Published in final edited form as:

Pharmacol Biochem Behav. 2006 October ; 85(2): 441–447.

Regulation of 5-HT_{2A/C} Receptors and DOI-Induced Behaviors by Protein Kinase C γ

Barbara J Bowers^{a,*}, Jill Miyamoto-Ditmon^a, and Jeanne M Wehner^{a,b}

^a Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309

^b Department of Psychology, University of Colorado, Boulder, CO 80309

Abstract

Protein kinase C γ (PKC γ) null mutant mice demonstrate increased behavioral impulsivity and ethanol consumption. Pharmacological studies have shown that 5-HT_{2A/C} receptors modulate impulsivity and ethanol consumption in rodents and that PKC can regulate 5-HT_{2A/C} receptors. To determine whether PKC γ plays a selective role in 5-HT_{2A/C} receptor regulation, biochemical and behavioral experiments were performed in PKC γ mutant and wild-type mice. DOI-stimulated phosphoinositol hydrolysis and [¹²⁵I]-DOI saturation binding in the PFC, and quantitative autoradiography of [¹²⁵I]-DOI binding sites in 15 brain regions were analyzed. DOI-induced head twitch responses (HTR) were measured in naïve mice after an acute 2.5 mg/kg injection of DOI. Results indicated that DOI-induced HTR was significantly greater in mutant mice compared to wild-type mice. Results of the phosphoinositol hydrolysis, membrane binding, and autoradiography experiments indicated that in mutant mice, increased HTR was associated with increased 5-HT_{2A/C} receptor function in the PFC, but not increased receptor number or affinity suggesting that PKC γ regulates receptor function but not receptor number. These data support a role for 5-HT_{2A/C} receptors in the PFC in mediating some of the behavioral differences observed between PKC γ mutant and wild-type mice.

Keywords

PKC γ ; mice; phosphoinositol hydrolysis; quantitative autoradiography; head twitch response; DOI; 5-HT_{2A/C} receptors

1. INTRODUCTION

Null mutant mice lacking the neuronal-specific gamma isotype of protein kinase C (PKC γ) are more impulsive in an appetitive signaled-nosepoke task and consume more ethanol in a free-choice paradigm when compared to wild-type control mice (Bowers and Wehner, 2001). The neural mechanism by which PKC γ impacts these behaviors is most likely complex. However, PKC γ is primarily located post-synaptically; therefore, it is possible to limit the investigation of mechanisms to post-synaptic processes. A logical neurotransmitter candidate is the serotonin (5-HT) system, as numerous human and animal studies have shown that dysfunction of the central serotonergic system is related to both increased behavioral disinhibition (Harrison et

*Corresponding author: Barbara J Bowers, PhD, Institute for Behavioral Genetics, UCB 447, University of Colorado, Boulder, CO 80309, Ph: (303) 735-0077, Fax: (303) 492-8063, e-mail: bbowers@colorado.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

al., 1997; 1999; Ho et al., 1998; Leyton et al., 2001) and increased ethanol consumption (Fils-Aimee et al., 1996; LeMarquand et al., 1994a, 1994b; McBride et al., 1995; Murphy et al., 1982).

The 5-HT receptor system is heterogeneous with at least 15 subpopulations of neuronal receptors from 7 distinct families (5-HT₁-5-HT₇), which are encoded by different genes (Barnes and Sharpe, 1999). One of these families, 5-HT_{2A/B/C}, has been shown to be associated with PKC such that agonist-stimulated receptor desensitization and down-regulation are regulated by PKC activity (Anji et al., 2001; Kagaya et al., 1990; Roth et al., 1998). A recent investigation of PKC isotype expression in neonatal rat spinal cord after 5-HT₂ receptor stimulation reported that gene expression for the PKC γ isotype was upregulated after treatment with the 5-HT_{2A/C} agonist, 2-5-dimethoxy-4-iodoamphetamine (DOI) (MacDonald et al., 2001). In addition, Anji et al. (2001) implicate PKC α and PKC γ in the regulation of 5-HT_{2A} receptor mRNA. This suggests a possible selective role of PKC γ in 5-HT₂ receptor regulation. Further support for this association is indicated by the shared brain regional localization of PKC γ and 5-HT₂ receptors, including prefrontal cortex, as well as their post-synaptic localizations (Backstrom et al., 2001; Naik et al., 2000; Pazos et al., 1985; Thomas and Everitt, 2001).

In the present study, experiments were designed to explore further the potential link between PKC γ , 5HT_{2A/C} receptors, prefrontal cortex (PFC), and altered behavioral responses in PKC γ mutants compared to wild-type mice. To test the hypothesis that PKC γ null mutant mice have a dysregulation in the function and/or number of 5-HT_{2A/C} receptors in the PFC, we evaluated DOI-stimulated phosphoinositol (PI) hydrolysis in slices isolated from the PFC of mutant and wild-type mice. In addition, we measured [¹²⁵I]-DOI binding using tissue extracts from the PFC followed by quantitative autoradiography of [¹²⁵I]-DOI binding sites in several brain regions including the PFC and other regions associated with drug reward. To confirm the role of 5-HT_{2A/C} receptors in regulating behavioral differences between PKC γ mutant and wild-type mice, we extended our previous studies of behavior to include a characterization of DOI-induced head twitch responses (HTR) because this response is regulated by the serotonin system in the PFC (Willins and Meltzer, 1997). The head twitch response is a behavioral manifestation of the functional activation of 5-HT₂ receptors (Darmani and Gerdes, 1995; Darmani et al., 1990; Darmani et al., 1992; Willins and Meltzer, 1997) and can be measured easily and quickly in mice.

2. MATERIALS AND METHODS

2.1 Animals

Male and female mice were 80–120 days of age at the time of testing and were housed in like-sex groups of 2–5. Mice were given food and water *ad libitum* and maintained on a 12 hr light/dark cycle (lights on at 7:00 AM). PKC γ null mutant mice were derived using gene-targeting and homologous recombination techniques (Abeliovich et al., 1993) and are currently bred on an F1 C57BL/6 X 129/S6 mixed genetic background at the Institute for Behavioral Genetics (Boulder, CO). The F1 generations are bred from heterozygous crosses from two congenic strains: C57BL/6.PKC γ and 129/S6.PKC γ . This breeding strategy produces homozygous mutant, heterozygous, and homozygous wild-type genotypes within a single litter, thereby providing wild-type littermate controls for each experiment. The PKC γ mutation is maintained in a heterozygous condition on C57BL/6 and 129/S6 inbred strains because homozygous null mutant mice do not survive on the C57BL/6 background (Bowers et al., 1999). All mice were genotyped prior to testing using DNA analysis of tail DNA as described previously (Bowers and Wehner, 2001). Groups of null mutant and wild-type littermate control mice used in the following experiments were derived from multiple litters. All animal use procedures were

performed in accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by the University of Colorado IACUC.

2.2 Phosphoinositol (PI) Hydrolysis

Male and female PKC γ mutant (n = 8) and wild-type mice (n = 8) were sacrificed by cervical dislocation; the brains were rapidly removed and placed in an ice-cold brain matrix where the prefrontal cortex (PFC) was removed for slicing. Tissue from PFC was sliced (400 nM thick) with a McIlwain tissue chopper. Assay conditions were based on a protocol described by Berridge et al. (1982) with the following modifications. Slices were labeled for 60 min with [3 H]-myoinositol (3uCi/tube) (Perkin Elmer, 18.5–25 Ci/mmo) in the presence of LiCl (10mM) and pargyline (10uM). Agonist stimulation was done by incubation for 60 min with DOI (10 uM final). This concentration was based on results from Toscano et al. (1999) who reported maximal stimulation by DOI at 10 uM. Inositol phosphates were bound using a resin slurry (1:1, AG 1-X8 resin, formate form/H $_2$ O; Bio Rad Laboratories, Hercules, CA) added to each sample tube. Tubes were vortexed for 4 min, then centrifuged for 5 min at 2600 rpm. Samples were washed 4X with cold 5 mM myoinositol to remove free [3 H]-myoinositol. Inositol monophosphates were eluted with a final wash using 1M formate/0.1M formic acid. After centrifugation aliquots of the supernatant were placed in vials containing scintillation fluid. Radioactivity was counted and agonist stimulation was calculated as a percent of basal stimulation. Proteins were measured using the method of Bradford (1976).

2.3 [125 I]-DOI Membrane Binding

Binding experiments were done according to McKenna et al. (1989b) with some modifications. Prefrontal cortices were isolated from naive mutant (n = 3) or wild-type (n = 3) mice. The tissue was immediately homogenized in 50 mM Tris buffer (pH 7.4) containing 0.1% ascorbate and 4 mM CaCl $_2$, then centrifuged. Proteins were determined by the method of Bradford (1976). Incubations were conducted in 96-well microtiter plates consisted of 20 ug protein and 0.02–5.0 nM of [125 I]-DOI (3 replicates/concentration). Non-specific binding was defined at each concentration with 1 uM ketanserin. Reactions were terminated by filtration through glass fiber filters (0.38 mm thickness, Inotech Biosystems International, Inc., Rockville, MD) presoaked in 0.1% polyethylenimine using an Inotech Cell harvester. Filters were washed 3X with ice-cold 50 mM Tris and were counted in a gamma counter.

2.4 [125 I]-DOI Quantitative Autoradiography

Mutant (n = 6) and wild-type (n = 6) mice were sacrificed by cervical dislocation and the brains were quickly removed and frozen in isopentane on dry ice. Brains were stored at -70°C until sliced. Tissue sections (14 uM thick) were cut in a cryostat at -20°C and were thaw mounted onto Fisher Superfrost slides (Fisher Scientific, Pittsburgh, PA). Coronal sections were taken from 3.20, 1.70, and 1.10 mm anterior to Bregma to examine prefrontal cortex, rostral and caudal striatum, respectively (Franklin and Paxinos, 1997). A comparison of rostral and caudal striatum was included based on the findings of Bowers et al. (2000) that DOI stimulates DA release in caudal, but not rostral, nucleus accumbens core and shell regions. Binding was done according to McKenna et al. (1989a) with some modifications. Slides were preincubated for 20 min at RT in 50 mM Tris buffer (pH 7.4) containing 0.1% ascorbate, 0.1% BSA, and 4 mM CaCl $_2$ followed by incubation for 90 min in the presence of 500 pM [125 I]-DOI. This concentration was chosen based on the membrane binding experiments where the signal to noise ratio was the strongest. Because the affinity of DOI for 5-HT $_2$ C receptors is only about 10-fold greater than the affinity for the 2A site (Appel et al., 1990; Knight et al., 2004; McKenna et al., 1989a), we make the assumption that both 2A and 2C receptor density was being measured. Non-specific binding was defined in the presence of 10 uM ketanserin. Slides were placed in x-ray cassettes opposite Super Resolution phosphor screens (Packard Bioscience,

Meriden, CT) for 7 hrs. [125 I]-standards were included in each exposure (GE Healthcare) Autoradiographic images were quantified using the Cyclone Storage Phosphor System (Packard Bioscience). Net binding density values (DLU/mm²; total binding minus nonspecific binding) obtained for a given brain region across six consecutive tissue sections were averaged for each mouse.

2.5 Behavioral Procedures

On experimental days, mice were placed in a quiet testing room for one hour before injections. Testing began by placing the mouse in a clear Plexiglas box (20 x 26 x 15 cm) layered with bedding for a 20 min habituation period. At 20 min, mice were injected with either saline (n = 6/group) or 2.5 mg/kg DOI (Sigma-Aldrich, St. Louis, MO) (n = 15/group). Separate groups of mice were used for each treatment group. DOI is classified as a phenalkylamine hallucinogen whose affinity for 5-HT_{2A/C} receptors is strongly correlated to its hallucinogenic potency (Glennon et al., 1984; Titeler et al., 1988). DOI was dissolved in 0.9% saline and injected intraperitoneally at 0.01ml/g body weight. This dose of DOI has been shown to elicit a robust frequency of head twitch responses in mice (Darmani et al., 1990). Mice were videotaped for 20 min and scoring began immediately after injection. The head twitch response is a very distinctive paroxysmal head-twitching behavior that is easily distinguished from head bobbing, lateral movements of the head, or grooming. Scoring was also divided into four 5 min bins to evaluate any response pattern differences within the 20 min test. Ear scratch responses were also observed after DOI treatment. The results were similar to the head twitch responses in that mutant mice exhibited a greater response (data not shown).

2.6 Data Analysis

Data from the phosphoinositol hydrolysis and quantitative autoradiography experiments were analyzed by Student's t-test. Autoradiography data were analyzed independently within each brain region. Some slides were omitted from analyses due to experimental error (two wild-type samples). Membrane binding data from PFC were evaluated using non-linear regression analysis (SigmaPlot 8.0) that provided B_{max} and K_d values. The head twitch experiments were analyzed by ANOVA with genotype, drug dose, and sex as between subjects factors. Initial analyses indicated that the sexes did not differ in any measure; therefore male and female data were combined for subsequent analyses.

3. RESULTS

3.1 Biochemical Characterization of naïve PKC γ mutant and wild-type mice

Phosphoinositol Hydrolysis—Figure 1 shows a comparison of 10 μ M DOI-stimulated phosphoinositol hydrolysis in the prefrontal cortices of mutant and wild-type mice indicating that 5-HT_{2A/C} function was significantly greater in mutant mice ($t_{14} = 2.58$, $p < 0.02$). DOI-stimulation over basal values averaged 125% in mutant PFC compared 108% in wild-type PFC.

[125 I]-DOI Binding—Studies using membrane preparations from prefrontal cortex were performed to determine B_{max} and K_d values. Results from the non-linear regression analysis of saturation binding in the prefrontal cortex of mutant and wild-type mice demonstrated that the genotypes did not differ in receptor number (B_{max}: 101.96 fmol/mg protein $\pm$ 0.91 and 111.60 fmol/mg protein $\pm$ 9.70; mutant and wild-type respectively) or affinity (K_d: 0.99 nM $\pm$ 0.26 and 1.29 nM $\pm$ 0.31; mutant and wild-type respectively). An analysis of the ratio of total binding over non-specific binding at each concentration indicated that the strongest signal to noise ratio (non-specific binding averaged 27.5% of total binding) was seen at 0.5 nM. Therefore, this concentration was selected for the quantitative autoradiography experiments.

[¹²⁵I]-DOI Quantitative Autoradiography—Because the saturation binding experiments did not reveal differences in the number of binding sites or affinity for DOI in the prefrontal cortex, autoradiographic analyses of [¹²⁵I]-DOI binding sites in other brain regions associated with impulsivity and drug reward were done. The amount and distribution of binding sites were evaluated in 15 brain regions (Table 1). Levels of [¹²⁵I]-DOI binding were not different between mutant and wild-type mice in any brain region. Non-specific binding was low and was consistent across brain regions. Specific binding in the PFC (33.09 ± 1.55 fmol/mg and 31.12 ± 1.13 fmol/mg; mutant and wild-type respectively) was in the range seen in the saturation binding experiment at 0.5 nM DOI (32.81 ± 1.65 fmol/mg and 25.42 ± 2.91 fmol/mg; mutant and wild-type, respectively). However, differences in the amount of binding were observed among the brain regions with high levels found in prefrontal cortex, claustrum, lateral striatum, dorsal endopiriform nucleus, lateral olfactory tract, and olfactory tubercle. Lower levels of binding were seen in the striatal areas with rostral and caudal caudate putamen exhibiting the least. This is in agreement with previous studies that found similar patterns of receptor distributions in mouse (Li et al., 2003) and rat brain (McKenna et al., 1989a; Saavedra and Himeno, 1991). In the present study, [¹²⁵I]-DOI binding was greater in rostral nucleus accumbens compared to caudal nucleus accumbens. This differs from what would have been predicted since Bowers et al. (2000) found that DOI-induced DA release was greater in caudal nucleus accumbens, suggesting an increased 5-HT_{2A/C} receptor density in this region. However, Appel et al. (1990) also found a gradient of decreasing receptor density from rostral to caudal regions.

3.2 The effects of DOI on Behavioral responses

Head Twitch Response—The DOI-induced head twitch response was included in this study to confirm the role of 5-HT_{2A/C} receptors in regulating behavioral differences between PKC γ mutant and wild-type mice. The results of an overall univariate ANOVA indicated significant main effects of genotype ($F_{1,42} = 9.20$, $p < 0.01$) and drug treatment ($F_{1,42} = 94.71$, $p < 0.0001$), and a significant interaction between genotype and drug treatment ($F_{1,42} = 5.15$, $p < 0.05$). These results are illustrated in Figure 2A which shows that 2.5 mg/kg DOI significantly increased HTR in both genotypes compared to saline; however, mutant mice exhibited a greater number of DOI-induced HTR compared to wild-type mice. Saline injections had little effect on the head twitch response in either genotype, and were not different between genotypes.

When the patterns of head twitch responses in 2.5 mg/kg DOI-treated mutant and wild-type mice were analyzed in 5 min bins, a repeated measures ANOVA indicated a significant main effect of bins ($F_{3,114} = 13.8$, $p < 0.0001$), genotype ($F_{1,38} = 32.3$, $p < 0.0001$), and a significant bins X genotype interaction ($F_{3,114} = 6.9$, $p < 0.0001$). Figure 2B illustrates that the interaction is due to the different pattern of responses in mutant mice across the 20 min compared to wild-type mice; i.e., mutant mice demonstrate an increase in responses in the last 15 min of the test compared to wild-type mice.

4. DISCUSSION

The primary objective of the present study was to determine whether the neuronal-specific isotype of PKC, PKC γ , regulates 5-HT_{2A/C} receptor number and function in the prefrontal cortex and whether there are behavioral correlates relating to this potential dysfunction. In support of this hypothesis the relevant findings of this study are: 1) in naive PKC γ null mutant mice 5-HT_{2A/C} receptor function, as measured by phosphoinositol hydrolysis, is increased in prefrontal cortex compared to wild-type mice; 2) receptor numbers are not different between the genotypes in PFC, or in several other brain regions; and 3) the head twitch response, a

behavioral response to 5-HT_{2A} receptor activation in the PFC, is increased in PKC γ null mutant mice compared to wild-type littermate controls after an acute injection of 2.5 mg/kg DOI.

4.1 Biochemical Assessments of 5-HT_{2A/C} Receptors

The results from the phosphoinositol hydrolysis experiment are indicative of an interaction of 5-HT_{2A/C} receptors with PKC γ , such that G protein-coupling may be regulated by PKC γ after agonist stimulation. This interaction is part of a constitutive negative feed-back mechanism between PKC and G protein-coupling that maintains a homeostasis of receptor function (Berg et al., 2001; Kagaya et al., 1990). Roth et al. (1998) describes a potential pathway of desensitization involving PKC phosphorylation of the receptor itself. Upon agonist stimulation, PKC is activated via phospholipase C-induced diacyl glycerol and Ca²⁺ release. Activated PKC phosphorylates the 5-HT_{2A/C} receptor which causes arrestins to bind to the receptor. Once arrestins are bound, receptor-G protein-coupling is compromised and the receptor loses function. We suggest that this negative feed-back desensitization process is dysregulated in PKC γ null mutant mice such that phosphorylation of the receptor does not occur resulting in heightened receptor function. This indicates a specific role for the gamma isotype in the phosphorylation of the 5-HT_{2A/C} receptor. The effects of this dysregulation would be increased sensitivity to endogenous serotonin and to agonist stimulation resulting in alterations in serotonin-mediated behaviors. The apparent desensitization effects of DOI in the present experiment were observed under experimental conditions described in the Methods section; therefore, non-specific effects by DOI at 10 μ M or incomplete desensitization after a 60 min incubation period cannot be ruled out. However, Toscano et al. (1999) have shown that at 1 μ M the potent 5-HT_{2A/C} antagonists, mesulergine and methysergide, completely block 10 μ M DOI-stimulated phosphoinositol hydrolysis in rat spinal cord slices indicating that at 10 μ M, DOI is specific for 5-HT_{2A/C} receptors. In addition, maximum 5-HT-induced desensitization of 5-HT_{2A} receptors occurs as early as 10 min and 5-HT_{2C} receptors by 60 min in CHO cells (Berg et al. 2001). Therefore, in the present study the 60 min incubation time would be sufficient for desensitization.

It was important to measure whether the number or affinity of 5HT_{2A/C} receptors differed as a function of genotype to provide a detailed picture of how the deletion of PKC γ could affect the 5HT system. The results from membrane binding experiments using [¹²⁵I]-DOI and quantitative autoradiography of [¹²⁵I]-DOI binding sites in naïve mice indicated that mutant and wild-type mice do not differ in the number or affinity of 5-HT_{2A/C} receptors in prefrontal cortex, or in 14 other brain regions associated with impulsivity and drug reward. *In vitro* experiments have shown that the integrity of 5-HT_{2A} receptor mRNA is regulated by a PKC-dependent mechanism (Ferry et al., 1994). However, the data from the present study suggest that PKC γ is not involved in this process and does not regulate receptor gene expression.

4.2 Behavioral Assessments of 5HT_{2A/C} receptors

In order to strengthen the argument that 5-HT_{2A/C} receptor function in PFC is dysregulated in PKC γ null mutant mice, mutant and wild-type mice were injected i.p. with DOI to produce HTR. Willins and Meltzer (1997) implicated the PFC as the primary brain region controlling 5-HT₂-mediated HTR by directly administering DOI into the medial prefrontal cortex of rat. DOI injections produced head twitch responses in both genotypes; however, HTR was greater in mutant mice compared to wild-type controls. This suggests that PKC γ regulates this 5-HT_{2A/C}-mediated behavior such that DOI produces a more robust response by acting at the more functionally sensitive receptor found in mutant mice. The magnitude of the phosphoinositol hydrolysis difference between the genotypes did not completely correspond to the substantial difference in the head twitch response. Since the behavioral adaptations appear to be mediated by PKC γ , it is possible that other neurotransmitter systems that are regulated by PKC γ are involved in these responses. For example, we have shown previously

that PKC γ regulates ethanol-stimulated GABA $_A$ receptor function in cortex (Harris et al., 1995). It has been shown that GABAergic drugs modulate head twitch behavior in rodents, perhaps through interaction with 5-HT $_2$ receptors (Handley and Singh, 1985; Moser and Redfern, 1988; Tadano et al., 2001). Feng et al (2001) reported that activation of 5-HT $_2$ receptors in the prefrontal cortex of rats increased *in vitro* PKC activity toward GABA $_A$ receptor γ_2 subunits. Moreover, the role of other neurotransmitter systems cannot be ruled out. Hayslett and Tizabi (2003, 2005) have shown that drugs acting at the nicotinic cholinergic and dopaminergic systems can regulate DOI-induced HTR.

The pattern of head twitch responses measured in 5 min bins suggests that acute desensitization does not occur in the mutant mice. *In vitro* studies have shown that 5-HT $_2A$ receptors are acutely desensitized, as measured by PI turnover, after as little as 15 min exposure to 5-HT (Kagaya et al., 1990; Van Oekelen et al., 2001).

4.3 Relevance of current findings to impulsivity and ethanol consumption in PKC γ mutants

PKC γ mutants show increased impulsivity in a signaled-nosepoke task (Bowers and Wehner, 2001). Impulsivity in humans and rodents has been associated with decreased serotonin levels in brain (Harrison et al., 1997; Leyton et al., 2001). However, Dalley et al. (2002) demonstrated that deficits in impulse control, using a simplified form of the 5 choice serial reaction task in rat, are positively associated with *elevated* levels of serotonin in the prefrontal cortex. This effect may be specifically associated with the 5-HT $_2A$ receptor (Winstanely et al., 2004). In rodents, treatment with the 5-HT $_{2A/C}$ agonist, DOI, increases impulsive responding (Evenden and Ryan, 1999; Evenden, 1998; Koskinen et al., 2000). If 5-HT $_2A$ receptor function is enhanced in PKC γ mutant mice, the impulsive responding that we observed in these mice may be regulated by increased sensitivity to endogenous serotonin. This idea is more compelling in relationship to the findings of Dalley et al. (2002) because the appetitive signaled-nosepoke task that was used to evaluate impulsivity in the PKC mice is based on impulse control to an auditory stimulus (Bowers and Wehner, 2001). Thus, we speculate that alterations in 5HT $_{2A/C}$ receptor function observed here may underlie the increased impulsivity phenotype in PKC γ mutants.

PKC γ mutants also show increased consumption of ethanol (Bowers and Wehner, 2001). Although decreased density of 5-HT $_2A$ receptors in the PFC have been associated with increased ethanol consumption (Blakley et al., 2001; Ciccocioppo et al., 1997; Ciccocioppo et al., 1999; McBride et al., 1993) a relationship between *increased* PI hydrolysis in this brain region and ethanol consumption has not been reported. However, using the selected rat lines, P and NP, Pandey et al. (1996) did show that in the high drinking P rats, 5-HT $_{2C}$ -mediated PI hydrolysis and receptor binding are increased in choroid plexus. Therefore, in a general sense, 5-HT $_{2A/C}$ receptor function may be important in regulating a genetic vulnerability to ethanol consumption. With this in mind, the enhanced PI hydrolysis observed in the PFC of PKC γ mutant mice supports the idea of a role for PKC γ that includes ethanol drinking. Experiments to test the direct effects of 5-HT $_{2A/C}$ drugs on impulsivity and ethanol consumption in PKC γ mice are in progress. The results of these experiments will give a more definitive explanation for the role of 5-HT $_{2A/C}$ receptors in these behaviors.

In conclusion, these results suggest a role for PKC γ in the functional regulation of the 5-HT $_{2A/C}$ receptor in the PFC as measured by PI hydrolysis. Therefore we speculate that a dysregulation of 5-HT $_{2A/C}$ receptor function occurs in the PFC of PKC γ null mutant mice and that this may underlie the increased impulsivity, ethanol consumption (Bowers and Wehner, 2001), and DOI-induced head twitch (present study) demonstrated in these mice.

Acknowledgements

This work was supported by NIH grants, AA13901 to BJB; and AA03527 and AA13018 to JMW.

References

- Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S. Modified hippocampal long-term potentiation in PKC γ -mutant mice. *Cell* 1993;75:1253–1262. [PubMed: 8269509]
- Anji A, Hanley NRS, Kumari M, Hensler JG. The role of protein kinase C in the regulation of serotonin-2A receptor expression. *J Neurochem* 2001;77:589–597. [PubMed: 11299321]
- Appel NM, Mitchell WM, Garlick RK, Glennon RA, Teitler M, De Souza EB. Autoradiographic characterization of ($\pm$)-1-(2,5-dimethoxy-4-[125 I]iodophenyl)-2-aminopropane ([125 I]DOI) binding to 5-HT $_2$ and 5-HT $_{1C}$ receptors in rat brain. *J Pharm Exp Ther* 1990;255:843–857.
- Backstrom JR, Miner LA, Sanders-Bush E, Sesack SR. Ultrastructural localization of the 5-HT $_{2A}$ receptor in the rat medial prefrontal cortex. *Soc Neurosci Abs*, P2001. Program Number, 380.19.
- Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharm* 1999;38:1083–1152.
- Berg KA, Stout BD, Maayani S, Clarke WP. Differences in rapid desensitization of 5-hydroxytryptamine $_{2A}$ and 5-hydroxytryptamine $_{2C}$ receptor-mediated phospholipase C activation. *J Pharm Exp Ther* 2001;299:593–602.
- Berridge MJ, Downes Cp, Hanley MR. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem J* 1982;206:587–595. [PubMed: 7150264]
- Blakley GG, Pohorecky LA, Benjamin D. Bidirectional changes in ethanol consumption in rats with site-specific antisense down-regulation of 5-hydroxytryptamine $_{2A}$ receptors in brain. *J Pharm Exp Ther* 2001;299:277–289.
- Bowers BJ, Owen EH, Collins AC, Abeliovich A, Tonegawa S, Wehner JM. Decreased ethanol sensitivity and tolerance development in γ -protein kinase C null mutant mice is dependent on genetic background. *Alcohol Clin Exp Res* 1999;23:387–397. [PubMed: 10195808]
- Bowers BJ, Henry MB, Thielen RJ, McBride WJ. Serotonin 5-HT $_2$ receptor stimulation of dopamine release in the posterior but not anterior nucleus accumbens of the rat. *J Neurochem* 2000;75:1625–1633. [PubMed: 10987844]
- Bowers BJ, Wehner JM. Ethanol consumption and behavioral impulsivity are increased in protein kinase C γ null mutant mice. *J Neurosci* 2001;21:RC180. [PubMed: 11606660]
- Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 1976;72:248–254. [PubMed: 942051]
- Ciccocioppo R, Angeletti S, Colomba G, Gessa GL, Massi M. Autoradiographic analysis of 5-HT $_{2A}$ binding sites in the brain of Sardinian alcohol-preferring and nonpreferring rats. *Eur J Pharm* 1999;373:13–19.
- Ciccocioppo R, Ge J, Barnes NM, Cooper SJ. Autoradiographic mapping of 5-HT $_{2A}$ binding sites in P and AA alcohol-preferring rats. *Brain Res Bull* 1997;44:33–37. [PubMed: 9288829]
- Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev* 2004;28:771–784. [PubMed: 15555683]
- Darmani NA, Gerdes CF. Temporal differential adaptation of head-twitch and ear-scratch responses following administration of challenge doses of DOI. *Pharm Biochem Behav* 1995;50:545–550.
- Darmani NA, Martin BR, Glennon RA. Withdrawal from chronic treatment with ($\pm$)-DOI causes supersensitivity to 5-HT $_2$ receptor-induced head-twitch behavior in mice. *Eur. J. Pharm* 1990;186:115–118.
- Darmani NA, Martin BR, Glennon RA. Behavioral evidence for differential adaptation of the serotonergic system after acute and chronic treatment with ($\pm$)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) or ketanserin. *J Pharm Exp Ther* 1992;262:692–698.
- Evenden JL, Ryan CN. The pharmacology of impulsive behavior in rats VI: the effects of ethanol and selective serotonergic drugs on response choice with varying delays of reinforcement. *Psychopharm* 1999;146:413–421.
- Evenden JL. The pharmacology of impulsive behavior in rats IV: the effects of selective serotonergic agents on a paced fixed consecutive number schedule. *Psychopharm* 1998;140:319–330.
- Feng J, Cai X, Zhao J, Yan Z. Serotonin receptors modulate GABA $_A$ receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. *J Neurosci* 2001;21:6502–6511. [PubMed: 11517239]

- Ferry RC, Unsworth CD, Artymyshyn RP, Molinoff PB. Regulation of mRNA encoding 5-HT_{2A} receptors in P11 cells through a post-translational mechanism requiring activation of protein kinase C. *J Biol Chem* 1994;269:31850–31857. [PubMed: 7989358]
- Fils-Aime M-L, Eckardt MJ, George DT, Brown GL, Mefford I, Linnoila M. Early-onset alcoholics have lower cerebrospinal fluid 5-hydroxyindoleacetic acid levels than late-onset alcoholics. *Arch Gen Psychiatry* 1996;53:211–216.
- Franklin, KBJ.; Paxinos, G. *The Mouse Brain in Stereotaxic Coordinates*. Academic Press; 1997.
- Glennon RA, Titeler M, McKenney JD. Evidence for 5-HT₂ involvement in the mechanism of action of hallucinogenic agents. *Life Sci* 1984;35:2505–2511. [PubMed: 6513725]
- Handley SL, Singh L. Modulation of 5-hydroxytryptamine-induced head-twitch response by drugs acting at GABA and related receptors. *Br J Pharm* 1985;86:297–303.
- Harris RA, McQuilkin SJ, Paylor R, Abeliovich A, Tonegawa S, Wehner JM. Mutant mice lacking the γ isoform of protein kinase C show decreased behavioral actions of ethanol and altered function of γ -aminobutyrate type A receptors. *Proc Nat'l Acad Sci* 1995;92:3658–3662.
- Harrison AA, Everitt BJ, Robbins TW. Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. *Psychopharm* 1997;133:329–342.
- Harrison AA, Everitt BJ, Robbins TW. Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced go/no-go conditional visual discrimination. *Behav Brain Res* 1999;100:99–112. [PubMed: 10212057]
- Hayslett RL, Tizabi Y. Effects of donepezil on DOI-induced head twitch response in mice: implications for Tourette syndrome. *Pharm Biochem Behav* 2003;76:409–415.
- Hayslett RL, Tizabi Y. Effects of donepezil, nicotine and haloperidol on the central serotonergic system in mice: Implications for Tourette's syndrome. *Pharm Biochem Behav* 2005;81:879–886.
- Ho M-Y, Al-Zahrani SSA, Al-Ruwaitea ASA, Bradshaw CM, Szabadi E. 5-hydroxytryptamine and impulse control: prospects for a behavioral analysis. *J Psychopharm* 1998;12:68–78.
- Kagaya A, Mikuni M, Kusumi I, Yamamoto H, Takahashi K. Serotonin-induced acute desensitization of serotonin₂ receptors in human platelets via a mechanism involving protein kinase C. *J Pharm Exp Ther* 1990;255:305–311.
- Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, Bickerdike M. Pharmacological characterization of the agonist radioligand binding site of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. *Naunyn-Schmied Arch Pharmacol* 2004;370:114–123.
- Koskinen T, Ruotsalainen S, Puumala T, Lappalainen R, Koivisto E, Männistö PT, Sirviö J. Activation of 5-HT_{2A} receptors impairs response control of rats in a five-choice serial reaction time task. *Neuropharm* 2000;39:471–481.
- LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: findings of animal studies. *Biol Psychiatry* 1994a;36:395–421. [PubMed: 7803601]
- LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: clinical evidence. *Biol Psychiatry* 1994b;36:326–337. [PubMed: 7993959]
- Leyton M, Okazawa H, Diksic M, Paris J, Rosa P, Mzengeza S, Young SN, Blier P, Benkelfat C. Brain regional α -[¹¹C]methyl-L-tryptophan trapping in impulsive subjects with borderline personality disorder. *Am J Psychiatry* 2001;158:775–782. [PubMed: 11329401]
- Li Q, Wichems CH, Ma L, Van de Kar LD, Garcia F, Murphy DL. Brain region-specific alterations of 5-HT_{2A} and 5-HT_{2C} receptors in serotonin transporter knockout mice. *J Neurochem* 2003;84:1256–1265. [PubMed: 12614326]
- MacDonald SC, Shay BL, Sawchuck MA, Hochman S, Buck CR. 5-HT₂ receptor stimulation changes protein kinase C expression in the neonatal rat spinal cord. *Soc Neurosci Abs*. 2001Program Number 718.6.
- McBride WJ, Bodart B, Lumeng L, Li T-K. Association between low contents of dopamine and serotonin in the nucleus accumbens and high alcohol preference. *Alcohol Clin Exp Res* 1995;19:1420–1422. [PubMed: 8749804]
- McBride WJ, Chernet E, Rabold JA, Lumeng L, Li T-KK. Serotonin-2 receptors in the CNS of alcohol-preferring and -nonpreferring rats. *Pharm Biochem Behav* 1993;46:631–636.

- McKenna DJ, Nazarali AJ, Himeno A, Saavedra JM. Chronic treatment with ($\pm$) DOI, a psychotomimetic 5-HT₂ agonist, downregulates 5-HT₂ receptors in rat brain. *Neuropsychopharm* 1989a;2:81–87.
- McKenna DJ, Nazarali AJ, Hoffman AJ, Nichols DE, Mathis CA, Saavedra JM. Common receptors for hallucinogens in rat brain: a comparative autoradiographic study using [¹²⁵I] LSD and [¹²⁵I] DOI, a new psychotomimetic radioligand. *Brain Res* 1989b;476:45–56. [PubMed: 2536576]
- Moser PC, Redfern PH. The effect of benzodiazepines on the 5-HT agonist-induced head-twitch response in mice. *Eur J Pharm* 1988;7:223–231.
- Murphy JM, McBride WJ, Lumeng L, Li T-K. Regional brain levels of monoamines in alcohol- preferring and –nonpreferring lines of rats. *Pharm Biochem Behav* 1982;16:145–149.
- Naik MU, Benedikz E, Hernandez I, Libien J, Hrabe J, Valsamis M, Dow-Edwards D, Osman M, Sacktor TC. Distribution of protein kinase M ζ and the complete protein kinase C isoform family in rat brain. *J Compar Neurol* 2000;426:243–258.
- Pandey SC, Lumeng L, Li T-K. Serotonin_{2C} receptors and serotonin_{2C} receptor-mediated phosphoinositide hydrolysis in the brain of alcohol-preferring and alcohol-nonpreferring rats. *Alcohol Clin Exp Res* 1996;20:1038–1042. [PubMed: 8892525]
- Pazos A, Cortés R, Palacios JM. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res* 1985;346:231–249. [PubMed: 4052777]
- Roth BL, Berry SA, Kroeze WK, Willins DL, Kristiansen K. Serotonin 5-HT_{2A} receptors: molecular biology and mechanisms of regulation. *Crit Reviews in Neurobiol* 1998;12:319–338.
- Saavedra, JM.; Himeno, A. Autoradiographic studies of 5-HT₂ receptors. In: Schwarcz, R., editor. *Kynurenine and Serotonin Pathways*. New York: Plenum Press; 1991. p. 107–113.
- Thomas KL, Everitt BJ. Limbic-cortical-ventral striatal activation during retrieval of a discrete cocaine-associated stimulus: a cellular imaging study with γ protein kinase C expression. *J Neurosci* 2001;21:2526–2535. [PubMed: 11264326]
- Titeler M, Lyon RA, Glennon RA. Radioligand binding evidence implicates the brain 5-HT₂ receptor as a site of action for LSD and phenylisopropylamine hallucinogens. *Psychopharm* 1988;94:213–216.
- Tadano T, Hozumi M, Satoh N, Oka R, Hishinuma T, Mizugaki M, Arai Y, Yasuhara H, Kinemuchi H, Nijima F, Nakagawasai O, Tan-no K, Kisara K. Central serotonergic mechanisms on head twitch responses induced by benzodiazepine receptor agonists. *Int J Exp Clin Pharm* 2001;62:157–162.
- Toscano E, Romero G, Oset C, Del Río J. Pharmacological characteristics and regulation of 5-HT receptor-stimulated phosphoinositide hydrolysis in the rat spinal cord. *Gen Pharm* 1999;32:351–358.
- Van Oekelen D, Jurzak M, Van de Wiel D, Van Hecke G, Luyten WHML, Leysen JE. Different regulation of rat 5-HT_{2A} and rat 5-HT_{2C} receptors in NIH 3T3 cells upon exposure to 5-HT and pipamperone. *Eur J Pharm* 2001;425:21–32.
- Willins DL, Meltzer HY. Direct injection of 5-HT_{2A} receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J Pharm Exp Ther* 1997;282:699–706.
- Winstanely CA, Dalley JW, Theobald DEH, Robbins TW. Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behavior. *Neuropsychopharm* 2004;29:1331–1343.

FIGURE 1.

DOI-stimulated phosphoinositol hydrolysis in PFC from mutant and wild-type mice. The percent of basal stimulation in mutant and wild-type mice in the presence of 10 μ M DOI is greater in mutant mice compared to wild-type mice (* $p < 0.02$).

FIGURE 2.

Head twitch responses (HTR) in mutant and wild-type mice after acute doses of saline or 2.5 mg/kg DOI. **A.** At 2.5 mg/kg DOI both mutant and wild-type mice had significantly greater responses than saline controls (* $p < 0.0001$). Mutant mice exhibited significantly greater DOI-induced HTR than wild-type mice (# $p < 0.01$). **B.** Patterns of HTR in mutant and wild-type mice in four 5 min bins on Day 0 after an acute injection of 2.5 mg/kg DOI. Response patterns differed between genotypes ($p < 0.0001$) which interacted with bins ($p < 0.0001$).

TABLE 1Comparison of regional densities of [125 I] DOI binding sites in PKC γ mutant and wild-type mice

Region	Specific Binding	
	Mutant	Wild-type
	<i>f mol/mg tissue equivalent $\pm$ SE</i>	
Prefrontal cortex *	33.09 $\pm$ 1.55	31.17 $\pm$ 1.13
Lateral olfactory tract	32.51 $\pm$ 2.06	26.70 $\pm$ 2.93
Cortex (rostral only)		
anterior cingulate	12.93 $\pm$ 1.15	14.69 $\pm$ 1.21
layer V	20.03 $\pm$ 0.79	20.18 $\pm$ 2.48
Clastrum	26.19 $\pm$ 1.26	26.05 $\pm$ 2.76
Dorsal endopiriform nucleus	24.11 $\pm$ 1.21	25.52 $\pm$ 2.90
Lateral striatum	27.99 $\pm$ 1.56	27.16 $\pm$ 3.11
Olfactory tubercle	25.44 $\pm$ 1.44	25.84 $\pm$ 2.25
Septum [‡]	9.59 $\pm$ 1.43	9.88 $\pm$ 2.42
Caudate putamen (rostral)	8.65 $\pm$ 0.86	8.46 $\pm$ 1.16
Caudate putamen (caudal)	4.73 $\pm$ 0.33	4.18 $\pm$ 0.58
Nucleus accumbens (rostral)		
shell	17.25 $\pm$ 1.06	17.24 $\pm$ 2.06
core	14.88 $\pm$ 0.98	15.39 $\pm$ 2.12
Nucleus accumbens (caudal)		
shell	13.68 $\pm$ 0.46	12.39 $\pm$ 1.36
core	9.58 $\pm$ 0.42	9.18 $\pm$ 1.25

* Prefrontal cortex densities include FrA, PrL, MO, LO, DLO, VO as published in Franklin KBJ, Paxinos G (1997)

[‡]Septum densities include DP, LSI, SHi, LSD as published in Franklin KBJ, Paxinos (1997)

Abbreviations: FrA, frontal association cortex; PrL, prelimbic cortex; MO, medial orbital cortex; LO, lateral orbital cortex; DLO, dorsolateral orbital cortex; VO, ventral orbital cortex; DP, dorsal pendicular nucleus; LSI, lateral septal nucleus, intermediate, SHi, septohippocampal nucleus; LSD, lateral septal nucleus, dorsal.