Protein Cell 2011, 2(11): 899–905
DOI 10.1007/s13238-011-1096-0

Protein & Cell

COMMUNICATION

Parkinson disease drug screening based on the interaction between D_2 dopamine receptor and beta-arrestin 2 detected by capillary zone electrophoresis

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Received July 10, 2011 Accepted August 5, 2011

ABSTRACT

Parkinson's disease is the second most common neurodegenerative disease in the world. Beta-arrestin-2 has been reported to be an important protein involved in D_2 dopamine receptor desensitization, which is essential to Parkinson's disease. Moreover, the potential value of pharmacological inactivation of G protein-coupled receptor kinase or arrestin in the treatment of patients with Parkinson's disease has recently been shown. We studied the interaction between D_2 dopamine receptor and beta-arrestin-2 and the pharmacological regulation of chemical compounds on such interaction using capillary zone electrophoresis. The results from screening more than 40 compounds revealed three compounds that remarkably inhibit the beta-arrestin-2/D_2 dopamine receptor interaction among them. These compounds are promising therapies for Parkinson's disease, and the method used in this study has great potential for application in large-scale drug screening and evaluation.

KEYWORDS drug screening, D_2 dopamine receptor, beta-arrestin-2, capillary zone electrophoresis, protein-protein interaction, Parkinson's disease

INTRODUCTION

G protein-coupled receptors (GPCRs) form a superfamily of heptahelical proteins that mediate various biological processes, ranging from neurotransmission and hormonal control of virtually all physiological responses to perception of taste, smell, light, and pain (Kobilka and Deupi, 2007; Rosenbaum et al., 2009). Upon stimulation, heptahelical receptors undergo conformational changes that allow binding of heterotrimeric G proteins, leading to the activation of different effectors and signaling pathways (Kobilka and Deupi, 2007). GPCRs also control their own responsiveness by activating mechanisms that result in their desensitization (Gainetdinov et al., 2004; Whalen et al., 2011). One of the first steps in this process involves the functional uncoupling of the G proteins from the receptors. This process is fairly rapid (from seconds to minutes) and depends on receptor phosphorylation by intracellular second-messenger-regulated kinases and GPCR kinases (GRKs) (Premont et al., 1995; Premont and Gainetdinov, 2007).

Second-messenger-regulated kinases can phosphorylate both active and inactive receptors, yielding heterologous desensitization. In the latter case, receptors that have not been activated may become desensitized by the activation of distinct receptors. However, GRKs specifically phosphorylate only agonist-occupied receptors, resulting in homologous desensitization (Reiter and Lefkowitz, 2006; Kelly et al., 2008).

GRK phosphorylation of receptors is not sufficient for desensitization and instead serves to create high-affinity sites to promote the binding of arrestin proteins, which in turn guarantees desensitization by preventing further coupling to G proteins (Reiter and Lefkowitz, 2006). Four distinct mammalian arrestin proteins are known, two of which (visual and cone arrestins) are restricted to the phototransduction pathway (Lefkowitz and Shenoy, 2005). The somatic isoforms β-arrestin-1 (arrestin-2) and β-arrestin-2 (arrestin-3) are ubiquitously expressed and assumed to regulate signaling as well as internalization of many heptahelical receptors

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(Reiter and Lefkowitz, 2006; DeWire et al., 2007; Ma and Pei, 2007; Whalen et al., 2011).

Dopamine receptors (DRs), which can be classified into D_1 and D_2 subtypes based on their pharmacological and biochemical characteristics, belong to the GPCR superfamily (Andersen et al., 1990; Neve et al., 2004). D_2DR has been implicated in the pathophysiology and treatment of movement disorders, schizophrenia, and drug addiction (Emilien et al., 1999; Goldman-Rakic et al., 2004; Mehler-Wex et al., 2006). Parkinson's disease is the second most common neurodegenerative disorder; it is caused by the degeneration of dopaminergic neurons in the substantia nigra (Olanow et al., 2006; Savitt et al., 2006). The lack of dopamine leads to disturbances in the basal ganglia circuitry and, consequently, to motor deficits. Dopamine replacement therapy with L-dopamine, the precursor of dopamine, is an effective way to correct motor abnormalities, but long-term therapy often results in reduced response to drug and motor complications, such as dyskinesia (Fahn et al., 2004; Dodd et al., 2005).

The desensitization of D_2DR is mediated by β-arrestin-2, which binds to the phosphorylated D_2DR to terminate the signal cascade (Oakley et al., 2000; Macey et al., 2004; Whalen et al., 2011). Suppression or inhibition of β-arrestin-2 could become an effective strategy to enhance the anti-nociceptive properties of analgesic morphine and reduce its side effects. In contrast, inactivation of the arrestin proteins could result in relatively amplified efficacy of endogenous or exogenous dopamine stimulation and is potentially helpful in restoring movement among patients with Parkinson's disease.

Capillary electrophoresis (CE) is an important and powerful method to study protein–protein interactions (Ding et al., 2005; Kostal et al., 2008). Capillary zone electrophoresis (CZE) is probably the most common mode of CE. CZE involves the electrophoretic separation of analytes under constant temperature and pH conditions in free solution within a capillary. Compared with other methods, CE can obtain more concrete results over a short period with small samples (in nanograms) (Holzgrabe et al., 2006; Kostal et al., 2008).

As β-arrestin-2 is one of the key elements in the desensitization of D_2DR, inhibition of its activity may enhance the signaling from the upstream signal pathway (DeWire et al., 2007). The dopamine theory of Parkinson's disease indicates that dopamine deficiency in the basal ganglia of the brain, which results in decreased signals from D_2DR, is the main cause of the disease (Obeso et al., 2008; Calabresi et al., 2009). Recent studies on the mechanism of receptor desensitization have proposed the use of β-arrestin-2 as a target for drug screening (Dromey and Pfleger, 2008; Levoye and Jockers, 2008). In this study, we used CZE to detect the interaction between D_2DR and β-arrestin-2. Large-scale drug screening indicated that three chemical compounds remarkably inhibited the said interaction, which renders them as potential drugs for the treatment of Parkinson's disease.

RESULTS AND DISCUSSION

Detection of the D_2DR/β-arrestin-2 complex

The desensitization of GPCR depends on the D_2DR/β-arrestin-2 interaction (Macey et al., 2004; Skinbjerg et al., 2009). We used β-arrestin-2 as a target protein for drug screening against Parkinson's disease. CE with laser-induced fluorescence (CE-LIF) has been proposed to detect and monitor protein–peptide and protein–protein interactions efficiently (García-Campaña et al., 2007; Yang et al., 2007). In this study, we detected a stable complex of FITC-arrestin/D_2DR using CE-LIF. The influence of different drugs on the complex could verify potential drugs for further screening. This method could be developed as an effective and simple instrument for large-scale drug screening and evaluation. Figure 1 shows the electropherograms of the FITC-arrestin/D_2DR complex detected by CE-LIF. CZE analysis of the single sample containing 2.71 μmol/L FITC-labeled β-arrestin-2 showed two peaks at 8.5 and 9.5 min, with the first peak representing FITC-arrestin and the second representing free FITC (Fig. 1A). However, CZE analysis of a mixture of 2.71 μmol/L FITC-labeled β-arrestin-2 with 1.26 μmol/L D_2DR identified three populations that migrated at 5.5, 8.5, and 9.5 min (Fig. 1B); the peak at 5.5 min represented the FITC-arrestin/D_2DR complex. As only β-arrestin-2 was labeled with FITC, FITC-labeled β-arrestin-2 (the peak at 8.5 min), free FITC (the peak at 9.5 min), and the FITC-arrestin/D_2DR complex (the peak at 5.5 min) were detected in chromatograms. CZE analysis of the mixture of FITC and D_2DR (at the same concentration but without incubation) was immediately performed to confirm whether the new peak, which appeared before the FITC-labeled β-arrestin-2, corresponded to the FITC-arrestin/D_2DR complex but not the FITC-labeled D_2DR. The results showed that FITC cannot bind to D_2DR in such a short period. Future experiments on the negative control protein of either mutant β-arrestin-2 or mutant D_2DR should demonstrate the specificity and functional relevance of this complex formation.

Pharmacological regulation of chemical compounds detected by CZE

More than 40 compounds, including phytic acid, 3-amino ethylamine, and 1,3-dichloro-2-propanol, were added to the mixtures to test the pharmacological regulation of the drugs on the D_2DR/β-arrestin-2 interaction. The mixtures were then injected into uncoated fused capillaries and separated by the different charges and sizes. We conveniently obtained information about the interaction from the electropherograms. Some compounds have acute effects on the native structures of the target proteins, whereas others have more moderate regulatory activities on the interaction, which showed more drug-like properties because the physiological regulations are usually reversible.

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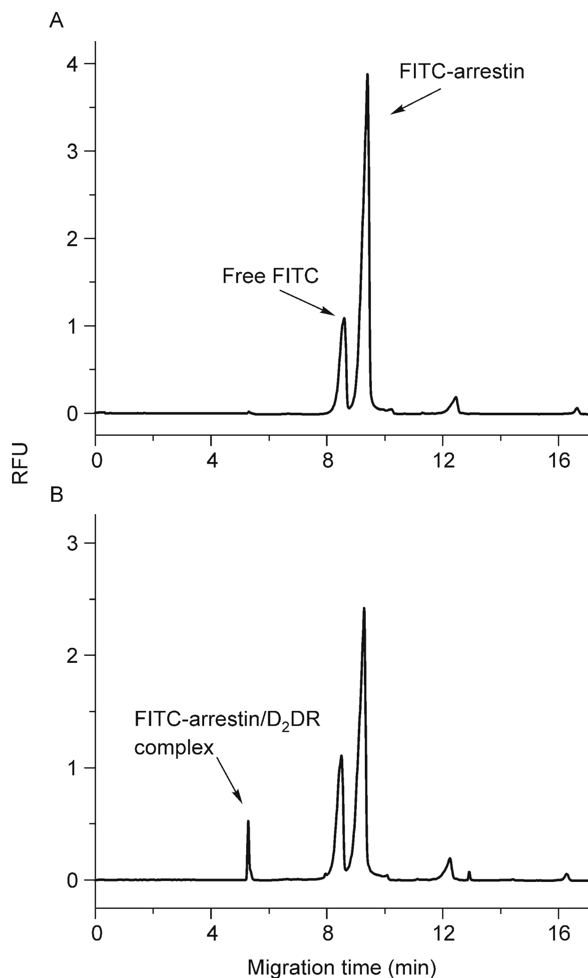

Figure 1. Electropherograms of 2.71 μmol/L FITC-labeled β-arrestin-2 (A) and 2.71 μmol/L FITC-labeled β-arrestin-2 incubated with D_2DR (B). Conditions: applied voltage, 15 kV; uncoated fused silica capillaries, 30 cm (effective length, 20 cm) × 25 μm i.d.; buffer, 25 mmol/L Na_3PO_4 at pH 7.4; injection, 0.5 psi for 5 s.

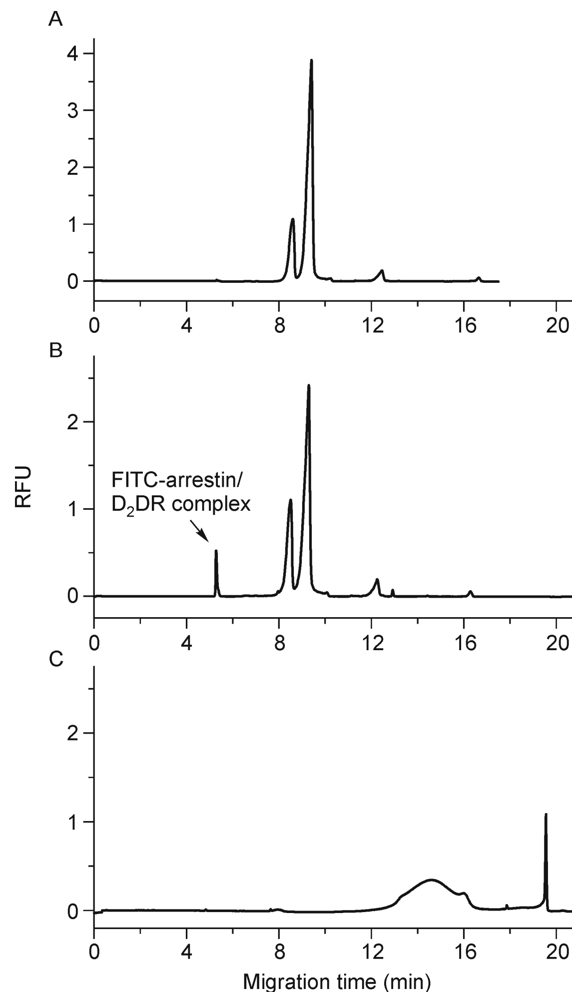

Figure 2. Electropherograms of 2.71 μmol/L FITC-labeled β-arrestin-2 (A), the mixture of 2.71 μmol/L FITC-labeled β-arrestin-2 and 1.26 μmol/L D_2DR (B), and 33 μmol/L 3-amino ethylamine added to the mixture of FITC-labeled β-arrestin-2 and D_2DR (C). Conditions: applied voltage, 15 kV; uncoated fused silica capillaries, 30 cm (effective length, 20 cm) × 25 μm i.d.; buffer, 25 mmol/L Na_3PO4 at pH 7.4; injection, 0.5 psi for 5 s.

3-amino ethylamine

Many chemical compounds, such as 3-amino ethylamine, acutely influence target proteins, but most irreversible actions to biomacromolecules could result in cell damage. Figure 2 shows the electropherograms of 33 μmol/L 3-amino ethylamine added to the mixture of 2.71 μmol/L FITC-arrestin and 1.26 μmol/L D_2DR. Although the FITC-arrestin/D_2DR complex was completely inhibited, the mobility of all peaks significantly increased, suggesting that either the characteristics of the capillary were modified or this chemical compound affected the structures of the target proteins. Therefore, the chemical compounds that influenced the FITC-arrestin/D_2DR complex similarly to 3-amino ethylamine have less drug-like properties.

Phytic acid

Phytic acid (myo-inositol hexaphosphoric acid) is an abundant plant constituent that comprises 1%–5% of edible legumes, cereals, oil seeds, pollens, and nuts. It has been reported to bind to photoreceptor arrestin and block its interaction with rhodopsin (Wilson and Copeland, 1997). Such an interaction predicts that phytic acid could alter GPCR desensitization. We investigated this inhibitory action using CZE. Phytic acid (33 μmol/L) was added to the mixture of 2.71 μmol/L FITC-labeled β-arrestin-2 and 1.26 μmol/L D_2DR (Fig. 3C). Compared with the data illustrated in panels A and B of Fig. 3, the FITC-arrestin/D_2DR complex was remarkably

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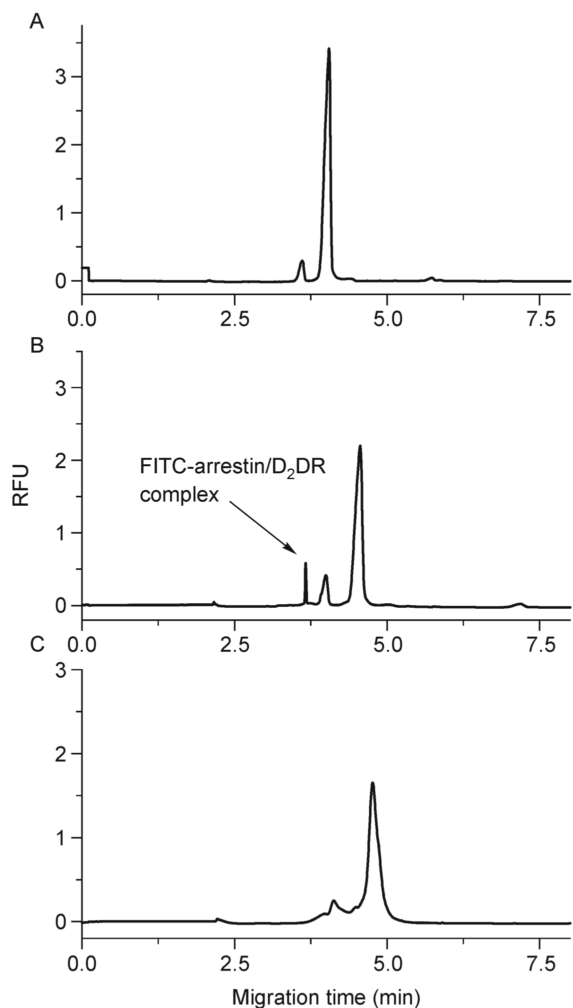

Figure 3. Electropherograms of 2.71 μmol/L FITC-labeled β-arrestin-2 (A), the mixture of 2.71 μmol/L FITC-labeled β-arrestin-2 and 1.26 μmol/L D_2DR (B), and 33 μmol/L phytic acid added to the mixture of FITC-labeled β-arrestin-2 and D_2DR (C). Conditions: applied voltage, 15 kV; uncoated fused silica capillaries, 30 cm (effective length, 20 cm) × 25 μm i.d.; buffer, 25 mmol/L Na_3PO_4 at pH 7.4; injection, 0.5 psi for 5 s.

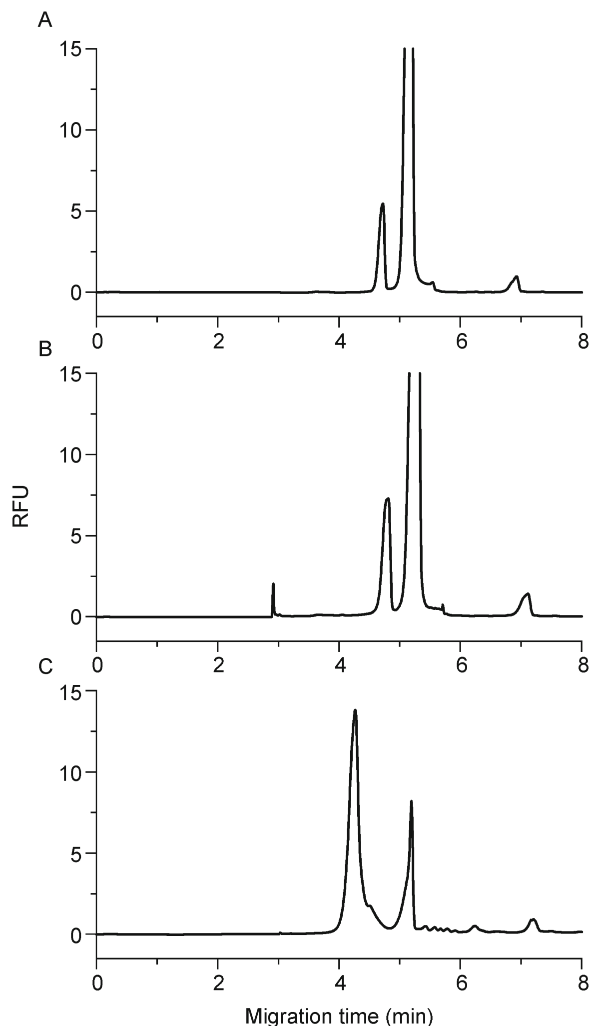

Figure 4. Electropherograms of 2.71 μmol/L FITC-labeled β-arrestin-2 (A), the mixture of 2.71 μmol/L FITC-labeled β-arrestin-2 and 1.26 μmol/L D_2DR (B), and 33 μmol/L ethylenediamine added to the mixture of FITC-labeled β-arrestin-2 and D_2DR (C). Conditions: applied voltage, 15 kV; uncoated fused silica capillaries, 30 cm (effective length, 20 cm) × 25 μm i.d.; buffer, 25 mmol/L Na_3PO_4 at pH 7.4; injection, 0.5 psi for 5 s.

inhibited by phytic acid, suggesting that phytic acid has a detectable interaction with β-arrestin-2 and thereby blocks further binding of β-arrestin-2 to D_2DR. Phytic acid is hence a promising therapy for Parkinson's disease.

Ethylenediamine and 1,3-dichloro-2-propanol

When 33 μmol/L ethylenediamine was added to the mixture of 2.71 μmol/L FITC-labeled β-arrestin-2 and 1.26 μmol/L D_2DR, the peak of FITC-arrestin/D_2DR complex in the electropherogram disappeared (Fig. 4C), indicating that ethylenediamine strongly inhibited the D_2DR/β-arrestin-2 interaction. Therefore, such chemical compounds as ethylenediamine that can inhibit the interaction have potential for use in the treatment of Parkinson's disease. The screening results demonstrated that 1,3-dichloro-2-propanol had inhibition properties similar to those of ethylenediamine. When 33 μmol/L or 67 μmol/L 1,3-dichloro-2-propanol (or 100 μmol/L 1,3-dichloro-2-propanol; data not shown) was added to the mixture of FITC-labeled β-arrestin-2 and D_2DR, the peak of FITC-arrestin/D_2DR complex in the electropherograms remarkably decreased; this peak decreased with increasing concentrations of the compound (Fig. 5C and 5D). Dose-response analysis (33–100 μmol/L) on the claimed inhibitor 1,3-dichloro-2-propanol, which is important for small-molecule inhibitor studies, was conducted. Overall, the results indi-

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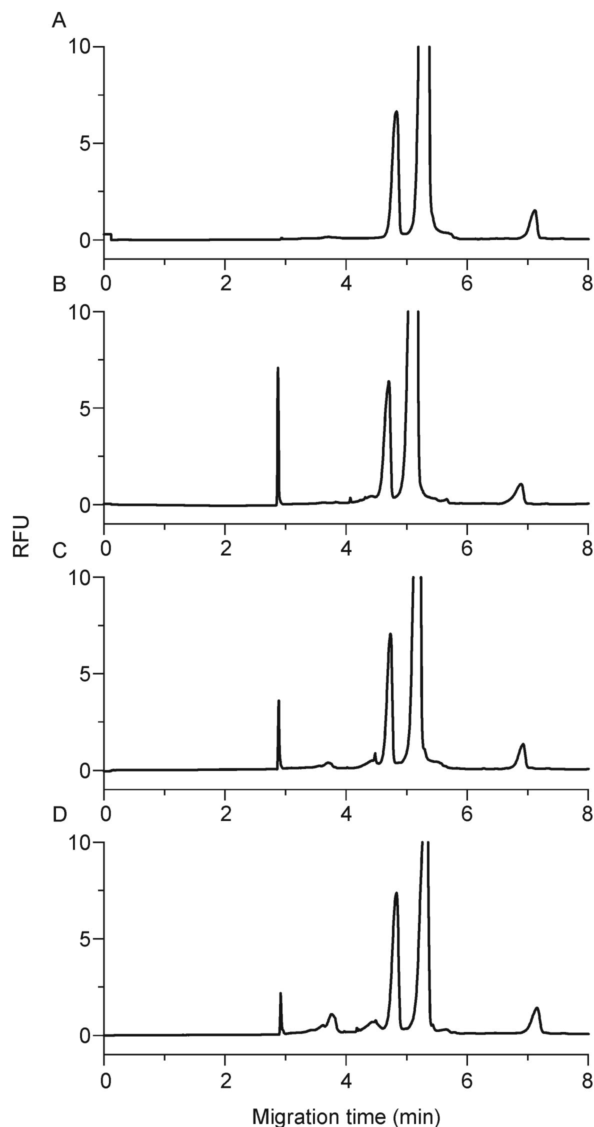

Figure 5. Electropherograms of 2.71 μmol/L FITC-labeled β-arrestin-2 (A), 2.71 μmol/L FITC-labeled β-arrestin-2 incubated with 1.26 μmol/L D_2DR (B), and 33 μmol/L (C) or 67 μmol/L (D) 1,3-dichloro-2-propanol added to the mixture of FITC-labeled β-arrestin-2 and D_2DR. Conditions: applied voltage, 15 kV; uncoated fused silica capillaries, 30 cm (effective length, 20 cm) × 25 μm i.d.; buffer, 25 mmol/L Na_3PO_4 at pH 7.4; injection, 0.5 psi for 5 s.

cated that 1,3-dichloro-2-propanol remarkably inhibited the D_2DR/β-arrestin-2 interaction and is a promising drug for the treatment of Parkinson's disease.

This study screened potential drugs for Parkinson's disease using CZE, which is based on the D_2DR/β-arrestin-2 interaction. Validation experiments using more traditional binding assays, such as immunoprecipitation and GST pull-down, should be conducted to demonstrate the effectiveness of the compounds in the cells. More data on the screened compounds at the cell and animal model levels are clearly needed.

CONCLUSIONS

Parkinson's disease is a chronic progressive neurodegenerative movement disorder (Jankovic, 2008). The typical drug screening strategy for it is based on the dopamine theory. Although L-dopamine-like drugs have improved the health of patients with Parkinson's disease, they are associated with many adverse effects (Calabresi et al., 2009; Dagher and Robbins, 2009). Specifically, research has shown that long-term treatment with L-dopamine may trigger symptoms of schizophrenia. Alternatively, pharmacological inactivation of GRK or arrestin could provide an effective approach to amplify the efficacy of endogenous or exogenous dopamine stimulation and will be helpful in restoring movement among patients with Parkinson's disease. Drug-designing targets in D_2DR-related proteins, such as arrestin or GRK, remain unexplored and should thus be evaluated. In this study, we utilized CZE to screen potential drugs and initially found three valuable chemical compounds that have significant dose-dependent effects on the D_2DR/β-arrestin-2 interaction. These compounds have promising applications in the treatment of Parkinson's disease with reduced side effects. Although these compounds need to be studied further, CZE proved to be an effective and convenient method of drug screening. It may be used for large-scale drug screening and evaluation.

MATERIALS AND METHODS

Reagents and materials

D_2DR was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemical compounds, such as 1,3-dichloro-2-propanol and ethylenediamine, and plasmid-encoded rat β-arrestin-2 were kindly provided by Prof. Xiang Zhou (College of Chemistry and Molecular Sciences, Wuhan University) and Prof. Robert J. Lefkowitz (Department of Medicine, Duke University Medical Center), respectively. β-arrestin-2 was expressed in *Escherichia coli* and purified according to published procedures (Xiao et al., 2004). All the other reagents used were of analytical grade.

Apparatus

CE experiments were performed in a Beckman P/ACE MDQ apparatus (Beckman-Coulter, Fullerton, CA, USA) with a LIF detector (excitation at 488 nm; emission at 520 nm) and a liquid cooling system. The uncoated fused silica capillaries (Yongnian Optic Fiber, China) measured 30 cm × 25 μm i.d. with a 20-cm distance from the detector.

Solutions and sample preparation

The running buffer in CE experiments was 10 mmol/L NaH_2PO_4-

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Na_2HPO_4 buffer (pH 8.0). All the solutions and deionized water were filtered through 0.22-μm cellulose acetate membrane filters and stored at 4°C. The concentrations of proteins were determined using Bio-Rad protein assays.

Preparation of FITC-labeled β-arrestin-2

FITC was dissolved in deionized water to a final concentration of 0.1 mmol/L. An aliquot (0.1 mL) of 0.1 mmol/L FITC solution was mixed with 0.9 mL of 0.1 mmol/L β-arrestin-2 (100 μL diluted in 800 μL of 20 mmol/L sodium bicarbonate buffer, pH 9.0). The solution was mixed thoroughly, left at 4°C and in the dark overnight, and then diluted to the desired concentrations with the running buffer prior to the CE experiments.

CE experiments

A new capillary was flushed with 1 mol/L NaOH for 60 min and then with deionized water for 60 min. Between measurements, the capillary was flushed with 1 mol/L NaOH for 2 min, with deionized water for 2 min, and with running buffer for 3 min. The capillary chamber temperature was controlled at 25°C by the liquid cooling system. The field strength of the capillary was 500 V/cm in the normal polarity mode. The samples were pressure-injected into the capillary at 0.5 psi for 5 s.

ACKNOWLEDGEMENTS

This work was supported by the National Key Basic Research Foundation of China (Grant No. 2006CB910301), the National Programs for High Technology Research and Development Program (863 Program) (No. 2004AA404260), the National Natural Science Foundation of China (Grant Nos. 30970599 and 30770421), and the Fundamental Research Funds for the Central Universities of China (Grant No. 1104006).

ABBREVIATIONS

CE, capillary electrophoresis; CE-LIF, capillary electrophoresis with laser induced fluorescence; CZE capillary zone electrophoresis; D_2DR, D_2 dopamine receptor; GPCR, G protein coupled receptors; GRKs, G protein-coupled receptor kinases

REFERENCES

Andersen, P.H., Gingrich, J.A., Bates, M.D., Dearry, A., Falardeau, P., Senogles, S.E., and Caron, M.G. (1990). Dopamine receptor subtypes: beyond the D1/D2 classification. Trends Pharmacol Sci 11, 231–236.

Calabresi, P., Mercuri, N.B., and Di Filippo, M. (2009). Synaptic plasticity, dopamine and Parkinson's disease: one step ahead. Brain 132, 285–287.

Dagher, A., and Robbins, T.W. (2009). Personality, addiction, dopamine: insights from Parkinson's disease. Neuron 61, 502–510.

DeWire, S.M., Ahn, S., Lefkowitz, R.J., and Shenoy, S.K. (2007). β-arrestins and cell signaling. Annu Rev Physiol 69, 483–510.

Ding, L., Zhang, X.X., Wei, P., Fan, K., and Lai, L. (2005). The interaction between severe acute respiratory syndrome coronavirus 3C-like proteinase and a dimeric inhibitor by capillary electrophoresis. Anal Biochem 343, 159–165.

Dodd, M.L., Klos, K.J., Bower, J.H., Geda, Y.E., Josephs, K.A., and Ahlskog, J.E. (2005). Pathological gambling caused by drugs used to treat Parkinson disease. Arch Neurol 62, 1377–1381.

Dromey, J.R., and Pfleger, K.D. (2008). G protein coupled receptors as drug targets: the role of β-arrestins. Endocr Metab Immune Disord Drug Targets 8, 51–61.

Emilien, G., Maloteaux, J.M., Geurts, M., Hoogenberg, K., and Cragg, S. (1999). Dopamine receptors—physiological understanding to therapeutic intervention potential. Pharmacol Ther 84, 133–156.

Fahn, S., Oakes, D., Shoulson, I., Kieburtz, K., Rudolph, A., Lang, A., Olanow, C.W., Tanner, C., and Marek, K., and the Parkinson Study Group. (2004). Levodopa and the progression of Parkinson's disease. N Engl J Med 351, 2498–2508.

Gainetdinov, R.R., Premont, R.T., Bohn, L.M., Lefkowitz, R.J., and Caron, M.G. (2004). Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 27, 107–144.

García-Campaña, A.M., Taverna, M., and Fabre, H. (2007). LIF detection of peptides and proteins in CE. Electrophoresis 28, 208–232.

Goldman-Rakic, P.S., Castner, S.A., Svensson, T.H., Siever, L.J., and Williams, G.V. (2004). Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology (Berl) 174, 3–16.

Holzgrabe, U., Brinz, D., Kopec, S., Weber, C., and Bitar, Y. (2006). Why not using capillary electrophoresis in drug analysis? Electrophoresis 27, 2283–2292.

Jankovic, J. (2008). Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79, 368–376.

Kelly, E., Bailey, C.P., and Henderson, G. (2008). Agonist-selective mechanisms of GPCR desensitization. Br J Pharmacol 153, S379–S388.

Kobilka, B.K., and Deupi, X. (2007). Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 28, 397–406.

Kostal, V., Katzenmeyer, J., and Arriaga, E.A. (2008). Capillary electrophoresis in bioanalysis. Anal Chem 80, 4533–4550.

Lefkowitz, R.J., and Shenoy, S.K. (2005). Transduction of receptor signals by β-arrestins. Science 308, 512–517.

Levoye, A., and Jockers, R. (2008). Alternative drug discovery approaches for orphan GPCRs. Drug Discov Today 13, 52–58.

Ma, L., and Pei, G. (2007). b-arrestin signaling and regulation of transcription. J Cell Sci 120, 213–218.

Macey, T.A., Gurevich, V.V., and Neve, K.A. (2004). Preferential Interaction between the dopamine D2 receptor and Arrestin2 in neostriatal neurons. Mol Pharmacol 66, 1635–1642.

Mehler-Wex, C., Riederer, P., and Gerlach, M. (2006). Dopaminergic dysbalance in distinct basal ganglia neurocircuits: implications for the pathophysiology of Parkinson's disease, schizophrenia and attention deficit hyperactivity disorder. Neurotox Res 10, 167–179.

Neve, K.A., Seamans, J.K., and Trantham-Davidson, H. (2004). Dopamine receptor signaling. J Recept Signal Transduct Res 24, 165–205.

Oakley, R.H., Laporte, S.A., Holt, J.A., Caron, M.G., and Barak, L.S. (2000). Differential affinities of visual arrestin, β arrestin1, and β

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arrestin2 for G protein-coupled receptors delineate two major classes of receptors. J Biol Chem 275, 17201–17210.

Obeso, J.A., Marin, C., Rodriguez-Oroz, C., Blesa, J., Benitez-Temiño, B., Mena-Segovia, J., Rodríguez, M., and Olanow, C.W. (2008). The basal ganglia in Parkinson's disease: current concepts and unexplained observations. Ann Neurol 64, S30–S46.

Olanow, C.W., Obeso, J.A., and Stocchi, F. (2006). Drug insight: Continuous dopaminergic stimulation in the treatment of Parkinson's disease. Nat Clin Pract Neurol 2, 382–392.

Premont, R.T., and Gainetdinov, R.R. (2007). Physiological roles of G protein-coupled receptor kinases and arrestins. Annu Rev Physiol 69, 511–534.

Premont, R.T., Inglese, J., and Lefkowitz, R.J. (1995). Protein kinases that phosphorylate activated G protein-coupled receptors. FASEB J 9, 175–182.

Reiter, E., and Lefkowitz, R.J. (2006). GRKs and β-arrestins: roles in receptor silencing, trafficking and signaling. Trends Endocrinol Metab 17, 159–165.

Rosenbaum, D.M., Rasmussen, S.G., and Kobilka, B.K. (2009). The structure and function of G-protein-coupled receptors. Nature 459, 356–363.

Savitt, J.M., Dawson, V.L., and Dawson, T.M. (2006). Diagnosis and treatment of Parkinson disease: molecules to medicine. J Clin Invest 116, 1744–1754.

Skinbjerg, M., Ariano, M.A., Thorsell, A., Heilig, M., Halldin, C., Innis, R.B., and Sibley, D.R. (2009). Arrestin3 mediates $D_{(2)}$ dopamine receptor internalization. Synapse 63, 621–624.

Whalen, E.J., Rajagopal, S., and Lefkowitz, R.J. (2011). Therapeutic potential of β-arrestin- and G protein-biased agonists. Trends Mol Med 17, 126–139.

Wilson, C.J., and Copeland, R.A. (1997). Spectroscopic characterization of arrestin interactions with competitive ligands: study of heparin and phytic acid binding. J Protein Chem 16, 755–763.

Xiao, K., Shenoy, S.K., Nobles, K., and Lefkowitz, R.J. (2004). Activation-dependent conformational changes in β-arrestin 2. J Biol Chem 279, 55744–55753.

Yang, P., Whelan, R.J., Mao, Y., Lee, A.W., Carter-Su, C., and Kennedy, R.T. (2007). Multiplexed detection of protein-peptide interaction and inhibition using capillary electrophoresis. Anal Chem 79, 1690–1695.

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