Insulin resistance induced by reduced pH of extracellular fluid

Takeshi TAKAKU[a] Michihiro SUMIDA[b] Hiromichi OKUDA*[b] and Nobuji MAEDA[c]

[a]*Central Research Laboratory,* [b]*2nd Department of Medical Biochemistry and* [c]*2nd Department of Physiology, School of Medicine, Ehime University*

(*Received February 17, 1994. Accepted April 4, 1994.*)

Abstract

The pH of the extracellular fluid in the anterior tibial muscle of anesthetized rats was monitored in a dark box at a constant temperature (25±0.5°C). After placing the pH electrode (diameter : 1.1 mm) on the tissue, the pH of the extracellular fluid increased gradually to a constant value (pH=7.42±0.008) after 20 min. When the femoral artery was pressed with a finger to stop the blood flow, the pH of the fluid rapidly decreased, but was promptly restored on releasing the pressure. Inhalation of carbon dioxide (CO_2) also resulted in reduction of the pH of the fluid. These results suggested that the reduction of extracellular pH of muscle tissue induced by disturbance of the blood circulation was due to the accumulation of CO_2. Inhalation of CO_2 for more than 20 min with intravenous injection of glucose significantly elevated the serum insulin level, but caused no change in the serum glucose concentration from that of controls inhaling air. The mechanism of insulin resistance due to reduction of the pH of the extracellular fluid is discussed.

Key words extracellular pH, insulin resistance.

Introduction

In mammalian organs, peripheral cells located outside the blood vessels are surrounded by extracellular fluid. The components and gas of this fluid concentration deffers from those of circulating blood due to the blood vessel wall barrier. In fact, Lemieux *et al.* reported that the pH value of the extracellular fluid of rat skeletal muscle was reduced by graded hemorrhage.[1] Endotoxin shock caused the decrease of the extracellular pH value to about 6.96, while the pH of the circulation blood remained at 7.33.[2] This result indicated that the pH of the extracellular fluid was decreased more easily than that of the blood. Previously, we reported that insulin-sensitive glucose uptake of isolated rat skeletal muscle was inhibited by incubation in mediums with pH less than 7.0,[4,5] and suggested that this inhibition was due to reduction of Na^+/H^+ exchange activity. We also proposed that reduction of the pH value of extracellular fluid of muscle tissue *in vivo* would also induce inhibition of insulin-sensitive glucose uptake. In the present report, we provide evidence to support this possibility obtained by reducing the pH of the extracellular fluid of rats by inhalation of CO_2.

Materials and Methods

Animals : Male Wistar King rats weighing 150 to 250 g were used. The rats were anesthetized with urethane-α-chloralose (1.5 g/kg body weight) and were placed in a shaded box at constant temperature (25±0.5°C) during measurements of the pH value of the extracellular fluid.

Measurements of pH of the extracellular fluid : The anesthetized rats were fixed on a board with their face upward and a 5 mm incision was made in the left hind leg. A pH electrode (diameter, 1.1 mm, FET sensor, pH-2135 : Nihon Kohden Co., Tokyo) was

*〒 791-02 愛媛県温泉郡重信町志津川
愛媛大学医学部第二医化学教室 奥田拓道
Shigenobu, Onsen-gun, Ehime, 791-02, Japan

和漢医薬学雑誌 11, 90 − 94, 1994

inserted through the incision to the surface of the anterior tibial muscle. Care was taken to place the pH electrode on the muscle without causing bleeding. A KR-5000 instrument (Nihon Kohden Co., Tokyo) with a temperature corrector was used as a pH monitor and an RTA-1100M instrument (Nihon Kohden Co, Tokyo) as a recorder.

Measurements of serum glucose and insulin: Serum glucose was measured with a Toekoosupa GT-1610 instrument (Kodama Co., Tokyo). Blood was taken from a tail vein for serum glucose assay. Serum insulin levels were measured with a Glazyme Insulin-EIA test kit (Wako Co., Osaka). Blood was taken from the jugular vein.

Analysis of data: Student's *t*-test was used to determine the significance of differences.

Results

The time course of change in the pH of extracellular fluid on the surface of an anterior tibial muscle was detected with a pH electrode placed on the muscle. As shown in Fig. 1, the pH gradually increased with time after electrode setting to a constant value in 20 min. The pH-increase in the initial phase was probably due to previous pH-reduction caused by the stimulus of the operation. Thus, in experiments the pH of the extracellular fluid was taken as that 20 min after placing the pH electrode. When the femoral artery was pressed manually with a finger to stop the blood flow around the anterior tibial muscle, the pH value in the extracellular fluid decreased rapidly within 1 min, but it returned to the control level within 1 min on release of the pressure as shown in Fig. 2.

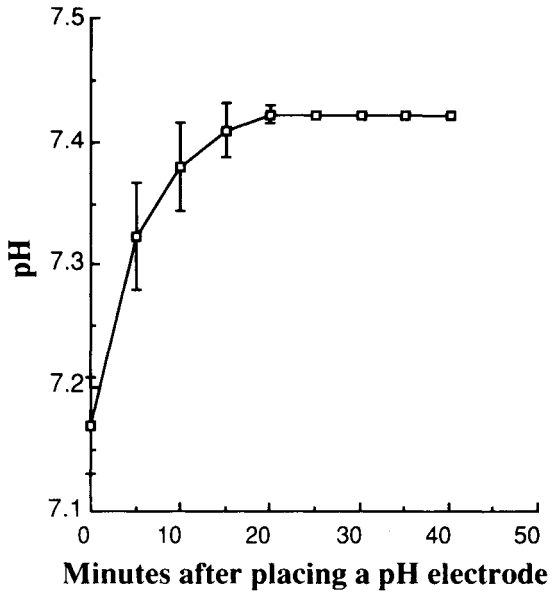

Fig. 1 Time course of change in pH in extracellular fluid on the surface of anterior tibial muscle of anesthetized rats determined with a pH electrode. Points and bars are means±S.E. for 5 separate experiments.

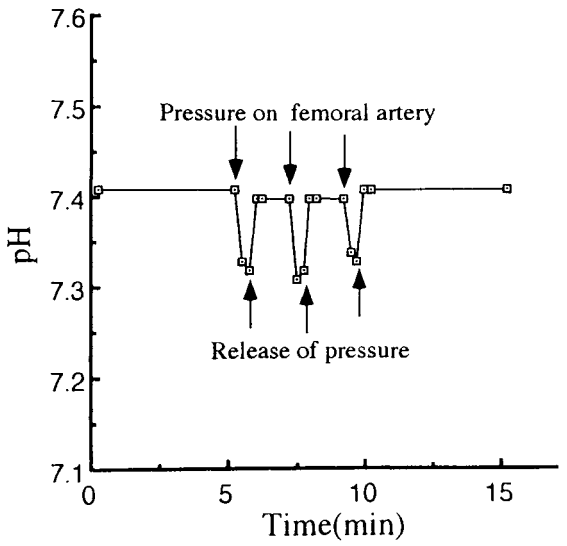

Fig. 2 Ischemia-induced pH reduction in the extracellular fluid on the surface of anterior tibial muscle. After stabilizing the pH level (Fig. 1), ischemia was induced by pressing the left femoral artery as indicated by arrows.

When inhalation of CO_2 was maintained continuously throughout experiments, the pH value in the extracellular fluid decreased (Fig. 3), reaching 7.17±0.01 within 20 min. When inhalation of CO_2 was terminated, the pH returned to approximately the original value (pH=7.42±0.007) in 20 min. When inhalation of CO_2 was prolonged, the pH value remained at the same level for up to 120 min (Fig. 4).

The effect of injection of glucose solution through the jugular vein at a dose of 0.25 g/kg body weight 20 min after the start of CO_2 inhalation was examined (Fig. 5). Although the pH value of the extramuscular fluid of rats was significantly reduced by inhalation of CO_2 (Fig. 4), the glucose level of their circulating blood was not significantly different from those of rats inhaling air (control) except at 60 min after glucose

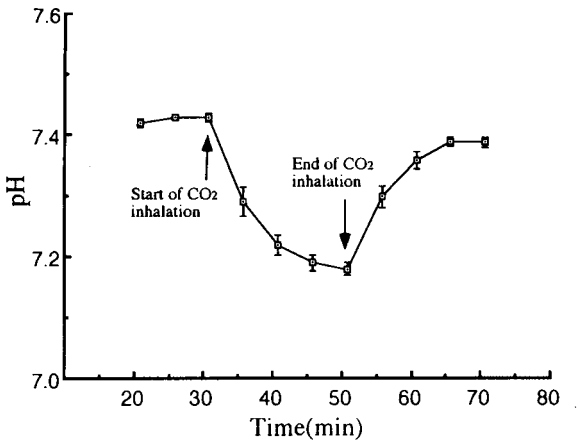

Fig. 3 Decrease in pH of the extracellular fluid on the surface of the anterior tibial muscle by CO_2 inhalation. After stabilizing the pH level (Fig. 1), the rats inhaled a mixture consisting of 10% CO_2, 21% O_2 and 69 % N_2 at 10 ml/min for 20 min. Points and bars are means±S.E. for 5 separate experiments.

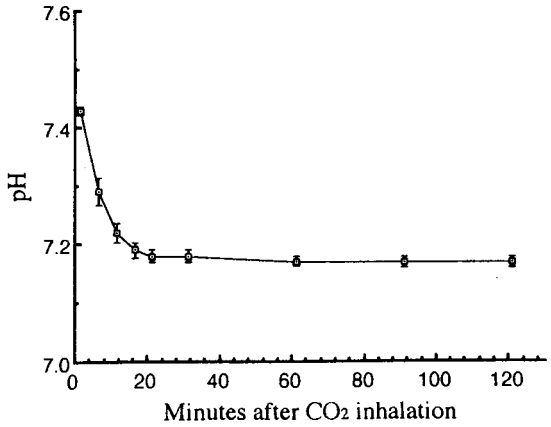

Fig. 4 Decrease in pH of the extracellular fluid on the surface of anterior tibial muscle by continuous CO_2-inhalation. Rats inhaled the CO_2-gas mixture described in Fig. 3 at a constant rate of 10 ml/min for up to 120 min.

injection. In contrast, the levels of serum insulin of rats inhaling CO_2 were significantly higher 20, 30, 60 and 90 min after glucose injection (188.8±2.5, 183.8±5.3, 137.4±9.1, 61.2±3.4 μU/ml) than those of control animals (152.6±4.7, 137.4±4.1, 67.0±4.4, 47.8±2.3 μU/ml), respectively. These results indicated that CO_2 inhalation inhibited insulin-stimulated glucose utilization in peripheral tissues.

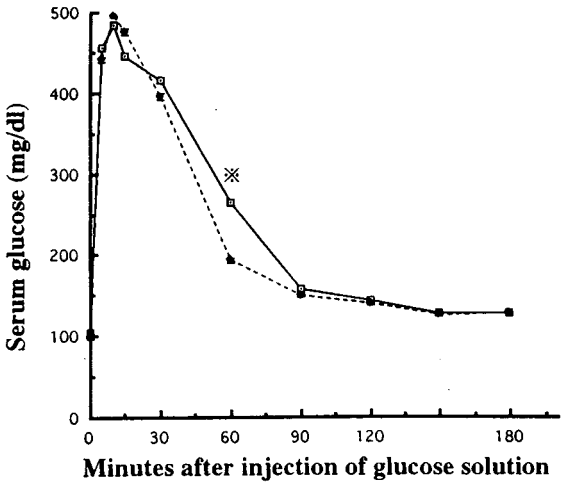

Fig. 5 Time course of change in serum glucose after intravenous administration of glucose under continuous CO_2-inhalation. After 20 min inhalation of the CO_2-gas mixture described in Fig. 4, glucose (50%) was injected into the jugular vein at a dose of 0.25 g/kg. Control rats (◆) inhaled air without CO_2, whereas experimental rats (□) inhaled the CO_2-gas mixture. Points and bars are means± S.E. for 5 separate experiments. ※ $p<0.05$

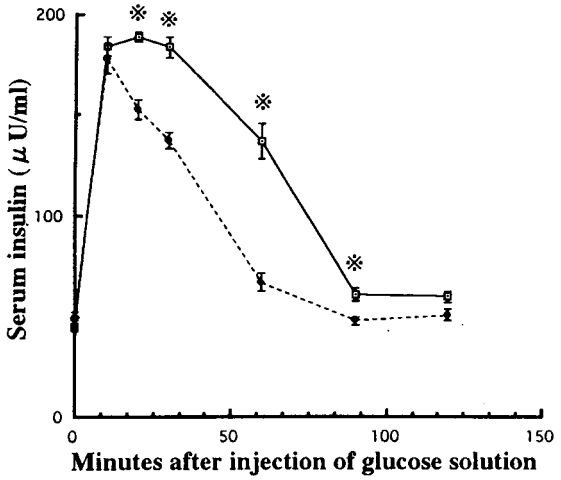

Fig. 6 Time course of serum insulin change after intravenous administration of glucose under continuous CO_2 inhalation. The experimental conditions were as described in the legend for Fig. 5. The control group (◆) inhaled air and the experimental group (□) inhaled the CO_2-gas mixture. Points and bars are means±S.E. for 5 separate experiments. ※ $p<0.05$

Discussion

In the present study we demonstrated that the pH of extracellular fluid of anterior tibial muscle is reduced by occlusion of the blood flow, or inhalation of CO_2. In peripheral tissues, various factors, such as CO_2, lactic acid,[3] free fatty acids and ketone bodies may decrease the pH of the extracellular fluid. We assumed that the decrease in pH of extracellular fluid in muscle tissue was induced by ischemia due to pressing the left femoral artery, and subsequent accumulation of CO_2 was produced by disturbance of the blood flow. This possibility was supported by the finding that inhalation of CO_2 reduced the pH of the extracellular fluid (Figs. 3 and 4). Previously we demonstrated that reduction of the pH of the extracellular fluid, inhibited insulin-sensitive glucose uptake by isolated tissue.[4,5] This inhibition was assumed to be mediated by reduction of Na^+/H^+ exchanger activity,[4,5] because the extracellular H^+ concentration was maintained by activity of the enzyme. We also suggested that insulin-stimulated 2-deoxy-D-glucose (2-DG) uptake by rats soleus tissue is closely related with Na^+/H^+ exchanger activity from the following observations[4,5]: (1) Insulin-stimulated 2-DG uptake by the tissue was inhibited by decrease in pH of the incubation medium. (2) Amiloride, a potent inhibitor of the Na^+/H^+ exchanger, decreased the effect of insulin on 2-DG uptake. (3) Insulin stimulated amiloride-sensitive Na^+ uptake in the muscle. (4) Insulin increased in the cytoplasmic pH of the muscle. (5) External Na^+ was essential for insulin-stimulated 2-DG uptake and could not be replaced by any other monovalent cations except Li^+. (6) The amiloride-sensitive Na^+ uptake was stimulated by insulin in both the presence and absence of external glucose. (7) The decreases in the effect on 2-DG uptake, amilorido-sensitive Na^+ uptake, and the specific Na^+ requirement for 2-DG uptake by decrease in pH of the medium could not be explained simply by decrease of specific binding of insulin to the tissue.

Based on these findings, we suggest that insulin stimulates glucose uptake in rat soleus muscle through an activity of the Na^+/H^+ exchanger as shown in the model in Fig. 7. Decrease in pH of the extracellular fluid would reduce insulin-stimulated glucose uptake by inhibiting the activity of the Na^+/H^+ exchanger. Therefore, it seems likely that disturbance of peripheral blood flow causes insulin resistance by reducing pH of the extracellular fluid. In the present study, we demonstrated that pH of the extracellular fluid is significantly lower in rats inhaling CO_2 than in those inhaling air (Figs. 3 and 4), and that during CO_2-inhalation, the concentration of serum glucose in the rats did not change although their serum insulin level was significantly higher than that of rats inhaling air.

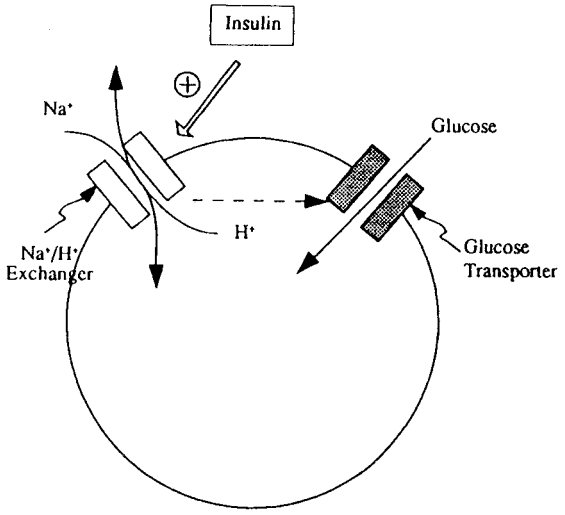

Fig. 7 Schematic model of insulin sensitive Na^+/H^+ exchanger and activation of glucose transport.

These results support the conclusion that reduction in pH of the extracellular fluid increases insulin resistance in peripheral tissues (Figs. 5 and 6).

和文抄録

細胞間質液 pH の測定法を確立した。麻酔をしたラットの前脛骨筋周辺の細胞間質液 pH を，遮光した恒温ボックス（25±0.5°C）で記録した。組織に pH 電極（直径 1.1 mm）を設置した後，細胞間質液 pH は，徐々に上昇し 20 分後には一定の値（pH=7.42±0.008）に到達した。指で大腿動脈を圧迫して血流を止めると，細胞間質液 pH は瞬時に減少し，指の圧迫を開放するとすぐに戻っ

た。また，炭酸ガス(CO_2)を吸入させると細胞間質液pHは低下した。このような結果は，血液の循環を妨害することにより，筋肉組織で生成された二酸化炭素が蓄積したため，細胞間質液pHが減少することを示すものである。炭酸ガスと空気を，それぞれ吸入させたラットにグルコースを静注すると，両群で血糖値には差は認められなかったが，血中インスリン値は炭酸ガス吸入群で高値を示し，インスリン抵抗性が出現した。このような成績をもとに細胞間質液pHの低下がインスリン抵抗性の出現を導く機序について考察した。

References

1) Lemieux, M.D., Smith, R. N. and Couch, N. P.: Surface pH and redox potential of skeletal muscle in graded hemorrhage. *Surgery* **65**, 457-461, 1969.

2) Kubo, M.: Muscle surface (interstitial) pH and hormonal action. *Ehime Igaku* **6**, 70-77, 1987.

3) Jain, R. K., Shah, S. A. and Finney, P. L.: Continuous noninvasive monitoring of pH and temperature in rat Walker 256 carcinoma during normoglycemia and hyperglycemia. *JNCI* **73**, 429-436, 1984.

4) Sekiya, K., Yamanouchi, T., Kubo, M., Kimura, S. and Okuda, H.: Effect of Na^+/H^+ exchanger inhibition on insulin stimulated lipogenesis on rat adipocytes. *Biomed. Res.* **10**, 191-196, 1989.

5) Yamanouchi, T., Sekiya, K., Okuda, H. and Kimura, S.: Role of Na^+/H^+ exchanger in insulin-stimulated glucose uptake into skeletal muscle. *Agressologie* **32**, 115-120, 1991.