bilirubin complexes with serum albumin. *Biochim Biophys Acta* 342: 54–59.
- Thase ME (2002). What role do atypical antipsychotic drugs have in treatment-resistant depression? *J Clin Psychiatry* 63: 95–103.
- Tournois C, Mutel V, Manivet P, Launay JM, Kellermann O (1998). Cross-talk between 5-hydroxytryptamine receptors in a serotonergic cell line: involvement of arachidonic acid metabolism. *J Biol Chem* 273: 17498–17503.
- Van de Kar LD, Javed A, Zhang Y, Serres F, Raap DK, Gray TS (2001). 5-HT_{2A} receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. *J Neurosci* 21: 3572–3579.
- Vial D, Piomelli D (1995). Dopamine D₂ receptors potentiate arachidonate release via activation of cytosolic, arachidonic-specific phospholipase A₂. *J Neurochem* 64: 2765–2772.
- Vijayaraghavan S, Huang B, Blumenthal EM, Berg DK (1995). Arachidonic acid as a possible negative feedback inhibitor of nicotinic acetylcholine receptors on neurons. *J Neurosci* 15: 3679–3687.
- Washizaki K, Purdon AD, DeGeorge JJ, Robinson PJ, Rapoport SI, Smith QR (1991). Fatty acid uptake and esterification by the *in situ* perfused rat brain. *Soc Neuroscience Abstr* 17: 864.
- Washizaki K, Smith QR, Rapoport SI, Purdon AD (1994). Brain arachidonic acid incorporation and precursor pool specific activity during intravenous infusion of unesterified [³H]arachidonate in the anesthetized rat. *J Neurochem* 63: 727–736.
- Westphal RS, Backstrom JR, Sanders-Bush E (1995). Increased basal phosphorylation of the constitutively active serotonin 2C receptor accompanied agonist-mediated desensitization. *Mol Pharmacol* 48: 200–205.
- Wettstein JG, Host M, Hitchcock JM (1999). Selectivity of action of typical and atypical anti-psychotic drugs as antagonists of the behavioral effects of 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI). *Prog Neuropsychopharmacol Biol Psychiatry* 23: 533–544.
- Wolf WA, Schutz LJ (1997). The serotonin 5-HT_{2C} receptors is a prominent serotonin receptor in the basal ganglia: evidence from functional studies on serotonin-mediated phosphoinositide hydrolysis. *J Neurochem* 69: 1449–1458.
- Wooten GF, Collins RC (1981). Metabolic effects of unilateral lesion of the substantia nigra. *J Neurosci* 1: 285–291.
- Yamazaki S, DeGeorge JJ, Bell JM, Rapoport SI (1994). Effect of pentobarbital on incorporation of plasma palmitate into rat brain. *Anesthesiology* 80: 151–158.