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LETTER TO THE EDITOR

Effect of cabergoline on increase of several ER stress-related molecules in 6-OHDA-lesioned mice

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We investigated the in vitro free radical scavenging and antioxidant activities of cabergoline as well as its glutathione (GSH)-, catalase-, and superoxide dismutase-activating effects and in vivo neuroprotective properties against 6-hydroxydopamine (6-OHDA) intracerebroventricularly (i.c.v.) in mice [1]. In addition, we found that cabergoline prevented the levodopa-induced abnormal increase in lipid peroxidation and caspase activities via increases in GSH content and inhibition of caspase activities in 6-OHDA-lesioned mice [2]. On the other hand, the molecular basis of dopaminergic cell death in Parkinson's disease (PD) is not fully understood. Actually, dysfunction of the ubiquitin–proteasome system (UPS), a major route for removal of oxidized, damaged, or misfolded proteins, is one of the factors related to the pathogenesis of PD [3, 4]. The endoplasmic reticulum (ER) is an intracellular compartment involved in calcium signaling and protein folding/processing. ER stress is now known as an important factor in the neuropathology of a wide variety of neurological disorders, including PD [3, 4]. The present study was conducted to assess the effects of cabergoline on 6-OHDA-induced ER stress in mice.

All other reagents and chemicals were of the highest commercial grade. Male ICR mice (Charles River), weighing 28–30 g, were provided with free access to food and water, while housed at constant room temperature (24 ± 1°C) and humidity (55%) with a 12-h light/12-h dark cycle (lights on at 07:00 h) for 1 week before the experiments. The principles of laboratory animal care and all experimental procedures were in strict accordance with the Guidelines for Animal Experiments of Shujitsu University School of Pharmacy. In all of the in vivo experiments, 25 mg/kg of desipramine was injected intraperitoneally (i.p.) and then, 30 min later, 6-OHDA (60 μg/2 μl of saline containing 0.05% ascorbic acid) or the same volume of saline was intracerebroventricularly (i.c.v.) injected under light ether anesthesia [1]. Twenty-four hours later, cabergoline (0.25 mg/kg) suspended in 0.25% methylcellulose (vehicle), or the same volume of vehicle alone, was administered intraperitoneally (i.p.) for 7 consecutive days. No further drugs were administered, and the mice were sacrificed 24 h later. After the mice were sacrificed by decapitation, the mouse striatal tissue was carefully dissected out and then preserved at −80°C until analysis. Expression levels of the caspase-12, Bip/GRP78 (Bip) or CHOP/GADD153 (CHOP) were examined by Western blot analysis with rabbit anti-mouse caspase-12 (Chemicon; 1:500 dilution), goat anti-mouse Bip (Santa Cruz; 1:100 dilution), or rabbit anti-mouse CHOP (Santa Cruz; 1:250 dilution) polyclonal antibody. For quantitative analysis, sample loading and transfer were normalized by using goat anti-human/mouse actin polyclonal antibody (Santa Cruz; 1:250 dilutions). The caspase-3 assay kit (Sigma) was used to determine caspase-3 activity as the index of apoptosis. We determined these activities by

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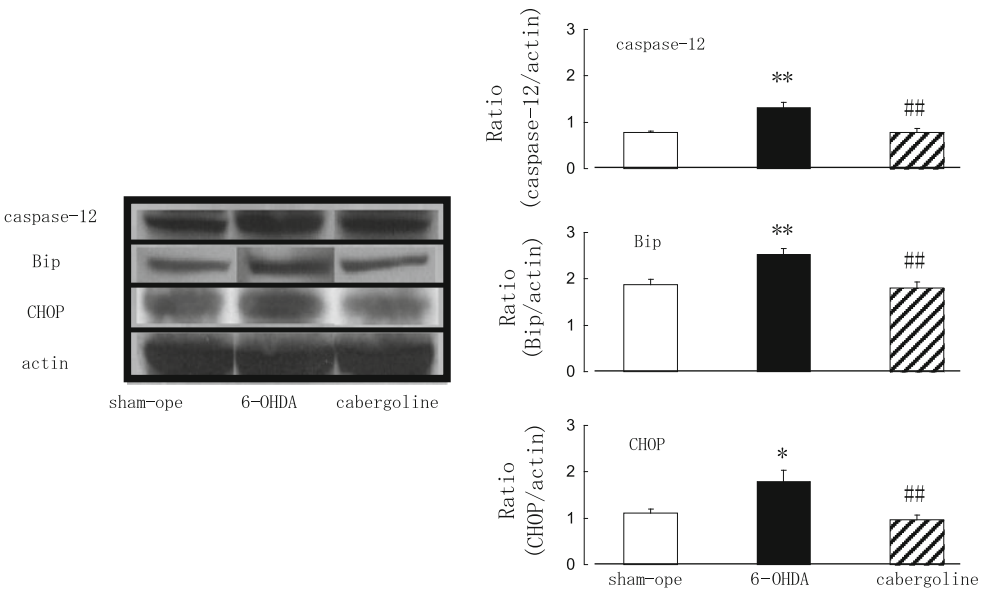

Fig. 1 Effects of cabergoline on each expression of caspase-12, Bip, or CHOP in the striatum. Representative typical immunoblotting of caspase-12, Bip, CHOP, or actin in the same experimental conditions. Quantitation of the density of the protein bands is shown next to panel. Each value is the mean ± SEM of 6–7 mice. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the vehicle + sham-operated mice (sham-ope). $^{\#\#}p < 0.01$ compared with the vehicle + 6-OHDA-injected mice (6-OHDA)

modifying the methods of the caspase-3 assay kit with Ac-DEVD-pNA, which is a colorimetric substrate for caspase-3. The protein concentration of striatal homogenates was determined using a Bio-Rad protein assay kit (Bio-Rad). Data are presented as mean ± SEM. Statistical analysis of biochemical data was performed by a one-way ANOVA followed by the post hoc Tukey–Kramer Multiple Comparisons test. A p value <0.05 denoted a statistically significant difference.

6-OHDA significantly increased the caspase-12, Bip, or CHOP expression compared with the sham-operated mice (Fig. 1). However, cabergoline significantly reduced the 6-OHDA-induced increase in the caspase-12, Bip, and CHOP expression in the striatum (Fig. 1). In addition, cabergoline prevented the 6-OHDA-induced increase in caspase-3 activity as an index of apoptosis in the midbrain mainly containing substantia nigra (Fig. 2).

Caspase-3, a typical effector caspase, is considered to play an important role in the final common pathway of

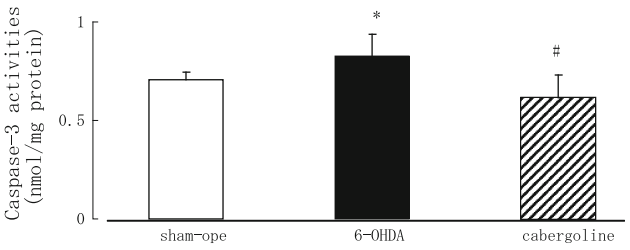

Fig. 2 Effects of cabergoline on caspase-3 activities in the substantia nigra. Each value is the mean ± SEM of 6–7 mice. $^{*}p < 0.05$ compared with the vehicle + sham-operated mice (sham-ope). $^{\#}p < 0.05$ compared with the vehicle + 6-OHDA-injected mice (6-OHDA)

apoptosis. Moreover, caspase-3 responds to direct activation by initiator caspases such as caspase-8, -9, and -12. Our previous results indicate that 6-OHDA induced caspase-3 activation mediated by the result of either Fas- or mitochondrial-pathways in the striatum [2]. On the other hand, caspase-12 has been proposed as an initiator caspase and also as the key molecule in the death-driving force in ER stress. Thus, cabergoline against 6-OHDA-induced caspase-3 activation may also be dependent on ER. In many studies about ER stress, we selected three molecules such as indices of UPR by ER stress, according to Rao's classification of ER stress-related molecular components [5]. Namely, each our three molecules were corresponded to a sensor (Bip), a modulator (CHOP), or an effector (caspase-12), in the ER stress-induced cell death [5]. Bip was found to attenuate protein folding in the ER and to block CHOP expression. Otherwise, 6-OHDA may be capable of activating ER stress signals through the accumulation of oxidized proteins, thereby inducing cell death in SH-SY5Y cells [6]. Furthermore, Holtz and O'Malley have reported that CHOP, a major target of the UPR pathway, was dramatically upregulated at the mRNA and protein levels by 6-OHDA treatment in MN9D cells [7]. Moreover, CHOP may also play a role as a mediator of apoptotic cell death in the 6-OHDA-treated adult CHOP null mice [8]. Considering this evidence, our present results suggest that the anti-apoptotic effects of cabergoline may be primarily dependent on the inhibition of 6-OHDA-induced increases in CHOP expression, although caspase-12 and Bip play an important role in the reduction of 6-OHDA-induced ER stress. It may, therefore, be important for the anti-apoptotic effect of cabergoline to ameliorate ER stress and ER stress-induced cell death.

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