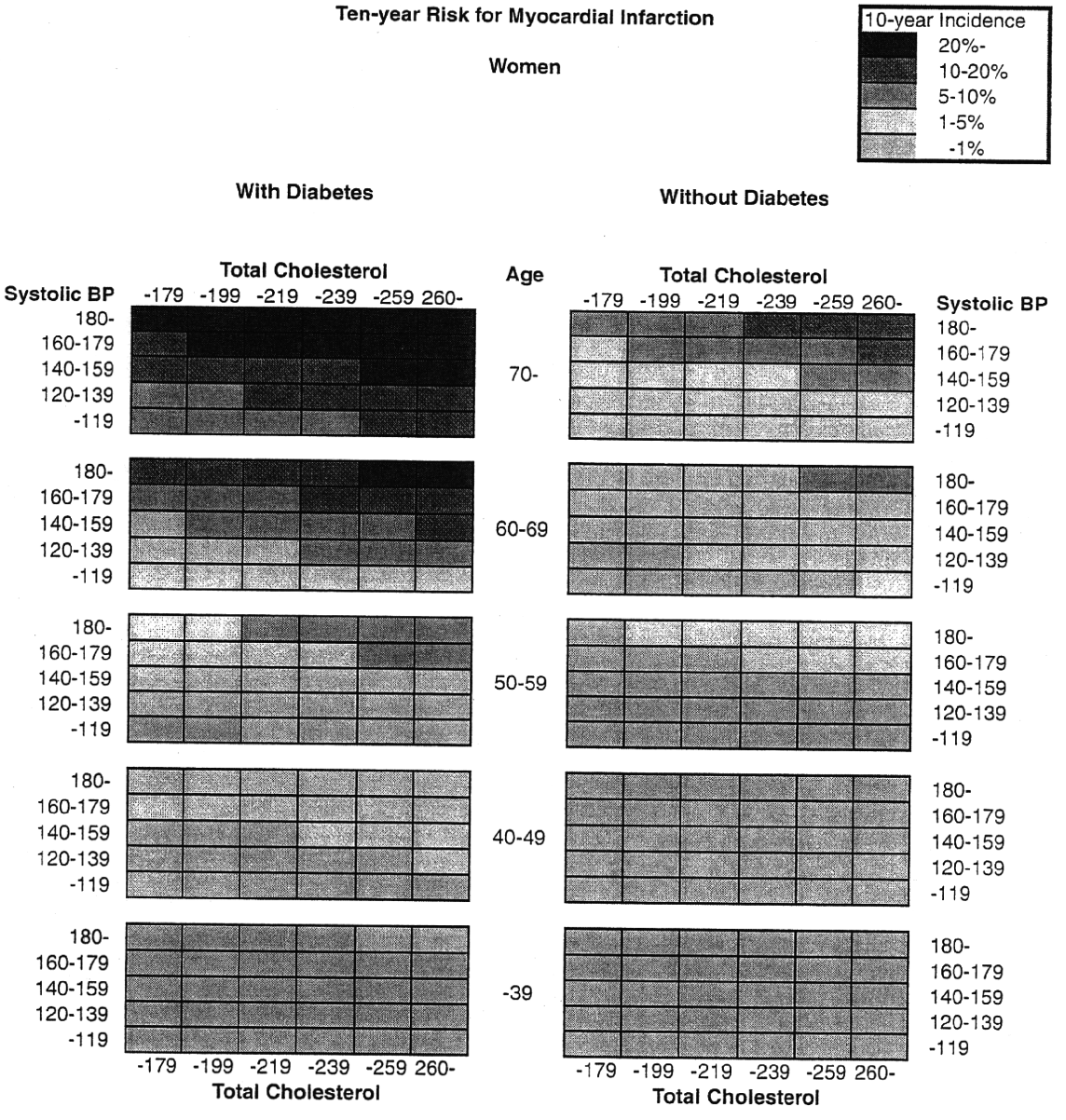

Figure 2. Chart showing 10-year risk for myocardial infarction in women

blood pressure. The charts should prove to be convenient and beneficial tools for health education. They can be used by both health professionals and individual patients, without the need for cumbersome calculations.

The data upon which the charts are based are derived from a Japanese community-based cardiovascular cohort study. The charts are thus more suitable for Japanese populations than charts based on non-Japanese populations, which possess very different genetic and environmental characteristics. The problems of applying the data from the Framingham study to populations other than North American white populations have been frequently described.[10,23–29] The major problem with using foreign risk charts is that incidences of cardiovascular events differ among populations. To address this problem in Europe, a multinational cardiovascular cohort study was conducted, and risk charts for MI were developed that were based on original incidence data from European populations.

In Japan, too, cardiovascular risk charts were created by using data from NIPPON DATA80, a 19-year follow-up study of Japanese populations.[16,17] The pattern of relations between conventional risk factors and coronary heart disease (color distribution) in the charts from the NIPPON DATA80 is largely similar to that shown in our charts. In both risk charts, most conventional risk factors increase the probability of coronary heart disease. The relations agree with the results of other cohort studies conducted in Japan.[3] However, the NIPPON DATA80 captured cardiovascular deaths, and in

that respect, differs from the JMS cohort study, where the outcome of interest was cardiovascular events. Somewhat surprisingly, the crude rate of coronary heart disease (CHD) in NIPPON DATA80 was higher than the incidence of MI in our study (0.74 vs. 0.68 per 1000 person-years),[16] even though the outcome of interest was cardiovascular death in the NIPPON DATA80. This result is probably due to the higher prevalences of risk factors in the NIPPON DATA80 cohort. Average systolic blood pressure in NIPPON DATA80, for example, is substantially higher than that in our study (men: 138.4 vs. 131.4 mm Hg; women: 133.9 vs. 128.2 mm Hg).[16]

However, despite the lower crude rate of MI in our study, the estimated 10-year risks of MI in our charts are mostly higher than the CHD risks in the NIPPON DATA80 charts. These seemingly contradictory results are largely attributable to differences between the 2 studies in hazard ratios for MI for some risk factors. In this study, as compared to the respective hazard ratios from NIPPON DATA80, the hazard ratio for MI for systolic blood pressure in women is six times as high in the present study, and those for female total cholesterol and male smoking were twice as high.[17]

The First and Second Joint Task Force of European and other Societies on Coronary Prevention adopted colored coronary risk charts and recommended that a 10-year absolute risk for MI of 20% be used as a threshold for intensified risk factor intervention, including lifestyle modification and selective use of proven drug therapies.[30] Although no such threshold of absolute risk is specified in the guidelines for the primary prevention of ischemic heart disease issued by the Japanese Circulation Society Joint Working Group (JCS 2006), the guidelines do recommend intervention for any modifiable factors known to be risks for coronary heart disease.[3] The present charts will assist health professionals in identifying high-risk individuals who require intervention to maintain good health. Indeed, these individuals themselves can also obtain beneficial information from the charts, such as the degree to which their MI risk decreases when they improve their risk factors.

There are some limitations in this study. First, this study focused only on MI and did not include angina, due to the difficulties in obtaining a definite diagnosis of the condition. Thus, the absolute risks calculated in this study are lower than would be if the risks of "coronary heart disease" had been considered in a broad sense. Second, this study did address stroke—the most prevalent cardiovascular event in Japan. The JMS Cohort Study did capture stroke events. Therefore, separate charts for estimating stroke risks based on the cohort data could and should be developed in the future. Third, most of the participants in the JMS Cohort Study were rural residents of retirement age or older. The incidence of MI in a group of participants can vary depending on living environment (rural vs. urban) and age studied (old vs. young populations). Use of the charts with different populations should be done with care.

ACKNOWLEDGEMENTS

This study was supported by a Scientific Research Grant from the Ministry of Education, Tokyo, Japan, and grants from the Foundation for the Development of the Community, Tochigi, Japan.

REFERENCES

1. World Health Organization. World Health Statistics 2007. Ogdensburg, NY: Renouf Pubulishing, 2007.
2. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare. Vital Statistics of Japan, 2000, Vol. 3. Tokyo: Health and Welfare Statistical Association, 2000.
3. Japanese Circulation Society Joint Working Group. Guidelines for the primary prevention of ischemic heart disease revised version (JCS 2006). 2006, available at http://www.j-circ.or.jp/guideline/pdf/JCS2006_kitabatake_h.pdf [accessed May 13, 2008].
4. Tanaka H, Date C, Chen H, Nakayama T, Yokoyama T, Yoshiike N, et al. A brief review of epidemiological studies on ischemic heart disease in Japan. J Epidemiol. 1996;6:S49–59.
5. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Arima H, Tanaka K, et al. Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community: the Hisayama study. Stroke. 2003;34:2349–54.
6. Anderson KM, Wilson PW, Odell PM, Kannel WB. An updated coronary risk profile. A statement for health professionals. Circulation. 1991;83:356–62.
7. Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. Am Heart J. 1991;121:293–8.
8. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837–47.
9. Second Joint Task Force of European and Other Societies. Prevention of coronary heart disease in clinical practice. Recommendations of the Second Joint Task Force of European and other Societies on coronary prevention. Eur Heart J. 1998;19:1434–503.
10. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003;24:987–1003.
11. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am Heart J. 1986;111:383–90.
12. Wolf PA, D'Agostino RB, Kannel WB, Bonita R, Belanger AJ. Cigarette smoking as a risk factor for stroke. The Framingham Study. JAMA. 1988;259:1025–9.
13. Levy D, Wilson PW, Anderson KM, Castelli WP. Stratifying the patient at risk from coronary disease: new insights from the Framingham Heart Study. Am Heart J. 1990;119:712–7.
14. Kodama K, Sasaki H, Shimizu Y. Trend of coronary heart disease and its relationship to risk factors in a Japanese population: a 26-year follow-up, Hiroshima/Nagasaki study. Jpn Circ J. 1990;54:414–21.

15. Chambless LE, Folsom AR, Sharrett AR, Sorlie P, Couper D, Szklo M, et al. Coronary heart disease risk prediction in the Atherosclerosis Risk in Communities (ARIC) study. J Clin Epidemiol. 2003;56:880–90.
16. NIPPON DATA80 Research Group. Risk assessment chart for death from cardiovascular disease based on a 19-year follow-up study of a Japanese representative population. Circ J. 2006;70:1249–55.
17. Kasagi F, Kodama K, Hayakawa T, Okayama A, Ueshima H. NIPPON DATA80 Research Group: Risk appraisal chart based on NIPPON DATA80: stroke and coronary heart disease. Japanese Journal of Cardiovascular Disease Prevention. 2005;40:20–6 (in Japanese).
18. Ishikawa S, Gotoh T, Nago N, Kayaba K. The Jichi Medical School (JMS) Cohort Study: design, baseline data and standardized mortality ratios. J Epidemiol. 2002;12:408–17.
19. Matsumoto M, Ishikawa S, Kajii E. Association of adiponectin with cerebrovascular disease: a nested case-control study. Stroke. 2008;39:323–8.
20. Ishikawa S, Shibano Y, Asai Y, Kario K, Kayaba K, Kajii E. Blood pressure categories and cardiovascular risk factors in Japan: the Jichi Medical School (JMS) Cohort Study. Hypertens Res. 2007;30:643–9.
21. WHO MONICA Project Principal Investigators. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. J Clin Epidemiol. 1988;41:105–14.
22. Ishikawa S, Kayaba K, Gotoh T, Nago N, Nakamura Y, Tsutsumi A, et al. Incidence of total stroke, stroke subtypes, and myocardial infarction in the Japanese population: the JMS Cohort Study. J Epidemiol. 2008;18:144–50.
23. D'Agostino RB Sr, Grundy S, Sullivan LM, Wilson P. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA. 2001;286:180–7.
24. Menotti A, Puddu PE, Lanti M. Comparison of the Framingham risk function-based coronary chart with risk function from an Italian population study. Eur Heart J. 2000;21:365–70.
25. Thomsen TF, McGee D, Davidsen M, Jorgensen T. A cross-validation of risk-scores for coronary heart disease mortality based on data from the Glostrup Population Studies and Framingham Heart Study. Int J Epidemiol. 2002;31:817–22.
26. Hense HW, Schulte H, Lowel H, Assmann G, Keil U. Framingham risk function overestimates risk of coronary heart disease in men and women from Germany–results from the MONICA Augsburg and the PROCAM cohorts. Eur Heart J. 2003;24:937–45.
27. Liu J, Hong Y, D'Agostino RB, Wu Z, Wang W, Sun J, et al. Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-Provincial Cohort Study. JAMA. 2004;291:2591–9.
28. Brindle P, Beswick A, Fahey T, Ebrahim S. Accuracy and impact of risk assessment in the primary prevention of cardiovascular disease: a systematic review. Heart. 2006;92:1752–9.
29. Haq IU, Ramsay LE, Yeo WW, Jackson PR, Wallis EJ. Is the Framingham risk function valid for northern European populations? A comparison of methods for estimating absolute coronary risk in high risk men. Heart. 1999;81:40–6.
30. Wood D, De Backer G, Faergeman O, Graham I, Mancia G, Pyorala K. Prevention of coronary heart disease in clinical practice. Summary of recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention. J Hypertens. 1998;16:1407–14.

J Epidemiol 2009;19(2):101-106
doi:10.2188/jea.JE20080092

Original Article

Risk Charts Illustrating the 10-year Risk of Stroke among Residents of Japanese Rural Communities: The JMS Cohort Study

Shizukiyo Ishikawa[1], Masatoshi Matsumoto[1], Kazunori Kayaba[2], Tadao Gotoh[3], Naoki Nago[4], Akizumi Tsutsumi[5], Eiji Kajii[1], and the Jichi Medical School (JMS) Cohort Study Group

[1]Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan
[2]School of Health and Social Services, Saitama Prefectural University, Koshigaya, Saitama, Japan
[3]Wara National Health Insurance Clinic, Gujo, Gifu, Japan
[4]Community Medicine Education Center, Japan Association for Development of Community Medicine, Tokyo, Japan
[5]Occupational Health Training Center, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan

Received September 9, 2008; accepted December 4, 2008; released online March 6, 2009

ABSTRACT

Background: Risk charts are used to estimate the risk of cardiovascular diseases; however, most have been developed in Western countries. In Japan, currently available risk charts are based on mortality data. Using data on cardiovascular disease incidence from the JMS Cohort Study, we developed charts that illustrated the risk of stroke.

Methods and Results: The JMS Cohort Study is a community-based cohort study of cardiovascular disease. Baseline data were obtained between 1992 and 1995. In the present analysis, the participants were 12 276 subjects without a history of stroke; the follow-up period was 10.7 years. Color-coded risk charts were created by using Cox's proportional hazards models to calculate 10-year absolute risks associated with sex, age, smoking status, diabetes status, and systolic blood pressure. The risks of stroke and cerebral infarction rose as age and systolic blood pressure increased. Although the risk of cerebral hemorrhage were generally lower than that of cerebral infarction, the patterns of association with risk factors were similar.

Conclusion: These risk charts should prove useful for clinicians and other health professionals who are required to estimate an individual's risk for stroke.

Key words: stroke; blood pressure; smoking; diabetes mellitus; cohort study

INTRODUCTION

Cardiovascular disease (CVD) and cerebrovascular disease are the second and third most common causes of death in Japan.[1] In most Western countries, the incidence of myocardial infarction (MI) is higher than that of stroke.[2,3] However, in Japan the incidence of stroke is much higher than that of MI.[1,4,5] Although stroke mortality[1] and incidence have declined in the last few decades,[1–3] stroke remains a significant healthcare burden for Japan.

Age, sex, blood pressure, smoking status, and diabetes status are considered the major factors in quantifying stroke risk.[4,6–11] Several models to predict the risk of CVD were developed after the Framingham risk estimates were reported.[12–19] However, most of these only assess the risk for coronary heart disease (CHD); only a few address the risk of stroke. Because the incidences of CHD and stroke differ between Japan and Western countries, risk assessment tools from the latter are not ideal for use in Japan.

Recently, a risk assessment chart for CVD was developed using data from the NIPPON DATA80 in Japan.[20,21] However, NIPPON DATA80 investigated stroke mortality only, ie, non-fatal strokes were not included in the analysis. Therefore, a risk chart constructed using these data would not be entirely suitable for predicting stroke incidence. The Jichi Medical School (JMS) Cohort Study is a multi-community prospective study that monitors residents of Japanese rural communities and captures CVD events. We used data from the JMS Cohort Study to develop charts that display the risk of stroke among Japanese.

METHODS

Study population

The JMS Cohort Study began in 1992. Its primary objective was to clarify associations between potential risk factors and CVD in 12 rural districts in Japan.[5,22]

Address for correspondence. Shizukiyo Ishikawa, MD, PhD, Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan (e-mail: i-shizu@jichi.ac.jp).

The baseline data of this cohort study were obtained between April 1992 and July 1995. If several sets of data were obtained for a single identical participant during that period, the first set was used as baseline. The baseline data were collected as a part of a national mass-screening program. In Japan, mass screening for CVD has been conducted since 1982, in accordance with the Health and Medical Service for the Aged Act of 1981. Local government offices in each community issued invitations to residents eligible for the mass screening, and personal invitations were also sent to all potential participants by mail. As a result, 12 490 participants were eligible (4913 males and 7577 females; age range, 19–93 years). The overall response rate among the 12 communities was 65.0%. Written informed consent to participate in the study was obtained individually from all the respondents to the mass screening.

Among the 12 490 participants, 95 (0.8%) who did not sign the agreement to participate in the study, 7 (0.06%) who had no follow-up data, and 112 (0.9%) who had a past history of stroke were excluded. Ultimately, 12 276 participants (4807 men and 7469 women) remained for analysis.

Measurement of baseline variables

To ensure uniform data collection, we established a central committee composed of the chief medical officers from all the participating districts. This committee developed a detailed manual for data collection. Systolic blood pressure and diastolic blood pressure were measured once with a fully automated sphygmomanometer, the BP203RV-II (Nippon Colin, Komaki, Japan), placed on the right arm of a seated participant who had rested in a sitting position for 5 minutes before measurement. Information about medical history and lifestyle was gathered by means of a written questionnaire.

Blood samples were drawn from the antecubital vein of seated participants, with minimal tourniquet use. Specimens were collected in siliconized vacuum glass tubes containing a 1/10 volume of 3.8% trisodium citrate for blood glucose, and no additives for lipids. Tubes were centrifuged at 3000g for 15 minutes at room temperature. After separation, the serum samples were stored at 4 °C in refrigerated containers if analysis was to be performed within a few days. Otherwise, the samples were frozen until analysis. Plasma samples were frozen as rapidly as possible to −80 °C for storage, until laboratory examination could be performed.

Total cholesterol was measured by using an enzymatic method (Wako, Osaka, Japan; interassay coefficient of variation (CV): 1.5%). Blood glucose was measured via an enzymatic method (Kanto Chemistry, Tokyo, Japan; interassay CV: 1.9%). In this study, blood samples of 5547 (45.0%) participants were collected after an overnight fast; all other samples were casual samples. Diabetic participants were defined as those with currently treated diabetes, plasma glucose ≥126 mg/dl after an overnight fast, or casual blood glucose ≥200 mg/dl. Participants were also asked whether they were current smokers or not.

Follow-up

A mass screening system was used to obtain baseline data and to follow the participants annually. Those examined were asked whether they had suffered a stroke after enrolling. Participants who did not come to the screening examination were contacted by mail or phone. Public health nurses also visited the participants to obtain pertinent information when necessary. In total, 100% of the participants were contacted. Those with a history of stroke were asked when the stroke had been diagnosed and where (at which hospital) they had been treated. Medical records at hospitals in the study areas were also checked to determine if these participants had been treated. If an incident was suspected, forms were filled out, and duplicates of the CT and/or MRI films of the case were obtained to confirm a diagnosis of stroke. Diagnoses were determined independently by a diagnosis committee comprising 1 radiologist, 1 neurologist, and 2 cardiologists. Stroke was defined as a focal, nonconvulsive neurological deficit of sudden onset that persisted for at least 24 hours. Stroke subtypes, ie, cerebral hemorrhage (CH), cerebral infarction (CI), and subarachnoid hemorrhage (SAH), were determined by using the criteria of the National Institute of Neurological Disorder and Stroke.[23] Symptomatic lacuna infarction was defined as a CI.

Statistical analysis

Statistical analyses were carried out using SAS version 8.2 (SAS Japan). Cox proportional hazards models were used to calculate the 10-year absolute risk of stroke for each risk factor. Under the Cox proportional hazards model, the survival probability S(T:X) of a person with a risk X at time T is defined as $S(T:X) = \{[S0(T)]\exp^{(BX)}\}^{\exp(B(X-Xm))}$, where S0(T) is survival probability corresponding to the standard hazard, B is the regression coefficient, and Xm is the population mean of risk X. The 10-year absolute risk of a person with risk X is thus 1-S(10:X).[21] Risk charts were created based on calculations of the absolute risk associated with 5 conventional cardiovascular risk factors: age, sex, smoking status, diabetes status, and systolic blood pressure. Age was grouped into 5 categories: less than 40, 40–49, 50–59, 60–69, and 70 years or older. Systolic blood pressure was also grouped into 5 categories: less than 120, 120–139, 140–159, 160–179, and 180 mm Hg or higher. The other risk factors were treated as dichotomous variables. The risk charts were color-coded so that users could easily estimate their probability of a stroke.

RESULTS

The mean age of participants at baseline was 55.2 years for men and 55.3 years for women. The mean duration of follow-up was 10.7 years (men: 10.6 years; women: 10.8 years).

Table 1. Participants from JMS Cohort Study included in the analysis of stroke risk

	Men	Women
Total Cohort Participants	4911	7579
Participants with Consent	4869	7519
Study Participants	4406	6817
Duration of follow-up (years)	10.6 ± 2.6	10.8 ± 2.2
Age (years)	55.2 ± 12.0	55.3 ± 11.2
Systolic Blood Pressure (mm Hg)	131.3 ± 20.5	128.0 ± 21.0
Smoker (%)	50.4	5.5
Diabetes (%)	4.5	2.2
Stroke	190	165
Cerebral hemorrhage	41	38
Cerebral infarction	136	87
Subarachnoid hemorrhage	13	39
Unclassified	0	1

Total incidence of stroke was 190 cases for men (CH: 41 [21.6%], CI: 136 [71.6%], SAH: 13 [6.8%]) and 165 cases for women (CH: 38 [23.0%], CI: 87 [52.7%], SAH: 39 [23.6%], Unclassified: 1 (0.6%]) (Table 1).

Figures 1 to 3 show the color-coded 10-year absolute risk for all stroke, CH, and CI for each of the combinations of risk factors. All charts were prepared in the same manner, according to diabetes status, smoking status, and systolic blood pressure in each sex. Initially, total cholesterol was included in the analysis; however, after it was determined that total cholesterol was not associated with stroke in either sex, it was excluded from the model. Figure 1 shows all-stroke risk in both sexes, Figure 2 shows CH risk, and Figure 3 shows the risk for CI. Risk can be read by matching the individual's age to the appropriate age group, and blood pressure to the nearest multiple of 20 mm Hg. The risks rose as systolic blood pressure and age increased. In addition, among men and women, current smokers and participants with diabetes were at higher risk for any stroke event and for CI. Although the 10-year risk of CH was lower than that of CI, the risk patterns were similar in men. The risk of SAH was higher in women than in men, and was positively associated with SBP in women, but not in men. The SAH data in the present study are not shown because the number of cases was small, especially among men, and thus the charts were not likely to be representative.

DISCUSSION

We developed risk charts for stroke based on data from the JMS Cohort Study. The charts show 10-year absolute risk for all stroke events and stroke subtypes associated with sex, age, smoking status, diabetes status, and systolic blood pressure. Because cholesterol was not associated with stroke, it was not

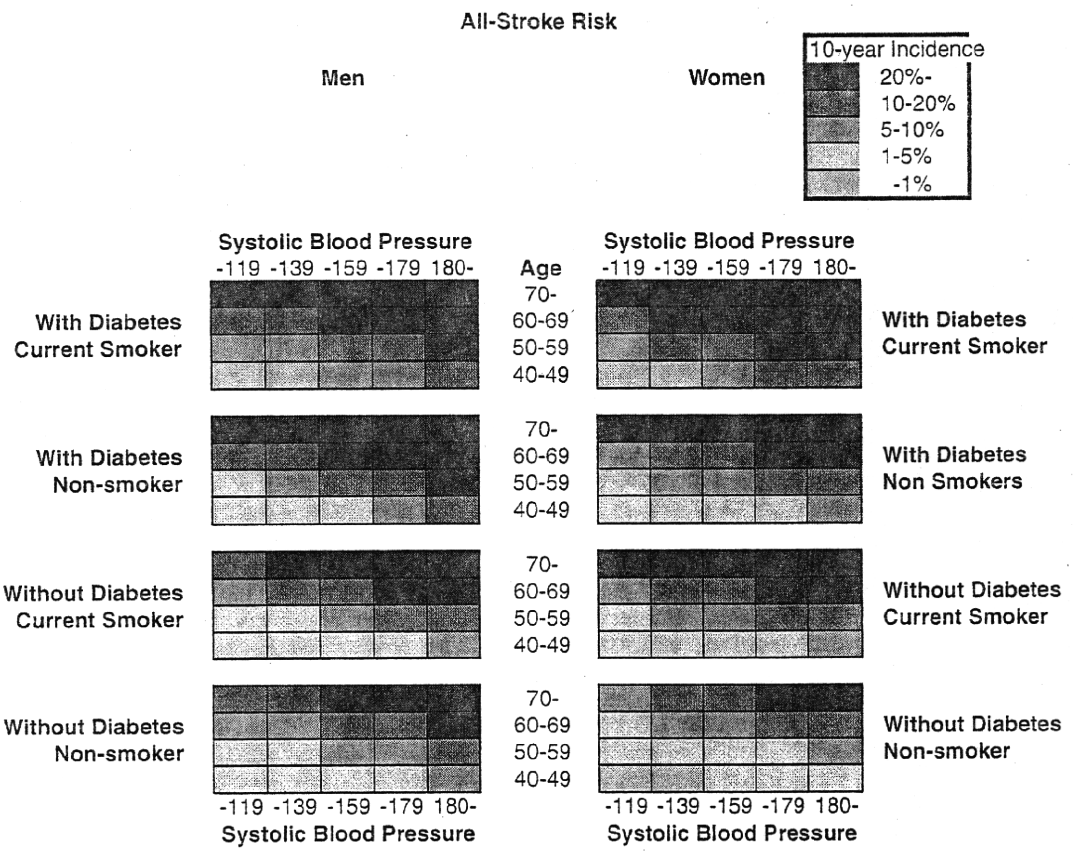

Figure 1. Chart showing 10-year all-stroke risk in men and women

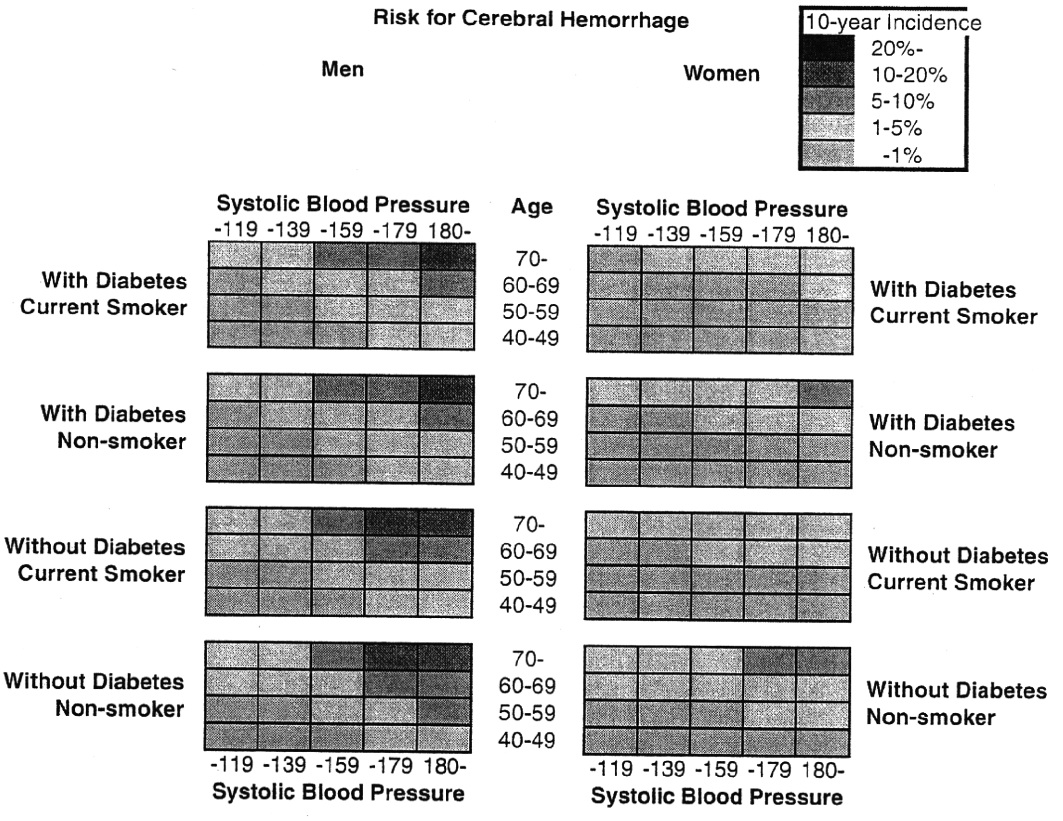

Figure 2. Chart showing 10-year risk for cerebral hemorrhage in men and women

included as an independent variable in the analysis. We believe that these charts will be useful for clinicians and other health professionals who are required to estimate an individual's risk for stroke.

The charts were developed using data from a Japanese community-based cardiovascular cohort study. In the past, a variety of risk charts were developed to estimate the probability of CVD. However, most used data from studies conducted in Western countries.[6,11–13,17–19,24–27] Risk profile charts that estimate the probability of stroke were developed using data from the Framingham Heart Study.[6,27] Although these risk estimates have been widely adopted in the formulation of clinical guidelines in the United States and elsewhere,[28–30] a number of problems in applying these estimates to other populations have been described, the most important of which is that incidences of CVD substantially differ by population.[7,31,32] More recently, tools have been developed that estimate cardiovascular risk in a number of specific populations.[11,13,17–19,25,26]

The Framingham estimates of stroke risk cannot be applied to Japanese because the incidence of stroke differs between the United States and Japan.[33–35] In Asia, tools for CHD risk prediction that are derived from Asian cohorts have been utilized[11]; however, there have been no instruments to predict the risk for stroke in an Asian population. In Japan, cardiovascular risk charts were developed using data from the NIPPON DATA80, which is a representative cohort study. However, the charts were based on mortality data.[20,21] The risk charts developed in the present study should be more accurate in predicting the risk for a stroke event, as opposed to stroke mortality, in Japanese.

We created sex-specific charts that showed risks associated with age, diabetes status, smoking status, and systolic blood pressure. A strong dose-response relationship between systolic blood pressure and stroke has been observed in Japan and other countries.[8–10,36] It is important to note that in the present study total cholesterol was excluded in the estimates of stroke risk because there was no association between total cholesterol and stroke. However, charts for MI risk that are based on data from the JMS Cohort Study have been produced, and these do include total cholesterol as a risk factor for MI.

We developed 10-year charts illustrating CH and CI risk, as well as all-stroke risk. The pattern for all-stroke risk resembled that of CI risk because more than half of the recorded stroke events were CI. The chart patterns for all-stroke, CH, and CI risk were similar, among both men and women.

There were some limitations in this study. The study participants were not randomly selected. The areas in which the study was conducted were primarily rural and the data may therefore not be generalizable to urban populations. In addition, a high-risk population may benefit from more

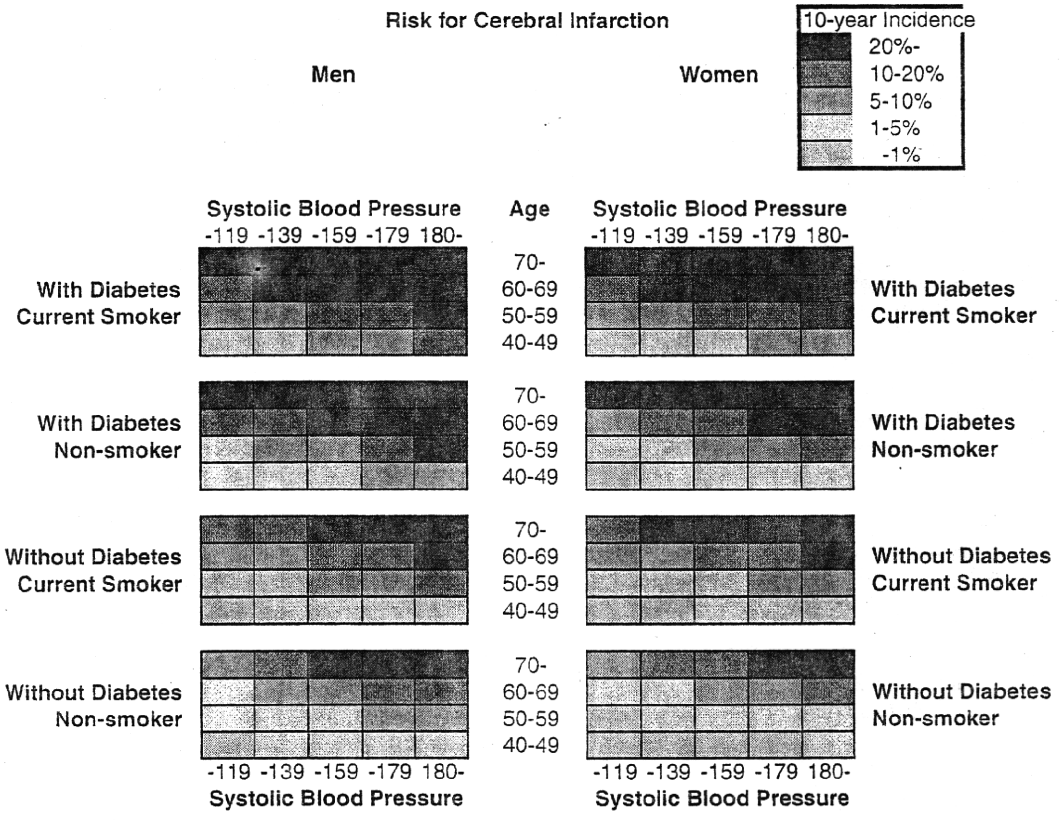

Figure 3. Chart showing 10-year risk for cerebral infarction in men and women

intensive monitoring and intervention than would a population at lower risk. This effect may have led to an underestimation of risks in the present study, because health promotion activities were held in the participating areas.

The strengths of the present study include its very high rates of response and follow-up. Furthermore, the present study was conducted in a standardized fashion in 12 geographically dispersed areas of Japan, and it comprised more than 12 000 men and women. These advantages substantially increased the reliability of the study results.

In conclusion, we used data from Japanese rural populations to develop risk charts that estimate the 10-year risk for stroke in individuals. However, these charts should be used with caution in non-rural populations.

ACKNOWLEDGMENT

This study was supported by a Scientific Research Grant from the Ministry of Education, Tokyo, Japan, and by grants from the Foundation for the Development of the Community, Tochigi, Japan.

REFERENCES

1. Vital Statistics of Japan, 2000, Vol. 3. Tokyo. Health and Welfare Statistical Association 2000.
2. Sytkowski PA, D'Agostino RB, Belanger A, Kannel WB. Sex and time trends in cardiovascular disease incidence and mortality: the Framingham Heart Study, 1950–1989. Am J Epidemiol. 1996;143:338–50.
3. Truelsen T, Mahonen M, Tolonen H, Asplund K, Bonita R, Vanuzzo D. Trends in stroke and coronary heart disease in the WHO MONICA Project. Stroke. 2003;34:1346–52.
4. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Arima H, Tanaka K, et al. Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community: the Hisayama study. Stroke. 2003;34:2349–54.
5. Ishikawa S, Kayaba K, Gotoh T, Nago N, Nakamura Y, Tsutsumi A, et al. Incidence of Total Stroke, Stroke Subtypes, and Myocardial infarction in Japanese Population: The JMS Cohort Study. J Epidemiol. 2008;18:144–50.
6. Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. Stroke. 1991;22:312–8.
7. D'Agostino RB Sr, Grundy S, Sullivan LM, Wilson P. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA. 2001;286:180–7.
8. Psaty BM, Furberg CD, Kuller LH, Cushman M, Savage PJ, Levine D, et al. Association between blood pressure level and the risk of myocardial infarction, stroke, and total mortality: the cardiovascular health study. Arch Intern Med. 2001;161:1183–92.
9. Prospective Studies Collabration. Age-specific relevance of

usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002;360:1903–13.
10. Asia Pacific Cohort Studies Collaboration. Blood pressure and cardiovascular disease in the Asia Pacific region. J Hypertens. 2003;21:707–16.
11. Asia Pacific Cohort Studies Collaboration. Systolic blood pressure, diabetes and the risk of cardiovascular diseases in the Asia-Pacific region. J Hypertens. 2007;25:1205–13.
12. Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. Am Heart J. 1991;121:293–8.
13. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003;24:987–1003.
14. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, et al. European guidelines on cardiovascular disease prevention in clinical practice: Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of eight societies and by invited experts). Eur Heart J. 2003;24:1601–10.
15. British Cardiac Society, British Hypertension Society, Diabetes UK, HEART UK, Primary Care Cardiovascular Society, The Stroke Association. JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. Heart. 2005;91:v1–52.
16. Menotti A, Lanti M, Agabiti-Rosei E, Carratelli L, Cavera G, Dormi A, et al. Riskard 2005. New tools for prediction of cardiovascular disease risk derived from Italian population studies. Nutr Metab Cardiovasc Dis. 2005;15:426–40.
17. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA. 2007;297:611–9.
18. Hippisley-Cox J, Coupland C, Vinogradova Y, Robson J, May M, Brindle P. Derivation and validation of QRISK, a new cardiovascular disease risk score for the United Kingdom: prospective open cohort study. BMJ. 2007;335:136–41.
19. Woodward M, Brindle P, Tunstall-Pedoe HSIGN group on risk estimation. Adding social deprivation and family history to cardiovascular risk assessment: the ASSIGN score from the Scottish Heart Health Extended Cohort (SHHEC). Heart. 2007;93:172–6.
20. Kasagi F, Kodama K, Hayakawa T, Okayama A, Ueshima H. Risk appraisal chart based on NIPPON DATA80: Stroke and coronary heart disease. Jpn J Cardiovasc Dis Prev. 2005;40:20–6 (in Japanese).
21. NIPPON DATA80 Research Group. Risk assessment chart for death from cardiovascular disease based on a 19-year follow-up study of a Japanese representative population. Circ J. 2006;70:1249–55.
22. Ishikawa S, Gotoh T, Nago N, Kayaba K. The Jichi Medical School (JMS) Cohort Study: design, baseline data and standardized mortality ratios. J Epidemiol. 2002;12:408–17.
23. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24:35–41.
24. Anderson KM, Wilson PW, Odell PM, Kannel WB. An updated coronary risk profile. A statement for health professionals. Circulation. 1991;83:356–62.
25. Menotti A, Lanti M, Puddu PE, Kromhout D. Coronary heart disease incidence in northern and southern European populations: a reanalysis of the seven countries study for a European coronary risk chart. Heart. 2000;84:238–44.
26. Assmann G, Cullen P, Schulte H. Simple Scoring Scheme for Calculating the Risk of Acute Coronary Events Based on the 10-Year Follow-Up of the Prospective Cardiovascular Munster (PROCAM) Study. Circulation. 2002;105:310–5.
27. Kannel WB, D'Agostino RB, Sullivan L, Wilson PW. Concept and usefulness of cardiovascular risk profiles. Am Heart J. 2004;148:16–26.
28. Pyorala K, De Backer G, Graham I, Poole-Wilson P, Wood D. Prevention of coronary heart disease in clinical practice. Recommendations of the Task Force of the European Society of Cardiology, European Atherosclerosis Society and European Society of Hypertension. Eur Heart J. 1994;15:1300–31.
29. WHO Guidelines Subcommittee. 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. J Hypertens. 1999;17:151–83.
30. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001;285:2486–97.
31. Liu J, Hong Y, D'Agostino RB Sr, Wu Z, Wang W, Sun J, et al. Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-Provincial Cohort Study. JAMA. 2004;291:2591–9.
32. Brindle P, Beswick A, Fahey T, Ebrahim S. Accuracy and impact of risk assessment in the primary prevention of cardiovascular disease: a systematic review. Heart. 2006;92:1752–9.
33. Shimamoto T, Iso H, Iida M, Komachi Y. Epidemiology of cerebrovascular disease: stroke epidemic in Japan. J Epidemiol. 1996;6:S43–7.
34. van den Hoogen PC, Feskens EJ, Nagelkerke NJ, Menotti A, Nissinen A, Kromhout D, et al. The Relation between Blood Pressure and Mortality Due to Coronary Heart Disease among Men in Different Parts of the World. N Engl J Med. 2000;342:1–8.
35. Stamler J, Elliott P, Dennis B, Dyer AR, Kesteloot H, Liu K, et al. INTERMAP: background, aims, design, methods, and descriptive statistics (nondietary). J Hum Hypertens. 2003;17:591–608.
36. Lawes CM, Bennett DA, Feigin VL, Rodgers A. Blood pressure and stroke: an overview of published reviews. Stroke. 2004;35:1024–33.

Endocrine Journal 2009, **56** (8), 945-950

Incomplete Deficiency of Hypothalamic Hormones in Hypothalamic Hypopituitarism Associated with an Old Traumatic Brain Injury

TOMOYUKI SAITO, NOBUAKI SATO, MIZUHO KIMOTO, TOMOKO ASANO, ATSUSHI AOKI, AKI IKOMA, HIDEO TOYOSHIMA, MASANOBU KAWAKAMI AND SAN-E ISHIKAWA

Department of Medicine, Jichi Medical University Saitama Medical Center, 1-847 Amanuma, Omiya-ku, Saitama, Saitama 330-8503 Japan

Abstract. A 62 year-old man was admitted to determine the pathogenesis of his hypoglycemia. He was unconscious and his plasma glucose level was 26 mg/dL. When he was 31 years old, he had a traffic accident and was unconscious for several days. Physical findings on admittance showed that the patient's BMI was 17.8 and blood pressure, 114/70 mmHg. He was alert. He had a hypogonadal face with a lack of beard, and he had an atrophic testis with a volume of 1 to 2 mL. Laboratory findings showed that his fasting plasma glucose was 73 mg/dL; serum sodium, 133 mmol/L; potassium, 4.1 mmol/L; serum insulin, less than 1.0 μU/mL; plasma ACTH, 45.8 pg/mL; serum cortisol, 5.2 μg/dL; and free cortisol urinary excretion, less than 4.5 μg/day; serum LH, 0.8 mIU/mL; serum testosterone, less than 0.05 ng/mL; serum TSH, 2.0 μIU/mL; free T_4, 0.7 ng/dL; free T_3, 1.5 pg/mL; and serum prolactin, 29.0 ng/mL. The levels of all the pituitary hormones were elevated in response to a mixture of exogenous corticotrophin-releasing hormone (CRH), luteinizing hormone- releasing hormone (LH-RH), thyrotropin-releasing hormone (TRH), and growth hormone-releasing hormone (GRH). However, there was no increased secretion of adrenocorticotropic hormone (ACTH) in response to hypoglycemia (induced by the administration of insulin) and there was no increased secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in response to the administration of clomiphene. Magnetic resonance imaging revealed an atrophied pituitary gland with an empty sella, but there were no abnormal findings of the hypothalamus. Hydrocortisone replacement at a dosage of 20 mg/day increased the patient's plasma glucose from 73 to 100 mg/dL and his serum sodium from 133 to 138 mmol/L. These findings therefore indicate a partial impairment in hypothalamic hormone release, resulting from a traumatic brain injury that the patient had received 31 years ago.

Key words: Hypothalamic hypopituitarism, Traumatic brain injury, Hypoglycemia, Hypogonadism

(*Endocrine Journal* **56**: 945-950, 2009)

TRAUMATIC brain injury is frequently involved in the dysregulation of the secretion of pituitary hormones during an acute phase [1], and about 30% of injured subjects have transient pituitary insufficiency, particularly growth hormone (GH) deficiency and gonadotropin deficiency [2]. The release of pituitary hormones normalizes a year after the brain injury in most clinical settings [2]. Injury to the hypothalamus or pituitary is not necessarily the mechanism by which pituitary hormone synthesis and/or release deteriorates in subjects with traumatic brain injury.

In the present study, we demonstrate a rare case of hypothalamic hypopituitarism, which appears to be associated with a traumatic brain injury 31 years ago. Here we exemplify hypothalamic hormone deficiency in the patient.

Received Jul. 14, 2009; Accepted Jul. 21, 2009 as K09E-202
Released online in J-STAGE as advance publication Jul. 29, 2009

Correspondence to: San-e ISHIKAWA, MD, PhD, Department of Medicine, Jichi Medical University Saitama Medical Center, 1-847 Amanuma Omiya-ku, Saitama, Saitama 330-8503 Japan.
E-mail: saneiskw@jichi.ac.jp

Case Report

A 62 year-old man had a transient disturbance of consciousness for approximately 30 minutes before breakfast in the beginning of August 2007. In end of

Table 1. Basal plasma or serum levels of various hormones and the urinary excretion of the hormones and their metabolites during the patient's hospitalization.

[Hormones associated with CRH]			[Hormones associated with GRH]		
ACTH	45.8	pg/mL	GH	1.4	ng/mL
Cortisol	5.2	μg/dL	IGF-I	24	ng/mL
Aldosterone	63	pg/mL			
Renin activity	0.7	ng/mL/hr	[Hormones associated with AVP]		
DHEA Sulfate	7	μg/dL	AVP	0.8	pg/mL
U. 17-OHCS	1.3	mg/day			
U. 17-KS	1.3	mg/day	[Hormones associated with insulin]		
U. cortisol	< 4.5	μg/day	Insulin	< 1.0	μU/mL
			CPR	0.3	ng/mL
[Hormones associated with LH-RH]			U.CPR	15.5	μg/day
LH	0.8	mIU/mL			
FSH	2.7	mIU/mL			
Testosterone	< 0.05	ng/mL			
[Hormones associated with TRH]					
TSH	2.0	IU/mL			
Free T4	0.7	ng/dL			
Free T3	1.5	pg/mL			
Prolactin	29	ng/mL			

August he did not have supper. The next morning, he was discovered in his bedroom as unconscious condition, and was urgently transferred to the local hospital. His plasma glucose level was 26 mg/dL and he was hospitalized. During the hospitalization, his plasma glucose levels fluctuated between 50 to 70 mg/dL. Therefore, he was referred to Jichi Medical University Saitama Medical Center in early October to have a further evaluation.

At the age of 28 years, he had undergone resection of his stomach because of a gastric ulcer. At the age of 31 years, he had a traffic accident and injured his head, resulting in a loss of consciousness lasting for several days. Several months after the accident, he had lost pubic and axillar hair. Before the traffic accident, he had had two children; the first, when he was 27 years old and the second, when he was 29 years old.

His physical findings on hospitalization were a height of 156.1 cm; body weight of 43.4 kg; and a body mass index of 17.8. His blood pressure was 114/70 mmHg (without a postural change); pulse rate, 72 beats/min (with a regular rhythm); and body temperature, 36.7°C. There were no abnormal findings in his head, neck, chest or abdomen. His face had a hypogonadic appearance with a beard loss and a female-like shape of the cheek and mandibula. No edema was noted in his legs or feet. Pubic and axillar hair were lacking. His testicular volume was markedly reduced at 1 to 2 mL.

Laboratory findings showed as follows: the white blood cell count was 4190/mm^3 (neutrophil 59.5%, lymphocyte 32.4%, monocyte 3.9%, eosinophil 1.4%, basophil 0.6%) ; red blood cell count, 292 x 10^4/mm^3; hemoglobin, 9.4 g/dL; hematocrit, 28.5%; and platelets, 47.7 x 10^4/mm^3. His serum electrolyte levels were: sodium (Na), 133 mmol/L; potassium (K), 4.1 mmol/L; and chloride, 100 mmol/L. His blood chemistry profile showed that his blood urea nitrogen was 8 mg/dL; serum creatinine, 0.5 mg/dL; and uric acid, 2.6 mg/dL. His fasting plasma glucose was 73 mg/dL; hemoglobin A_{1c}, 4.8%; total cholesterol, 195 mg/dL; and triglyceride, 86 mg/dL.

Table 1 summarizes the basal levels of various hormones. In assessing the CRH-ACTH- adrenal axis, we found the patient's plasma ACTH level was 45.8 pg/mL; serum cortisol level, 5.2 μg/dL; plasma aldosterone level, 63.0 pg/mL; urinary excretion of free cortisol, less than 4.5 μg/day; urinary 17-OHCS excretion, 1.3 mg/day; and urinary 17-KS excretion, 1.3 mg/day. The CRH challenge test showed prompt increases in ACTH. The peak value of plasma ACTH was greater than 300 pg/mL at 30 min (Fig. 1a). A

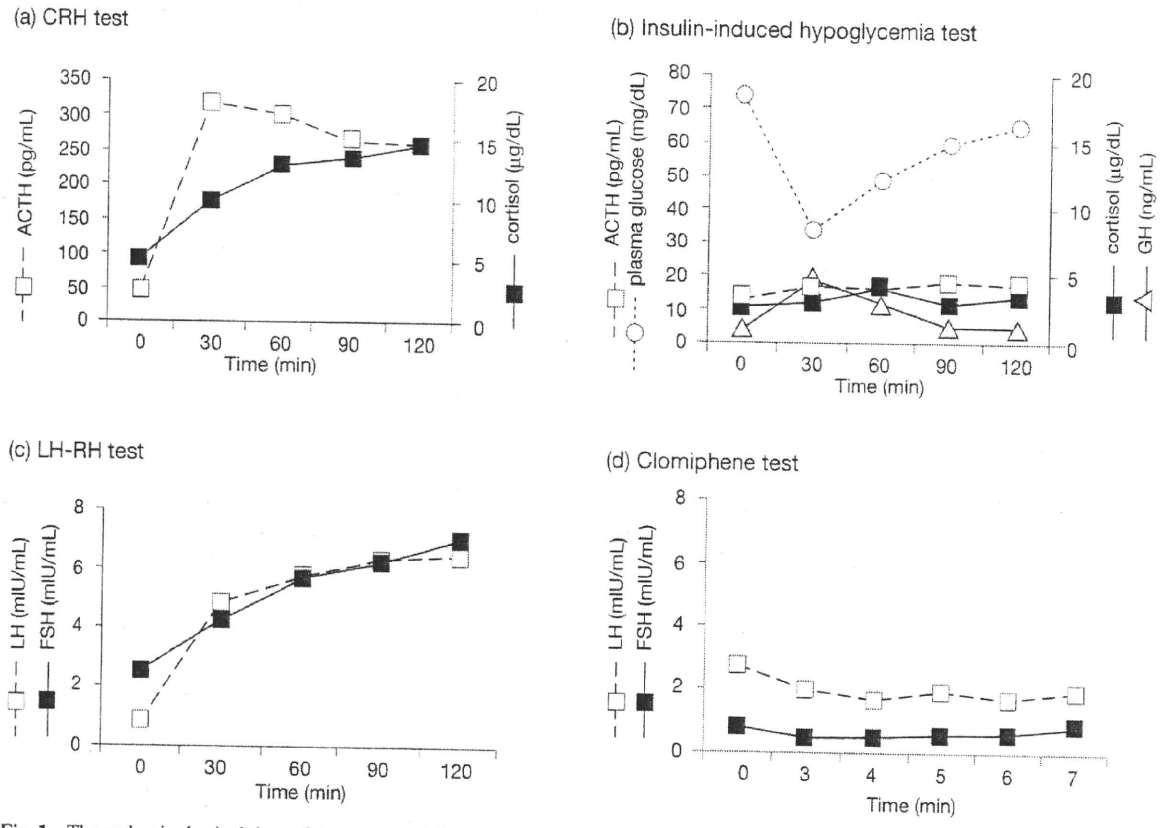

Fig. 1. The endocrinological data of the various challenge tests for pituitary hormones.
(a) CRH challenge test, (b) Insulin induced hypoglycemia test, (c) LH-RH challenge test, and (d) Clomiphene test.

rapid ACTH challenge test showed an increase in serum cortisol level (Table 2). However, there was no increase in the release of ACTH despite a marked reduction in plasma glucose which had been induced by the intravenous administration of insulin (Fig. 1b).

In assessing LH-RH and gonadotropin activity, laboratory findings showed that the serum LH was 0.8 mIU/mL; serum FSH, 2.7 mIU/mL; and serum testosterone level, less than 0.05 ng/mL. The LH-RH challenge test indicated delayed, but significant increases in serum LH and FSH levels (Fig. 1c). By contrast, there was no LH and FSH release in response to the oral administration of clomiphene (Fig. 1d). Serum TSH level was 2.0 μIU/mL; serum free T_4, 0.7 ng/dL; serum free T_3, 1.5 pg/mL; and serum prolactin level, 29.0 ng/mL. However, the serum TSH and prolactin levels were rapidly increased after the intravenous administration of TRH (Table 2). The patient's serum GH level was 1.4 ng/mL and serum insulin-like growth factor-1 (IGF-1) was 24 ng/mL. He had GH release in response to the GRH challenge. However, there was no increase in serum GH levels in response to insulin-induced hypoglycemia (Fig. 1b).

He had a normal urinary concentrating ability since his urine volume ranged from 900 to 1500 mL/day and his urinary osmolality was 493 mmol/kg. The plasma arginine vasopressin (AVP) level was 0.8 pg/mL, which was associated with a plasma osmolality of 269 mmol/kg.

When his plasma glucose level was 73 mg/dL, his serum insulin was less than 1.0 μU/mL and C-peptide immunoreactivity (CPR) was 0.3 ng/mL. Anti-insulin antibody was only 7%.

Brain imaging showed that there was no abnormal finding in the skull x-ray film. Brain magnetic resonance imaging (MRI) showed an atrophied pituitary gland with an empty sella. There was no abnormal finding in the hypothalamus around the third ventricle (Fig. 2).

Table 2. The endocrinological data of various challenge tests.

(a) Challenge tests by a mixture of TRH and GRH

	Time (min)				
	0	30	60	90	120
TSH (IU/mL)	4.3	15.8	17.2	17.2	16.1
GH (ng/mL)	1.5	6.5	25.9	22.7	16.1
Prolactin (ng/mL)	36.5	66.8	59.9	52.6	48.7

(b) ACTH challenge test

	Time (min)				
	0	30	60	90	120
Cortisol (μg/dL)	3.3	8.2	10.5	10.9	11.7

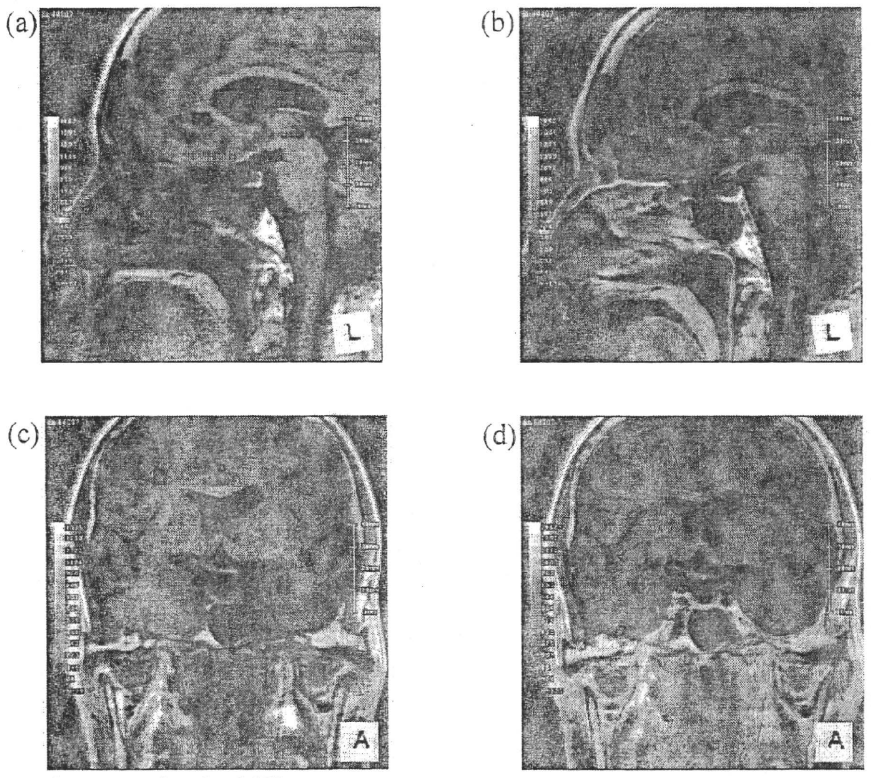

Fig. 2. Brain magnetic resonance imaging (MRI).
(a) Plain MRI, sagittal view, (b) Enhanced MRI, sagittal view, (c) Plain MRI, coronal view, and (d) Enhanced MRI, coronal view.

Clinical course

We performed extensive laboratory tests to determine the pathogenesis of his severe hypoglycemia. We excluded the diagnoses of reactive hypoglycemia, insulin autoimmune syndrome and insulinoma. Because the patient had a hypogonadal face, atrophied testis, and a low serum cortisol level, we focused on dysfunction of the anterior pituitary gland. As noted earlier, he had hypothalamic hypopituitarism, including insufficient secretion of CRH, LH-RH, TRH and GRH. Since the patient was 62 years old, we began hydrocortisone replacement therapy at a dosage of 20 mg/day. After initiating hydrocortisone replacement therapy, the patient's plasma glucose levels increased to the normal value ranging from 90 to 110 mg/dL and

his serum Na level increased to 138 mmol/L. Three months later in the outpatient clinic, thyroxin replacement therapy was added at a dosage of 50 μg/day. Six months later, his body weight had increased from 43.4kg to 48.2kg.

Discussion

The patient was admitted to our medical center for the evaluation of his hypoglycemia. His serum insulin level was below 1.0 μU/mL for a plasma glucose level of 73 mg/dL. His anti-insulin antibody was only 7%. Thus, we excluded the involvement of insulin in the pathogenesis of his hypoglycemia. However, his facial features suggested hypogonadism - he had the lack of beard and had a female-like cheek and mandibula. We focused on dysfunction of the hypothalamo-pituitary axis. The serum levels of cortisol, testosterone, free T_3 and free T_4 were all reduced. The basal level of serum gonadotropin was markedly decreased, but other pituitary hormones were in the normal ranges. Challenge tests, which were performed by administering exogenous hypothalamic hormones, clearly showed the pituitary had a good secretory capability for ACTH, gonadotropin, TSH and GH. However, insulin-induced hypoglycemia, which is mediated via the hypothalamus, did not stimulate ACTH and GH release. The clomiphene test did not increase LH and FSH release at all. These findings therefore indicated an impaired release of the hypothalamic hormones in the patient.

It is important to consider when the hypothalamic disorder occurred. He had two children (the first when he was 27 years old and the second when he was 29 years old). At 31 years old, he had a severe traumatic brain injury due to a traffic accident. He lost consciousness for several days after the accident. However, the details of his brain injury are unclear. He had lost his pubic and axillar hair several months after the traumatic brain injury. Thus, hypopituitarism might have occurred at least 31 years ago. Except for the loss of pubic and axillar hair, he had no symptoms or signs associated with hypopituitarism.

Hypoglycemia was initially noted several years ago. Brain MRI showed an atrophied pituitary gland, but without any trace of organic impairment. Generally, the release of pituitary hormones does not occur in response to a single administration of exogenous hypothalamic hormones in such cases. As noted earlier, challenge tests (i.e., the administration of exogenous hypothalamic hormones) promptly increased the release of ACTH, TSH, GH, and gonadotropin. Taken together, these findings indicated that the release of hypothalamic hormones in the patient was impaired, but still partially preserved.

The association between the severity of traumatic brain injury and the incidence of pituitary impairment is controversial [1, 3-10]. In the literature, acute pituitary insufficiency has been frequently noted after traumatic brain injury [1]. People with pituitary insufficiency have a high incidence of GH and LH/FSH deficiency, but a low incidence of ACTH and TSH deficiency [2-5]. During a 12-month follow-up period patients recover and alterations in hormonal release in the very early stage are not associated with long-term post-traumatic hypopituitarism [2, 4, 5]. There are several cases in the literature of pituitary insufficiency occurring many years after a traumatic brain injury [6, 7, 11]. There are few studies that investigate whether hormone insufficiency is primarily linked to hypothalamic hormones or to pituitary hormones. On the contrary, we proved that hypothalamic hormone deficiency was present approximately 31 years after a traumatic brain injury in our patient. This abnormality is quite a unique observation.

As noted above, there were no abnormal findings on the MRI. We only found the insufficient secretion of hypothalamic hormones. Other hypothalamic functions such as posterior pituitary hormone levels, food intake, thirst, and temperature control were intact. Therefore, we surmise that the traumatic damage would be located in a restricted region of hypothalamus. Since the secretory capability of the anterior pituitary deteriorated without any disturbance of arginine vasopressin release, we suspect there may be diffuse axonal and vascular injury to the hypothalamic region either around the third ventricle or to the portal circulation to the anterior pituitary gland.

In summary, we have described a case of hypothalamic hypopituitarism in the absence of neurohypophysial dysfunction that occurred approximately 31 years after a traumatic brain injury. Hypoglycemia was the presenting symptom. He secreted anterior pituitary hormones in response to the exogenous administration of hypothalamic hormones. The present findings indicate that the long-term, insufficient release of hypothalamic hormones had persisted after a traumatic brain injury.

References

1. Agha A, Rogers B, Mylotte D, Taleb F, Tormey W, Phillips J, Thompson CJ (2004) Neuroendocrine dysfunction in the acute phase of traumatic brain injury. *Clin Endocrinol* 60: 584-591.
2. Giordano G, Aimaretti G, Ghigo E (2005) Variations of pituitary function over time after brain injuries: The lesson from a prospective study. *Pituitary* 8: 227-231.
3. Bondanelli M, De Marinis L, Ambrosio MR, Monesi M, Valle D, Zatelli MC, Fusco A, Bianchi A, Ferneti M, degli Uberti EC (2004) Occurrence of pituitary dysfunction following traumatic brain injury. *J Neurotrauma* 21: 685-696.
4. Aimaretti G, Ambrosio MR, Di Somma C, Fusco A, Cannavo S, Gasperi M, Scaroni C, De Marinis L, Benvenga S, degli Uberti EC, Lambardi G, Mantero F, Martino E, Giordano G, Ghigo E (2004) Traumatic brain injury and subarachnoid hemorrhage are conditions at high risk for hypopituitarism: Screening study at 3 months after brain injury. *Clin Endocrinol* 61: 320-326.
5. Aimaretti G, Ambrosio MR, Di Somma C, Gasperi M, Cannavo S, Scaroni C, Fusco A, Del Monte P, De Menis E, Faustini-Fustini M, Grimaldi F, Logoluso F, Razzore P, Rovere S, Benvenga S, degli Uberti EC, De Marinis L, Lombardi G, Mantero F, Martino E, Giordano G, Ghigo E (2005) Prospective study about brain injury-induced hypopituitarism: Residual pituitary function may improve or more rarely worsen over 12 month follow-up. *J Clin Endocrinol Metab* 90: 6085-6092.
6. Lieberman SA, Oberoi AL, Gilkinson CR, Masel BE, Urban RJ (2001) Prevalence of neuroendocrine dysfunction in patients recovering from traumatic brain injury. *J Clin Endocrinol Metab* 86: 2752-2756.
7. Kelly DF, Gaw Gonzalo IT, Cohan P, Berman N, Swerdloff R, Wang C (2000) Hypopituitarism following traumatic brain injury and aneurismal subarachnoid hemorrhage: A preliminary report. *J Neurosurg* 93: 743-752.
8. Greenwald BD, Burnett DM, Miller MA (2003) Congenital and acquired brain injury. 1. Brain injury: Epidemiology and pathophysiology. *Arch Phys Med Rehabil* 84: S3-S7.
9. Marshall LF, Marshall SB, Klauber MR, Van Berkum Clark M, Eisenberg H, Jane JA, Luerssen TG, Marmarou A, Foulkes MA (1992) The diagnosis of head injury requires a classification based on computed axial tomography. *J Neurotrauma* 9: S287-S292.
10. Bruns J Jr, Hauser WA (2003) The epidemiology of traumatic brain injury: A review. *Epilepsia* 44: 2-10.
11. Benvenga S, Campenni A, Roggeri R, Trimarchi F (2000) Hypopituitarism secondary to head trauma. *J Clin Endocrinol Metab* 85: 1353-1361.

FEBS Letters 583 (2009) 2225–2230

ELSEVIER

FEBS Letters

journal homepage: www.FEBSLetters.org

Regulation of voltage-gated K^+ channels by glucose metabolism in pancreatic β-cells

Masashi Yoshida [a], Katsuya Dezaki [b], Shiho Yamato [a], Atsushi Aoki [a], Hitoshi Sugawara [a], Hideo Toyoshima [a], San-e Ishikawa [a], Masanobu Kawakami [a], Masanori Nakata [b], Toshihiko Yada [b], Masafumi Kakei [a,*]

[a] First Department of Medicine, Saitama Medical Center, Jichi Medical University School of Medicine, Omiya 1-847, Saitama 337-8503, Japan
[b] Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, Tochigi 329-0423, Japan

ARTICLE INFO

Article history:
Received 17 April 2009
Revised 28 May 2009
Accepted 29 May 2009
Available online 3 June 2009

Edited by Maurice Montal

Keywords:
Potassium channel
Kv-channel
Pancreatic β-cell
Insulin secretion
Glucose metabolism

ABSTRACT

Regulation of delayed rectifier-type K^+ channels (Kv-channels) by glucose was studied in rat pancreatic β-cells. The Kv-channel current was increased in amplitudes by increasing glucose concentration from 2.8 to 16.6 mM, while it was decreased by 2.8 mM glucose in a reversible manner (down-regulation) in both perforated and conventional whole-cell modes. The current was decreased by FCCP, intrapipette 0 mM ATP or AMPPNP. Glyceraldehyde, pyruvic acid, 2-ketoisocaproic acid, and 10 mM MgATP prevented the down-regulation induced by 2.8 mM or less glucose. The residual current after treatment with Kv2.1-specific blocker, guangxitoxin-1E, was unchanged by lowering or increasing glucose concentration. We conclude that glucose metabolism regulates Kv2.1 channels in rats β-cells via altering MgATP levels.

1. Introduction

Potassium channels in pancreatic β-cells play a pivotal role in glucose-stimulated insulin secretion. The resting potential of β-cells is determined by activity of ATP-sensitive K^+ channels (K_{ATP} channel) [1–3]. Elevation of external glucose concentration depolarizes β-cell membrane by closure of K_{ATP} channels, thereby inducing bursting spike-like action potentials that are produced by orchestrated openings of voltage-dependent Ca^{2+} channels (VDCCs) and voltage-gated K^+ channels (Kv-channels). Kv channels are composed of delayed rectifier K^+ channels that are slowly activated upon depolarization [4,5] and a distinct class of Kv channels with fast and transient activation during depolarization, being defined as A-current [6,7]. The delayed rectifying current is the major component of Kv-channel currents in β-cells from human [8,9] and rodents [4,5]. Among these Kv channels, Kv2.1 is a predominant component of the delayed rectifying current identified in mammalian β-cells [7,8]. As the other delayed rectifying Kv channels, Kv3.2, Kv8.1 and Kv9.3 are reportedly present in β-cells [8] and furthermore Kv1.5 and Kv1.6 in human islets [7]. mRNAs of Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv3.1 and Kv3.2 channels were detected in INS-1 cells [10]. Apart from these channels, Kv1.4, Kv3.3, Kv3.4 and Kv4.2 show the feature of A-current [7]. Pharmacological, metabolic and hormonal regulations of Kv-channel current are thus expected to shorten or prolong action-potentials elicited by glucose stimulation, thereby influencing Ca^{2+} entry through VDCCs and eventually insulin secretion [11–16]. Recently it was reported that electrical activity of human β-cells during glucose stimulation was little influenced by Kv2.1 inhibition with a specific blocker, stromatoxin [17]. In the present report we demonstrate a novel regulation of delayed rectifier-type Kv-channel by glucose metabolism in rat pancreatic β-cells. The Kv-channel activity increases with an elevation of glucose concentration.

2. Materials and methods

Male Wistar rats were housed according to our institutional guidelines and for animal care. Approval of animal experiments by institutional committee of ethics was obtained. Islets of Langerhans were isolated by collagenase digestion from the rats aged 8–10 weeks, as previously reported [14,18]. Collected islets were dispersed into single cells and maintained in short-term culture for up to 3 days in Eagle's minimal essential medium containing 5.6 mM glucose supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin in 95% air with 5% CO_2 at 37 °C. The cells were superfused with control HEPES-Krebs-Ringer bicarbonate buffer (HKRB) solution containing 2.8 mM glucose.

* Corresponding author. Fax: +81 48 648 5188.
E-mail address: mkakei@jichi.ac.jp (M. Kakei).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.05.050

Perforated whole-cell clamped currents were recorded using a pipette solution containing nystatin (150–200 μg/ml) dissolved in 0.1% DMSO, as previously reported [14,18]. Membrane currents were recorded using an amplifier (Axopatch, 200B, Foster, CA) in a computer using pCLAMP10.2 software. The resistances of patch pipettes ranged from 3 to 5 MΩ.

For perforated whole-cell clamp, pipette solution contained (in mM): K_2SO_4 40, KCl 50, $MgCl_2$ 5, EGTA 0.5 and HEPES 10 at pH 7.2 with KOH. For conventional whole-cell clamp experiments, pipette solution contained (in mM): KCl 50, K_2SO_4 35, $MgCl_2$ 5, EGTA 11, $CaCl_2$ 1, HEPES 11 and ATP-2Na (Rosh Diagnostic, Tokyo, Japan) 5 at pH 7.2 with KOH. The HKRB solution contained (in mM): NaCl 129, $NaHCO_3$ 5.0, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.0, $MgSO_4$ 1.2 and HEPES 10 at pH 7.4. Glucose was added to this solution at required concentrations. Glycolytic intermediates: D,L-glyceraldehyde (Nacarai Tesque, Tokyo, Japan), pyruvic acid (Wako Pure Chemical Industries Ltd., Tokyo, Japan), or 2-ketoisocaproic acid (KIC, Nacarai Tesque, Tokyo, Japan) were applied from either external or internal side as indicated in text. Guangxitoxine-1E was from Peptide Institute Inc. (Tokyo, Japan). *p*-Trifluoromethoxyphenylhydrazone (FCCP) was from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Nystatin, Adenosine 5′-(β,γ-imido)triphosphate (AMPPNP), β-nicotinamide adenine dinucleotide phosphate hydrate (NADPH) and β-nicotinamide adenine dinucleotide phosphate (NADP) were from Sigma–Aldrich (Tokyo, Japan). The experiments were performed at room temperature (25 °C) or 36 °C (Fig. 3B). To identify whether the voltage-clamped cell is an insulin-producing cell, the cell was fixed with 4% paraformaldehyde after electrophysiological experiment and incubated for one hour with rabbit polyclonal anti-insulin antibody (MP Biomedicals, OH, USA) at a dilution of 1:250 at 36 °C followed by Alexa Fluor 488-labeled goat anti-rabbit IgG (Molecular Probes Inc., Eugene, USA). Negative control immunofluorescence was performed by omitting anti-insulin antibody (data not shown).

Data represent the mean ± S.E.M. Statistical analyses were performed using the Student's *t*-test or one-way ANOVA as indicated

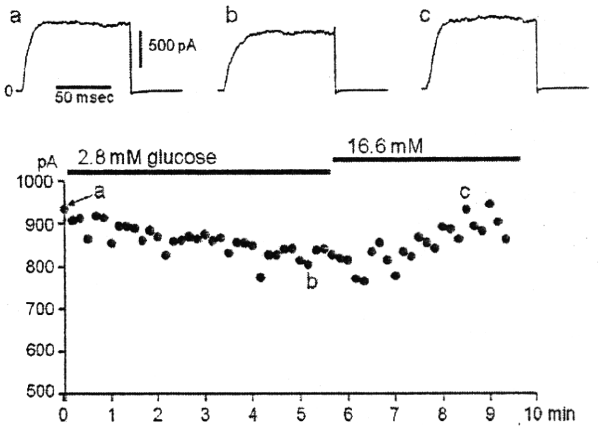

Fig. 2. Kv-channel current is down-regulated by low glucose and restored by high glucose. Amplitudes of Kv-channel current recorded from time zero in the same protocol as in Fig. 1 were plotted by measuring currents at the end of step pulses with 100 ms duration to +20 mV in the presence of 2.8 mM glucose and subsequent exposure to 16.6 mM glucose. Current traces depicted on upper panel (a–c) were obtained at the time points indicated in lower panel.

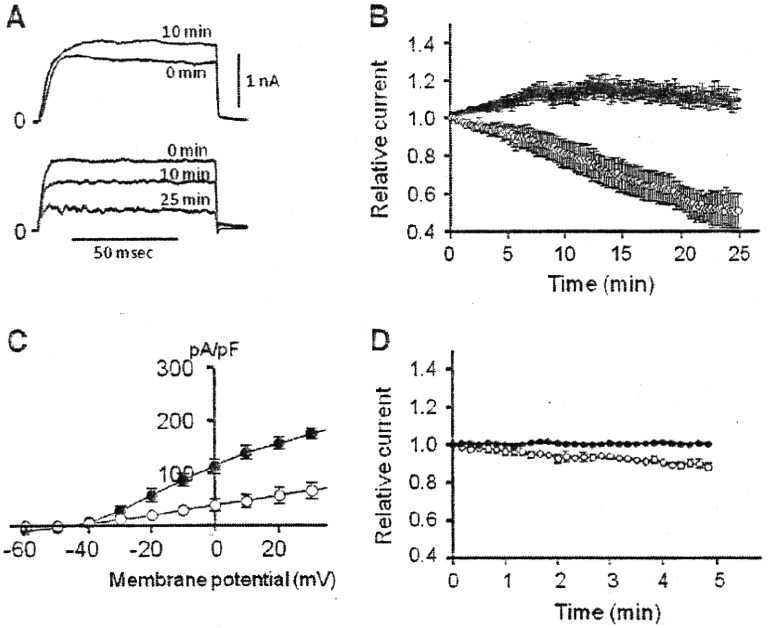

Fig. 1. Effects of glucose on the Kv-channel currents. (A) Current traces in response to depolarization to 0 mV with 100 ms duration from a holding potential of −70 mV at intervals of 10 s were recorded after formation of perforated whole-cell clamp in the presence of 2.8 mM glucose in HKRB solution. Tolbutamide at 100 μM was then added to the solution to inhibit K_{ATP} channels. At 3 min after exposure to this solution the time was reset to 0 min, the solution was changed to HKRB containing 16.6 mM glucose and recordings were initiated (upper traces). In the experiments for lower traces, the solution was continuously superfused with HKRB containing 2.8 mM glucose from time 0. Original current traces at time indicated were depicted. (B) The Kv-channel current measured at the end (a current level averaged between 90 and 99 ms) of depolarized pulses was normalized to that at time 0 and plotted against time in HKRB solution with 2.8 mM (open circles) or 16.6 mM glucose (closed circles). Data were from perforated whole-cell mode and expressed by mean ± S.E.M. (C) Current–voltage relations recorded in the presence of 2.8 mM (open symbols, $n = 6$) or 16.6 mM (closed symbols, $n = 7$) in perforated mode at 25 min. In comparison of the current densities between 2.8 and 16.6 mM glucose, P was 0.18 at −30 mV and <0.05 at more positive potentials (unpaired test). (D) Time courses of the relative Kv-channel current obtained with conventional whole-cell mode, while other protocol was the same as that in (B). Open symbols indicate data recorded from the cells exposed to 2.8 mM ($n = 4$) and closed symbols those to 11.2 mM glucose ($n = 6$).

with a software package of GrafPad Prism ver. 3.02. *P*-values below 0.05 were considered statistically significant.

3. Results

3.1. Regulation of β-cell Kv-channel current by changes in glucose concentrations

After formation of the perforated whole-cell clamp mode in control HKRB solution containing 2.8 mM glucose, the solution was changed to the HKRB solution containing 100 μM tolbutamide and either 2.8 mM or 16.6 mM glucose. Current recording was then commenced and voltage-gated activation of the currents was observed as depicted in Fig. 1A. These voltage-gated currents (Kv-channel currents) were consistent with delayed rectifying K^+ currents [4,5,7,9,14,15]. Amplitudes of Kv-channel currents gradually increased with time during exposure to 16.6 mM glucose (Fig. 1A, upper panel), while they decreased in the persistent presence of 2.8 mM glucose (down-regulation, lower panel). The relative Kv-channel currents decreased during continuous exposure to 2.8 mM glucose in a time-dependent fashion; by contrast a rise in external glucose concentration to 16.2 mM increased amplitudes of the current with time (Fig. 1B).

The relative Kv-channel current 2 min after exposure to 16.6 mM glucose was 1.04 ± 0.01 ($n = 9$, $P = 0.02$ vs. time 0 at membrane voltage of +20 mV, paired test). This was also significantly greater than that measured at the same time after exposure to 2.8 mM glucose, 0.94 ± 0.01 ($n = 10$, $P = 0.0002$ vs. 16.6 mM glucose at +20 mV, unpaired test). In terms of comparison of current levels after superfusion of 2.8 and 16.6 mM glucose, the Kv-channel current measured at 12 min were 111.9 ± 17.6 and 164.4 ± 16.3 pA/pF ($P < 0.05$), respectively. Current–voltage relations obtained 25 min after superfusion with 16.6 or 2.8 mM glucose showed glucose concentration-dependent increase in Kv-channel currents (Fig. 1C). Similar results were observed in conventional whole-cell mode (Fig. 1D). In perforated whole-cell mode the relative Kv-channel current was continuously decreased to 0.46 ± 0.08 at 25 min exposure time (Fig. 1B, open circles, $n = 5$), but it was 0.73 ± 0.01 (Supplementary Fig. 1, $n = 4$, $P = 0.03$) in conventional whole-cell mode at 2.8 mM glucose. We observed these decrease and increase in Kv-current in response to changes in glucose concentrations in all the cells examined as far as we successfully performed voltage clamp experiments for 20 min or more ($n = 52$). Furthermore, the cell in which the Kv-current decreased in the presence of 2.8 mM glucose in perforated mode was shown to be immunoreactive to insulin (Supplementary Fig. 2). These observations suggest that Kv channels of β-cells are regulated by changes in external glucose concentration.

3.2. Reversibility of down-regulated Kv-channel current by high glucose

The Kv-channel current that was down-regulated by continuous exposure to 2.8 mM glucose was restored to a control level by increasing the glucose concentration to 16.6 mM (Fig. 2), suggesting that down-regulation of the channel activity by low glucose

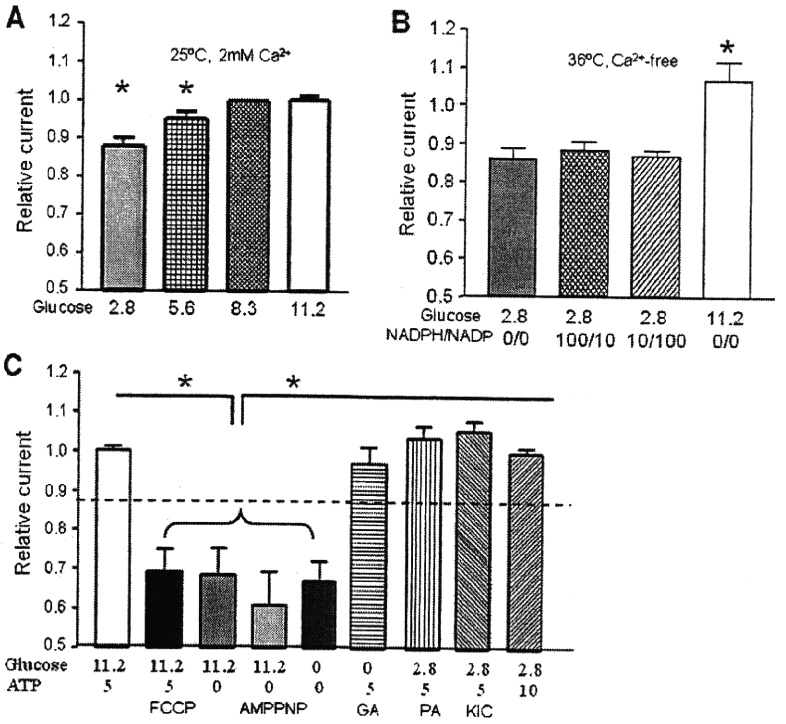

Fig. 3. Effects of glucose, temperature, ratio of cytosolic NADPH/NAD, glycolytic intermediates, metabolic inhibitions on the Kv-channel currents. All data were obtained in conventional whole-cell mode at membrane potential of +20 mV depolarized from −70 mV. (A) The relative current measured 5 min after initiation of recording with 2.8, 5.6, 8.3 or 11.2 mM glucose. *$P < 0.05$ vs. 11.2G (unpaired test). Number of data in each bar was 4–6. (B) Relative Kv-channel current measured 5 min after initiation of each recording during exposure to 2.8 or 11.2 mM glucose in Ca^{2+}-free HKRB solution at 36 °C. To test effects of NADPH/NADP ratio in the pipette solution on Kv-channel current, 100 μM NADPH and 10 μM NADP (100/10) or 10 μM NADPH and 100 μM NADP (10/100) were included in standard pipette solution, which resulted in the relative currents of 0.89 ± 0.02 ($n = 7$) and 0.87 ± 0.02 ($n = 11$), respectively. *$P < 0.05$ vs. the other bars by ANOVA. (C) Relative Kv-channel currents measured 5 min after initiation of each recording during exposure to 1 μM FCCP, 5 mM AMPPNP or glycolytic intermediates: 11.2 mM glyceraldehyde (GA), 10 mM pyruvic acid (PA) and 10 mM 2-ketoisocaproic acid (KIC) in the presence of different concentrations of glucose and ATP. Number of data in each bar was 5–10. Glucose and intrapipette ATP concentrations were indicated in mM in the graph. Dotted line indicated a level of the relative current at 2.8 mM glucose with 5 mM ATP as in (A). *$P < 0.05$ by ANOVA. Data were obtained at the membrane potential of +20 mV.

may be physiological but not due to cytotoxicity induced by insufficient energy supply. We observed similar results in 3 other experiments.

3.3. Down-regulation of Kv channels is independent of temperature, external Ca^{2+} and changes in cytosolic NADPH/NADP ratio

Relative Kv-channel currents at 5 min after exposure to solutions containing varying glucose concentrations were 0.88 ± 0.02 (n = 4) at 2.8 mM glucose, 0.95 ± 0.02 (n = 4) at 5.6 mM, 1.00 ± 0.001 (n = 4) at 8.3 mM and 1.00 ± 0.01 (n = 6) at 11.2 mM (Fig. 3A). These changes in Kv-current amplitude in response to glucose were further explored in terms of temperature- and extracellular Ca^{2+}-dependence (Fig. 3B). Relative Kv-channel currents were 0.86 ± 0.03 (n = 7) at 2.8 mM glucose (36 °C and in Ca^{2+}-free HKRB; P = 0.65 vs. 2.8G in Fig. 3A: 25 °C and 2 mM Ca^{2+}). Under the same condition, relative Kv-channel currents were 1.07 ± 0.05 by continuous exposure to 11.2 mM glucose for 5 min (n = 6, P = 0.003 vs. 2.8 mM glucose in Fig. 3B). Kv2.1 channels in β-cells are also thought to be regulated by the cytosolic NADPH/NADP ratio [13]. We compared effects of different ratio of cytosolic 100:10 or 10:100 NADPH/NADP in whole-cell mode on Kv-channel current during continuous exposure to 2.8 mM glucose at 36 °C and Ca^{2+}-free in HKRB solution. The relative currents after 5 min exposure to 2.8 mM glucose were not altered by these different ratios of NADPH/NADP.

3.4. MgATP mediates the down-regulation of Kv channels

In the presence of FCCP, an uncoupler of electron transport of mitochondria [19], relative Kv-channel current measured at 5 min after exposure decreased to 0.69 ± 0.06 despite the presence of 11.2 mM glucose in HKRB solution (P < 0.002 vs. 11.2 mM glucose, open bar, n = 8, Fig. 3C). Glycolytic intermediates: 11.2 mM glyceraldehyde (GA; bath application), 10 mM pyruvic acid (PA; intrapipette use) and 10 mM 2-ketoisocaproic acid (KIC; intrapipette use) were tested at 0 or 2.8 mM glucose and all these prevented the low glucose-induced down-regulation of the Kv-channel currents. The relative Kv currents with 2.8 mM glucose and 5 mM ATP, indicated as a dotted line in Fig. 3C, were lower than those observed with GA (P = 0.029), PA (P = 0.004) and KIC (P = 0.002). Likewise, increase in MgATP to 10 mM in the pipette also prevented the down-regulation of Kv-channel current (P = 0.001). Omission of MgATP in the pipette with or without 11.2 mM glucose and non-hydrolysable ATP analogue, AMPPNP mimicked the effects of FCCP.

3.5. Kv2.1 channels as the major component of the down-regulation

The current was substantially reduced in the presence of a Kv2.1-specific inhibitor [20,21], guangxitoxin-1E (Fig. 4A). The current that remained after guangxitoxin-1E treatment exhibited a fast activating and slowly decaying kinetics, the properties known for A-current [6,9] and was insensitive to the change in glucose concentration to 11.2 or 2.8 mM (Fig. 4B). These results suggested that the component down-regulated by prolonged exposure to 2.8 mM glucose was Kv2.1 channel current.

3.6. Changes in activation and inactivation kinetics during down-regulation of Kv channels

We examined whether activation and inactivation kinetics of the Kv2.1 channels after down-regulation were influenced. We observed a leftward shift of the half-maximal conductance ($G_{1/2}$) in the conductance–voltage (G–V) relations from −7.5 ± 1.2 mV (n = 5) to −16.6 ± 3.0 mV (n = 4) after down-regulation in the continuous presence of 2.8 mM glucose (Fig. 5A). The half-maximal effect for membrane potential ($V_{i1/2}$) of steady-state inactivation was −36.9 ± 2.6 mV in control and −39.6 ± 2.6 mV after 5 min exposure to 2.8 mM glucose (n = 4, Fig. 5B). The reduction of Kv-channel current at low glucose was use-dependent (Fig. 5C). Voltage pulses to +20 mV at intervals of 10 s for 3 min followed by 5 min pause at a holding potential of −70 mV were repeated during the continuous exposure to 2.8 mM glucose. The current amplitudes during repetitive pulses decreased with time and partially restored after the pauses. This use-dependent down-regulation was not observed in the presence of 11.2 mM glucose (Fig. 5D). The relative currents were increased during exposure to higher glucose concentration.

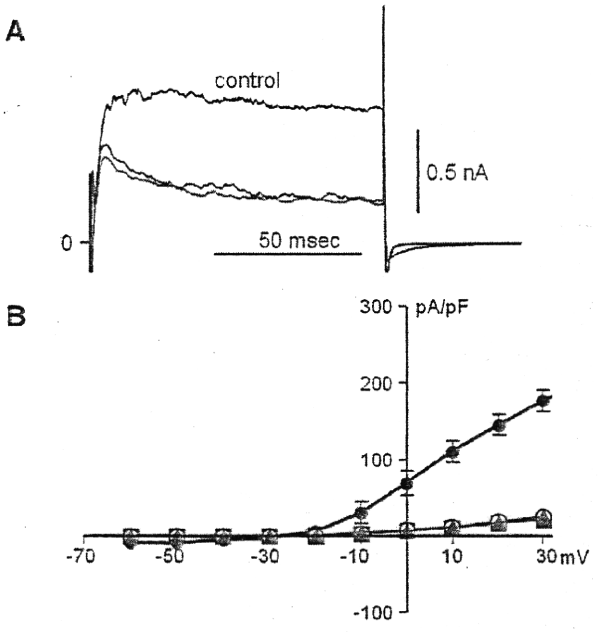

Fig. 4. Selective regulation of Kv2.1 channels by glucose metabolism. (A) After adding tolbutamide to HKRB solution, the current traces at +20 mV (control) and those in the presence of 30 nM guangxitoxin-1E before (black) and at 5 min after (red line) exposure to 2.8 mM glucose were shown. (B) Current densities in 2.8 mM glucose (control, closed circles) were decreased by exposure to 30 nM guangxitoxine-1E (open circles). The current–voltage relations after the guangxitoxin-1E were not influenced by 5 min exposure to low glucose (2.8 mM; closed squares) and high glucose (11.2 mM; closed triangles). These three current–voltage relations were completely overlapped. Number of data in each group was 4.

4. Discussion

In the present report we demonstrated a regulation of the Kv-channel current by glucose metabolism in pancreatic β-cells. The Kv-channel current increased in response to elevation of glucose concentration in a physiological range and it decreased upon prolonged exposure to low glucose in both perforated and conventional whole-cell clamp modes (down-regulation of the channel current). These glucose effects may be due to modulation of the channel activity by glucose metabolism, whereas changes in NADPH/NADP ratio, temperature or extracellular Ca^{2+} concentration did not influence time-course and magnitude of down-regulation of the channel current. Increase of osmotic pressure by an addition of 8.4 mM sucrose in the presence of 2.8 mM glucose did not mimic the effect of 11.2 mM glucose on the Kv-channel activity; the relative Kv-channel current 5 min after exposure was 0.70 ± 0.03 in the former (n = 4, P < 0.0001; unpaired test vs. 11.2 mM glucose). Thus, glucose metabolism but not osmotic pres-

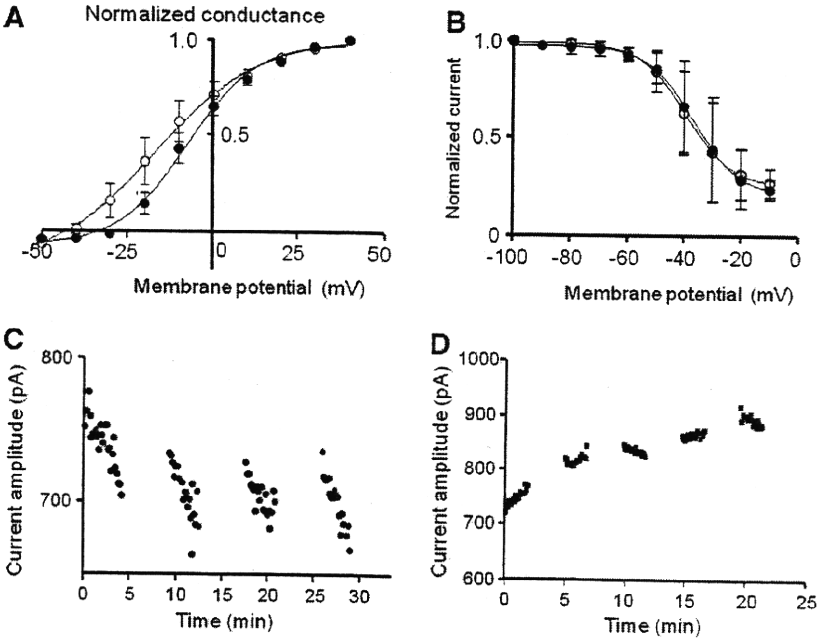

Fig. 5. (A) Cord conductance–voltage (G–V) relations before and after down-regulation of Kv channels. Membrane potential was depolarized to +50 mV in 10 mV step with an interval of 20 s between pulses from a holding potential of −70 mV. Cord conductances were calculated by dividing the current amplitudes measured at the end of each test pulse with a difference between the test potential and E_K that was calculated from the Nernst equation (−80.7 mV in our solutions). Cord conductances were normalized to that obtained at +50 mV and plotted against membrane potentials. The Boltzmann equation was used to fit these data. Closed and open circles were data before and after the down-regulation that was produced by exposure to 2.8 mM glucose for 5 min, respectively. (B) Steady-state inactivation curves before (closed circles) and after (open circles) the down-regulation of Kv-channel current. In terms of voltage protocol, prepulses of various potentials between −100 and −10 mV in 10 mV step with a long duration (10 s) were applied to inactivate the channels, and followed by a short hyperpolarization (−100 mV) of 10 ms with subsequent depolarizing pulse to +20 mV of 100 ms to measure a relative level of steady-state inactivation. Normalized relative currents were plotted and Boltzmann equation was also used to fit the data. (C) Use-dependent down-regulation of Kv channels at low glucose. The Kv-channel currents were measured at the end of test pulses to +20 mV at 10 s intervals with the same voltage protocol as used in previous figures and plotted against time. The depolarized pulses were applied for 3 min and paused for 5 min. These intermittent stimulations and pause were repeated as illustrated. The cells were continuously superfused with HKRB solution containing 2.8 mM glucose throughout the experiment. The down-regulation was partially restored after pauses. (D) Kv-channel current were increased by exposure to 11.2 mM glucose. Voltage protocol for intermittent stimulations and pauses was same as in (C) except for continuous exposure to high glucose. Date from whole-cell mode and 25 °C.

sure is crucial in Kv-channel regulations. External application of glyceraldehyde and intracellularly dialysed intermediates, pyruvic acid or KIC, mimicked the effect of high glucose and prevented the channel down-regulation in the presence or absence of 2.8 mM glucose. Application of KIC to β-cell at 0 mM glucose-stimulated insulin secretion by increasing the intracellular ATP levels [22]. Glyceraldehyde also was used as an intermediate product of glycolysis [23]. Both of the intermediates inhibited openings of K_{ATP} channels activated under conditions of metabolic inhibition in pancreatic β-cells [18]. Thus, glycolysis as well as mitochondrial metabolism may contribute to maintenance of the Kv-channel activity. Increase in internal MgATP concentration to 10 mM in whole-cell pipette solution also stabilized the channel activity, while FCCP and 0 mM ATP with or without 11.2 mM glucose produced down-regulation of the Kv channel. Non-hydrolysable analogue of ATP, AMPPNP, did not mimic the ability of high glucose to prevent the Kv-channel down-regulation (Fig. 3C). These results suggest that MgATP plays a pivotal role in maintaining the activity of the channel.

This Kv-channel regulation by glucose metabolism was specific for Kv2.1 channels but not for A-current, because the residual channel current after inhibition of Kv-channels by guangxitoxin-1E remained unaffected during superfusion with 2.8 or 11.2 mM glucose. Guangxitoxin-1E is a blocker selective to Kv2.1 and Kv2.2 channels with a half-maximal concentration of ~1 nM [20,21]. In rat β-cells, Kv2.1 but not Kv2.2 channels are reportedly present [7]. Accordingly, Kv2.1 channel appears to be the primary Kv-channel subtype that is targeted by glucose.

Direct phosphorylation of Kv2.1 channels influences channel kinetics [24–26]. Kv2.1 channels are highly phosphorylated and graded dephosphorylation shifted $G_{1/2}$ in the G–V relations toward more negative potentials as compared to that in control [26]. In the present paper, we observed similar leftward shift of $G_{1/2}$ from −7.5 ± 1.2 to −16.6 ± 3.0 mV after down-regulation in the continuous presence of 2.8 mM glucose (Fig. 5A). $V_{i1/2}$ of steady-state inactivation was −36.9 ± 2.6 mV in control and −39.6 ± 2.6 mV after 5 min exposure to 2.8 mM glucose (Fig. 5B). Dephosphorylated Kv2.1 channels reportedly showed a shift of $V_{i1/2}$ toward negative potential [26]. Although this small shift of the $V_{i1/2}$ was insignificant, dephosphorylation of Kv-channels might be in part involved in the down-regulation of Kv-channel currents during exposure to low glucose. The ineffectiveness of low-glucose exposure on inactivation kinetics suggests that changes in one or more of the following parameters: maximum open probability of Kv channels, availability of number of the channels and recovery from inactivation between pulses may be involved. ATP depletion and dephosphorylation as a consequence of low MgATP levels as substrate may mechanistically contribute to down-regulation of Kv channels.

We also found that intracellular energy metabolism is not chemically clamped in conventional whole-cell mode and influenced by changes in external metabolic environment such as glucose concentrations. Reduction of the Kv-channel currents by 2.8 mM glucose in the whole-cell mode was less pronounced than that in the perforated whole-cell mode (Fig. 1B and Supplementary Fig. 1). Therefore, it is suggested that cytoplasmic ATP levels can