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Neuroprotective mechanism of glial cell line-derived neurotrophic factor on dopamine neurons: role of antioxidation

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Abstract

Recombinant human GDNF was infused into the rat striatum either acutely or subchronically. Its effects and its interactions with MPP^+ on antioxidant enzyme activities were examined. Results indicated that acute GDNF infusion significantly increased glutathione peroxidase, superoxide dismutase and catalase activities. Subchronic GDNF treatment decreased the DA level and enhanced DA turnover. Pre-treatment with GDNF markedly protected DA neurons against MPP^+-induced toxicity. These results suggest that GDNF protects DA neurons through its activation of the antioxidant enzyme systems.

Keywords: Glial cell line-derived neurotrophic factor; Glutathione peroxidase; Superoxide dismutase; Catalase; Dopamine; 1-Methyl-4-phenylpyridinium.

The onset of oxidative stress resulting from the excess formation of free radicals is thought to lead to the damage of dopamine (DA) neurons in the substantia nigra (SN). Under oxidative stress, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are believed to be the major antioxidant enzymes in the cell for removing reactive oxygen species. Based on this concept, one of the hypotheses underlying Parkinson's disease (PD) is that the antioxidant defense mechanism is impaired or inadequate in the brain of parkinsonian patients (for review, see Jenner and Olanow, 1996).

Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor superfamily which has been suggested to be a specific neurotrophic factor for midbrain DA neurons (Lin et al., 1993). Both in vitro and in vivo studies have shown that GDNF protects DA neurons against toxin-induced damage (for review, see Lapchak et al., 1997). Some therapeutic agents for PD are also found to increase GDNF mRNA expression in the striatum, such as (−)-deprenyl (Tang et al., 1998). However, although there are many reports showing the neuroprotective effects of GDNF on DA neurons, the mechanism underlying this protection has been rarely examined. In the present study, we investigated whether one of the protective mechanisms of GDNF is through an enhancement of antioxidant enzyme activity. The DA neuron toxin 1-methyl-4-phenylpyridinium (MPP^+) was used. Recombinant human GDNF was infused into the rat striatum. Its effects and its interactions with MPP^+ on various antioxidant enzyme activities, DA level and DA turnover were measured in rat striatum.

Adult male Sprague–Dawley rats (weighing 250–300 g) were used in the present study. The cannulae were implanted bilaterally into the striatum through stereotaxic surgery. For the acute injection study, 1 μg of GDNF (Pepro Tech EC Ltd., London, England) was infused into one side of the striatum and saline infused into the other side. This design was based on our preliminary results that when GDNF was infused into

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one side of the striatum, these antioxidant enzyme activities did not change on the other side of the striatum (unpublished observations). Animals were sacrificed 4 h later. For the subchronic injection study, one group of animals received bilateral infusions of saline and the other group received 0.75 μg GDNF for 5 days continuously and they were sacrificed on the sixth day. The infusion volume was 2 μl each side. For the GDNF–MPP^+ interaction study, animals were divided into three groups: S + S group received two saline infusions; S + M group received saline followed by MPP^+ infusions (25 μg); G + M group received GDNF (1 μg) followed by MPP^+ infusions (25 μg). Each infusion volume was 1 μl and the interval between two infusions was 4 h. Rats were sacrificed 5 days after the last infusion and the striatal tissue was used for various biochemical assays. The methods used for measuring SOD, GPx, glutathione (GSH) and CAT activity are described elsewhere (Hung and Lee, 1998). Briefly, GPx activity was measured by a coupled enzyme procedure with glutathione reductase using GSH, NADPH and H_2O_2 as substrates. GSH was measured according to the enzymatic recycling method with DTNB, NADPH and GSH reductase. SOD activity was assayed based on the ability of SOD to inhibit the autoxidation of epinephrine by xanthine oxidase. Catalase has been shown to be another major enzyme which defends oxidative stress (Desagher et al., 1996) and was determined by the initial decay rate of H_2O_2 absorbance at 240 nm (ε value of 0.0394 mM^{-1} cm^{-1} for H_2O_2). Each assay mixture consisted of a cell sample of approximately 40 μg protein per ml and an initial concentration of 10 mM H_2O_2. DA and homovanillic acid (HVA) were determined by HPLC with electrochemical detection.

As shown in Table 1, results indicated that acute GDNF infusion markedly increased GPx, SOD and CAT activities in the striatum ($t = 8.26$, $P < 0.05$; $t = 8.97$, $P < 0.01$ and $t = 37.85$, $P < 0.01$, respectively). Further analyses revealed that GDNF mainly increased CuZnSOD activity ($t = 5.51$, $P < 0.05$), but not MnSOD activity ($t = 0.28$, $P > 0.05$). Similarly, subchronic GDNF treatment also increased GPx activity in the striatum ($t = 8.37$, $P < 0.05$); but it also increased GSH level ($t = 6.52$, $P < 0.01$). Superoxide dismutase activity was not altered ($t = 0.05$, $P > 0.05$); the DA level was decreased ($t = 9.40$, $P < 0.05$) while DA turnover was increased by this treatment ($t = 35.68$, $P < 0.01$). In another experiment, intra-striatal MPP^+ infusion markedly increased MnSOD activity ($t = 1.52$, $P < 0.05$), decreased CuZnSOD activity ($q = 0.62$, $P < 0.01$) and the DA level ($q = 1245$, $P < 0.01$); but it increased DA turnover ($q = 0.05$, $P < 0.01$) in the striatum. Pre-treatment with GDNF markedly protected DA neurons by causing a lesser degree of DA depletion and a smaller increase in DA turnover ($q = 1058$, $P < 0.05$ and $q = 0.04$, $P < 0.05$

Table 1
Effects of acute or subchronic GDNF, and GDNF/MPP^+ treatments on GPx and SOD activities, GSH content, CAT activity, the DA level and DA turnover, as expressed by HVA/DA, in the striatum of rats[a]

Treatment	GPx activity (U mg protein^{-1})	GSH	SOD activity (U mg protein^{-1})			CAT activity (U mg protein^{-1})	DA (ng g tissue^{-1})	HVA/DA
			Total SOD	MnSOD	CuZnSOD			
Acute								
Saline	0.115 ± 0.006	155 ± 10	7.10 ± 0.41	4.49 ± 0.35	2.61 ± 0.40	4.51 ± 0.51	074 ± 856	0.057 ± 0.006
GDNF	0.170 ± 0.018[b,d]	167 ± 6	11.10 ± 1.27[b,e]	5.24 ± 1.35	5.86 ± 1.33[b,d]	8.56 ± 0.41[b,e]	8470 ± 884	0.079 ± 0.009
Subchronic								
Saline	0.147 ± 0.011	190 ± 5	8.94 ± 0.46	5.57 ± 0.35	3.36 ± 0.52	4.35 ± 0.44	9210 ± 1033	0.058 ± 0.005
GDNF	0.212 ± 0.022[b,d]	234 ± 16[b,d]	8.76 ± 0.71	5.50 ± 0.46	3.26 ± 0.62	5.33 ± 0.48	5552 ± 381[b,d]	0.105 ± 0.006[b,e]
GDNF/MPP^+								
S+S	0.118 ± 0.005	137 ± 7	7.97 ± 0.38	5.73 ± 0.35	2.26 ± 0.04	3.95 ± 0.23	11664 ± 214	0.039 ± 0.003
S+M	0.210 ± 0.019[b,d]	130 ± 5	7.84 ± 0.60	7.32 ± 0.64[b,d]	0.52 ± 0.11[b,e]	4.14 ± 0.42	2483 ± 201[b,e]	0.130 ± 0.014[b,e]
G+M	0.182 ± 0.047	122 ± 11	8.52 ± 0.42	5.06 ± 0.33[c,d]	3.46 ± 0.19[c,e]	3.63 ± 0.39	3557 ± 272[c,e]	0.082 ± 0.003[c,e]

[a] Data are expressed as mean ± SEM. ($n = 10$ for GPx and SOD activity assays and $n = 6$ for GSH, CAT and DA, HVA assays in the acute and subchronic GDNF experiments. For the GDNF/MPP^+ experiment, $n = 6$ in each group). Statistical significance was evaluated by Student's t-test or one-way analysis of variance followed by the Newman–Keuls method.

[b] When compared with the corresponding control group.

[c] When compared with the S+M group. S+S group: saline injection twice, S+M: saline injection followed by MPP^+injection, G+M: GDNF injection prior to MPP^+ injection. Unit for CAT activity: 1 μmol H_2O_2 depleted per min; for SOD: the amount that reduces the absorbance change by 50% per min; for GPx: 1 μmol NADPH oxidized per min. GSH concentration is expressed as nmol mg protein^{-1}.

[d] $P < 0.05$.

[e] $P < 0.01$.

when comparing the G + M group with the S + M group). Although there was not an overall significant effect on GPx activity, further analysis indicated that intra-striatal MPP^+ infusion produced a marked increase in GPx activity when compared with the saline controls by Dunnett's t-test ($tD = 2.0$, $P = 0.05$).

Results of the present study indicated that both acute and subchronic GDNF treatments elevated GPx activity and acute GDNF treatment also increased SOD and CAT activities in rat striatum. In addition, subchronic GDNF treatment decreased the DA level while it increased DA turnover. It is postulated that the increase in SOD activity without a concomitant increase in GPx/CAT activity may result in hydroxyl radical formation through the Fenton reaction which is believed to cause lipid peroxidation and cell damage (Huang et al., 1992). In the present study, acute GDNF infusion increased SOD, GPx and CAT activities simultaneously. These results suggest that GDNF treatment can not only maintain a balance between these enzyme systems, but also remove reactive oxygen species more efficiently. Further analyses revealed that the acute effect of GDNF on SOD was mainly caused by its enhancing effect on CuZnSOD activity; instead of MnSOD activity. The reason for this selective action of GDNF is unknown. However, this result is consistent with the report that increased CuZnSOD activity in transgenic mice is resistant to MPTP-induced toxicity (Przedborki et al., 1992). This result is also consistent with our previous observation that the mesolimbic dopaminergic pathway is more resistant to environmental insult than the nigrostriatal dopaminergic pathway does due to a higher basal level of CuZnSOD activity in the ventral tegmental area compared with the SN (Hung and Lee, 1998). On the other hand, subchronic GDNF treatment did not affect SOD activity. The reason for this is also unknown; while in a time-course study, we found that this enzyme activity was transiently increased by GDNF but it returned to the control level in about 16 h (unpublished observations). Therefore, the effects of subchronic GDNF infusion on SOD activity may have disappeared 24 h after the last GDNF infusion. Further, acute GDNF infusion increased CAT activity, but the same effect was not seen with subchronic GDNF treatment. This may be due to the short half-life of the CAT protein (~ 1.5 days); while the exact reason awaits to be investigated.

In the present study, subchronic GDNF administration also significantly decreased the DA level and increased DA turnover in the striatum. This is possibly due to enhanced DA neuron activity and increased DA release caused by GDNF; therefore, the cellular content of DA is decreased. This suggestion is consistent with the report of Hudson et al. (1995) that intracranial GDNF administration increases DA turnover in rat striatum. Results from the GDNF–MPP^+ interaction study are consistent with most of the literature reporting that MPP^+ produces a marked decrease in the DA level, while GDNF protects against MPP^+-induced DA depletion. Moreover, the decrease in CuZnSOD activity may be due to direct damage to DA neurons caused by MPP^+; while the increase in MnSOD activity may be due to a compensatory defensive mechanism against MPP^+ toxicity to the mitochondria respiratory chain (Thiffault et al., 1995). The increase in GPx activity by MPP^+ may be explained in a similar way. GDNF also reversed these effects. Altogether, the present results demonstrate that one of the cellular mechanisms underlying the neuroprotective action of GDNF is through its activation of the antioxidant enzyme systems.

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References

Desagher, S., Glowinski, J., Premont, J., 1996. Astrocytes protect neurons from hydrogen peroxidase toxicity. J. Neurosci. 16, 2553–2562.

Huang, T., Carlson, E.J., Leadon, S.A., Epstein, C.J., 1992. Relationship of resistance to oxygen free radicals to CuZn-superoxide dismutase activity in transgenic, transfected, and trisomic cells. FASEB J. 6, 903–910.

Hudson, J., Granholm, A., Gerhardt, G.A., Henry, M.A., Hoffman, A., Biddle, P., Leela, N.S., Mackerlova, L., Lile, J.D., Collins, F., Hoffer, B.J., 1995. Glial cell line-derived neurotrophic factor augments midbrain dopaminergic circuits in vivo. Brain Res. Bull. 36, 425–432.

Hung, H.C., Lee, E.H.Y., 1998. MPTP produces differential oxidative stress and antioxidative responses in the nigrostriatal and mesolimbic dopaminergic pathways. Free Radic. Biol. Med. 24, 76–84.

Jenner, P., Olanow, C.W., 1996. Oxidative stress and the pathogenesis of Parkinson's disease. Neurobiology 47, S161–S170.

Lapchak, P.A., Gash, D.M., Collins, F., Hilt, D., Miller, P.J., Araujo, D., 1997. Pharmacological activities of glial cell line-derived neurotrophic factor (GDNF): prelinical development and application to the treatment of Parkinson's disease. Exp. Neurol. 145, 309–312.

Lin, L.F., Doherty, D.H., Lile, J.D., Bektesh, S., Collins, F., 1993. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260, 1130–1132.

Przedborki, S., Kostic, V., Jackson-Lewis, V., Naini, A.B., Simonetti, S., Fahn, E., Carlson, E., Epstein, C.J., Cadet, J.L., 1992.

Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. J. Neurosci. 12, 1658–1667.

Tang, Y.P., Ma, Y.L., Chao, C.C.and, Lee, E.H.Y., 1998. Enhanced GDNF mRNA expression upon (−)-deprenyl and melatonin treatment. J. Neurosci. Res. 53, 593–604.

Thiffault, C., Aumont, N., Quirion, R., Poirier, J., 1995. Effects of MPTP and L-deprenyl on antioxidant enzymes and lipid peroxidation levels in mouse brain. J. Neurochem. 65, 2725–2733.