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ORIGINAL INVESTIGATION

Cabergoline, a dopamine receptor agonist, has an antidepressant-like property and enhances brain-derived neurotrophic factor signaling

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Abstract
Rationale Dopamine agonists have been implicated in the treatment of depression. Cabergoline is an ergot derivative with a high affinity to dopamine D_2-like receptors; however, there have been few preclinical studies on its antidepressant-like effects.
Materials and methods Behavioral effects of cabergoline were examined in rats using forced swimming (FST), novelty-suppressed feeding (NST), open field (OFT), and elevated-plus maze (EPT) tests. In a single treatment paradigm, behaviors of rats were analyzed 4 h after single injection of cabergoline (s.c., 0–4 μmol/kg). In a repeated-treatment paradigm, OFT, EPT, and FST were conducted on days 11, 12, and13–14, respectively, during daily cabergoline injections (s.c., 0.5 μmol/kg), and then hippocampus was removed 24 h after the last injection. NST was conducted in a separate experiment at day 14. Western blotting was used for the analysis of the protein levels of brain-derived neurotrophic factor (BDNF) and the activation of intracellular signaling molecules.
Results Single injection of cabergoline demonstrated decreased immobility in FST and distance traveled during 0–10 min in OFT, while time spent and entry into open arms were increased at 4 μmol/kg. When cabergoline was repeatedly administered, immobility in FST and the latency of feeding in NSF were significantly reduced, while vertical movement was increased in OFT. The time in closed arms was tended to be decreased in EPT. Expression of BDNF and activation of extracellular signal-regulated kinase 1 were up-regulated after the chronic administration of cabergoline.
Conclusions Cabergoline exerts antidepressant- and anxiolytic-like effects, which may be mediated by potentiation of intracellular signaling of BDNF.

Keywords Antidepressant · Anxiety · Brain-derived neurotrophic factor (BDNF) · Cabergoline · Depression · Dopamine receptor agonist · Extracellular signal-regulated kinase (ERK) · Locomotor activity

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Introduction

The current first-line treatments of depressive disorder are serotonin-selective reuptake inhibitors (SSRI), serotonin–noradrenalin reuptake inhibitors (SNRI), and tricyclic antidepressants; however, a substantial proportion of depressed patients are refractory to such treatments (Keller 2005). Dopamine receptor agonists have been thought as one of the promising candidates to improve outcomes of patients with treatment-resistant and nonremitting depression (Dunlop and Nemeroff 2007). Cabergoline is an ergot

derivative and dopamine D_2 receptor-like agonist with a lower affinity to D_1-like, adrenergic, and serotonergic receptors (Millan et al. 2002). Its agonistic effect on dopamine receptors has been utilized in therapies for Parkinson's disease and hyperprolactinaemia. Depression is one of the common complications in Parkinson's disease (Yamamoto 2001), and decreased dopamine transmission has been suggested as one of the causes of this phenomenon (Lemke 2008). Indeed, dopamine receptor agonists such as pramipexole and pergolide demonstrated their effectiveness both on depression and motor functioning in patients with Parkinson's disease (Rektorová et al. 2003; Lemke et al. 2005, 2006; Leentjens et al. 2009). Patients with treatment-resistant depression were also subjected to administration of dopamine receptor agonist in addition to contemporary antidepressants, and favorable results were reported with regard to pramipexole (Lattanzi et al. 2002), bromocriptine (Inoue et al. 1996), and pergolide (Izumi et al. 2000). Takahashi et al. (2003) reported that cabergoline was effective in two cases of refractory depression as a supplementation therapy to an SNRI milnacipran. However, detailed mechanism underlying antidepressant-like effects of dopamine receptor agonists including cabergoline is still unclear.

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins and thought to play an important role both in the etiopathology of depression and the action of antidepressants (Castrén et al. 2007; Duman and Monteggia 2006; Adachi and Kunugi 2008). BDNF binds to its high-affinity receptor TrkB and low-affinity receptor p75 (Huang and Reichardt 2001) and exerts its biological effects through various intracellular pathways including ERK and inositol trisphosphate kinase (PI3K)-Akt signalings (Huang and Reichardt 2001; Kaplan and Miller 1997). BDNF is suggested to be involved in neuronal differentiation, survival, and synaptic plasticity (Poo 2001). Recently, we reported that its important roles in the regulation of synaptic transmission in cultured hippocampal and cortical neurons (Kumamaru et al. 2008; Numakawa et al. 2009). Changes in the expression of BDNF in patients with depression have been reported in the hippocampus, which is one of the important regions in mood regulation. For example, mRNA and protein levels of BDNF were decreased in the postmortem hippocampus of depressive patients (Dunham et al. 2009) and animal models of depression (Grønli et al. 2006). Treatment with antidepressants up-regulates the expression of BDNF in the hippocampus of patients with depression (Chen et al. 2001) and animals (Nibuya et al. 1995). The BDNF up-regulation was also observed after other antidepressive treatments including chronic electroconvulsive therapy (Nibuya et al. 1995). These studies suggest that BDNF may be involved in the final common pathway in the action of the antidepressive therapies.

Dopamine signaling may play a significant role in the regulation of BDNF expression. Dopamine receptor agonists are shown to increase the BDNF levels in cultured astroglial (Ohta et al. 2003) and neuronal cells (Küppers and Beyer 2001; Du et al. 2005). In contrast, haloperidol, an antipsychotic drug which antagonizes D_2 receptor, decreased the expression of BDNF in the brain (Angelucci et al. 2000; Dawson et al. 2001), although some atypical antipsychotics were reported to increase BDNF levels (Fumagalli et al. 2004; Parikh et al. 2004). Ohta et al. (2004) found that cabergoline induced BDNF up-regulation in cultured astrocytes. However, it is unclear whether administration of cabergoline induces the expression of BDNF in vivo.

These previous studies prompted us to test the hypothesis that cabergoline has an antidepressant-like property that is mediated through enhanced BDNF expression in the hippocampus. Here, we investigated the effects of acute/chronic systemic cabergoline administration in the behavioral tests, which are useful for screening of antidepressants and anxiolytics [i.e. forced swim test (FST), novelty-suppressed feeding test (NST), open field test (OFT), and elevated-plus maze test (EPT)]. Antidepressant-like effect was also analyzed using Wistar-Kyoto rats, an innate animal model of depression (Paré 1989). Furthermore, we tried to elucidate the possible molecular mechanism underlying the action of cabergoline by examining changes in the expression of BDNF, TrkB, and p75, as well as activation of ERK and Akt, downstream signalings of TrkB.

Materials and methods

Animals and experimental design

All the experimental procedures were approved by the ethics review committee for animal experimentation at the National Institute of Neuroscience, Japan and done with every effort to minimize the number of animals used and their sufferings. Male Wistar and Wistar-Kyoto rats were purchased from Charles River Japan (Yokohama, Japan) at 6 weeks old and housed in standard laboratory condition (22–24°C, 40–60% humidity, 3:00 PM light on, 3:00 AM light off). Rats were kept in polycarbonate cages in groups, and laboratory chaws and water were available ad libitum. All rats were handled daily for a few minutes from 7 weeks of age. Cabergoline (Mylan Pharmaceutical, Tokyo, Japan) and fluvoxamine (Meiji-seika, Tokyo, Japan) were dissolved in 0.5% carboxymethyl cellulose (Sigma, St. Louis, MO, USA) in sterilized water. In the acute treatment paradigm, behaviors of rats were analyzed 4 h after single injection (from 8:00 to 10:00 AM) of cabergoline (0, 0.25, 0.5, 1, 2, and 4 μmol/kg BW, s.c.) or 1 h after fluvoxamine treatment (138 μmol/kg BW, p.o.). In the chronic treatment

paradigm, OFT, EPT, and FST were conducted at days 11, 12, and 13–14, respectively, during repeated injections of cabergoline (s.c. s.i.d. at 3:00PM, 0.5 μmol/kg BW). The hippocampus was removed from the rat 24 h after the last injection of cabergoline in the chronic treatment regimen. The sample was quickly frozen on dry ice after the removal and stored at −80°C until used. NST was conducted at day 14 of the chronic treatment regimen in a separate experiment. The doses of cabergoline were according to a previous study on an animal model of Parkinson's disease (Miyagi et al. 1996). The schedule of repeated administration was chosen because 2 weeks are needed for antidepressants to change behaviors in several animal models of the depression (Gambarana et al. 2001).

Behavioral tests

FST A modified version of FST (Detke et al. 1995), which consisted of two swim sessions, was carried out. In the first swim session, the rat was introduced into a plastic cylinder (40 cm depth, 20 cm diameter) filled with 25 cm deep water of 23–25°C and forced to swim for 15 min. Twenty-four hours after the first session, the rat was reintroduced into the same cylinder, and their 5-min swimming was observed. The behavior of the rat was recorded on videorecorder (SONY, Tokyo, Japan). After each swim session, the rat was removed from the cylinder, dried with paper towels, placed in the resting cages for 20 min, and then returned to its home cage. Water in the cylinder was renewed between sessions.

Analysis was done on data from the second session of the FST in the acute, and day 14 (FST) in the chronic treatment regimen. Behavioral measures of the rat were defined as follows: (1) immobility—floating in the water without active moving of its limbs and making only slight movements necessary to keep its head above water; (2) swimming—moving more than necessary to keep its head above water and its forelimbs being in the water; (3) climbing—actively trying to climb the wall of the cylinder with its forelimbs above the water; (4) diving—diving into water and its entire body being submerged. The time was manually recorded when each one of the behaviors had started. The time a rat displayed one of the behaviors was calculated by subtraction of the start time from the end time of the behavior.

NST Rats were deprived from food for at least 16 h before the experiment and introduced into an open-field apparatus (100 cm×100 cm×40 cm) with the rat chow on the filter paper placed in the center of the field. Behavior was recorded from the charged-coupled device (CCD) camera above the field. Latency of feeding was measured manually. If the feeding did not happen within 10 min, the latency was recorded as 10 min.

OFT Voluntary movements during 30-min test were monitored by introducing the rat into the open-field apparatus using a CCD camera, and images were captured on Macintosh computer by the Image OF software (modified software based on the NIH image program developed at the U.S. National Institute of Mental Health; modified by O'Hara & Co., Tokyo, Japan). Distance traveled and time spent in the central square that was enclosed by the peripheral zone 20 cm from the wall were automatically calculated by the Image OF software. Rearing and grooming behaviors were also recorded.

EPT Elevated-plus maze has two closed arms that have 50-cm high walls around the arm (10 cm width, 50 cm length; east and west) and two open arms that have 0.5-cm ridges around the arms (10 cm width, 50 cm length; north and south). The maze was elevated 40 cm from the floor. The rat was introduced into the eastern arm of the closed arms and allowed to move freely for 15 min. Behaviors were monitored with a CCD camera and recorded on the Macintosh computer with the Image-EP software (modified software based on the NIH image program developed at the U.S. National Institute of Mental Health; modified by O'Hara & Co., Tokyo, Japan). Total duration of the time spent and entries into each arms were obtained with the Image-EP software. Rearing and grooming behaviors were also recorded.

Behaviors were assessed by a rater who was blind to the cabergoline- or vehicle-treatment status of each rat.

Western blotting

The separated hippocampus was homogenized in lysis buffer, and the protein concentration in the sample was determined before the western blotting assay as previously reported (Numakawa et al. 2003, 2004, 2009). The equivalent amounts of total protein were assayed for each immunoblotting. Primary antibodies were used at the following dilutions: anti-Akt (1:1,000, Cell Signaling, Danvers, MA, USA), anti-pAkt (1:1,000, Cell Signaling), anti-ERK (1:1,000, Cell Signaling), anti-pERK (1:1,000, Cell Signaling), anti-p75 (1:1,000, Promega, Madison, WI, USA), anti-TrkB (1:1,000, BD Biosciences, San Jose, CA, USA), and anti-BDNF (1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) antibodies.

Data analysis

All data were expressed as mean ± standard error (SEM). Behavioral data were analyzed with repeated analysis of variance (ANOVA) or one-way ANOVA, followed by post hoc Student's *t* test or Dunnett method if appropriate. Data obtained from western blotting were analyzed by Student's

t test. Outliers were removed if the Smirnov–Grubbs test was significant. The R software (version 2.7.2, R Foundation for Statistical Computing, Vienna, Austria) was used for the statistical analysis. The statistical significance was considered when p value was less than 0.05.

Results

Behavioral effects of single cabergoline administration

In FST, Wistar rats showed more active climbing as the dose of the drug increased [$F(1,38)=6.9$, $p=0.013$; Fig. 1a] and exhibited significantly longer duration of climbing at the doses of 1.0 (167±23 s; $t=3.5$, $p=0.005$) and 2.0 μmol/kg BW (154±16 s; $t=2.9$, $p=0.022$) in comparison with the vehicle-treated group (87±13 s). The total duration of swimming was not significantly different after treatment with any dose of cabergoline [$F(1,38)=1.0$, $p=0.32$]. The cabergoline-treated rats showed significantly less immobility compared with that of the vehicle-treated group in a dose-dependent manner [$F(1,38)=15.3$, $p<0.001$; 75±14 s, $t=2.6$, $p=0.045$ at 0.5 μmol/kg; 42±12 s, $t=4.2$, $p<0.001$ at 1.0 μmol/kg; 39±13 s, $t=4.3$, $p<0.001$ at 2.0 μmol/kg]. In addition, the latency of immobile posture was increased in a dose-dependent fashion [$F(1,38)=4.9$, $p=0.032$] and significantly longer at 1.0 μmol/kg (160±23 s, $t=3.0$, $p=0.017$; Fig. 1b) than vehicle treatment (59±12 s). A few rats (3/40) displayed diving, and no dose-dependent response was observed.

As expected, Wistar-Kyoto rats showed significantly shorter climbing [$F(2,1,46)=75$, $p<0.001$; Fig. 1c] and swimming [$F(2,1,46)=35$, $p<0.001$], and longer immobility [$F(2,1,46)=174$, $p<0.001$] in comparison with Wistar rats. Cabergoline treatment in Wistar-Kyoto rats demonstrated significantly increased climbing (28±8 s; $t=2.4$, $p=0.047$) and a trend for reduced immobility (217±10 s; $t=2.0$, $p=0.097$), although no significant changes in immobility ($p=0.49$) were observed in fluvoxamine-treated group. In Wistar rats, reduction in the immobility by cabergoline (61±16 s, $t=3.1$, $p=0.01$) and a trend of the same direction by fluvoxamine (78±26 s, $t=2.1$, $p=0.08$) were observed.

In NST, non-significant effect was shown in the Wistar rats after single cabergoline administration on the latency of feeding in the novel environment (597±3 s in vehicle, 526±52 s in cabergoline group; $t=1.4$, $p=0.22$; Fig. 1d).

In OFT, Wistar rats were treated with cabergoline at three doses (0, 0.25, and 1.0 μmol/kg BW), and its behavior was monitored for 30 min. Distance traveled (horizontal movement; Fig. 2a) and the number of rearing (vertical movement; Fig. 2b) decreased as the time passed [$F(5,10,75)=27.3$; $p<0.001$ for distance; $F(5,10,75)=31.4$, $p<0.001$ for rearing]. There was an interactive effect between dose and time [$F(5,10,75)=2.66$, $p=0.008$], and distance was decreased in the 1.0 μmol/kg BW dose group during 0–5 (9.1±2.1 m, $p=0.037$) and 5–10 min (7.1±1.2 m, $p=0.023$) periods compared with the vehicle-treatment group (16.5±2.1 m, 13.1±1.9 m during 0–5 and 5–10 min periods, respectively). However, no significant effect of cabergoline was observed

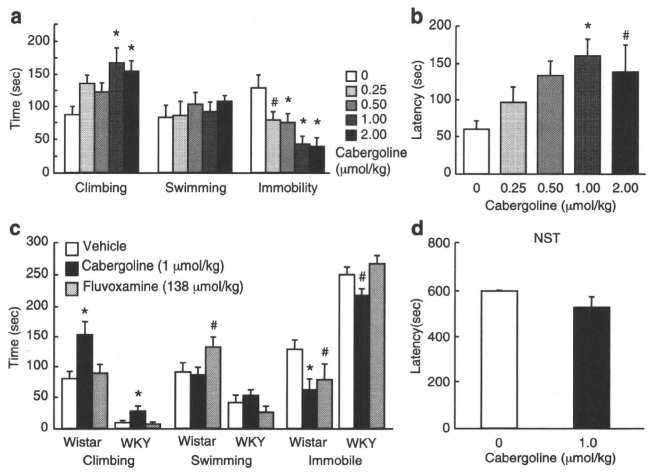

Fig. 1 Effects of single administration of cabergoline observed in FST and NST **a** duration of climbing, swimming, and immobility in Wistar rats in FST ($n=8$); **b** latency of immobile posture in Wistar rats in FST; **c** duration of climbing, swimming, and immobility in Wistar and Wistar-Kyoto (WKY) rats after cabergoline or fluvoxamine treatment in FST ($n=7$–10); **d** latency of feeding of Wistar rats in NST ($n=8$). *Columns* and *bars* represent mean±SEM. *$p<0.05$, #$p<0.1$ vs. vehicle

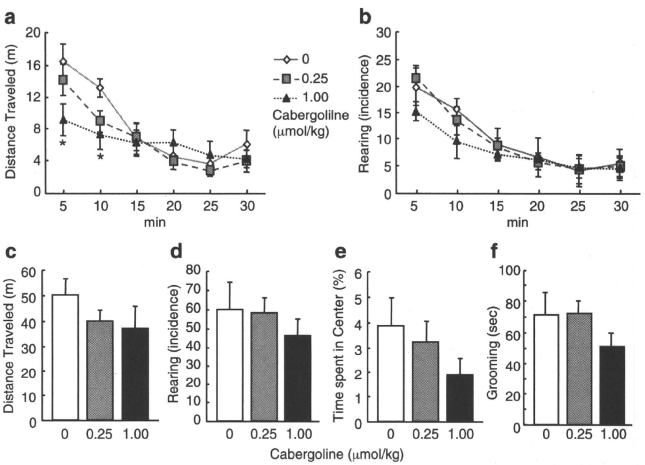

Fig. 2 Effects of single administration of cabergoline of different doses observed in OFT **a** time course curves of distance traveled for rats receiving different doses of cabergoline, **b** the number of rearing, **c** total distance traveled, **d** total number of rearing, **e** time spent in center, and **f** grooming. *Symbols/columns* and *bars* represent mean±SEM. $n=6$ for each group. *$p<0.05$ vs. vehicle

in the total distance traveled during the 30-min test [$F(1,16)=1.5$, $p=0.24$; Fig. 2c]. Cabergoline had no significant effect on total number of rearing [$F(1,16)=1.0$, $p=0.32$; Fig. 2d], time spent in center [$F(1,15)=0.13$, $p=0.72$; Fig. 2e], or grooming behaviors [$F(1,16)=0.51$, $p=0.54$; Fig. 2f].

In EPT, the cabergoline-treated animals (4 μmol/kg BW) spent more time in open arms (163±45 s for cabergoline vs. 61±13 s for vehicle, $t=3.2$, $p=0.008$; Fig. 3a) and less time in closed arms (515±82 s for cabergoline vs. 744±30 s for vehicle, $t=3.6$, $p=0.003$) compared with the vehicle control. The cabergoline treatment slightly, but not significantly, reduced the number of entries into open arms at two doses (1.4±0.4 and 2.2±0.5 at 0.25 and 1 μmol/kg, respectively; Fig. 3b). A remarkable increase in open arm-entry was observed in the 4 μmol/kg group compared with vehicle (9.4±3.0 for cabergoline vs. 3.2±0.5 for vehicle, $t=3.2$, $p=0.007$). There was a significant interaction between time and doses on distance traveled [$F(1,1,76)=5.1$, $p=0.027$; Fig. 3c]. Clear reductions in the distance traveled were observed in cabergoline-treated group from 0 to 5 ($t=2.5$ $p=0.047$ in 0.25 and $t=3.1$ $p=0.011$ in 1 μmol/kg) and from 5 to 10 min ($t=2.6$, $p=0.035$ in 1 μmol/kg). Total distance [$F(1,36)=0.81$, $p=0.37$; Fig. 3f] and the number of rearing were not different between groups [$F(1,36)=0.28$, $p=0.60$; Fig. 3d, g]. There was a trend toward reduced percentage of entries into open arms at 0.25 μmol/kg ($t=2.1$, $p=0.09$; Fig. 3e). A decrease in grooming behavior was also observed at 4 μmol/kg group ($t=4.3$, $p<0.001$; Fig. 3h).

Behavioral effects of chronic cabergoline administration

In FST, there were significant reduction in immobility (99±14 for cabergoline vs. 143±15 s for vehicle; $t=-2.3$, $p=0.035$; Fig. 4a) and significant increase in swimming (116±14 for cabergoline vs. 71±9 s for vehicle; $t=3.0$, $p=0.01$). Latency of the immobile behavior was not significantly different between the two groups ($t=-0.15$, $p=0.88$; Fig. 4b).

In NST, Wistar rats demonstrated a significant reduction in the latency of feeding in the novel environment after the chronic administration of cabergoline (562±38 s in vehicle, 395±63 s in cabergoline group; $t=2.3$, $p=0.04$; Fig. 4c).

In OFT, the cabergoline-treated rats showed slightly longer distance traveled compared with the vehicle-treated rats, although the difference did not reach statistical significance [$F(1,14)=1.0$, $p=0.33$; Fig. 5a, c]. The cabergoline treatment also induced significant increase in the number of vertical movements [$F(1,14)=8.9$, $p=0.01$; Fig. 5b]. Total number of rearing was increased after the treatment (94±10 vs. 55±9; $t=3.0$, $p=0.01$; Fig. 5d). No significant differences were observed in the time spent in the center area ($t=1.2$, $p=0.29$; Fig. 5e) or grooming ($t=-0.85$, $p=0.41$; Fig. 5f).

In EPT, cabergoline-treated rats demonstrated trends toward a decrease in the time in closed arms ($t=-2.0$, $p=0.071$; Fig. 6a), as well as an increase in the time in center ($t=1.7$, $p=0.096$) and total frequency of entry ($t=2.1$, $p=0.051$; Fig. 6b). There was a tendency of more active locomotion during the 15-min test [$F(1,14)=3.3$, $p=0.09$; Fig. 6c, f]. No significant difference was found in the

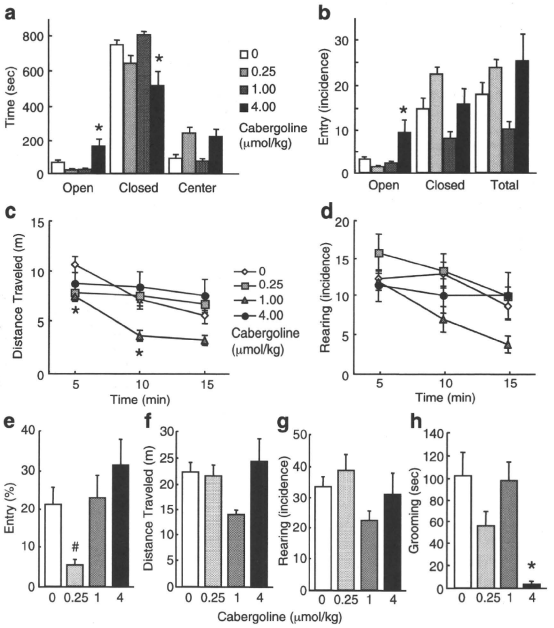

Fig. 3 Effects of single administration of cabergoline observed in EPT **a** time spent in open arm, closed arm, and center in cabergoline- and vehicle-treated rats; **b** the number of entries into arms; **c** time course curves of distance; **d** time course curves of rearing; **e** the percentage of entries into open arms per total entries; **f** total distance traveled; **g** total number of rearing; and **h** the time of grooming behavior. *Symbols/columns* and *bars* represent mean±SEM. n=7–10 for each group. *p<0.05, #p<0.1 vs. vehicle

number of rearing (t=0.47, p=0.65; Fig. 6d, g) and percentage of entries into open arms per total number (t=0.83, p=0.42; Fig. 6e). There was a trend for decreased time of grooming by the treatment (t=−1.89, p=0.079; Fig. 6h).

Effects of chronic cabergoline administration on BDNF protein expression and its related signaling

As shown in Fig. 7a, b, the chronic cabergoline treatment induced a 1.6-fold increase in the expression level of BDNF (t=4.6, p=0.002) in the homogenates from hippocampus. In contrast, no significant differences were detected in the expression levels of either BDNF receptor, i.e., p75 (t=1.4, p=0.21) or TrkB (t=1.3, p=0.22). When we examined downstream signals of TrkB, marked activation (phosphorylation) of ERK1 (pERK1, t=3.5, p=0.008; Fig. 7c, d) was observed (1.9 times higher than vehicle control). The same increasing tendency was caused by chronic cabergoline in the pERK2 level (t=2.0, p=0.074). On the other hand, no significant difference was observed in the pAkt that is another downstream signal of TrkB (t=0.8, p=0.44). In our

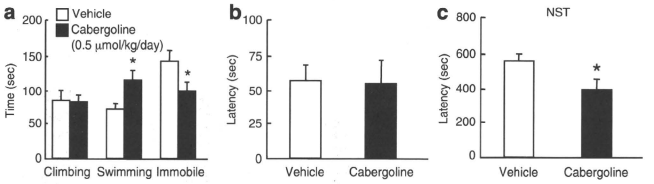

Fig. 4 Effects of chronic (14 days) administration of cabergoline (0.5 μmol/kg BW) observed in FST and NST **a** time in climbing, swimming, and immobility in cabergoline- and vehicle-treated rats in FST; **b** latency of the immobile behavior in FST; **c** latency of feeding in the NST. *Columns* and *bars* represent mean±SEM. n=8 for each group. *p<0.05 vs. vehicle

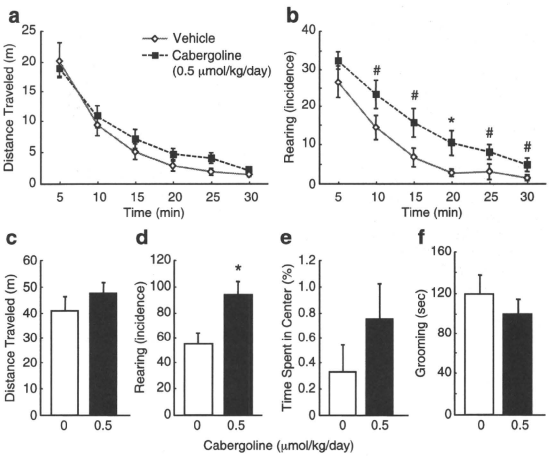

Fig. 5 Effects of chronic administration of cabergoline observed in OFT **a** time course curve of distance traveled, **b** time course curve of the number of rearing, **c** total distance traveled, **d** total number of rearing, **e** time spent in center, and **f** time of grooming. *Symbols/columns* and *bars* represent mean±SEM. $n=8$ for each group. $*p<0.05$, $\#p<0.1$ vs. vehicle

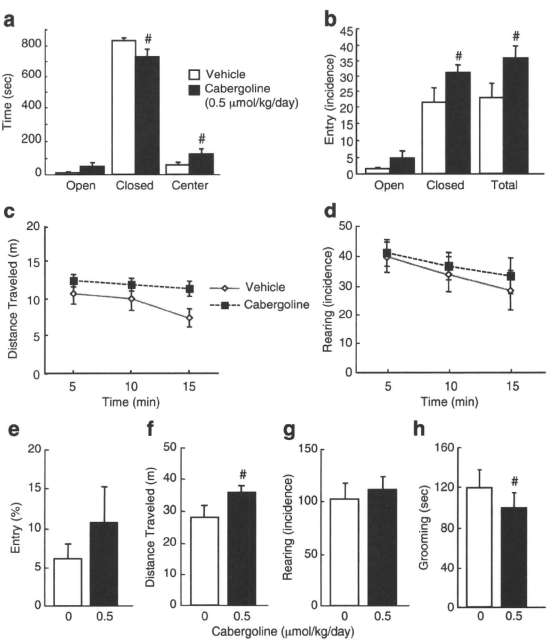

Fig. 6 Effects of chronic administration of cabergoline observed in EPT **a** time spent in each arm, **b** number of entries into each arm, **c** time course of distance traveled, **d** time course of the number of rearing, **e** the percentage of entries into open arms per total number of entries, **f** total distance traveled, **g** total number of rearing, and **h** time of grooming. *Symbols/columns* and *bars* represent mean±SEM. $n=8$ for each group. $*p<0.05$, $\#p<0.1$ vs. vehicle

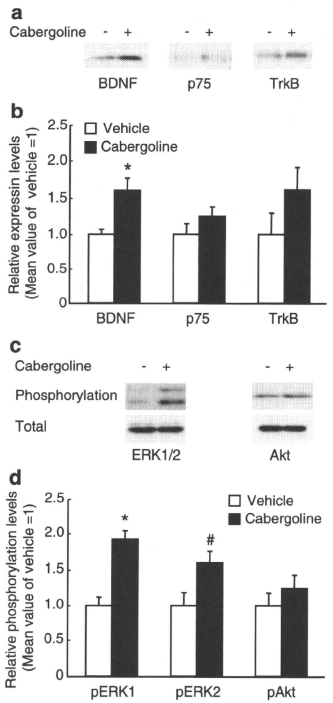

Fig. 7 Effects of chronic administration of cabergoline on the expression and activation of BDNF-related signals **a** representative blotting of BDNF (*left*), p75 (*center*), and TrkB (*right*); **b** densitometrically quantified values of BDNF and its receptors; **c** representative blotting of pERK1/2 (*left*) and pAkt (*right*); **d** densitometrically quantified values of BDNF-related signals. *Columns* and *bars* represent mean±SEM. $n=6$ for each group. $*p<0.05$, $\#p<0.1$ vs. vehicle

system, total expression of ERK1/2 or Akt was not altered by chronic cabergoline (Fig. 7c).

Discussion

The present study demonstrated that cabergoline reduced immobility in both acute and chronic treatment regimen. In NST, the delay in the first feeding was shortened by the repeated administration of this substance. Single treatment with low-dose cabergoline decreased distance traveled in OFT/EPT, while the high-dose treatment increased the time spent as well as the number of entries into open arms in EPT. Chronic treatment with cabergoline increased the number of rearing in OFT and tended to increase the distance traveled in EPT. In addition, our system showed that the 2-week treatment with cabergoline increased the protein level of BDNF and activation of ERK1 in hippocampus.

Our results corroborated the previous reports about antidepressant-like effect of dopamine agonists in the FST (Millan et al. 2004a; Basso et al. 2005) that has been considered as a useful test to measure the antidepressant-like properties of substances (Porsolt et al. 1977). Basso et al. (2005) demonstrated that agonists for D_2/D_3 dopamine receptors (quinpirole and PD128907), but not D_4 receptor (PD168077 and CP226269), reduced the immobility and induced the climbing behavior in the FST. Cabergoline has relatively high affinities to human D_2/D_3 dopamine receptors (Millan et al. 2002); therefore, its agonistic effect to these receptors may be attributable to the behavioral changes in the FST. In addition, chronic treatment with cabergoline reduced the latency of feeding in the novel environment that has been reported to be sensitive by the chronic antidepressant administration (Bodnoff et al. 1989). To our knowledge, this is the first evidence that dopamine agonist stimulates feeding behavior in the novel environment, although further studies are needed to elucidate its mechanism of action. Nevertheless, these results support the possibility that cabergoline has an antidepressant-like property.

Wistar-Kyoto rats displayed 2.5 times longer length of immobility in comparison with Wistar rats. In Wistar-Kyoto rats, the length of climbing behavior was increased, and that of immobility tended to be attenuated by cabergoline, but not by fluvoxamine administration. These results are consistent with previous findings that fluoxetine (SSRI) as well as 8-OH-DPAT (5-HT_{1A} agonist) were ineffective in Wistar-Kyoto rats (López-Rubalcava and Lucki 2000). Tejani-Butt et al. (2003) also showed that chronic treatment with nomifensine (a norepinephrine and dopamine reuptake inhibitor), but not with paroxetine (SSRI), reduced the immobility in FST. Interestingly, the expression level of D_2 receptor in Wistar-Kyoto rats is higher than Wistar in the ventral tegmental area where this receptor functions as an autoreceptor (Yaroslavsky et al. 2006). Swimming stress has been shown to enhance the metabolism of dopamine and 5-HT in the prefrontal cortex of Wistar-Kyoto, but not in Wistar rats (De La Garza and Mahoney 2004). These reports together with our study support the possibility that aberrant dopamine transmission and/or metabolism is involved in hyper-responsiveness to stress in Wistar-Kyoto rats and that dopamine agonists including cabergoline are effective to this strain which is refractory to SSRI.

Cabergoline at low doses demonstrated an anxiogenic effect slightly, but not significantly, while it showed a

striking anxiolytic-like effect at 4 μmol/kg BW. Other dopamine agonists such as ropinirole (Rogers et al. 2000), 7-OH-DPAT (Rogóz et al. 2004), and S32504 (Millan et al. 2004a) have been reported to demonstrate a dose-responsive anxiolytic-like property, suggesting that anxiolytic-like effect may somewhat differ between cabergoline and the other dopamine agonists. This may be attributable in part to the differential affinities for D_2 and D_3 receptors; cabergoline has similar affinities for the two receptors, while the other dopamine agonists have relatively higher affinity for D_3 over D_2 receptors (Millan et al. 2002, 2004a). Especially, preferential D_3 receptor agonist 7-OH-DPAT exerts its anxiolytic-like effect at lower doses than its optimum dose needed for antidepressant-like action (Rogóz et al. 2004). Therefore, it is possible that the action through D_3 receptor may explain the differential anxiolytic-like effects between dopamine agonists. Importantly, the chronic treatment negated the difference in the optimum doses for antidepressant and anxiolytic-like effects of cabergoline. Changes in the action of dopamine agonist after repeated administration were reported previously. The acute stimulation of D_2-like receptor by quinpirole has a dose-dependent bi-phasic effect that reduces the locomotor activity in lower doses (Eilam and Szechtman 1989). Another D_2-like agonist S32504 was also found to inhibit activities in a novel environment by a single administration (Millan et al. 2004b). On the other hand, repeated quinpirole treatment increases locomotor activity in a time-dependent manner (Szechtman et al. 1994; Rowlett et al. 1995). Our results are consistent with these observations. The time-dependent changes in the behavior might be mediated by down-regulation of D_2 autoreceptor by repeated treatment with its agonists as discussed by Szechtman et al. (1994). Another possibility is synaptic neuroadaptation after dopamine transmission. Koeltzow et al. (2003) reported similarities in dopamine outflow in the nucleus accumbens between acute and chronic quinpirole administrations, and suggested the lack of necessity of subsensitized D_2 autoreceptor for behavioral activation after the chronic treatment. From the results of the current study, it is possible that this adaptation by chronic dopamine agonist treatment is mediated by up-regulated BDNF, an important molecule for the modulation of synaptic transmission as discussed below.

We found that repeated administration of cabergoline induced the up-regulation of BDNF and activation of ERK1 in the hippocampus. Many studies found the relationship between ERK, a downstream signaling via TrkB stimulated by BDNF, and the pathophysiology of depression. Dwivedi et al. (2001) reported the inactivation and reduced expression of ERK1/2 in the prefrontal cortex and hippocampus of postmortem brains of individuals with depression. Such a reduced phosphorylation of ERK1/2 was observed in the prefrontal cortex and hippocampus of depression-model rats (Feng et al. 2003; Qi et al. 2006). In contrast, inverse phenomena were confirmed in rats after chronic treatments with fluoxetine (Qi et al. 2008), imipramine (Fumagalli et al. 2005), and mood stabilizers (Einat et al. 2003). BDNF exerts its effects on cell survival (Hetman et al. 1999) and synaptic transmission (Ying et al. 2002) via ERK signaling. Recently, we found that BDNF enhances synaptic maturation via TrkB/ERK signaling and triggers release of glutamate, an excitatory neurotransmitter, in vitro (Kumamaru et al. 2008; Numakawa et al. 2009). Furthermore, we previously showed that antidepressants (imipramine and fluvoxiamine) reinforced the BDNF-triggered glutamate release (Yagasaki et al. 2006). These findings together with the present results suggest that BDNF and ERK signaling are involved in the molecular basis of the action of antidepressants, including cabergoline, which can be attributable to synaptomodulation by BDNF.

In conclusion, the present study suggests that cabergoline has antidepressant- and anxiolytic-like properties in both acute and chronic treatment regimen. Because BDNF signaling is involved in the action of antidepressants and modulates synaptic maturation and transmission, these actions of cabergoline may be mediated by the increased BDNF/ERK signaling. In addition, we found that cabergoline showed the trend for antidepressant-like action in an innate depression-model-rat strain of Wistar-Kyoto that is known to be refractory to SSRI treatments. These results suggest that cabergoline is a promising drug candidate for the treatment of patients with depression who are refractory to SSRI.

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うつ病研究紹介

治療抵抗性うつ病に対するドパミン受容体作動薬の有用性に関する検討

功刀　浩[*1)]，千葉秀一[*2)]，堀　弘明[*3)]，沼川忠広[*4)]

治療抵抗性うつ病への対策には種々の方法があるが，その一つとして抗パーキンソン病薬として適応承認されているものの気分障害に対しては未承認であるドパミン作動薬の有用性が指摘されている。しかし，いまだに前臨床，臨床研究ともにエビデンスが不十分である。われわれはラットを用いた前臨床研究によって，カベルゴリンが単独で抗うつ効果，抗不安効果をもち，海馬における脳由来神経栄養因子シグナルを増加させることを明らかにした。また，非麦角系ドパミン作動薬の１つプラミペキソールの治療抵抗性うつ病に対するオープン試験を行い，有効であることを示唆する予備的結果を得たので紹介する。

Ⅰ．はじめに

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) などの大規模臨床研究によって，現在，第一選択薬として用いられている選択的セロトニン再取り込み阻害薬（SSRI）やセロトニン・ノルアドレナリン再取り込み阻害薬（SNRI）に反応しない，あるいはこれらの薬物では寛解に至らず，意欲低下，集中力低下，無快感症などの症状が残る患者が少なくないことが明らかにされている。意欲，集中力，快感などの情動はドパミンに制御されており，これらの治療抵抗性患者の少なくとも一部にはドパミン系の機能低下があり，セロトニンやノルアドレナリンに作用する薬物では治療効果が十分に上がらず，ドパミン再取り込み阻害薬やモノアミン酸化酵素阻害薬，ドパミン受容体作動薬などが有効であると考えられる[1)]。これらのドパミン系を活性化する薬物のうち，ドパミン受容体作動薬には，ブロモクリプチン，ペルゴリド，カベルゴリン，プラミペキソール，ロピニロールなどがあり，抗パーキンソン病薬として臨床使用されているものの，うつ病に対する保険適応は今のところ承認されていない。しかし，わが国では北海

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道大学のグループの一連の研究があり[2)-6)]，難治性うつ病に対する増強療法の1つの選択肢として有用であることが示唆されている。今後，適応拡大に向けて検討を行う価値があると考えられる。そこで，われわれもカベルゴリン単剤投与の抗うつ効果に関する前臨床研究を行ったほか[7)]，プラミペキソールを用いた治療抵抗性うつ病患者に対する臨床試験(オープン試験)を開始したので紹介したい。

Ⅱ．カベルゴリン単剤投与による前臨床研究

カベルゴリンは，麦角アルカロイド誘導体であり，D_2，D_3，5-HT_{2B}受容体に高い親和性を示すが，他のドパミン受容体への親和性は比較的低い。半減期は60～80時間と比較的長い。パーキンソン病や高プロラクチン血症に適応がある。ドパミン作動薬はSSRIなどと併用した際の増強効果に関するエビデンスは比較的多いが，単剤使用の抗うつ効果に関する検討は少なく，カベルゴリンの単剤投与に関する前臨床検討は，筆者らの知る限りほとんどなかった。

そこで我々は，Wistar系および内因性うつ病モデルとして知られるWistar-Kyoto系ラットを用い，一連の行動実験を行い，同薬の抗うつ薬様・抗不安薬様作用を検討した[7)]。単回投与実験では，8週齢のラットに対して，カベルゴリン(0～4μmol/kg)を皮下投与し，その4時間後に強制水泳試験（FST)，novelty-suppressed feeding試験(NSF)，オープンフィールド試験(OFT)および高架式十字迷路試験(EPM)を行った。反復投与実験では，同週齢のWistarラットにカベルゴリン(0，0.5μmol/kg)を1日1回投与し，投与開始から11，12，13，および14日後にそれぞれOFT，EPM，FST(2日間)を行った。別実験で投与14日後にNSFを行った。

その結果，Wistar系ラットにおいて，カベルゴリン単回投与はFSTにおける無動時間の合計と潜時をと

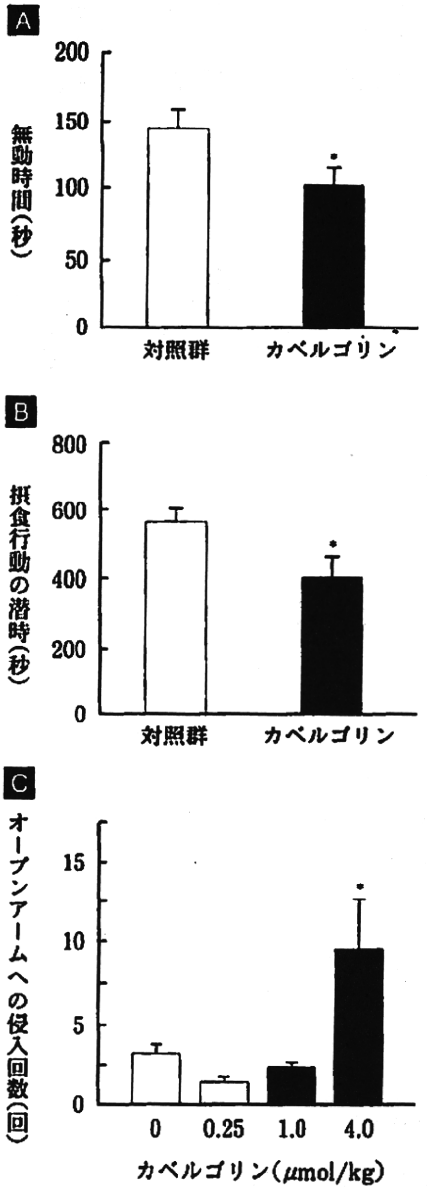

図1　カベルゴリンの抗うつ薬・抗不安薬様作用
A)カベルゴリン反復投与後のFSTにおける無動時間の合計，B)カベルゴリン反復投与後のNSFにおける摂食行動の潜時，C) カベルゴリン単回投与後のEPMのオープンアームへの侵入回数。値は平均±標準誤差。*は対照群と$p < 0.05$で有意な差があることを示す。
下線の略語説明は脚注に示す。　(文献7より)

STAR*D (The Sequenced Treatment Alternatives to Relieve Depression)
SSRI (選択的セロトニン再取り込み阻害薬)
SNRI (セロトニン・ノルアドレナリン再取り込み阻害薬)
NSF (novelty-suppressed feeding試験；新奇環境下摂食行動抑制試験)
FST (強制水泳試験)　OFT (オープンフィールド試験)　EPM (高架式十字迷路試験)

もに用量依存的に減少させ，その効果は1μmol/kgで最大であった。同処置を受けたWistar-Kyoto系ラットにおいても，FSTにおける無動時間の減少傾向が認められた。また，カベルゴリン反復投与を受けたWistar系ラットにおいても無動時間が減少した（図1A）。NSFにおいて，Wistar系ラットの摂食行動の潜時は，単回投与では有意な差が認められなかったものの，反復投与によって有意に減少した（図1B）。FSTの無動時間は，いわゆる"絶望"を意味するものと考えられており，既存の抗うつ薬の投与によって減少することが知られている。一方，NSFにおける摂食行動は，抗うつ薬の慢性投与によって促進されることが知られている。従って，カベルゴリンは単独でも抗うつ作用をもつ可能性が示唆された。

また，単回投与後のOFT，EPMにおいて，開始から10分間の活動量はカベルゴリン（1μmol/kg）の単回投与により減少した。EPMのオープンアーム侵入回数は0.25および1μmol/kgでは有意な影響が見られなかったものの，4μmol/kgの用量で有意に増加した（図1C）。一方，反復投与後ではOFTにおける総移動量の増加やEPTにおけるオープンアーム滞在時間の増加傾向が認められた。OFTの中心部やEPMのオープンアームは周囲に壁がないことから，動物が不安を感じる場所とされる。ベンゾジアゼピン等の抗不安薬は，同所での滞在時間や侵入頻度を減少させることが知られている。我々の結果から，カベルゴリンは抗不安薬様の作用を有することが示唆されるものの，その発現には抗うつ薬様作用より高い濃度や長期的な作用が必要であると考えられた。

以上の行動学的解析から，特に反復投与（14日間）によりカベルゴリンの抗うつ・抗不安薬様作用が示された。この長期的作用は脳内のどのような変化が関与するのであろうか。我々は，うつ病の脳由来神経栄養因子（BDNF）仮説に基づいて[8,9]，反復投与実験後の海馬を採集し，ウエスタンブロット法によりBDNF関連シグナルタンパク質の発現・活性を解析した。その結果，BDNF発現量の上昇が認められた一方で，BDNFの受容体であるTrkBやp75の発現量には変化が見られなかった（図2）。また，TrkB下流のシグナルタンパクのリン酸化（活性化）を定量したところ，同処置によってAktのリン酸化には影響が見られなかったものの，細胞外シグナル制御キナーゼ1（extracellular signal-regulated kinase 1：Erk1）のリン酸化上昇が認められた。

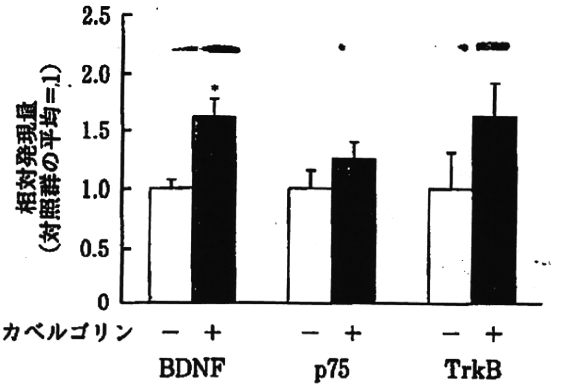

図2　カベルゴリン反復投与後の海馬におけるBDNF関連タンパクの発現変化

図上部の写真は左からBDNF（脳由来神経栄養因子），BDNFの低親和性受容体p75，BDNFの高親和性受容体TrkBの代表的なブロットを示す。同一の写真の中で，左は対照群，右はカベルゴリン投与群のブロットである。図下部のグラフはブロットを定量したものである。値は平均±標準誤差。

*は対照群と$p < 0.05$で有意な差があることを示す。

（文献7より）

以上の結果から，カベルゴリンの反復投与による行動変化にはBDNFシグナルの増強が関与している可能性が支持された。

興味深いことに，Wistar-Kyoto系ラットはSSRI投与に対して，FSTの無動時間の変化が乏しいことが知られている。本研究においてもフルボキサミン（60mg/kg）によってFSTの無動時間が有意に変化しないことを確認した。しかし，カベルゴリンは同系統に対してもFSTの無動時間を減少させる傾向が見られたことから，SSRI抵抗性のうつ病患者に対して有効である可能性が示唆された。

BDNF（脳由来神経栄養因子）

Ⅲ．治療抵抗性うつ病患者に対するプラミペキソールの効果 ～オープン試験の予備的報告～

上記の抗パーキンソン薬として適応が承認されているドパミン作動薬のうち，ブロモクリプチンは，うつ病に対する効果に関するエビデンスがわが国を含めて比較的多く，二重盲験比較試験で有効性を示す結果も得られているが，経口バイオアベイラビリティが低いために高用量を要する[10]。また，麦角系であり，ペルゴリドやカベルゴリンと共に，稀ではあるが心臓弁膜症の危険性があるという問題点がある。プラミペキソールとロピニロールは非麦角系であるため，心臓弁膜症の危険性はないが，突発性睡眠および傾眠の危険がある。麦角系の薬剤は定期的な心エコー検査を要することもあり，我々はまず非麦角系について検討することにした。非麦角系の２剤のうち，うつ病に対するエビデンスはプラミペキソールの方が多く，双極性うつ病に対する二重盲験比較試験で有効性を示す結果も複数得られているが[11]，我々が知る限りわが国における報告は少ないため，まずプラミペキソールについてオープン試験を行った。ただし，現在も症例数を増やしており，以下に述べる結果は予備的段階のものである。

１．対象

対象はこれまでに１種類以上の十分な抗うつ薬治療に反応しない患者であり，ハミルトンうつ病評価尺度21項目（HAMD-21）で15点以上のうつ状態を呈する患者とした。なお，突発性睡眠の危険性があるため，運転を行う者など，不適切な患者は除外した。研究について十分に説明したのち，文書で同意を得た。本研究は，国立精神・神経医療研究センター倫理委員会の承認を得ている。本稿執筆時点（2010年８月）で16例のエントリーがあった（男性６例，女性10例；平均年齢36〔±９〕歳）。DSM-IVに基づく臨床診断（主診断）は，大うつ病８例，気分変調症ないし大うつ病とのdouble depressionが４例，双極Ⅱ型障害４例であった。実際にエントリーした患者のほとんどは２剤以上の抗うつ薬が無効であった難治例であり，ThaseとRushの治療抵抗性うつ病のレベル分類[12]では，全例がstage 2（作用機序の異なる２つの抗うつ薬による適切な治療に反応しない）以上であり，11例がstage 3（stage 2に加えて１つの三環系抗うつ薬による適切な治療に反応しない）以上の難治例であった。

２．試験概要

プラミペキソールはこれまでの治療薬にadd onする形で処方し，0.25mg/日から開始し，効果をみながら２週間ごとに最大３mg/日まで増量し（0.25→0.50→1.0→2.0→3.0），12週間の経過観察を行った。試験期間は併用薬の変更は原則として行わなかった。重症度については，２週間ごとにHAMD-21，臨床概括重症度について評価した。なお，有害事象についても２週間ごとに聴取した。

３．結果

２例は初回のみの投与で脱落した。１例（症例P3）は境界性人格障害を伴う気分変調症の女性で，２回目以降理由を告げずに来院しなくなった。他の１例はやはり気分変調症を主診断として非定型病像の大うつ病のdouble depressionを呈していた女性で，プラミペキソール服薬後に食欲が高まり，元来肥満傾向にあったため，薬物の中止を希望した。残り14例のHAMDの平均点の経時変化を**図３**に示す。**図３**にみられるように，効果は４週間以内に現れることが多く，その後はゆるやかな改善を示した。また，本稿執筆時点で９例は12週間の経過観察が終了しており，12例は８週間以上の経過観察が終了していた。この12例についてみると，最終的なプラミペキソールの１日投与量は0.25mg～３mgであり，その平均は，1.5±0.8mgであった。HAMDの平均点は治療前19.7±4.4から治療後6.8±5.5へと大きく改善し（$p = 0.00007$，paired t-test），HAMDスコアで50%以上の改善を示したレスポンダーは８例（67%）であり，うち７例（58%）が寛解（HAMD ≦ 7）に至った。臨床概括評価

HAMD-21（ハミルトンうつ病評価尺度21項目）

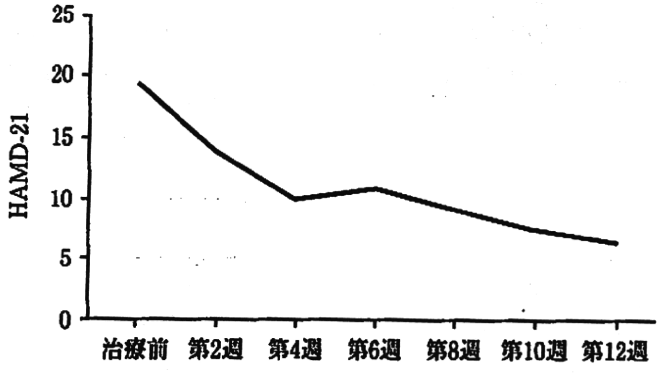

図3　治療抵抗性うつ病に対するプラミペキソール投与後のハミルトンうつ病評価尺度（HAMD-21）スコアの平均値の時間経過（N＝14）

全体の平均でみると，効果は4週間以内に現れ，スコアがおよそ半分程度までに改善し，その後はゆるやかな改善を示した。　（筆者作成）

では「著明改善」6例，「かなり改善」3例，「やや改善」3例，「不変」0，「悪化」0であった。初回で脱落した症例P3以外の15例における副作用は，嗚気2例，空腹1例，起立性低血圧2例，眠気2例，不眠2例であったが，1例の患者に比較的頑固な嗚気がみられたほかは，軽度なものにとどまっていた。

4．考察

本結果から，プラミペキソールは治療抵抗性うつ病相に有効であり，stage 3ないしそれ以上の難治性うつ病に対しても有効性が高く，大うつ病のほか，双極Ⅱ型障害や慢性化による病像変化を来している例でも有効であることが示唆された。しかし，気分変調症に対しては概して有効性が乏しいと考えられた（4例中，2例は脱落，1例はやや改善，1例は著明改善）。これについては，さらに多数例での検討が必要であろう。プラミペキソールの至適用量は症例によって0.5mg～3 mgと幅広かった。従って，少量で有効なこともあるが，3mg程度まで増量しないと効果判定できない症例もあったことから，少量で有効性がみられなくても少なくとも3 mgまで増量する価値があると考えられる。

以上のプラミペキソールの効果に関する我々の結果は，これまでの海外での検討とも合致する。Lattanziら[13]は，37例の治療抵抗性うつ病（16例が単極型，21例が双極型）を対象にプラミペキソール0.375～1 mg/日を16週間投与したところ，Montgomery-Asberg Depression Rating Scale（MADRS）スコアが33.3±8.4から13.9±11.5に低下し（$p < 0.001$），67.7%がMADRSスコアにおいて50%以上の減少を示すレスポンダーであった。Zarateら[14]による21例の双極Ⅱ型障害を対象としたプラセボとの二重盲験比較試験では，実薬がプラセボより有意に勝っており，10例の実薬群（プラミペキソール平均1.7±0.9mg/日）のうち，60%がMADRSで50%以上の減少を示すレスポンダーであった。Goldbergら[15]による双極型うつ病22例に対するプラセボとの二重盲験比較試験検討でも，プラミペキソール（平均投与量1.7±1.3mg/日）はプラセボと比較して有意に効果が高く，実薬群の67%がHAMDスコアで50%以上の改善を示した。以上のように投与量や改善率の高さは我々の結果と概ね一致した。

副作用では，頑固な嗚気が1例にみられ，また，中止の動機となった空腹感が出現した例もあったが，これは過食傾向があったこの症例に対しては「有害事象」であったが，食欲低下を呈するような症例では抗うつ

MADRS (Montgomery-Asberg Depression Rating Scale)

効果とみなすこともできるだろう。なお，眠気は2例にみられたが，突発性睡眠とは性質の異なるものであった。以上から，安全性，忍容性も比較的高いと思われた。

Ⅳ．おわりに

ドパミン作動薬の抗うつ薬に関する前臨床研究と臨床研究に関する我々の取り組みを紹介した。どちらにおいても，ドパミン作動薬が治療抵抗性うつ病に有効であることを示唆する結果が得られた。臨床試験では，従来の抗うつ薬に反応しない症例に対しても有効な患者の頻度が予想外に高かった。今後は，さらに症例数を増やすとともに，長期効果や単剤での有効性についての検討，もう1つの非麦角系ドパミン作動薬ロピニロールに関する検討などを行っていきたいと考えている。また，今回の試験から，プラミペキソールには適応承認をめざした治験を行う価値があることが示唆された。うつ病に対する保険適応が承認されれば，ドパミン作動薬の恩恵を受けるうつ病患者は非常に多い可能性がある。

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3. 双極性障害と注意障害

精神障害における認知障害というテーマは，歴史的に統合失調症で最も注目されてきたものであり，気分障害が対象として取り上げられるようになったのは比較的最近のことである．近年に至るまで気分障害に伴う認知障害が重要視されてこなかった大きな理由の一つとして，Kraepelin によって確立された躁うつ病概念の影響が色濃く残っていたのではないか，という指摘がなされている．

Kraepelin はかつてその有名な教科書で，早発痴呆（dementia praecox）では感情・意欲・認知の障害が段階的に進展し最終的に荒廃状態へと至るのに対して，躁うつ病（manisch-depressive Irresein）では原則的に認知障害は病期に限ったものであり機能低下を残さず完全に治癒すると唱えた．この近代精神医学の嚆矢たる定義は精神科医の無意識に根深く残り，その後の精神障害における認知障害研究を方向づけたともいえる．

1950 年代から 1960 年代にかけて気分障害の概念は，それまでの一元論から，単極性うつ病（unipolar depression：UP）と双極性障害（bipolar disorder：BD）の二元論へと変遷する．その後，脳機能研究の隆盛と歩を同じくするように 1970 年代頃から単極性うつ病における認知機能障害研究が散見されるようになるが，双極性障害に関しての報告が集積し始めるのは双極スペクトラム障害という大きな疾患概念が生まれ始めた 1970 年代以降，1980 年代頃からのことである．

さらに近年，神経生物学的観点からみた注意障害概念の充実に伴って精神障害における注意障害への関心が高まるなか，双極性障害の認知障害研究はその疾患概念とともに新たな注目を浴びつつある．一方，臨床的には，発達論的観点から双極性障害の併存障害として，注意欠如・多動性障害（ADHD*1）の罹患率の高さが指摘されている．こういった背景の下，本項では双極性障害における認知障害をめぐる研究報告から，注意障害に関するものに焦点を当て，それらについて概説する．

*1 ADHD：attention-deficit/hyperactivity disorder.

注意

「注意」（attention）は認知の根幹をなす概念であるが，認知機能における注意の定義は諸説ありいまだ定まらないのが現状である．本項では「注意」を持続性（durability）・選択性（selectivity）などを機能特徴とした情