Central $\alpha_2$-Adrenergic Stimulation Increases Neurointermediate Lobe Immunoreactive $\beta$-Endorphin in Spontaneously Hypertensive Rats

KENICHI YASUNARI, YOSHIHARU KANAYAMA, MASAKAZU KOHNO, KOICHI MURAKAWA, TAKAHIKO KAWARABAYASHI, AND TADANAO TAKEDA

SUMMARY A possible influence of the central $\alpha_2$-adrenergic system on $\beta$-endorphin was examined in rat anterior pituitary, neurointermediate lobe, and plasma. The concentration of $\beta$-endorphin in anterior pituitary, neurointermediate lobe, and plasma was determined by radioimmunoassay 15 minutes after subcutaneous injection of clonidine in 14-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Clonidine reduced the concentration of the plasma $\beta$-endorphinlike immunoreactivity in SHR and to a lesser extent in WKY. No significant changes in the concentration of $\beta$-endorphinlike immunoreactivity were observed in anterior pituitary. Clonidine increased the concentration of neurointermediate lobe $\beta$-endorphinlike immunoreactivity in SHR in a dose-related manner but did not affect the concentration in WKY. Administration of yohimbine (1 mg/kg) completely blocked the clonidine-induced increase of neurointermediate lobe $\beta$-endorphinlike immunoreactivity in SHR, while prazosin (1 mg/kg) had no effect. These data suggest that the central $\alpha_2$-adrenergic activation increases the neurointermediate lobe concentration of $\beta$-endorphinlike immunoreactivity in SHR by suppressing $\beta$-endorphin release from the neurointermediate lobe into the circulation. (Hypertension 9: 566-570, 1987)

KEY WORDS • clonidine • acute effect • prazosin • yohimbine

The interaction of clonidine, an $\alpha_2$-adrenergic antihypertensive drug, with opiate systems of the brain has been demonstrated in several reports. The hypotensive action of clonidine appears to involve opiate receptors, especially in spontaneously hypertensive rats (SHR) and in patients with essential hypertension, since naloxone inhibits the hypotensive action in SHR, but not in Wistar-Kyoto rats (WKY), and in hypertensive patients, but not in normotensive controls. Clonidine releases $\beta$-endorphin from brainstem and hypothalamus slices of SHR but not of WKY. Therefore, central $\alpha_2$-adrenergic stimulation, which may be mediated by $\beta$-endorphin, is believed to initiate the bulbospinal inhibitory system that decreases the peripheral sympathetic neuron activity and blood pressure.

On the other hand, the physiological role of circulating $\beta$-endorphin is rather controversial. Clonidine has been reported to release $\beta$-endorphin from anterior pituitary (AP) and increase the plasma level of $\beta$-endorphin in normotensive male Sprague-Dawley rats. Long-term clonidine administration has been reported to increase the plasma level of $\beta$-endorphin only in patients with essential hypertension, without affecting the pattern of the diurnal rhythm. However, plasma $\beta$-endorphin has been reported to play no role in the hypotensive action of short-term clonidine administration in WKY and in SHR. In addition, Farsang et al. found that plasma $\beta$-endorphin did not rise in a subgroup of patients with essential hypertension. Recently, we showed that central $\alpha_2$-adrenergic activation induced by short-term clonidine administration reduces the plasma level of $\beta$-endorphin in patients with essential hypertension.

To elucidate the physiological roles of circulating $\beta$-endorphin and the mechanism of the release of $\beta$-endorphin at the central level by central $\alpha_2$-adrenergic activation, we measured the concentration of $\beta$-endor-
phin in the AP, neurointermediate lobe (NIL), and plasma of SHR and WKY after short-term clonidine administration.

Materials and Methods

Animals

Fourteen-week-old male SHR, each weighing 240–280 g, and age-matched WKY, each weighing 260–300 g, were housed (light on from 0700 to 2100) with free access to chow and tap water. Animals were allowed to become accustomed to daily handling for at least 3 days before each experiment. All procedures of animal handling were in accordance with guidelines of the Animal Research Committee of Osaka City University Medical School.

Sample Collection

Rats were injected subcutaneously with saline, 1 ml/kg, or clonidine hydrochloride (Boehringer-Ingelheim, Tokyo, Japan), 100, 250 or 500 μg/kg and decapitated 15 minutes later. The heads were radiated with microwaves (900 W for 30 seconds), and the pituitary was removed. The AP and NIL were dissected under a stereomicroscope and weighed. The AP and NIL then were homogenized in 1 N acetic acid (2 and 1 ml, respectively). The homogenate was then centrifuged at 16000 g for 10 minutes, and the supernatant was kept at −80°C until assay. The concentration of β-END in the AP and NIL was expressed per gram of wet tissue weight.

Blood samples were collected into chilled, siliconized disposable glass tubes containing aprotinin (300 kallikrein inactivation units/ml) and ammonium EDTA (1 mg/ml) before and 15 and 30 minutes after subcutaneous injection of clonidine (100, 250 or 500 μg/kg) or saline (1 ml/kg). Plasma was separated by centrifugation at 4°C, and plasma samples were kept at −80°C until assay. The β-EpLI was extracted from rat plasma using a silica gel (Wako Pure Chemical Industries, Osaka, Japan) and then eluted by 0.1 N HCl acetone. Extraction efficiency for plasma containing β-END was 72.6 ± 1.1%.

Radioimmunoassay

The concentration of β-EpLI in the supernatant of AP, NIL, and plasma extract was measured by radioimmunoassay using human β-END (Protein Research Foundation, Osaka, Japan) as a standard. The antibody was raised in rabbits by weekly immunization of synthetic human β-END, which was not conjugated with any carrier protein, in complete Freund’s adjuvant. The assay showed less than 5% cross-reactivity with human β-lipotropin (Peninsula Laboratories, San Carlos, CA, USA), and no tracer replacement by α-END, α-melanocyte stimulating hormone, or methionine and leucine enkephalin (Protein Research Foundation). The antibody cross-reacted 73.3% with N-acetyl-β-END (Peninsula Laboratories), the inactive form of β-END. The minimum detectable concentration was 10 pg/tube. The intra-assay and interassay coefficients of variation were 5 and 13%, respectively.

Statistics

Data were analyzed statistically using Duncan’s multiple-range test for multiple comparisons preceded by analysis of variance, p values of less than 0.05 were considered significant. Results are expressed as means ± SD.

Results

Pituitary Lobe Weight

As shown in Table 1, there were no significant differences in the weight of AP and NIL between each group.

Effects of Clonidine on Endorphinlike Immunoreactivity

As shown in Figure 1, short-term clonidine administration significantly reduced the concentration of plasma β-EpLI in a dose-related manner in SHR. Clonidine administration also reduced the plasma concentration of β-EpLI in WKY, but to a lesser extent. The basal concentration of plasma β-EpLI in SHR was about twofold higher than that in WKY. Yohimbine pretreatment reversed the decrease of plasma β-EpLI induced by short-term clonidine administration.

As shown in Figure 2, the basal concentration of β-EpLI in AP in WKY was the same as that in SHR. Short-term clonidine administration had no effect on the concentration of β-EpLI in the AP of WKY or SHR.

As shown in Figure 3, the basal concentration of NIL β-EpLI in SHR was much greater than that in WKY. Although short-term clonidine administration did not change the concentration of NIL β-EpLI in WKY, it increased the concentration of NIL β-EpLI in SHR in a dose-related manner.

Pretreatment with prazosin (1 mg/kg i.p.) 30 minutes before the administration of clonidine did not influence the clonidine-induced increase of NIL β-EpLI in SHR; however, pretreatment with yohimbine (1 mg/kg i.p.) 60 minutes before the administration of clonidine completely blocked the clonidine-induced increase of NIL β-EpLI in SHR (see Figure 3).

<table>
<thead>
<tr>
<th>Variable</th>
<th>AP (mg)</th>
<th>NIL (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8.75 ± 0.33</td>
<td>1.47 ± 0.06</td>
</tr>
<tr>
<td>Clonidine, 500 μg/kg</td>
<td>8.62 ± 0.30</td>
<td>1.53 ± 0.09</td>
</tr>
<tr>
<td>Saline</td>
<td>8.59 ± 0.38</td>
<td>1.47 ± 0.07</td>
</tr>
<tr>
<td>Clonidine, 100 μg/kg</td>
<td>8.52 ± 0.36</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>Clonidine, 250 μg/kg</td>
<td>8.23 ± 0.37</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>Clonidine, 500 μg/kg</td>
<td>8.41 ± 0.48</td>
<td>1.54 ± 0.10</td>
</tr>
</tbody>
</table>

Values are means ± SD. AP = anterior pituitary; NIL = neurointermediate lobe.
FIGURE 1. Effect of short-term clonidine administration on plasma β-endorphinlike immunoreactivity (β-EpLJ) in WKY and SHR. Numbers in parentheses indicate number of animals. Single (p<0.05) and double (p<0.01) asterisks indicate significant difference compared with respective values for saline.

WKY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SD</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>221 ± 24</td>
<td>10</td>
</tr>
<tr>
<td>Clonidine 500μg/kg</td>
<td>472 ± 91</td>
<td>10</td>
</tr>
<tr>
<td>Clonidine 100μg/kg</td>
<td>369 ± 60</td>
<td>10</td>
</tr>
<tr>
<td>Clonidine 250μg/kg</td>
<td>324 ± 35</td>
<td>7</td>
</tr>
<tr>
<td>Clonidine 500μg/kg</td>
<td>263 ± 124</td>
<td>7</td>
</tr>
</tbody>
</table>

SHR

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SD</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>456 ± 107</td>
<td>8</td>
</tr>
<tr>
<td>Clonidine 500μg/kg</td>
<td>389 ± 48</td>
<td>9</td>
</tr>
</tbody>
</table>

FIGURE 2. Effect of short-term clonidine administration on anterior pituitary β-endorphinlike immunoreactivity (β-EpLJ) in WKY and SHR. Number of rats is shown in parenthesis.

Serial Changes in Plasma and Neurointermediate Lobe Concentration of β-Endorphinlike Immunoreactivity

As shown in Figure 4A, the administration of clonidine induced a significant decrease in the plasma concentration of β-EpLJ within 15 minutes; however, plasma β-EpLJ returned to basal levels within 30 minutes after clonidine administration. The concentration of NIL β-EpLJ was significantly increased after 15 minutes and remained elevated at 30 minutes after clonidine administration (Figure 4B).

Discussion

Recent findings have shown that both anterior and intermediate rat pituitary lobes contain cells that secrete β-endorphin, and together these constitute the principal source of circulating β-endorphin, although different mechanisms are operative in the process from common precursor, pro-opiomelanocortin, and secretion. In the AP, β-endorphin release is mainly under the stimulatory control of corticotropin-releasing factor, while in the NIL it is under inhibitory dopaminergic control.

We have found that clonidine-induced central α2-adrenergic activation decreases the plasma level of β-EpLJ in both WKY and SHR. These results confirm our previous studies in humans and indicate that peripheral β-endorphin does not contribute to the hypotensive action of clonidine. Because short-term administration of clonidine has been reported to decrease plasma adrenocorticotropic hormone (ACTH) levels in humans and dogs and because β-endorphin is secreted concomitantly with ACTH from AP, it is reasonable to assume that short-term administration of clonidine inhibits the release of β-endorphin from AP. Since the increase of NIL β-EpLJ concentration and the decrease of plasma β-EpLJ concentration induced by short-term clonidine administration were much greater in SHR, it is plausible that clonidine also inhibits the release of β-EpLJ from NIL. Therefore, the decrease of plasma β-EpLJ can be considered a concerted result of inhibition of β-endorphin release from both AP and NIL, although it is difficult to determine which is the predominant contributory factor.

Clonidine is thought to cause the release of β-EpLJ from cell cultures of the anterior lobes and NIL of rat pituitary. However, the results of our in vivo study differ from these in vitro studies. Since epinephrine has been reported to have an additive effect on the corticotropin releasing hormone-induced ACTH release, and since clonidine inhibits the centrally mediated stimulatory adrenergic influences and lowers not only plasma but also cerebrospinal fluid level of catecholamines, the mechanisms of inhibition of β-endorphin release may be due to clonidine-induced de-
crease of corticotropin releasing hormone, which may partly explain the differences between in vivo and in vitro studies. We have shown a higher plasma β-EpLI level in SHR than in WKY in an unstimulated condition, which is consistent with the findings of Ramirez-Gonzalez et al. Morris and Sundberg reported that dopamine concentration and biosynthetic activity were reduced in SHR. Since β-endorphin release from NIL is under inhibitory tonic dopaminergic control, reduced dopamine concentration and biosynthetic activity may release β-endorphin from NIL, thus inducing plasma β-EpLI elevation.

NIL β-EpLI concentration in SHR was increased more than twofold compared with that in WKY. This finding is consistent with the results of Hutchinson et al. Enkephalin, another opioid peptide in NIL is also reported to be higher in SHR than in WKY. These observations are in line with the proposal that modulation of the differential release of neurohypophysial hormones by opioid peptides is altered in SHR.

In contrast to our results, Pettibone and Mueller reported that clonidine administration increased plasma β-EpLI in Sprague-Dawley rats. They later reported that clonidine induced a 1.8-fold β-EpLI increase. However, with the use of gel chromatography, they found that β-lipotropin was increased by 220%, while β-endorphin was increased by only 15%. Moreover, they found that a specific α,-antagonist, prazosin, completely blocked clonidine's effect on plasma β-EpLI. Although it is difficult to explain why our results differ from theirs, it should be noted that the...
specificity of antibodies used for the β-endorphin radioimmunoassay was different. The antibody for β-endorphin that we used showed cross-reactivity with β-lipotropin of less than 5%, while their antibody exhibited equal cross-reactivity.32,33

The physiological importance of an increase in NIL β-EpLI induced by α-adrenergic activation in SHR remains to be elucidated. β-Endorphin in NIL is reported to inhibit vasopressin release tonically,32,33 and nalorexsignificantly elevates the plasma concentration of vasopressin in SHR but not in WKY.29 Thus, the increased amount of NIL β-EpLI may increase tonic inhibition of vasopressin release from the pituitary. Because the role of vasopressin in maintaining the blood pressure is well known,34 the inhibition of the release of vasopressin from the pituitary may contribute to the hypotensive action of clonidine in SHR.

References

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