Effects of Insulin on Vasoconstriction Induced by Thromboxane A$_2$ in Porcine Coronary Artery

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To elucidate the role of insulin in the control of coronary artery tone, its effects on porcine coronary artery contraction evoked by thromboxane A$_2$ (TXA$_2$) were studied in vitro. Ring preparations of porcine proximal coronary artery were suspended in a Magnus apparatus filled with Tyrode's solution at 37$^\circ$C and aerated with 100% O$_2$, and the isometric tension of the contractions was measured. Insulin itself caused neither contraction nor relaxation. Insulin had no significant effect on the coronary artery contractions evoked by 20 mM K$^+$, norepinephrine, histamine, and serotonin; however, 120 minutes of preincubation with a physiological concentration of insulin (30–300 $\mu$units/ml) significantly accentuated coronary artery contractions evoked by STA$_2$ (10$^{-11}$ to 10$^{-7}$ M), a stable analogue of TXA$_2$ that is known to act on TXA$_2$/prostaglandin H$_2$ receptors (141.4±10.9% of the control at 10$^{-7}$ M STA$_2$ in the presence of 300 $\mu$units/ml insulin; $p<0.01$). The enhancing effects of insulin on the STA$_2$-induced contractions were affected by extracellular glucose or magnesium ion concentrations. The enhancing effects of insulin were observed only at the glucose concentrations of 100–300 mg/dl and magnesium concentrations of 0.5–1.5 mM. Therefore, insulin was suspected of enhancing TXA$_2$-induced contraction through a process that depends on extracellular glucose and Mg$^{2+}$. (Circulation 1990;81:1654–1659)

Although the pathogenesis of coronary vasoconstriction has been studied extensively, its precise mechanisms have not been fully clarified. It is well known that various neurohumoral factors and substances derived from blood cells such as acetylcholine, histamine, serotonin, thromboxane A$_2$ (TXA$_2$), and leukotrienes evoke vasoconstriction in porcine, canine, and human coronary arteries.

Several clinical studies have provided evidence that high circulating levels of insulin can promote the development of atherosclerotic vascular diseases like coronary heart disease. It has been reported that hypertension is closely related to hyperinsulinemia, and insulin is thought to cause hypertension through its vasoconstricting action. Other studies have revealed that insulin infusion reduces renal or retinal blood flow in diabetic patients and normal men. In animal experiments, insulin restored vascular contraction that was attenuated in the diabetic state.

These results suggest that insulin enhances vasoconstriction, although conflicting results have also been reported.

In this study, we examined the effect of insulin on porcine coronary artery contraction evoked by TXA$_2$ in vitro and demonstrated that insulin significantly enhanced TXA$_2$-induced coronary artery contraction. Furthermore, we studied the effects of extracellular glucose and magnesium ions on the action of insulin to determine the mechanisms of its action.

Methods

Coronary arteries excised from porcine hearts (57 hearts) were studied. The hearts were incubated in ice-cold Tyrode's solution (pH 7.4), containing (mM): NaCl 136.5, KCl 5.4, CaCl$_2$ 1.8, MgCl$_2$ 0.53, glucose 5.5, and HEPES-NaOH buffer 5.5. The proximal portion of the left anterior descending coronary artery was isolated at a portion about 2 cm from the coronary ostium. Connective and fat tissues were removed under a dissecting microscope, and coronary arteries were dissected into 2-mm-long segments. The segment was suspended in a Magnus apparatus filled with 30 ml Tyrode's solution and incubated at 37$^\circ$C with aeration of 100% oxygen. The isometric tension was measured by an isometric tension transducer (FD pickup, TB 611T, Nihon...
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Kohden, Tokyo, Japan), and recorded with a thermal pen recorder (WT-685G, Nihon Kohden, Tokyo, Japan). In each experiment, an initial tension load of 0.5 g was applied to the segment, which was allowed to equilibrate for 1 hour. Each preparation was suspected of not having intact endothelium because the preparation did not relax with a low dose of acetylcholine (10^-8 to 10^-7 M).

First, 20 mM K^+ was administered to the organ bath to evoke tonic contraction of ring segments. The tension of tonic contraction evoked by 20 mM K^+ had a constant ratio to that of maximal tonic contraction evoked by 100 mM K^+ in the segments obtained from the same coronary artery. Therefore, the contractions evoked by test agents were expressed as percentages of the contraction evoked by 20 mM K^+ in the absence of insulin.

After several washings with normal Tyrode’s solution, the preparations were restored to their basal state. They were then incubated with several physiological concentrations of insulin (30–300 μunits/ml) for 120 minutes, followed by the administration of 9,11-epithio-11,12-methano TXA2 (STA2), a stable analogue of TXA2. STA2 was dissolved in 30 μl

**FIGURE 1.** Tracings and plotting showing effect of insulin on STA2-induced contraction. Upper panel: Typical traces of experimental records showing effect of insulin on vasoconstriction evoked by STA2 in porcine coronary artery. Upper trace is contraction in absence of insulin, and lower trace is contraction in presence of insulin (300 μunits/ml). Preparations were incubated with insulin for 120 minutes before STA2 treatment. Lower panel: Dose-response curves for contractions evoked by STA2 in presence (○--○) and absence (○—○) of insulin (300 μunits/ml). Ordinate: Relative tension of contraction expressed as percentage of 20-mM K^+-induced contraction. Each point represents mean of eight experiments; bars indicate ±SEM.
Tyrode’s solution containing ethanol (less than 10⁻⁴ M), which evoked neither contraction nor relaxation. STA2 was cumulatively administered to the bath to attain a final STA2 concentration of 10⁻¹¹ to 10⁻⁷ M.

Control experiments were conducted concurrently with a preparation obtained from a neighboring portion of the same coronary artery. The preparation was incubated in Tyrode’s solution containing no insulin, and STA2 was administered in the same manner.

Similarly, the contractions evoked by norepinephrine, serotonin, histamine, and 20 mM K⁺ were studied. After the first 20-mM K⁺-induced contraction, preparations were preincubated for 120 minutes with or without insulin. After the preincubation period, these agonists were administered to the preparations, respectively. In the administration of norepinephrine, preparations were preincubated with 10 μM propranolol for 10 minutes.

To study the effect of extracellular glucose on the action of insulin, Tyrode’s solution containing various concentrations of glucose (0–900 mg/dl) were substituted for Tyrode’s solution alone, and the preparations were incubated for 120 minutes with or without insulin. The effect of Mg²⁺ on the action of insulin was also investigated by adding Mg²⁺ (0.2–4.5 mM) to Tyrode’s solution.

Insulin (Actrapid Human Monocomponent Insulin 40) was purchased from Novo Industries (Copenhagen, Denmark). STA2 was kindly provided by Ono Pharmaceutical Co (Osaka, Japan). Norepinephrine, propranolol, serotonin, and histamine were purchased from Sigma Chemical Co. (St. Louis, Missouri). The results were expressed as mean±SEM. Differences between treatments were tested by one-way analysis of variance for repeated measures, and p values less than 0.05 were considered statistically significant.

Results

Effect of Insulin on Porcine Coronary Artery Contraction

Insulin caused neither contraction nor relaxation of porcine coronary artery at a concentration of 30–300 μunits/ml (Figure 1, upper panel).

Effect of Insulin on Coronary Artery Contraction Evoked by 20 mM K⁺, Norepinephrine, Histamine, and Serotonin

The contractions evoked by 20 mM K⁺, norepinephrine, histamine, and serotonin were examined with or without preincubation of insulin for 120 minutes. Table 1 shows the relative tension of these contractions in the presence and absence of insulin. The contractions evoked by these agonists in the presence of insulin were not significantly different from those evoked in the absence of insulin (Table 1).

Effect of Insulin on STA2-Induced Coronary Artery Contraction

The effect of pretreatment with insulin (300 μunits/ml) on STA2-induced porcine coronary artery contraction is shown in Figure 1 (upper panel), and the corresponding dose-response curves of STA2 in the presence and absence of 300 μunits/ml insulin are presented in Figure 1 (lower panel). After 120 minutes of incubation with or without insulin, STA2 evoked tonic contraction of the porcine coronary artery in a dose-dependent manner. STA2-evoked contraction was observed at concentrations above 10⁻¹⁰ M, and the maximum contraction was obtained at about 10⁻⁷ M STA2. The ED₉₀ value of STA2, which evoked half the maximal contraction, was about 5×10⁻⁹ M. The contraction evoked by STA2 was remarkably stable, showing no decline until STA2 was washed from the incubation solution. Insulin significantly augmented the STA2-induced contractions at all examined dose levels of STA2 and increased the maximum level of STA2-induced contractions by about 28% (118.0±8.4% to 146.0±6.3%; n=8, p<0.01). Insulin, however, had no effect on the ED₉₀ value of STA2 (Figure 1, lower panel).

The effect of the duration of insulin preincubation on the enhancement of STA2-induced contraction by insulin was also studied (Figure 2). Preincubation with insulin (100 μunits/ml) for less than 30 minutes had no influence on subsequent STA2-induced contraction. As the duration of preincubation increased, the subsequent contraction evoked by 10⁻⁷ M STA2 increased gradually. The enhancement of STA2-induced contractions by insulin reached plateau levels after 120 minutes of preincubation with insulin.

As shown in Figure 3, insulin (30–300 μunits/ml) applied for 120 minutes increased the contractions evoked by 10⁻⁷ M STA2 in a dose-dependent manner. This effect was maximal at 300 μunits/ml insulin (141.4±10.9% of the STA2-induced contraction in the absence of insulin; n=7; p<0.01), whereas about 60 μunits/ml insulin caused half the maximal effect (Figure 3).

Effect of Extracellular Glucose Concentration on Effect of Insulin

Figure 4 shows the effect of various concentrations of extracellular glucose on coronary artery contraction in the presence and absence of 100 μunits/ml insulin. As the concentrations of extracellular glucose increased from 0 to 900 mg/dl, STA2-induced contractions increased irrespective of the presence or absence of insulin.

| Table 1. Effects of Insulin on Porcine Coronary Artery Contractions Evoked by Norepinephrine, Histamine, Serotonin, and 20 mM K⁺ |
|-----------------|-----------------|-----------------|
| Agonist         | Ins(-)          | Ins(+)          |
| Norepinephrine (10⁻³ M) (%) | 26.9±5.4 | 30.0±6.0† |
| Histamine (10⁻⁴ M) (%)          | 186.0±46.1 | 183.5±33.5‡ |
| Serotonin (10⁻³ M) (%)          | 67.0±21.7 | 67.3±15.9‡ |
| 20 mM K⁺ (%)                  | 112.3±9.4 | 109.2±1.8‡ |

*In the presence of 10⁻⁵ M propranolol; †300 μunits/ml insulin; ‡100 μunits/ml insulin; n=6.
Insulin significantly enhanced STA₂-induced contractions at an extracellular glucose concentration of 100 mg/dl (100.0±10.1% to 124.4±8.8%; n=5; p<0.05). Insulin did not have a significant effect at an extracellular glucose concentration of 300 mg/dl (138.8±17.5% to 160.7±15.0%; n=5; p=NS). The enhancing effect of insulin, however, was not observed in solutions containing either no glucose or a very high concentration of glucose (900 mg/dl).

Effects of Extracellular Magnesium Ion Concentration on Effect of Insulin

Figure 5 shows the effect of insulin on STA₂-induced contractions in solutions containing various concentrations of Mg²⁺ (0.2–4.5 mM). In the absence of insulin, STA₂-induced contractions decreased as the concentration of extracellular Mg²⁺ was increased from 0.2 to 4.5 mM. In the presence of insulin (300 μunits/ml), STA₂-induced contractions did not decrease as the concentration of extracellular Mg²⁺ was increased from 0.2 to 0.5 mM and then did decrease as the Mg²⁺ concentration was increased from 0.5 to 4.5 mM.
At low (0.2 mM) and high (4.5 mM) concentrations of Mg\(^{2+}\), insulin enhancement of STA\(_2\)-induced coronary artery contractions was not observed. The enhancement was demonstrated only at the Mg\(^{2+}\) concentrations of 0.5 mM (118.8±8.8% to 146.3±6.3%; n = 5; p < 0.05) and 1.5 mM (95.7±7.5% to 116.3±17.5%; n = 5; p = NS) but was not statistically significant at Mg\(^{2+}\) concentrations of 1.5 mM.

**Discussion**

TXA\(_2\) is formed in activated platelets and promotes platelet aggregation and vasoconstriction. STA\(_2\) was reported to cause rabbit coronary artery contraction in vitro and to cause diffuse coronary vasospasm and temporary occlusion by intracoronary infusion in vivo.\(^3\) The TXB\(_2\) concentration was reported to be increased in the coronary sinus blood of patients with vasospastic angina, and TXA\(_2\) has been suggested as a possible mediator of vasospasm.\(^4\)

In this study, we demonstrated that physiological concentrations of insulin (30–300 μunits/ml) specifically enhanced porcine coronary artery contraction induced by STA\(_2\) although insulin had no effect on vasoconstriction and had no influence on contractions evoked by other agonists (i.e., 20 mM K\(^+\), norepinephrine, histamine, and serotonin). Thus, insulin was suspected of exerting a modulating effect on coronary vascular tone by increasing TXA\(_2\)-induced contraction (Figures 1 and 3).

Previous reports concerning the effect of insulin on various vessels have presented inconsistent results.\(^12\)–\(^18\) Although the reasons for the differences in the action of insulin demonstrated in these reports have not been fully elucidated, differences in animal species, vessels, and the vasoconstricting agents used should be considered.\(^13\)\(^,\)\(^17\) Furthermore, most of these previous studies were performed as in vivo studies. Because insulin enhances sympathetic nervous activities and renal sodium reabsorption,\(^19\) it might be difficult to analyze the mechanisms by which insulin affects vascular tone using in vivo studies.

The significant enhancing effect of insulin was observed only after an incubation period of more than 120 minutes (Figure 2). In a previous study, the uptake of glucose in bovine mesenteric artery induced by insulin increased with the length of exposure to insulin (0–180 minutes) and was inhibited by a protein synthesis inhibitor, puromycin. Therefore, it has been suggested that protein synthesis is involved in the time-dependent increase.\(^20\) In our study, the effect of insulin on the STA\(_2\)-induced contraction also increased time dependently, and some synthetic process might be involved because puromycin effectively inhibited the enhancement of STA\(_2\)-induced contraction by insulin (data not shown).

The dose-response curve of STA\(_2\) in the presence and absence of insulin showed that insulin increased the maximum contraction evoked by STA\(_2\) but did not change the EC\(_{50}\) value of STA\(_2\) (Figure 1, lower panel). From this result, insulin is considered to increase the number of contraction units but not the sensitivity of the coronary artery to STA\(_2\).

In a previous study by Pennington et al,\(^21\) insulin increased the incorporation of inositol into phosphatidylinositol dose and time dependently and to enhance phosphatidylinositol turnover induced by phenylephrine in fat cells. Insulin has also been reported to increase protein kinase C activity by continuous activation of de novo phospholipid synthesis in myocytes.\(^22\)

Concerning the mechanism of action of TXA\(_2\), it has been reported that STA\(_2\) binds to TXA\(_2\)/prostaglandin (PG)\(_H_2\) receptors and produces inositol triphosphate and diacylglycerol through hydrolysis of phosphatidylinositol in vascular smooth muscle as well as platelets.\(^23\)\(^,\)\(^24\) Therefore, it is possible that insulin enhances STA\(_2\)-induced vasoconstriction by increasing the synthesis of phosphatidylinositol or related substances.

On the other hand, insulin had no effect on contractions evoked by norepinephrine, histamine, and serotonin, which were also reported to be mediated by the process involving phosphatidylinositol turnover (Table 1). Therefore, it was speculated that insulin acted on the synthesis of substances involved in processes from binding of STA\(_2\) to TXA\(_2\)/PGH\(_2\) receptors to activation of phospholipase. If, however, a thromboxane A\(_2\)-specific phosphatidylinositol pool exists,\(^25\) increased synthesis of phosphatidylinositol by insulin might explain the enhancement of STA\(_2\)-induced vasoconstriction.

Results of our studies on the effect of extracellular glucose and Mg\(^{2+}\) have shed some light on another characteristic of insulin. The increase in extracellular glucose concentration potentiated STA\(_2\)-induced vasoconstriction. The action of insulin was diminished in the presence of high concentrations of glucose or in the absence of glucose (Figure 4). These results suggest that STA\(_2\)-induced vasoconstriction was, at least partly, a glucose-dependent process and that insulin might enhance STA\(_2\)-induced vasoconstriction by stimulating the uptake of glucose into the cells.

Increased extracellular Mg\(^{2+}\) inhibited STA\(_2\)-induced vasoconstriction dose dependently (Figure 5). This action of Mg\(^{2+}\) is reportedly because of its calcium antagonistic action.\(^26\) Our results indicated that an optimal dose of magnesium ion was required for insulin to enhance vasoconstriction. The regulatory role of magnesium ion was discussed earlier by Gould and Chaudry,\(^27\) who reported insulin-induced enhancement of glucose uptake by rat soleus muscle cells. Thus, insulin was suspected of acting on vascular smooth muscle through a process modulated by magnesium ions, for example, glucose uptake and its intracellular metabolism.

The signal transduction systems functioning in the action of insulin on target cells have not been well understood. Insulin is known to stimulate both phosphorylation and dephosphorylation of cellular proteins.\(^25\) Insulin reportedly inhibits adenylate
cyclase activity,28 increases intracellular Ca2+ ions,29 and enhances phosphatidylinositol turnover evoked by agonists.21 These actions of insulin might explain the enhancement mechanism of STA2-induced contraction by insulin. On the other hand, other studies have shown that insulin stimulates the Na+-K+ pump and increases intracellular potassium concentration and hyperpolarization of the cell membrane.30 This effect of insulin should tend to antagonize the contraction evoked by STA2 and might limit the magnitude of the observed response; however, further studies are necessary to clarify the exact mechanisms by which insulin acts to enhance STA2-induced vasoconstriction.

Acknowledgment
We are indebted to Dr. Yoshihisa Kurachi for providing equipment used in this study.

References

Key Words • coronary vascular tone • hyperinsulinemia • glucose • magnesium ion
Effects of insulin on vasoconstriction induced by thromboxane A2 in porcine coronary artery.
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Circulation. 1990;81:1654-1659
doi: 10.1161/01.CIR.81.5.1654

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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