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Original Article

Impact of Manipulation of Energy Substrates on Sinus Nodal Rhythm

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ABSTRACT

Background: The high energy demand of the heart is supported by metabolic flexibility in the utilization of energy substrates for adenosine triphosphate (ATP) synthesis. To investigate the roles of glucose and long-chain fatty acids (FA) as energy substrates in sinus nodal rhythm, we developed an isolated mouse atrial preparation and studied the effects of manipulating these energy substrates on atrial beating rate.

Methods: The spontaneous beating rate of isolated atria from mouse was measured at resting tension in a modified Tyrode solution that was gassed with 100% O_2 at 37℃ and contained 1 of the following sets of energy substrates: 10 mM glucose plus 0.4 mM palmitate (control), 5 mM glucose plus 0.4 mM palmitate (low glucose), or 10 mM glucose without palmitate (FA (−)). Atria were subjected to hypoxic conditions for 10 min followed by re-oxygenation.

Results: The spontaneous beating rate measured under low glucose conditions was significantly lower than that measured under control conditions, which was associated with concomitant reductions in ATP level and glucose consumption. In contrast, in FA (−) conditions, beating rate was significantly lower than that under control conditions, but the reduction in beating rate was not accompanied by significant changes in ATP level or glucose consumption. To investigate change in energy substrate preference under hypoxic conditions, atria were subjected to hypoxia in 3 modified Tyrode solutions. Under low glucose conditions, beating rate during hypoxia decreased to almost the same level as that of control, while recovery of beating rate during re-oxygenation was compromised. Under FA (−) conditions, exposure to hypoxia revealed a shift of energy substrate to glucose.

Conclusions: ATP produced by glucose metabolism is required in order to maintain sinus nodal rhythm. FA may be involved in regulating beating rate not only as an energy substrate but also through a novel mechanism.

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KEYWORDS: sinus node, isolated atria, glucose, fatty acid (FA), energy metabolism, adenosine triphosphate (ATP)

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The heart continually generates adenosine triphosphate (ATP) to supply the high energy demands required to maintain its functions. ATP has important roles as an energy source and as a modulator of membrane transporters and signaling events. ATP is involved in regulating spontaneous pacemaker activity in the sinoatrial node as a substrate for adenosine 3',5'-cyclic phosphate (cAMP).[1] As compared with ventricular myocytes, a greater level of cAMP is catalyzed from ATP by adenylyl cyclase (AC) in sinoatrial node cells.[1–3] cAMP/protein kinase A (PKA)-dependent phosphorylation of Ca^{2+}-cycling proteins such as sarcoplasmic reticulum (SR)/endoplasmic reticulum (ER) Ca^{2+}-ATPase (SERCA2), phospholamban (PLB), ryanodine receptors (RyR), and L-type Ca^{2+} channels facilitates generation of pacemaker action potentials in sinoatrial node cells.[1, 2, 4]

The ATP-sensitive potassium channel (K_{ATP} channel) links myocardial energy metabolism to membrane electrical activity. The K_{ATP} channel is activated when intracellular ATP concentration decreases with a concomitant increase in adenosine diphosphate (ADP) in cardiac myocytes under hypoxic/ischemic conditions.[5] The opening of the sarcolemmal K_{ATP} channel shortens the action potential duration by accelerating phase III repolarization, which reduces cardiac contractility and thus reduces energy demand and prevents Ca^{2+} overload during ischemia.[6] A study using *Kir6.2* knockout mice showed that the K_{ATP} channel contributes to inhibition of pacemaker activity in sinoatrial node cells during hypoxia, which is important for cardioprotection.[7]

In addition, cardiac L-type Ca^{2+} channels were reported to be regulated through direct allosteric modulation by intracellular ATP derived from glycolysis.[8] This mechanism may also contribute to reducing pacemaker activity and limiting cardiac energy expenditure during ischemia.[9]

In normal adult heart, 60% to 80% of ATP production depends on β-oxidation of long-chain fatty acids (FA); the remainder is accounted for by oxidation of carbohydrates (glucose and lactate) and ketone bodies.[10] However, the contribution of FA β-oxidation to total energy metabolism varies dynamically according to the energy demands of the heart and the supply of energy substrates or oxygen.[11] Although the contribution of energy substrates and their perturbation under ischemic conditions in ventricular myocytes is well understood, the role of energy substrates in regulating pacemaker activity remains to clarified. To determine the contributions of glucose and FA as energy substrates for ATP production in sinus nodal function, we studied the impact of glucose and FA deprivation on sinus nodal pacemaker activity in isolated mouse atrial preparation under oxygenated and hypoxic conditions. To assess sinus nodal function, the spontaneous beating rate of mouse isolated atria was measured.[12–14]

Methods

Measurement of developed force and spontaneous beating rate in isolated atria

All experiments were performed with the approval of the Animal Care and Use Committee of Toho University and were conducted according to the Act on Welfare and Management of Animals and the Guide for the Care and Use of Laboratory Animals in Japan. Wild type (WT) male mice (C57BL/6J, Clea Japan, Inc., Tokyo, Japan), age 9 to 25 weeks, were killed with an overdose of pentobarbital (50 mg/kg, ip). The heart was excised and immediately placed in ice-cold Ca^{2+}-free Tyrode solution containing (in mmol/l) NaCl, 137; KCl, 5.4; $CaCl_2$, 0; HEPES, 10; $MgCl_2$, 1; and glucose, 10 (pH 7.4 with NaOH). The heart was retrogradely perfused through the aorta with Ca^{2+}-free Tyrode solution, and the atria were dissected from the ventricle under a microscope. The atria were immersed in Krebs-Henseleit solution containing (in mmol/l) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $NaHCO_3$, 25; and glucose, 11.1, gassed with 5% CO_2/95% O_2 at 37℃, in an organ bath and then attached to an isometric force transducer to measure spontaneous contraction of the atria. Developed force and spontaneous beating rate were measured at a resting tension of 0.15 g and a sampling rate of 4 kHz using a PowerLab data-acquisition system. Data were entered into PowerLab Chart 7 computer software (AD Instruments Japan Inc., Nagoya, Japan). Sinus nodal function in response to β-adrenergic receptor stimulation with isoproterenol (ISO) (10^{-12}-10^{-6}M) was then examined.

Modification of energy metabolism

Developed force and spontaneous beating rate of atria isolated from mice (age, 9-23 weeks) were measured at a resting tension of 0.15 g in normal Tyrode solution containing 2.5 mM Ca^{2+} gassed with 100% O_2 at 37℃. After stabilization of developed force and beating rate, atria were immersed for more than 20 min in normal Tyrode solution gassed with 100% O_2 at 37℃ and containing 2.5 mM Ca^{2+}, 1 ng/ml insulin, 1% FA-free bovine serum albumin (BSA, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 1 of the following sets of energy substrates—(A) control con-

dition: 10 mM glucose, 0.4 mM sodium palmitate, (B) low glucose condition: 5 mM glucose, 0.4 mM sodium palmitate, (C) FA (−) condition: 10 mM glucose, 0 mM sodium palmitate.

Insulin was added to mimic serum insulin level,[15] and BSA was added to bind palmitate. FA concentration was based on the reported normal circulating FA concentration, which ranges from 0.2 to 0.6 mM.[11, 16, 17] After stabilization, oxygenation of the atria was discontinued for 10 min (hypoxic condition) followed by re-oxygenation for 20 min.

Metabolome analysis

Atrial preparations from male mice (age, 9-18 weeks) underwent measurement of developed force and beating rate in the modified Tyrode solutions were then rapidly frozen in liquid nitrogen for measurement of ionic metabolite levels, which were measured by a capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) system.[18] Atria from 3 different mice in each group were combined for normalization. The metabolic pathway map was produced using public-domain software.

Measurement of glucose consumption

A BioVison Glucose Assay Kit (BioVision, Inc., Milpitas, CA, USA) was used to measure glucose concentration in the 3 modified Tyrode solutions containing spontaneous contracting atria subjected to oxygenation and hypoxia for 5 min and 10 min.

Statistical analysis

Data are shown as the mean ± standard error of the mean. Statistical analysis was carried out with either the paired *t*-test, 1-way repeated-measure analysis of variance, or Bonferroni's multiple *t*-test. A p value of less than 0.05 was considered to be statistically significant. Regression analysis was used to investigate and model the relationship between 2 variables.

Results

Response of spontaneous beating rate and developed force to β-adrenergic stimulation with isoproterenol in atria isolated from mice

We developed an isolated mouse atrial preparation for analysis of sinus nodal rhythm. To examine spontaneous beating rate in atria isolated from mice, developed force and spontaneous beating rate were measured at a resting tension of 0.15 g (the minimum tension needed to obtain developed force and beating rate) in Krebs-Henseleit solution gassed with 5% CO_2/95% O_2 at 37℃.

We examined the response of the sinus node to β-adrenergic stimulation with ISO (Fig. 1A). The spontaneous beating rate of mouse atria increased in relation to ISO concentration (10^{-12} to 10^{-6}M). The maximal beating rate response was evoked at an ISO concentration of 10^{-7}M (Fig. 1B).

Developed force was affected by beating rate, due to the negative staircase effect. Therefore, we did not use developed force to evaluate response to ISO.

Deprivation of FA and glucose significantly reduced spontaneous beating rate

To investigate the contribution of energy substrates in sinus nodal rhythm, we measured the spontaneous beating rate in the modified Tyrode solution, which was gassed with 100% O_2 at 37℃ and contained 1 of the following sets of substrates: (A) control condition, 10 mM glucose with 0.4 mM sodium palmitate, (B) low glucose condition, 5 mM glucose with 0.4 mM palmitate, or (C) FA (−) condition, 10 mM glucose without sodium palmitate. Beating rate measured under partial deprivation of glucose was significantly lower than that measured in the presence of 10 mM glucose (control condition). In addition, beating rate measured in the absence of FA was significantly lower than that measured in the presence of FA (control condition). Beating rate values measured under control, low glucose, and FA (−) conditions were (in beat per minute) 309 ± 4, 258 ± 5, and 263 ± 5, respectively ($p<0.05$ vs control conditions; n = 28-30).

No significant differences between control, low glucose, and FA (−) conditions in levels of glycolytic and tricarboxylic acid (TCA) metabolites in atria

Under normal physiological conditions, FA and carbohydrates including glucose and lactate are used as the primary metabolic fuels to maintain cardiac function, and more than 95% of ATP production is attributable to mitochondrial oxidative phosphorylation.[11] In the present study, deprivation of energy substrates significantly reduced beating rate. To examine the effect of substrate deprivation on energy metabolism in spontaneously contracting atria, we used a CE-TOFMS system to measure ionic metabolite levels in atria under control, low glucose, and FA (−) conditions. There were no significant differences in levels of glycolytic metabolites between control, low glucose, and FA (−) conditions. Levels of TCA cycle metabolites were similar under control, low glucose, and FA (−) conditions (Fig. 2).

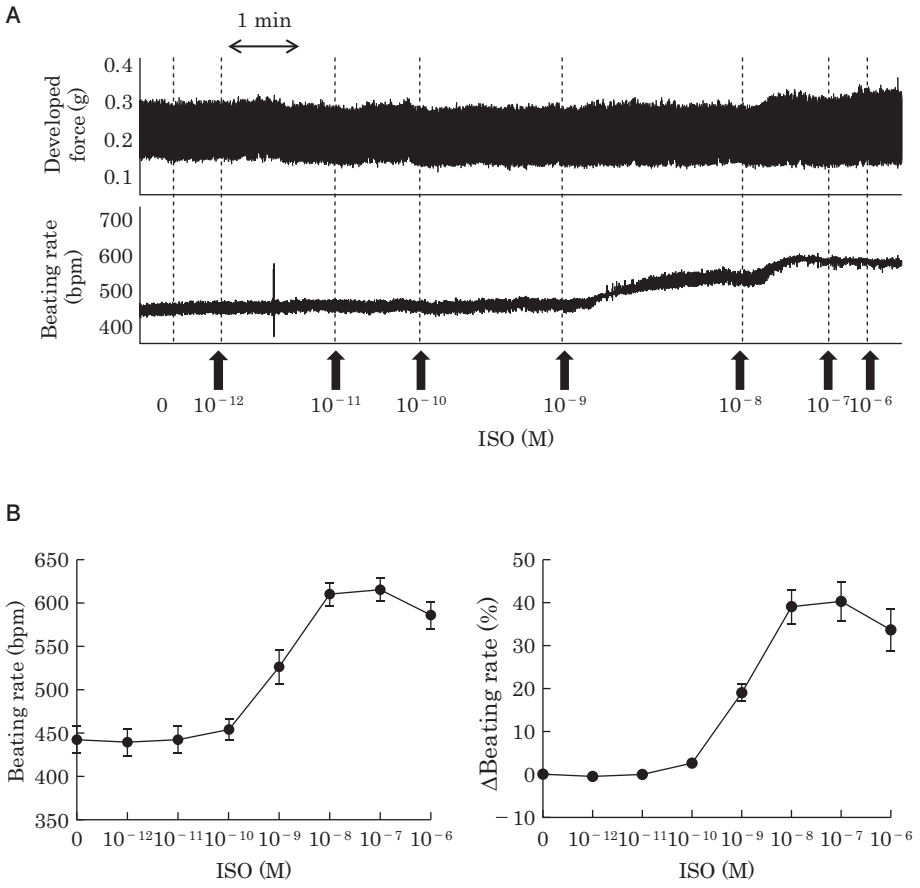

Fig. 1 Concentration-dependent effects of isoproterenol (ISO) on spontaneous beating rate in isolated mouse atria. (A) Representative example of developed force and beating rate. (B) Beating rate increased with the concentration of isoproterenol, *i.e.*, from 10^{-12} to 10^{-7} M (n = 10).

ATP level measured under partial deprivation of glucose was lower than in control but was maintained in the absence of FA

High cardiac energy demand is supported by hydrolysis of ATP to ADP and AMP. Under low glucose conditions, ATP level in atria was lower than that in control conditions (Fig. 3). In contrast, under FA (−) conditions, ATP was maintained at a level similar to that of control conditions. However, ADP and AMP levels under low glucose and FA (−) conditions were similar to those of control conditions. Levels of ATP-degradation products such as adenosine, adenine, inosine, and hypoxanthine were not significantly different between the control, low glucose, and FA (−) conditions (Fig. 3).

Reduction of spontaneous beating rate is associated with a decrease in ATP level in atria under low glucose conditions but not under FA (−) conditions

As shown in Fig. 4A, beating rate and ATP level measured under control and low glucose conditions were linearly correlated. ADP/ATP ratio is used as an index of the energy state of cardiac myocytes.[5] A higher ADP/ATP ratio represents either a decrease in ATP supply or an increase in ATP consumption. ADP/ATP ratio measured under low glucose conditions was larger than that under control conditions and was associated with a lower beating rate. Therefore, ADP/ATP ratio and beating rate were inversely correlated (Fig. 4B). These results indicate that beating rate depends on ATP generated via glucose metabolism. Thus, reduction in beating rate under partial glucose deprivation is due to a shortage of ATP supply.

In contrast, under control and FA (−) conditions, beating rate and ATP level were inversely correlated (Fig. 4C). ADP/ATP ratio measured in the absence of FA tended to be slightly lower than in the control, and beating rate was not correlated with ADP/ATP ratio (Fig. 4D). These results indicate that the reduction in beating rate measured under FA (−) conditions does not reflect ATP level.

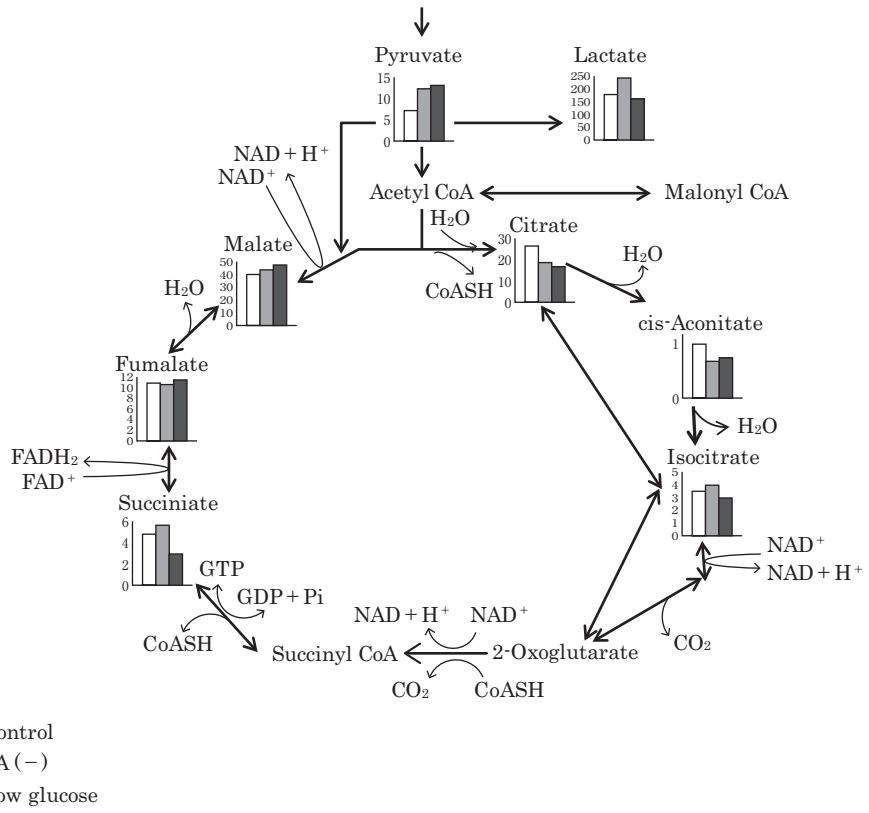

Fig. 2 Glycolytic and tricarboxylic acid (TCA) metabolites (nmol/g) in isolated mouse atria bathed in 3 modified Tyrode solutions. There were no significant differences in levels of glycolytic and TCA metabolites between control, low glucose, and fatty acid (FA) (−) conditions (control, n = 2; low glucose, n = 4; FA (−), n = 4).
NAD: nicotinamide adenosine dinucleotide, FAD: flavine adenine dinucleotide, $FADH_2$: 1,5-dihydro-flavine adenine dinucleotide, GTP: guanosine 5'-triphosphate, GDP: guanosine 5'-diphosphate, CoASH: coenzyme A, FA: fatty acid

During hypoxia, deprivation of FA and glucose did not compromise spontaneous beating rate as compared with control conditions

Because we expected that sinus nodal pacemaker activity would be susceptible to alterations in the supply of energy substrates and oxygen, we examined changes in sinus nodal rhythm during hypoxic insult. After stabilization in oxygenated modified Tyrode solution, atria were subjected to hypoxia for 10 min followed by re-oxygenation for 20 min. Under control conditions, beating rate declined during hypoxia in a time-dependent manner (Fig. 5). During re-oxygenation, beating rate recovered to the rate measured under oxygenation. During partial glucose deprivation, beating rate decreased time-dependently during hypoxia, to the level measured under control conditions (Fig. 5A, B). However, in contrast to control conditions, recovery of beating rate during re-oxygenation was slightly but significantly attenuated. Under FA (−) conditions, beating rate declined during hypoxia to the level measured under control conditions (Fig. 5C, D). During re-oxygenation, under FA (−) conditions beating rate recovered to a level close to that measured under oxygenation. Beating rates measured under oxygenation versus re-oxygenation and recovery (% of oxygenated level) were as follows: control conditions, 319 ± 11 versus 310 ± 15, 97 ± 10% (n = 7); low glucose conditions, 233 ± 6 versus 219 ± 4, 94 ± 2% (n = 7, $p < 0.05$); FA (−) conditions, 251 ± 10 versus 236 ± 6, 97 ± 4% (n = 6).

Reduction of spontaneous beating rate was associated with decreased glucose consumption in atria under low glucose conditions but not FA (−) conditions

FA and glucose have a reciprocal relationship in oxidative metabolism (glucose/FA cycle).[11] Under aerobic conditions, most cardiac energy demand is supplied by oxidation of FA and carbohydrates, while during ischemia, the reliance shifts toward glycolysis.[11] We hypothesized that

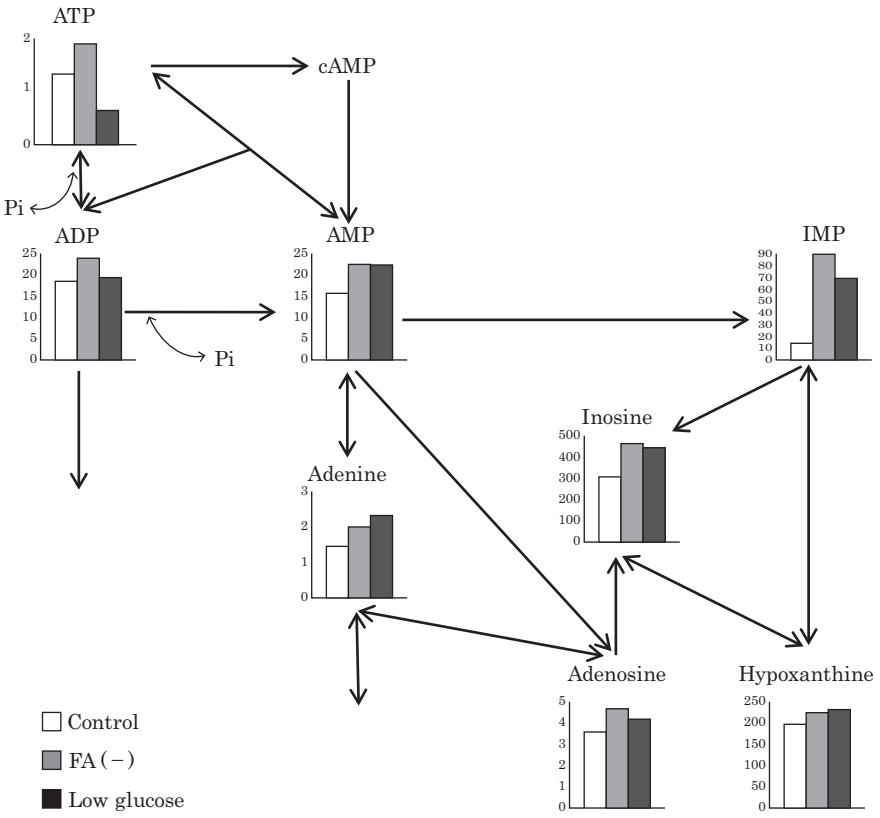

Fig. 3　Levels of ATP, ADP, and AMP (nmol/g) and related metabolites in isolated mouse atria bathed in 3 modified Tyrode solutions (control, n = 2; low glucose, n = 4; FA (−), n = 4).
ATP: adenosine triphosphate, ADP: adenosine diphosphate, AMP: adenosine monophosphate, cAMP: adenosine 3',5'-cyclic phosphate, IMP: inosine monophosphate, FA: fatty acid

beating rate would change in parallel with glucose expenditure, since beating rate was correlated with ATP level depending on the supply of glucose (Fig. 4A, C). We also hypothesized that glucose expenditure would be higher under FA (−) conditions than under control conditions, since ATP level was maintained and loss of β-oxidation of FA would be compensated by oxidation of glucose (Fig. 4 C, D).

To assess glucose consumption in spontaneously contracting atria, we measured glucose concentration in the 3 modified Tyrode solutions, in which atria were suspended under oxygenation followed by hypoxia for 5 min and 10 min. During hypoxia, under control conditions glucose consumption increased time-dependently to a level significantly higher than that measured under oxygenation (Fig. 6A, B). Under FA (−) conditions, the complete reliance on glucose as an energy substrate could facilitate glucose consumption during hypoxia. Under FA (−) conditions, glucose consumption was significantly greater during hypoxia for 5 min and 10 min as compared with that during oxygenation (Fig. 6B). Glucose consumption increased more rapidly under FA (−) conditions than under control conditions (Fig. 6B). However, under partial deprivation of glucose, glucose consumption was markedly lower than in control conditions (Fig. 6A), which is consistent with a reduction of ATP level under low glucose conditions (Fig. 3). Glucose consumption under control and low glucose conditions was linearly correlated with beating rate (Fig. 6C). This indicates that glucose is required as an energy substrate to maintain sinus nodal pacemaker activity. Contrary to our working hypothesis, glucose consumption in atria was not higher in the absence of FA than in the presence of FA (Fig. 6B). Accordingly, glucose consumption measured under control and FA (−) conditions was poorly correlated with beating rate (Fig. 6D). These results suggest that FA deprivation reduced beating rate through a mechanism independent from changes in glucose expenditure and ATP production in atria. This reduction in beat-

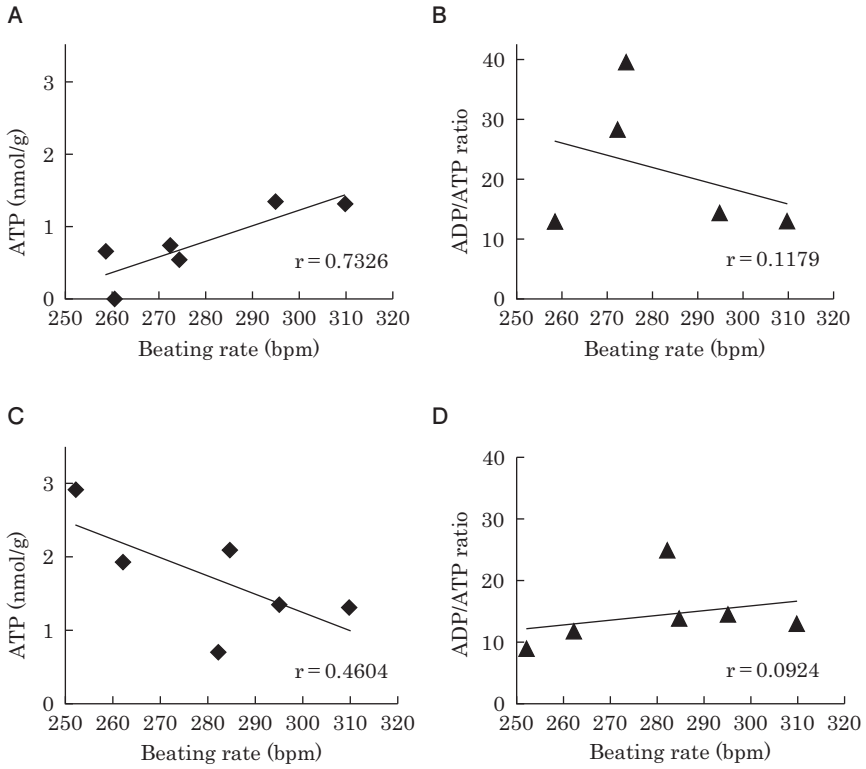

Fig. 4 Relationship between spontaneous beating rates and adenosine triphosphate (ATP) measured under control, low glucose, and fatty acid (FA) (−) conditions. (A) Correlation between beating rates and ATP levels measured under control and low glucose conditions (control, n = 2; low glucose, n = 4). (B) Correlation between beating rates and adenosine diphosphate (ADP)/ATP ratio measured under control and low glucose conditions (control, n = 2; low glucose, n = 4). (C) Correlation between beating rates and ATP levels measured under control and FA (−) conditions (control, n = 2; FA (−), n = 4). (D) Correlation between beating rates and ADP/ATP ratio measured under control and FA (−) conditions (control, n = 2; FA (−), n = 4).

ing rate may have limited consumption of ATP and glucose under FA (−) conditions.

Discussion

Under normal physiological conditions, FA and carbohydrates (glucose and lactate) serve as the primary metabolic fuels in maintaining cardiac contractile function, and more than 95% of ATP production is attributable to mitochondrial oxidative phosphorylation.[11] Genetic impairment of the FA β-oxidation mechanism has been implicated as a cause of cardiac arrhythmia.[19, 20] However, the contributions of energy metabolites such as long-chain FA and glucose to the regulation of pacemaker activity is poorly understood. Therefore, we investigated the relationship between energy metabolism of the sinus node and pacemaker activity.

Experimental manipulation of glucose concentration in the modified Tyrode solutions revealed that spontaneous beating rate was positively correlated with ATP level and glucose consumption in atria (Fig. 4A, 6C). Partial deprivation of glucose reduced ATP level. Under low glucose conditions, the contribution of FA β-oxidation could have been increased as compared with that of control conditions. However, as to the efficiency for ATP synthesis through oxidative phosphorylation, long-chain FA such as palmitate are a less "oxygen-efficient" energy substrate than glucose, because FA generates more ATP, which requires more oxygen than is required with glucose.[11] In addition, cardiac mechanical efficiency, defined as the ratio of contractile power to cardiac energy expenditure of the left ventricle, is lower when FA rather than glucose is used as an energy substrate.[11] Likewise, in atria, FA may be less efficient than glucose as an energy substrate. Our results indicate that ATP derived from glucose metabolism is re-

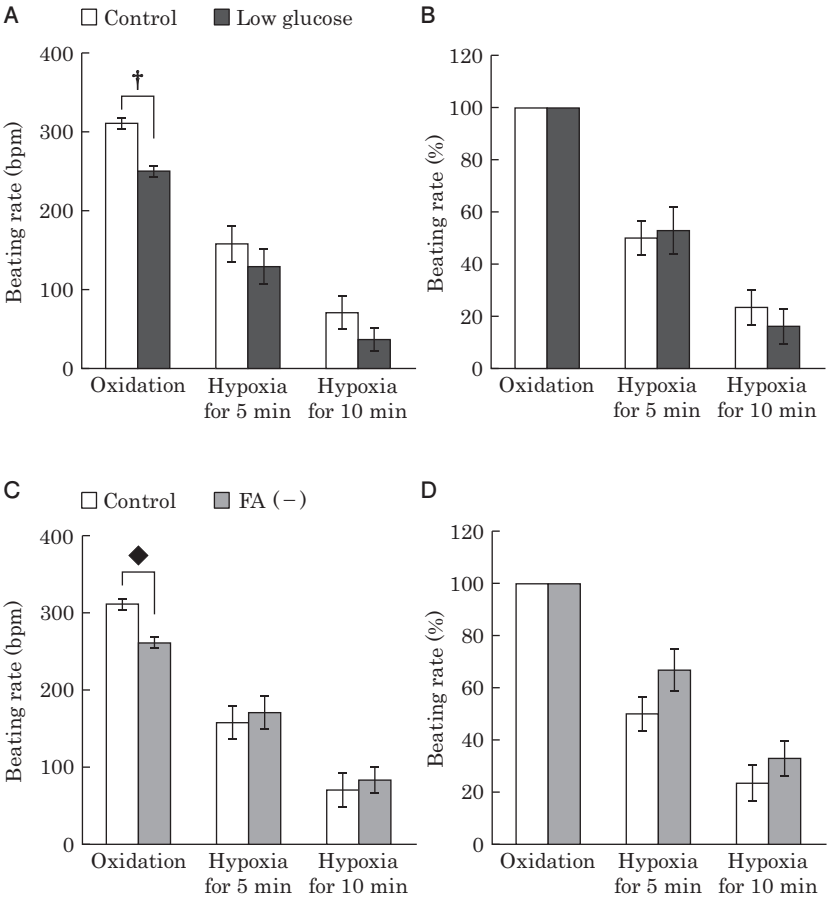

Fig. 5　Change in spontaneous beating rates of isolated mouse atria measured under oxygenated conditions and during hypoxia for 5 min and 10 min. (A) Effect of hypoxia on beating rates measured under control and low glucose conditions (control, n＝13; low glucose, n＝13). (B) Beating rates normalized to rates measured under oxygenated conditions, represented as 100% (control, n＝13; low glucose, n＝13). (C) Effect of hypoxia on beating rates measured under control and fattty acid (FA) (−) conditions (control, n＝13; FA (−), n＝13). (D) Beating rates normalized to that measured under oxygenated conditions, represented as 100% (control, n＝13; FA (−), n＝13).
◆: $p<0.05$ control vs FA (−), †: $p<0.05$ control vs low glucose.

quired in order to maintain beating rate.

In sharp contrast, under FA (−) conditions, reduction of beating rate was not associated with decreased ATP level and glucose expenditure (Fig. 4C, 6D). These results imply that lack of FA reduces beating rate through a mechanism independent from ATP shortage in atria. The reduction in beating rate may limit energy expenditure and preserve ATP under FA (−) conditions. In addition to its role as an energy substrate, FA may have a role in the signaling pathway that regulates sinus nodal pacemaker activity. Another possible explanation is that, as compared with working atrial myocytes (which mostly contribute to measured ATP level), sinoatrial pacemaker cells are more dependent on FA as an energy substrate. This possibility should be investigated in a future study.

The balance between oxidation of FA and glucose is perturbed under ischemia and ischemic-reperfusion in the heart.[11] Ischemic insult results in a rapid decline in ATP synthesis through the oxidative phosphorylation of energy substrates.[5, 11] It has been reported that sinoatrial node cells have a high density of mitochondrial protein and a high basal oxygen consumption, which reflects the rate of ATP production in mitochondria.[1, 8] Therefore, we hypothesized that sinus nodal pacemaker activity would be susceptible to alteration in the supply of energy substrates and oxygen. Under low glucose conditions, beating rate

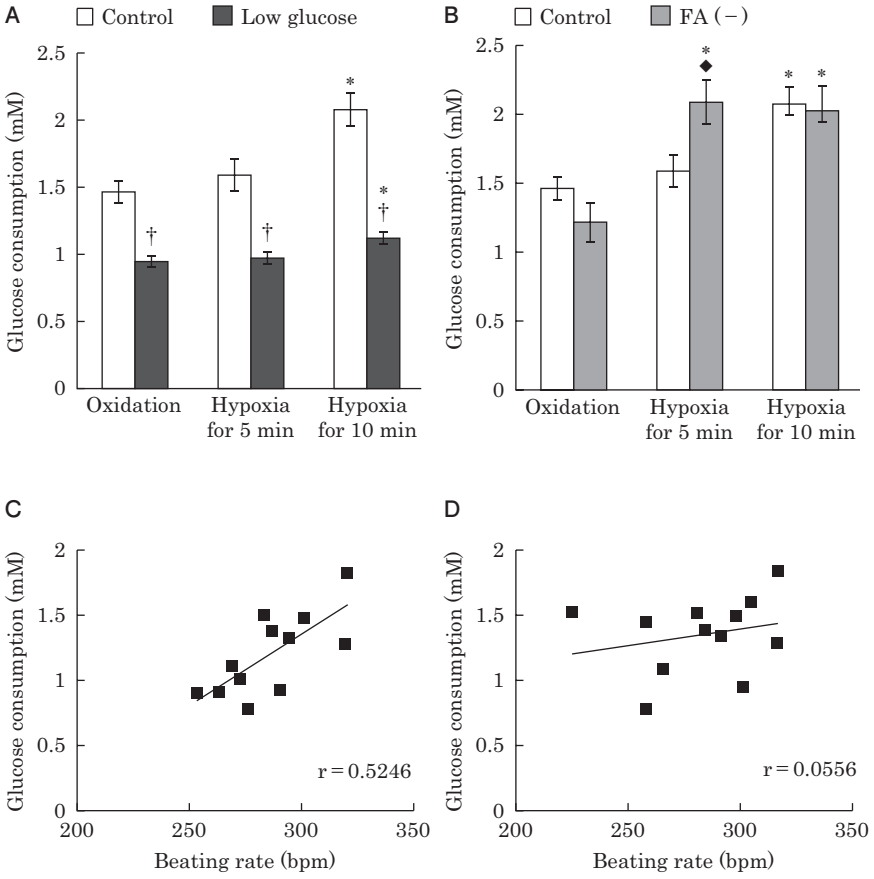

Fig. 6 Assessment of glucose consumption in isolated mouse atria measured under oxygenated conditions and during hypoxia for 5 min and 10 min. (A) Effect of hypoxia on glucose consumption measured under control and low glucose conditions (control, n = 6; low glucose, n = 6). (B) Effect of hypoxia on glucose consumption measured under control and fatty acid (FA) (−) conditions (control, n = 6; FA (−), n = 6). (C) Relationship between beating rates and glucose consumption in oxygenated mouse atria measured under control and low glucose conditions (control, n = 6; low glucose, n = 6). (D) Relationship between beating rates and glucose consumption in the oxygenated mouse atria measured under control and FA (−) conditions (control, n = 6; FA (−), n = 6). ◆: $p < 0.05$ control vs FA (−), †: $p < 0.05$ control vs low glucose, *: $p < 0.05$ oxygenated vs hypoxia.

during hypoxia decreased to the level observed in control conditions (Fig. 5A, C), whereas the recovery of beating rate during re-oxygenation was attenuated and accompanied by a marked decrease in glucose expenditure (Fig. 6 A). A previous report found that a high rate of FA β-oxidation contributed to ischemic injury by inhibiting glucose oxidation.[11] Under low glucose conditions, compensative increase in the contribution of FA β-oxidation may lead to insufficient recovery of sinus nodal rhythm during re-oxygenation after hypoxic insult. Under FA (−) conditions, although glucose expenditure was not higher than that under control conditions, exposure to hypoxia markedly increased glucose consumption in atria, thus revealing a shift in the energy substrate used for ATP synthesis, from FA to glucose, during hypoxia (Fig. 6B). Since cardiac mechanical efficiency is higher when glucose rather than FA is used as a substrate,[11] beating rate is expected to be more tolerant to hypoxia under FA (−) conditions as compared with control conditions. If beating rate were modulated by FA through a mechanism independent of energy metabolism, the reduction in beating rate due to FA deprivation might limit ATP consumption, thus protecting sinus nodal rhythm from hypoxia and re-oxygenation insult.

In summary, the present study demonstrated that ATP produced by glucose metabolism was required in order to maintain sinus nodal pacemaker activity. FA appears to be

involved in regulating beating rate not only as an energy substrate but also through a novel mechanism.

Clinical implications

Alterations in energy metabolism of the myocardium can affect heart rate.[21] Bradycardia after resuscitation from cardiac arrest was associated with poor prognosis.[22] In the setting of bradyasystole, energy metabolism in cardiac myocytes and endogenous substances such as adenosine may affect sinus nodal pacemaker activity.[23, 24] Conduction disorders and atrial tachycardias were observed in cases of FA β-oxidation disorders, such as in patients with deficient long-chain FA transport across the mitochondria (*i.e.*, carnitine palmitoyl transferase Type II deficiency and carnitine-acylcarnitine translocase deficiency) and those with trifunctional protein deficiency.[19]

Our findings suggest that shifting the balance between FA β-oxidation and glucose oxidation in atria may increase efficiency of ATP production and thus protect sinus nodal function against metabolic disorders.

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洞結節リズムに対するエネルギー基質の影響

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要約

背景と目的：心臓の高いエネルギー需要は，複数のエネルギー基質を adenosine triphosphate（ATP）産生に利用できる代謝調節により満たされている．グルコースと長鎖脂肪酸がエネルギー基質として洞結節リズムに果たす役割を明らかにするために，マウスの摘出心房筋標本を用いて，心房の拍動数に及ぼす影響を明らかにする．

対象および方法：マウスの摘出心房筋の自発的な拍動数を，以下のエネルギー基質を含み，37℃ に保温し酸素飽和させた改変 Tyrode 溶液中で静止張力を掛けて測定した．10 mM のグルコースと 0.4 mM パルミチン酸を含んだ Tyrode 溶液をコントロールとし，グルコースを 5 mM に半減した低グルコース溶液とパルミチン酸を除いた fatty acid（FA）欠損溶液を作成した．心房筋を 10 分間低酸素に曝した後，再酸素化した．

結果：低グルコース条件下では，コントロールに比べて自発的な拍動数は有意に低く，ATP レベルの低下およびグルコース消費量の低下との間に相関が認められた．一方，FA 欠損条件下では，拍動数はコントロールに比べ有意に低下したが ATP レベルまたはグルコース消費量の有意な変化を伴わなかった．低酸素条件におけるエネルギー基質利用の変化を調べるために，3 種類の改変 Tyrode 溶液中で心房筋を低酸素に曝した．低グルコース条件下では，低酸素条件下において，拍動数はコントロールとほぼ同等のレベルまで低下したが，再酸素化による回復は悪かった．FA 欠損条件下では，低酸素暴露により，エネルギー基質のグルコースへのシフトが露呈した．

結論：本研究により，グルコース代謝により産生される ATP が洞結節リズムの維持に必要であることを明らかにした．脂肪酸は，エネルギー基質としてのみならず，新たな調節機構を介して拍動数の制御に関与しているという可能性を見いだした．

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