East And Central African Journal Of Pharmaceutical Sciences
3 (2000) 2-7

The Involvement of Tissue Kallikrein in the Kinin release in the Experimental Myocardial Ischaemia in the Rat

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Occlusion of the coronary artery in the rat brought about a progressive rise in kinin levels from control, (before occlusion of the artery), to 30 minutes of the occlusion. The values were 246.38±28.86, 248.75±25.59, 415.23±45.93, and 514.11±41.64pcg/ml blood for control, pre-occlusion, 15 and 30 minutes after occlusion respectively. Aprotinin (1.52mg/kg body weight) which is a tissue and glandular kallikrein inhibitor, (but not an inhibitor of plasma kallikrein), significantly ($p<0.001$) prevented the release of kinins. However, Soybean trypsin inhibitor, (SBTI) an inhibitor of kallikrein and other serine proteases, in a dose of 3.03 and 9.1 mg/kg and Hexadimethrine, (which inhibits the activation of plasma pre-kallikrein and Hageman factor and therefore causing inhibition of kinin formation), in doses of 0.091 and 0.91mg/kg, did not prevent the increase in the levels of kinins after the occlusion of the coronary artery for 30 minutes.

These results seem to indicate that the tissue kallikrein rather than the plasma kallikrein is involved in the release of kinins during myocardial ischaemia in the rat.

Key Words: ischaemia, kallikrein, proteases inhibitors, aprotonin, hexadimethrine, Soybean trypsin inhibitor

INTRODUCTION

Early experiments demonstrated the stimulation of the kallikrein kinin system during an induced myocardial infarction in animals [1,2]. Clinical observations have also shown that coronary artery infarct is followed by the interplay of the kinin-kallikrein system [3,4]. In addition, other workers [5] have described the activation of kinin system after thrombolytic therapy in patients with acute myocardial infarction. Apart from the plasma, the possibility of the presence of this system has been demonstrated in the isolated hearts of the dog [6] and the rat [7].

The pharmacologically active agent of this system, called kinin, has been found to lower blood pressure [8] and cause immense pain [9,10].Its other pharmacological roles include local regulation of renal function [11], blunting the effects of pressor agents [11], participation in a variety of inflammatory disorders [12,13], and the involvement in shock [14].

Because of the kinin propensity to cause pain, the relationship between the system and infarction is of clinical importance, probably associated with angina pectoris [15]. This has lead to the suggestion that kinin generation might influence vascular tone and leucocyte function and thus be involved in the pathophysiological alterations occurring in patients with recurrent angina at rest [15]. Related to this, Liubimova and Popov [16] demonstrated the activation of kallikrein-kinin system when the infarcted heart is exposed to electromagnetic field. Additionally, favourable effects of the myocardial function have been observed when it is exposed to kinins [17].

Two enzymes are responsible for the release of kinins, one being from the pre-cursor in secretory cells and the other from a plasma pre-cursor (Fletcher Factor). The bigger enzyme from the plasma releases kinin from the plasma and activates kininogen II while the smaller Glandular (Tissue) kallikrein activates both Kininogen I and II.

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While the studies above have demonstrated the excitement of kinin system in general, and although possible evidence exists for the presence of this system in the isolated dog and rat hearts, none has so far shown which enzyme is responsible for the kinin release during myocardial ischaemia. We suggest that this information should help to identify the best option for intervention during myocardial ischaemic pain in patients.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 250-350gm were used. Sodium pentobarbitone anaesthesia, 60mg/kg, i.v. was used with artificial ventilation (1.5cc/100gm rat weight and 54 strokes per minute). Anaesthesia was maintained by occasional intraperitoneal administration of sodium pentobarbitone, 10-25mg/kg body weight. Artificial respiration was instituted with a small animal pump (Nihon Kohden 21).

Cannulation Procedure

After full anaesthesia was effected, the right femoral vein was cannulated with a polyethylene cannula (P.E 50). The injection of aprotonin, hexadimethrine and infusion of soybean trypsin inhibitor were given through this cannula. The left femoral and carotid arteries were cannulated with a polyethylene cannula (P.E 60). These were for the recording of systemic blood pressure and collection of blood samples respectively.

Production of Ischaemia

After the achievement of anaesthetic effect, cannulation were done as above. A left thoracotomy was performed at the 4th or 5th intercostal space, 3-4 cm from the sternum and a positive artificial ventilation with room air was immediately started using a Howard small animal respirator, 15-25cm Hg at a rate of 54 strokes per minute, 1.5cc/100g of rat. After sectioning the 4th and 5th ribs, the pericardium was incised to facilitate access to the heart.

The heart was exteriorised by exerting a gentle downward pressure on the ribs and sternum. A 6/0 braided silk suture attached to a 10mm reverse cutting needle (Mersilk, W812, Ethicon) was placed under the left main coronary artery at a point close to its origin. The heart was returned to the cavity and blood pressure (BP) and the electrocardiogram (ECG) was allowed to stabilise for 15 minutes. Any animal which produced arrhythmias or sustained fall in BP to less than 70mm Hg by this procedure alone was discarded from the study at this point.

Measurement of Blood Pressure and Heart Rate

The mean systemic blood pressure was measured by a pressure transducer (MPU - 0.5, Nihon Kohden, Tokyo, Japan) attached to a cannula inserted into the right femoral artery and this parameter was recorded on a polygraph (RM-85 Nihon Kohden, Tokyo, Japan). The heart rates and ECG were recorded by a force-displacement transducer.

Experimental Design

There were 5 groups of animals in this study. In addition each animal served as its own control.

Group 1(Control): The animals were only operated and blood samples withdrawn after stabilisation of blood pressure and ECG for 15 minutes. Blood samples were withdrawn immediately before occlusion, 15 and 30 minutes after the coronary artery occlusion.

Group 2: The animals were given soybean trypsin inhibitor through the left femoral vein before the coronary artery occlusion. Further blood samples were taken 15 and 30 minutes after the occlusion.

Group 3: Consisted of 5 animals treated in the same way as in group 2 above. In addition, hexadimethrine was administered iv and blood samples were taken before coronary artery occlusion. After the coronary artery occlusion, blood samples were taken at 15 and 30 minutes.

Group 4: Consisted of five animals which received aprotinin iv after which blood samples were taken before coronary artery occlusion. After the occlusion, blood samples were again taken at 15 and 30 minutes.

Group 5: The animals were treated in the same way as the others, except that there was no coronary artery occlusion and administration of drugs. Blood samples were drawn before opening the chests and at 15 and 30 minutes of opening the chests. Each animal therefore served as its own control.

Doses of the Drugs

Soybean trypsin inhibitor (SBTI) was given in doses of 3.3 and 9.1mg/kg body weight, the number of animals used at each dose level was 5. Hexadimethrine was used in a dose of 0.091 and 0.91mg/kg of the rat, with five animals at each dose level. Aprotinin was given in a dose of 1.52mg/kg rat, to 5 rats.

In all the cases, 1 ml of blood was drawn from the left carotid artery by a sterile plastic syringe and placed in ice-cold polyethylene tubes containing 4 ml of absolute ethanol and 0.2 ml of the previously made enzyme inhibition mixture. The tubes and contents were then heated at 70°C and spun for 15 minutes at 1500g at 4°C. One ml aliquotes of the supernatant were each transferred to triplicate tubes and dried by a slow stream of nitrogen gas at 70°C, then frozen at -20°C until they were radio immunoassayed.

Measurement of kinins

The Standardisation of Radioimmunoassay Procedure for Kinins

Rabbit anti-bradykinin antibody commercially obtained was reconstituted with 11ml of phosphate buffer, pH 7.4. Standard bradykinin and T-kinin serial dilutions were made with this buffer, from 160 to 80,000 pg/ml. In each polypropylene assay tubes, 0.1ml bradykinin or T-kinin and 0.1 ml of reconstituted buffer were added and incubated for between 18 and 24 hours at 4°C. Labelled antigen, ^{125}I-Bk, (0.1ml), having approximately 15000 dpm (displacements per minute), was added. Further incubation was done for 48 hours at 4°C. Separation of bound and unbound kinins was done by addition of 20ml of normal rabbit serum, lml of propanol and shaking vigorously. All separation processes were done at 0°C. The mixture was then centrifuged for 15 minutes at 3000g and 4°C. The supernatant was discarded and the pellets were counted in a gama counter.

Radioimmunoassay of Samples

The dried samples were dissolved in the buffer. Then 0.lml was added to the assay tube containing 0.lml of antibody and 0.1 ml of buffer. This gave the net count reading. It then underwent the same process as the standard. Included in the assay were tubes containing the ^{125}I-Bk without the unlabelled compound and antibody. This represented the total radioactivity present in each sample. The tubes with the ^{125}I-Bk, antiserum and buffer without the unlabelled compound indicated the maximum binding in the absence of the standard or sample. There were tubes also containing only buffer and the labelled traces. This represented a non-specific (blank) count.

Results

Figure 1 shows a typical marked ST depression which serves as the evidence for myocardial ischaemia event which in most cases occurred after 5-7 minutes of the coronary occlusion.

After coronary artery occlusion in the rat, kinin levels increased as follows: 246.38±28.86, 248.75 ±25.59, 415.23 ±45.93 and 514.11 ±41.64, for control, pre-occlusion, fifteen and thirty minutes after occlusion of the coronary artery (Table 1).

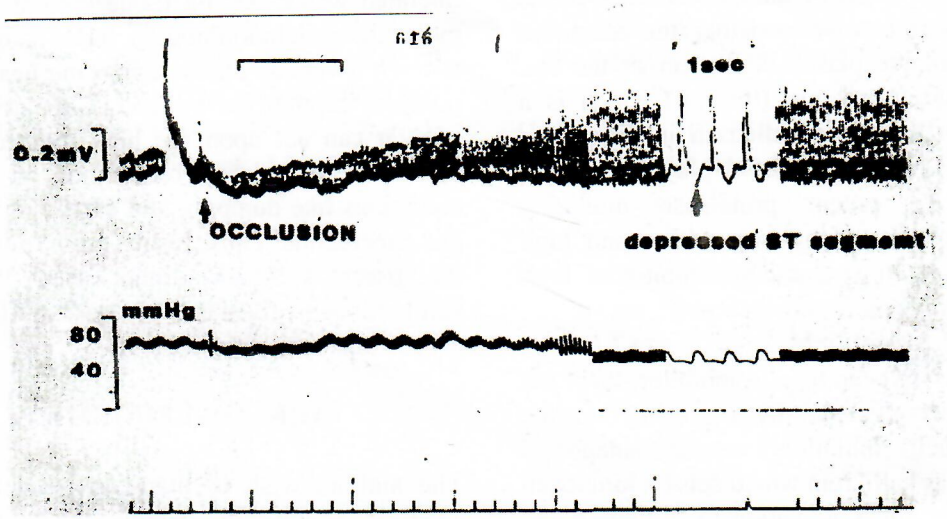
Table 1: Release of kinins after coronary artery occlusion experiment Comparison between control, Soybean trypsin inhibitor and Hexadimethrine

	Control	Pre-Occlu	15Min	30Min
Control	246.38	248.75	415.23	514.11
	±31.21	±28.31	±33.18	±36.91
SBTI	253.12	246.23	415.67	515.91
(9.1mg/kg)	±16.45	±23.51	±48.85	±38.08
SBTI	267.62	295.51	430.37	536.62
(3.03mg/kg)	±18.39	±24.81	±30.56	±39.35
HXDM	265.53	265.18	413.21	542.65
(0.091mg/kg)	±33.88	±19.82	±27.65	±31.88
HXDM	248.51	256.21	418.51	522.89
(0.91mg/kg)	±32.21	±23.41	±22.89	±28.38

Soybean Trypsin Inhibitor

Compared with control experiments, soybean trypsin inhibitor (SBTI), 3.03 and 9.1mg/kg body weight of the rat did not prevent the activation of the kinin system after the occlusion of the coronary arteries in rats (Table 1).

FIG 1

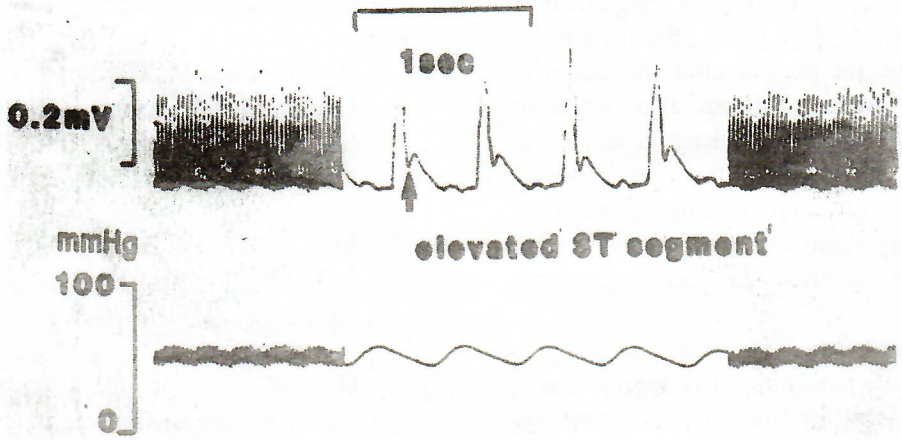


Hexadimethrine in 0.091 and 0.91mg/kg did not prevent the increase in the levels of kinins after the occlusion of the coronary artery for 30 minutes (Table 1). Aprotinin (1.51mg/kg) which is a tissue and glandular kallikrein inhibitor, (but not an inhibitor of plasma kallikrein), compared with the controls, significantly ($p<0.001$) prevented the increased release of kinins seen with the coronary artery occlusion. The levels of kinins released were 225.03±12.00, 238.37±16.32, 238.83±19.91, 249.45±10.97 for control, pre-occlusion, 15 minutes and 30 minutes after the occlusion which matched closely those of the sham operation which were 228.12±12.00, 239.95±19.51, 244.49±27.43 and 235.97±19.35 (Fig3).

DISCUSSION AND CONCLUSION

Figure 1 shows characteristic lead II changes in the electrocardiogram (ECG) 15 minutes after the coronary artery ligation. It was represented by the development of deep Q-wave and marked S-T segment elevation which was previously depressed from the onset of occlusion (Figure 2). The distortion of the S-T segment of the ECG pattern indicates myocardial ischaemia. Since complete polarisation of the membrane cannot occur during this time, strong currents of injury flow from the infarcted area of the ventricles, leading to, first, depression and later, elevation of the S-T segment.

FIG. 2



Since the measurement of kinins was made in the plasma, a question arises whether the identified kinin was from the plasma kallikrein or the one from the tissue. Aprotonin (trasylol) which is a tissue, but not a plasma kallikrein inhibitor was used to assess which kallikrein played the part. Aprotinin is a potent proteinase inhibiting compound. It is stable against acidity and high temperature [19]. It is a strong inhibitor of both kallikrein and trypsin.

Apart from the soybean trypsin inhibitor, there are a great number of naturally occurring enzyme inhibitors. Such inhibitors would antagonise enzymes such as kallikrein which acts on kininogen to release kinin. Such activation by kallikrein can occur at anytime of the experimental process and increase the level of kinins. Activation of Factor X11 can also occur which then gives rise to the general activation of the kallikrein-kinin system. The activation would therefore give rise to increased levels of kinins, and therefore the requirement for the sham operation. We used hexadimethrine, an anticoagulant that inhibits Factor X11 from initiating coagulation and formation of Permeability Factor (PF). This prevents the increase in kinin levels, which comes about during the process of dilution. It has also been shown that hexadimethrine inhibits the generation of kinins in plasma induced by gland activation [18].

Soybean trypsin inhibitor, apart from inhibiting Factor X11, is also a potent inhibitor of plasma kallikrein, but not of tissue kallikrein [19]. However, it does not remarkably inhibit the glandular kallikrein. The fact that SBTI and hexadimethrine did not modify significantly ($p>0.05$), even in two dose levels, the increase in kinin levels in the rat plasma after the coronary artery occlusion would indicate that whatever happens during the coronary ischaemia in the rat, Hageman Factor (Factor X11) was not involved. On the other hand, aprotinin, 1.52mg/kg rat, at the dose used here very significantly prevented the rise in the kinin levels after the coronary artery occlusion ($p< 0.05$).

Following the above reasoning, it is appropriate to suggest here that after the tissue injury by ischaemia, an enzyme, probably tissue kallikrein, is liberated which acts on tissue kininogen (the low molecular weight kininogen). It can also be washed through a venous drainage into the heart and then pumped through the systemic circulation. In this way, it can act upon the high molecular weight kininogen to release bradykinin as in certain conditions like dumping and carcinoid syndromes, and pancreatitis. These results seem to indicate that the tissue kallikrein rather than the plasma kallikrein is involved in the release of kinins during myocardial ischaemia in the rat.

ACKNOWLEDGEMENT

The authors wish to thank the Commonwealth Medical fellowship, which made this study possible.

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