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The application of glucose biosensor in studying the effects of insulin and anti-hypertensive drugs towards glucose level in brain striatum

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ABSTRACT

The mechanisms involving insulin and anti-hypertensive drugs regulation for in vivo cerebral glucose metabolism are not well-understood. This might be due to lack of direct means of measuring cerebral glucose. It is known that the continuous delivery of glucose to the brain is critical for its normal metabolic function. In this study, we report the effect of insulin and anti-hypertensive drugs on glucose level in the striatum of rats. The rats were divided into two groups, i.e. hyperglycemia (14.8 ± 0.3 mM plasma glucose) and diabetic (10.8 ± 0.2 mM plasma glucose). A custom-built glucose microsensor was implanted at coordinates A/P 1.0 from bregma, M/L +2.5 and D/V −5.0 (from dura) in the striatum. The amperometric response obtained at +0.23 V vs. Ag|AgCl corresponded to the glucose level in striatum. By varying the concentrations of protaminc zinc insulin infused into the rats, striatum glucose level was found to remain constant throughout, i.e. 9.8 ± 0.1 and 4.7 ± 0.1 mM for hyperglycemic rats and for diabetic rats, respectively. However, infusion of valsartan and felodipine has lowered the striatum glucose level significantly. These findings agreed with the hypothesis that suggested striatum glucose uptake do not depend on insulin but is clearly dependant on anti-hypertensive drugs administration.

1. Introduction

Impaired glucose tolerance was previously considered a risk factor for the development of diabetes. Only recently, much attention has been given to the fact that impaired glucose tolerance is rather more of a significant risk factor for cardiovascular disease. Hypertension is prevalent in individuals with diabetes, particularly when nephropathy is present, which exacerbates all the vascular complications [1–3]. It should be appreciated that patients with diabetic nephropathy is the second most common cause of death after cardiovascular disease. Thus, the prescription of hypertensive drugs is vital to patients with diabetes and cardiovascular disease. However, little has been studied on the effects of these drugs towards the glucose level in the brain.

Glucose is the primary fuel for energy generation in the brain [4]. The administration of insulin that causes blood glucose levels to drop below 3 mM for several minutes will result in subtle brain dysfunction. Therefore, the brain has to rely upon continuous supply of glucose to maintain normal metabolic function. However, the role of insulin and hypertensive drugs in the regulation of cerebral glucose is still not understood. Previous results [5–12] have gathered enough evidence that insulin regulates the entry of glucose into the cerebral system. However, the studies have flaws in that they failed to directly register cerebral glucose concentration. In addition, there is no report on the effect of hypertensive drugs on cerebral glucose level. Both grey and striatum in the brain rely heavily on continuous glucose supply for its metabolic function. It was reported [12,13] that striatum metabolizes glucose at a different rate from the gray matter. The correlation between cerebral glucose concentration and different glucose metabolization rate as well as the role of insulin in regulating regional cerebral glucose metabolism in humans has yet to be determined. This was not feasible as there were no direct methods to measure brain glucose concentrations. Recently, a few methods have been developed for measuring cerebral glucose concentrations [14–17]. But these methods suffer either from interference signals or incapable of measuring glucose concentration directly or limited model assumptions used for data analysis and other complications.

One of the approaches to measure brain glucose concentration is by means of glucose sensor. Several enzyme-based glucose sensors [18–20] have been applied for the brain glucose measurement with some degree of success. These enzyme-based sensors are governed by the ability of the immobilized enzyme on the electroactive surface to exchange redox equivalents with the electroactive

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surface at an acceptable electron transfer rate. The simplest electron transfer pathway would be direct electron transfer (DET) between the enzyme's active site and the electroactive surface. For a glucose sensor, the DET would involve flavin adenosine dinucleotide (FAD), the active site in glucose oxidase (the immobilized enzyme) and the electrode surface. The FADs, which were deeply buried inside the protein shell resulting the electron transfer rate to be negligible [21]. Since the FAD centers were oxidized by diffused O_2, the glucose concentrations could then be determined from the detection of either O_2 consumed or H_2O_2 produced. However, this type of measurement produces errors as the O_2 concentration fluctuates in the microenvironment of the brain. Thus, more promising approaches through the employment of redox polymer to shuttle electron were reported [18,22]. This provides a very efficient electron transfer via electron hopping mechanism from the immobilized enzyme to the redox polymer bound at the electrode surface. This so-called mediated electron transfer (MET), has several advantages such as, faster electron transfer rate, independent from the interference of O_2 in the microenvironment of the brain and the regeneration of oxidized mediator from its reduce state.

Previous work from this laboratory is mainly on fabricating implantable microsensors for the subcutaneous glucose monitoring [23]. The mediated electrical communications between redox enzymes and the chemically modified microelectrode were shown to be possible. In the present paper, direct measurement of cerebral glucose concentration utilizing a chemically modified microelectrode is proposed. We also investigate the effects of exogenous insulin, AIIRB (angiotensin II receptor blockers) and CCB (calcium channel blockers) towards the glucose level in the striatum of the brain in diabetic and hyperglycemia rats.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx) from asperigillus niger (EC 1.1.3.4, Boehringer Mannheim catalogue no. 646 431, grade II lyophilised, 100,000 i.u.), Triton® X-100, i.e. polyethylene glycol *tert*-octylphenyl ether (PEG), 10% (w/v) were from Boehringer Mannheim GmbH, Germany. The 4-vinylpyridine (4VP) monomer (~96%), tetrabutylammonium perchlorate, (TBAP), lactic acid and 4-acetaminophenol were from Fluka Chemika, Switzerland. Acetonitrile (MeCN) from Romil Chemicals, England; Araldite® epoxy resin from Ciba Geigy, Switzerland; Uric acid was from Matheson, Coleman and Bell, USA. Mercury (extra pure, catalogue no. RdH 10008) was from Fluka Chemical, Swizerland. Streptozotozin (STZ), protamine zinc insulin from bovine pancreas, β-D-glucose, glutaraldehyde (25%, v/v) and mineral oil were all products of Sigma Chemicals, USA. Nafion® (5%, w/v) (equivalent mass, 1100 g) in a mixture of methanol and 10% water was obtained from Aldrich (Milwaukee, WI, USA). Bovine serum was diluted to 80% with phosphate-buffered saline (PBS), pH 7.4 prior to use.

All other reagents were of the highest grade available and were used as received. Glucose stock solutions were prepared daily in PBS solution, by dissolving 2.754 g of NaCl, 2.081 g of KH_2PO_4 and 0.477 g of NaOH in 1000 mL of pure water and then pH adjusted to 7.4 with 0.1 M NaOH. Pure water was used for preparation of all aqueous solutions, and was obtained by passing distilled water through a Milli-Q Plus from Millipore Corp., USA. The oxygen-free nitrogen (OFN) was obtained from Nissan-IOI, Malaysia. The stock solution of Nafion® (0.5%, w/v) was prepared in a low molecular weight alcohol. The electrode material, i.e., composite graphite powder from 2B pencil lead, Mars Lumograph 100, of Staedtler, Germany was cleaned under continuous reflux in methanol for 8 h. The composite graphite paste was a mixture of composite graphite powder (0.88 g), mineral oil (0.5 mL) and PEG (0.01 mL).

2.2. Microsensors fabrication

Platinum fiber (0.02 mm in diameter, 6.4 mm long, Lot #20590, of Goodfellow Cambridge Limited, Cambridge, England) was sealed into a pulled (micropipette puller PB–7, Narishige, Japan) glass capillary (1 mm × 90 mm GD–1, Narishige, Japan). The microelectrodes were polished at 45° with a micropipette grinder (EG–4, Narishige, Japan) on an extra-fine diamond abrasive plate to produce elliptical platinum disk. Magnification of microelectrode was seen under a stereomicroscope (SMZ-2T, Nikon, Japan). Electrical contact was established by filling the capillary with mercury (0.5 mL) and then with carbon paste. A copper wire was then inserted in the paste. The upper part of the capillary was finally sealed with Araldite® epoxy. The microelectrode was cured at 70 °C for two days after sitting at room temperature overnight.

The 4VP was anodically deposited onto the platinum fiber as poly 4-vinylpyridine (P4VP) at applied potential of +0.40 V vs. Ag/AgCl. The P4VP-modified microelectrode was then washed with acetone. The GOx and glutaraldehyde mixture was then immobilized, by dip coating method, on the polymer surface. The microelectrodes were dip into 2 mL of aqueous buffer containing 300 i.u. mL^{-1} GOx and glutaraldehyde for 5 times (each dip lasted around 15–30 min between dips). The microelectrodes were then allowed to dry in air for an hour. The dropwise addition of 10 μL of stock Nafion® solution was later applied onto the GOx/P4VP. The GOx/P4VP microelectrodes were then conditioned in buffer solution at 4 °C.

2.3. Electrochemistry

Most electrochemical studies and analysis were done using a Potentiostat/Galvanostat 273A, Princeton Applied Research, USA. An IBM PS/2 model 80 computer with software developed in house, was used for controlling the potentiostat and for data acquisition. Amperometry measurements were made using Autoranging Microvolt DMM (Model 197A, Keithley Ins., Cleveland, Ohio, USA). A custom-made platinum plate electrode (1 cm^2) and a Ag/AgCl electrode (Russell pH, UK) were used as counter and reference electrodes, respectively. The microsensors were operated at +0.23 V vs. Ag/AgCl. The electrodes were mounted in the outlet of the custom made cell. Unless otherwise stated, all the experiments were performed at 36 ± 3 °C in a temperature-regulated oven. Temperature was monitored using a thermocouple placed in the cell, as near as possible to the microelectrodes.

2.4. In vitro evaluation

Ten microsensors were prepared for in vitro glucose evaluation. All experiments were performed in PBS solution at pH 7.0. The in vitro sensitivity at a given glucose concentration was obtained by dividing the observed current, after subtraction from background current, with the respective glucose concentration [22]. Usual parameters such as linearity towards glucose concentrations, Michaelis–Menten value, effects of interference and life span of the microsensor were determined.

2.5. In vivo techniques

Diabetes was induced in a 285 ± 5 g adult male Sprague–Dawley rat, ten days before study, by injecting intravenously STZ (50 mg/kg body weight in 200 mL citrate buffer, pH 4.3) in the penis vein. Four

days after STZ infusion, the rats received daily injections of protamine zinc insulin at a dosage sufficient enough to maintain body weight but insufficient to prevent chronic hyperglycemia. The control (non-diabetic) rats received either saline infusion (euglycemia, $n = 5$) or a variable rate of glucose infusion (10 mg glucose each time) to raise and maintain plasma glucose at diabetic level. In order to check the blood sugar level, blood samples were taken at a regular interval in the ear vein (vena auricularis posterior).

During the night before experiment, the rats were fasted, but given access to water. At the beginning of the experiment, anaesthesia with chlorolase/urethane (ethyl carbamate, 1500 mg/kg, i.p.) was induced and the animals were tracheotomised and breathed spontaneously. A femoral vein was cannulated for the continuous intravenous infusion of saline (150 mM NaCl) initiated at 3 mL h^{-1} and the anaesthetic (urethane/chlorolase, 180 mg/kg and 12 mg/kg, respectively, i.v.) at 3 mL h^{-1}. In addition, a femoral artery was also cannulated for monitoring blood pressure and heart rate. The rat was then positioned onto a stereotaxic frame. The implantation site was cleanly shaved before any surgeries for microelectrode implantation. The skull was then surgically exposed and small holes were drilled for microsensor implantation. To measure striatum glucose, the microsensor was implanted at the striatum at coordinates A/P 1.0 from bregma, M/L +2.5 and D/V −5.0 (from dura). The reference electrode was placed in the cortex. All dimensions were in millimeters according to the atlas of Paxinos and Watson [24]. The microsensors were then connected to Autoranging Microvolt DMM Model 197A (Keithley Ins., Cleveland, Ohio, USA). Insulin (1 mg/kg), valsartan (1 mg/kg) and felodipine (1 mg/kg) were injected (i.p.) at 5 mL h^{-1}. All experiments involving rats have been conducted according to the ethical guidelines approved [25].

3. Results and discussion

A redox mediator underwent a one step recycling process at the electrode. In this process, the mediator (MED) underwent a redox cycle whereby the produced MED_{red} can be electrooxidized when $E_{appl} > E^{\circ\prime}_{MEDox/MEDred}$. Therefore, the redox cycle of MED_{red}/MED_{ox} formed the basic principle of MET between the electrode and the enzyme. The e^- generated from redox cycle of MED_{red}/MED_{ox} was utilized in the analytical detection of glucose. The efficiency of MET in the analytical detection of glucose in the brain is assured as the measured current does not correlate to the concentration of O_2 or H_2O_2 produced. The amount of H_2O_2 normally found in the microenvironment of the brain is ≈100 μM [26]. Thus, elimination of the interference from H_2O_2 is vital to ensure that the amperometric signals produced were of GOx catalysis alone. During the immobilization, the GOx molecules were not properly orientated to the electrode surface. This resulted in that not all the GOx molecules underwent DET; thus the MET pathway, facilitated by MED will be increasingly more dominant.

In this study, 4VP was utilized as the electroactive material at the platinum fiber surface. Efficient electron transfer was exhibited in the cyclic voltammogram produced when the microsensor was scanned in a 10 mM glucose solution in PBS at pH 7.0. The difference in reduction and oxidation peaks, $\Delta E \approx 100$ mV, followed the relationship similar to those reported [27–35] for molecular diffusion.

To confirm that an enzymatically coupled reaction is occurring, it is useful to plot the current function vs. the potential scan rate. This allows the effect of scan rate of the diffusional process to be separated from its effects on the kinetics. The current function, however, is given by

$$I_p = 0.4463nFA\left(\frac{D_0 nFv}{RT}\right)^{1/2} C_0 \quad (1)$$

The scan rate was fixed at 200 mV s^{-1} and the sweep took about 5 s to achieve steady state. Cyclic voltammetry was also performed on insulin, valsartan and felodipine, separately, with the potential window applied was from −0.5 V to +1.00 V vs. Ag/AgCl. No oxidation/reduction peaks were observed other than those of the redox polymers. However, when 10 mM of glucose was spiked into the sample, reduction and oxidation peaks typical of glucose were observed. This showed that insulin, valsartan and felodipine did not interfere in the determination of glucose.

3.1. Response of the microelectrode towards glucose

Fig. 1A and B indicated that a good linearity was obtained towards the glucose concentration range studied. Fig. 1A was constructed using Eq. (1). At glucose concentration (>30 mM), the amperometric response had decreased by as much as 35% when repeated samples ($n = 10$) of glucose were spiked into the solution. At glucose concentration of 30 mM, a slow electrode process was observed, indicating that the enzyme was denatured. The $\Delta E_p = 240$ mV obtained was an indicator of irreversible redox process.

The kinetic parameters, K_m and i_{max}, was calculated from the Michaelis–Menten equation

$$I = i_{max} - K_m\left(\frac{I}{C}\right) \quad (2)$$

where I is the steady state catalytic current, i_{max} the maximum current under saturating substrate conditions, K_m the apparent Michaelis–Menten constant and C is the concentration of glucose in solution.

The apparent K_m value, 5.8 ± 0.5 μM ($n = 5$), obtained from the slope of the Eadie–Hofstee plot in which, the electrode response was linear. The kinetic behavior of the microelectrodes was evaluated from the amperometric response at $E_{app} = +0.23$ V vs. Ag/AgCl. The i_{max} was 0.88 ± 0.03 μA and the effective current density, j_c, was in the range of 80–1000 μA cm^{-2}. The i_{max} and j_c were calculated based on the area of the hole at the tip of the recess. Replotting the calibration curves as Eadie–Hofstee was preferred as this plots offers wider substrate range.

The response of the microsensors towards glucose in the presence of interference was also investigated. The interferents studied were those normally found in biological fluid in the microenvironment of the brain. Additional volume of 10 mL of 10 mM of glucose when injected into a solution containing 5 mM of uric acid, 4-acetaminophenol, ascorbic acid, 5 mM of lactic acid and 100 μM of H_2O_2 produced a current of 85 ± 2 nA. That indicates that the microsensors were not affected by any of the interferences. The outer layer of Nafion® has effectively blocked all the interferences from reaching the electroactive centre [28,36].

The amperometric response of the microsensor, kept in a PBS buffer solution at 4 °C, towards glucose remained fairly constant for 15 days. After that, a decrease of 9% in current response was observed which could be the result of enzyme denaturation. Investigation on its reproducibility performed in 10 mM glucose indicated that the relative error of the response was less than 1% with measurements being carried out ($n = 10$) every 2 days after the day of fabrication.

3.2. Glucose detection in vivo

Under normal circumstances, a mixture of α- and β-D-glucopyranose (D-glucose) is the only fuel for brain energy metabolism. On the other hand, the breakdown of D-glucose is regulated by complex mechanisms that influenced by the activities of phosphofrucktokinase and hexokinase. The metabolism depends

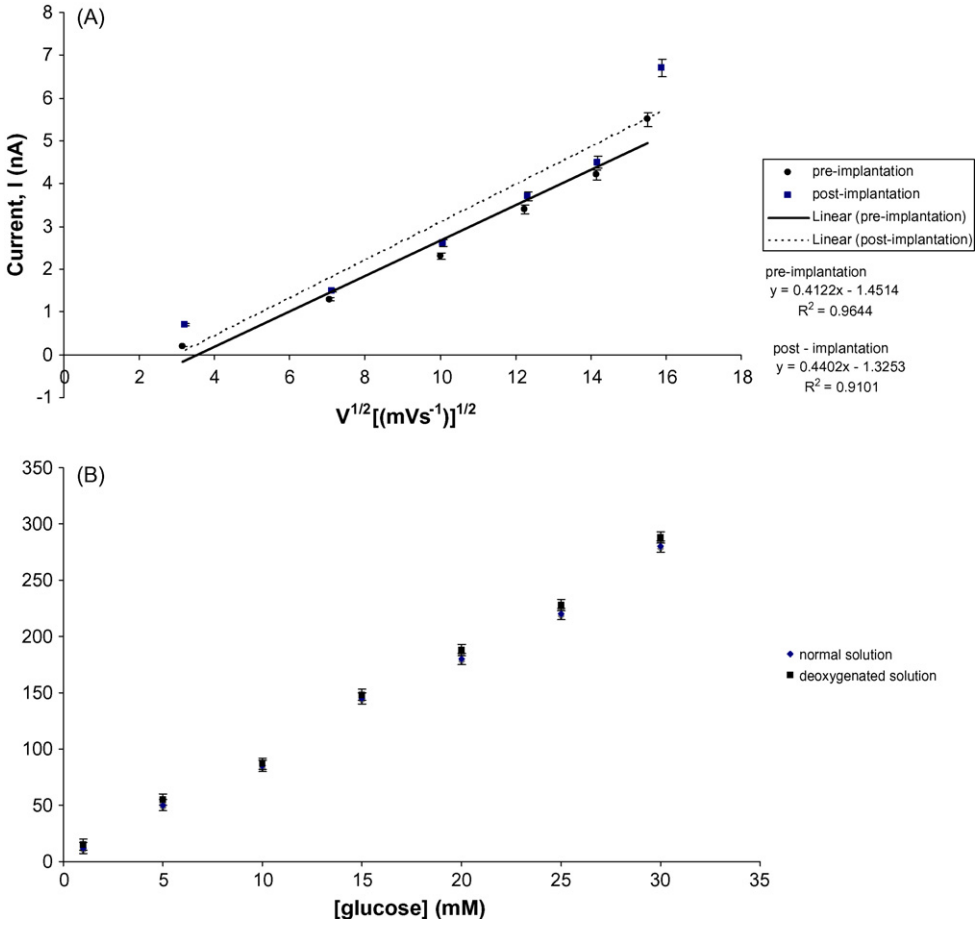

Fig. 1. (A) (♦) Plot of I vs. $v^{1/2}$ for pre-implantation glucose microsensors. (■) Plot of I vs. $v^{1/2}$ for post-implantation glucose microsensors. (B) Variation of current response as a function of glucose concentration (entire) for pre-implantation glucose microsensors ($n = 5$) in PBS solution at pH 7.0. The applied potential was +0.23 V vs. Ag/AgCl. The correlation coefficient value obtained with O_2 and without O_2 solution was 0.9793 and 0.9765, respectively.

indirectly on the glucose concentration of the brain intracellular and interstitial fluids. Since the tissue glucose concentration, in turn, depends directly on the phosphorylation rate, the blood brain transfer assumes a pivotal role in the supply of glucose to the brain. The concept of facilitated diffusion evolved in parallel with the study of the transport of electrolytes across cellular membranes. The nature of the specific glucose-transporting properties of all membranes remained a mystery until the discovery of insulin heralded the study of facilitated diffusion. Although it was apparent that human erythrocytes must posses certain properties for blood glucose transfer to the brain, the relation to insulin, if any, remained obscure. Recently, with high fatality rate of diabetic patients due to cardiovascular disease, hypertensive drugs and insulin has been prescribed to diabetic patients with aims to lower cardiovascular risk and blood sugar level.

In this study, the glucose microsensor was implanted in the striatum of the rat's brain for both animal groups. The region was known to contain high concentration of glucose [4,37]. Peak currents of 84 ± 4 nA and 128 ± 5 nA (Fig. 2) were obtained for diabetic and hyperglycemia rats, respectively. The amperometric responses remain constant for more than 2 h of measurement. This was oxidation of glucose by O_2 in blood vessel [13]. Dissolved O_2 concentration formed a gradient, which was highest at the capillary wall and lowest at the site of metabolic consumption. However, at the implantation sites, we observed no significant differences in glucose level indicating that the glucose level in the area of striatum was constant throughout. Additionally, the current generated from the catalytic reaction was dependent on the orientation of the implanted microelectrode with respect to the bloodstreams and metabolically active sites. Peak current, I_p, of 298 ± 3 nA for a diabetic rat was detected at +0.23 V vs. Ag/AgCl at coordinate A/P +1.0 from bregma, M/L +2.5 and D/V −5.0 (from dura), when death of the animals occurred by inducing an overdose of anaesthetics. This indicated the high concentration of glucose in the brain and was in agreement to the above explanation that there is no oxidation of glucose at zero concentration of O_2 when blood flow stopped [38].

The level of glucose in the striatum after the administration of AIIRB or CCB and insulin was investigated by implanting the microsensor at coordinates, skull leveled between bregma and lambda; A/P 1.0 (from bregma), M/L +2.5 and D/V −5.0 (from dura) for both diabetic and hyperglycemia rats. In both cases the reference electrode was placed in the cortex. Valsartan, Felodipine and protaminc zinc insulin was infused at the tenth day from the intravenous injection of STZ. Fig. 3A and B shows the glucose level in striatum and blood pressure in diabetic and hyperglycemia rats after the infusion of protaminc zinc insulin followed by valsartan. For both groups of rats, administration of insulin resulted in a significant drop in arterial blood glucose while glucose level in striatum remained constant. However, the administration of valsartan reduced ($P < 0.05$) the striatum glucose level and blood pressure for both groups of rats. The striatum glucose level and blood pressure in both groups of rats returned to initial readings as the effect of valsartan faded.

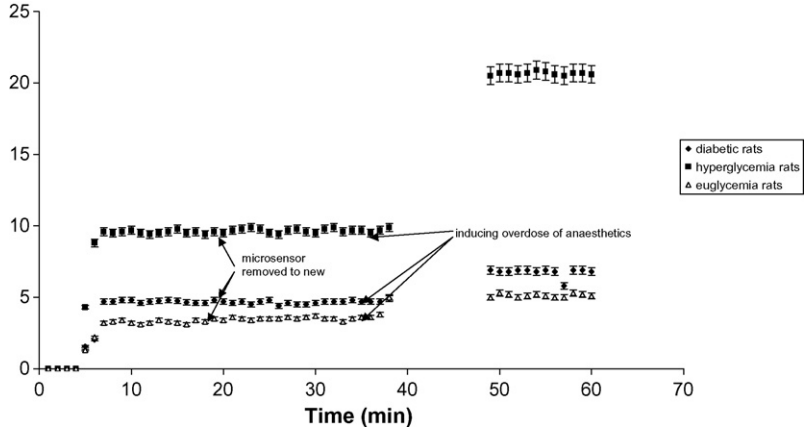

Fig. 2. Glucose measurements in the right striatum of the rat's brain for (♦) diabetic rats, (■) euglycemic rats and (Δ) hyperglycemic rats. The plot was constructed from amperometric measurements made at 25 min intervals. The potential applied was +0.23 V vs. Ag/AgCl.

Fig. 4A and B meanwhile shows the level of glucose in striatum and blood pressure after the infusion of protaminc zinc insulin followed by felodipine. The glucose level in striatum and arterial blood glucose followed a similar pattern as observed in Fig. 3. Administration of reduced ($P < 0.05$) the arterial blood glucose in both groups of rats studied. However, the glucose level in striatum for diabetes and hyperglycemia rats remained constant. Administration of felodipine resulted in a slight drop ($P < 0.05$) of glucose level in striatum. A sharp ($P < 0.05$) drop in blood pressure for diabetes rats and hyperglycemia rats were observed after the administration of felodipine.

With a sample size of ten for each rat's group and confidence limit of 95%, such a value represents 6.0% mean brain glucose level measured during the experiment and is unlikely of any major physiology significance. Although a small effect of insulin on glucose transport across blood–brain barrier cannot be completely eliminated with our findings, we feel confident that insulin does not play a clinically relevant role in regulating the brain-glucose uptake. The role of insulin in the regulation of brain-glucose metabolism has long been an area of controversy. Most investigators have concluded that insulin was without effect on glucose transport across the blood–brain barrier [7,39]. However, both insulin receptors [40] and

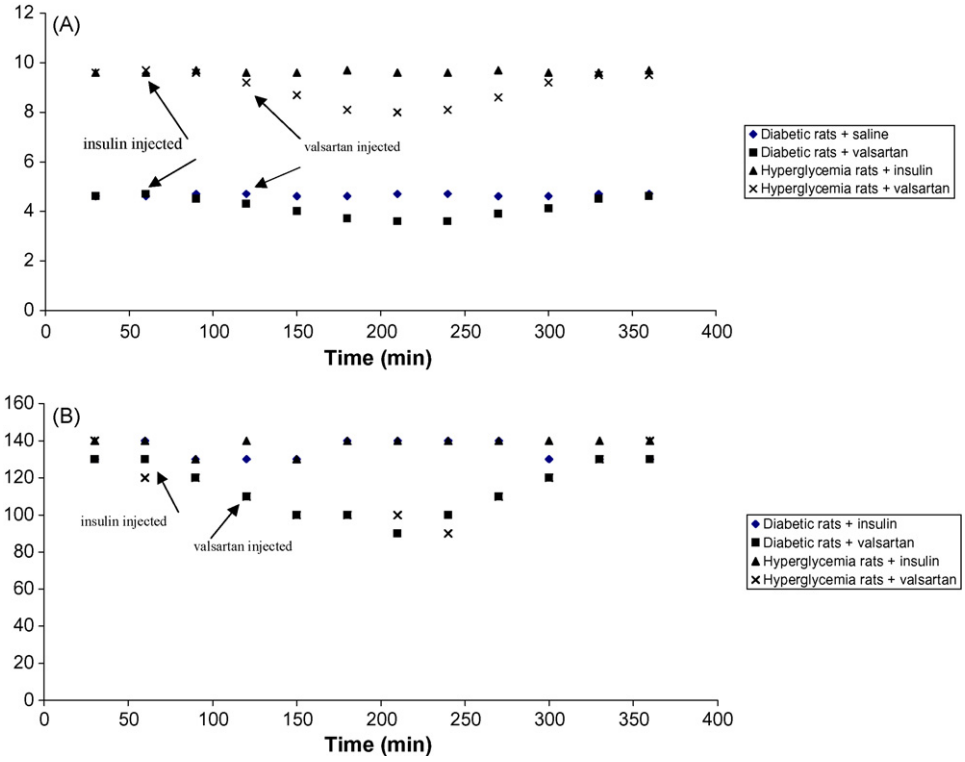

Fig. 3. (A) Striatum glucose level after the infusion of protaminc zinc insulin [(♦) for diabetic and (▲) for hyperglycemia rats] and valsartan [(■) for diabetic and (×) for hyperglycemia rats] as a function of time (minutes). Striatum glucose level was measured at coordinates A/P 1.0 from bregma, M/L +2.5 and D/V −5.0 (from dura) and the reference electrode was placed in the cortex. (B) Blood pressure level after the infusion of protaminc zinc insulin [(♦) for diabetic and (▲) for hyperglycemia rats] and valsartan [(■) for diabetic and (×) for hyperglycemia rats] as a function of time (minutes).

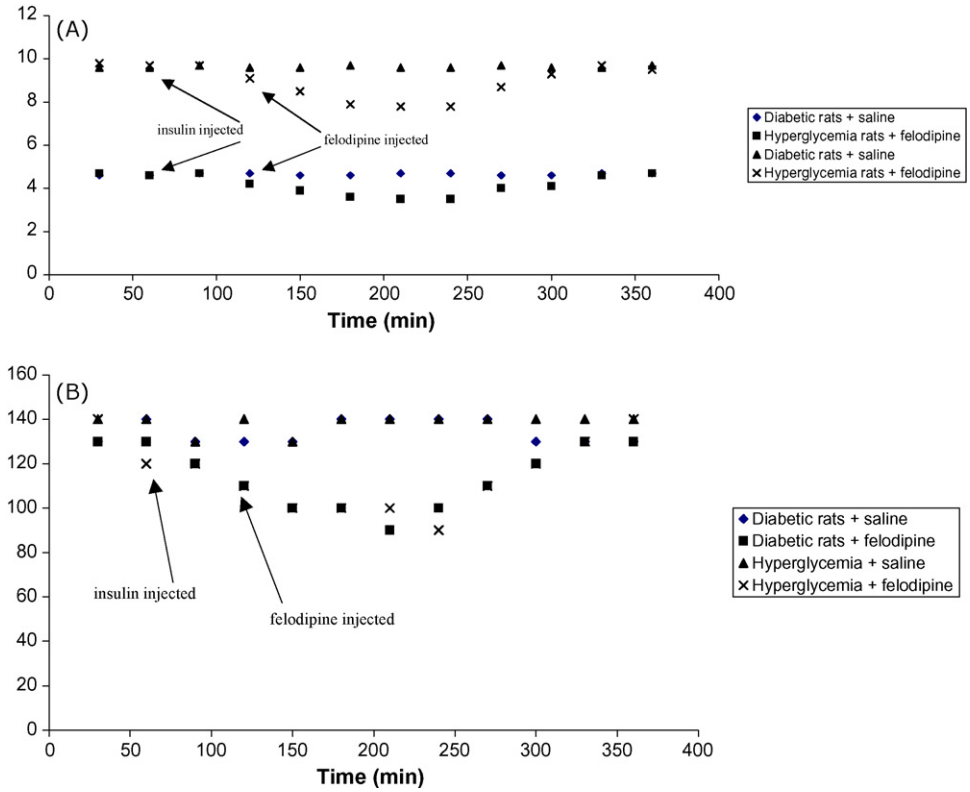

Fig. 4. (A) Striatum glucose level after the infusion of protaminc zinc insulin [(♦) for diabetic and (▲) for hyperglycemia rats] and felodipine [(■) for diabetic and (×) for hyperglycemia rats] as a function of time (minutes). Striatum glucose level was measured at coordinates A/P 1.0 from bregma, M/L +2.5 and D/V −5.0 (from dura) and the reference electrode was placed in the cortex. (B) Blood pressure level after the infusion of protaminc zinc insulin [(♦) for diabetic and (▲) for hyperglycemia rats] and felodipine [(■) for diabetic and (×) for hyperglycemia rats] as a function of time (minutes).

insulin sensitive glucose transporter (GLUT 4) [41], has been found at the blood–brain barrier, showing that insulin was an important modulator of the autonomic response to hypoglycemia. Insulin crosses the blood–brain barrier via receptor-mediated transcytosis. However, the function of insulin upon surpassing the barrier was yet to be fully understood. CCB, meanwhile, blocked the voltage sensitive calcium channels in the heart and blood vessels. This has prevented calcium level in the cell as much when stimulated, leading to less contraction. This lowered the total peripheral resistance by dilating the blood vessels and decreases the cardiac output by lowering the force of contraction. Therefore, the decreased in blood pressure has enabled higher level of oxygen being supplied to the brain. AIIRB selectively bind the angiotensin II type 1 receptor (AT1) preventing the effects of angiotensin II. This created an insurmountable blockade of angiotensin II at the AT1 receptors forming a more complete blockade of the renin–angiotensin system without affecting bradykinin.

Recalibration of the implanted microsensor, after 10 h, revealed that its amperometric response has a good correlation ($R^2 = 0.9765$) with the pre-implantation ($R^2 = 0.9957$) (Fig. 1A). This strongly implicates that membrane biofouling and fibrous encapsulation is either minimal or possibly has no effect at all towards the "stained" microsensor even after 10 h of implantation.

4. Conclusion

This study has demonstrated that the glucose microsensor used is capable of measuring directly the level of glucose in the striatum before and after the administration of insulin and anti-hypertensive drugs. There is no significant effect by insulin towards glucose concentration in the striatum for diabetic and hyperglycemia rats. However, the administration of anti-hypertensive drugs, i.e. valsartan and felodipine, has lowered ($P < 0.05$ for both valsartan and felodipine) the glucose level in the striatum. The anti-hypertensive drugs have also lowered ($P < 0.05$ for both valsartan and felodipine) the blood pressure by blocking the voltage sensitive calcium channels or rennin–angiotensin system. The increased in oxygen supply to the brain has, indirectly, increased the oxidation of glucose in the brain. The passivation layer formed immediately upon implantation has minimal or possibly no effect at all on the ability of the micro sensor for longterm implantation. The ability of the micro sensor is, however, limited upon implantation in a blocked blood vessel. This study will be discussed in future paper.

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