Alpha-Methyladrenaline: A Possible Active Metabolite of Alpha-Methyldopa in the Rat Brain

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Accepted June 7, 1985

Abstract—To determine whether or not α-methyladrenaline (MA) is an active metabolite of α-methyldopa, a centrally-acting hypotensive compound, we measured MA in the rat brain using the high-performance liquid chromatographic electrochemical detection method. After five daily treatments of α-methyldopa given twice daily a dose of 40 mg/kg, we found trace amounts of MA in the hypothalamus and C1–C2 area (hypothalamus, 23.7 ± 2.3 picomole/g, n=7; C1–C2 area, 5.4 ± 0.4 picomole/g, n=4), as well as large amounts of α-methylnoradrenaline (MNA) (Hypothalamus, 16.6 ± 0.4 nanomole/g, n=7; C1–C2 area, 7.0 ± 0.2 nanomole/g, n=4). In these brain areas, the amount of endogenous adrenaline was reduced to 10.6% and 16.1% of the control values, respectively. The amounts of MA were only 9.0% and 6.2% of that of endogenous adrenaline in these respective areas whereas MNA was detected at approximately the same level as endogenous noradrenaline. These findings indicate that MA is synthesized from α-methyldopa to a very minute extent in the hypothalamus and C1–C2 area, and a large amount of MNA was synthesized in these areas. These are of interest considering the changes of endogenous adrenaline and noradrenaline. Our results raise doubts about the participation of MA on the main determinant of the central hypotensive effect of α-methyldopa.

The mechanism of the hypotensive effect of α-methyldopa, a widely used centrally-acting antihypertensive drug, is still poorly understood. Central adrenaline neurons whose cell bodies are located in the C1 and C2 areas of the lower brainstem (1) are known to participate in the regulation of blood pressure (2–4). Tung et al. (5) recently reported that α-methyladrenaline (MA), a candidate for one of the in vivo metabolites of α-methyldopa, reduced blood pressure in rats when administered into the cerebral ventricle and nucleus of the solitary tract. Decrease in adrenaline contents in the rat hypothalamic nuclei was observed after five days of treatment with α-methyldopa (40 mg/kg given twice a day) (6). Thus, it is postulated that central adrenaline neurons convert α-methyldopa to MA, and the hypotensive action of α-methyldopa may be dependent on the production of MA in the central nervous system.

Using a highly sensitive high-performance liquid chromatographic electrochemical detection method for catecholamines (7–9), we quantitated α-methylated catecholamines, i.e., MA, α-methylnoradrenaline (MNA) and α-methyldopamine (MDA), as well as endogenous catecholamines in the rat brain, and we found a substantial amount of MA in the hypothalamus and C1–C2 area among the areas of the brain after the five daily treatments of α-methyldopa given twice daily, in a dose of 40 mg/kg.

Materials and Methods

Rats and drug treatment: Adult male Wistar rats weighing 300–350 g were fed a standard diet (F-2, Funabashi Farm Co., Chiba, Japan) and water ad libitum and housed at 24°C.
Alpha-methyldopa was given orally in a dose of 40 mg/kg twice daily (9:00-10:00 am and 21:00-22:00 pm) for five days.

**Sample preparations:** Four hours after the last treatment, all rats were decapitated, the brains immediately removed, and the cortex and hypothalamus dissected out by the method of Glowinski and Iversen (10). The noradrenergic A1-A2 and adrenergic C1-C2 areas in the lower brainstem were separated in accordance with the data of Chamba et al. (11) on an ice-cooled glass plate. Briefly, after the removal of the cerebellum, tissue blocks were isolated from the lower brainstem by vertical cuts at 2.5 mm rostral and 2.5 mm caudal to the obex. The isolated tissues were then divided into two parts by a vertical cut of the obex. The rostral and caudal portions were labelled the C1-C2 and A1-A2 areas, respectively. The thoracic spinal cords (Th2-5) were removed by laminectomy. All the isolated tissues were placed on dry ice and stored at -30°C until assayed.

**Determination of α-methylated and endogenous catecholamines:** The catechols of the cortex, hypothalamus, spinal cord, A1-A2 and C1-C2 areas were quantitated in the homogenates pooled from 5-10 rats. Tissues were homogenized in 4 volumes of 0.4 N HClO₄, containing 3,4-dihydroxybenzylamine (DHB, 250 pg/ml) and epinine (N-methyldopamine, 50 ng/ml). Epinine was used as an internal standard for MNA, MDA, α-methyldopa, noradrenaline and dopamine, and DHB was used for MA and adrenaline. Homogenization was performed in an ice-water bath for 45 sec using a Polytron homogenizer (PT 10, Kinematica, Switzerland), and homogenates were centrifuged at 15,000 rpm, 4°C for 15 min. The supernatants were transferred to conical reaction vials containing 100 mg of alumina, 100 μl of 0.1 M EDTA-2Na, 100 μl of 1 M sodium bisulfite and 8 ml of 3 M Tris-HCl buffer, pH 8.6. Catechols in the supernatants were absorbed onto the alumina by shaking for 30 min. After washing with 40 ml of 5.0 mM Tris-HCl buffer, pH 8.6, the alumina was transferred to a MF-1 microfilter (Bioanalytical systems, U.S.A.). Catechols were eluted with 250 μl of 0.1 N HClO₄. Twenty μl of the HClO₄ eluent for adrenaline and 150 μl for the other catechols were injected into the high-performance liquid chromatograph equipped with an electrochemical detector (LCEC).

The LCEC was composed of a 6000 A pump, U6K universal injector, π-Bondapak C18 reverse phase column (30×0.39 cm), radial compression separation system (RCM-100 and Nova Pak C18 cartridge column) (Waters Assoc., U.S.A.) and an electrochemical detector (LC-4A, Bioanalytical System, U.S.A.) with a glassy carbon electrode (TL-5). The working electrode was maintained at +0.7 V versus the Ag/AgCl reference electrode. The mobile phase, 0.15 M monochloroacetic acid-NaOH buffer, pH 3.0, containing 0.2 mM sodium octylsulfate (PIC B-8, Waters Assoc.) was pumped at a rate of 0.8 ml/min at 25°C.

The concentration of each compound was calculated from the peak height ratio to the respective internal standard and compared with authentic compounds which were run through the whole sequence of procedures.

**Drugs and other materials:** Alpha-methyldopa, obtained from the Merck-Banyu Co., Japan, was suspended in 0.5% carboxymethyl cellulose at a concentration of 20 mg/ml. Alpha-methyldaprenaline was a kind gift from Dr. John A. Oates, Department of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee, U.S.A. Alpha-methylnoradrenaline and α-methyldopamine were obtained from Sterling-Winthrop Research Inst., NY, U.S.A. 3,4-dihydroxybenzylamine (DHB) and epinine HCl were purchased from the Aldrich Chemical Co., U.S.A. Alumina (Aluminium oxide, Woelm neutral, Grade 1; M. Woelm, West Germany) was activated by the method of Anton and Sayre (12). All other drugs were obtained from the Sigma Chemical Co., U.S.A.

Values were expressed as the average ±S.E.M. of picomole or nanomole per g wet weight of tissue. The statistical significance of differences between mean values was analyzed using Student's t-test.

**Results**

The five daily treatments of α-methyldopa given twice daily in a dose of 40 mg/kg
reduced systolic blood pressure significantly from 124±4 mmHg to 97±7 mmHg (n=5, P<0.01) by the tail plethysmographic method.

Figure 1 shows typical chromatograms obtained from standard solutions containing known amounts of authentic noradrenaline, MNA, dopamine, α-methyldopa, epinine, MDA, adrenaline, DHB and MA. As shown in Figs.3 and 4, since the levels of adrenaline and MA in the brain areas were too small to quantitate with other catechols simultaneously, we used two internal standards, DHB and epinine, and applied two different sensitivities of the LCEC system. The chromatograms of B and C were illustrated at a high sensitivity, that is 20 times higher than that of the chromatogram of A. Under the conditions described in the section of Materials and Methods, noradrenaline, MNA, adrenaline, DHB, MA, dopamine, α-methyldopa, epinine and MDA were eluted from a reverse phase μ-Bondapak C18 column and a Nova Pak C8 cartridge column in the LCEC system with retention times of 15.7, 24.3, 26.2, 33.3, 48.4, 54.9, 67.7, 76.6 and 154.5 min, respectively. Although the separation between adrenaline and MNA was not complete, strict linearity in the standard curve for adrenaline was obtained between 2–20 picomoles per sample, and that for MA was obtained between 1–20 picomoles per sample, as indicated in the upper portion of the figure.

The chromatograms at the low sensitivity obtained from the hypothalamus and C1–C2 area of control rats (Fig. 2, control) and α-methyldopa-treated rats (Fig. 2, treated) illustrate a significant decrease of endogenous noradrenaline and dopamine. We detected a large amount of MNA and MDA in these areas.

Figure 3 shows typical chromatograms of adrenaline obtained from the hypothalamus and C1–C2 area. The decreases of endogenous adrenaline are illustrated in the chromatograms after α-methyldopa treatment.

Figure 4 shows typical profiles of the MA

Fig. 1. Typical chromatograms of authentic noradrenaline (NA), α-methylnoradrenaline (MNA), dopamine (DA), α-methyldopa (MDP), epinine (EPI, internal standard), α-methyladrenaline (MDA), adrenaline (AD), 3,4-dihydroxybenzylamine (DHB, internal standard) and α-methyladrenaline (MA) extracted as described in "Materials and Methods". NA, MNA, AD, DHB, MA, DA, α-methyldopa, epinine and MDA were eluted from a reverse phase μ-Bondapak C18 column and a Nova Pak C8 cartridge column in the LCEC system with retention times of 15.7, 24.3, 26.2, 33.3, 48.4, 54.9, 67.7, 76.6 and 154.5 min, respectively. The strict linearity in calibration curves of NA, MNA, DA, MDA, α-methyladrenaline, AD and MA are illustrated in the upper portion of the figure.
Fig. 2. Typical chromatograms of noradrenaline (NA), \( \alpha \)-methylnoradrenaline (MNA), dopamine (DA), \( \alpha \)-methyldopa (MDP), epine (EPI, internal standard) and \( \alpha \)-methyldopamine (MDA) extracted as described in "Materials and Methods" from the hypothalamus and C1–C2 area. The decreases of the peak height of NA and DA are illustrated in the chromatograms (treated) after the \( \alpha \)-methyldopa treatment.

Fig. 3. Typical chromatograms of adrenaline (AD) extracted from the hypothalamus and C1–C2 area. Noradrenaline (NA), \( \alpha \)-methylnoradrenaline (MNA), AD and 3,4-dihydroxybenzylamine (DHB, internal standard) were eluted from a reverse phase \( \mu \)-Bondapak C18 column and a Nova Pak C18 cartridge column in the LCEC system with retention times of 15.7, 24.3, 26.2 and 33.3 min, respectively. The decreases of the peak height of AD are illustrated in the chromatograms (treated) after the \( \alpha \)-methyldopa treatment.

Chromatograms in the hypothalamus and C1–C2 area. No peaks were observed at the retention time of MA in the chromatograms of control rats, and there was no splitting of the MA peak in the chromatograms (treated + authentic MA) when known amounts of authentic MA were simultaneously injected with the HClO4 eluent to the LCEC system. Thus, our LCEC system allowed for quantitation of a trace amount of adrenaline and
Fig. 4. Typical chromatograms of α-methyladrenaline (MA) extracted from the hypothalamus and C1–C2 area. Noradrenaline (NA), α-methylnoradrenaline (MNA), adrenaline (AD), 3,4-dihydroxybenzylamine (DHB, internal standard) and MA were eluted from a reverse phase μ-Bondapak C₁₈ column and a Nova Pak C₁₈ cartridge column in the LCEC system with retention times of 15.7, 24.3, 26.2, 33.3 and 48.4 min, respectively. No peaks of MA are illustrated in the chromatograms of control rats (control). No splitting of the peaks of MA are illustrated in the chromatograms (treated + authentic MA) when known amounts of authentic MA and HClO₄ eluent were simultaneously injected to the LCEC system.

Table 1. Changes in endogenous dopamine (DA), noradrenaline (NA) and adrenaline (AD) contents in four brain regions and thoracic spinal cord of control and α-methylldopa-treated rats

<table>
<thead>
<tr>
<th>Region</th>
<th>DA</th>
<th>NA</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (7)</td>
<td>207±33</td>
<td>1931±45</td>
<td>–</td>
</tr>
<tr>
<td>treated (7)</td>
<td>65±2*</td>
<td>162±13*</td>
<td>–</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (7)</td>
<td>3219±83</td>
<td>11580±340</td>
<td>264±10</td>
</tr>
<tr>
<td>treated (7)</td>
<td>1037±66*</td>
<td>608±45*</td>
<td>28±3*</td>
</tr>
<tr>
<td>C1–C2 area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (7)</td>
<td>486±12</td>
<td>4332±90</td>
<td>87±6</td>
</tr>
<tr>
<td>treated (4)</td>
<td>256±5*</td>
<td>185±21*</td>
<td>14±1*</td>
</tr>
<tr>
<td>A1–A2 area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (7)</td>
<td>419±13</td>
<td>2914±121</td>
<td>24±2</td>
</tr>
<tr>
<td>treated (4)</td>
<td>252±15*</td>
<td>188±17*</td>
<td>–</td>
</tr>
<tr>
<td>Thoracic spinal cord</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (7)</td>
<td>250±8</td>
<td>1640±63</td>
<td>11±1</td>
</tr>
<tr>
<td>treated (7)</td>
<td>175±12*</td>
<td>41±5*</td>
<td>–</td>
</tr>
</tbody>
</table>

Treated rats were given α-methylldopa (40 mg/kg, p.o., twice a day, for five days). Values are the average±S.E.M. of picomole per g wet weight of tissue. Numbers of determinations are given in parenthesis. *P<0.001, when compared with control value. (–): non-detectable.
Changes in endogenous catecholamines contents are summarized in Table 1. A trace amount of endogenous adrenaline was quantitated in the hypothalamus, thoracic spinal cord, A1–A2 and C1–C2 areas of the control rats. The amount of adrenaline was only 0.7 to 0.8% of the noradrenaline content in the A1–A2 area and thoracic spinal cord. We detected a relatively larger amount of adrenaline in the hypothalamus and C1–C2 area, where the adrenaline levels were about 2.0% of those of NA. The adrenaline content in the hypothalamus and C1–C2 area decreased to 10.6% and 16.1%, respectively, after five daily doses of α-methyldopa (40 mg/kg, p.o., twice a day). Although we observed a decrease of adrenaline content in the A1–A2 area and thoracic spinal cord, the level of adrenaline was under the detection limit (<2–4 picomole/ a sample) after the α-methyldopa treatment. The endogenous noradrenaline and dopamine in all areas studied decreased significantly.

We quantitated a small amount of MA in the hypothalamus and C1–C2 area; however, the levels of MA were only 9.0% and 6.2% of the endogenous adrenaline amounts, respectively (Table 2). Although we observed a decrease of adrenaline content in the A1–A2 area and thoracic spinal cord, no peaks of MA were detected in the chromatograms of these areas. The accumulation of MNA was in proportion to the levels of endogenous noradrenaline. It should be noted that MNA was almost the same level as the endogenous noradrenaline, whereas MDA was 3- to 8-fold higher than the endogenous dopamine (Tables 1 and 2).

<table>
<thead>
<tr>
<th>脑区</th>
<th>α-甲基多巴</th>
<th>MDA</th>
<th>MNA</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>前额叶 (7)</td>
<td>34.7±3.2</td>
<td>538±20</td>
<td>2415±71</td>
<td>-</td>
</tr>
<tr>
<td>下丘脑 (7)</td>
<td>19.4±3.3</td>
<td>1330±1330</td>
<td>16580±420</td>
<td>23.7±2.3</td>
</tr>
<tr>
<td>C1–C2区域 (4)</td>
<td>30.9±3.6</td>
<td>3972±393</td>
<td>6856±172</td>
<td>5.4±0.4</td>
</tr>
<tr>
<td>A1–A2区域 (4)</td>
<td>22.1±6.1</td>
<td>3183±288</td>
<td>3863±161</td>
<td>-</td>
</tr>
<tr>
<td>胸部脊髓 (7)</td>
<td>20.0±3.5</td>
<td>1663±109</td>
<td>2519±84</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Accumulations of α-甲基多巴, α-甲基多巴胺 (MDA), α-甲基去甲肾上腺素 (MNA) and α-甲基肾上腺素 (MA) in four brain regions and thoracic spinal cord of α-甲基多巴-treated rats

Treated rats were given α-甲基多巴 (40 mg/kg, p.o., twice a day, for five days). Values are the average±S.E.M. of nanomole (α-甲基多巴) or picomole (MDA, MNA and MA) per g wet weight of tissue. Numbers of determinations are given in parenthesis. (-): non-detectable.

Discussion

It is widely accepted that α-甲基多巴 is a centrally-acting hypotensive drug, mainly based on the data that MNA produced in the central noradrenergic neurons stimulates the vasodepressor areas (13–15). On the other hand, there is evidence suggesting that MA might also be responsible for the hypotensive mechanism of α-甲基多巴. Exogenous MA reduced blood pressure when injected into the cerebroventricular fluid and nucleus of the solitary tract (5). MA could bind to α-receptors (16), and chronic α-甲基多巴 treatment decreased the endogenous adrenaline content in the hypothalamic nuclei (6). Therefore, it was deemed necessary to find and quantitate precisely the amount of MA produced in the central nervous system after the systemic administration of α-甲基多巴.

We chose a high-performance liquid chromatographic method, since various derivatives of α-甲基多巴 may be produced in the brain. Actually, our simple method of LCEC enabled quantitation of nine catechols simultaneously. Jonsson et al. (9) quantitated adrenaline contents in rat brain regions (hypothalamus, 62±3.2 ng/g; pons-medulla, 9.4±1.4 ng/g) by the LCEC method. Our present values of endogenous adrenaline in the brain are compatible with these data.

Tung et al. (5) reported that exogenous MA had a 5- to 10-fold greater depressor effect than MNA. We could measure a small amount of de novo MA in the hypothalamus and C1–C2 area after α-甲基多巴 administration. These amounts were only 0.14% and 0.08% of those of MNA, respectively. On the
basis of our results, it seems unlikely that MA plays a significant role in reducing blood pressure. However, the present study was done on a relatively wide brain area, i.e., the hypothalamus and C1–C2 area, and the sensitivity of our LCEC method used here was limited to detect smaller amounts of MA. The sensitivity of LCEC has to be increased so that we can detect metabolites of α-methyldopa in anatomically-restricted areas, such as regions punched out by the method of Palkovits (17). Furthermore, we measured MA at only one point after the five daily doses of α-methyldopa. Thus, until more data are obtained with regard to the precise localization and the time course of MA accumulation, the role of MA in blood pressure remains speculative.

Whereas endogenous adrenaline levels of the hypothalamus and C1–C2 area were reduced after α-methyldopa to 10.6% and 16.1%, respectively, the ratios of MA to endogenous adrenaline in these areas were 9.0% and 6.2%, respectively. This is of particular interest when compared with changes of endogenous noradrenaline and accumulation of MNA. The ratios of MNA to endogenous noradrenaline were a little over 100% in these areas, indicating that MNA replaced endogenous noradrenaline in the store sites. This has been already pointed out (18). Taken in conjunction with the previous data that α-methyldopa is taken up and converted into MNA by central noradrenergic neurons and MNA is stored in the storage vesicles of noradrenaline (19), our data lead to the question of whether the central adrenergic neurons take up α-methyldopa and convert it to MA. The finding that phenylethanolamine-N-methyltransferase was not decreased after the administration of 6-hydroxydopamine (20) suggests a different uptake mechanism in central adrenergic neurons. Saavedra et al. (21) considered that in the hypothalamus, adrenaline may be stored in the noradrenergic nerve endings after production by postsynaptically-located phenylethanolamine-N-methyltransferase. This has been recently confirmed and extended (22). Thus, it could be speculated that MNA produced in the hypothalamic noradrenergic nerve endings and released to synaptic clefts might be converted to MA by postsynaptically-located phenylethanolamine-N-methyltransferase, after which the MA is taken up and stored in the nerve endings of noradrenergic neurons. Therefore, endogenous adrenaline in the hypothalamic noradrenergic nerve endings may be replaced by both MNA and MA. In addition, MNA is suggested to be a poor substrate for phenylethanolamine-N-methyltransferase (23). These would explain the discrepancy between the ratios of MA and MNA to endogenous adrenaline and noradrenaline.

Acknowledgement: We thank Dr. Juan M. Saavedra, Laboratory of Clinical Science, National Institute of Mental Health, Washington, D.C., U.S.A. for valuable discussions, M. Ohara for advice on the manuscript and K. Kato for manuscript preparation.

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