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Glucose concentration-dependent potentiation of insulin secretion by a new chemical entity, KCP256

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Abstract

The insulinotropic activity of KCP256 [(*R*)-8-benzyl-2-cyclopentyl-7, 8-dihydro-4-propyl-1H-imidazo[2,1-i]purin-5(4H)-one hydrochloride] was examined using MIN6 cells (a pancreatic β-cell line) and pancreatic islets isolated from rats. Unlike sulfonylurea anti-diabetic drugs, KCP256 dose-dependently (0.1–10 μM) enhanced insulin secretion from MIN6 cells and its insulinotropic effect was exerted only at high concentrations of glucose (8.3–22 mM) but not at low concentrations of glucose (3.3–5.5 mM). Furthermore, the action mechanism of KCP256 was different because, unlike sulfonylurea drugs, KCP256 did not displace the binding of [^{3}H]glibenclamide, and did not inhibit the $^{86}Rb^+$ efflux nor K_{ATP} channel activity. In isolated islets, KCP256 also enhanced insulin secretion in a dose- and a glucose-concentration-dependent manner. Plasma levels of insulin after glucose challenge in KCP256-administrated rats were higher than those in vehicle-administrated animals, indicating that KCP256 can enhance insulin secretion in vivo. Since the insulinotropic activity of KCP256 only occurs at high concentrations of glucose, this novel drug may exhibit a decreased risk of drug-induced hypoglycemia compared with sulfonylurea drugs when treating patients with diabetes.
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Keywords: KCP256; Sulfonylurea; K_{ATP} channel; Insulin secretion; MIN6 cell; Pancreatic islet

1. Introduction

Glucose is a major inducer of insulin secretion from pancreatic β cells under physiological conditions (Ashcroft et al., 1984; MacDonald, 1990). The energy provided by the metabolism of glucose is used in the ADP to ATP reaction and the resulting increase in the concentration of ATP causes closure of ATP-sensitive K^+ channels (K_{ATP} channel). Since K_{ATP} channels are pivotal in determining the membrane potential in β cells, closure of K_{ATP} channels results in membrane depolarization that consequently increases insulin secretion.

Sulfonylurea drugs, such as glibenclamide and tolbutamide, are insulin secretagogues widely used for the treatment of type-2 diabetes (Ferner and Neil, 1988). sulfonylurea drugs bind to the sulfonylurea receptor subunit (SUR1) of the K_{ATP} channel in pancreatic β cells causing closure of the channel (Aguilar-Bryan et al., 1995).

However, sulfonylurea drugs have defects, one of which is that they cause hypoglycemia (Ferner and Neil, 1988; Asplund et al., 1983) which is attributable to their action mechanism. Therefore, sulfonylurea drugs depolarize the plasma membrane of the β cells to stimulate insulin secretion, irrespective of glucose concentrations. Hypoglycemia occurs because sulfonylurea drugs exert their effect even when blood glucose is normal or subnormal. These conditions occur frequently in diabetic patients, e.g., when a meal is missed or when patients overdose with the sulfonylurea drugs.

Encouraged by the data generated with wortmannin (Hagiwara et al., 1995) and believing that compounds with glucose concentration-dependent insulinotropic profiles would offer distinct advantages as anti-diabetic drugs, we tried to find an insulin secretion potentiator with a new chemical structure. Intensive screening of compounds from various chemical sources, including synthetic and natural libraries, was undertaken. A series of imidazopurine derivatives were identified as new insulin secretion potentiators. In this paper, the insulinotropic properties of this new chemical class, represented by KCP256,

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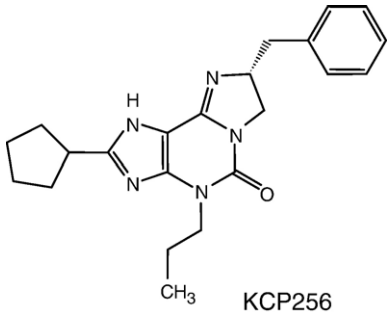

Fig. 1. Chemical structure of KCP256.

[(*R*)-8-benzyl-2-cyclopentyl-7,8-dihydro-4-propyl-1H-imidazo [2,1-I]purin-5(4H)-one hydrochloride], are described and experiments revealing that the action mechanism of KCP256 is different from that of sulfonylureas are reported (Fig. 1).

2. Materials and methods

2.1. Chemicals

KCP256, mitiglinide, and nateglinide were synthesized in the chemistry laboratories of Kyowa Hakko Kogyo Co., Ltd. (Shizouka, Japan). Tolubutamide was purchased from Wako Pure Chemical Industries (Osaka, Japan). Glibenclamide was purchased from Sigma (St. Louis, MO). [^{125}I]Insulin, [^{3}H]glibenclamide, and ^{86}RbCl were purchased from Amersham Biosciences (Buckinghamshire, UK).

2.2. Cell culture

MIN6 cells were cultured as described previously (Miyazaki et al., 1990). Briefly, MIN6 were cultured in Dulbecco's modified Eagle's medium containing 25 mM glucose supplemented with 15% fetal bovine serum (JRH, Lenexa, KS), 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2/95% air. No change in glucose-stimulated insulin secretion by MIN6 cells was observed up to passage 40 and all cells used for experimentation were harvested before passage 40.

2.3. Animals

Male Wistar rats were purchased from CLEA Japan (Tokyo, Japan). The animals received standard laboratory chow, FR-2 (Funabashi Farms, Chiba, Japan) and water ad libitum. They were housed in a temperature (19–25 °C)-, humidity (30–70%)- and light (diurnal time; 0700–1900)-controlled room. The Bioethical Committee of the Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd, approved all the experimental protocols.

2.4. Pancreatic islets

Islets were isolated from male Wistar rats (9- to 11-week-old) according to the method of Sutton et al. (1986) with slight modifications. In brief, the abdomens of the rats were opened under pentobarbitone-anesthesia, then, collagenase (Worthington, Lakewood, NJ) solution (0.7 mg/ml) dissolved in Hank's balanced salt solution (HBSS) containing 10 mM HEPES–NaOH (pH7.4) was injected into the bile duct to digest the pancreas. The pancreata were then removed and incubated for 30 min at 37 °C with shaking. After disruption of digested tissues by vigorous shaking, islets were filtrated through a mesh, sequentially washed in HBSS containing 10 mM HEPES, and purified in a single layer of histopaque-1077 (Sigma, St. Louis, MO) by centrifugation at 2000 ×*g* for 20 min. Five to ten islets were transferred into separate plastic tubes for insulin secretion assays.

2.5. Insulin secretion

MIN6 cells were seeded onto 24-well plates with a density of 1×10^6 cells per well. Seven days after plating, cells were washed twice and the preincubated with Krebs–Ringer HEPES buffer containing 119 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 10 mM HEPES–NaOH (pH7.4), 2 mM glucose, 0.1% bovine serum albumin (BSA) for 60 min at 37 °C. These cells were then incubated in Krebs–Ringer HEPES buffer containing various concentrations of glucose and test compounds for 45 min at 37 °C. Pancreatic islets were also preincubated in Krebs–Ringer HEPES buffer containing 2 mM glucose for 15 min at 37 °C, and insulin secretion assays were performed in Krebs–Ringer HEPES buffer supplemented with various concentrations of glucose and test compounds for 60 min at 37 °C. For experiments with both MIN6 cells and islets, all test compounds were added in dimethyl sulfoxide in a volume not exceeding 0.1% of dimethyl sulfoxide in the final incubation mixture; this concentration of solvent did not affect any of the biochemical processes examined. Insulin secreted from MIN6 cells and islets were measured by radioimmunoassay using a rat anti-insulin antibody (Linco, St. Charles, MO). The dynamic range and sensitivity of the radioimmunoassay system were comparable to those of commercially available kits.

2.6. [^{3}H]glibenclamide binding assay

Binding of [^{3}H]glibenclamide to MIN6 microsomal membrane fraction was measured according to the method of Akiyoshi et al. (1995) with slight modifications. In brief, subconfluent cultures of MIN6 cells were harvested and homogenized in an ice-cold binding buffer containing 100 mM NaCl, 2 mM EDTA, 5 μM leupeptin, 1 μg/ml aprotinin, and 10 mM Tris–HCl (pH7.4), and then centrifuged at 1250 ×*g* for 10 min. The supernatant was centrifuged further at 100,000 ×*g* for 30 min to obtain the microsomal membrane fraction; the resultant pellet was suspended in binding buffer. An aliquot of the microsomal suspension (1 μg) was incubated with 1 nM [^{3}H] glibenclamide and various concentrations of test compounds for 1 h at room temperature. Incubation was terminated by filtration through GF/B glass fiber filter and the filter was washed 4 times with binding buffer. The residual radioactivity on the filter was measured using a liquid scintillation counter.

The non-specific binding was determined in the presence of 10 μM unlabeled glibenclamide. Inhibition of binding (%) was calculated from the specific binding of [^{3}H]glibenclamide in the presence and absence of test compounds.

2.7. $^{86}Rb^+$ efflux assay

The $^{86}Rb^+$ efflux assay is one of the methods used to determine the gating state of K^+ channels in intact cells (Henquin, 1977). A $^{86}Rb^+$ efflux assay was performed according to the method of Schmid-Antomarchi et al. (1987) with slight modifications. In brief, MIN6 cells cultured in a 24-well plate were incubated with Krebs–Ringer HEPES buffer containing ^{86}RbCl (0.25 μCi/well) for 60 min at 37 °C. Then, the cells were washed and incubated with Krebs–Ringer HEPES buffer containing 1.2 μg/ml oligomycin, 1 mM 2-deoxy-D-glucose, 2 mM glucose and various concentration of KCP256 or glibenclamide for 7.5 min at 37 °C. After incubation, the supernatant was removed, and the cells were solubilized with 1N NaOH. The radioactivity recovered in the supernatant and the cell lysate was measured using a γ counter. $^{86}Rb^+$ efflux (%) was calculated as follows: (Radioactivity in the supernatants)/(Total radioactivity in the supernatants and cell lysates) × 100.

2.8. Electrophysiological analysis of K_{ATP} channel

Electrophysiological activity of K_{ATP} channel was measured in rat pancreatic β cells by the whole cell patch clamp technique. The pipette solution contained 135 mM KCl, 4 mM $MgSO_4$, 1 mM Na_2ATP, 1 mM Na_2GTP, 0.5 mM EGTA, and 10 mM HEPES–KOH (pH7.2) and the bath solution contained 135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, and 10 mM HEPES–NaOH (pH7.2). Currents during repetitive ramp voltage sweeps between −100 to −60 mV were recorded using an Axopatch 200 A patch clamp amplifier (Axon Instruments, CA, USA) controlled by Pclamp6 software (Axon Instruments, CA, USA).

2.9. Oral glucose tolerance test

Male Wistar rats (9-week-old) were used for the experiments. After 20-h overnight fast, a test compound or vehicle was administered orally. Glucose (2 g/kg) was given orally to the rats 60 min after the administration of test compound. Blood was withdrawn 2 min before and at 5 and 15 min after glucose loading. Blood was centrifuged immediately to obtain plasma and the blood glucose concentration was determined by the glucose oxidase method. Also, plasma insulin was measured using a commercial kit for rat insulin (Shibayagi, Gunma, Japan).

2.10. Statistical analysis

Statistical analyses were performed with SAS software (Release 6.12, SAS Institute Inc, Cary, NC, USA). Statistical significance between two groups was determined using *F*-test followed by Student's *t* test or Aspin–Welch test. When the experimental design involved more than two groups, statistical analysis was performed by one-way analysis of variance and further post hoc analysis using the Dunnett test. *P* values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of KCP256 on insulin secretion by MIN6 cells

MIN6 cells are a pancreatic β cell line that secretes insulin in response to physiological concentrations of glucose thus mimicking the β cells in pancreatic islets (Miyazaki et al., 1990). Therefore, in this study, MIN6 cells were used as a model cell to evaluate glucose-dependent responses of pancreatic β cells. In the presence of a stimulating concentration of glucose (16.7 mM), KCP256, at concentrations as low as 0.01 μM, enhanced insulin secretion; the enhancement was maximal at 1 μM of KCP256 (Fig. 2A). KCP256 (1 μM) significantly enhanced insulin secretion in the presence of high glucose concentrations (8.3, 14.5, and 22 mM) whereas the compound did not increase the secretion of insulin in the presence of low concentrations of glucose (3.3 and 5.5 mM; Fig. 2B). On the other hand, glibenclamide significantly stimulated insulin secretion from MIN6 cells regardless of the glucose concentrations examined (Fig. 2C).

3.2. Effect of KCP256 on the binding of [^{3}H]glibenclamide to MIN6 cell microsomes

MIN6 cells were reported to express SUR1 in their plasma membranes (Sakura et al., 1999). In the present study, binding of [^{3}H]glibenclamide to the microsomal fraction of the cell line was demonstrated (K_d: 0.61 nM and B_{max}: 8.7 pmol/mg protein) as reported (Akiyoshi et al., 1995). Glibenclamide, nateglinide, and mitiglinide inhibited [^{3}H]glibenclamide binding in a concentration-dependent manner (Fig. 3), suggesting that these compounds share the same binding site in the membranes of MIN6 cells, confirming a previous report (Mogami et al., 1994). However, KCP256, even at concentrations as high as 100 μM, did not displace the binding of [^{3}H]glibenclamide (Fig. 3).

3.3. Effect of KCP256 on the functions of K_{ATP} channels in MIN6 cells

The activities of K_{ATP} channels were measured by two different methodologies; (i) $^{86}Rb^+$ efflux assays and (ii) electrophysiological recordings.

$^{86}Rb^+$ efflux was observed from MIN6 cells in settings where glucose metabolism was completely blocked. Thus, efflux of $^{86}Rb^+$ was suppressed by a K_{ATP} channel inhibitor, glibenclamide; 1 μM glibenclamide caused 78% inhibition (Fig. 4), suggesting that under the experimental settings used in the present study, $^{86}Rb^+$ is released from MIN6 cells mainly through K_{ATP} channels. However, at concentrations (0.01–1 μM) that are insulinotropic, KCP256 did not affect the efflux of $^{86}Rb^+$ (Fig. 4). Higher concentrations (10 μM) of KCP256

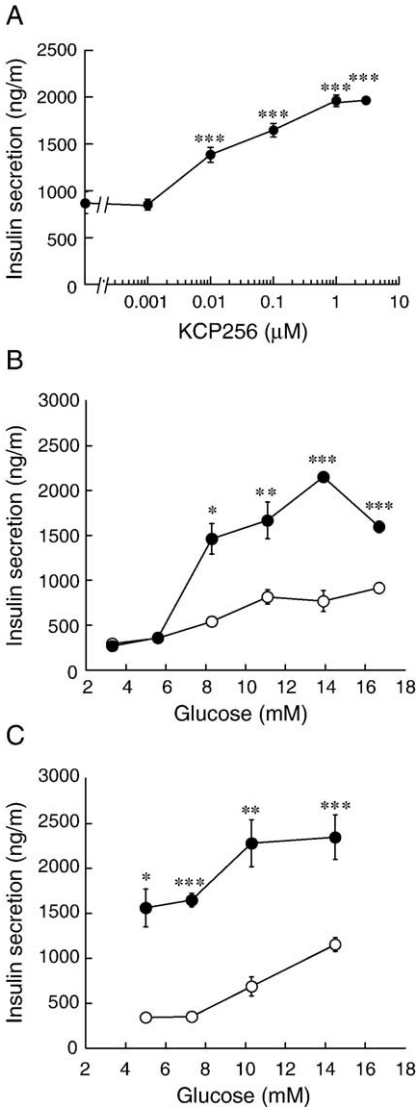

Fig. 2. Glucose-concentration-dependent insulinotropic effects of KCP256 in MIN6. MIN6 cells were preincubated with Krebs–Ringer HEPES buffer containing 2 mM glucose for 60 min at 37 °C and then were incubated with Krebs–Ringer HEPES buffer containing 16.7 mM glucose and various concentrations of KCP256 for 45 min at 37 °C (A), or were incubated with Krebs–Ringer HEPES buffer containing various concentrations of glucose with (●) or without (○) either 1 μM of KCP256 (B) or 0.1 μM of glibenclamide (C) for 45 min at 37 °C. Insulin in the supernatants was measured as described in Materials and methods. Values represent the mean±S.E.M. of three to six determinations in the same experiment. $^{**}P<0.01$, $^{***}P<0.001$.

inhibited efflux by 20%, suggesting a weak effect of KCP256 on K_{ATP} channels.

A linear voltage–current relationship and a reverse potential around −80 mV (Fig. 5) demonstrated that the K_{ATP} current was predominant under the experimental conditions described in Materials and methods. A sulfonylurea drug, tolbutamide (100 μM) completely suppressed the current (Fig. 5A, B), which strongly suggests that the observed current is the K_{ATP} current. KCP256 at 1 (Fig. 5C) and 3 μM (not shown) did not inhibit the current measured at −100 mV, but at 10 μM (Fig. 5D) KCP256 did inhibit the current by 18%. The effect of KCP256 was diminished after wash-out, indicating that the

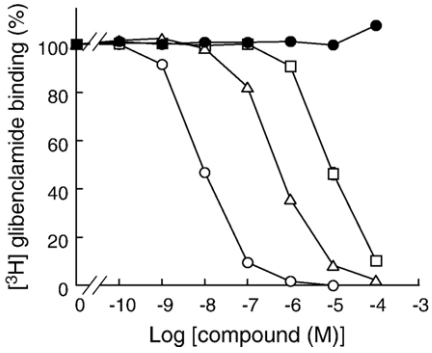

Fig. 3. The effects of KCP256 on [^{3}H]glibenclamide binding to MIN6 plasma membrane. MIN6 microsomal membranes were incubated with 1 nM of [^{3}H] glibenclamide and various concentrations of either KCP256 (●), glibenclamide (○), nateglinide (□) or Mitiglinide (Δ) for 1 h at room temperature. Then, membrane fractions were filtrated, washed, and the residual radioactivity on the filter was measured. Values represent the mean of three determinations±S.E.M. in the same experiment.

slight inhibition was not due to run-down phenomena. These data correspond well with those obtained in $^{86}Rb^+$ efflux experiments, although isolated β cells were used for the patch clamp experiments whereas MIN6 cells were used for the $^{86}Rb^+$ efflux experiments.

3.4. Effects of KCP256 on pancreatic islets

Since MIN6 is a clonal cell line, the biochemical machinery involved in insulin secretion may be altered during culture and subsequent passages. Therefore, the insulinotropic effect of the compound was examined in isolated pancreatic islets. KCP256 stimulated insulin secretion in the islets isolated from male Wistar rats at a high concentration of glucose (16.7 mM, Fig. 6A). KCP256 at concentrations as low as 0.3 μM significantly enhanced insulin secretion and the enhancement reached maximum at 1 μM (Fig. 2A). The glucose-concentration dependency of this insulinotropic effect was also confirmed in isolated rat islets (Fig. 6B); KCP256 did not increase secretion of

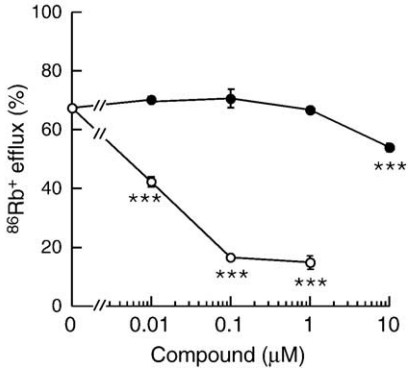

Fig. 4. The effects of KCP256 on $^{86}Rb^+$ efflux from MIN6. MIN6 cells loaded with ^{86}RbCl were incubated with Krebs–Ringer HEPES buffer containing oligomycin, 2-deoxyglucose, 2 mM glucose and either various concentrations of KCP256 (●) or glibenclamide (○) for 7.5 min at 37 °C. After the incubation, supernatant was collected and cells were solubilized. Radioactivity in the supernatant and in the cell lysate was measured using a γ counter. $^{86}Rb^+$ efflux (%) was calculated as described in Materials and methods. Results are the mean±S.E.M. of three determinations in the same experiment. $^{***}P<0.001$.

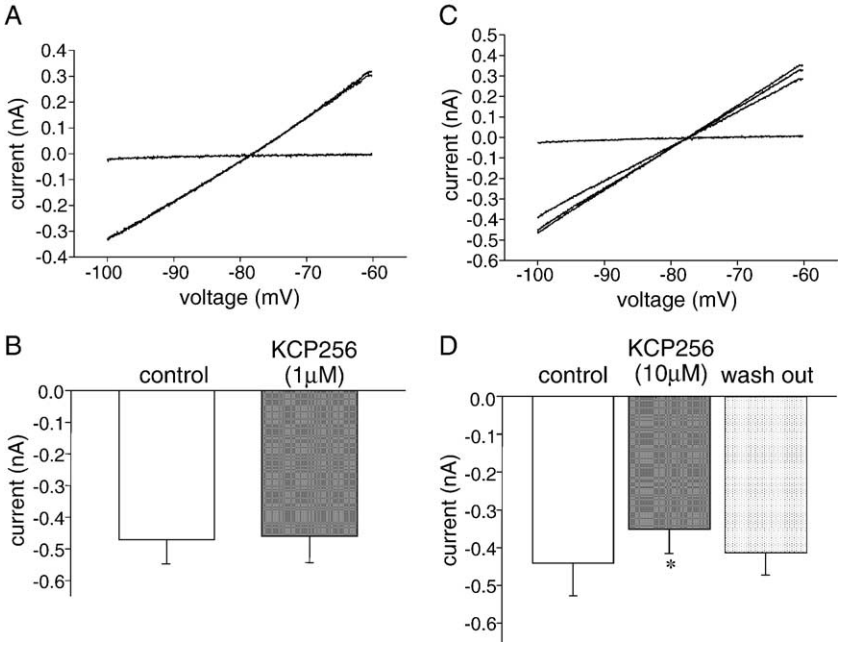

Fig. 5. The effects of KCP256 on K_{ATP} channel current in rat pancreatic β cells. Representative traces of K_{ATP} current obtained in the absence (Control) or the presence of either KCP256 (1 μM : A, 10 μM : B) or Tolbutamide (100 μM) on K_{ATP} channel. The effects of KCP256 (1 μM : C, 10 μM : D) on K_{ATP} current measured at −100 mV in the ramp voltage clamp experiments. Results are the mean±S.E.M. of three determinations in the same experiment. *$P<0.05$.

insulin in the presence of 3.3 mM glucose and was minimal with 8.3 mM glucose in isolated rat islets whereas the effect was significant with 14.5 and 22 mM glucose.

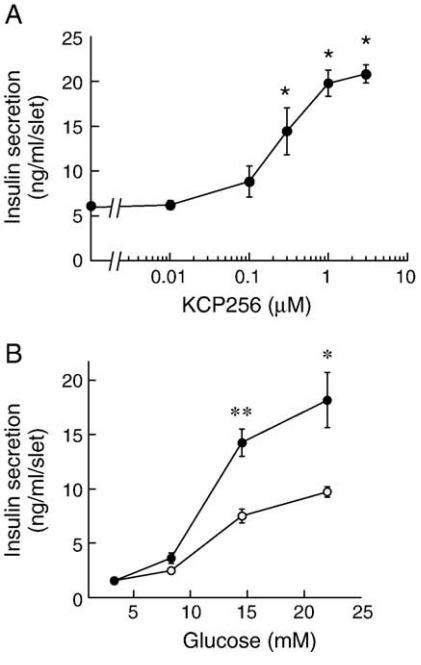

Fig. 6. Glucose-concentration-dependent insulinotropic effects of KCP256 in rat islets. Islets isolated from male Wistar rats were preincubated with Krebs–Ringer HEPES buffer containing 2 mM of glucose for 15 min at 37 °C. (A) Islets were incubated with Krebs–Ringer HEPES buffer containing 16.7 mM glucose and various concentrations of KCP256. (B) Islets were incubated with Krebs–Ringer HEPES buffer containing various concentrations of glucose in the presence (●) or absence (○) of 1 μM of KCP256. Insulin in the supernatants was measured as described in Materials and methods. Values represent the mean±S.E.M. of three determinations in the same experiment. *$P<0.05$, **$P<0.01$.

3.5. KCP256 increases insulin secretion and reduces blood glucose in vivo

Plasma levels of insulin and blood glucose before the glucose challenge were not significantly different between vehicle and KCP256 groups (Fig. 7), indicating that insulin and glucose levels were not affected by administration of KCP256 in the absence of glucose challenge. After glucose challenge, both plasma insulin and blood glucose rapidly increased. In the KCP256 group, plasma insulin measured 5 min after glucose challenge was significantly higher compared with that in the vehicle group (Fig. 7A). Correspondingly, the blood glucose measured 15 min after glucose challenge was significantly

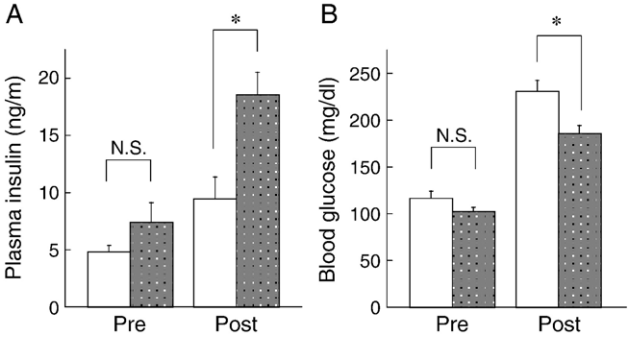

Fig. 7. Effect of KCP256 on insulin secretion and blood glucose in vivo. Wistar rats were orally challenged with glucose (2 g/kg) 60 min after administration of KCP256 (30 mg/kg, p.o.). Blood was withdrawn 2 min before (Pre), and 5 (plasma insulin), 15 min (blood glucose) after glucose challenge (Post). Plasma insulin (A) and blood glucose (B) were measured as described in Materials and methods. Open and shaded columns show data for rats treated with vehicle only and KCP256, respectively. Results are the mean±S.E.M. of six determinations in the same experiment. N.S.—Not Significant, *$P<0.05$.

lower in the KCP256 group compared with that in the vehicle group (Fig. 7B).

4. Discussion

In this study, we have demonstrated that KCP256, at concentrations as low as 0.01 μM, enhanced insulin secretion in a pancreatic β cell line under conditions of high glucose concentrations (8.3 mM or higher), but the compound was ineffective at low glucose concentrations (5.5 mM or lower). Type 2 diabetes is diagnosed as follows: blood glucose more than 126 mg/ml (7 mM) in a fasting state or 200 mg/ml (11.1 mM) after 75 g glucose challenge. Consequently, the concentrations of glucose required to reveal the insulinotropic effect of the compound were similar to the plasma glucose concentrations in diabetic patients. The insulinotropic effect of KCP256 was observed not only in the cell line, but also in pancreatic islets isolated from Wistar rats. In the isolated islets, the effect of KCP256 was also glucose concentration-dependent. Furthermore, oral administration of KCP256 increased insulin secretion in rats that had elevated blood glucose produced by oral glucose challenge. However, this enhancement was not observed in KCP256-administered animals when the blood glucose was low (fasting level). These results suggest that KCP256 is effective in cells, tissues, and whole animals as a glucose concentration-dependent insulin secretion potentiator.

The effective concentrations of KCP256 were higher in isolated rat islets than in the mouse cell line, MIN6 (Fig. 2A vs. Fig. 6A). Quantitative differences were also observed in the experiments in which glucose concentration dependency was examined; the insulinotropic effect of KCP256 was detected in MIN6 cells in the presence of 8.3 mM glucose (Fig. 2B), whereas the effect was minimal in isolated islets at the same glucose concentration (Fig. 6B) and a significant effect was only apparent at 16.7 mM glucose. The reason for this quantitative variation is unknown but it may be attributable to differences between rats and mice and/or differences between cultured cells and freshly isolated tissues.

The action mechanism of KCP256 was compared with that of insulinotropic antidiabetic drugs. Insulinotropic antidiabetic agents used clinically are sulfonylurea drugs and newly developed drugs with no sulfonylurea moiety, such as nateglinide (Kikuchi, 1996) and mitiglinide (Misawa et al., 2001). In the present study, we demonstrated that these classic insulinotropic compounds displaced the binding of [^{3}H]glibenclamide to microsomal fractions of MIN6 cells (Fig. 3) and that the concentrations required for the displacement were comparable to those required for stimulation of insulin secretion. These data confirmed that the insulinotropic effect of nateglinide and mitiglinide is attributed to the inhibition of K_{ATP} channel, and that these drugs share the same binding site and the same action mechanism as sulfonylurea drugs. In contrast, KCP256, at concentrations as high as 100 μM, did not displace the binding of [^{3}H]glibenclamide to the microsomes (Fig. 3), suggesting that the site of action of KCP256 is different from that of sulfonylurea drugs. Experiments undertaken to examine the function of K_{ATP} channels further distinguished the activities of KCP256 and sulfonylurea drugs as follows: (i) KCP256 did not inhibit $^{86}Rb^+$ efflux from MIN6 cells at 1 μM or lower concentrations, whereas glibenclamide inhibited the efflux in a concentration-dependent manner (Fig. 4), (ii) K_{ATP} current measured on MIN6 cells using a patch-clamp technique was not inhibited by 1 μM of KCP256, a concentration which is sufficient to stimulate insulin secretion, whereas tolbutamide did suppress the current (Fig. 5). However, high concentration of KCP256 may affect the K_{ATP} channel, since the $^{86}Rb^+$ efflux and the K_{ATP} current was inhibited slightly by 10 μM of KCP256 (Figs. 4 and 5).

Several insulinotropic reagents with a glucose-concentration-dependent action have been described, such as RX871024 (Zaitsev et al., 1996), efaroxane (Mourtada et al., 1997), LY389382 (Mest et al., 2001), pimobendan (Fujimoto et al., 1998), L-686,398 (Leibowitz et al., 1995), and JTT-608 (Itabashi et al., 2001). It was reported that RX871024 (Efendic et al., 2002) and efaroxan (Chan et al., 2001) stimulate insulin secretion via K_{ATP} channel-dependent and K_{ATP} channel-independent mechanisms. LY389382, a new improved insulin secretion potentiator, does not stimulate insulin secretion under low glucose concentrations even with high concentrations of the compound (Mest et al., 2001). These three compounds all contain an imidazoline moiety in their chemical structures. Although the action mechanism of insulinotropic imidazolines is not fully elucidated, these compounds have reported to act through imidazoline binding sites and to produce glucose-dependent and K_{ATP} channel independent effects on insulin secretion (Bleck et al., 2004). The action mechanism of KCP256 is less likely the same with that of RX871024, efaroxan and LY389382 because the binding of [^{3}H]KCP256 was just slightly displaced by these compounds only at much higher concentrations compared with those required for insulin secretion (data not shown). KCP256, however, contains an imidazoline like moiety, therefore, further investigations are required to elucidate whether KCP256 act through imidazoline binding sites. Compounds L-686,398, JTT-608 and pimobendan are from different chemical classes but they all inhibit the activities of a phosphodiesterase, probably phosphodiesterase III (Fujimoto et al., 1998; Leibowitz et al., 1995; Itabashi et al., 2001). KCP256 did not affect cAMP phosphodiesterase activities in MIN6 cell homogenates (data not shown), indicating that the insulinotropic action of KCP256 was not exerted via inhibition of phosphodiesterase. All these results demonstrate that KCP256, an imidazopurine compound, increases secretion of insulin via a unique mechanism, although further studies are required to clarify the precise action mechanism of KCP256.

In conclusion, KCP256 is a new glucose-dependent insulin secretion potentiator with an action mechanism different from that of sulfonylurea drugs. Since the insulinotropic activity of KCP256 only occurs at high concentrations of glucose, this novel drug may exhibit a decreased risk of drug-induced hypoglycemia compared with sulfonylurea drugs when treating patients with diabetes.

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