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Imaging Brain Phospholipase A_2-Mediated Signal Transduction in Response to Acute Fluoxetine Administration in Unanesthetized Rats

Ying Qu*[,1], Lisa Chang[1], Justin Klaff[1], Ruth Seemann[1], and Stanley I Rapoport[1]

[1]*Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, USA*

Fluoxetine, a selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor, is used widely to treat depression and related disorders. By inhibiting presynaptic 5-HT reuptake, fluoxetine is thought to act by increasing 5-HT in the synaptic cleft, thus 5-HT binding to postsynaptic 5-$HT_{2A/2C}$ receptors. These receptors can be coupled via a G-protein to phospholipase A_2 (PLA_2), which when activated releases the second messenger arachidonic acid from synaptic membrane phospholipids. To image this activation, fluoxetine (10 mg/kg) or saline vehicle was administered i.p. to unanesthetized rats, and regional brain incorporation coefficients k^* of intravenously injected radiolabeled arachidonic acid were measured after 30 min. Compared with vehicle, fluoxetine significantly increased k^* in prefrontal, motor, somatosensory, and olfactory cortex, as well as in the basal ganglia, hippocampus, and thalamus. Many of these regions demonstrate high densities of the serotonin reuptake transporter and of 5-$HT_{2A/2C}$ receptors. Brain stem, spinal cord, and cerebellum, which showed no significant response to fluoxetine, have low densities of the transporters and receptors. The results show that it is possible to image quantitatively PLA_2-mediated signal transduction *in vivo* in response to fluoxetine.
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INTRODUCTION

Fluoxetine is widely used to treat depression, obsessive–compulsive disorder, panic disorder, and bulimia (Fuller, 1995). It is a selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor, enhancing postsynaptic serotonergic neurotransmission by increasing the concentration of 5-HT in the synaptic cleft (Fuller and Wong, 1977; Wong *et al*, 1995).

Acute administration of fluoxetine to rodents elicits anxiogenic effects in social interaction and maze tests (Kurt *et al*, 2000; To *et al*, 1999), potentiates defensive reactions such as flight and biting (Griebel *et al*, 1995), and potentiates amphetamine-induced locomotor activity (Sills *et al*, 1999). Acute drug also stimulates secretion of pituitary ACTH and adrenal corticosterone (Dinan, 1996; Li *et al*, 1993). It triggers transcription of the gene encoding the corticotrophin-release factor and its type 1 receptor (Torres *et al*, 1998), thus affecting the hypothalamic–pituitary–adrenal axis that is considered hyperactive in depressed patients (Leonard, 2001).

Acute administration of fluoxetine also exerts inhibitory effects. It reduces spontaneous motor activity in 6- to 8-month-old rats, and to a lesser extent in aged rats (Stanford *et al*, 2002). It reduces regional cerebral metabolic rates for glucose ($rCMR_{glc}$) in awake rats (Freo *et al*, 2000) as well as in humans (Cook *et al*, 1994). Similar reductions in $rCMR_{glc}$ in rats have been noted in response to the 5-$HT_{2A/2C}$ receptor agonist ($\pm$)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) (Freo *et al*, 1991).

5-$HT_{2A/2C}$ receptors can be coupled via G-proteins to phospholipase A_2 (PLA_2) (Berg *et al*, 1998; Felder *et al*, 1990; Kim *et al*, 1999; Qu *et al*, in press), which when activated will release arachidonic acid (20:4 n-6) from the stereospecifically numbered (*sn*)-2 position of phospholipids (Axelrod, 1990). Both arachidonate and its eicosanoid metabolites are important second messengers (Fitzpatrick and Soberman, 2001). Much of the released arachidonate is not metabolized to eicosanoids, however, but rapidly re-esterified into synaptic membrane phospholipids, together with arachidonate derived from the plasma. The re-esterification process can be imaged in unanesthetized rats by injecting radiolabeled arachidonic acid intravenously following drug, and measuring its regional brain incorporation with quantitative autoradiography (Chang *et al*, 1997;

*Correspondence: Dr Y Qu, Present address: Neuroscience Johnson & Johnson Pharmaceutical Research and Development, 3210 Merryfield Row, San Diego, CA 92121, USA, Tel: +1 858 320 3418, Fax: 858 450 2040, E-mail: yqu1@prdus.jnj.com
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DeGeorge *et al*, 1991; Jones *et al*, 1996; Rapoport, 2001; Robinson *et al*, 1992).

In this regard, we reported that radiolabeled arachidonate could be used to image brain PLA_2 activation in rats administered the 5-$HT_{2A/2C}$ receptor agonist, DOI (Qu *et al*, 2001a). Significant increases in tracer incorporation, noted in brain areas with high densities of 5-$HT_{2A/2C}$ receptors, could be blocked by pretreatment with the 5-HT_2 antagonist mianserin. The increases were considered to follow the binding of DOI to 5-$HT_{2A/2C}$ receptors, and activation of the PLA_2 to which these receptors are coupled.

We thought it of interest to see if fluoxetine also would increase incorporation of labeled arachidonate into the brain, as might be expected from its ability to increase 5-HT in the synaptic cleft and thus 5-HT occupancy of 5-$HT_{2A/2C}$ receptors (see above). To test this expectation, we decided to measure the incorporation of radiolabeled arachidonate from plasma into the brain of unanesthetized rats, 30 min after giving fluoxetine 10 mg/kg i.p. At 30–60 min, this dose increases 5-HT levels in the rat brain, and the levels remain elevated for 3 h (Guan and McBride, 1988). Additionally, after 30 min, fluoxetine at doses of 4 mg/kg and 40 mg/kg i.p., respectively, has been reported to reduce $rCMR_{glc}$ in 28 of 66 regions (mean global decrease was 23%) and 37 of 66 (mean global decrease was 33%) regions examined (Freo *et al*, 2000).

MATERIALS AND METHODS

Materials

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

Animals

Male Fischer-344 rats (Charles River Laboratories, Wilmington, MA), weighing 290–320 g and 12-weeks old, were housed under standard laboratory conditions with a 12 h light–12 h dark cycle, with ready access to 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). Rats were divided into two groups, eight animals in each: (1) those administered fluoxetine, 10 mg/kg i.p. in 1 ml saline, 30 min before tracer infusion and (2) controls administered 1 ml i.p. saline 30 min before tracer infusion.

Arterial and Venous Catheterization

The *in vivo* fatty acid method to image brain PLA_2 signaling has been described elsewhere (DeGeorge *et al*, 1991; Hayakawa *et al*, 2001; Qu *et al*, 2003). Briefly, rats in each of the two 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 using a rectal thermometer and a feedback heating device.

Drug Administration and Tracer Infusion

After a rat was allowed to recover from anesthesia for 4 h, 125 μl arterial blood was withdrawn to measure pH, pO_2, and pCO_2. At 30 min after the administration of i.p. saline or fluoxetine (see above), 1.75 mCi/kg [^{3}H]arachidonate 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 canula with an infusion pump (Harvard Instruments, 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. The brain was immediately removed and frozen in 2-methylbutane at −70°C for subsequent quantitative autoradiography. Plasma was separated from arterial blood by centrifugation, and its lipids were extracted by the method of Folch (Folch *et al*, 1957). Radioactivity in the organic fraction was measured by liquid scintillation counting.

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 three sections were exposed together with [^{3}H]methylmethacrylate autoradiographic standards (Amersham, Arlington Heights, IL) to [^{3}H]phosphor imaging plates (Fuji Medical Systems, Stamford, CT) for 7 days, which were then scanned by a BAS 5000 scanner system (West Lafayette, IN) following the manufacturer's instructions. An adjacent section was collected and stained with cresyl violet to identify brain regions using a rat-brain atlas (Paxinos and Watson, 1987).

Regional brain radioactivity was measured in sextuplicate by quantitative densitometry using phosphor-imaging software (Image Gauge V3.45, Fuji). Regional brain incorporation coefficients k^* were calculated as

$$k^* = \frac{c^*_{\text{brain}}(20\,\text{min})}{\int_0^{20} c^*_{\text{plasma}}\,dt} \quad (1)$$

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

Data were compared statistically using Prism software for the Macintosh (Abacus Concepts, Berkeley, CA) and are reported as means ± SEM. Student's t-test tests were used

to evaluate statistical significance between experimental and control means. $P \leqslant 0.05$ was taken as indicating statistically significance.

RESULTS

Table 1 summarizes the mean physiological parameters in unanesthetized control and fluoxetine-treated rats. The control means are similar to published values (Qu *et al*, 2003) and were not changed significantly by drug.

Figure 1 presents representative autoradiographs of coronal brain sections from a rat administered fluoxetine i.p. and a control rat. Fluoxetine 10 mg/kg i.p. caused significant widespread increases in k^* for [^{3}H]arachidonate. The mean regional incorporation coefficients k^* corresponding to such increases are presented in Table 2.

As illustrated by Table 2, 30 min after fluoxetine administration, 42 of 85 brain regions had significantly increased mean values for k^* (by about 30%) compared with control values. Significant increases were evident in prefrontal frontal, motor and somatosensory cortex, and olfactory and pyriform cortex, but not in the auditory or visual cortex. Regions in the caudate-putamen, nucleus accumbens, globus pallidus, amygdala, septum, thalamus, and hypothalamus also showed significant increments in k^*, whereas significant changes generally were absent in regions of the brain stem, spinal cord, and cerebellum. The hippocampus showed elevations in CA1, CA2, and CA3 areas, but not in the dentate gyrus. The choroid plexus, where the control value of k^* was much greater than in the brain parenchyma, demonstrated a 40% increase in k^* in response to fluoxetine. White matter incorporation of [^{3}H]arachidonate was unaffected by fluoxetine.

DISCUSSION

At 30 min following administration to unanesthetized adult male rats of fluoxetine (10 mg/kg i.p.), the incorporation coefficient k^* for [^{3}H]arachidonate was increased significantly compared with control in 45 of 85 brain regions that were examined. Significant increases were noted in regions of the prefrontal and frontal cortex, motor and somatosensory cortex, and of the basal ganglia, septum, hippocampus, thalamus, and hypothalamus, but not of the auditory or visual cortex, white matter, brain stem, spinal cord, or cerebellum (Table 2) (Appel *et al*, 1990; Li *et al*, 2001; Pazos and Palacios, 1985).

Table 1 Physiological Parameters of Rats after Surgery

	Saline	Acute fluoxetine
Body temperature[a] (°C)	36.1 ± 0.3[b]	36.1 ± 0.3
Arterial blood pressure (mm Hg) (systolic pressure/diastolic pressure)	125 ± 2/78 ± 1	129 ± 5/74 ± 3
Heart rate (beats/min)	416 ± 4	413 ± 13
Arterial pH	7.41 ± 0.02	7.41 ± 0.02
Arterial blood gas (pCO_2)	41.9 ± 1.3	39.6 ± 2.0
Arterial blood gas (pO_2)	99.8 ± 5.0	101 ± 3.0

[a]Temperature was measured with a rectal thermoprobe.
[b]Mean ± SEM.
Animal number = 8.

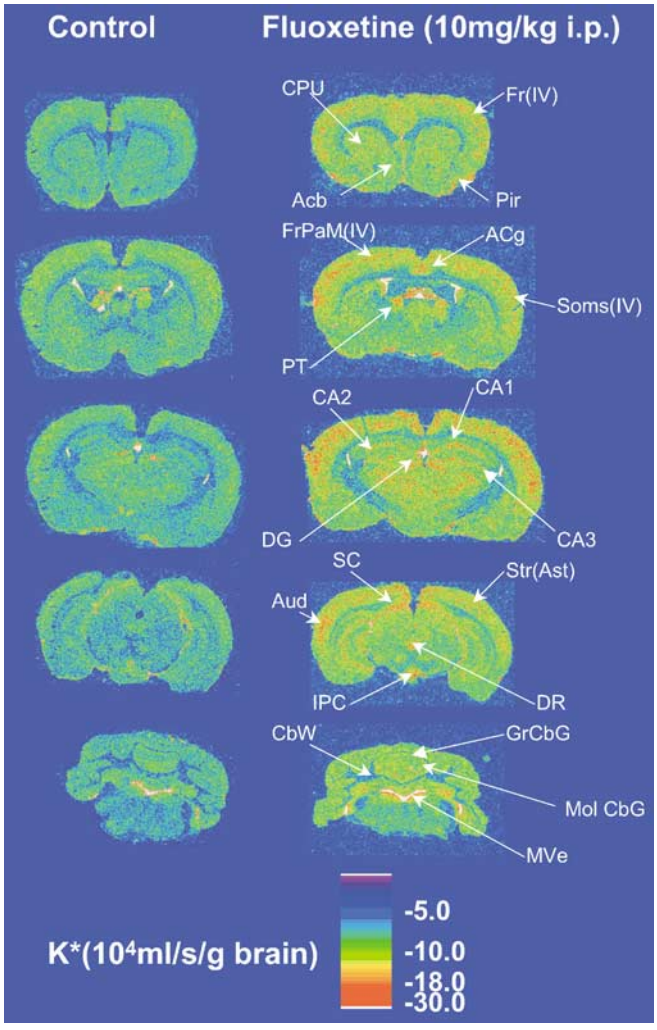

Figure 1 Coronal autoradiographs demonstrating [^{3}H]arachidonate incorporation coefficients k^* from brains of (a) control rat and (b) rat acutely administered fluoxetine (10 mg/kg i.p.). k^* is color coded.

Fluoxetine, a high–affinity, high-selectivity antagonist of the serotonin reuptake transporter (Wong *et al*, 1991) promotes accumulation of 5-HT in the synaptic cleft. Acutely administered fluoxetine has been reported to increase extracellular 5-HT in many brain areas, including the striatum (Perry and Fuller, 1993; Rutter and Auerbach, 1993), thalamus (Dailey *et al*, 1992), diencephalon (Rutter *et al*, 1993), hypothalamus (Perry *et al*, 1993), and nucleus accumbens. Increases were evident within 30–60 min and last for up to 3 h (Guan *et al*, 1988). In light of our observations that DOI increases k^* for [^{3}H]arachidonate and that the increases can be blocked by a 5-HT_2 antagonist (Qu *et al*, 2003), our results with fluoxetine (Table 2) suggest that increased synaptic cleft 5-HT caused by the drug indirectly increased the 5-HT occupancy of 5-$HT_{2A/2C}$ receptors, which are coupled to PLA_2, so as to release arachidonic acid (Berg *et al*, 1998; Felder *et al*, 1990; Kim *et al*, 1999). The regional incorporation coefficient k^* for [^{3}H]arachidonate reflects this release (see Introduction) (Rapoport, 2001; Robinson *et al*, 1992).

While many brain areas with reported high densities of the serotonin reuptake transporter and of 5-$HT_{2A/2C}$

Table 2 Regional [^{3}H]Arachidonic Acid Incorporation Coefficients k^* (ml/s/g brain $\times 10^4$) in Rat Brain in Response to Acute Fluoxetine

Brain region[a]	Saline control	Fluoxetine
Cerebral cortex		
Prefrontal cortex IV	9.1 ± 0.3	12.0 ± 1.2*
Frontal cortex layer II–III	9.5 ± 0.5	12.0 ± 1.0*
Frontal cortex layer IV	10.6 ± 0.5	13.5 ± 1.1*
Motor cortex layer II–III	9.1 ± 0.4	12.1 ± 1.2*
Motor cortex layer IV	10.5 ± 0.5	14.1 ± 1.3*
Motor cortex layer V–VI	8.9 ± 0.4	12.3 ± 1.0*
Somatosensory cortex layer II–III	9.5 ± 0.5	12.7 ± 1.2*
Somatosensory cortex layer IV	10.2 ± 0.5	14.0 ± 1.4*
Somatosensory cortex layer V–VI	8.9 ± 0.4	11.9 ± 1.0*
Anterior cingulate cortex	9.8 ± 0.4	11.8 ± 1.4
Auditory cortex layer II–III	10.3 ± 1.2	11.6 ± 1.4
Auditory cortex layer IV	11.9 ± 1.5	13.1 ± 1.2
Auditory cortex layer V–VI	9.6 ± 1.1	10.1 ± 0.8
Visual cortex layer II–III	10.0 ± 1.0	11.6 ± 1.1
Visual cortex layer IV	10.7 ± 1.0	12.3 ± 1.1
Visual cortex layer V–VI	10.0 ± 0.9	13.6 ± 2.4
White matter		
Corpus callosum	4.4 ± 0.4	5.4 ± 0.3
Internal capsule	4.2 ± 0.4	5.2 ± 0.3
Anterior commissure	5.3 ± 0.5	6.5 ± 0.4
Olfactory system		
Olfactory cortex	11.1 ± 0.5	14.6 ± 1.1**
Pyriform cortex	9.7 ± 0.4	14.5 ± 1.2**
Basal ganglia and related areas		
Nucleus accumbens	8.1 ± 0.4	10.9 ± 0.9*
Caudate-putamen dorsal	8.1 ± 0.3	10.5 ± 0.7*
Caudate-putamen ventral	8.3 ± 0.3	10.4 ± 0.9
Caudate-putamen lateral	8.0 ± 0.4	10.6 ± 0.8*
Caudate-putamen medial	7.9 ± 0.3	9.5 ± 0.8
Bed nucleus stria preoptic nucl.	7.4 ± 0.3	9.7 ± 0.7*
Suprachiasmatic nucleus	7.7 ± 0.3	10.9 ± 0.6*
Bed nucleus stria terminalis	6.6 ± 0.4	8.6 ± 0.7*
Entopeduncular nucleus	6.2 ± 0.5	7.9 ± 0.7
Globus pallidus	6.2 ± 0.5	8.0 ± 0.7*
Subthalamic nucleus	7.5 ± 0.4	9.8 ± 0.8*
Amygdala basolateral/basomedial nucl.	6.6 ± 0.3	9.2 ± 1.0*
Substantia nigra		
Pars reticulata	8.5 ± 0.8	9.6 ± 0.7
Pars compacta	8.2 ± 0.8	7.9 ± 0.9
Septum		
Lateral septal nucleus	6.5 ± 0.3	8.1 ± 0.7
Medial septal nucleus	7.3 ± 0.4	10.0 ± 0.8*
Dorsal diagonal band	7.6 ± 0.5	9.9 ± 0.8*
Ventral diagonal band	7.6 ± 0.4	9.9 ± 0.7*
Hippocampal formation		
Ammon's horn CA1	9.2 ± 0.3	12.1 ± 1.0*
Ammon's horn CA2	8.5 ± 0.4	12.3 ± 1.3*
Ammon's horn CA3	8.7 ± 0.5	12.3 ± 1.0**
Dentate gyrus	9.9 ± 1.2	11.6 ± 1.2
Thalamus and related areas		
Paratenial nuclei	8.2 ± 0.3	11.2 ± 1.1*
Anteroventral nuclei	10.2 ± 0.4	13.0 ± 0.8**
Anteromedial nuclei	8.5 ± 0.3	10.9 ± 0.7*
Reticular nuclei	8.4 ± 0.5	10.8 ± 0.9*
Paraventricular nuclei	7.3 ± 0.6	10.0 ± 0.6**
Ventroposterior medial nucleus	7.9 ± 0.3	10.3 ± 0.8*
Ventroposterior lateral	7.7 ± 0.3	9.6 ± 0.8
Lateral habenular nucleus	8.6 ± 0.3	11.9 ± 1.2*
Medial habenular nucleus	9.3 ± 0.4	12.5 ± 1.2*
Medial geniculate nucleus	10.4 ± 1.0	11.3 ± 1.2
Dorsolateral geniculate nucleus	8.4 ± 0.4	10.9 ± 0.8*
Parafascicular nucleus	7.84 ± 0.30	10.3 ± 0.75*
Inferior colliculus	11.9 ± 0.97	12.9 ± 0.99
Superior colliculus	11.7 ± 1.9	12.4 ± 1.5
Hypothalamus		
Supraoptic nucleus	13.0 ± 2.5	16.1 ± 2.8
Subfornical organ	9.1 ± 0.6	12.9 ± 1.3*
Lateral nuclei	6.7 ± 0.5	8.7 ± 0.7*
Anterior nuclei	6.8 ± 0.8	9.3 ± 0.9*
Periventricular nucleus	8.0 ± 0.5	9.2 ± 0.8
Arcuate nucleus	6.8 ± 0.4	8.2 ± 0.8*
Ventromedial nucleus	6.5 ± 0.4	8.9 ± 0.9*
Posterior nucleus	7.6 ± 0.3	10.1 ± 0.8*
Medial forebrain bundle	6.8 ± 0.4	9.2 ± 0.8
Mammillary body	7.4 ± 0.4	10.7 ± 1.0*
Median eminence	14.0 ± 2.5	13.5 ± 2.6
Brainstem and spinal cord		
Raphe magnus nuclei	7.5 ± 0.6	8.9 ± 1.2
Raphe pallidus nuclei	8.5 ± 0.7	9.6 ± 1.4
Raphe median nuclei	8.5 ± 0.8	10.2 ± 1.2
Raphe dorsal nuclei	9.1 ± 0.7	10.4 ± 0.8
Locus coeruleus	9.9 ± 1.0	10.5 ± 0.7
Cochlear nucleus	14.0 ± 2.5	16.7 ± 3.8
Vestibular nucleus (medial)	11.7 ± 0.8	13.8 ± 1.2
Pretectal area	12.6 ± 1.5	15.1 ± 2.0
Pedunculopontine nucleus	7.2 ± 0.7	8.2 ± 0.8
Deep layers of superior colliculus	13.0 ± 1.8	14.8 ± 1.6
Interpeduncular nucleus	10.3 ± 0.8	18.0 ± 6.1
Spinal Tract V nucleus	8.3 ± 0.8	8.8 ± 0.7
Cerebellum		
Cerebellar gray matter	10.4 ± 0.9	11.5 ± 0.8
Molecular layer, gray matter	10.7 ± 0.9	11.9 ± 0.8
Granular layer, gray matter	11.9 ± 0.7	14.3 ± 1.2
Flocculus	10.3 ± 0.7	12.3 ± 1.3
Cerebellar white matter	5.3 ± 0.6	5.5 ± 0.3
Choroid plexus	46.9 ± 3.1	66.6 ± 5.3**

k^* values are means ± SEM ($n = 8$). k^* in rats given fluoxetine (10 mg/kg i.p.) was compared with k^* in rats given i.p. saline (controls).
Mean significantly different from control mean, *$p < 0.05$; **$p < 0.01$
[a]From Paxinos (1987).

receptors demonstrated significant increases in k^* for [^{3}H]arachidonate in response to fluoxetine (Table 2), exceptions were evident. High densities of the serotonin reuptake transporter have been noted in the olfactory tubercle, lateral septal nucleus, olfactory tubercle, lateral septal nucleus, hypothalamic and thalamic nuclei, globus pallidus, central gray, superior colliculus, substantia nigra, interpeduncular nucleus, dorsal and lateral raphé, locus coeruleus. Lesser but nevertheless high densities are present in the frontal cortex, caudate-putamen, ventral pallidum, and hippocampus (Choi *et al*, 2000; De Souza and Kuyatt, 1987; McReynolds and Meyer, 1998; Moll *et al*, 2000). k^* was elevated significantly in many of these regions, but not in all of them (eg lateral septal nucleus, superior colliculus, median, and dorsal raphé). High 5-HT$_2$ receptor densities are reported in the cerebral cortex (particularly layer IV),

olfactory and pyriform cortex, nucleus accumbens, caudate-putamen body and tail (Appel *et al*, 1990; Li *et al*, 2001; Pazos and Palacios, 1985). Frontal and motor cortical regions have higher densities than other cortical regions. Densities are not as high in the caudate-putamen head, globus pallidus, red nuclei, septal nuclei, and most parts of the hippocampus, thalamus, and hypothalamus. Spinal cord, brain stem, and cerebellum have few transporter or 5-$HT_{2A/2C}$ receptor sites, and as expected k^* was not elevated significantly in these areas.

Increases in k^* may not occur at all brain sites containing the serotonin reuptake transporter because transporter sites and postsynaptic 5-$HT_{2A/2C}$ receptors are not always colocalized. The transporter can be found at axons distant from 5-HT_2 synapses (Zhou *et al*, 1998), and some 5-HT_2 synapses may lack the transporter altogether (Brown and Molliver, 2000). Additionally, fluoxetine can increase brain dopamine levels (Yoshino *et al*, 2002) to activate PLA_2 at dopaminergic D_2 receptor sites rather than at 5-$HT_{2A/2C}$ receptor sites (Hayakawa *et al*, 2001; Vial and Piomelli, 1995). Finally, fluoxetine can noncompetitively inhibit cholinergic muscarinic (Owens *et al*, 1997) and nicotinic receptors (Fryer and Lukas, 1999) and thereby reduce release of arachidonic acid.

Indeed, PLA_2 can be activated via a G-protein when an agonist binds to cholinergic muscarinic receptors and dopaminergic D_2 receptors as well as to 5-$HT_{2A/2C}$ receptors (Bayon *et al*, 1997; Cooper *et al*, 1996; DeGeorge *et al*, 1991; Felder *et al*, 1990; Hayakawa *et al*, 2001; Kim *et al*, 1999; Vial *et al*, 1995). PLA_2 also may be activated by Ca^{2+} entry into cells when glutamate acts at NMDA receptors or acetylcholine acts at nicotinic receptors. Thus, baseline k^* for [^{3}H]arachidonate in control rats represents the sum of baseline activation of PLA_2 via these different receptor subtypes, as well as incorporation because of membrane synthesis (particularly relevant for white matter myelin) (Rapoport *et al*, 1997).

A 10-fold higher baseline value of k^* for [^{3}H]arachidonate in the choroid plexus, as well as a significant increment in k^* in response to DOI or fluoxetine (Table 2) are consistent with high densities of 5-HT_{2C}-binding sites in the choroid plexus (Kaufman *et al*, 1995; Li *et al*, 2001; Qu *et al*, 2003) and with activation of PLA_2 via these receptors. Fluoxetine has been reported to bind to 5-HT_{2C} sites in the choroid plexus (Palvimaki *et al*, 1996). The high baseline k^* in the choroid plexus is consistent with high rates of uptake of radiolabeled fatty acids into median eminence, subfornical organ, pineal gland, adenohypophysis, and neurohypophysis (Noronha *et al*, 1990; Noronha *et al*, 1989; Qu *et al*, 2003). These regions lack the continuous capillary bed of the blood–brain barrier (Rapoport, 1976), allowing access of labeled fatty acid both in its unbound unesterified form (as for the brain parenchyma), and when bound to albumin (Robinson *et al*, 1992). High values of k^* for labeled arachidonate also have been noted in the heart (Murphy *et al*, 2000).

Fluoxetine can induce hyperthermia in rats (Lin *et al*, 1998). The activity of PLA_2 as well that of other enzymes might be expected to increase with an increase in temperature (Bell *et al*, 1996). However, it is unlikely that hyperthermia accounted for the findings in this paper, as increased values for k^* were not found in regions having low densities of the serotonin reuptake transporter, and because body temperature was maintained between 36 and 37°C in this study (see Materials and methods). Additionally hyperthermia has been shown to produce widespread increases in $rCMR_{glc}$ in awake rats (Mickley *et al*, 1997), whereas fluoxetine, when administered under the same conditions in our study, has been shown either to not change or to reduce $rCMR_{glc}$ (Freo *et al*, 2000).

Serotonin release from presynaptic elements can be controlled by two types of autoreceptors (Cerrito and Raiteri, 1980). 5-HT_{1A} autoreceptors are found at the somatodendritic region of cell bodies in midline and raphé nuclei in the pons and upper brain stem, whereas 5-HT_{1B} autoreceptors are located at axon terminals, where they control local 5-HT synthesis and release (Cooper *et al*, 1996; Hervas *et al*, 2000; Martin and Sanders-Bush, 1982). Acutely administered fluoxetine has been reported to inhibit electrical activity of serotonin neurons by activating somatodendritic 5-HT_{1A} autoreceptors (Czachura and Rasmussen, 2000; Fuller, 1995), thus reducing 5-HT released into the synaptic cleft. With chronic fluoxetine, inhibition of 5-HT unit activity is less because of desensitization of the 5-HT_{1A} autoreceptor. Desensitization and recovery of firing of 5-HT neurons develop slowly and concurrently, and may contribute to the delayed onset of therapeutic efficacy (Bergqvist *et al*, 1999; Blier and De Montigny, 1983).

The widespread increments in k^* for [^{3}H]arachidonate in response to acute fluoxetine (Table 2) contrast with reports of no change or decrements in $rCMR_{glc}$ under comparable experimental conditions (Freo *et al*, 2000). A similar discrepancy between increased radiolabeled arachidonate incorporation but decreased or no change in $rCMR_{glc}$ follows DOI or methiothepin administration to unanesthetized rats (Freo *et al*, 1991; Qu *et al*, 2001b; Ricchieri *et al*, 1987). These discrepancs likely arise because [^{3}H]arachidonate incorporation localizes the *postsynaptic* PLA_2-mediated release of arachidonic acid at the serotonergic neuron, whereas $rCMR_{glc}$ represents ATP consumption by the downstream firing of *presynaptic* axon terminals of that neuron (Ashby *et al*, 1990; Purdon and Rapoport, 1998; Qu *et al*, 2003; Sokoloff, 1999).

The frontal cortex and hippocampus have been implicated as sites of action of antidepressant drugs (Duman *et al*, 1997; Jacobson and Sapolsky, 1991). In this study (Table 2), incorporation coefficients k^* for [^{3}H]arachidonate were increased by fluoxetine in these regions, suggesting that to signaling via PLA_2 at these sites contributes to fluoxetine's antidepressant action. In major depression, serotonergic neurotransmission may be disturbed and processes of cellular immunity may be activated, suggesting a relation between the two processes. Indeed, there appears to be a constant 'crosstalk' between the immune endocrine, central, and peripheral nervous systems which can involve serotonin and arachidonic acid metabolites (eg prostaglandins) (Leonard, 2001; Maier and Watkins, 1998).

In conclusion, we have demonstrated in unanesthetized rats that acute fluoxetine activates PLA_2 signaling and increases labeled arachidonate incorporation from plasma into brain regions having high serotonin reuptake transporter densities, with some exceptions. Increased tracer incorporation likely arises because fluoxetine indirectly

increases binding of 5-HT to PLA_2-coupled 5-$HT_{2A/2C}$ receptors. Identifying the exact mechanisms of fluoxetine's acute action will entail using specific receptor antagonists with it (Hayakawa *et al*, 2001; Mazzola-Pomietto *et al*, 1997; Rabiner *et al*, 2002), or studying 5-HT_{2A} or 5-HT_{2C} knockout mice (Lira *et al*, 2001).

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