Effects of Bromocriptine on Dopamine Turnover with or without Levodopa

N Ogawa¹, M Asanuma¹, K Tanaka¹, K Matsura, K Iida¹ and M Yamamoto²

¹Department of Neuroscience, Institute of Molecular and Cellular Medicine, Okayama University Medical School, Okayama 700, Japan;
²Department of Neurology, Kagawa Central Hospital, Takamatsu 760, Japan

Bromocriptine, a dopamine agonist, alleviates symptoms of Parkinson's disease, even when administered alone, and is used for its treatment. Better therapeutic effects are, however, achieved when bromocriptine is used in combination with levodopa. In this study, we examined the biochemical changes caused by bromocriptine administration with and without levodopa, and evaluated the effects of the treatments on dopamine turnover in the mouse striatum. Results show that dopamine turnover is suppressed by the administration of bromocriptine alone with a slight decrease in the amount of dopamine, and dopamine turnover is very strongly promoted by the administration of levodopa. When the two drugs are administered together, bromocriptine enhances the levodopa-induced increase in dopamine turnover in the striatum. These findings indicate that bromocriptine therapy in combination with levodopa enhances the dopaminergic function and suggest that the combination therapy of bromocriptine and levodopa shows good efficacy. The results of this study may, thus, provide a theoretical basis for the combination therapy of bromocriptine and levodopa.

KEY WORDS: Bromocriptine; Levodopa; Combination therapy; Dopamine turnover; Dopamine; Metabolite
INTRODUCTION
Levodopa therapy markedly alleviates symptoms of Parkinson's disease and improves the survival of patients to a level comparable to that of healthy individuals. However, since introducing this therapy in 1976, various problems associated with long-term administration of levodopa have been noted, for example, wearing-off, on-off phenomenon and dyskinesia. Hence, the establishment of improved therapies is warranted.

Levodopa is believed to act on dopamine receptors after it has been transported into the brain and metabolized to dopamine. Progress in dopamine-receptor research has led to the development of dopamine agonists. Bromocriptine, a dopamine agonist that alleviates symptoms of Parkinson's disease, is one of the fruits of this research.

When administered alone, bromocriptine alleviates symptoms of Parkinson's disease and is used as an anti-Parkinsonian drug. However, since it was first used to treat this condition, numerous reports have indicated excellent therapeutic effects when bromocriptine is given in combination with levodopa. There are also numerous studies on the pharmacological actions of bromocriptine itself, but there is insufficient biochemical data to explain the synergic effects of combined therapy.

In this study, we aimed to clarify the mechanism of enhancement of the therapeutic effect by combined use of bromocriptine and levodopa by examining dopamine turnover in the mouse striatum.

MATERIALS AND METHODS
Male ICR mice (aged 7 weeks, body weight 30 – 37 g) were used in the study, and bromocriptine (Sandoz Co. Ltd, Basel, Switzerland) or levodopa/carbidopa (Wako, Tokyo, Japan/RBI, MA, USA) suspended in 0.5% methylcellulose, or 0.5% methylcellulose alone, were administered to the mice intraperitoneally.

Control mice were injected daily for 1 week with 0.3 ml of 0.5% methylcellulose only. Other mice were injected with either bromocriptine, 0.5, 1.5 or 5 mg/kg body weight, levodopa/carbidopa 7.5/0.75 or 25/2.5 mg/kg body weight, or a bromocriptine/levodopa/carbidopa combination, 1.5/25/2.5 or 5/25/2.5 mg / kg body weight, daily for 1 week. All mice were killed 1 h after the last injection by exposure to microwave irradiation. The striatum was immediately isolated. Striatal levels of dopamine and its metabolites were determined by high performance liquid chromatography (model JMD-501, Yanagimoto, Kyoto, Japan), as previously described.

STATISTICAL ANALYSIS
Data are presented as the mean ± SEM, and are analysed by one-way ANOVA followed by Duncan's post-hoc multiple comparison test or the Mann-Whitney U-tests.

RESULTS
The concentrations of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the mouse striatum after the administration of the control vehicle, bromocriptine alone, levodopa/carbidopa alone and both drugs together were determined (Table 1). After administration of bromocriptine alone at 0.5, 1.5 or 5 mg/kg, the dopamine level in the mouse striatum tended to decrease, but no significant difference was observed at 0.5 and 5 mg/kg bromocriptine compared with the control group (Table 1). The concentrations of DOPAC and HVA...
were similar in the group given bromocriptine compared with the control mice. Bromocriptine decreased the HVA levels dose-dependently, but the reduction in the HVA level was not significant compared with the controls. Dopamine turnover \((\text{DOPAC} + \text{HVA})/\text{dopamine}\) in the mouse striatum was suppressed by the administration of bromocriptine alone at 5 mg/kg (Fig. 1).

Administration, however, of levodopa/carbidopa at 25/2.5 mg/kg increased DOPAC and HVA without influencing level of dopamine (Table 1). Dopamine turnover was increased significantly by the administration of levodopa/carbidopa at 7.5/0.75 and 25/2.5 mg/kg (Table 1; Fig. 1). When bromocriptine (1.5 mg/kg) and levodopa/carbidopa (25/2.5 mg/kg) were administered together, the

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Dopamine</th>
<th>DOPAC</th>
<th>HVA</th>
<th>DOPAC + HVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (control)</td>
<td>13</td>
<td>35.86 ± 1.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.69 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.93 ± 0.26</td>
<td>0.212</td>
</tr>
<tr>
<td>Levodopa/carbidopa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5/0.75</td>
<td>8</td>
<td>35.87 ± 3.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.67 ± 0.76&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.260</td>
</tr>
<tr>
<td>25/2.5</td>
<td>10</td>
<td>35.29 ± 1.72</td>
<td>4.97 ± 0.28&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>15.27 ± 0.73&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
<td>0.574</td>
</tr>
<tr>
<td>Bromocriptine (0.5)</td>
<td>8</td>
<td>28.71 ± 2.82</td>
<td>1.35 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.27 ± 0.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.265</td>
</tr>
<tr>
<td>Bromocriptine (1.5)</td>
<td>12</td>
<td>28.07 ± 1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.23 ± 0.40</td>
<td>0.221</td>
</tr>
<tr>
<td>Bromocriptine (5)</td>
<td>10</td>
<td>28.75 ± 1.81</td>
<td>0.97 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.87 ± 0.44</td>
<td>0.168</td>
</tr>
<tr>
<td>Levodopa/carbidopa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25/2.5 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bromocriptine (1.5)</td>
<td>12</td>
<td>37.08 ± 1.87&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>5.43 ± 0.33&lt;sup&gt;a,b,c,d&lt;/sup&gt;</td>
<td>19.61 ± 0.94&lt;sup&gt;a,b,c,d&lt;/sup&gt;</td>
<td>± 0.675</td>
</tr>
<tr>
<td>Levodopa/carbidopa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25/2.5 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bromocriptine (5)</td>
<td>10</td>
<td>42.51 ± 2.49&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>5.75 ± 0.31&lt;sup&gt;a,b,c,d&lt;/sup&gt;</td>
<td>18.53 ± 0.88&lt;sup&gt;a,b,c,d&lt;/sup&gt;</td>
<td>0.571</td>
</tr>
</tbody>
</table>

DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.
All treated mice were killed 1 h after the last of 7 daily injections of vehicle, bromocriptine, levodopa/carbidopa or bromocriptine, levodopa, carbidopa combination.
Numbers in parentheses indicate doses of drugs administered (mg/kg).
Values (ng/mg protein) are means ± SEM.
<sup>a</sup><sup>P</sup> < 0.01 and <sup>b</sup><sup>P</sup> < 0.05 vs vehicle; <sup>c</sup><sup>P</sup> < 0.01 and <sup>d</sup><sup>P</sup> < 0.05 vs levodopa/carbidopa (25/2.5); <sup>e</sup><sup>P</sup> < 0.01 and <sup>f</sup><sup>P</sup> < 0.05 vs bromocriptine (1.5); <sup>g</sup><sup>P</sup> < 0.01 and <sup>h</sup><sup>P</sup> < 0.05 vs bromocriptine (5).
levels of DOPAC, HVA and the [DOPAC + HVA]/dopamine ratio increased markedly (Table 1; Fig. 1). The dopamine level in the mouse-striatum did not increase after co-administration of bromocriptine and levodopa/carbidopa; rather, the change was similar to that seen with levodopa/carbidopa alone (Table 1). The increases in the levels of HVA and in the [DOPAC+HVA]/dopamine ratio after administration of levodopa (25/2.5 mg/kg) with bromocriptine (1.5 mg/kg) were even greater than those after administration of levodopa/carbidopa alone (Table 1; Fig. 1).

DISCUSSION
Bromocriptine, a dopamine agonist, has a positive effect on Parkinson's disease by acting directly on dopamine receptors in the brain. The results of a number of studies on the pharmacological actions of bromocriptine suggest that the drug alleviates the symptoms of Parkinson's disease by acting on D2-receptors among dopamine-receptor subtypes. Furthermore, bromocriptine is known to have diverse regulatory activity on neurotransmission in the cholinergic and serotonergic nervous systems as well as the dopaminergic system.

The anti-Parkinsonian action of bromocriptine is believed to be mediated by postsynaptic D2-receptors. Therefore, it should also theoretically be effective in advanced stage Parkinson's disease, in which pre-synaptic dopamine neurons are markedly degenerated. However, clinical studies have demonstrated that greater effects can be
achieved by using bromocriptine with levodopa, and animal experiments on the combination confirm this effect. Indeed, the presence of pre-synaptic dopamine is necessary for bromocriptine to be effective: that is, the effects of bromocriptine are suppressed when dopamine is reduced by pretreatment with agents such as reserpine and \( \alpha \)-methyl-
\( p \)-tyrosine. These studies support the finding that bromocriptine in combination with levodopa is more effective than bromocriptine alone.

Biochemical changes in the striatum induced by concomitant administration of bromocriptine and levodopa have not as yet been described. Similarly, data that provide a biochemical basis for the enhanced effect of the combination of bromocriptine and levodopa have not been reported.

When bromocriptine is administered alone to mice, dopamine turnover \( ([DOPAC+HVA]/dopamine) \) is slightly suppressed (Fig. 1). This phenomenon can be explained by an early report that bromocriptine suppresses the activity of dopamine neurons by acting on autoreceptors at the pre-synaptic nerve terminals, i.e. those that have the greatest affinity for dopamine agonists. The administration of levodopa increases the amount of dopamine, with a consequent enhancement of dopamine turnover. Despite our expectations that dopamine turnover would be normalized by the concomitant use of the two drugs, bromocriptine markedly and synergistically increased the enhancement of dopamine turnover in the striatum by levodopa (Table 1; Fig. 1). This is the first biochemical evidence for the increased therapeutic effects of the combination therapy of bromocriptine and levodopa which have long been known from clinical experience and behavioural animal studies. This enhancement of the effects of levodopa by bromocriptine is explained by the relationship between D1- and D2-receptors in motor functions of the extrapyramidal system. The effects of D2-receptor stimulators are markedly increased by pre-stimulating D1-receptors. Similarly, the agonist action of bromocriptine on D2-receptors is considered to be markedly increased by co-administration of levodopa because the large amount of dopamine produced by levodopa administration stimulates both D1- and D2-receptors.

The present results suggest that a greater effect on Parkinson’s disease may be obtained by bromocriptine therapy in combination with levodopa administration, providing a theoretical basis for combination therapy with bromocriptine and levodopa in human studies.

**ACKNOWLEDGEMENT**
This work was supported in part by Grants-in-Aid from the Japanese Ministry of Health and Welfare. The authors would like to thank Miss H Danjo for her excellent technical support.

**REFERENCES**


3 Marsden CD, Parkes JD: Success and


21 Jenkins OF, Jackson DM: Bromocriptine enhances the behavioural effects of apomorphine and dopamine after systemic or


---

**N Ogawa, M Asanuma, K Tanaka et al.**

**Bromocriptine on dopamine turnover**

---

**Address for correspondence**

**N OGAwa MD**

Department of Neuroscience, Institute of Molecular and Cellular Medicine, Okayama University Medical School, 2-5-1 Shiktaocho, Okayama 700, Japan.
The Journal of International Medical Research 1996; 24: 387

ERRATA

Effects of Bromocriptine on Dopamine Turnover with or without Levodopa
N Ogawa, M Asanuma, K Tanaka, K Matsuura, K Iida and M Yamamoto
In Table 1, page 273, the effect of Levodopa/carbidopa (25/2.5) + bromocriptine (1.5) on HVA levels should read ‘19.61 ± 0.94 a,b,c,d’ across column 5.

Partial Effective Time (teff\textsubscript{p7}): A new Spirometric Parameter for Lung Function Assessment
G Tatsis and J Jordanoglou
In Figure 1, page 286, the part of the graph on the right hand side of the Figure should have been moved down slightly so that the two pairs of horizontal lines relating to 60 – 70% FVC\textsubscript{N} align across the page. In addition, the time axis should read ‘100% RV, 100% FVC’ and not ‘100% RV, 0% FVC’. The correct Figure is shown below.

EXPIROGRAM OF FORCED VITAL CAPACITY WITH TIME ILLUSTRATING CALCULATION OF THE PARAMETER \textit{teff}_{p7}.

FIGURE 1