Altered Platelet $\alpha_2$-Adrenoceptors in Patients With Ischemic Heart Disease

Kazuaki Uchino, M.D., Satoshi Umemura, M.D.*, Hisao Ochiai, M.D.*
Yoshihiro Ishikawa, M.D.*, Tohyoh Nihei, M.D.*
and Masao Ishii, M.D.*

We evaluated the characteristics of platelet $\alpha_2$-adrenoceptors in 12 patients with effort angina pectoris, 11 patients with variant angina pectoris and 11 normal control subjects. $\alpha_2$-Adrenoceptors were quantified using a radioligand binding assay with radiolabelled rauwolscine, an $\alpha_2$-selective antagonist. In addition, plasma norepinephrine concentrations were measured by high performance liquid chromatography. The mean value of the maximal number of binding sites ($B_{\text{max}}$) in patients with effort angina ($205.1 \pm 11.3$ fmol/mg protein) was significantly lower than that in control subjects ($293.0 \pm 10.2$ fmol/mg protein). $B_{\text{max}}$ did not differ between patients with variant angina ($322.9 \pm 45.4$ fmol/mg protein) and control subjects. There was no significant difference in the dissociation constant ($K_d$) among the 3 groups.

The plasma norepinephrine concentration tended to be higher in patients with effort angina or variant angina than in normal controls, but this difference was not statistically significant. In addition, studies in another group of young volunteers (n=20) revealed a negative correlation ($r=-0.50$, $p<0.05$) between the $B_{\text{max}}$ of $^2$H-rauwolscine binding to platelets and the percent change in the plasma norepinephrine concentration when subjects moved from the supine to the standing position. This suggests a functional correlation between platelet $\alpha_2$-adrenoceptors and those located at presynaptic sites. If platelet $\alpha_2$-adrenoceptors correlate with presynaptic $\alpha_2$-adrenoceptors, the current findings of decreased $\alpha_2$-adrenoceptor density in platelets from patients with effort angina could represent attenuated negative feedback of norepinephrine by presynaptic $\alpha_2$-adrenoceptors.

(Received April 25, 1994; accepted March 14, 1995)
Department of Cardiology, Sagamihara National Hospital, 18-1 Sakuradai, Sagamihara 228, Japan
*Second Department of Internal Medicine, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236, Japan
Mailing address: Kazuaki Uchino, M.D., Department of Cardiology, Sagamihara National Hospital, 18-1 Sakura-dai, Sagamihara city, Kanagawa 228, Japan

Key words: Effort angina, Variant angina, $\alpha_2$-adrenoceptors, Norepinephrine concentration

$\alpha$ and $\alpha_2$-Adrenoceptors may play an important role in modulating coronary artery tone in humans as well as in experimental animals. Coronary artery constriction has been elicited by infusing norepinephrine or by stimulating sympathetic nerve, and has been inhibited by $\alpha_2$-blocking with rauwolscine in dogs. Coronary $\alpha_2$-adrenoceptors have been identified in humans by radioligand binding assay. Since human coronary artery material is not readily available, platelets have been used to study $\alpha_2$-adrenoceptors in humans. Platelet $\alpha_2$-adrenoceptor density has been reported to be decreased in patients with unstable angina or symptomatic angina pectoris, whereas $\alpha_2$-adrenoceptor density is increased in variant angina. $\alpha_2$-Adrenoceptor
<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Kd</th>
<th>Bmax</th>
<th>PNE</th>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Kd</th>
<th>Bmax</th>
<th>PNE</th>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Kd</th>
<th>Bmax</th>
<th>PNE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>M</td>
<td>1.236</td>
<td>242.1</td>
<td></td>
<td>1</td>
<td>75</td>
<td>M</td>
<td>1.961</td>
<td>282.6</td>
<td>104.2</td>
<td>1</td>
<td>61</td>
<td>M</td>
<td>1.479</td>
<td>303.5</td>
<td>310.2</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>M</td>
<td>1.451</td>
<td>189.5</td>
<td>484.6</td>
<td>2</td>
<td>32</td>
<td>M</td>
<td>1.730</td>
<td>170.4</td>
<td>299.5</td>
<td>2</td>
<td>67</td>
<td>M</td>
<td>1.572</td>
<td>289.4</td>
<td>306.2</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>M</td>
<td>1.075</td>
<td>223.8</td>
<td>452.6</td>
<td>3</td>
<td>52</td>
<td>M</td>
<td>2.016</td>
<td>530.4</td>
<td>160.0</td>
<td>3</td>
<td>61</td>
<td>M</td>
<td>1.412</td>
<td>273.6</td>
<td>156.1</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>M</td>
<td>1.267</td>
<td>205.8</td>
<td>202.6</td>
<td>4</td>
<td>44</td>
<td>F</td>
<td>3.401</td>
<td>683.9</td>
<td>156.9</td>
<td>4</td>
<td>59</td>
<td>M</td>
<td>2.203</td>
<td>285.6</td>
<td>45.5</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>F</td>
<td>2.611</td>
<td>274.4</td>
<td>312.7</td>
<td>5</td>
<td>52</td>
<td>M</td>
<td>1.859</td>
<td>349.5</td>
<td>247.7</td>
<td>5</td>
<td>61</td>
<td>M</td>
<td>1.779</td>
<td>229.7</td>
<td>91.7</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>M</td>
<td>1.350</td>
<td>181.7</td>
<td>253.1</td>
<td>6</td>
<td>56</td>
<td>M</td>
<td>1.081</td>
<td>262.5</td>
<td>262.0</td>
<td>6</td>
<td>52</td>
<td>M</td>
<td>1.582</td>
<td>314.0</td>
<td>232.2</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>M</td>
<td>1.449</td>
<td>200.0</td>
<td></td>
<td>7</td>
<td>47</td>
<td>M</td>
<td>1.481</td>
<td>247.2</td>
<td>621.0</td>
<td>7</td>
<td>59</td>
<td>F</td>
<td>0.969</td>
<td>318.0</td>
<td>141.3</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>M</td>
<td>2.049</td>
<td>179.1</td>
<td>212.8</td>
<td>8</td>
<td>59</td>
<td>M</td>
<td>1.350</td>
<td>181.8</td>
<td>275.0</td>
<td>8</td>
<td>46</td>
<td>M</td>
<td>3.348</td>
<td>265.5</td>
<td>69.5</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
<td>M</td>
<td>1.546</td>
<td>150.0</td>
<td>265.0</td>
<td>9</td>
<td>47</td>
<td>F</td>
<td>2.380</td>
<td>246.0</td>
<td>105.4</td>
<td>9</td>
<td>55</td>
<td>M</td>
<td>1.292</td>
<td>389.0</td>
<td>189.2</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>M</td>
<td>1.035</td>
<td>171.0</td>
<td>52.6</td>
<td>10</td>
<td>60</td>
<td>F</td>
<td>1.435</td>
<td>382.5</td>
<td>180.0</td>
<td>10</td>
<td>54</td>
<td>M</td>
<td>4.482</td>
<td>283.4</td>
<td>291.1</td>
</tr>
<tr>
<td>11</td>
<td>59</td>
<td>M</td>
<td>1.845</td>
<td>275.0</td>
<td>223.1</td>
<td>11</td>
<td>67</td>
<td>F</td>
<td>1.295</td>
<td>215.1</td>
<td>224.6</td>
<td>11</td>
<td>58</td>
<td>M</td>
<td>1.715</td>
<td>317.3</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>64</td>
<td>M</td>
<td>1.080</td>
<td>168.3</td>
<td>189.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>53.8</td>
<td></td>
<td>1.500</td>
<td>205.1</td>
<td>264.9</td>
<td>mean</td>
<td>53.7</td>
<td></td>
<td>1.817</td>
<td>322.9</td>
<td>239.7</td>
<td>mean</td>
<td>55.7</td>
<td></td>
<td>1.988</td>
<td>293</td>
<td>183.3</td>
</tr>
<tr>
<td>SE</td>
<td>2.2</td>
<td></td>
<td>0.129</td>
<td>11.3</td>
<td>38.0</td>
<td>SE</td>
<td>3.4</td>
<td></td>
<td>0.195</td>
<td>45.1</td>
<td>41.1</td>
<td>SE</td>
<td>2.3</td>
<td></td>
<td>0.298</td>
<td>10.2</td>
<td>29.7</td>
</tr>
</tbody>
</table>

Kd: Dissociation constant (nmol/L), Bmax: Maximum under of binding site (fmol/mg protein), PNE: Plasma norepinephrine concentration (pg/ml), SE: Standard error of the mean, F: female, M: male.

Affinity for epinephrine is increased in patients with unstable angina pectoris, while no change in affinity has been reported in those with stable angina pectoris.\(^8\,^9\) Plasma norepinephrine concentrations in these patients do not differ from those in control subjects.\(^8\) Consequently, there have been many studies of platelet \(\alpha_2\)-adrenergceptor in many forms of angina pectoris. However, especially in the classification of effort angina and variant angina, changes in \(\alpha_2\)-adrenergceptor density and affinity have not been defined and the significance of such changes has not been clarified. \(\alpha_2\)-Adrenergceptors exist in both pre- and post-synaptic sites\(^10\) and platelet \(\alpha_2\)-adrenergceptors have been suggested as a model for \(\alpha_2\)-adrenergceptors on adrenergic neurons, including sympathetic nerve terminals\(^11,\,12\), where \(\alpha_2\)-adrenergceptors regulate norepinephrine release.

The purpose of this study was to determine the platelet \(\alpha_2\)-adrenergceptor density and plasma concentration of norepinephrine in patients with either effort angina pectoris or variant angina pectoris and in control subjects. These groups were classified by clinical symptoms, and electrocardiogram and coronary angiography findings. The properties of platelet \(\alpha_2\)-adrenergceptor and plasma norepinephrine levels in both groups of patients and control subjects were measured. In addition, plasma norepinephrine concentrations were also measured in young volunteers before and after standing stress.

**Patients and Methods**

Patients

Twenty-three patients with ischemic heart diseases, including 12 patients with effort angina and 11 patients with variant angina, were studied. These patients were compared with a control group of 11 subjects (10 men and one woman) who were hospitalized mainly for gastrointestinal diseases. Subjects were excluded from the control group if they had diabetes mellitus, hormonal disorders, hypotension, syndrome X or chest pain syndrome.\(^13\) All of the subjects had been admitted consecutively to Yokohama City University Hospital. Effort angina was defined as chest pain on exertion with significant stenosis in more than one coronary artery, as evaluated by coronary angiography. Patients whose effort angina pectoris was recognized as unstable angina and/or those who had a history of old myocardial infarction were excluded from the effort angina group. Variant angina was defined as chest pain at rest with transient ST-segment elevation on electrocardiogram during the pain and/or coronary artery spasm confirmed by ergonovine provocation testing during coronary angiography. Three patients with variant angina had chest pain during this.
hospitalization and were considered unstable. All drugs (including nitrate, calcium antagonist, \(\beta\)-adrenergic blockade and aspirin) were discontinued 5 days before the study began. None of the patients were taking any other drugs known to modify \(\alpha_2\) adrenoceptors (such as antidepressants or other adrenergic agents). The average age was 53.8 \(\pm\) 2.2 years in the effort angina group, 53.7 \(\pm\) 3.4 years in the variant angina group and 55.7 \(\pm\) 2.3 years in the control subjects (Table I). Informed consent was obtained from all of the patients. Twenty normal healthy young volunteers (22.0 \(\pm\) 1.2 years) were also studied to evaluate the relationship between platelet \(\alpha_2\)-adrenoceptors and plasma norepinephrine concentrations at rest and after standing for 30 min.

**Blood Sampling and Platelet Membrane Preparation**

Following bed rest for 30 min, 20 ml of blood was obtained from each subject and mixed with 4 ml acid citrate dextrose in polyethylene tubes. All blood samples were taken between 9 and 10 am. Platelet membranes were prepared and a radioligand binding assay for \(\alpha_2\)-adrenoceptors was performed by the method described by Daiguiji et al\(^{14}\) with slight modifications\(^{15}\). Briefly, samples were centrifuged at 400 \(\times\) g for 10 min at 25°C and plasma was recentrifuged at 16,000 \(\times\) g for 10 min to obtain platelet pellets. The pellets were resuspended in 10 ml ice-cold 50 mmol/L Tris-HCl buffer (pH 7.5) containing 0.11 mol/L NaCl and 0.02 mol/L Na\(_2\) EDTA, and recentrifuged at 1000 \(\times\) g for 10 min. The supernatant was discarded and 5 mM Tris-HCl (pH 7.5) with 5 mmol/L Na\(_2\) EDTA was added to the pellets. The pellets were disrupted by a Polytron (Brinkman, setting no. 5) for 30 sec and recentrifuged at 39,000 \(\times\) g for 10 min at 4°C. The supernatant was discarded and 50 mmol/L Tris-HCl buffer (pH 7.5) was added and homogenized with the pellet. This platelet membrane suspension was used on the day of preparation for the radioligand binding assays.

**Radioligand Binding Assay**

An \(\alpha_2\)-adrenoceptor antagonist, \(^3\)H-rauwolscine (New England Nuclear), specific activity 78.8–88.8 Ci/mmol, was used for binding assays as the radioligand. One hundred-microliter aliquots of the fresh platelet membranes were incubated in duplicate at 25°C with 6 concentrations (0.4–15 nmol/L) of \(^3\)H-rauwolscine for 60 min to determine total binding. Non-specific binding was determined in the presence of 10 \(\mu\)mol/L phentolamine. Specific binding was calculated from the difference between total and non-specific binding. Incubations were terminated by adding 4 ml of 50 mmol/L Tris-HCl buffer followed by filtration through Whatmann GF/C glass fiber filters using vacuum aspiration. The filters were washed twice with 4 ml of 50 mmol/L Tris-HCl buffer and dried in air; scintillator (Aquazol II, New England Nuclear) was added and radioactivity was determined by scintillation spectroscopy. Protein concentrations in the platelet membranes were measured by the method of Lowry et al\(^{16}\). The maximal number of binding sites (B\(_{max}\)) and the dissociation constant (K\(_d\)) were determined by Scatchard plot analysis of saturation curves\(^{17}\).

**Plasma Norepinephrine Assay**

Plasma norepinephrine concentrations were determined by high performance liquid chromatography\(^{18}\). All blood samples were collected via an indwelling intravenous
Fig. 2. Kinetic analysis of $^3$H-rauwolscine binding to human platelet membranes. The association curve (Fig. 2a) was determined by incubation with $^3$H-rauwolscine (3–4 mmol/L) in a total volume of 150 mmol/L at 25 °C for the time indicated. The dissociation curve (Fig. 2b) was determined by adding phenolamine to a final concentration of 10 mmol/L after incubating the ligand and the membranes for 60 min. Each value is expressed as a percentage of the binding at equilibrium in association experiments and at time zero in dissociation experiments. Association curve inset: kinetic plots of specific binding; Beq is the specific binding at equilibrium and Bt is the binding at time t. The apparent rate constant, Kap, is given by the gradient of the plot ln |Beq/(Beq−Bt)| against time. The association rate constant $K_a$ is calculated from $K_a = (Kap−K_d)/[^3]$H-rauwolscine concentration. The dissociation rate constant $K_d$ was derived from dissociation experiments. Dissociation curve inset: first-order rate plot of the dissociation of the receptor-ligand complex. The dissociation rate constant $K_d$ is equal to the slope of the line relating ln (Bt/Bo) and time, where Bo is the specific binding at time zero and Bt is the binding at time t. The slope of the line was determined by linear regression analysis.

catheter after patients had been recumbent for at least 30 min. Blood was collected in acid citrate dextrose and the serum was immediately frozen at −70 °C. A second blood sample was taken after standing for 30 min.

Statistical Analyses

Values are given as the mean ± SE. Student’s t-test was used to determine the significance of differences between groups. A probability of less than 0.05 was considered statistically significant.

RESULTS

The specific binding was 75–95% (Fig. 1) in preliminary experiments. The binding of $^3$H-rauwolscine to platelet membranes at 25 °C was rapid and reversible (Fig. 2a, 2b). $^3$H-Rauwolscine dissociated from the membranes in the presence of 10 μmol/L phenolamine (Fig. 2b). Kap, $K_a$ and $K_d$ (Fig. 2a,
Fig. 4. $B_{\text{max}}$ (A) and $K_d$ (B) of $^3$H-rauwolscine binding to platelet membranes from patients with effort angina, patients with variant angina, and control subjects.

Fig. 5. Plasma norepinephrine concentrations in patients with effort angina, patients with variant angina, and control subjects.

2b) were determined according to the method described by Williams et al.\textsuperscript{19} The $K_1$: $K_2$ ratio yielded a value similar to that determined by the saturation study. The specific binding of $^3$H-rauwolscine to platelet membranes from patients and controls was saturable and consistent with a single site (Fig. 3). The data and individual profiles of the patients are shown in Table I. $B_{\text{max}}$ was significantly lower in patients with effort angina than in normal controls (Fig. 4a, Table I). Three of the 11 patients with variant angina had increased $B_{\text{max}}$. However, the mean $B_{\text{max}}$ in variant angina was not different from that in the control group (Fig. 4a). There was no significant difference in $K_d$ among the 3 groups (Fig. 4b). Plasma norepinephrine concentrations in effort angina and variant angina tended to increase, but this tendency was not statistically significant (Fig. 5).

In 20 normal volunteers, the plasma norepinephrine concentration, either at rest or after standing, did not correlate with platelet $\alpha_2$-adrenoceptor $B_{\text{max}}$. However, the percent change in plasma norepinephrine after
Fig. 6. The percent change in plasma norepinephrine after standing and platelet $\alpha_2$-adrenoceptor density in young volunteers. NE: plasma norepinephrine concentration. $\Delta$NE: (plasma norepinephrine after standing−plasma norepinephrine at rest)/plasma norepinephrine at rest.

standing $\Delta$ norepinephrine = [(plasma norepinephrine after standing−plasma norepinephrine at rest)/plasma norepinephrine at rest] was negatively correlated with platelet $\alpha_2$-adrenoceptor density ($r = -0.50$, $p < 0.05$) (Fig. 6).

**DISCUSSION**

Both clinically and pathophysiologically, angina pectoris can be classified into 2 types: effort angina pectoris and variant angina pectoris. In this study, we grouped our patients based on clinical symptoms, electrocardiogram findings and coronary angiography results. This approach differed from those in previous reports concerning platelet $\alpha_2$-adrenoceptors in angina pectoris.

The current study shows that platelet membrane $\alpha_2$-adrenoceptors were decreased in patients with effort angina, but were unchanged in patients with variant angina. The results for effort angina are consistent with previous reports of symptomatic angina pectoris or unstable angina pectoris, although the clinical conditions of the patients in those reports were different. It has been suggested that a possible mechanism of this decreased platelet $\alpha_2$-adrenoceptor density might be down-regulation secondary to the increased plasma norepinephrine concentrations. However, this is unlikely since many reports have shown that plasma norepinephrine concentrations tend to increase, but not significantly, in angina pectoris, as we have also confirmed in this study. In addition, it has been reported that circulating catecholamines have a negligible effect on the number of platelet $\alpha_2$-adrenoceptors. Therefore, we evaluated a different group and found that the percent change in plasma norepinephrine after standing was inversely correlated with platelet $\alpha_2$-adrenoceptor density. Recently, this correlation has also been demonstrated by other investigators. These findings suggest that platelet $\alpha_2$-adrenoceptors may correlate with presynaptic $\alpha_2$-adrenoceptors. Therefore, human blood platelet has been suggested as a model for adrenergic neurons where $\alpha_2$-adrenoceptors exist on presynaptic nerve terminals, and decreased $\alpha_2$-adrenoceptors in the presynapses could enhance norepinephrine release following nerve stimulation. This hypothesis is further supported by a report that platelet and presynaptic $\alpha_2$-adrenoceptors consist of identical $\alpha_2A$-adrenoceptors in rabbits and humans. Decreased $\alpha_2$-adrenoceptor density in patients with effort angina could therefore permit a transient increase in norepinephrine released from neurons during exercise, thus resulting in an anginal attack.

In variant angina, the mean $\alpha_2$-adrenoceptor density was not increased compared to that in the control group. This result differs from a report showing increased platelet $\alpha_2$-adrenoceptors in variant angina. However, 3 of our patients with variant angina had very large numbers of platelet $\alpha_2$-adrenoceptors. These patients had episodes of chest pain during hospitalization and were considered to have unstable variant angina pectoris. It has been reported that platelets taken from patients with variant angina showed obvious platelet hyperactivity to epinephrine at concentrations lower than 0.1 $\mu$mol/L. In contrast, platelets taken from patients with effort angina did not show a different aggregation threshold for either epinephrine or adenosine diphosphate as compared with control subjects. Thus, further studies are required to evaluate whether the increased $\alpha_2$-adrenoceptors seen in some of the patients with variant...
angina in this study, perhaps of the unstable type, might be related to the hypersensitivity of platelets to epinephrine. In this study, the affinities of patients with either effort angina or variant angina for the antagonist were not different from those of the controls. However, further study using an agonist may also be required, since a difference in affinity for an agonist, but not an antagonist, has been reported.

In summary, we found decreased platelet α₂-adrenoceptor density in patients with effort angina, but not in those with variant angina pectoris. In addition, we observed a negative correlation between the number of platelet α₂-adrenoceptors and the percent change in plasma norepinephrine after standing. If platelet α₂-adrenoceptors correlate with presynaptic α₂-adrenoceptors, the present results may suggest a role for presynaptic α₂-adrenoceptors in patients with effort angina pectoris, since decreased α₂-adrenoceptor density might lead to transient norepinephrine release and possible anginal attack. However, the precise role of these changes in α₂-adrenoceptor density is not clear and further studies are needed.

REFERENCES

4. Bassenge E, Holtz J, Sommer O, Saedel M: Vascular α₂-adrenoceptors mediate coronary constrictrions induced by norepinephrine and by sympathet


Japanese Circulation Journal Vol. 59, October 1995

24. Trendelenburg AU, Limberger N, Starke K: Presynaptic \( \alpha_2 \)-autoreceptors in brain cortex: \( \alpha_2D \) in the rat and \( \alpha_2A \) in the rabbit. Arch Pharmacol 1993; 348: 35–45