Protective effect of aripiprazole against glutamate cytotoxicity in dopaminergic neurons of rat mesencephalic cultures.

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Keywords: Aripiprazole; Dopamine; Glutamate cytotoxicity; Intracellular dopamine content; Neuroprotective effect

Abstract

Aripiprazole, a dopamine D_2 receptor partial agonist, is used to treat schizophrenia. Although aripiprazole has been reported to protect non-dopaminergic neurons, its effect on dopaminergic neurons has yet to be investigated. In the present study, we examined whether aripiprazole protected dopaminergic neurons against glutamate-induced cytotoxicity in rat mesencephalic cultures. Pretreatment with aripiprazole protected dopaminergic neurons in a concentration-dependent manner. The neuroprotective effect was not attenuated by sulpiride, a dopamine D_2 receptor antagonist, suggesting that the effect is independent of dopamine D_2 receptors. Aripiprazole reduced intracellular dopamine content in a concentration-dependent manner. In addition, its neuroprotective effect was partially inhibited when dopamine was added. These results suggest that aripiprazole protects dopaminergic neurons against glutamate cytotoxicity partly by reducing intracellular dopamine content.

Introduction

Aripiprazole, a dopamine D_2 receptor partial agonist, has been used as an atypical antipsychotic [1, 10]. It has been reported that aripiprazole protected against kainic acid-induced striatal lesions by stimulating serotonin 5-HT_{1A} receptors [3], inhibited excessive glutamate release in isolated nerve terminals (synaptosomes) from the prefrontal cortex [23] and increased glutathione peroxidase activity in the cortex [5]. These reports suggest that aripiprazole is neuroprotective, although its effects on dopaminergic neurons have not been examined.

Glutamate excitotoxicity has been implicated in several neurodegenerative processes, including ischemia and Parkinson's disease [2]. We previously found that excessive exposure to glutamate caused dopaminergic neuronal death in mesencephalic cultures [9, 20]. We also demonstrated that dopamine D_2 receptor agonists had protective effects against the dopaminergic neurotoxicity [7, 21]. For example, bromocriptine protected mesencephalic neurons by scavenging free radicals via the stimulation of dopamine D_2 receptors [21], while pramipexole, bromocriptine and 7-OH-DPAT protected dopaminergic neurons by reducing intracellular dopamine content independently of dopamine D_2 receptors [7]. Since aripiprazole also acts as an agonist at presynaptic dopamine D_2 receptors [10], we examined whether aripiprazole has protective effects against glutamate-induced dopaminergic neurotoxicity in this study. We found that aripiprazole protected dopaminergic neurons against glutamate cytotoxicity in mesencephalic cultures by reducing intracellular dopamine content independently of dopamine D_2 receptors.

Materials and Methods

Aripiprazole was kindly provided by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Sulpiride was purchased from Sigma (St. Louis, MO). Dopamine was obtained from Nacalai Tesque (Kyoto, Japan). GBR12909 was purchased from Tocris Cookson Ltd. (Bristol, UK).

Rat mesencephalic cultures were established according to methods described previously [9]. Briefly, the ventral two-thirds of the mesencephalon was dissected from rat embryos on the 16th day of gestation. The tissues were then chemically and mechanically dissociated into single cell suspensions. Cells were plated onto 0.1% polyethyleneimine-coated plastic coverslips at a density of 1.3×10^5 cells/cm^2. Cultures were maintained in Eagle's minimum essential medium (EMEM) containing 10% fetal calf serum [1-4 days *in vitro* (DIV)] or horse serum [5-12 DIV]. Cultures were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The animals were treated in accordance with the guidelines of the Kyoto University animal experimentation committee and of the Japanese Pharmacological Society.

For the evaluation of neurotoxicity, mesencephalic cultures were exposed to glutamate for 24 h at 9 DIV. Treated cultures were incubated in EMEM containing 10% horse serum without drugs for a further 48 h and fixed for immunocytochemistry. Following fixation with 4% paraformaldehyde for 30 min, they were incubated with 0.2% Triton X-100 for 10 min. They were then incubated with anti-tyrosine hydroxylase (TH; Chemicon/Millipore, Temecula, CA) antibody for 2 h, with biotinylated secondary antibody for 1.5 h and with avidin-biotinylated horseradish peroxidase complex (Vector Laboratories Inc., Burlingame, CA) for 1.5 h. Finally, they were reacted with diaminobenzidine (Dojindo Laboratories, Kumamoto, Japan)

solution for 8 min. For the evaluation of viability, TH-immunoreactive cells were counted in 20 randomly selected fields (858×1287 μm^2/field). Counts were made by an observer blinded to the experimental treatments. The viability of neurons was calculated as a percentage of the number of sham-treated neurons.

Intracellular dopamine content was measured by high performance liquid chromatography with electrochemical detection (HPLC-ED) as described previously [9]. Mesencephalic cultures were exposed to drugs at 8 DIV. To measure intracellular dopamine content after treatment with the drugs for 24 h, cells were scraped, centrifuged and resuspended in extraction buffer (200 μL) (0.1 N perchloric acid containing 10 mM sodium disulfite and 1 mM EDTA). After sonication, the lysate was centrifuged at 17,000 *g* for 30 min. The supernatant (50 μL) was analyzed by HPLC-ED. The intracellular dopamine content in sham-treated cells was expressed as 100%. The HPLC system consisted of an automatic sample injector (Model 231, Gilson, Villiers-le-Bel, France), a pump (EP-300, Eicom, Kyoto, Japan), a degasser (DG-300, Eicom), a reverse-phase column (Eicompak CA-5ODS, inner diameter: 2.1 mm, length: 150 mm, Eicom), a column oven (ATC-300, Eicom) and an electrochemical detector (ECD-300, Eicom) with a working electrode versus an Ag/AgCl reference electrode. The working electrode was maintained at an oxidative potential of +750 mV. The mobile phase consisted of 0.1 M acetate-citrate buffer (pH 3.7) containing 300 mg/L sodium octylsulfate, 5 mg/L EDTA-2Na and 16% methanol, and the flow rate was set at 0.23 mL/min.

The statistical significance of the difference among three or more groups of individual data was analyzed by the one-way analysis of variance (ANOVA) and post hoc multiple comparison using Tukey's test. Statistical significance was defined as $p <$

0.05. Data were expressed as the mean ± S.E.M.

Results

In agreement with our previous report [9], the exposure of mesencephalic cultures to glutamate (100 μM) for 24 h at 9 DIV reduced the viability of dopaminergic neurons. The glutamate-induced dopaminergic neurotoxicity was significantly inhibited by pretreatment with aripiprazole (10 μM) for 24 h and/or co-administration of aripiprazole (10 μM) with glutamate (Fig. 1A). Since the pretreatment by itself was sufficient to protect dopaminergic neurons, it was chosen in subsequent experiments. Aripiprazole (10-30 μM) showed the neuroprotective effect in a concentration-dependent manner (Fig. 1B), which was not suppressed by sulpiride, a dopamine D_2 receptor antagonist (Fig. 1C).

We have reported that glutamate-induced dopaminergic neurotoxicity was mediated by intracellular dopamine [9]. To investigate the mechanism underlying the neuroprotective effect of aripiprazole, we examined intracellular dopamine content after treatment with aripiprazole for 24 h by HPLC-ED. Aripiprazole reduced intracellular dopamine content in a concentration-dependent manner (Fig. 2A). The reduction in intracellular dopamine content by treatment with aripiprazole (30 μM) for 24 h was not suppressed by co-administration of sulpiride (30 μM) for 24 h, and a significant reduction was observed after treatment with aripiprazole for just 0.5 h (Fig. 2B and C).

To confirm that the reduction in intracellular dopamine content contributes to the protective effect of aripiprazole against glutamate cytotoxicity in dopaminergic neurons, dopamine (100 μM) was applied for 3 h after 24-hour pretreatment with aripiprazole (30 μM), and then the cultures were exposed to glutamate (100 μM) in the presence of dopamine (100 μM). The neuroprotective effect of aripiprazole against

glutamate cytotoxicity was significantly attenuated by the addition of dopamine, which was cancelled by GBR12909 (1 μM), an inhibitor of dopamine transporter (Fig. 3).

Discussion

We found that aripiprazole, a dopamine D_2 receptor partial agonist, protected dopaminergic neurons against glutamate cytotoxicity. We previously reported that dopamine D_2 receptor agonists had protective effects against glutamate-induced dopaminergic neurotoxicity dependently or independently of dopamine D_2 receptors [7, 21]. The neuroprotective effect of aripiprazole was not mediated by dopamine D_2 receptors because it was not suppressed by sulpiride.

Endogenous dopamine affects the susceptibility of dopaminergic neurons to Pael receptor-, rotenone- and α-synuclein-induced toxicity [11, 19, 24]. We also demonstrated that glutamate-induced dopaminergic neurotoxicity was mediated by intracellular dopamine [9]. In addition, dopamine D_2 receptor agonists protected dopaminergic neurons against glutamate cytotoxicity by reducing intracellular dopamine content independently of dopamine D_2 receptors [7]. Aripiprazole also reduced intracellular dopamine content in a concentration-dependent manner and the neuroprotective effect of aripiprazole was attenuated by addition of dopamine. These results suggest that the protective effect is derived from the reduction in intracellular dopamine content.

The neuroprotective effect was also seen when aripiprazole was administered together with glutamate, although the effect was smaller than that when it was administered before glutamate exposure. This result seems reasonable because the treatment with aripiprazole for 0.5 h significantly reduced intracellular dopamine content but the reduction was smaller than that after treatment for 24 h. We have previously reported that dopamine receptor agonists and antagonists, including aripiprazole, reduced intracellular dopamine content by inhibiting vesicular

monoamine uptake in PC12 cells [8, 14]. Thus, it is likely that aripiprazole also reduced intracellular dopamine content by inhibiting vesicular monoamine uptake in mesencephalic cultures, although the mechanisms involved are not examined in the present study. The reduction of intracellular dopamine content by aripiprazole in mesencephalic cultures was observed at lower concentrations than that by pramipexole, bromocriptine and 7-OH-DPAT [7]. It is suggested that aripiprazole inhibits vesicular monoamine uptake more potently than other dopamine receptor agonists.

The protective effect of aripiprazole against glutamate-induced dopaminergic neurotoxicity was not fully suppressed when dopamine was added to the mesencephalic cultures, although intracellular dopamine content was recovered by the addition of dopamine (data not shown). This result suggests that other mechanisms are also involved in the neuroprotection by aripiprazole. It has been reported that the neuroprotective effect of aripiprazole against kainic acid-induced striatal lesions *in vivo* was related to the activation of serotonin 5-HT_{1A} receptors [3]. The mechanism underlying neuroprotection by stimulation of serotonin 5-HT_{1A} receptors is still not fully understood but might involve neuronal hyperpolarization [15, 16]. Since serotonin 5-HT_{1A} receptors are expressed in dopaminergic neurons of the ventral tegmental area [4], it is possible that the protective effect of aripiprazole against glutamate cytotoxicity in dopaminergic neurons is also brought by the stimulation of serotonin 5-HT_{1A} receptors. In fact, it has been reported that 8-OH-DPAT, a serotonin 5-HT_{1A} receptor agonist, protected against NMDA-induced apoptotic cell death in rat mesencephalic cultures and the neuroprotective effect was inhibited by a 5-HT_{1A} antagonist [13]. In addition, it has been reported that aripiprazole increased brain-derived neurotrophic factor (BDNF) levels and glutathione peroxidase activity [5,

17]. Therefore, it is possible that aripiprazole protects dopaminergic neurons against glutamate cytotoxicity not only by reducing intracellular dopamine content but also by stimulating serotonin 5-HT_{1A} receptors and increasing levels of neuroprotective factors. However, further experiments will be required to examine whether serotonin 5-HT_{1A} receptors and these factors contribute to the protective effect of aripiprazole on dopaminergic neurons.

Some reports suggest that aripiprazole may be useful for reducing psychosis and improving mood and cognition in patients with Parkinson's disease [6, 12, 22]. As glutamate cytotoxicity has been suggested to contribute to the continued degeneration of dopaminergic neurons in the progression of Parkinson's disease [18], aripiprazole may have the potential to inhibit dopaminergic neuronal degeneration in Parkinson's disease.

In conclusion, aripiprazole protected dopaminergic neurons against glutamate cytotoxicity by reducing intracellular dopamine content. Our results provide a novel insight into the neuroprotective effect of aripiprazole on dopaminergic neurons.

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Figure legends

Figure 1 Effect of aripiprazole on glutamate cytotoxicity in dopaminergic neurons of mesencephalic cultures. (A) Aripiprazole (10 μM) was applied for 24 h before and/or during exposure to glutamate (100 μM) for 24 h. Co; co-administration of aripiprazole with glutamate for 24 h. Pre; pretreatment with aripiprazole for 24 h and exposure to glutamate for 24 h. Pre + Co; pretreatment with aripiprazole for 24 h and co-administration of aripiprazole with glutamate for 24 h. (B) After treatment with aripiprazole (10-30 μM) for 24 h, cultures were exposed to glutamate (100 μM) for 24 h. (C) After treatment with aripiprazole (30 μM) in the presence or absence of sulpiride (30 μM) for 24 h, cells were exposed to glutamate (100 μM) for 24 h. Data shown are mean values ± S.E.M. (n=8). ***p < 0.001, compared with sham. $^{###}p$ < 0.001, compared with glutamate (100 μM). n.s.; not significant.

Figure 2 Effect of aripiprazole on intracellular dopamine content in mesencephalic cultures. (A) After treatment with aripiprazole (1-30 μM) for 24 h, intracellular dopamine content was determined by HPLC-ED. (B) After treatment with aripiprazole (30 μM) in the presence or absence of sulpiride (30 μM) for 24 h, intracellular dopamine content was determined by HPLC-ED. (C) After treatment with aripiprazole (30 μM) for 0.5, 1 or 3 h, intracellular dopamine content was determined by HPLC-ED. Data shown are mean values ± S.E.M. (n=3). $^{###}p$ < 0.001, compared with sham. n.s.; not significant.

Figure 3 Effect of exogenous dopamine on aripiprazole-induced protection in dopaminergic neurons of mesencephalic cultures. After treatment with aripiprazole (30

μM) for 24 h, dopamine (100 μM) was applied for 3 h and then cultures were exposed to glutamate (100 μM) in the presence of dopamine (100 μM) for 24 h. GBR12909 (1 μM) was coadministered with dopamine (100 μM). Data shown are mean values ± S.E.M. (n=8). ***p < 0.001, compared with sham. $^{###}p$ < 0.001, compared with glutamate (100 μM). n.s.; not significant.

Figure 1

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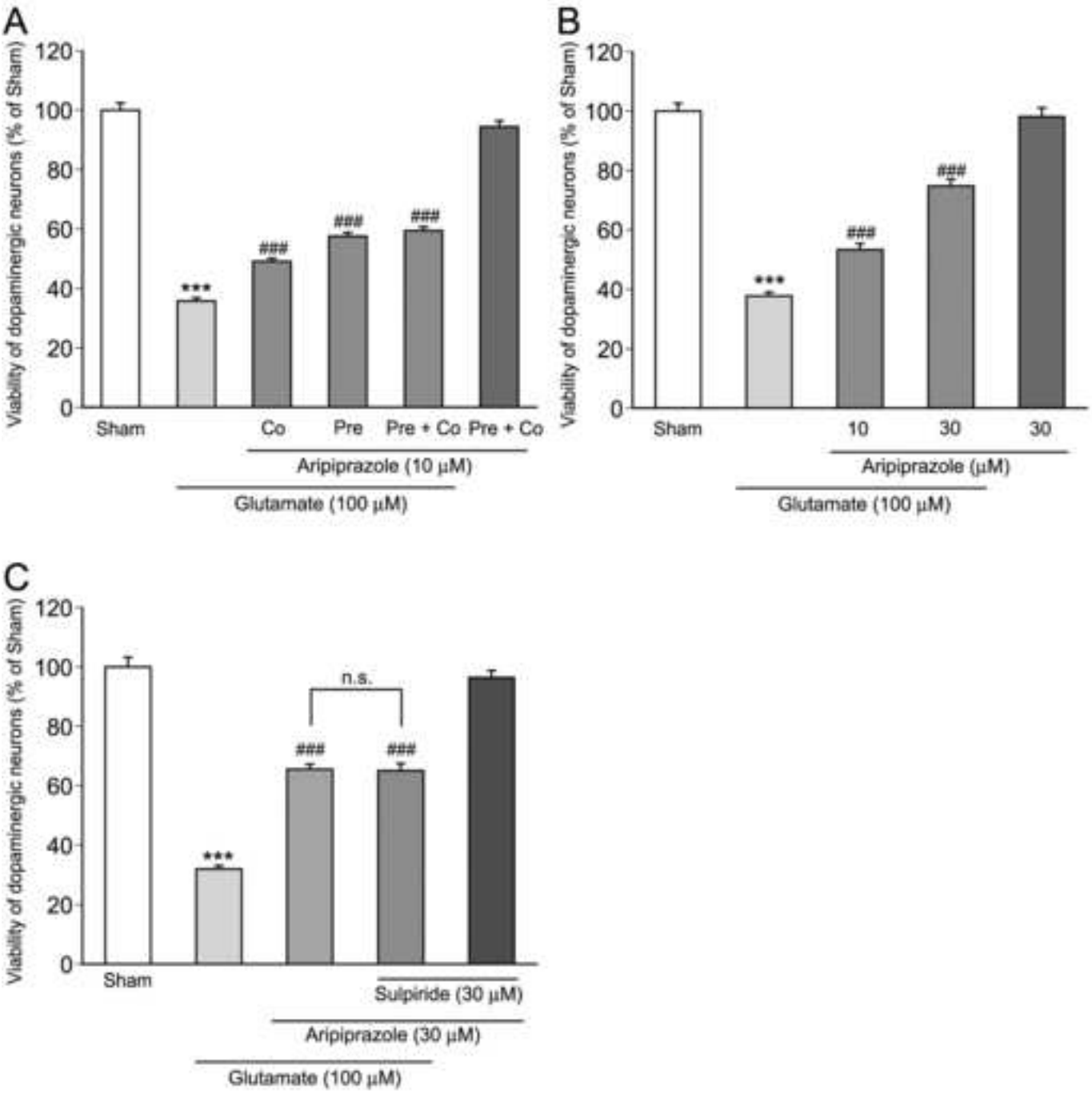

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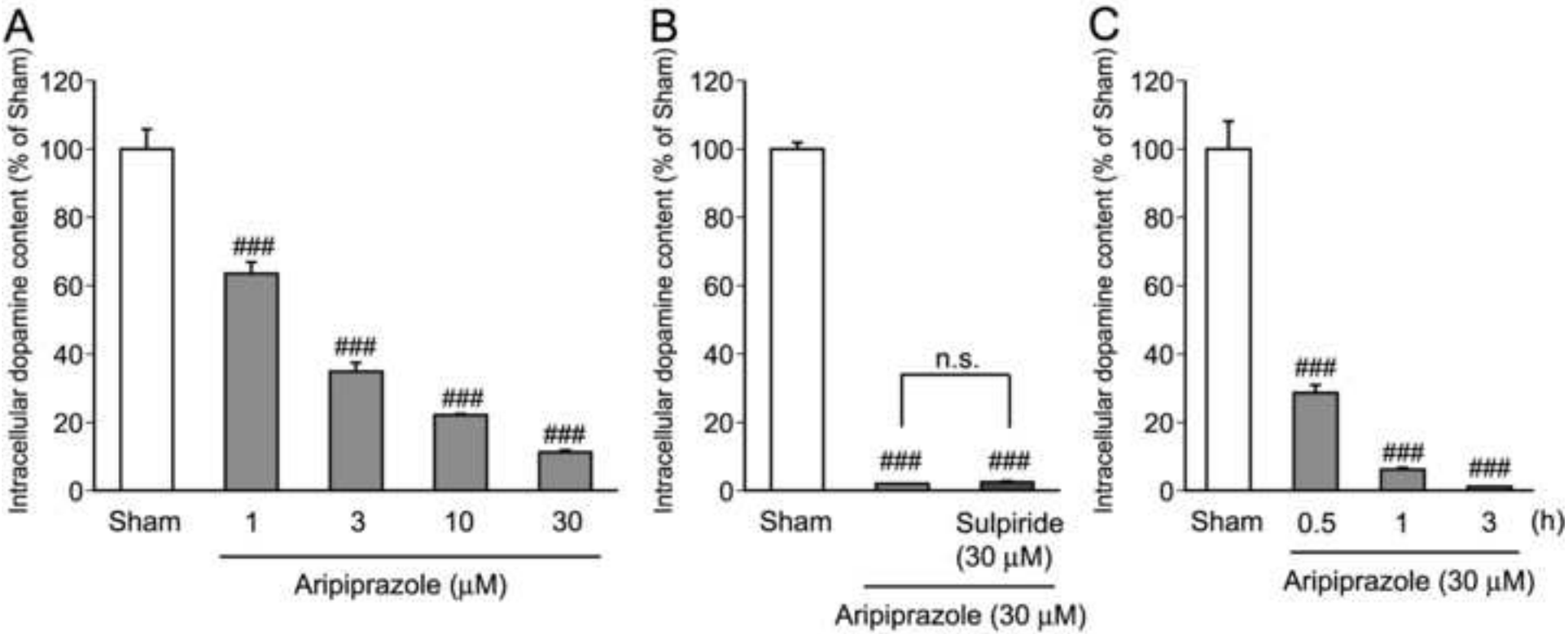

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Figure 3
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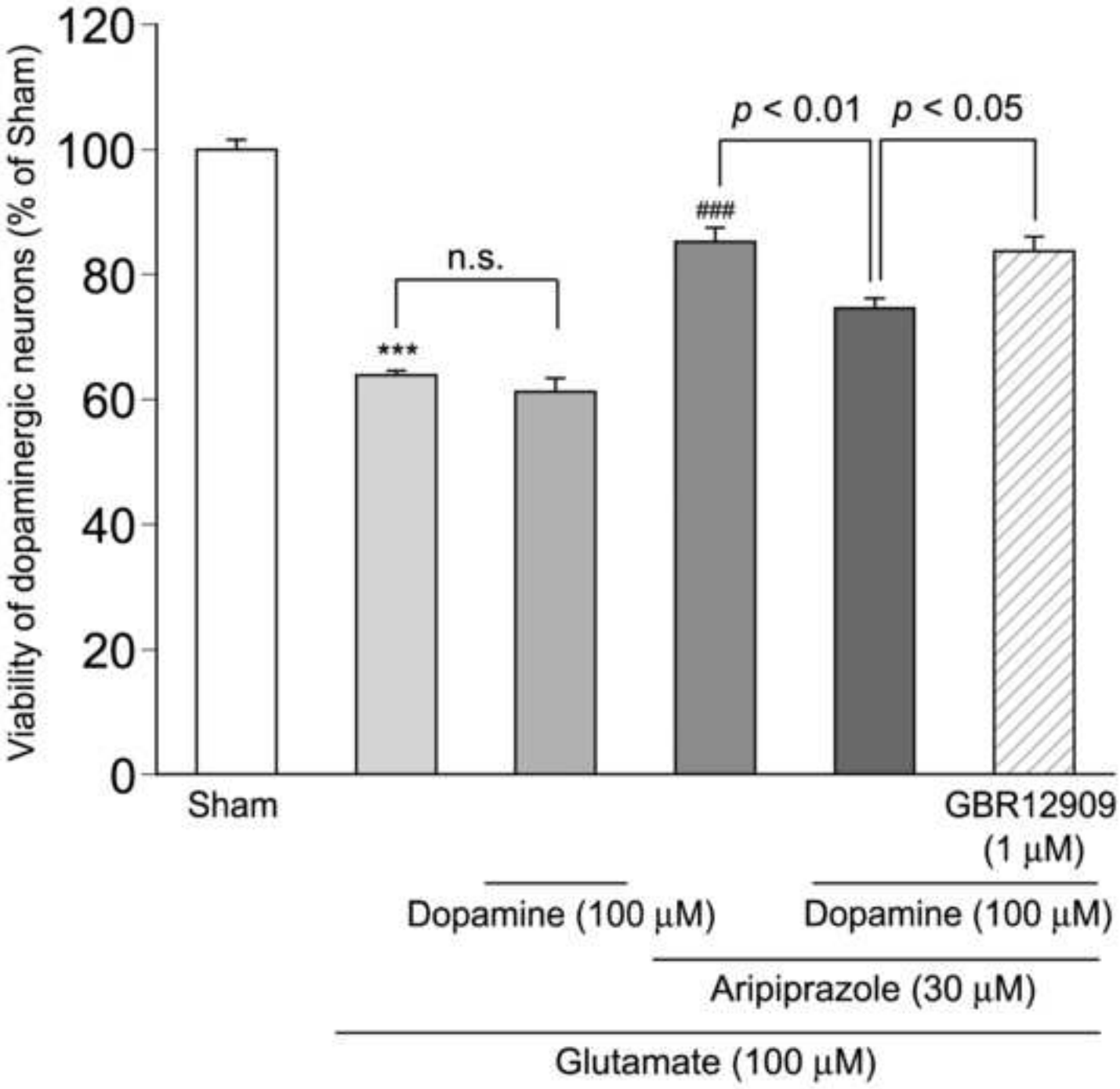

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