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Haloperidol and Clozapine Differentially Affect the Expression of Arrestins, Receptor Kinases, and Extracellular Signal-Regulated Kinase Activation

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Abstract

Dopamine and other G protein-coupled receptors (GPCRs) represent the major target of antipsychotic drugs. GPCRs undergo desensitization via activation-dependent phosphorylation by G protein-coupled receptor kinases (GRKs) followed by arrestin binding. Arrestins and GRKs are major regulators of GPCR signaling. We elucidated changes in expression of two arrestins and four GRKs following chronic (21 days) treatment with haloperidol (1 mg/kg i.p.) or clozapine (20 mg/kg i.p.) 2 or 24 h after the last injection in 11 brain regions. Haloperidol decreased GRK3 in ventrolateral caudate-putamen and transiently down-regulated GRK5 in globus pallidus and caudal caudate-putamen. Clozapine also caused a short-term suppression of the GRK5 expression in the caudal caudate-putamen and globus pallidus, but, unlike haloperidol, elevated GRK5 in the caudal caudate-putamen after 24 h. Unlike haloperidol, clozapine decreased arrestin2 and GRK3 in hippocampus and GRK3 in globus pallidus but increased arrestin2 in the core of nucleus accumbens and ventrolateral caudate-putamen and GRK2 in prefrontal cortex. Clozapine, but not haloperidol, induced long-term activation of extracellular signal-regulated kinase (ERK) 2 in ventrolateral caudate-putamen and transient in prefrontal cortex. The data demonstrate that haloperidol and clozapine differentially affect the expression of arrestins and GRKs and ERK activity, which may play a role in determining their clinical profile.

The analysis of the pharmacological profile of antipsychotic drugs (APDs) or neurochemical consequences of antipsychotic treatment has been widely used to understand the pathophysiology of schizophrenia. Based on their propensity to induce extrapyramidal symptoms (EPSs), ADP drugs can be classified as typical or atypical (Tandon and Jibson, 2003; Meltzer, 2004). The molecular mechanisms responsible for the differences in the clinical profile of typical and atypical APDs remain unclear. Both groups of APDs are quite diverse in their pharmacological properties. Lower incidence of EPSs with atypical APDs has been attributed to their lower affinity and lower in vivo occupancy of D2 dopamine receptors (Abi-Dargham and Laruelle, 2005). Substantial antagonism at serotonin receptors may be related to superior efficacy of atypical APDs toward negative and cognitive symptoms (Meltzer, 2004).

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The studies of molecular mechanisms of APD action have long concentrated on the effects of APDs on gene expression. The pattern of gene expression induced by acute or chronic APD administration is predictive of the propensity of drugs to generate EPSs (McClung et al., 2004; Maheux et al., 2005). Typical and atypical APDs differentially alter the expression of signaling proteins (Merchant et al., 1994; Dwivedi et al., 2002; Alimohamad et al., 2005) and the concentrations of neurotransmitter receptors (Burnet et al., 1996; Steward et al., 2004). Modulation of signaling via G protein-coupled receptors (GPCRs) may represent an important molecular mechanism of antipsychotic action. The signaling is regulated via interaction of GPCRs with regulatory proteins. Two groups of such proteins, G protein-coupled receptor kinases (GRKs) and arrestins, are involved in receptor desensitization. After activation, GPCRs are phosphorylated by a GRK. Subsequent arrestin binding stops receptor-G protein interaction and induces receptor internalization followed by either recycling or degradation (for review, see Gurevich and Gurevich, 2006). Therefore, arrestins and GRKs are important regulators of GPCR-mediated signaling.

Drugs that cause persistent stimulation or blockade of GPCRs often alter the expression of arrestins and GRKs (Hurlé, 2001; Díaz et al., 2002; Fan et al., 2002; Miralles et al., 2002). The direction of changes is not always predictable based on whether stimulation or blockade is employed. Simple logic would suggest that agonists should up-regulate arrestins and/or GRKs as a compensatory measure aimed at reducing excessive signaling, and antagonists should have the opposite effect. Indeed, chronic or even acute agonist treatment can increase the concentration of arrestins and/or GRK, which may lead to tolerance to drugs (Hurlé, 2001; Díaz et al., 2002; Fan et al., 2002). However, treatment with both opioid agonists and antagonists increases the expression of arrestin2 and GRK2 in the brain (Hurlé, 2001; Díaz et al., 2002). Changes in arrestin/GRK expression elicited by in vivo application of drugs are quite complex, involving several proteins in multiple brain regions. The mechanisms of these effects are unclear and may involve changes in transcription, posttranscriptional regulation, and degradation (for review, see Penela et al., 2003). Most importantly, numerous data prove that cellular concentration of arrestins and GRKs is a major regulatory factor affecting GPCR signaling both in vitro and in vivo (Bohn et al., 2003; Gainetdinov et al., 2004).

It is conceivable that chronic treatment with APDs alters the expression of arrestins and GRKs in the brain. Such alterations may modify receptor trafficking, thereby contributing to changes in receptor densities after APD treatment, and significantly affect signaling pathways. However, the expression of arrestins or GRKs after APD treatment has never been studied. In this work, we sought to compare the effect of a widely used typical APD haloperidol and a prototypical atypical drug clozapine on the arrestin/GRK expression in terms of the regional pattern, magnitude and direction of effects, and specific arrestin/GRK subtypes targeted. To detect changes in downstream signaling possibly associated with altered expression of arrestins and/or GRKs, we compared the levels of ERK phosphorylation after antipsychotic treatment. Understanding how APDs modulate the receptor regulation machinery may prove essential for targeted design of APDs with desired clinical profiles and provide insights into the pathophysiology of schizophrenia.

Materials and Methods

Animals and Tissue Preparation

All experiments were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Vanderbilt University Animal Care and Use Committee. Male Sprague-Dawley rats (275–300 g weight at the beginning of the experiment) were housed at 12-/12-h light/dark cycle with food and water available ad libitum. Drugs were purchased from Sigma-Aldrich (St. Louis, MO). Rats received injections with vehicle, haloperidol (1 mg/kg i.p.), or clozapine (20 mg/kg i.p.) once a day for 21 days.

These doses induce the expression of various genes (Dwivedi et al., 2002; Alimohamad et al., 2005; Maheux et al., 2005). The animals were sacrificed after 2 or 24 h after the last injection. One short washout period and one long washout period were chosen to separate short-term and longer lasting effects. Transcription factors are induced 1 h after administration of APDs (Merchant et al., 1994; Maheux et al., 2005), and the expression of other genes tends to be altered 2 to 7 h after drug injection (Merchant et al., 1994). Limited pharmacokinetic data indicate that in rats, clozapine and haloperidol are still present in the brains 2 h after the injection (Baldessarini et al., 1993). The drugs are rapidly eliminated from the rat brain (half-life, 1.5 h) (Baldessarini et al., 1993; Kapur et al., 2003 and references therein). It is noteworthy that the drug metabolites are low or undetectable in the brain, and chronic drug treatment does not lead to accumulation of the drug (Baldessarini et al., 1993; Kapur et al., 2003). Therefore, short-term effects should be detectable 2 h after the drug administration, but mostly chronic effects should remain after 24 h. Brains were rapidly removed, frozen on dry ice, and stored at -80°C until used. There were 10 rats in each experimental group.

The brain areas of interest were rapidly isolated from 100- μm -thick coronal cryosections at -20°C . Brain structures were identified and named according to Paxinos and Watson (1998). Eleven different brain regions were analyzed. These included the following cortical areas: 1) prefrontal cortex (PFC), which included prelimbic and infralimbic cortices (plates 6–10 in Paxinos and Watson, 1998); 2) cingulate cortex (areas 1 and 2, plates 11–14); and 3) dorsal hippocampus (dHipp) (plates 28–31). The rostral caudate-putamen, encompassing plates 11 to 15, was subdivided into four quadrants: 1) dorsolateral, 2) ventrolateral (VL), 3) dorsomedial, and 4) ventromedial. The core (ACc) and shell regions of the nucleus accumbens were collected separately, ACc from plates 9 to 15 and nucleus accumbens shell from plates 10 to 15. The region of caudal caudate-putamen (cCPu) corresponded to plates 21 to 24, and the globus pallidus (GP) was collected from the same plates.

The tissue was immediately immersed into 200 μl of lysis solution (Ambion, Austin, TX). The lysis solution effectively lyses the tissue while inhibiting all enzymatic activity. This ensures good preservation of proteins. Protein concentration in the samples was measured with Bradford reagent (Bio-Rad, Hercules, CA). Samples were then precipitated with 90% (v/v) methanol, and protein was pelleted by centrifugation and dissolved in SDS sample buffer at the final concentration of 0.25 μg of protein/ μl .

Western Blotting

Electrophoresis and transfer onto Immobilon-P (Millipore, Bedford, MA) membrane were performed essentially as described previously (Gurevich et al., 2002; Gurevich et al., 2004). Arrestins were detected with arrestin2-specific (1:9000) or arrestin3-specific (1:900) affinity-purified rabbit polyclonal antibodies. We used rabbit polyclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) to quantify GRK2 (1:500), GRK3 (1:300), GRK5 (1:500), and GRK6 (1:300). To detect phosphorylated ERK, phosphospecific mouse monoclonal antibody (1:2000; Cell Signaling Technology, Danvers, MA) was used, and total ERK was detected with rabbit antibody (1:1000; Cell Signaling Technology). For quantification of arrestins, dilutions of standards containing 1:1 mix of *Escherichia coli*-expressed purified bovine arrestin2 and arrestin3 (Gurevich et al., 2002, 2004) in sample buffer were loaded onto each gel along with samples. For quantification of GRKs, we used bovine GRK2 and GRK3, human GRK5, and GRK6 purified as described previously (Gurevich et al., 2004). For quantification of phospho- and total ERK, respective purified ERK standards were used. ERK2 was expressed in *E. coli* and purified to homogeneity, as described previously (Waas et al., 2003). Purified ERK2 was phosphorylated by recombinant purified MEK1 in vitro, as described. Protein concentration was determined by absorption at 280 nm, using the molar extinction coefficient of 52,067 M/cm (Waas et al., 2003). Appropriate dilutions of

purified proteins were loaded onto each gel alongside the samples to generate calibration curves. Calibration curves generated with protein standards on each blot allowed for quantification of the proteins in absolute units (nanograms per milligram or picomoles per milligram of total protein). All statistically significant results obtained during initial analysis were validated twice in separate experiments based on specific hypotheses.

Data Analysis

For Western blots, the gray values of the bands were measured on X-ray film using the Versadoc system (Bio-Rad). The optical densities of the bands corresponding to arrestins and GRKs were converted into nanograms of the respective protein based on calibration curves produced using purified proteins. Calibration curves were fitted to linear equations using Prism 4.0 (GraphPad Software, San Diego, CA). To convert the values into picomoles per milligram of protein, the following values for molecular masses were used: arrestin2, 47 kDa; arrestin3, 45 kDa; GRK2, 80 kDa; GRK3, 83 kDa; GRK5, 67.6 kDa; and GRK6, 65.9 kDa. For the statistical analysis, StatView software (SAS Institute, Cary, NC) was used. The Western blot data were analyzed by two-way analysis of variance with treatment (saline, haloperidol, and clozapine) and time (2 and 24 h) as main factors. Analysis was performed for each brain region separately followed by Student-Newman-Keuls post hoc test. When treatment $\times$ time interaction was significant, separate analysis for each time point (2 and 24 h postinjection) was also performed. The value of $p < 0.05$ was considered significant. Because the experimental design involves multiple comparisons of values (11 regions for four arrestin and GRK isoforms plus ERK) that can potentially generate false-positive results, we verified each original significant finding in two separate independent experiments.

Results

Expression of Key GPCR Regulators, Arrestin and GRKs, in the Rat Brain

We used previously characterized antibodies to arrestin2 and arrestin3 for their selectivity to rat arrestin subtypes (Gurevich et al., 2002). As reported previously (Gurevich et al., 2002, 2004), both arrestin2 and arrestin3 are ubiquitously expressed throughout the brain. Arrestin2 is the major arrestin subtype in the adult rat brain. In various brain regions, the excess of arrestin2 concentration over that of arrestin3 was 9 to 40-fold (Fig. 1). There are five nonvisual GRKs, GRK2, 3, 4, 5, and 6. GRK4 was not analyzed in this work because it has a very limited distribution in the brain. First, we tested the antibodies for selectivity and sensitivity to rat GRK subtypes using recombinant purified GRK proteins as standards. The antibodies to GRK2 and GRK5 were selective for the respective subtypes, labeling essentially one band on the blot (Fig. 2, A and C). The antibody for GRK3 is not subtype selective because it labels GRK2 as well as GRK3. However, its sensitivity to GRK3 is much higher than to GRK2, and the bands corresponding to the two isoforms are easily separated by size on 8% gel (Fig. 2B). The antibody to GRK6 labels a number of extraneous bands, but the one corresponding to GRK6 was identified with purified GRK6 standard (Fig. 2D). As shown in Fig. 1, the expression levels of various GRK subtypes differed significantly among brain regions. GRK6 is expressed at the highest level in the basal ganglia as well as in the cortical areas examined, although its expression exceed that of the second most abundant GRK subtype, GRK2, by less than 2-fold. At the same time, the GRK6 concentration in most brain regions exceeded that of GRK5 severalfold (Fig. 1). GRK3 is overall expressed at the lowest level but is relatively enriched in cortical regions (Fig. 1).

Effect of Antipsychotic Drugs on Arrestin Expression

Treatment with haloperidol produced no detectable changes in the expression of either arrestin2 or arrestin3. In contrast, atypical antipsychotic clozapine significantly reduced the arrestin2 expression in dHipp [$F(2,44) = 5.1$, $p < 0.02$; Fig. 3A] compared with both saline- and

haloperidol-treated groups. There was no significant effect of time, indicating that the changes in the arrestin2 expression were similar after 2- and 24-h washout periods (by 32 and 25.6%, respectively). The effect of clozapine in the basal ganglia regions was the opposite of that in the dHipp. Clozapine induced an up-regulation of arrestin2 in VL [$F(2,44) = 5.2, p < 0.01$; average change by 37%] and ACc [$F(2,44) = 4.3, p < 0.02$; by 23%] (Fig. 3A) compared with saline- and haloperidol-treated groups. There was no significant effect of time or treatment $\times$ time interaction, indicating that the effects were similar after both washout periods. Clozapine treatment did not alter the arrestin3 expression (data not shown).

Effect of Antipsychotic Drugs on GRK Expression

Haloperidol and clozapine differentially affected the expression of GRK subtypes in the brain regions studied. The concentration of GRK2 in PFC was significantly affected by treatment [$F(2,44) = 4.8, p < 0.02$], but there was no significant effect of time or treatment $\times$ time interaction. GRK2 expression was increased by clozapine by 30.6 and 38.6% after 2 and 24 h, respectively. Haloperidol did not affect GRK2 expression in PFC (Fig. 3B). In other brain regions, the expression of GRK2 was unchanged by either drug. In contrast, the expression of GRK3 was reduced by both haloperidol and clozapine but in different brain regions. Haloperidol significantly reduced the expression of GRK3 in VL [$F(2,45) = 3.8, p < 0.05$; by 24% both 2 and 24 h after the last injection] but not in other regions (Fig. 4A). Clozapine treatment significantly (by 22–23%) reduced the GRK3 expression in dHipp [$F(2,43) = 4.0, p < 0.05$] compared with control and in GP [$F(2,45) = 3.9, p < 0.05$] compared with control and haloperidol independent of the interval after the last injection (Fig. 4A).

Drug treatment significantly affected the expression of GRK5. The expression of GRK5 was significantly altered by treatment in cCPu [$F(2,45) = 3.64, p < 0.05$] and GP [$F(2,45) = 3.3, p < 0.05$]. There was also significant effect of time in GP [$F(1,45) = 8.3, p < 0.01$] and treatment $\times$ time interaction in both regions. The level of GRK5 in saline-treated animals was measurably higher at 2 h than 24 h after the last injection. Both APDs suppressed the GRK5 expression 2 h after the last treatment in both brain regions (Fig. 4B). It is interesting to note that clozapine increased the GRK5 concentration 24 h after the last drug administration in cCPu, but there was no such effect in GP (Fig. 4B). Neither drug altered the expression of GRK6 in any brain region examined (data not shown).

Effect of Chronic Antipsychotic Treatment on ERK Activation

Arrestins and GRKs have been linked to mitogen-activated protein kinase (MAPK) signaling pathways, where arrestins serve as scaffolds for multiprotein complexes assembled on activated phosphorylated GPCRs (Shenoy and Lefkowitz, 2003). Acute treatment with clozapine has been shown to activate ERK but not c-Jun NH₂-terminal kinase or p38 (Browning et al., 2005). We have asked whether chronic treatment with APDs affects ERK activation and whether ERK is activated in the same brain regions where antipsychotics alter the expression of arrestins and GRKs. The level and pattern of ERK phosphorylation varied among brain regions (Figs. 5 and 6). In all regions, the major phosphorylated ERK subtype was ERK2 (42 kDa) (Fig. 5, A and C), and the concentration of total ERK2 was higher than that of ERK1 in all regions (Fig. 5, B and D). We have found that ERK1 phosphorylation was significantly enhanced only by clozapine in two of 11 brain regions examined (Fig. 6). In PFC, the effect of clozapine was transient; the concentration of phosphorylated ERK2 was elevated 2 h (by 75%) but not 24 h after the last injection [$F(2,44) = 5.4, p = 0.008$ for group effect and $F = 4.9, p = 0.0125$ for group $\times$ time interaction]. In contrast, in the VL subdivision of the striatum, enhanced ERK2 phosphorylation was detected 2 h (by 118%) as well as 24 h (by 77%) after the last injection [$F(2,44) = 6.8, p = 0.0027$] (Fig. 6). There were no differences in the levels of total ERK1,2 or phosphorylated ERK1 in any of the brain regions examined (data not shown).

Discussion

We have surveyed changes in expression of the key components of the GPCR desensitization machinery after chronic treatment with the classic typical antipsychotic haloperidol and the atypical antipsychotic clozapine in 11 brain regions linked to pathophysiology of schizophrenia and actions of APDs. The data show that the two drugs elicited differential changes in the expression of arrestins and GRKs (Table 1). The changes observed in this study were modest in magnitude (20–30%). However, even relatively small changes in the availability of arrestins and GRKs may be functionally important. For example, mice hemizygous for arrestin and GRK knockouts, which have roughly 50% less protein than wild-type mice, often demonstrate behavioral phenotype nearly identical to that of corresponding knockout mice (Gainetdinov et al., 2004; Premont and Gainetdinov, 2007). Both APDs transiently down-regulated GRK5, and haloperidol decreased GRK3 in the striatal regions. These effects are strongly reminiscent of the down-regulation of GRK3 and 5 caused by dopamine depletion in the basal ganglia (Ahmed et al., 2007) and, thus, may be mediated by blockade of striatal dopamine receptors. Haloperidol did not alter the expression of either arrestin subtype in any brain region and did not increase the concentration of any protein studied. In contrast, clozapine increased the expression of arrestin2 and GRK2 in specific brain regions (Table 1). It is interesting to note that neither haloperidol nor clozapine affected the expression of GRK6, the kinase that has been shown to specifically regulate dopamine receptors (Gainetdinov et al., 2004). Clozapine affected more brain regions than haloperidol, and only clozapine affected cortical areas such as PFC and dHipp (Table 1). Haloperidol had no effect on ERK2 activation in any region, whereas clozapine enhanced ERK2 activation. It is interesting to note that the clozapine-induced ERK activation in PFC, although transient, was not desensitized during chronic treatment, as is often the case with repeated drug administration. The magnitude of the effect seen here (75% elevation) is comparable with that reported previously for acute clozapine treatment, although a lower dose of clozapine (10 mg/kg) was used in that study (Browning et al., 2005). Previous research has demonstrated that haloperidol and clozapine cause similar (Alimohamad et al., 2005) as well as differential changes in the expression and activity of signaling proteins (Dwivedi et al., 2002). Other ADPs also can alter signaling pathways (Singh et al., 2007). Chronic treatment with atypical antipsychotic olanzapine, but not with haloperidol, has been found to enhance ERK activation in PFC (Fumagalli et al., 2006) in a manner similar to that we detected with clozapine. It is unfortunate that only a limited number of signaling effects of a few APDs have been examined so far. In this work, only two APDs, haloperidol and clozapine, were tested. Thus, no prediction can be made as to whether or how other typical or atypical APDs would change the arrestin/GRK expression or ERK activation. Some specific effects observed in this study could be unique for haloperidol or clozapine, whereas others may be shared by all or some typical or atypical drugs. Additional studies with multiple APDs are required to identify the changes in the arrestin2/GRK2 expression and ERK activation characteristic for typical versus atypical APD classes.

APDs interact with multiple neurotransmitter receptors, thereby affecting intracellular signaling pathways. The receptor activity may alter transcription, post-transcriptional regulation, or degradation of arrestins and GRKs. Persistent GPCR stimulation or blockade often leads to enhanced or suppressed expression of arrestin/GRKs, respectively (Hurlé, 2001; Díaz et al., 2002; Fan et al., 2002). It is conceivable that haloperidol and clozapine decreased the expression of GRKs in striatal regions via blockade of dopamine D2 and/or D3 receptors. A more widespread effect of clozapine on the arrestin/GRK expression may be related to a wider pharmacological profile of the drug (Roth et al., 2004; Abi-Dargham and Laruelle, 2005; Miyamoto et al., 2005). The action of clozapine in the dHipp may be mediated by 5-hydroxytryptamine 1A receptors abundant in hippocampal pyramidal cells. Clozapine is a partial agonist to 5-hydroxytryptamine 1A and several other GPCRs (Roth et al., 2004; Miyamoto et al., 2005), which may contribute to the observed molecular effects. In addition,

clozapine, but not haloperidol, also stimulates dopamine, norepinephrine, and acetylcholine release in the cortex (Ichikawa et al., 2002; Pira et al., 2004), which adds another layer of complexity to the molecular mechanisms of the clozapine action. The elevation of the GRK2 expression and ERK2 activation by clozapine in PFC may be linked, considering that ERK-mediated phosphorylation of GRK2 regulates the function of GRK2 as well as its transcription and degradation (Penela et al., 2003). Molecular events leading from the drugs' interactions with specific GPCRs to alterations in the arrestin/GRK expression are likely to be complex, involving multiple receptors and signaling pathways. The design of this study did not allow for elucidation of molecular mechanisms mediating the drugs' effects on the arrestin/GRK expression. Additional research is required to uncover molecular mechanisms underlying observed changes in the arrestin/GRK expression caused by haloperidol or clozapine treatment.

The current paradigm of the arrestin-dependent GPCR desensitization and trafficking posits that upon activation, a receptor is phosphorylated by a GRK, and arrestin binding to the phosphorylated receptor precludes further receptor-G protein interaction (for review, see Gurevich and Gurevich, 2006). Due to the mode of arrestin action, e.g., 1:1 interaction with receptors, the availability of arrestins in cells has a profound effect on receptor sensitivity. Indeed, numerous data show that overexpression of arrestins facilitates GPCR trafficking, whereas reduced arrestin concentration leads in most cells to deficits in GPCR desensitization and exaggerated signaling via affected receptors (Bohn et al., 2003; Gainetdinov et al., 2004). Similar to arrestins, up- or down-regulation of GRKs facilitates or impedes, respectively, GPCR desensitization in vitro and in vivo (Gainetdinov et al., 2004). Concomitant modulation of arrestin and GRK concentrations has a particularly strong effect on GPCR trafficking (Kim et al., 2001; Pan et al., 2003). Thus, simultaneous down-regulation of arrestin2 and GRK3 in dHipp by clozapine might conceivably result in an enhanced signaling via specific GPCRs important for cognition and/or affective functions, which may contribute to superior efficacy of clozapine toward negative and cognitive symptoms. The clozapine-induced up-regulation of the arrestin2 expression in the striatal and nucleus accumbens subterritories may act in concert with the blockade of dopamine and other receptors to further inhibit G protein-mediated signaling in these regions. Arrestins, in addition to their role in GPCR desensitization, serve as scaffolding proteins coupling GPCRs to the MAPK signaling pathways (for review, see Lefkowitz and Whalen, 2004). Arrestin-mediated activation of MAPK is enhanced by arrestin overexpression and inhibited by arrestin depletion (Tohgo et al., 2002; Ahn et al., 2003). Thus, enhanced ERK activation by clozapine observed in VL may be linked to up-regulation of arrestin2 expression also detected in this region. Taken together, the data demonstrate that haloperidol and clozapine elicit specific molecular modifications of the GPCR regulation machinery in multiple brain areas, accompanied in the case of clozapine by changes in downstream signaling. The arrestin/GRK-mediated desensitization of GPCRs is a feedback mechanism designed to regulate the GPCR signaling. The availability of arrestins and GRKs is a critical factor that determines how this feedback functions. Changes in the arrestin/GRK concentration induced by drug treatment would alter the GPCR availability and/or signaling and impact upon the drug activity. Thus, arrestin and GRKs are perfectly positioned as components of neuroplastic changes that are increasingly recognized as the foundation of the therapeutic activity of APDs (Frost et al., 2004). Therefore, designing APDs with defined abilities to modulate arrestins and/or GRKs may prove beneficial in the treatment of schizophrenia.

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ABBREVIATIONS

APD	antipsychotic drug
EPS	extrapyramidal symptom
GPCR	G protein-coupled receptor
GRK	G protein-coupled receptor kinase
ERK	extracellular signal-regulated kinase
PFC	prefrontal cortex
dHipp	dorsal hippocampus
VL	ventrolateral caudate-putamen
cCPu	caudal caudate-putamen
GP	globus pallidus
MAPK	mitogen-activated protein kinase

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Fig. 1.

Comparative expression levels of arrestin and GRK subtypes in the control rat brains. Bar graph, means $\pm$ S.E.M. Conditions for Western blotting and antibody labeling were as described under *Materials and Methods*. ACsh, shell subdivision of the nucleus accumbens; Cg, cingulate cortex; DL, dorsolateral subdivision of the striatum; DM, dorsomedial subdivision of the striatum; VM, ventromedial subdivision of the striatum.

Fig. 2.

Representative Western blot demonstrating antibody specificity and the expression of GRKs in the rat brain. A, expression of GRK2; B, labeling of GRK3 compared with GRK2 by the antibody directed against GRK3. Right lanes show the indicated amount of purified GRK2 and GRK3 run on the same gel; C, expression of GRK5; D, antibody specificity and expression of GRK6. Left lanes show standards containing the indicated amounts of purified GRKs. The following lanes show bands of GRK subtypes detected in the rat brain. To detect GRK2 and GRK5, 0.625 μ g of total protein was loaded per lane; to detect GRK3, 1.25 μ g protein/lane; and to detect GRK6, 5 μ g protein/lane. Antibodies to GRK2 and GRK5 (in most regions) selectively label one band corresponding to the appropriate kinase. The antibody against GRK3 also labels GRK2, but the two kinases are clearly separated on 8% polyacrylamide gel electrophoresis. The antibody directed against GRK6 labels extraneous bands, but the band corresponding to GRK6 is clearly separated from others on 8% polyacrylamide gel electrophoresis. Abbreviations are the same as in Fig. 1.

Fig. 3.

Expression of arrestin2 (A) and GRK2 (B) in the rat brain after chronic treatment with haloperidol or clozapine. Drug treatment was as described under *Materials and Methods*. Bar graphs show the results of quantitative analysis of Western blots as percentages (mean $\pm$ S.E.M.) from corresponding (2 or 24 h) control values. Note that for statistical analysis, absolute values were used. Also note that S.E.s for ratios are more than 2-fold larger than S.E.s of absolute values. Between 0.45 and 1.25 μ g protein/lane (less for cortical regions) were used. The data were statistically analyzed separately for individual brain regions by two-way analysis of variance with group (control, haloperidol, clozapine) and time (2 and 24 h) as main factors followed by Student-Newman-Keuls post hoc test. *, $p < 0.05$; **, $p < 0.01$ to control; #, $p < 0.05$ to the haloperidol (both clozapine groups are different from both haloperidol groups). Co2h and Co24h, control groups treated with saline and sacrificed 2 or 24 h after the last injections; HAL-2h and HAL-24h, groups treated with haloperidol; CLZ-2h and CLZ-24h, groups treated with clozapine. Abbreviations for the brain regions are the same as in Fig. 1.

Fig. 4.

Expression of GRK3 (A) and GRK5 (B) in the rat experimental groups presented as percentages from corresponding (2 or 24 h) control values. GRK3 was measured using 2.5 μ g protein/lane, and GRK5 was measured using 1.25 μ g protein/lane. The data were statistically analyzed and are presented as described in the legend to Fig. 3 and *Materials and Methods*. *, $p < 0.05$ to control; #, both clozapine groups are different from both haloperidol groups with $p < 0.05$; \$, $p < 0.05$ to the corresponding haloperidol group. Abbreviations are as listed in legends to Figs. 1 and 3.

Fig. 5.

Representative Western blots illustrating the levels of ERK phosphorylation and expression in the PFC and dorsolateral striatum (DL) of rats treated with haloperidol (HAL) and clozapine (CLZ). A and C, phospho-ERK in PFC (A) and VL (C); B and D, total ERK in PFC (B) and VL (D). Abbreviations are as listed in legends to Figs. 1 and 3.

Fig. 6.

The concentration of phosphorylated ERK2 (p42 MAPK) in the rat brain after chronic treatment with haloperidol or clozapine shown as percentages from corresponding (2 or 24 h) control values. The data were statistically analyzed as described in the legend to Fig. 3 and *Materials and Methods*. *, $p < 0.05$ to control; #, $p < 0.05$ to haloperidol (both clozapine groups are different from both haloperidol groups). Abbreviations are as listed in legends to Figs. 1 and 3.

TABLE 1

Summary of the changes in the expression of arrestins and GRKs induced by the treatment with haloperidol or clozapine

Brain Region	Haloperidol	Clozapine
Cortical		
Prefrontal cortex	↔	GRK2↑ pERK↑
Cingulate cortex	↔	↔
Dorsal hippocampus	↔	ARR2↓ GRK3↓
Basal ganglia		
Nucleus accumbens core	↔	ARR2↑
Nucleus accumbens shell	↔	↔
Caudatoputamen dorsomedial	↔	↔
Caudatoputamen dorsolateral	↔	↔
Caudatoputamen ventromedial	↔	↔
Caudatoputamen ventrolateral	GRK3↓	ARR2↑ pERK↑
Caudal caudatoputamen	GRK5↓(2h)	GRK5↓(2 h) ↑(24 h)
Globus pallidus	GRK5↓(2h)	GRK5↓(2 h) GRK3↓

↔, no significant differences; ↓ or ↑, a protein was significantly decreased or increased in the drug-treated as compared with the vehicle-treated group; (2 h) or (24 h), the protein was altered 2 or 24 h after the last injection; otherwise, the changes were detected with both washout periods.