Effect of Buflomedil [4-(1-Pyrrolidinyl)-1-(2,4,6-Trimethoxyphenyl)-1-Butanone Hydrochloride] on Neurotransmitters in the Striatum and Substantia Nigra

Hirohumi KODA, Tsuneichi HASHIMOTO and Kinya KURIYAMA
Department of Pharmacology, Kyoto Prefectural University of Medicine,
Kamikyo-ku, Kyoto 602, Japan
Accepted March 26, 1988

Abstract—Effect of buflomedil [4-(1-pyrrolidinyl)-1-(2,4,6-trimethoxyphenyl)-1-butanolone hydrochloride] administration on central monoaminergic systems was investigated using male Wistar rats. Single administration of buflomedil (300 mg/kg, p.o.) induced a significant increase in the content of homovanillic acid without altering the content of dopamine (DA) and also caused a significant decrease in choline acetyltransferase (ChAT) activity in the corpus striatum. In addition, both L-glutamic acid decarboxylase activity (GAD) and γ-aminobutyric acid (GABA) content showed a significant decrease in the substantia nigra following a single administration of buflomedil. These results indicate that buflomedil enhances DA turnover in the nigro-striatal DA pathway, possibly by activating nigro-striatal DA neurons, or by suppressing striatal cholinergic interneurons and/or striato-nigral GABAergic neurons.

Buflomedil [4-(1-pyrrolidinyl)-1-(2,4,6-trimethoxyphenyl)-1-butanone hydrochloride] is known as a vasoactive agent which improves circulation in peripheral and central blood vessels. For example, it has been shown that intravenous administration of buflomedil to anesthetized dogs induces the increase of femoral and cutaneous blood flows (1). In isolated dog arteries, it has also been shown that buflomedil reversibly blocks α-adrenoceptors and possesses an anti-nicotine action (2). Similarly, buflomedil was found to increase the blood flow of forefoot skin in patients suffering from peripheral arterial diseases as well as that of calf muscle (3). Courbier et al. (4) also reported that buflomedil improved the vasomotor reactions to cold stimulus in Raynaud’s patients. In addition, it has been found that buflomedil improves various neuropsychiatric symptoms associated with chronic cerebrovascular diseases in elderly patients (5, 6). In spite of these effectivenesses of buflomedil in the treatment of patients with peripheral and cerebral vascular diseases, the action of buflomedil on the metabolism and function of cerebral tissues has not been clarified. Therefore, the present study was designed to examine the effect of buflomedil on the metabolism and function of cerebral neurotransmitters using the corpus striatum and substantia nigra as an experimental model.

Materials and Methods

Animal used: Male Wistar rats (180–230 g) were used for this study. These animals were kept with free access to laboratory chow (MF: Oriental Yeast, Co., Ltd.) and tap water. All experiments were started at 10:00 a.m. in order to avoid possible circadian variation in the functional states of central monoamine neurons.

Drug administration: Buflomedil hydrochloride (Dainabot Co., Ltd.) was dissolved in distilled water, and the final volume given to the animal was adjusted to 1 ml/100 g body weight. For single oral administrations, 120, 200 or 300 mg/kg of buflomedil was used, while 30 mg/kg/day of buflomedil was given orally for 7 days to examine the effect of continuous administrations of this drug. The same volume of vehicle was given orally
to each control group. One hour after the last administration, rats were killed by focussed microwave irradiation (5 kW, for 0.7 sec) for determining cerebral monoamines, their metabolites and neuroactive amino acids. On the other hand, animals were killed by decapitation when brains were subjected to the determination of enzyme activities.

Measurements of cerebral monoamines and their metabolites: The brain was dissected on a chilled plastic plate, according to the method of Glowinski and Iversen (7). Monoamines and their metabolites were extracted from brain tissues with 0.05 M perchloric acid (PCA) and added with 3,4-dihydroxybenzylamine (DHBA) as an internal standard. In the case of determination of 3-methoxy-4-hydroxyphenyl ethyleneglycol (MHPG), a norepinephrine (NE) metabolite, it was extracted from brain tissue with ethylacetate and determined using 3-hydroxybenzyl alcohol (3HBA) as an internal standard, according to the method of Towell and Erwin (8). The contents of monoamines and their metabolites were determined using a Yanaco L-4000W HPLC with a VM D-101 A electrochemical detector and a Yanapack ODS-A reverse-phase column (particle size of 10 μm, 25×0.4 cm, i.d.) (Yanaco Ltd., Kyoto, Japan), according to the method of Wagner et al. (9) with some modifications. The mobile phase was 0.1 M potassium dihydrogen phosphate/phosphoric acid buffer (pH 3.2) containing 10 μM EDTA, 2.5 mM 1-octane sulfonic acid sodium and methanol (10–15%). The flow rate of mobile phase and detector potential were set at 1 ml/min. and 0.8 V against an Ag/AgCl electrode, respectively.

Measurement of neuroactive amino acids: Neuroactive amino acids were extracted from the substantia nigra with 5% trichloroacetic acid, and determined by an HPLC system (Model LC 3A; Shimadzu Seisakusho Ltd., Kyoto, Japan) with the column (15×0.4 cm, i.d.) packed with cation-exchange resin, ISC-07/S1504, according to the method of Ida and Kuriyama (10).

Measurements of enzyme activity: The activity of tyrosine hydroxylase (TH) was measured by a modification of the assay procedure of Hendry and Iversen (11). The reaction mixture, containing 0.4 M potassium phosphate buffer (pH 6.0), 1.25 mM 6,7-dimethyl-5,6,7,8-tetrahydropterine (DMPT), 125 mM 2-mercaptoethanol, 0.1 mM benzserazide (Ro 4-4602), 7.5 μM L-[2,3-3H]-tyrosine and enzyme preparation, was incubated at 37°C for 20 min. The reaction was terminated by the addition of 0.25 ml of 0.4 N PCA. Following centrifugation of the suspension, 100 mg of activated alumina was added to the resultant supernatant, which was subsequently adjusted to pH 8.6 with 1 ml Tris solution (100 mM Tris, 50 mM EDTA, 75 mM NaOH). The stirred mixture was packed into a column (0.6 cm diameter), which was then washed with 20 ml of 5 mM Tris-HCI buffer (pH 8.6). The [3H]3,4-dihydroxyphenylalanine (DOPA) formed was eluted with 1.5 ml of 0.5 M acetic acid and transferted to a counting vial containing 10 ml Triton-toluene scintillant (0.5% 2,5-diphenyloxazole (PPO), 0.03% 1,4-di[5-phenyloxazolyl]benzene (POPPO) and 33% Triton X-100 in toluene), and the radioactivity in each vial was measured by a Packard 3379 liquid scintillation spectrometer, which was also utilized for all determinations of enzyme activity.

The decarboxylation of DOPA decarboxylase (DDC) was measured by the formation of 14CO2 from radiolabelled DL-DOPA according to the method of Lloyd and Hornykiewicz (12).

The decarboxylation of L-glutamic acid by L-glutamic acid decarboxylase (GAD) was measured by the formation of 14CO2 from 1-[14C]-L-glutamic acid according to the method of Kimura and Kuriyama (13).

Choline acetyltransferase (ChAT) activity was measured by the method of Fonnum (14).

Measurement of protein content: Protein content in each enzyme preparation was determined by the method of Lowry et al. (15), using bovine serum albumin as a standard.

Statistical analysis: Results were expressed as values of the mean±S.E.M., and statistical significance was determined using Student's t-test.

Drugs used: Buflomedil hydrochloride was obtained from Dainabot Co., Ltd. (Osaka, Japan). 6,7-Dimethyl-5,6,7,8-tetrahydro-
pterine monohydrochloride, physostigmine sulfate, Hyamine 10X-OH, PPO and POPOP were obtained from Nakarai Chemicals Co. (Kyoto, Japan). PLP was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 1-Octanesulfonic acid sodium salt was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.) and benserazide (Ro 4-4602) was a gift from Nippon Roche, Japan. Activated alumina was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). L-[2,3-3H]tyrosine (16 Ci/mmol), DL-3,4-[alanine-1-14C]DOPA (50.0 mCi/mmol), L-[1-14C]glutamic acid (47.3 mCi/mmol) and [1-14C]acetyl CoA (54 mCi/mmol) were obtained from New England Nuclear (Boston, MA, U.S.A.).

Results

Changes in body weight and behavior in buflomedil-treated rats: Continuous administration of buflomedil (30 mg/kg x 7 days) had no significant effects on body weight and behavior of rats, although larger doses (120, 200, 300 mg/kg) of buflomedil induced a sedation.

Effect of buflomedil on the contents of monoamines and their metabolites in various cerebral regions: Continuous administration of buflomedil (30 mg/kg x 7 days) induced a significant increase in the content of homovanillic acid (HVA), a main metabolite of dopamine (DA), in the striatum without altering that of DA (Table 1). The contents of NE, MHPG, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), however, showed no significant changes in all cerebral regions following continuous administration of buflomedil. Similarly, single administration of buflomedil (200 and 300 mg/kg) induced a significant increase in the striatal content of HVA without altering that of DA (Fig. 1). These data indicate that buflomedil is a drug capable of inducing the facilitation of DA turnover rate specifically in the striatum following single and continuous administrations. Therefore, the effects of buflomedil on nigro-striatal DA neurons, cholinergic interneurons in the corpus striatum and striato-nigral GABAergic neurons (16-18) were investigated to clarify the neurochemical mechanisms underlying this buflomedil-induced increase of DA turnover rate in the striatum.

Effect of buflomedil on the activities of TH, DDC and GAD and ChAT in the nigrostriatal pathway: TH, DDC and GAD activities were measured in both the corpus striatum and substantia nigra, while ChAT activity was measured only in the corpus striatum (Table

---

**Fig. 1.** Effect of single oral administration of buflomedil (Buf) on striatal dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) contents in rats. Rats received orally various doses (120, 200, 300 mg/kg) of Buf. Each bar represents the mean±S.E.M. from 5 separate experiments. **P<0.02, ***P<0.01, compared with each control value.
Table 1. Effect of continuous oral administration of buflomedil on dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) contents in brain of rats

<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Buflomedil</td>
<td>Control</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>523.61±153.17</td>
<td>722.52±219.31</td>
<td>93.96±14.80</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>42.48±9.64</td>
<td>44.94±14.20</td>
<td>12.86±3.11</td>
</tr>
<tr>
<td>Midbrain</td>
<td>454.01±108.88</td>
<td>338.95±86.10</td>
<td>77.59±16.04</td>
</tr>
<tr>
<td>Pons-Medulla</td>
<td>61.62±19.61</td>
<td>60.80±10.51</td>
<td>18.92±1.75</td>
</tr>
<tr>
<td>Striatum</td>
<td>5909.45±1541.14</td>
<td>7241.49±2024.67</td>
<td>630.60±72.26</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>594.29±211.09</td>
<td>329.10±146.92</td>
<td>132.84±19.02</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>183.58±37.60</td>
<td>143.90±44.77</td>
<td>26.08±2.97</td>
</tr>
</tbody>
</table>

Rats received orally 30 mg/kg of buflomedil once daily for 7 days. Each content is expressed as ng/g wet weight. Values given are the mean±S.E.M. from 4 to 5 separate experiments. *P<0.05, represents significant difference from the control value.
2). TH and DDC activities, involved in DA biosynthesis, showed no changes in both the corpus striatum and substantia nigra following single (300 mg/kg) and continuous (30 mg/kg \times 7 days) administrations of buflomedil. In contrast, single administration of buflomedil (300 mg/kg) induced a significant decrease in striatal ChAT activity, the rate limiting enzyme of ACh biosynthesis, although this decrease in ChAT activity was not observed following the continuous administration of buflomedil.

GAD activity, the rate limiting enzyme for GABA biosynthesis, showed a significant decrease in the substantia nigra. On the other hand, continuous administration of buflomedil had no effect on GAD activity in both the corpus striatum and substantia nigra.

**Effect of buflomedil on the contents of neuroactive amino acids in substantia nigra:**

The nigral contents of neuroactive amino acids (taurine, aspartic acid, glutamic acid, glycine and GABA) were also investigated following the single administration of buflomedil (300 mg/kg) (Fig. 2), since the single administration of buflomedil exhibited a significant decrease in GAD activity of this cerebral region.

Table 2. Effect of oral administration of buflomedil on tyrosine hydroxylase (TH), dopa decarboxylase (DDC), choline acetyltransferase (ChAT) and L-glutamic acid decarboxylase (GAD) activities in striatum and substantia nigra

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Buflomedil (300 mg/kg)</th>
<th>Control</th>
<th>Buflomedil (30 mg/kg \times 7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td>2.41±0.21</td>
<td>2.65±0.56</td>
<td>2.32±0.44</td>
<td>2.93±0.67</td>
</tr>
<tr>
<td>DDC</td>
<td>13.63±0.56</td>
<td>9.33±1.95</td>
<td>11.15±1.30</td>
<td>15.61±3.56</td>
</tr>
<tr>
<td>GAD</td>
<td>31.10±2.28</td>
<td>28.40±2.04</td>
<td>28.20±3.30</td>
<td>31.50±3.90</td>
</tr>
<tr>
<td>ChAT</td>
<td>245.87±12.42</td>
<td>184.32±17.80*</td>
<td>229.41±13.46</td>
<td>203.73±7.64</td>
</tr>
<tr>
<td><strong>Substantia nigra</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td>3.57±0.70</td>
<td>3.33±0.81</td>
<td>1.61±0.48</td>
<td>1.74±0.30</td>
</tr>
<tr>
<td>DDC</td>
<td>10.45±1.75</td>
<td>12.80±1.64</td>
<td>12.74±2.00</td>
<td>16.06±2.68</td>
</tr>
<tr>
<td>GAD</td>
<td>53.40±4.91</td>
<td>40.10±2.23*</td>
<td>47.99±7.56</td>
<td>41.43±4.81</td>
</tr>
</tbody>
</table>

All enzyme activities are expressed as amoles/mg protein/hour. Values given are the mean±S.E.M. from 5 to 10 separate experiments. *P<0.05, represents significant difference from each control value.

**Fig. 2.** Effect of single oral administration of buflomedil (Buf) on nigral contents of neuroactive amino acids in rats. Rats received orally 300 mg/kg of buflomedil. Each bar represents the mean±S.E.M. from 5 separate experiments. Abbreviation used: GABA=\(\gamma\)-aminobutyric acid. **P<0.02, compared with each control value.
GABA without altering those of other neurotransactive amino acids (Fig. 2).

**Discussion**

There are many experimental or clinical reports suggesting the possible therapeutic value of buflomedil on various neuropsychiatric disorders associated with cerebrovascular diseases (1–6). The mechanism of action for such effects of buflomedil, however, is unknown at present.

In the present study, it has been found that continuous administration of buflomedil (30 mg/kg x 7 days) induces the increase of striatal content of HVA, a metabolite of DA, without altering that of DA. Furthermore, this acceleration of DA turnover rate was also observed in the striatum following single administration of large doses of buflomedil (200, 300 mg/kg). In addition, it has been found that NE, 5-HT and their respective metabolites examined in various brain areas do not alter under the same experimental conditions. These results suggest that buflomedil may accelerate selectively the striatal turnover rate of DA. Therefore, the effect of buflomedil on the nigro-striatal system was further investigated to clarify the mechanism underlying buflomedil-induced enhancement of DA turnover rate.

Single administration of buflomedil (300 mg/kg) exhibited no change in the activities of TH and DDC in the corpus striatum and substantia nigra. This result suggests that the buflomedil-induced enhancement of striatal DA turnover rate may not be due to the activation of DA biosynthesizing enzymes but due to the alterations in other functions of nigro-striatal DA neurons such as uptake and release of DA. On the other hand, the nigral GABA content as well as GAD activity and the ChAT activity in the corpus striatum showed a significant decrease following a single administration of buflomedil (300 mg/kg). These results suggest that buflomedil may inhibit the function of striato-nigral GABAergic neurons and cholinergic interneurons in the corpus striatum.

It is well-known that the nigro-striatal dopaminergic neurons have an inhibitory regulation on the striatal cholinergic interneurons (16, 17), and these cholinergic interneurons further act as an excitatory regulator on the striato-nigral GABAergic neurons (18). Furthermore, the striato-nigral GABAergic neurons act as an inhibitory regulator on nigro-striatal dopaminergic neurons (18). Considering these facts, it is probable that buflomedil may accelerate the striatal turnover rate of DA by inducing the hyperfunction of nigro-striatal dopaminergic neurons or the hypofunction of striatal cholinergic interneurons and/or striato-nigral GABAergic neurons. Continuous administration of a smaller dose of buflomedil (30 mg/kg x 7 days), however, did not induce a significant decrease in nigral GAD and striatal ChAT activities in spite of the increase of striatal DA turnover rate. This result suggests that either large doses of buflomedil or a longer period of administration may be required to induce the changes in ChAT and GAD activities in the nigro-striatal system or a long-term enhancement of striatal DA turnover rate by buflomedil may alter the functions of striato-nigral GABAergic neurons and striatal cholinergic interneurons. These points should be clarified in future studies.

In conclusion, buflomedil seems to be a drug capable of enhancing the striatal turnover rate of DA through the activation of nigro-striatal DA neurons, or the inhibition of striatal cholinergic neurons and/or striato-nigral GABAergic neurons.

**References**


4. Courbier, R., Bergeon, P. and Fouque, R.:


