Changes in Hemodynamics and Bradykinin Concentration in Coronary Sinus Blood in Experimental Coronary Artery Occlusion

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SUMMARY

The following parameters were studied before and after acute occlusion of the anterior descending branch of the left coronary artery in 17 dogs: bradykinin (BK) in the coronary sinus blood, heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular max dp/dt (LV max dp/dt), and an index of myocardial contractility (LV max dp/dt/IP).

BK levels increased, reaching a maximum of 30±13 ng/ml 2 min after coronary ligation, accompanied by a significant elevation of LVEDP, and lowering of the myocardial contractility index. HR and LV max dp/dt showed no significant changes. A positive correlation obtained between the level of BK and LVEDP, as well as a negative correlation between the level of BK and of both LVSP and myocardial contractility index. Pretreatment with aprotinine (Trasylol), an inhibitor of kinin forming enzyme, prevented the increase in both BK and LVEDP after coronary artery ligation and caused an elevation of myocardial contractility index. These results suggest that BK formed within ischemic myocardium exerts a negative inotropic action on the heart.

Additional Indexing Words:
Cardiogenic shock Left ventricular max dp/dt/IP Aprotinine
Myocardial contractility Catecholamines

BRADYKININ, which has strong pain producing and hypotensive actions, has been suggested as a possible cause of anginal pain\textsuperscript{1,2} and of cardiogenic shock.\textsuperscript{3,4} Sicuteri et al\textsuperscript{5} have postulated that nervous, biochemical and hemodynamic factors play an important role in the occurrence of shock

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in myocardial infarction. Specifically, these authors consider bradykinin as one of the biochemical factors involved, since in patients with myocardial infarction the observed decrease in arterial blood pressure is accompanied by a marked decrease in kininogen levels in peripheral venous blood. Finding that the kininogen decrease is especially pronounced in the presence of shock, they concluded that a bradykinin-mediated peripheral vasodilation may be superimposed on the diminished cardiac contractility observed in so-called cardiogenic shock due to acute myocardial infarction.

Wiegershausen et al. have found that blood kininogen levels remained unchanged during the first 24 hours after the attack, but then declined over the following 7 days, with the lowest level observed on the second day. Since according to Dissman et al. it is the second or third day post-infarct that the peripheral vasodilation occurs, Wiegershausen et al. like Sicuteri et al. inferred a participation of bradykinin in that event.

Whereas those authors based their conclusions indirectly on decreases in blood kininogen levels, we reported in a previous paper a marked increase in bradykinin in parallel with a decrease in kininogen in the coronary sinus blood of dogs immediately after coronary artery occlusion. From this it appears that bradykinin is produced at and released from the site of myocardial ischemic injury.

The present study was undertaken to investigate whether the increase in bradykinin seen after coronary artery ligation plays a role in the development of shock following acute myocardial infarction.

**Materials and Methods**

Seventeen dogs, weighing 15 to 20 kg were anesthetized with sodium pentobarbital, 30 mg/Kg i.v. Under respiratory control with Bird’s respirator, a right thoracotomy was performed through the fourth intercostal space. After incising the pericardium, a vinyl catheter of 5 mm O.D. was inserted through the right atrium into coronary sinus to obtain blood samples. Anticoagulants were not employed. To prevent coagulation the catheter was filled with physiologic saline when blood samples were not taken.

An 8F polyethylene catheter was inserted via the right femoral artery into the left ventricle and connected to an electric sphygmomanometer (Nippon Koden RH-1) for the continuous measurement of heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and LV max dp/dt. Myocardial contractility was expressed as an index LV max dp/dt/IP where IP is the left ventricular developed pressure at the moment of max dp/dt (Veragut et al.).

The heart was rotated and the anterior descending branch of the left coronary artery was completely ligated about 3 cm distal from its origin. Blood samples were obtained immediately before and 1, 2, 5, and 10 min after the ligation and
bioassayed for bradykinin according to the method of Abe et al.,\textsuperscript{7)} using the isolated perfused guinea-pig ileum. In 8 out of 17 dogs aprotinin (Trasylol\textsuperscript{*}), an inhibitor of kinin forming enzyme, was injected intravenously at a dose of $2 \times 10^4$ unit/Kg 10 min prior to coronary artery ligation.

To study the hemodynamic effect of the inhibitor itself, the same dose of aprotinin was administered to 3 control dogs who had been prepared surgically in a similar fashion but without coronary ligation.

**Results**

1. Effect of coronary occlusion on bradykinin concentration in the coronary sinus blood.

Fig. 1 shows the changes in bradykinin levels in coronary sinus blood after the ligation of a coronary artery. As shown in the left panel, the concentration of bradykinin increased within minutes after ligation in all the non-pretreated animals. The mean concentration of bradykinin at 2 min after the ligation was significantly higher ($p<0.05$) than the mean per-ligation levels.

As shown in the right panel, no significant increase in bradykinin occurred following coronary ligation in the animals which were pretreated with aprotinin.

* Trasylol, product of Bayer AG, Wuppertal, West Germany.
Table I. The Influence of Aprotinine on Hemodynamic Changes in 3 Intact Dogs

<table>
<thead>
<tr>
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<th>Before</th>
<th>After Infusion</th>
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<tr>
<td></td>
<td></td>
<td>10 Min.</td>
</tr>
<tr>
<td>Heart Rate ( /min.)</td>
<td>193 ± 7</td>
<td>187 ± 12</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>160 ± 10</td>
<td>165 ± 11</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>9 ± 3</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>LV max dp/dt (mmHg/sec)</td>
<td>2400 ± 150</td>
<td>2600 ± 400</td>
</tr>
<tr>
<td>LV max dp/dt/IP (sec⁻¹)</td>
<td>25 ± 4</td>
<td>25 ± 3</td>
</tr>
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</table>

All values are given as mean ± S.D.

2. Hemodynamic parameters.
   a) Effect of aprotinine in control dogs.

Table I shows the results obtained in the experiments in which aprotinine was given to 3 control dogs without coronary ligation. No significant changes in HR, LVSP, LVEDP, LV max dp/dt, and LV max dp/dt/IP were noted after the administration of the drug, indicating that aprotinine alone has no effect on cardiac function.

b) Effect of coronary occlusion.

The HR did not show significant changes after ligation in both non-
pretreated and aprotinine-pretreated animals.

As seen in Fig. 2, an initial rise in LVSP was observed 1 min after ligation in most of the non-pretreated animals (left panel) whereas aprotinine apparently blocked this LVSP change following ligation (right panel).

As shown in Fig. 3, LVEDP rose in all non-pretreated animals (left panel) whereas aprotinine apparently blocked this change following ligation (right panel).
panel) 1 min after ligation and was maintained at this level throughout the observation period. In every case a statistically significant difference (p<0.05) was found between the pre- and postligation values of LVEDP. In the aprotinine-pretreated animals (right panel), no marked change in LVEDP was noted after ligation.

As shown in Fig. 4, LV max dp/dt increased 1 min after ligation in all non-pretreated animals except one in which it was decreased (left panel). After 5 min it decreased beyond the pre-ligation value. In the aprotinine-pretreated animals (right panel) LV max dp/dt tended to decrease until 5 min after ligation, and recovered after 10 min. These post-ligation changes, however, were statistically not significant in either group.

Fig. 5 shows the changes in LV max dp/dt/IP. In all non-pretreated animals (left panel), a decrease in LV max dp/dt/IP was noted 1 min after ligation followed in most instances by return to normal level. The decrease at 1 min after ligation was statistically significant (p<0.05). In the aprotinine-pretreated animals (right panel) LV max dp/dt/IP was significantly increased 1 min after ligation in all animals, and it tended to decrease but still remained above the baseline value.

3. Relationships between bradykinin concentration in coronary sinus blood and hemodynamic parameters.

There was a significant positive correlation of r=+0.576 (p<0.05) between high bradykinin levels and high LVEDP. Weak but significant nega-
Table II. Summary of Experimental Results

<table>
<thead>
<tr>
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<th>After Ligation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 Min.</td>
</tr>
<tr>
<td>Level</td>
<td>↑ or ↓ shows significant increase or decrease when compared with preligation value.</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>↑ or ↓ shows tendency to increase or decrease when compared with preligation value.</td>
</tr>
<tr>
<td>LVSP</td>
<td>→ shows no change when compared with preligation value.</td>
</tr>
<tr>
<td>LVEDP</td>
<td>* and ** show p value less than 5% and 1% respectively, when student t tests were done between aprotinine-pretreated and non-pretreated values in the same post-ligation period.</td>
</tr>
</tbody>
</table>

Positive correlations of $r = -0.358$ (p<0.05) and $r = -0.413$ (p<0.05) were observed between high bradykinin levels and low LVSP as well as low LV max dp/dt/IP, respectively.

4. Differences between the hemodynamic parameters in non-pretreated and aprotinine-pretreated animals.

The results of the differences between the hemodynamic parameters in non-pretreated and aprotinine-pretreated animals are summarized in Table II. In the non-pretreated animals, a decrease in LV max dp/dt/IP and an increase in LVEDP were observed after coronary artery ligation, along with a rise in bradykinin levels in coronary sinus blood. A transient rise in LVSP was also seen. In the aprotinine-pretreated animals, no changes in bradykinin level and in LVEDP were noted following ligation, while the LV max dp/dt/IP increased. LVSP tended to decrease 2 min after ligation. In both the non-pretreated and the aprotinine-pretreated animals no significant changes were noted in HR and LV max dp/dt.
DISCUSSION

As shown, coronary artery ligation in non-pretreated dogs caused a significant increase in bradykinin in the coronary sinus blood, and a variety of hemodynamic changes. The magnitude of the observed bradykinin increase is similar to that observed in our previous experiments.²) The increase in bradykinin in the coronary sinus blood may be explained by a release at the site of myocardial ischemia. At the same time there was a marked decrease in myocardial contractility as indicated by elevation of LVEDP and a lowering of LV max dp/dt/IP. Pretreatment of animals with aprotinin prevents both the release of bradykinin and the above-mentioned hemodynamic changes.

These results suggest that there is a causal interrelationship between the increase in bradykinin and the changes in hemodynamic parameters. The rise of LVEDP showed a positive correlation with the bradykinin concentration in the coronary sinus blood, while the lowering of LV max dp/dt/IP showed a negative one. These data would indicate that bradykinin has a negative inotropic action.

Our findings do not bear out the current pharmacological concept that bradykinin has a positive inotropic action.⁸⁻¹²) It is generally said that bradykinin increases the cardiac output and causes a fall in blood pressure.⁸⁻¹⁶) Two reasons have been advanced for postulating that bradykinin increases cardiac output. One refers to its direct positive inotropic action to the heart, demonstrated by the observation that the increase in cardiac output in response to intravenous injection of bradykinin is seen even after the administration of reserpine.⁸,⁹) The other reason is the increase in myocardial contractility via catecholamines release secondary to the fall in blood pressure associated with the peripheral vasodilation caused by bradykinin.¹⁰⁻¹²)

Contrary to the first theory, Harrison et al.¹³) have found no change in myocardial contractility measured with a strain gauge even if the cardiac output is increased by bradykinin administration. They concluded that the increase in cardiac output after bradykinin infusion was due to the decrease in peripheral vascular resistance and the resultant increase in venous return. The second theory is supported by Freitas et al.¹¹) They ascribed the increase in cardiac output to the lowering of peripheral resistance, since the cardiac output increase after bradykinin administration was proportional to the fall in blood pressure associated with an increase in stroke volume. They furthermore believe that the reflex increase in myocardial contractility due to carotid sinus pressure decrease contributes to these phenomena. In their view, augmentation of these effects by catecholamines released from the adrenal gland also adds to the increase in cardiac output.
Contrary to these postulates, Nakano\textsuperscript{10} has reported that a transient negative inotropic action is observed when bradykinin is injected intravenously after reserpine administration. He also observed no change in myocardial contractility in isolated atrial muscle following a small dose of bradykinin, whereas a large dose produced a marked negative inotropic effect.

In our experiment, the concentration of bradykinin in coronary sinus blood was found to be considerably lower than the concentration one would expect following a large dose (0.25 $\mu$g/ml) such as used by Nakano.\textsuperscript{10} However, in view of the short biological half-life of bradykinin in the blood, a considerable amount of the bradykinin produced locally in the myocardium in our experiments may have been destroyed prior to reaching the coronary sinus. In addition, since there was little blood flow through the ischemic portion, it may be assumed that the bradykinin observed by us was released from the peripheral portion of the lesion. Furthermore, since the amount of bradykinin releasable from 1 ml plasma corresponds to 1,500 to 6,000 ng of synthetic bradykinin,\textsuperscript{17} it appears that a relatively large quantity of bradykinin could be produced at the site of ischemia.

It is therefore conceivable that the amount of bradykinin produced by coronary ligation is sufficient to exert a direct negative inotropic effect on the myocardium.

It is, however, necessary to consider whether myocardial depressant factor (MDF)\textsuperscript{18\textendash}20 plays a role in the depression of myocardial contractility in our experiments. According to Lefer et al\textsuperscript{18} MDF is also inhibited by aprotinine. However, since Glenn et al\textsuperscript{20} observed that the MDF level in the blood reached its peak after 5 hours of coronary artery occlusion, a significant contribution by this substance is not very likely in our experiments. In addition, the site of production of MDF is said to be the ischemic pancreas, but there was no indication in our animals of a state of splanchnic hypoperfusion. However, in order to further elucidate this problem, carboxypeptidase B, which inactivates bradykinin but not MDF, may have to be used instead of aprotinine in further experiments.

If the post-ligation decrease in max dp/dt/IP observed in the non-pretreated animals was caused by bradykinin alone, one should have expected that the administration of aprotinine would at most restore the pre-ligation value. But surprisingly, the value of max dp/dt/IP increased following the administration of aprotinine. It therefore might appear that aprotinine exerts an inotropic effect on its own. But, as indicated in Table I, our studies have shown that aprotinine has no significant hemodynamic effects in dogs subjected to sham coronary artery ligation. Similar findings have been reported by Lefer et al.\textsuperscript{18} More likely then, the additional inotropic effect observed
is caused by catecholamines, which reportedly are released from the myocardium following acute coronary artery occlusion\textsuperscript{21–23}. A close relationship between bradykinin and catecholamines, with either one causing a release of the other, has been reported\textsuperscript{24,25}.

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REFERENCES