East and Central African Journal of Pharmaceutical Sciences
Vol. 4 (2001) 18 - 24

Coronary Circulation and Kinin Release after Experimental Obstruction of the Left Anterior Descending (LAD) Coronary Artery in the Pig

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A 30-minute occlusion of the Left Anterior Descending (LAD) coronary artery in the pig had no significant effect on the heart rate, left ventricular and aortic pressures or resting coronary flow. However there was a significant increase in kinin levels in the blood sample drawn from the coronary sinus, but not the one drawn from the aorta. The levels in the sinus were significantly ($p<0.05$) different from the one in the aorta. The concentrations of blood gases also showed differences in the sinus and the aorta: Oxygen (O_2) was gradually decreasing in the sinus with a corresponding rise in Carbon dioxide CO_2 tension from zero to 30 minutes of the occlusion. There were however no differences in the values of Haematocrit, Hemoglobin and pH in both the sinus and aorta samples after the occlusion.

These results tend to indicate that the magnitude of flow reduction is related to the size of ischaemia. It also indicates that kinin measurement in the sinus may be used as a marker to demonstrate flow characteristics.

Key words: Kinin release coronary circulation, occlusion.

INTRODUCTION

Over the decades, it has been acknowledged that the most outstanding cause of the coronary artery disease is coronary artery blockade, leading to an insufficient perfusion of the heart muscle [1]. This lack of perfusion then leads to a cascade of biochemical events which are responsible for the irreversible myocardial injury which itself involves various mechanisms that change the properties of the cell membrane [2], and release of various substances [3]. The local needs, including oxygen demand by the myocardium is the single most important factor for control of the coronary perfusion. However other endogenous substances have been found to play important roles. An array of early and recent studies has all demonstrated a major role for kinins in the control of cardiovascular system. Longhurst and Cardway showed that bradykinin can stimulate gallbladder receptors to induce significant reflex activation of the cardiovascular system [4]. In cerebral circulation, Kamitani and coworkers found that activation of brain kallikrein and subsequent formation of kinin from brain kininogen may be important in modulation of cerebral blood flow or generation of cerebral edema [5]. In clinical terms, observations have also shown that bradykinin introduced externally is beneficial in improving myocardial performance during heart surgery [6]. A number of other studies have strongly indicated that kinin participates in the control of blood pressure [7,8,9], as well as in the structural preservation of the heart muscle [10]. In addition, it has been suggested that central kinins may be involved in the modulation of cardiovascular function as in the protection of arterial hypertension [11]. In contrast however, the presence of bradykinin B2 receptors in the cardiovascular regulatory centers of the brain is responsible for increase in mean arterial pressure and heart rate after intracerebroventricular injections of bradykinin, and this has been

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postulated to be mediated via stimulation of sympathetic nervous system. [12].

The involvement of kinins in homeostasis of cardiovascular system has been postulated to be through the involvement of other substances, notably nitrous oxide [13], linking other vasoactive agents with nitrous oxide [14]. While a connection between an induced coronary ischaemia and circulatory disorders has long been elucidated in a number of laboratory settings, as in the dog [15] and rat [16], our present investigation was motivated by the previous observations that pigs (*landrace breed)* posses a collateral vessels almost matching those in the primates and humans [17,18,19].

MATERIALS AND METHODS

Animal Preparation

Pigs (*landrace* breed), weighing between 25–35kg were anaesthetized with intravenous chloralose, 30mg/kg. Additional doses were given as required. After intra-tracheal intubation and mechanical respiration with room air, a mid-sternal thoracotomy was performed. Rectal temperature was monitored and maintained at 37.5°C by a heating pad and a servomechanical controller.

Instrumentation

Left ventricular and arterial systolic and end diastolic pressures were recorded using a 7F dual pressure transducer-tipped polyethylene catheters (SPR-227, Milar, Houston, Texas) passed through the left carotid artery into the left ventricle and ascending aorta, and another in the femoral artery. A catheter was also manipulated into the coronary sinus after being introduced through the jugular vein by the aid of an x-ray process using a screen monitor. Then thoracotomy was performed at the left 5^{th} intercostal space, the lung was retracted and the heart was suspended in a pericardial cradle. The origin of the left anterior descending artery (1.0–1.5cm segment) was carefully dissected from the surrounding tissue and a screw clump was placed at this point for ligation. The electrocardiogram (ECG) (limb lead II), heart rate, phasic and mean aortic and left ventricular systolic and end diastolic pressures, phasic and mean coronary flows were continuously recorded on a polygraph (model 7, Grass instrument Co. Quincy, Massachusetts).

Experimental Protocol and Collection of Samples

Each animal served as its own control. After surgical procedure, the blood pressure and other parameters were allowed to stabilize for 30 minutes. Blood samples as well as other baseline data were taken for minute 0. Then ischaemia was produced by clumping the left coronary artery, close to its origin, as already indicated above, for 30 minutes. During this period other samples for blood gases, kinin, pH and hemoglobin measurements were obtained by the cannulae in the femoral artery and the coronary sinus at 10 and 30 minutes

Other Parameter Measurement

The blood pH, pO_2 and haemoglobin content were determined by a micro blood gas/pH meter 413 (instrumental Laboratory. Kinin determination was measured as previously described (Adome and Anokbonggo [in print], *East and Central African Journal of Pharmaceutical Sciences*) by use of radio-immuno-assay.

Statistical Analysis

When variances were not heterogeneous, student's t-test was used to evaluate the significance of differences. Otherwise statistical analysis was performed by Wilcoxin's rank sum test.

RESULTS

Haemodynamic Characteristics

Heart rate (HR), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), mean diastolic blood flow (MDBF) mean systolic blood flow (MSBF) and mean arterial blood flow (MABF) are shown in the table 1 below. Heart rate and mean aortic pressure were similar at zero time and at 30 min of LAD occlusion.

Table 1: Haemodynamic characteristics after the occlusion of LAD

Control		Occlusion
Heart Rate	113± 3	116± 3
MAP (mm Hg)	98± 4	95± 4
LVSP(mmHg)	30 ±5	135± 7
LVDP(mmHg)	15 ±4	15± 6
MDBF(ml/min)	54± 7	15± 6
MSBF(ml/min)	6± 3	16± 3
MABF (ml/min)	41 ± 5	40± 7

Effects of Coronary Artery Occlusion on kinin Concentration in the Pig

The release of kinin after the LAD occlusion is shown in figure 1. In the sinus, kinin concentration increased significantly ($p<0.01$) from 132pcg/ml blood to 241 pcg/ml of blood after 10 minutes of the coronary artery occlusion. There was no significant change ($p>0.05$), in the samples taken from the femoral artery. After 30 minutes, the levels of kinin in the sinus blood returned to the level of pre-occlusion. The values in the sinus were 132.183 ± 6.93, 143.46±12.13, 241.26± 19.69 and 140.07 ± 11.69 pcg/ml blood, for control, pre-occlusion, 10 and 30 minutes after occlusion respectively (Fig 1). For pig femoral arterial blood, the values were: 131.62±9.12, 135.08± 9.88, 131.21± 6.72 and 150.86± 18.81

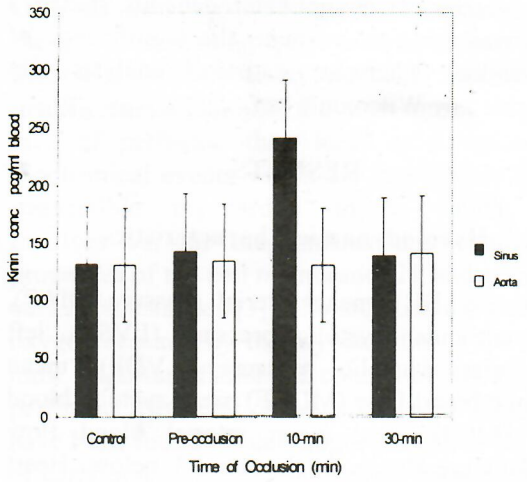

Fig 1: Concentration of kinins in the sinus and aorta after the occlusion of LAD

pcg kinin/ml blood for control, pre-occlusion, 10 and 30 minutes of the coronary artery occlusion respectively (Fig 1).

Oxygen and carbon dioxide concentration

There was a gradual decrease in the sinus blood oxygen from the control (before occlusion) to 30 minutes after the coronary artery occlusion (Fig. 2), which was significant ($p<0.05$). The gradual decrease of oxygen tension in the sinus blood indicates anoxia, which is well correlated with ischaemia. The values in the sinus were 43.27±2.02, 40.37±5.25; 28.36±2.19; and 26.7±5.24 for control, pre-occlusion, 10 and 30 minutes after the occlusion, respectively. In the femoral arterial blood, the values were 127.13±17.32; 118.56±13.54; 122.2±15.63 and 128.5±25.3, for control, pre-occlusion, 10 and 30 minutes after the coronary artery occlusion respectively.

Carbon dioxide tension was observed to increase at 10 minutes of the occlusion and then at 30 minutes in the sinus blood sample (Fig. 2).

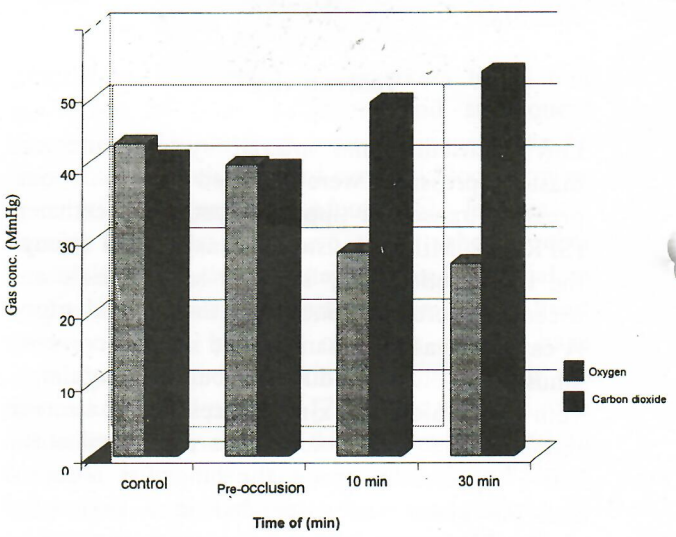

Fig. 2: Concentration of oxygen and carbon dioxide in the sinus after LAD occlusion

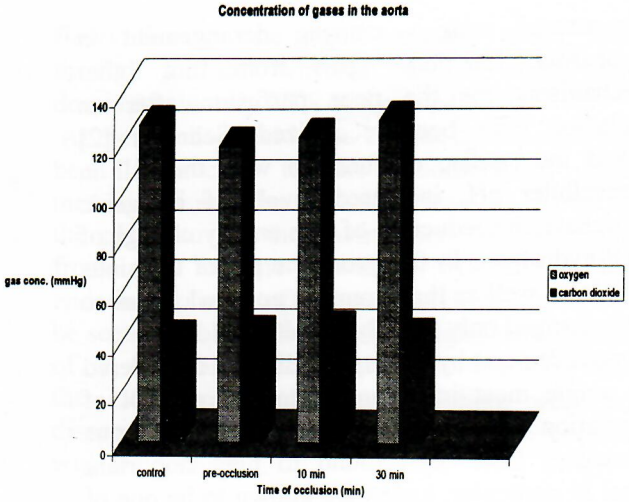

Fig. 3: Concentration of oxygen and carbon dioxide in aorta

There was not much change in the sample taken from the femoral artery. Values in the sinus samples were 41.33 ± 2.33, 40.05 ± 2.68, 49.16 ± 1.92 and 43.33 ± 3.18. For control, pre-occlusion, 10 and 30 minutes of the coronary artery occlusion respectively. For the arterial samples, the values were: 41.26 ± 2.45, 43.09 ± 5.64, 44.98 ± 8.88 and 42.03 ± 6.01 for control, pre-occlusion, 10 and 30 minutes of the occlusion respectively (Fig.3).

Hemoglobin and pH values

Table 2: Values of pH Haemoglobin (Hb)and haematocrit (hrt) in samples from the sinus blood

Para-meter	Control	Pre Occl.	10 MIN	30 MIN
Hb	7.43 ± 0.13	7.43 ± 0.05	7.39 ± 0.04	7.37 ± 0.03
Hb	8.1 ± 0.9	0.9 ± 1.3	8.82 ± 1.5	8.92 ± 1.23
HRT	27.13 ± 1.25	28.25 ± 1.8	29.22 ± 2.3	28.92 ± 1.95\

Table 3: Values of haemoglobin (Hb), pH haematocrit (hrt) in samples from femoval artery.

Para-meter	Control	Pre Occl.	10 MIN	30 MIN
Hb	9.25 ± 1.17	9.8 ± 1.19	9.49 ± 1.16	9.44 ± 2.19
PH	7.4 ± 0.23	7.43 ± 0.27	7.39 ± 0.21	7.41 ± 0.04
HRT	29.31 ± 3.5	27.91 ± 4.41	29.1 ± 3.59	28.92 ± 1.9

As can be seen in tables 2 and 3, there was no significant change ($p>0.05$) in hemoglobin values either in the sinus or arterial samples. Similar observation was made as regards the values for pH after the occlusion of the LAD in both the sinus and aorta.

DISSCUSSION

Previous work using animal models for studying myocardial dysfunction include the dog [20,21], the cat [22] and the rat [23]. Many other studies have employed the guinea pig [24]. Here we have employed the pig, *landrace* breed, because of its haemodynamic characteristics, which resemble that of man [19].

In this work major haemodynamic parameters did not show significant changes. The heart rate, mean arterial pressure, the left ventricular diastolic pressure and all blood flows were not markedly changed (Table 1). In addition there was no significant fall of pressure in the sinus as well as in the aorta. The distribution of resting coronary blood flow was unchanged after the occlusion. Changes observed were the increase in the left ventricular systolic pressure from 30±5, before to 135±7 mmHg after the occlusion. There was also an increase of systolic blood flow from 6±3 before to 16±3 after the occlusion. *Mehta et al* [25] have shown that in the dog vasodilator response of vessels reduce after occlusion, perhaps due to the loss of EDRF. This may come about in the following manner: compressive forces like the occlusion cause edema, which leads to destruction of the endothelium-resident EDRF, which is known as a potent vasodilator agent. The destruction of this agent therefore narrows the vessels thereby reducing flow. In this work we did not see, this perhaps because of the short period of observation (30 min), and perhaps due to species variation. Earlier work showed that in prolonged ischaemia following hypoxia, the

resultant process of infarction creates necrosis in an area of ischaemia in tissue adjacent to the necrotic core [26]. The necrosis causes an irreversible loss of contractile function with the eventual scar formation. The occlusion itself results in an asymmetric distribution of dis-ordered contractility, which alters the symmetry and synchrony of ventricular contraction and therefore reduces the formation of the myocardial pump. The heart becomes incapable of contracting with sufficient force to pump enough blood into the arterial tree and death can occur because of low perfusion of the vital organs. We observed that oxygen content in the sinus fell progressively as the carbon dioxide level increased and this phenomena served as evidence for ischaemia and hypoxia. On the other hand these values did not change in the aorta samples with the LAD occlusion (Figs.2 and 3).

Other events have been noted with experimental occlusive arterial study. After an occlusion, safety mechanism exists that attempts to prevent immediate fatality. In a number of studies, the improvement in regional myocardial function because of dilatation of collateral blood vessels after myocardial ischaemia is well documented [27, 28, 29]. This improvement is related to the development of collateral circulation as a safety mechanism. When there is an interruption in the circulation in the main arteries, an efficient development of collateral circulation occurs in the area to the extent that blood from the shunt perfuses the surrounding tissues. This ability to develop collateral circulation is variable among different species of experimental animals, and can influence the effects of a coronary artery occlusion. The previous work on the dog model reported highest peak of collateral development within the first 3 to 6 minutes [20]. The pig has been noted to have less developed collaterals than the dog and cat.

We have before[1] reported the development of arrhythmias after the occlusion of the LAD in the rat. Here we did not observe any arrhythmia. However other workers [30] using the dog noted that uncontrolled arrhythmia brings about increased myocardial oxygen consumption and the resultant hypoxia depresses automaticity in the sino-arterial node - causing derangement in myocardial function. Apart from this, other mechanisms for the poor perfusion after an occlusion have been recognized. Schrader [2] linked the cardiac dysfunction with the fall in intracellular pH, increased levels of inorganic phosphate and reduction of free energy change of ATP-hydrolysis. In this work the pH of the sinus sample as well as that from the aorta was seen to have changed only slightly (Table 1).

Oxygen demand by the myocardium is considered the single most important factor for control of circulation. However other endogenous substances have been found to play important roles. In particular, kinins are known to be one of the most potent vasodilating agents. Clinically, drugs such as ACE inhibitors: captopril, lisinopril, etc are known to be beneficial in controlling blood pressure by mobilizing the kallikrein-kinin system [31]. A recent study also indicates that nifedipine mediates coronary vasodilatation – thus improving myocardial ischaemia, through bradykinin [14]. In this study, the concentration of kinins in the sinus sample rose and fell from the pre-occlusion level to the 30-minute time of occlusion. The levels were 132.183 $\pm$ 6.93, 143.46$\pm$12.13, 241.26$\pm$ 19.69 and 140.07 $\pm$ 11.69 pcg/ml blood, for control pre-occlusion, 10 and 30 minutes after occlusion respectively. This rise and fall of kinin levels was not observed in the samples obtained from the aorta.

The difference in kinin release in the manner that was observed follows what is known about circulation within the heart. In the heart, most of the venous blood flowing from the left ventricles leaves by way of the coronary sinus, which is equal to about 75% of the total coronary blood flow. Most of the blood from the right ventricle flows through the small anterior cardiac veins, which empty directly into the right atrium and are not connected with the coronary sinus. A small amount of coronary blood flows back into the heart through the thebessian veins, which empty directly into all chambers of the heart. The left coronary artery supplies mainly the left ventricle and the right arteries supplies mainly the right ventricle, but usually also a small part of the left ventricle as well. In most humans more blood

flows through the right coronary artery than through the left whereas the left artery predominates in only 20% of the population.

At the biochemical levels cardiac dysfunction has been connected to the disorders of lipid metabolism and free radical formation, as well as the pH status of the environment [32] resulting from the enzyme xanthine oxidase at the endothelial lining of the vessels. However, as can be seen in table 2, there was no significant change of the value of pH both in the sinus and aorta with the LAD occlusion. Likewise there were no discernable changes in the heamoglobin content with the occlusion.

ACKNOWLEDGEMENT

This work was supported by the Commonwealth Medical Fellowship of 1990 to whom the authors are very grateful.

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