科学研究費助成事業 研究成果報告書



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機関番号: 24303 研究種目: 挑戦的萌芽研究 研究期間: 2013~2014 課題番号: 25670111

研究課題名(和文)ISFETpHセンサーを用いた間質液pH連続測定とインスリン抵抗性発症機構解明

研究課題名(英文)Continuous pH measurement of interstitial fluid and clarification of pathogenic mechanism of insulin resistance by ISFET pH sensor

研究代表者

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交付決定額(研究期間全体):(直接経費) 2,900,000円

研究成果の概要(和文): 間質液の摂取法の開発および間質液部分へのアプローチ開発: 2日間、ラット背側間質部へ外径0.80 mm/内径0.57 mmの注射針を留置した。今回使用した注射針で物理的根詰まりが生じたので、実際の間質液pH測定は不可能であった。マイクロpH ISFETはラット間質液のpH測定では安定した測定結果を得ることが不可能であった。アンチモン製pH電極を用いて、pH測定を実施した結果、間質液において安定したpH測定を行なうことが可能であった。糖尿病ラット間質液pH 7.21±0.03 、非糖尿病ラット間質液pH 7.36±0.02 (n=6; p<0.05) という測定結果を得た。

研究成果の概要(英文): A) Developments of collection methods of interstitial fluid and methods approaching to interstitial fluid. We succeeded in two-day insertion of syringe with outer diameter of 0.80 mm and inner diameter of 0.57 mm in interstitial fluid space. B) pH measurements of interstitial fluid. Even if we tried to obtain the interstitial fluid using the syringe, we could not get the interstitial fluid due to clogged inner spaces of the syringe. Therefore, we tried to directly measure pH of interstitial fluids using micro pH meters, ISFET pH sensor and antimony pH sensor. We could not obtain any stable pH value of interstitial fluid using ISFET pH sensor, however stable values of interstitial fluid pH were obtained by antimony pH sensor. Using antimony pH sensor, we obtained pH of 7.21 \pm 0.03 in diabetic rats and pH of 7.36 \pm 0.02 in non-diabetic rats (n = 6; p < 0.05).

研究分野: イオン環境と生体機能

キーワード: pH 糖尿病 組織間質液

1.研究開始当初の背景

申請者は糖尿病において間質液 pH が低下し ていること、また間質液 pH 低下がインスリン抵抗性を生み出すことも見出した。さらに、 糖尿病での間質液 pH 低下が糖尿病発症初期 において観察されることも明らかにした。これらのことから、間質液 pH を測定すること は糖尿病発症早期診断に重要であることが 明らかとなった。しかしながら、間質液 pH 測定は侵襲が大きく低侵襲的測定法の開発 が待ち望まれている。

2.研究の目的

低侵襲的間質液 pH 測定法を開発することを 目的とし、ISFET (イオン感応性電解効果 型トランジシタ) pH センサー(下記図2参 照)を用いた培養液・間質液 pH 連続測定の 開発を行なう。

3.研究の方法

- A) 細胞・分子レベル実験:
 - ・ 骨格筋モデル L6 細胞を培養し、培養 液 pH は上記方法にて培養期間中連続 的に測定する。
 - 種々の pH を有した培養液中で培養し た L6 紬胞を用いて、インスリン作用 の細胞外液 pH との関連性を明らかに する。
- B) モデル動物実験:
 - 2型糖尿病モデルラットである **OLETF** (Otsuka Long-Evans Tokushima Fatty) ラットを用いて、 腹腔内間質液 pH を連続的に測定する。
 - 血糖値、血圧、血中インスリン活性を 測定することにより、間質液 pH とイ ンスリン抵抗性との関係を明らかに する。

4. 研究成果

- A) 間質液の摂取法の開発および間質液部 分へのアプローチ開発
 - ・ 2日間、ラット腹側間質部および背側 間質部へ外径 0.80 mm/内径 0.57 mm の 注射針を留置した。
 - ・注射針の腹側間質部への留置は、ラッ トの四肢による注射針抜針という事 態が起きることにより、2日間の留置 は困難であった。背側間質部への2日 間の注射針の留置は成功した。
- B) 組織間質液 pH 測定法開発結果
 - a) 組織間質液摂取による pH 測定法の開 発を実施した。
 - ・ 今回使用した外径 0.80 mm / 内径 0.57 mm の注射針で物理的根詰まり が生じたので、実際の間質液 pH 測定は不可能であった。 従って、間質液摂取による体外での
 - pH 測定は不可能であった。
 - b) 間質液部分へ pH 電極挿入法の開発 および pH 測定
 - マイクロ pH ISFET (ISFETCOM (株) 製)をラット背側間質部分に留置し た。

- ・2 日間の pH 電極留置期間中は大きな 炎症反応は観察されなかった。
- ・マイクロ pH ISFET (ISFETCOM (株) 製)は、人工電解質溶液 pH 測定に おいては問題がなかった。
- ・しかしながら、ラット間質液の pH 測定では安定した測定結果を得るこ とが不可能であった。
- ・従って、アンチモン製 pH 電極 (ケミカル機器 (株)製)を用いて、pH 測定を実施した結果、人工電解質溶 液および間質液両方において安定 した pH 測定を行なうことが可能で あった。
- ・その結果、糖尿病ラット間質液 pH 7.21 ± 0.03 、非糖尿病ラット間 質液 pH 7.36 ± 0.02 (n = 6; p < 0.05)という測定結果を得た。

5 . 主な発表論文等

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[図書](計0件)

[産業財産権]

出願状況(計0件)

取得状況(計0件)

〔その他〕

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