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Protein kinase C in rat brain cortex and hippocampus: effect of repeated administration of fluoxetine and desipramine

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1 Recent evidence indicates that changes in the activity of cyclic AMP-dependent protein kinase may be involved in neuroadaptive mechanisms after chronic treatment with antidepressants. The aim of this study was to investigate the effect of repeated administration of fluoxetine (FL) and desipramine (DMI) on the distribution and activity of protein kinase C (PKC) in subcellular fractions of rat cortex (Cx) and hippocampus (Hc) under basal conditions and in response to a single *in vivo* administration of 5-$HT_{2A/2C}$ agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI).

2 Rats were treated for 21 days with FL (5 mg kg^{-1} day^{-1}, i.p.) or DMI (10 mg kg^{-1} day^{-1}, i.p.). DOI was injected to groups of rats receiving repeated doses of antidepressants or to control rats 1 h before *ex vivo* PKC assay. Distribution of PKC was determined by [^{3}H]-phorbol-12,13-dibutyrate ([^{3}H]-PDBu) binding and PKC activity by the Amersham enzyme assay system.

3 Autoradiography of tissue sections revealed decreased [^{3}H]-PDBu binding in CA_1 region of hippocampus (by 18%) and paraventricular thalamic nucleus (by 28%) of rats after repeated administration of FL.

4 *In vitro* exposure of brain sections to 50 μM FL resulted in significant decreases (by 23-32%) of [^{3}H]-PDBu binding in six out of seven regions examined; exposure to 100 μM FL reduced [^{3}H]-PDBu binding (by 36-52%) in all regions. In contrast, exposure of brain sections to 100 μM DMI failed to alter specific [^{3}H]-PDBu binding in brain sections.

5 The activity of PKC in subcellular fractions of Cx and Hc was significantly (by 40-50%) decreased in rats given repeated doses of FL or DMI. A single administration of either drug was without effect.

6 A single *in vivo* administration of DOI to control rats resulted in reduced PKC activity (by 30-40%) in the particulate fraction of both Cx and Hc. This response to DOI was similar in DMI-treated rats but was not seen in rats given repeated doses of FL. A single administration of DOI to animals given repeated doses of FL resulted in PKC activities higher than those seen in rats treated with FL alone.

7 The results indicate that repeated administration of FL and DMI produced similar changes in basal PKC activity but differentially affected the PKC response to the 5-$HT_{2A/2C}$ receptor agonist, DOI. The effect on basal PKC activity may result from a post-receptor action of antidepressants; the alteration of PKC response to DOI after fluoxetine could be due to receptor-mediated desensitization of the signalling system.

Keywords: Protein kinase C; fluoxetine; desipramine; rat brain; 5-$HT_{2A/2C}$ receptor; [^{3}H]-PDBu binding

Introduction

Fluoxetine is a selective 5-hydroxytryptamine(5-HT) reuptake inhibitor widely used as an effective antidepressant drug which, in contrast to tricyclic antidepressants, lacks significant affinity to various neurotransmitter receptors and does not have significant sedative, anticholinoceptor and/or cardiovascular effects (Beasley *et al.*, 1992). Most antidepressant drugs ultimately affect the 5-hydroxytryptaminergic transmission by their important effects on several processes in 5-hydroxytryptaminergic neurones, including neuronal activity, synthesis, enzymatic degradation, release and reuptake of 5-HT. Chronic treatment with some antidepressants induces homologous desensitization of 5-HT-stimulated phosphoinositide hydrolysis as well as simultaneous downregulation of 5-HT_{2A} sites (Peroutka & Snyder, 1980; Kendall & Nahorski, 1985; Conn & Sanders-Bush, 1987; Sanders-Bush *et al.*, 1989). However, the new selective 5-HT uptake inhibitors such as fluoxetine, fluvoxamine and citalopram have not been shown to alter consistently the number of 5-HT_{2A} receptors (Sanders-Bush *et al.*, 1989). Our earlier autoradiographic study (Hrdina & Vu, 1993) has shown that repeated treatment of rats with fluoxetine increases the density of 5-HT_{2A} receptors in several brain regions. Changes in 5-HT_{2A} receptor density has not always been correlated with changes in phosphoinositide turnover after chronic antidepressant treatment. For example, sertraline, a potent 5-HT uptake inhibitor, causes no down-regulation of 5-HT_{2A} receptors and yet produces a decrease in [^{3}H]-inositol phosphate formation upon stimulation with 5-HT (Sanders-Bush *et al.*, 1989).

Since receptor changes represent only the first step in the action of antidepressants, recent studies have been focused on neuronal signal transduction processes beyond the receptor level as potential targets for the action of antidepressants. Recagni *et al.* (1992) has shown that chronic treatment with desipramine and fluoxetine alters the adenosine 3':5'-cyclic monophosphate (cyclic AMP) dependent phosphorylation system in rat brain associated with the microtuble fraction and suggested that this system could represent an intracellular target involved in the biochemical mechanism of action of antidepressant drugs. Protein kinase C (PKC) is another pivotal enzyme in phosphorylation of cellular proteins and its activity has been associated with regulation of 5-HT receptor-triggered signals (e.g. 5-$HT_{2A/2C}$), neurotransmitter release and neuronal plasticity (Nishizuka, 1988; Wang & Friedman, 1990). Recently, imipramine was reported to prevent *in vitro* the inhibitory effect of the PKC activator, phorbol ester, on noradrenaline (NA)-induced accumulation of inositide phos-

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phates in brain slices (Nalepa & Vetulani, 1991). The role of PKC in the chronic effects of antidepressant drugs has not yet been studied. The aim of the present experiments was to investigate the effect of repeated administration of antidepressants on PKC localization and activity, and on PKC responses to 5-HT receptor stimulation by a 5-$HT_{2A/2C}$ receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (Appel *et al.*, 1990) in subcellular fractions of rat brain tissue.

Methods

Treatments

Male Sprague-Dawley rats, weighing 150 to 175 g, were injected i.p. daily for 21 days with fluoxetine (5 mg kg^{-1}), desipramine (10 mg kg^{-1}) or the equivalent volume of 0.9% saline as a vehicle. Animals were killed 48 h later in the case of fluoxetine or 24 h later in the case of desipramine. The time periods between the last daily dose of drugs and death were chosen to minimize residual drug interference with the assays, and were based on the difference in plasma half-lives between the two drugs. Other groups of animals received a single dose of fluoxetine or desipramine and were used for PKC activity determination 48 h or 24 h later. In experiments in which the PKC responses to 5-$HT_{2A/2C}$ receptor stimulation *in vivo* were studied, the 5-$HT_{2A/2C}$ agonist, DOI was injected i.p. in a dose of 10 mg kg^{-1} 1 h before the *ex vivo* PKC assay. Control rats were treated with corresponding volumes (2.5 ml kg^{-1}) of saline solution.

[^{3}H]-phorbol-12,13-dibutyrate ([^{3}H]-PDBu) binding

[^{3}H]-PDBu binding in homogenates was determined as described by Horsburgh *et al.* (1991). Rats were killed by decapitation, their brains dissected out and quickly removed. Cortical or hippocampal tissue was homogenized in 10 volumes of buffer (0.32 M sucrose, 5 mM benzamidine, 2 mM dithiothreitol, 3 mM EGTA, 0.5 mM $MgSO_4$, 0.5 mM $ZnSO_4$, 0.1 mM phenylmethylsulphonylfluoride, 0.1 mg ml^{-1} leupeptin, 0.05 mg ml^{-1} pepstatin and 0.1 mg ml^{-1} aprotinin) for 10 s in a Polytron (setting 6). Homogenates were then centrifuged at 10,000 *g* and 4°C for 8 min to precipitate nuclei and cytoskeleton. The resulting supernatant was then centrifuged at 100,000 *g* at 4°C for 1 h. The resulting pellet, resuspended in the original volume of buffer, and supernatant constituted the particulate and soluble fractions of the tissue, respectively. Assay tubes (final volume of 1 ml) contained the incubation buffer (50 mM Tris-HCl, 10 mM $(CH_3COO)_2Mg.4H_2O$, 1.4 mM $CaCl_2$, 0.4 mM EGTA, 50 mM KCl, 4 mg ml^{-1} bovine serum albumin (BSA) and 100 μl ml^{-1} phosphatidylserine, pH 7.5) without or with 2 μM cold PDBu (for determination of total and non-specific binding, respectively) and 2.5 nM [^{3}H]-PDBu. Incubation (in triplicate) was started by the addition of 20 μl homogenate, continued at 4°C for 2 h, and was terminated by the addition of 5 ml ice-cold buffer (20 mM Tris-HCl, 1 mM $CaCl_2$ and 10 mM $(CH_3COO)_2Mg.H_2O$, pH 7.5), followed by subsequent filtration through Whatman GF/B filter discs (dipped in ice-cold 20 mM Tris-HCl buffer containing 0.1% polyethyleneimine) and 4 additional washes. The filters were dried and the retained radioactivity was determined by liquid scintillation spectrometry. The specific binding of [^{3}H]-PDBu accounted for 80–95% of total binding.

[^{3}H]-PDBu binding in brain sections was determined by quantitative autoradiography as described by Worley *et al.* (1986). Coronal brain sections (15 μm thick) were prepared as described earlier (Hrdina *et al.*, 1990) and were incubated for 1 h at 23°C in a buffer (50 mM Tris-HCl, 1 mM $CaCl_2$ and 100 mM NaCl; pH 7.7) containing 2.5 nM [^{3}H]-PDBu. To determine the non-specific binding, adjacent sections were incubated in the presence of 1 μM cold PDBu. Specific binding in sections accounted for 80–90% of total binding. For autoradiography, the sections were dried, apposed to tritium-sensitive Hyperfilm (Amersham, Des Plaines, IL, U.S.A.) and kept at 4°C for 4 days. Every tenth section was stained with cresyl violet to facilitate the identification of brain structures by the atlas of Paxinos & Watson (1986). Autoradiograms were quantified by use of a Microcomputer Imaging Device (Hrdina *et al.*, 1990).

Protein kinase C activity

PKC activity in subcellular tissue fractions was measured with the Amersham enzyme assay system. A PKC-specific target peptide and all necessary co-factors were provided in the kit. Soluble and particulate fractions of cortical or hippocampal tissues were prepared as described above for [^{3}H]-PDBu binding. Soluble fractions were subsequently diluted in 8 volumes and particulate fractions in 4 volumes of homogenate buffer. Assay tubes (with final incubation volumes of 75 μl) contained 25 μl of component mixture (3 mM $Ca(C_2H_3O_2)_2$, 2 mol% L-α-phosphatidyl-L-serine, 6 μg ml^{-1} phorbol 12-myristate 13-acetate, 225 μM peptide and 7.5 mM dithiothreitol in 50 mM Tris-HCl containing 0.05 volumes sodium azide, pH 7.5) and 25 μl homogenate (blank tubes representing non-specific phosphorylation contained 25 μl homogenate buffer instead). The reaction was initiated by the addition of 25 μl of Mg-ATP buffer (10 μCi ml^{-1} [^{32}P]-ATP, 150 μM ATP and 45 mM $(CH_3COO)_2Mg.4H_2O$ in 50 mM Tris-HCl containing 0.05 volumes sodium azide, pH 7.5) to each tube. Incubation proceeded for 15 min at room temperature (25°C) and was terminated by the addition of 100 μl 'stop' reagent (dilute acidic reaction-quenching reagent) to each tube. An aliquot of solution from each tube (125 μl) was blotted onto individual peptide-binding papers which were then placed in a 5% acetic acid bath (10 ml/paper) for 10 min at room temperature. This solution was then decanted and replaced with fresh 5% acetic acid for a second 10 min wash. Papers were dried and the retained radioactivity determined by liquid scintillation spectrometry. The number of pmol phosphate transferred per minute by PKC to the PKC-specific peptide substrate was calculated. The inter-assay coefficient of variation was 19% ($n=14$) for PKC determination in soluble fraction from cortex of control animals. The design of experiments allowed for parallel measurements of samples from control and treated animals.

Protein content

In order to avoid interference of reagents present in the homogenate buffer with the protein assay, a modified version of the Lowry protein assay (1951) was used. Aliquots of homogenate were made up to a volume of 950 μl with distilled water and 50 μl of absolute trichloroacetic acid was added to each tube to precipitate proteins. The tubes were then centrifuged for 5 min. The resulting supernatants were aspirated and discarded, and the resulting pellets were resuspended in 200 μl of 0.1 M NaOH. The method of Lowry *et al.* (1951) was subsequently used to determine the protein content of each tube.

Drugs used

Labelled phorbol-12,13-dibutyrate ([^{3}H]-PDBu; specific activity, 18.6 Ci $mmol^{-1}$) and [^{32}P]-ATP tetra(triethylammonium) salt (specific activity, 3000 Ci $mmol^{-1}$) were purchased from New England Nuclear (Boston, MA, U.S.A.). PDBu was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), (±)-DOI hydrochloride from Research Biochemicals Inc. (Natick, MA, U.S.A.) and the protein kinase C enzyme assay system from Amersham (Des Plaines, IL, U.S.A.). Fluoxetine hydrochloride was generously donated by Eli Lilly & Co. (Indianapolis, IN, U.S.A.) and desipramine hydrochloride by Ciba-Geigy Canada Ltd. Other chemicals used were of purest grade available.

Statistics

Data are expressed as mean ± s.e.mean. Two-way ANOVA with *post hoc* Neuman-Keuls test for group differences and Student's *t* test (two-tailed) were used to determine the statistical difference between the various means.

Results

PKC activity

The mean activity of PKC in the soluble and particulate fractions from cerebral cortex and hippocampus of control rats (Table 1) and the relative distribution of activity between the two fractions observed in our study are similar to those reported by Wieloch *et al.* (1991). Two-way ANOVA with acute DOI and repeated administration of antidepressants as the two factors showed a highly significant effect of antidepressants on PKC activity in both fractions of cortex and hippocampus ($F=14.3$ and $F=11.7$, $P<0.0001$, in soluble and particulate fractions, respectively of cortex; $F=7.15$, $P<0.003$ and $F=12.0$, $P<0.0001$ for the respective fractions from hippocampus). There was also a highly significant interaction between single DOI and repeated antidepressant administration on PKC activity in both cortical fractions ($F=18.8$ and $F=11.8$, $P<0.0001$ for soluble and particulate fraction, respectively) as well as in the hippocampus ($F=7.15$, $P<0.003$ and $F=27.3$, $P<0.0001$ for the respective fractions). Repeated administration of fluoxetine produced significant decreases in basal PKC activity in both soluble and particulate fraction of the cerebral cortex and hippocampus (Figures 1 and 2). In comparison, repeated desipramine administration resulted in significant reduction of PKC activity in both fractions from cortex but only in particulate fraction from the hippocampus. Single administration of either fluoxetine or desipramine had no significant effect on PKC activity in subcellular fractions from cortex or hippocampus (Table 1). A single injection of the 5-$HT_{2A/2C}$ agonist, DOI (10 mg kg^{-1}, i.p.) resulted in a significant reduction of cytosolic PKC activity in cortex (by 40%) but not in hippocampus. Unexpectedly, the PKC activity in particulate fractions from both cortex and hippocampus was found to be significantly decreased (by about 60%) 1 h after DOI injection (Figures 1 and 2). The PKC response to DOI challenge was similar, even more pronounced, in rats given repeated doses of desipramine and given DOI 24 h after the last injection of the antidepressant (Figures 1 and 2). However, in animals treated with repeated doses of fluoxetine, a single injection of DOI did not produce the suppression of PKC activity in soluble fraction of cortex seen with DOI alone, and the reduction of PKC activity in particulate fraction of both cortex and hippocampus was reversed (Figures 1 and 2). In fact, PKC activity in response to DOI was higher in these animals than in those receiving only fluoxetine.

Table 1 Effect of a single administration of fluoxetine and desipramine (DMI) on basal protein kinase C (PKC) activity in rat cerebral cortex and hippocampus

Region / *treatment* / *Cerebral cortex*	*PKC activity* (nmol min^{-1} mg^{-1} protein) *Soluble*	*Particulate*
Control	3.05 ± 0.17	1.06 ± 0.05
Fluoxetine	2.70 ± 0.11	0.71 ± 0.12
DMI	3.00 ± 0.49	0.92 ± 0.18
Hippocampus		
Control	3.73 ± 0.17	1.11 ± 0.10
Fluoxetine	4.05 ± 0.09	0.96 ± 0.06
DMI	3.32 ± 0.21	0.85 ± 0.01

The values are the means ± s.e.mean from four animals in each group. Fluoxetine (5 mg kg^{-1}, i.p.) and desipramine (10 mg kg^{-1}, i.p.) were given in a single dose and *ex vivo* PKC assay (in duplicate) was done 1 h later. No statistically significant differences between the groups were found by ANOVA.

[^{3}H]-PDBu binding

Repeated administration of fluoxetine failed to produce significant changes in [^{3}H]-PDBu binding in subcellular fractions from rat cerebral cortex homogenate (Table 2). The

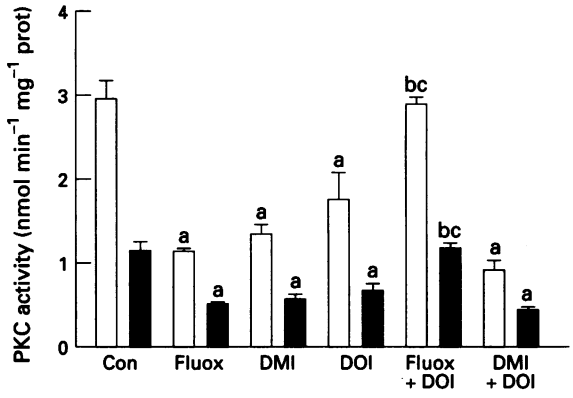

Figure 1 Effect of repeated administration of fluoxetine (Fluox; 5 mg kg^{-1}, i.p. for 21 days) and desipramine (DMI; 10 mg kg^{-1}, i.p. for 21 days) on basal protein kinase C (PKC) activity and PKC responses to a single injection of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 10 mg kg^{-1}, i.p.) in soluble (open columns) and particulate (solid columns) fractions of rat brain cortex. Each column represents the mean value ± s.e.mean from 10 animals in the control group (Con), 9 animals in the DOI group, 5 animals in the Fluox, DMI and DMIO + DOI groups and 4 animals in the Fluox + DOI group. Assays were performed in duplicate. Results were analysed by two-way ANOVA (with single DOI and repeated antidepressant drug administration as the two factors) and by *post-hoc* Neuman-Keuls test for group differences. $^aP<0.01$ vs Con; $^bP<0.01$ vs DOI; $^cP<0.01$ vs Fluox.

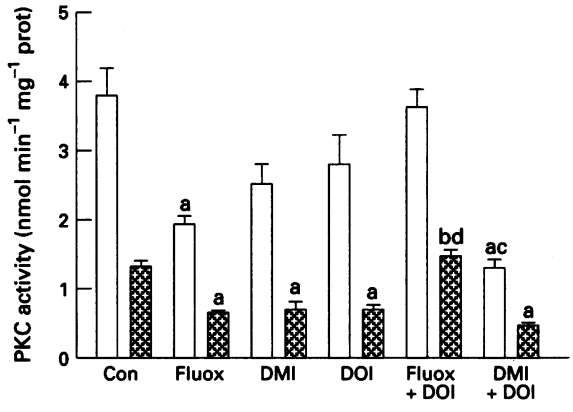

Figure 2 Effect of repeated administration of fluoxetine (Fluox; 5 mg kg^{-1}, i.p. for 21 days) and desipramine (DMI; 10 mg kg^{-1}, i.p. for 21 days) on basal protein kinase C (PKC) activity and PKC responses to a single injection of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 10 mg kg^{-1}, i.p.) in soluble (open columns) and particulate (cross-hatched columns) fractions of rat hippocampus. Each column represents the mean value + s.e.mean from 10 animals in the control group (Con), 9 animals in the DOI group, 5 animals in the Fluox, DMI and DMI + DOI groups and 4 animals in the Fluox + DOI group. Assays were performed in duplicate. Results were analysed by two-way ANOVA (with single DOI and repeated antidepressant drug administration as the two factors) and by *post-hoc* Neuman-Keuls test for group differences. $^aP<0.01$ vs Con; $^bP<0.01$ vs DOI; $^cP<0.05$ vs DOI; $^dP<0.01$ vs Fluox.

autoradiography of [^{3}H]-PDBu binding in rat brain sections has shown a differential distribution of the label among the 7 regions analyzed with highest concentration in CA_{2-3} region of hippocampus and lowest in thalamic nuclei (Table 3). In rats chronically treated with fluoxetine, the [^{3}H]-PDBu binding which reflects the amount of membrane bound enzyme (Worley *et al.*, 1986) was significantly decreased in CA_1 region of hippocampus (by 18%) and paraventricular thalamic nucleus (by 28%; Table 3). *In vitro* exposure of brain sections to 10 μM fluoxetine failed to alter significantly the distribution of [^{3}H]-PDBu binding in the regions examined. However, a higher concentration of the drug (50 μM) produced significant decreases in [^{3}H]-PDBu binding (by 23 to 32%) in all but one region and the highest concentration (100 μM) of fluoxetine markedly reduced (by 36–52%) [^{3}H]-PDBu binding in all regions examined (Table 3). In contrast, exposure of brain sections to 100 μM DMI did not alter the relative amount of specific [^{3}H]-PDBu binding to brain sections (93.7 ± 0.7% vs 94.3 ± 0.3% in controls, $n = 3$).

Discussion

One of the main findings of this study was that repeated administration of fluoxetine, a selective 5-HT uptake inhibitor significantly suppressed the basal activity of PKC in subcellular fractions from rat brain cortex and hippocampus, and significantly altered the PKC response to a 5-$HT_{2A/2C}$ agonist, DOI. The effect of fluoxetine on basal PKC activity could be a part of neuroadaptive changes after repeated administration of this antidepressant since it was not seen after a single dose of the drug. Regulation of 5-$HT_{2A/2C}$ receptor-triggered signalling is associated with PI turnover and the PKC activity (Conn & Sanders-Bush, 1987; Nishizuka, 1988). The decrease in PKC activity observed in the present study could therefore be a result of receptor-mediated downregulation of signalling mechanisms after prolonged exposure of 5-$HT_{2A/2C}$ receptors to increased concentrations of 5-HT subsequent to the inhibition of its neuronal reuptake by fluoxetine. However, the observation that chronic treatment with desipramine which does not appreciably block 5-HT uptake resulted in similar changes in PKC activity would argue against this possibility. On the other hand, desipramine has been reported to downregulate 5-HT_{2A} receptors (Peroutka & Snyder, 1980) and thus the reduction of basal PKC activity and PKC response to DOI after repeated DMI administration could be related to this effect.

The two antidepressant drugs may affect PKC activity by a direct post-receptor action. This notion is supported by our finding that both fluoxetine and desipramine showed similar effects, by a demonstrated inhibitory effect in fluoxetine on Ca^{2+}- and calmodulin-regulated protein kinase system (Silver *et al.*, 1986) and by the finding of an enhanced activity of cyclic AMP-dependent phosphorylation system associated with cerebrocortical microtubule fraction after chronic treatment with desipramine (Perez *et al.*, 1989). It is conceivable that both major phosphorylation systems (PKA- and PKC-linked) are intracellular targets involved in the biochemical mechanism of action of antidepressants drugs.

The reason for the decreases in both the cytosolic and particulate PKC activity after repeated administration of antidepressants is not immediately apparent. The possibilities include a suppression of enzyme synthesis, increased turnover with subsequent degradation, sequestration by endosomes or partial translocation to the nucleus as shown for PKA after chronic imipramine treatment (Nestler *et al.*, 1989). However, a direct effect of the residual drug on PKC cannot be excluded, particularly in the case of fluoxetine which is known to accumulate significantly in cerebral cortex and hippocampus after repeated administration (Caccia *et al.*, 1992). In fact, as shown by these authors, hippocampal fluoxetine levels measured in rats 24 h after 21 day administration at 15 mg kg^{-1} twice daily were 91 nmol g^{-1} and one week later the active metabolite, norfluoxetine was still present at 109 nmol g^{-1}. It is therefore likely that in our experiments fluoxetine was still present in brain in significant concentrations 48 h after the last dose. Due to its long elimination half-life (which is even longer for its

Table 2 Effect of repeated fluoxetine and desipramine (DMI) administration on [^{3}H]-phorbol-12, 13-dibutyrate ([^{3}H]-PDBu) binding in subcellular fractions from rat cortex homogenate

	[^{3}H]-PDBu binding (pmol mg^{-1} protein)	
Treatment	*Soluble*	*Particulate*
Control	57.7 ± 1.3	13.6 ± 1.5
Fluoxetine	58.6 ± 1.2	11.6 ± 0.4
DMI	60.4 ± 1.2	11.6 ± 1.4

Fluoxetine (5 mg kg^{-1}) and desipramine (10 mg kg^{-1}) were given i.p. for 21 days and [^{3}H]-PDBu binding (in triplicate, at 2.5 nM concentration) was determined 48 h and 24 h, respectively after the last dose in subcellular fractions of homogenate from cerebral cortex. $n = 4$ for each group. No significant difference between the groups was found by ANOVA.

Table 3 [^{3}H]-phorbol-12, 13-dibutyrate ([^{3}H]-PDBu) binding in rat brain sections from control and fluoxetine-treated rats and after *in vitro* exposure to fluoxetine

	[^{3}H]-PDBu binding (pmol mg^{-1} protein)				
		Chronic	In vitro *Fluoxetine*		
Region	*Control*	*fluoxetine*	10 μM	50 μM	100 μM
Cortex					
Layer 1-3	17.1 ± 1.6	14.0 ± 0.6	21.3 ± 0.8	13.1 ± 0.5*	7.7 ± 0.7**
Hippocampus					
CA1	19.0 ± 1.1	15.7 ± 0.8*	19.9 ± 1.5	16.7 ± 1.7	12.2 ± 1.5**
CA2-3	22.2 ± 1.4	18.9 ± 0.8	21.6 ± 1.0	15.7 ± 1.1**	11.4 ± 1.2**
Dentate gyrus	14.1 ± 1.4	11.0 ± 0.7	17.4 ± 0.9	10.7 ± 0.8*	6.8 ± 0.5**
PV med.thalamic n.	10.3 ± 0.9	7.4 ± 0.5*	11.3 ± 0.4	7.4 ± 1.0*	5.9 ± 0.7**
Caudate-putamen	16.6 ± 1.6	13.6 ± 0.8	19.7 ± 1.0	11.4 ± 1.2**	8.6 ± 0.8**
Amygdala	18.3 ± 2.1	14.7 ± 0.6	19.4 ± 1.5	12.5 ± 1.9*	9.2 ± 1.1**

Rats were treated with fluoxetine (5 mg kg^{-1}, i.p. for 21 days) or saline (controls). Binding sites were labelled by incubating adjacent sections with 2.5 nM [^{3}H]-PDBu. Values are means ± s.e.mean of measurements from 4 rats in each group with three bilateral determinations made from each of 2–3 sections for each area in each brain. For determination of *in vitro* effect of fluoxetine, the drug was added in 10, 50 or 100 μM concentration to 2–3 adjacent sections from 4 control brains 15 min before exposure to [^{3}H]-PDBu. The anatomical terminology is derived from Paxinos & Watson (1986). *$P < 0.05$; **$P < 0.01$ when values were compared to corresponding values in control rats (Student's t test, two tailed).

metabolite, norfluoxetine) and high (94%) protein binding (Beasley *et al.*, 1992), a significant amount of drug could persist in tissue despite repeated washing. The above evidence and our finding of reduced [^{3}H]-PDBu binding after *in vitro* exposure of tissue sections to fluoxetine (similar in pattern to that seen after repeated administration of the drug) would indicate that residual drug present in the tissue after repeated treatment could have been responsible for the changes in PKC.

In vitro exposure of rat cortical slices to DOI was shown to translocate PKC activity from the cytosolic to membrane fraction (Wang & Friedman, 1990). This effect appears to be due to a selective 5-HT_{2A} receptor stimulation since it is prevented by 5-HT_{2A} receptor antagonists. We found that *in vivo* administration of DOI produced significant decrease of PKC activity in particulate fraction of cortical and hippocampal tissue. We have assayed PKC activity 1 h after DOI injection based on the time course of behavioural effects of DOI (Berendsen & Boekkamp, 1991) and on the fact that maximal translocation of PKC activity *in vitro* was only seen after 20 min of exposure to DOI (Wang & Friedman, 1990). We cannot exclude the possibility that an initial increase in particulate PKC activity after DOI injection did occur but might have terminated due to increased turnover rate and subsequent degradation of the activated enzyme, while the decrease in cytosolic PKC activity persisted. The decrease in membrane-bound PKC activity 1 h after DOI injection was not seen in rats chronically treated with fluoxetine. This might have been due to receptor-mediated desensitization of the signalling system involving PKC. Chronic block of 5-HT uptake by fluoxetine and prolonged availability of the transmitter for action at receptors could have resulted in decreased sensitivity to acute challenge such as DOI injection. Indeed, a substantial down-regulation (by 60–75%) of 5-HT_{2A} receptor number in rat cortex was observed after chronic treatment with the 5-$HT_{2A/2C}$ receptor agonist, DOI (Pranzatelli, 1991). The above suggestion is supported by our observation that no significant alteration of PKC response to DOI challenge was found in rats repeatedly treated with desipramine, a tricyclic antidepressant that does not inhibit 5-HT uptake to a significant degree.

Another possible explanation for the differential effect of fluoxetine and DMI on PKC responses to DOI administration is that fluoxetine may directly interfere with pharmacokinetics of DOI and/or its action at the receptor level. Fluoxetine is a potent inhibitor of cytochrome CPY2D6, a major enzyme catalysing the metabolism of several important drugs including tricyclic antidepressants (Brosen & Skjelbo, 1991). It is however, not known whether fluoxetine influences the disposition of DOI after *in vivo* administration. Furthermore, fluoxetine was found to have an appreciable affinity (pK_i of 6.57) for 5-HT_{2C} receptors in bovine choroid plexus (Wang *et al.*, 1991). It could thus compete with DOI at 5-HT_{2C} receptors and influence the activity of the PI-PKC signalling system in response to DOI, and also modify the basal activity of this system. However, it is not known whether fluoxetine has the same affinity for 5-HT_{2C} receptors in rat brain tissue as for those in bovine choroid plexus.

In conclusion, antidepressant drugs of different pharmacological profile, fluoxetin and desipramine, produce after repeated administration significant effects on PKC activity in subcellular fractions of cortical and hippocampal tissue and differentially affect PKC responses to 5-HT_{2A} receptor stimulation by *in vivo* DOI injection. This novel and possibly post-receptor action of these drugs may be part of an adaptive neuronal changes seen after repeated administration of antidepressants.

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