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The Double-edged Effect of Insulin on the Neuronal Cell Death Associated with Hypoglycemia on the Hippocampal Slice Culture

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It is well known that the central nervous system (CNS) is vulnerable to hypoglycemia and hyperglycemia. Insulin is indispensable for serum glucose control and diabetes patients are on the relative or absolute deficient state of insulin. The role of insulin on the CNS, however, has not been fully elucidated, yet. To reveal the role of insulin on the neuronal survival, we have used in vitro system of an organotypic hippocampal slice culture from rat, and examine the neuronal cell death at the various glucose concentrations in the presence or absence of insulin. When glucose concentrations is varied to 0, 1, 3, 5 and 30mM in the incubation medium, the neuronal cell death was minimum at 5mM, and no neuronal survival was observed under 1mM on the CA1. On the dentate gyrus granule cells (DG), on the other hand, the significant neuronal survival was observed even as low as 1mM. In the presence of 1nM concentration of insulin, the neuronal cell death curve showed the U-shape, and the minimum death point was 3-5mM glucose concentrations at the CA1. At the DG, insulin did not show the protective effect up to 48 hours culture regardless of glucose concentration. In the absence of glucose, insulin accelerated the neuronal cell death both in the CA1 and DG. We concluded that insulin has a double-edged effect on the neuronal cell death dependent on glucose concentration, and that the CA1 and the DG have a different sensitivity to insulin in terms of cell survival.

It has been well known that the central nervous system is vulnerable to hypoglycemia. Insulin regulates a blood glucose level and its deficiency causes diabetes. An action of insulin on the central nervous system has not been enough elucidated, yet. Recent reports have suggested that the type 2 diabetes is one of the risk factors for the decay of cognitive function and the blockade of insulin signal cascade may be involved for its pathology (17, 21, 22). And after ischemic events, insulin acts directly on the brain to reduce ischemic brain necrosis independent of hypoglycemia (26). Hypoglycemia causes lethal consequences during insulin treatment. Clinically, it is mandatory to avoid nocturnal hypoglycemia especially in case of treating elderly and the type1 diabetic patients, and it is known that 2 to 4% of the type1 diabetic patients die by an excess of insulin administration (15). The harm effects of hypoglycemia, therefore, are well known and the protection method is mainly to keep blood glucose in adequate levels. The insulin action per se during hypoglycemia against the CNS, however, is merely understood.

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In this paper, we examined the direct action of insulin on the neuronal cell death at a variety of glucose concentrations by using cultured hippocampal slices. Our experimental results suggested that the CNS damage during hypoglycemia would exaggerate by insulin therapy itself and the caution may be necessary not only the glucose levels but also the insulin therapy itself during hypoglycemia.

MATERIALS AND METHODS

The experiments were conducted according to the guidelines for animal experimentation at the Kobe University School of Medicine, and conform to the relevant National Institution of Health guidelines.

Preparation of organotypic hippocampal slices

Hippocampal slices were made from the septal half of the hippocampus using a standard method (20). Briefly, 9- to 11-days Wistar rats (Hartley, SLC, Japan) were anesthetized with 98% diethyl I ether and decapitated. The hippocampus were rapidly dissected at 4-6 °C and cut into 450μm slices using a McIllwain Tissue Chopper (Mickle Laboratory Engineering Co., Ltd, UK). Slices were then transferred onto membranes (pore diameter: 30μm, Millicell-CM, Millipore, Bedford, MA, USA), and placed into a six-well microplate (Costar Corning Inc, NY, USA) with 1mL of slice culture medium per well. The culture medium contained 50% Eagle's minimal essential medium (MEM) (Gibco, CA, USA), 25% Hanks' Balanced Salt Solution (HBSS)(Gibco, CA, USA), 25% heat-inactivated horse serum (Gibco, CA, USA) containing 1% penicillin/streptomycin. The medium was changed every 3 days. Slices were kept in culture for 14 days before study and the six-well microplates were stored at 37 °C in a 5% CO2 incubator under a 95% humidity atmosphere (Sanyo, Tokyo, Japan).

Treatment of hippocampal slices

Slices in the six-well microplates at day 14 were washed, and the basic medium was replaced with various agents for the treatment. The basic medium contained 90mM NaCl, 4mM KCl, 0.1mM MgCl2, 0.1mM KH2PO4, 0.5mM MgSO4, 0.1mM Na2HPO4, 0.5mM NaH2PO4, 14mM NaHCO3, 1.2mM CaCl2, 10mM glucose, about 2mM essential and non-essential amino acids, and 0.02mM vitamins. In order to investigate the changes in neuronal toxicity due to the glucose concentration, various glucose concentrations (0mM, 1mM, 3mM, 5mM, and 30mM) were added to the medium that was used to treat the slices. Moreover, the change in the neuronal death rate was investigated both with and without insulin loading at a concentration of 1nM. (insulin: Humulin® R, Eli Lilly, Indiana, USA)

Assessment of cell death in hippocampal slices

The propidium iodide (PI) method was used in the assessment of neuronal death in hippocampal slices at 24h, 48h, and 72h after each treatment in the CA1 and the dentate gyrus granule cells (DG) of the hippocampus. To label the nuclei of dead neurons, 4.6μg/mL of PI (Sigma, St. Louis, Mo, USA) was added to the wells of the culture microplates for 15min. PI is a polar compound that only enters cells with damaged cell membranes, where it binds to nucleic acids within the cells and develops a bright red fluorescence. The dye is basically non-toxic to neurons, and is used as an indicator of neuronal integrity and cell viability (11). Thus the intensity of fluorescence correlates with the cell death fraction. After 15 min, digital images of the PI fluorescence were obtained with an inverted fluorescence microscope (4×objective) equipped with a digital camera (Olympus IX70, Tokyo, Japan). After the final image, all the neurons were killed by adding 10 μ M N-methyl-D-aspartic acid (NMDA) and the final PI fluorescence intensity was

calibrated as 100% cell death. The mean intensity (green values) of the PI fluorescence were measured using an image program MacScope (Ver 2.6.1, Mitani Inc, Osaka, Japan).

Statistical analysis

Values were expressed as mean ± standard error of the mean (SEM) from three independent experiments. The statistical significance was established by ANOVA followed by a post-hoc test, and then the non-paired t-test was employed using StatView software (v.5.0.1.0; SAS Institute Inc., Cary, NC, USA). $p<0.05$ was considered to be statistically significant.

RESULTS

CA1 neuronal cell death in the presence or absence of serum

Serum is widely used for maintenance of cultured neuronal cell viability. To know the extent of nerve protection effects of serum in our experimental settings, the neuronal cell death was evaluated in the presence or absence of the heat inactivated horse serum (Gibco, CA, USA) in the culture medium. The glucose concentration in the medium was kept at 30mM, the concentration that is usually commercially available. After 72 hour culture, the neuronal cell survival was better in the presence of serum (n=30) in comparison with the absence of serum (n=11) and the cell death rate was 22.7 ± 6.3% and 40.8 ± 6.2%, respectively (non-paired t-test; $p<0.05$) (Fig.1) .

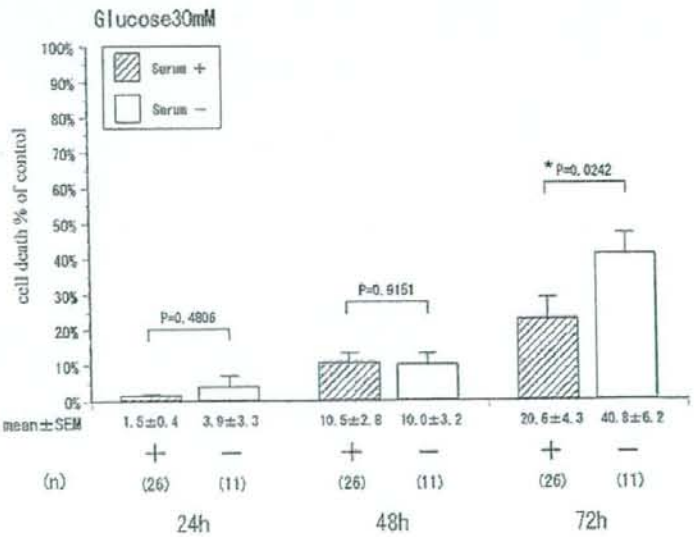

Fig. 1. CA1 neuronal cell death in the presence or absence of serum.
The glucose concentration in the medium was kept at 30mM, the concentration that is usually commercially available. After 72-hour culture, the neuronal cell survival was better in the presence of serum (n=30) in comparison with the absence of serum (n=11) and the cell death rate was 22.7±6.3% and 40.8±6.2%, respectively (*: non-paired t-test; $p<0.05$.)

The CA1 neuronal cell death during low glucose

The difference in cell death rates was examined in various glucose concentrations (0mM, 1mM, 3mM, 5mM, and 30mM) under the environment lacking serum on the CA1 pyramidal cell. Glucose 0mM (n=9) and 1mM (n=14) resulted prominently high cell death rates after 48 hour culture and the cell death rates were 57.0±6.5% and 53.7±7.4%, respectively. After 72 hour, the rate further increased and the each rate was 83.6± 4.9% and 92.9±1.2%, respectively. The cell death rates showed the U-shaped curve against the glucose concentrations and neuronal cell death was minimum at 5mM glucose (n=9) (Fig. 2)(One-way ANOVA; $p<0.0001$, Scheffe's F test; $p<0.01$).

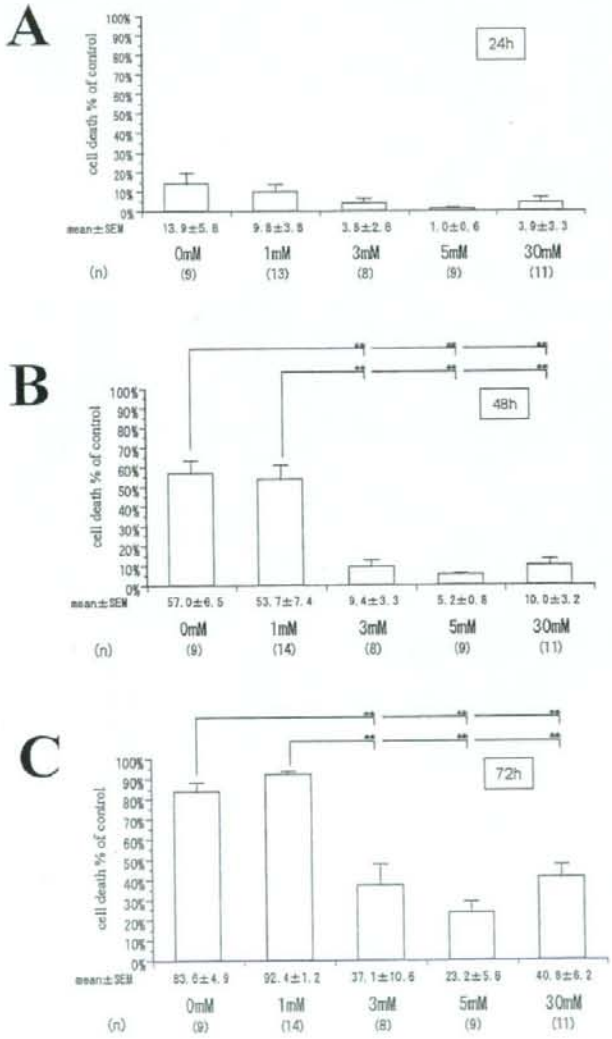

Fig. 2. The CA1 neuronal cell death during low glucose.

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The DG granule cell death during low glucose

The difference in cell death rates was examined in various glucose concentrateions (0mM, 1mM, 3mM, 5mM, and 30mM) under

the environment lacking serum on the DG granule cell. The cell death rates increased maximally at 0mM glucose and the cell death rate after 48 hours was 59.2±5.3%. The DG granule cell was kept relatively well alive even in 1mM glucose condition and the death rate after 48 hours was only 14.5 ± 5.0%. The cell death rates did not show the U-shaped curve against the glucose concentrations and neuronal cell death was most inhibited at 3mM glucose (one-way ANOVA; $p<0.0001$, Scheffe's F test; $p<0.01$) (Fig. 3).

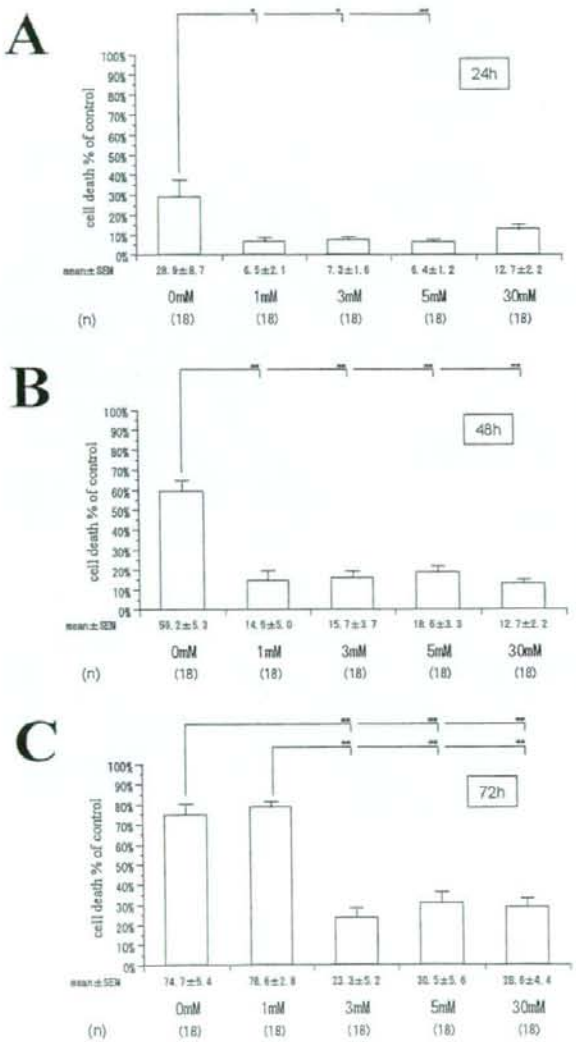

Fig. 3. The DG granule cell death during low glucose.

Various glucose concentrations (0mM, 1mM, 3mM, 5mM, and 30mM) under the environment lacking serum on the DG granule cell. The cell death rates increased maximally at 0mM glucose and the cell death rate after 48 hours was 59.2±5.3%. The DG granule cell was kept relatively well alive even in 1mM glucose condition and the death rate after 48 hours was only 14.5 ± 5.0%. The cell death rates did not show the U-shaped curve against the glucose concentrati-onns and neuronal cell death was most inhibited at 3mM glucose (one-way ANOVA; $p<0.0001$, Scheffe's F test; $p<0.01$) (Fig. 3).

CA1 neuronal cell death in the presence or absence of insulin

The difference in the cell death rates on the CA1 pyramidal neuron was examined in the presence or absence of 1nM insulin during treatment with a variety of glucose concentrations (0mM, 3mM, and 30mM). In the presence or absence of insulin at 3mM glucose, the observed cell death rates after 48 hours were 10.3±1.2% and 38.1±9.1%, respectively, and after 72 hours were 22.4±3.8% and 54.5±8.2%, respectively ($p<0.05$) (Fig.4B). Thus, the presence of insulin significantly improved the cell survival at 3mM glucose concentrations up to 72hours. In the case of 0mM glucose, the insulin addition surprisingly deteriorated the cell survival and the cell death rates of the presence or absence of insulin were 67.0 ± 10.5% and 37.3 ± 8.9%, respectively ($p<0.05$) (Fig. 4A). At 30mM glucose condition, the insulin addition did not give any significant effects on the cell survival ($p=0.789$) (Fig. 4C).

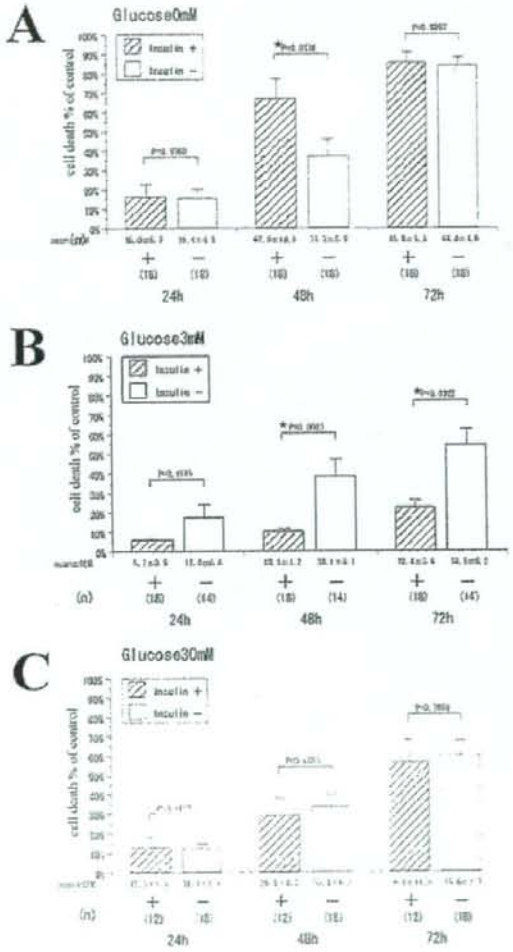

Fig. 4. CA1 neuronal cell death in the presence or absence of insulin.

The presence or absence of 1nM insulin during treatment with a variety of glucose concentrations (0mM, 3mM, and 30mM). In the presence or absence of insulin at 3mM glucose, the observed cell death rates after 48 hours were 10.3±1.2% and 38.1±9.1%, respectively, and after 72 hours were 22.4±3.8% and 54.5±8.2%, respectively (*: non-paired t-test; $p<0.05$) (Fig.4B). Thus, the presence of insulin significantly improved the cell survival at 3mM glucose concentrations up to 72hours. In the case of 0mM glucose, the insulin addition surprisingly deteriorated the cell survival and the cell death rates of the presence or absence of insulin were 67.0 ± 10.5% and 37.3 ± 8.9%, respectively (*: non-paired t-test; $p<0.05$) (Fig. 4A). At 30mM glucose condition, the insulin addition did not give any significant effects on the cell survival (non-paired t-test; $p=0.789$) (Fig. 4C).

The DG granule cell death in the presence or absence of insulin

The difference in cell death rates on the DG granule cell was examined in the presence or absence of 1nM insulin. The presence and absence of insulin at 3mM glucose showed no significant difference after 48 hours (22.1± 3.2 % and 25.5±3.6%, respectively) (p=0.4963). After 72 hours, the insulin showed minor protective effect against the cell death (31.8 ± 4.2% and 46.6 ± 4.1%, respectively) (p=0.0161). At 0mM glucose, the insulin addition deteriorated the cell survival and the cell death rates after 48 hours in the presence or absence of insulin were 47.2 ± 9.2% and 23.9 ± 3.7%, respectively (p<0.05) (Fig. 5A) . At 30mM glucose condition, no significant difference was observed between the insulin and non-insulin groups (p=0.2074) (Fig. 5C).

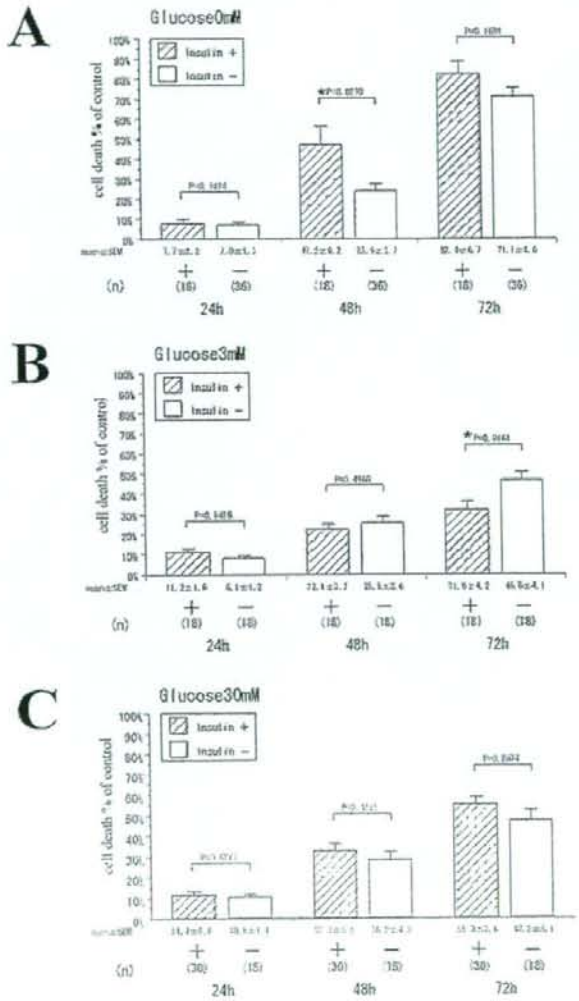

Fig. 5. The DG granule cell death in the presence or absence of insulin.

The presence or absence of 1nM insulin. The presence and absence of insulin at 3mM glucose showed no significant difference after 48 hours (22.1± 3.2 % and 25.5±3.6%, respectively) (non-paired t-test; p=0.4963). After 72 hours, the insulin showed minor protective effect against the cell death (31.8 ± 4.2% and 46.6 ± 4.1%, respectively) (*: non-paired t-test; p=0.0161). At 0mM glucose, the insulin addition deteriorated the cell survival and the cell death rates after 48 hours in the presence or absence of insulin were 47.2 ± 9.2% and 23.9 ± 3.7%, respectively (*: non-paired t-test; p<0.05) (Fig. 5A) . At 30mM glucose condition, no significant differrence was observed between the insulin and non-insulin groups (non-paired t-test; p=0.2074) (Fig. 5C).

DISCUSSION

In the present experiment, we examined the insulin effects on the neuronal cell death during low glucose condition, and found that the insulin protected neuronal cell with low glucose, but increased neuronal cell death in case of glucose free condition (Fig. 4A, Fig. 5A). Moreover, while the DG had more tolerant against low glucose, the neuroprotective effect of insulin during low glucose had more prominent on the CA1 than the DG.

Neurotrophic effects of serum

When serum is contained, a low neuronal cell death rate was observed in comparison with addition of insulin only (1nM), suggesting that a variety of factors contained in serum work for neuronal cell protection in addition to insulin (Fig. 1). Neurotrophic factors such as NGF (nerve growth factor) or BDNF (brain derived neurotrophic factor), and vitamin B family in serum are supposed to restrain apoptosis and promote neuronal survival (18, 27). Our results were coincident to these previous results.

The CA1 vulnerability to hypoglycemia

Selective vulnerability has been well known to date and particularly the CA1 is one of the most vulnerable sites in the CNS against a stress. It is commonly believed that glutamate excitotoxicity relates to selective vulnerability. Glutamate level in the hippocampus of mouse after ischemic stress was greater in the CA1 than that of the DG (6, 14, 25). Also it has been known that the extracellular glutamate level rises in a glucose-free condition (24). As for the functional selectivity, the field potential was reported to be well maintained with low glucose concentration on the DG compared with the CA1. Li et al explained these selectivity as the differential activity of phosphofructokinase (PFK), the key enzyme for glycolysis. And the DG, indeed, has a high PFK activity than that of the CA1 (10). We found that the DG showed more tolerance to hypoglycemia than the CA1 at 1mM glucose concentration (Fig. 2, Fig.3), indicating the lower dependency of the DG granule neurons on glucose for their survival. Albeit it is a well-known phenomenon, the precise mechanism of the difference of glucose sensitivity between the CA1 and the DG neurons will need more exploration.

The protective effect of insulin during low glucose

The culture medium was adjusted to prepare insulin at 1nM concentration. This concentration corresponds to a blood insulin level following a hypodermic injection of about 27 units of insulin as a conversion to a 50 kg human body (8). This amount is nearly equal to that used in a clinical treatment. There is a report on the experiment in that 4nM insulin successfully worked for the suppression of cell apoptosis in the CNS (23). Our results showed even the smaller dose of insulin could affect the neuronal cell death. In CA1, 3mM glucose with insulin treatment inhibited prominently the neuronal cell death (Fig. 4B). A question arose whether lower than 3mM glucose concentration with insulin might alter the cell death rate. We conducted the experiment at the condition of 1.5mM and 2mM glucose, and obtained an advantageous result for survival of the neuronal cell (data not show). In case of the DG, the insulin treatment did not inhibit the neuronal cell death (Fig. 5B, Fig. 5C). Insulin takes glucose actively in the cell through the GLUT4 translocation to convert ca. 50% of glucose to energy. Furthermore, insulin activates MAP kinase (mitogen-activated protein kinase) working as a cell propagation factor to support the neuronal cell (12). In addition, insulin induces the expression of BDNF (29). A cooperation of these factors takes probably an important role for survival of neurons. In the present study, the prominent inhibition of the neuronal cell death was found only in the CA1. The levels of mRNA of GLUT4 were found to express in higher degree in the CA1 than the DG (2). Therefore, the CA1 neurons will be affected more influence by insulin than the DG neurons, at least on

glucose transport. Moreover, it has been reported that the depression of the insulin signal in the CNS increases GSK activity (Glycogen Synthase Kinase-3), that may lead to induce the neuronal cell death (1). GSK-3 expresses more in the CA1 pyramidal neurons and these preference in the CA1 may a least partially explain the insulin-sensitive selective vulnerability of the CA1 (4).

The acceleration of cell death by insulin during glucose deprivation

It has long been alleged that a possible secondary action of insulin includes affecting an amino acid metabolism and a lipid metabolism to enhance protein synthesis and lipid synthesis resulting in inhibiting the use of a substrate other than glucose by the cell. Thus, the environment lacking an enough amount of glucose may allow insulin to work negatively for cell survival (7). AMPK (AMP-activated protein kinase) that enhances the glucose transportation in a hypoxia tissue, is reduced by insulin treatment in an ischemic heart muscle (3, 5, 9, 19, 28). It is possible that insulin may block the induction of AMPK during glucose deprivation, and thus result in increase of cell death. Interestingly, an in vivo experiment reported that the neuronal protection effect of insulin showed the U-shaped curve, having a maximum peak in 6 to 7mM of the glucose level, and insulin rather accelerated the neuronal cell death at 2 to 3mM or the lower concentration of glucose (30).

CONCLUSION

Insulin therapy is now a common strategy for diabetic treatment, and caution for its therapy has been paid mainly on the treatment related hypoglycemia. Our study indicated that in central nervous system, insulin indeed has double-edged effects, and while neuronal survival is promoted in the presence of the adequate concentrations of glucose, the hazard effect of hypoglycemia may be accelerated by the presence of insulin. The selective vulnerability did not exist in this hypoglycemia-related insulin neuronal toxicity. The further study for this mechanism especially on the molecular cascade may lead to the better clinical management for diabetic care particularly on the prevention of the CNS complication.

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アルツハイマー病

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チアゾリジン誘導体

Thiazolidine derivatives

櫻井 孝　横野浩一

Key words：チアゾリジン誘導体，アルツハイマー病，糖尿病

はじめに

チアゾリジン誘導体(TZD)はインスリン抵抗性改善薬であり，世界的にはpioglitazone (Actos)とrosiglitazone(Avandil)の2種類が発売されており，我が国では前者のみが承認されている．TZDはブドウ糖・脂質代謝を改善するのみではなく，抗炎症作用，抗動脈硬化作用が報告されており，最近ではアルツハイマー病(AD)の治療薬としても期待されている．一方，TZDには副作用として，浮腫，体重増加，心不全があり，その使用にあたっては慎重な管理を要する．今日，我が国は超高齢社会にあり，後期高齢者になって寝たきりとなる原病をたどるとその多くは生活習慣病である．サクセスフルエイジングのためにもTZDを有効に利用し，生活習慣病や認知症の進展を抑制することが重要である．

本稿では特にAD治療薬としてのTZDについて，最近の知見をまとめ紹介したい．

1. チアゾリジンの作用機序

ペルオキシゾーム増殖因子活性化受容体(peroxisome proliferators-activated receptor: PPAR)は，核内受容体スーパーファミリーの一つで，体内および食品に存在する低分子量の脂溶性生理活性物質(15-deoxy $\Delta^{12,14}$ prostaglandin J2など)をリガンドとしている．PPARsは主に糖・脂質代謝にかかわる遺伝子群の発現制御を行い，PPARにはα，β/δ，γの3つのタイプが同定されている．PPARαアゴニストであるフィブラート系薬剤は高脂血症治療薬として，PPARγアゴニストであるTZDは糖尿病治療薬として臨床応用されている．PPARγには更に2つのアイソフォームがあり，脂肪組織にPPARγ2が，マクロファージおよび血管にPPARγ1が存在する．PPARγ2は脂肪細胞の分化を促進し，糖取り込みやインスリン感受性にかかわる分子の発現を亢進させる．また同時にアディポネクチンの発現を亢進させる．PPARγ1はNF-κBとダイマーを形成し，その作用を阻害することで，MCP-1，VACM-1，TNF-α，COX-2，CRPなどの炎症性マーカーを抑制する[1]．

脳ではPPARγは，神経細胞，グリア細胞および脳血管に存在する．TZDはPPARγ活性化により炎症物質の発現を抑制する．神経変性疾患，虚血を問わず，炎症は神経細胞死を誘導する因子であり，このためPPARγはADのみならず，グルタミン酸による神経障害，脳虚血，パ

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ーキンソン病，多発性硬化症などの疾患でも，治療の新たなターゲットとして期待されている．

2. 糖尿病とアルツハイマー病

糖尿病では非糖尿病に比して認知機能障害が認められる．Strachanらの総説では，注意-集中力の低下，前頭葉-遂行機能の障害，視覚性記憶また言語性の記憶低下，精神運動性知能の低下，一般的な知能検査の成績が低下している[2]．しかし糖尿病にみられる認知機能低下は，日常生活に支障を来す程度のものではないと考えられ，これまで多くの関心を集めることはなかった．

ところが近年の疫学調査により，高齢者糖尿病では認知症との合併が多いことが明らかになってきた．糖尿病におけるAD，血管性認知症の相対危険度は，各々1.3-2.3倍，2.0-3.5倍程度とされる[3]．最も信頼性の高い研究とされるRotterdam研究では，インスリン使用者で認知症の相対危険度が4.3倍と高いことが報告された[4]．Honolulu-Asia研究では，ADの遺伝的危険因子であるApo E ε4を保持する高齢者2型糖尿病で相対危険度は更に高いこと，また海馬に老人斑，神経原線維変化が出現すると相対危険度が2.5-3倍高値であることが示され，これまでの臨床解析を裏づける病理成績が示された[5]．現在，どのような高齢者糖尿病で認知機能が低下し，認知症の発症が多いかについて，世界中で研究が進められている．糖尿病の血管性，代謝性要因の中でも，高インスリン血症はADの発症機構の根幹にかかわる可能性があり，以下に述べたい．

3. アルツハイマー病における高インスリン血症の関与

インスリンは，脳血管関門を通過し，海馬，大脳皮質，視床下部などに分布するインスリン受容体に結合する．脳内でもインスリンは少量産生される．高インスリン血症では，脳のインスリン取り込みがdown-regulationを受け，長期的には脳内のインスリンシグナルが低下する可能性が提唱されている[6,7]．実際，ADではインスリン受容体が増加しており，インスリン受容体以降のシグナルであるチロシンキナーゼ活性が低下している．

脳においてインスリンは糖エネルギー代謝を調整するばかりではなく，アセチルコリンやノルエピネフリンなどの神経伝達物質の合成を調節し，シナプスの可塑性，記憶や学習に深くかかわる[7]．

一方，インスリンはアミロイドβやタウの代謝にも作用する．インスリンは神経細胞内のアミロイドβの細胞外への分泌を刺激し，またアミロイドβの消化酵素の一つであるインスリン分解酵素(insulin degrading enzyme: IDE)の発現を調整している．ADでは海馬でのIDEの発現が低下しており[8]，IDE関連遺伝子の異常も指摘されている．このため脳内インスリン作用が低下すると，アミロイドβの分解が低下し，アミロイドβの神経細胞内での蓄積が促進される[7]．また，脳から末梢循環中に排出されたアミロイドβのクリアランスが，高インスリン血症により低下する可能性も指摘されている．

更に高インスリン血症では炎症が惹起され，脳脊髄液中のIL-1β，IL-6，TNF-αが増加していることが報告されている[7]．これらの作用を介して高インスリン血症は脳機能を低下させ，AD発症のリスクになると考えられている(図1)．最近ではADにおける脳のインスリン作用不足に伴う代謝異常を3型糖尿病と呼ぶ論文もみられる[9]．

4. アルツハイマー病のチアゾリジンによる治療

ADの予防に消炎鎮痛薬(NSAIDs)が有効である可能性が以前より指摘されていたが，NSAIDsのターゲットがPPARγの活性化にあることが明らかとなった[10]．この発見を契機に，また上述のADと高インスリンの関連から，TZDがAD治療に有効であることが期待されている．TZDが炎症性サイトカインを抑制し，アミロイド前駆体蛋白の代謝酵素であるBACE1の発現を軽減させること，アミロイドβの脳内でのクリアランスを亢進させること，またADモデル

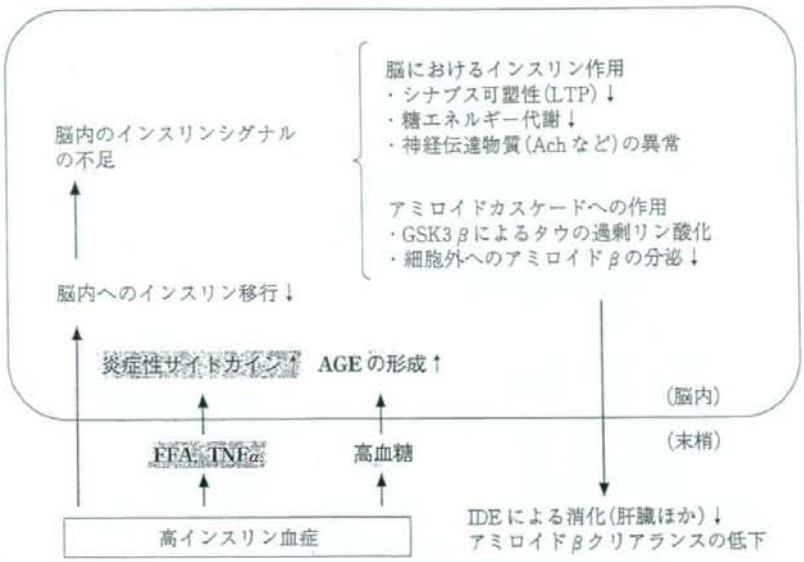

図1 高インスリン血症とアルツハイマー病

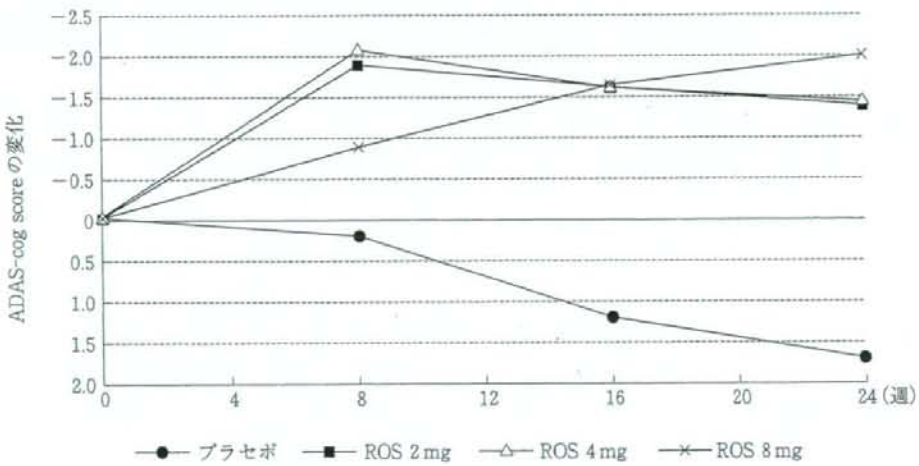

図2 アルツハイマー病に対するロシグリタゾンの効果
Apo E ε4非保持者のみ表示.(文献[11]より改変)

動物で経口投与されたTZDがアミロイドβの蓄積を減少させるなど,基礎的データが蓄積されつつある[10].

最近,軽症のADやamnestic MCIを対象として,rosiglitazoneとプラセボの無作為二重盲検試験が報告された[11].その結果,全体解析では両群間に差はみられなかったが,Apo E ε4非保持者ではrosiglitazone投与群で,記憶や注意力が改善していた(図2).Apo E ε4非保持者のADでは,脳脊髄液/血液インスリン比が低値であるという[7].またrosiglitazone,pioglitazoneは,少量であるが脳へ移行するとの報告もみられ[12],TZDは高インスリン血症改善による作用,抗炎症作用または直接作用により脳機能を改善したものと考えられる.現在,TZDはADのモデュレーターとして治療の選択肢に考えられつ

つあり，米国ではrosiglitazoneを用いた第三相臨床試験が進行している．

5. 投与上の注意とまとめ

TZDの副作用として浮腫は重要であり，心不全を惹起するとの報告もある．2003年にはAHAとADAによる共同声明が[13]，2007年にはrosiglitazoneによる心筋梗塞リスクが記載された[14]．ここでは我が国におけるpioglitazoneの市販後調査であるPRACTICAL(糖尿病を対象)の結果を基に説明する[15]．

TZDが浮腫を来す機序として，PPARγ刺激を介した腎尿細管ナトリウム再吸収の促進作用が知られている．浮腫の発現頻度は男性4.2%，女性で12.1%であり，女性に多く，用量依存性に増加する．浮腫のリスクとして，①女性，②糖尿病合併症あり，③糖尿病罹病期間が長い，④高血圧の合併，⑤BMI高値，⑥高齢者があげられる．一方，心不全に進行するリスク要因としては，心不全・心筋梗塞・冠動脈疾患の既往，高血圧，左室肥大，弁膜症，高齢者，糖尿病罹病歴10年以上，インスリンの併用，慢性腎疾患(クレアチニン2.0mg/dl以上)などがある．よって，基本姿勢として投与前後に胸部X線，心エコー，BNP値で心不全・体液貯留をチェックすることが重要である．集合尿細管におけるナトリウムチャネル阻害薬であるトリアムテレン(カリウム保持性利尿薬)が浮腫に有効と考えられるが，実際にはループ利尿薬の使用による浮腫のコントロールが一般的である．

一方，pioglitazoneは心血管障害・脳卒中の予防に有効であることが既に証明されている(PROactive)．同じPPARγ作動薬でありながらrosiglitazoneとpioglitazoneとの差異については不明な点も残るが，現状では慎重な投与が望まれる．

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新時代の糖尿病学（4）

—病因・診断・治療研究の進歩—

G. 糖尿病に起因する合併症　V. 慢性合併症—その他—

糖尿病における認知機能障害の概念・特徴・診断・治療

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糖尿病における認知機能障害の概念・特徴・診断・治療

Cognitive impairment in diabetes—concept, feature, diagnosis, therapy—

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Key words：糖尿病，認知機能障害，Alzheimer病，アミロイドβオリゴマー

はじめに

ブドウ糖は脳の主たるエネルギー代謝の基質であり，糖代謝異常は脳機能に影響する．急性高血糖では脳機能は可逆性に低下し，2型糖尿病および耐糖能異常でも認知機能は低下することが知られている．近年，欧米を中心とした疫学研究により，2型糖尿病やインスリン抵抗性を伴った高齢者にアルツハイマー型認知機能障害の合併頻度が高いことが報告され[1]，45–64歳の壮年層を対象に認知機能を6年間追跡調査した研究においても空腹時インスリン値12.2 μU/LとHOMA指数2.6以上のインスリン抵抗性が存在すると，6年後には認知機能が低下する頻度が高いことが報告されている[2]．我が国の久山町研究では高血圧が血管性認知症のリスクであるのに対して，Alzheimer病では高血圧より耐糖能異常がより強い危険因子であることが示されている．明らかな脳梗塞所見を伴わずに認知機能障害を呈する頻度が糖尿病で高いことが報告されており，非血管性認知機能障害として注目される．

本稿では糖尿病における認知機能障害および認知症について最近の知見をまとめて概説する．

1．高齢者糖尿病と認知症の疫学

近年の疫学的研究により，高齢者糖尿病では認知症の合併が多いことが報告されている．表1では高齢者糖尿病と，認知症の2大原因である血管性認知症とAlzheimer病の合併リスクを相対危険度で示した[3]．1996年の久山町研究に始まり，いずれの報告でも血管性認知症の相対危険度は2–3.5程度であり，統計学的な検定でも有意であった．またAlzheimer病に関しても，相対危険度は1.3–2.3と高値であり，統計学的に有意とする報告が多い．なかでも1999年に発表されたRotterdam研究では，インスリン使用者で認知症の相対危険度が4.3と高いことが指摘されている．またHonolulu–Asia研究では，Alzheimer病の遺伝的危険因子であるApo Eε4を保持する高齢者2型糖尿病で相対危険度は更に高いこと，また海馬に老人斑，神経原線維変化が出現する相対危険度が2.5–3倍高値であることが示され，これまでの臨床解析を裏付ける病理成績が示された．更にIrieらはApo Eε4を保持する高齢者2型糖尿病での相対危険度は，Alzheimer病と混合型(アルツハイマー型＋血管性)認知症で有意に高値となるが，血管性認知症では有意差はなかったと報告している[4]．現在，どのような高齢者糖尿病で認知機能が低下し，認知症の発症が多いかについて，世界中で研究が進められている．

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表1 糖尿病における認知症の相対危険度

	症例数		relative risk(95%CI)		
	糖尿病(+)	糖尿病(−)	認知症全て	血管性認知症	Alzheimer病
久山町研究(1995) (観察期間7年)	753[a]		記載なし	2.8(2.6-3.0)[b]	2.2(1.0-4.9)[b]
Leibsonら(1997) (観察期間6.85年)	1,455	記載なし	1.7(1.3-2.0)	記載なし	M2.3(1.6-3.3)[b] F1.4(0.9-2.0)[b]
Rotterdam study(1999) (観察期間2.1年)	689	4,532	1.9(1.3-5.8)[c]	記載なし	記載なし
Luchsingerら(2001) (観察期間4.3年)	255	1,007	記載なし	3.4(1.7-6.9)[d]	1.3(0.8-1.9)[e]
Honolulu-Asia study(2002) (観察期間2.9年)	900	1,674	1.5(1.0-2.2)[f]	2.3(1.1-5.0)[f]	1.8(1.1-2.9)[f]
Canadian study(2002) (観察期間4-6年)	503	5,071	1.2(0.9-1.7)[g]	2.2(1.3-3.8)[g]	1.2(0.7-1.8)[g]
Hassingら(2004) (観察期間6年)	38	220	2.1(0.99-4.4)[h]	記載なし	記載なし
Arvanitakisら(2004) (観察期間5.5年)	127	697	記載なし	記載なし	1.6(1.1-2.5)[i]

[a]総数のみ，[b]年齢で補正，[c]年齢，性別で補正，[d]性別，教育歴，高血圧，心疾患，LDLレベル，喫煙，民族で補正，[e]性別，教育歴，民族，Apo Eε4の有無で補正，[f]性別，教育歴，喫煙，アルコール歴，糖尿病歴，Apo Eε4の有無で補正，[g]年齢，性別，教育歴で補正，[h]原著のデータより計算，[i]年齢，性別，教育歴およびこれらの経過時間で補正．

(文献[9]より引用)

表2 2型糖尿病における認知機能障害(53-80歳)

	低下あり (%)	低下なし (%)	ND (%)
注意-集中力	31.6	26.3	42.1
前頭葉-遂行機能	15.8	26.3	57.9
視覚性記憶	26.3	26.3	47.4
言語性記憶	47.4	31.6	21.1
精神運動性知能	15.8	36.8	47.4
MMSE	15.8	—	84.2

(文献[5]より改変)

2. 高齢者糖尿病における認知障害

a. 認知機能障害の特徴

認知症を合併しない糖尿病患者は，非糖尿病者に比して認知機能障害が認められる．過去19編の文献(1985-95)から内容を整理したStrachenらの報告(平均年齢53-80，n=20-140)によると，認知症を合併しない糖尿病患者の認知機能障害では，注意-集中力の低下，前頭葉-遂行機能の障害，視覚性記憶また言語性の記憶低下，精神運動性知能の低下，MMSE(一般的な知能検査)の低下が指摘されている(表2)[5]．そのなかでも言語性記憶障害，注意-集中力障害の頻度が高く，次いで前頭葉-遂行機能障害が多い．これらの結果は，我が国における高齢者糖尿病の認知機能に関する研究とも，おおむね結果は一致している．表3では，上記の認知機能障害を検査する方法として，代表的な神経心理検査を示した[6]．これらの症候と糖尿病のコントロール状況との関連については，語想起，引き算の成績が糖尿病コントロールと関連したとの報告がある[7]．

b. 形態的特徴

1) 脳萎縮について

加齢に伴う認知機能の低下では，言語性記憶・視覚性記憶の低下，注意力の低下がみられるが，言語能力は比較的良く保存される[6]．海馬は記憶の形成に枢要な部位として知られている

表3　認知機能検査

	WAIS-R	MMSE	HDS-R	ADAS	CERAD	RBMT	WMS-R
言語 側頭葉 頭頂葉 前頭葉	類似，理解， 知識，単語	物品呼称 文の復唱 口頭命令 書字命令 書字	語想起	手指呼称 口頭命令 口頭言語能力 聴理解 喚語困難	物品呼称 文の復唱 口頭命令 書字命令 書字 語想起		
エピソード記憶 側頭葉		単語再生 (直後・遅延)	単語再生 (直後・遅延) 物品再生	単語再生 教示再生	単語再生 (直後・遅延 再生・再認)	物語再生 (直後・遅延) 持ち物場所再生 道順再生，用事再生 顔写真再認と氏名再生 線画再認 (直後・遅延)	物語再生 (直後・遅延) 単語対連合 (直後・遅延) 図形対連合 (直後・遅延) 図形再生 (直後・遅延) 図形再認
注意遂行 前頭葉 頭頂葉	数唱，算数， 符号	数唱 連続引き算 見当識(時) 見当識(所)	連続引き算 見当識(時) 見当識(所)	見当識(時) 見当識(所) 観念運動	連続引き算 見当識(時) 見当識(所)	見当識(時)	数唱 見当識(時) 見当識(所)
構成 頭頂葉	積木，組合せ (符号)	図形模写		図形模写 (観念運動)	図形模写		
視空間認知 後頭葉	絵画完成 絵画配列						

WAIS-R: ウェクスラー成人知能検査改訂版，MMSE: Mini-Mental State Examination，HDS-R: 改訂長谷川式簡易知能評価スケール，ADAS: Alzheimer's Disease Assessment Scale，CERAD: Consortium to Establish a Registry for Alzheimer's Disease，RBMT: リバーミード行動記憶検査，WMS-R: ウェクスラー記憶評価検査改訂版．

(文献[9]より改変)