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Effect of a non-sulphonylurea hypoglycaemic agent, KAD-1229 on hormone secretion in the isolated perfused pancreas of the rat

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1 We examined the cooperative effect of a newly synthesized oral hypoglycaemic agent, KAD-1229 with glucose on insulin, glucagon and somatostatin secretion in the isolated perfused pancreas of the rat.

2 KAD-1229 stimulated concentration-dependently the first phase of insulin secretion without the second phase in the presence of 2.8 mM glucose, while it stimulated both the first and the second phase of insulin release in the presence of 5.6 mM glucose. It was confirmed that the first phase of insulin release is depolarization-induced release with no other additional signal transduction.

3 KAD-1229 also enhanced insulin release evoked by 16.7 mM glucose, a concentration known to inhibit the ATP-sensitive K^+ current completely.

4 A low concentration (2.8 mM) of glucose stimulated somatostatin release transiently, while a higher concentration (16.7 mM) of glucose exerted a sustained stimulation. KAD-1229 stimulated somatostatin secretion in a concentration-dependent manner irrespective of glucose concentrations.

5 When glucagon release was stimulated with 2.8 mM glucose, KAD-1229 inhibited this hypoglycaemia-induced glucagon secretion.

6 When pancreata from rats pretreated with streptozotocin (STZ) 60 mg kg^{-1} were perfused, the basal secretion of glucagon was markedly elevated, and the glucagon response to the low glucose was abolished. Further, the insulin and somatostatin responses to KAD-1229 were largely attenuated. KAD-1229 showed transient enhancement followed by inhibition of the glucagon release from the STZ-pretreated rat pancreas.

7 We conclude that KAD-1229 stimulates insulin and somatostatin release, while it inhibits glucagon release following transient stimulation.

Keywords: KAD-1229; rat perfused pancreas; insulin; glucagon; somatostatin

Introduction

Recently, a new synthesized non-sulphonylurea hypoglycaemic agent, calcium (2*S*)-2-benzyl-3-(*cis*-hexahydro-2-isoindolinylcarbonyl)propionate dihydrate (KAD-1229), has been developed (Ohnota *et al.*, 1994) (Figure 1). This agent exerts a rapid but short-lasting hypoglycaemic effect, and controls postprandial plasma glucose without extra hypoglycaemia (Ohnota *et al.*, 1994; 1995b). KAD-1229 has been reported to displace the binding of [^{3}H]-glibenclamide to the microsomal fraction from β-cell lines, inhibit ATP-sensitive K^+ current, and increase the intracellular calcium concentration (Mogami *et al.*, 1994). Its insulinotropic effect has been reported to be pharmacologically indistinguishable from sulphonylureas in that its effect is eliminated in the presence of diazoxide, inhibited by noradrenaline, and the maximum effect is not enhanced by the addition of a sulphonylurea, gliclazide (Ohnota *et al.*, 1995a). Its insulinotropic effect was delineated in the presence of 5.6 mM glucose, but it has been poorly investigated in the presence of the lower and the higher concentrations of glucose. In addition, the effect of this non-sulphonylurea compound on glucagon and somatostatin secretion is unknown. In the present study, we investigated the cooperative effect of KAD-1229 with glucose on pancreatic hormone secretion in the perfused pancreas of the rat. We also undertook the perfusion experiment using streptozotocin (STZ)-treated pancreata in order to examine the effect of the intra-islet interaction of hormones.

Methods

Perfusion of the rat pancreas

The perfusion of the rat pancreas was performed according to the modified method previously described (Kanno, 1972; Ohnota *et al.*, 1995a). Briefly, normally-fed Sprague-Dawley rats (SLC, Shizuoka, Japan) weighing 300–400 g were anaesthetized with 50 mg kg^{-1} pentobarbitone (Abbott, Illinoi, U.S.A.). All blood vessels located between the pancreas and other organs were ligated and amputated with little bleeding. Both of the superior mesenteric and the celiac arteries were cannulated, and used as the inlet of the perfusion medium. The portal vein was used as the outlet. The common bile duct was also cannulated. The pancreas with an adjacent 5–6 cm of duodenum was excised and transferred to a container maintained at 37°C. Perfusion was begun at a flow rate of 2.0 ml min^{-1}. The perfusion medium was modified Krebs-Ringer bicarbonate buffer containing (mM): NaCl 118, KCl 4.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, dextran T-70 4% (Pharmacia, Uppsala, Sweden) and 0.2% bovine serum albumin (Sigma, St. Louis, U.S.A.). After a 20 min

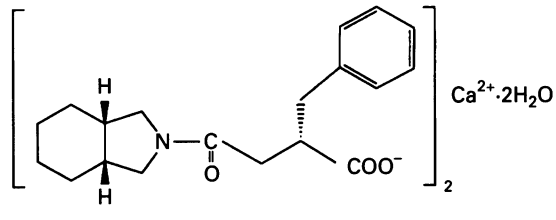

Figure 1 Chemical structure of KAD-1229.

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stabilizing period, experimental perfusion was initiated. The effluent was collected into ice cold tubes. After a 5 min basal perfusion with buffer containing 5.6 mM glucose, the pancreas was perfused for 30 min with the test substance, followed by a 10 min perfusion with basal buffer. The data are expressed with the dead volume uncorrected. Therefore, fractions collected between 7 and 36 min contained perfusate from the pancreas exposed to the test substance. When appropriate rats were treated with 60 mg kg^{-1} STZ (Sigma) administered intravenously 30 min before the operation for the isolation of the pancreas. Preparation of the pancreas for perfusion was performed as described above.

Materials and statistics

KAD-1229 was synthesized in our company. Streptozotocin was purchased from Sigma. Insulin was determined by a radioimmunoassay kit (Eiken, Tokyo, Japan) using rat insulin (Novo, Denmark) as a standard (Komatsu *et al.*, 1991). Glucagon (Daiichi, Tokyo, Japan) and somatostatin (Incstar, Minnesota, U.S.A.) were also determined by commercial kits according to the manufacturer's instructions (Nishino *et al.*, 1981; Arimura *et al.*, 1978). Statistical analyses were performed by Student's *t* test when necessary. $P<0.05$ was considered as significant.

Results

Insulinotropic effect of KAD-1229 (Figure 2)

In the presence of 2.8 mM glucose, KAD-1229 exerted a concentration-dependent stimulation of the first phase of insulin release, and its effect was comparable to that induced by 16.7 mM glucose (Figure 2a, c). During the second phase period (11–36 min), the insulinotropic effect of KAD-1229 did not exceed that of the pre-conditioning period with 5.6 mM glucose, although a small enhancement of basal insulin release was observed (Figure 2a). On removal of the drug, a paradoxical enhancement of insulin release was observed due to the return of glucose concentration to the basal level.

In the presence of 5.6 mM glucose, 0.1–10 μM KAD-1229 concentration-dependently stimulated both the first and the second phase of insulin release (Figure 2b). The removal of KAD-1229 induced a prompt decrease in insulin release.

When pancreata were stimulated with 16.7 mM glucose, the maximal stimulation of the first and second phase of insulin release was observed. In the presence of high glucose, KAD-1229 enhanced the second phase of insulin release with no effect on the first phase (Figure 2c), and its effect was apparently maximum at the concentration of 0.1 μM.

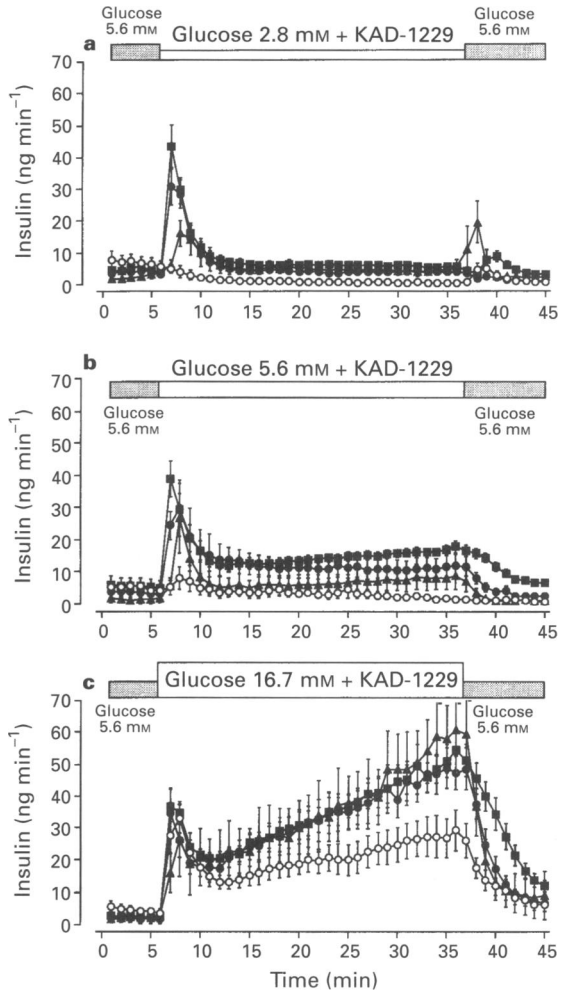

Figure 2 Effect of KAD-1229 on insulin release from the rat perfused pancreas in the presence of 2.8 (a), 5.6 (b), and 16.7 (c) mM glucose. The isolated pancreas of the rat was perfused as described in Methods, and stimulated with KAD-1229 in the presence of various concentrations of glucose between 7–36 min. Released insulin was determined by a radioimmunoassay kit. (○) Control; (▲) KAD 0.1 μM; (●) KAD 1 μM; (■) KAD 10 μM. Data were expressed as means ± s.e. ($n=3-7$).

Effect of KAD-1229 on glucagon secretion (Figure 3)

When the pancreas was perfused with 2.8 mM glucose, glucagon release was markedly stimulated (Figure 3a). Simultaneous application of 0.1–10 μM KAD-1229 with low glucose eliminated the hypoglycaemia-induced glucagon secretion. In the presence of 5.6 mM and 16.7 mM glucose, no change in glucagon secretion was observed with or without the drug (Figure 3b, c).

Effect of KAD-1229 on somatostatin release (Figure 4)

When the rat pancreas was stimulated with 2.8 mM glucose, a transient enhancement of somatostatin release was observed. KAD-1229 stimulated somatostatin release in the presence of 2.8, 5.6 and 16.7 mM glucose.

Effect of KAD-1229 on hormone secretion in the STZ-treated rat pancreas (Figure 5)

We examined the effects of glucose and KAD-1229 on hormone secretion in the STZ-treated rat pancreas in order to elucidate the paracrine effect of insulin on glucagon and somatostatin release. In the STZ-treated rat pancreas, insulin release induced by KAD-1229 was largely abolished, and basal glucagon secretion in the presence of 5.6 mM glucose was enhanced (Figure 5a, b). A glucagonotropic response to the stimulation with low glucose was not observed. KAD-1229 induced a transient increase in glucagon secretion and subsequently suppressed basal enhanced glucagon release (Figure 5b). The effect of KAD-1229 on the transient increase was not concentration-related, while later inhibition was concentration-dependent. Somatostatin release induced by KAD-1229 in the STZ-treated rat pancreas was also attenuated (Figure 5c).

Discussion

We have investigated the effect of the non-sulphonylurea, KAD-1229 on hormone secretion using the isolated pancreas of the rat. We used 5.6 mM basal glucose concentration for the pre-conditioning of the pancreas because it is close to the fasting plasma glucose level, and because a low glucose concentration is known to stimulate glucagon and somatostatin release. Several previous reports on hormone release by sulphonylureas in perfused rat pancreas have been published

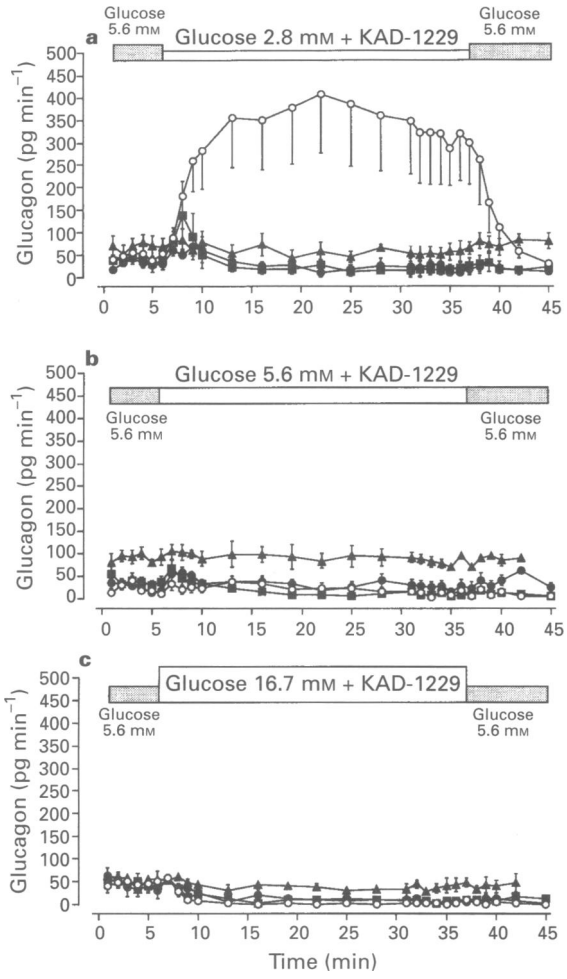

Figure 3 Effect of KAD-1229 on glucagon release from the rat perfused pancreas in the presence of 2.8 (a), 5.6 (b), and 16.7 (c) mM glucose. The pancreas was stimulated with KAD-1229 between 7–36 min. Released glucagon was determined by a radioimmunoassay kit. (○) Control; (▲) KAD 0.1 μM; (●) KAD 1 μM; (■) KAD 10 μM. Data were expressed as means ± s.e. ($n = 3-7$) in percentage of initial values.

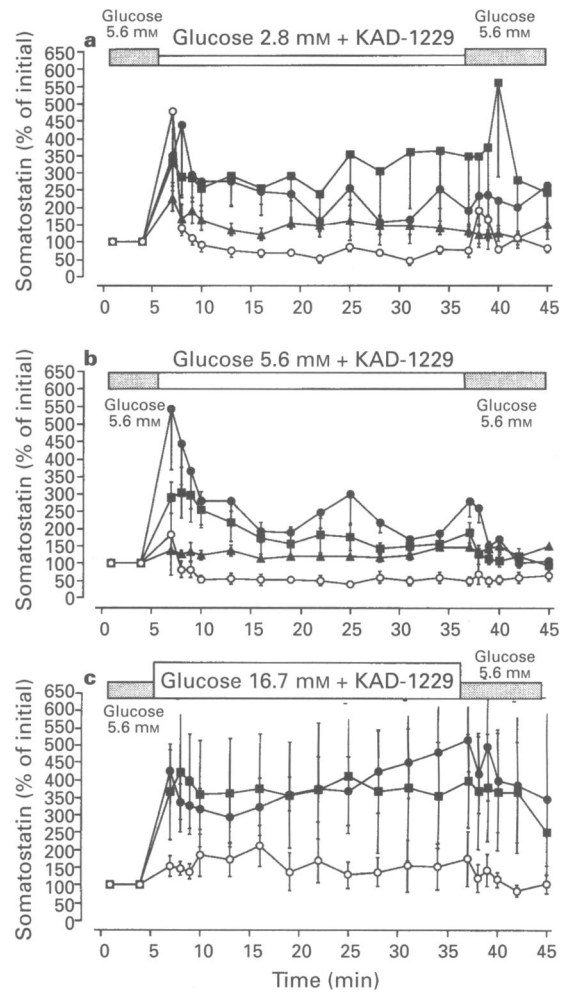

Figure 4 Effect of KAD-1229 on somatostatin release from the rat perfused pancreas in the presence of 2.8 (a), 5.6 (b), and 16.7 (c) mM glucose. The pancreas was stimulated with KAD-1229 between 7–36 min. Released somatostatin was determined by a radioimmunoassay kit. (○) Control; (▲) KAD 0.1 μM; (●) KAD 1 μM; (■) KAD 10 μM. Initial values of the control were 29.4 ± 8.7 pg min^{-1} in (a), 99.5 ± 54.0 pg min^{-1} in (b) and 41.4 ± 13.5 pg min^{-1} in (c). Data are expressed as means ± s.e. ($n = 3-7$).

(Gotfredsen, 1976; Grodsky *et al.*, 1977; Efendić *et al.*, 1979; Östenson *et al.*, 1986).

Efendić and coworkers described the effect of glibenclamide on insulin release in detail in the presence of various glucose concentrations using perfused rat pancreas (Efendić *et al.*, 1979). They reported that in the presence of low glucose (0–3.3 mM), glibenclamide stimulated both the first and the second phase of insulin release, while our present data show that KAD-1299 induces the first phase but not the second phase in the presence of 2.8 mM glucose. Our results are compatible with the report by Gotfredsen (1976) who demonstrated that, in the presence of 5.6 mM glucose, glibenclamide exerted a larger stimulation of the second phase than that induced by other sulphonylureas. Glibenclamide is known to exert a potentiating effect on insulin secretion in the pancreatic β-cells (Zawalich, 1991), while KAD-1229 is not known so far to exert any alternative effect other than the inhibition of ATP-sensitive K^+ channels. Therefore, in the present study, we showed that the first phase of insulin release in the perfused rat pancreas is a depolarization/Ca immobilization-associated insulin release. Namely, the first phase of insulin release reflects the insulinotropic effects of calcium influx (including resultant down stream signal transduction) without additional potentiating signal such as IP_3 turnover (Zawalich & Zawalich, 1988), cyclic AMP production (Ämmälä *et al.*, 1993) and glucose metabolism (Matschinsky *et al.*, 1991; Ghosh *et al.*, 1991). It is consistent with the work by Zawalich who showed that A23187, a calcium ionophore, mimicked the first phase of insulin release by glucose without the second phase in perifused rat islets (Zawalich *et al.*, 1983).

Another point we note is that KAD-1229 enhanced the second phase of insulin release in the presence of 16.7 mM glucose. This concentration of glucose inhibits ATP-sensitive K^+ current completely in patch-clamped β-cells (Soria *et al.*, 1991). Efendić *et al.* (1979) reported that glibenclamide had no effect on the insulin release induced by 16.7 mM and 33.3 mM glucose. However, in the present study, we conclude differently. When the pancreas was stimulated with a high concentration of glucose, each β-cell repeats depolarization and repolarization, and produces pulsatile insulin secretion (Atwater *et al.*, 1989). Therefore it is possible that KAD-1229 shifts the polarization/depolarization balance to the direction of depolarization by inhibiting ATP-sensitive K^+ current. In other words, it is suggested that activation of ATP-sensitive K^+ current involves repolarization of β-cells in the presence of high concentration of glucose.

With somatostatin release, 2.8 mM glucose induced a transient increase in somatostatin secretion, while 16.7 mM glucose showed sustained small enhancements. KAD-1229 stimulated somatostatin release in the perfused rat pancreas as reported

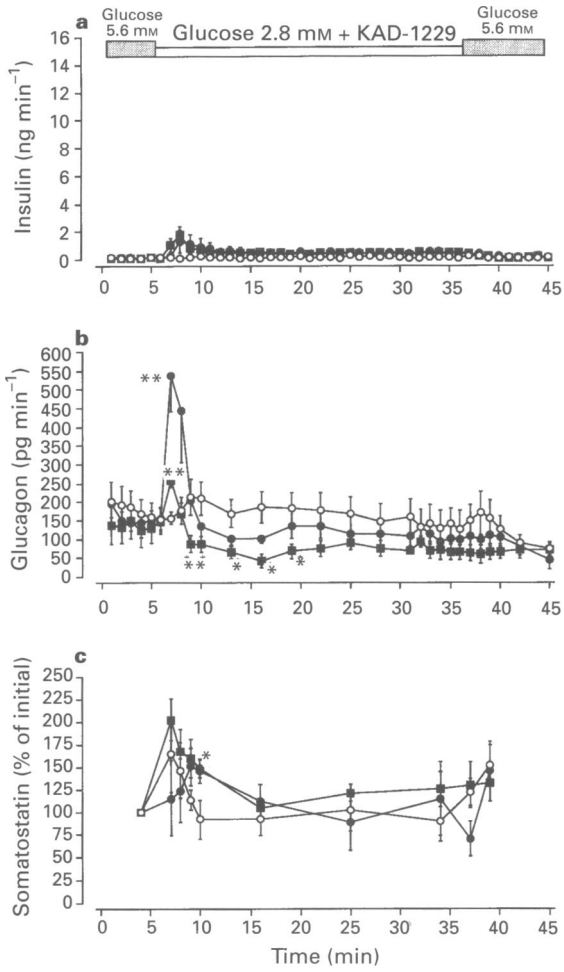

Figure 5 Effect of KAD-1229 on insulin (a), glucagon (b) and somatostatin (c) release from STZ-treated rat pancreas in the presence of 2.8 mM glucose. STZ (60 mg kg^{-1}) was administered intravenously 30 min before the operation for the isolation of the pancreas. Insulin, glucagon and somatostatin were determined by radioimmunoassay kits. (○) Control; (●) KAD 1 μM; (■) KAD 10 μM. The initial value of somatostatin in the control was 6.0 ± 0.9 pg min^{-1} (c). Data are expressed as means ± s.e. ($n = 4-5$). Significant difference from the control with $^{*}P < 0.05$ and $^{**}P < 0.01$.

with sulphonylureas previously (Ipp *et al.*, 1977; Efendić *et al.*, 1979). Its effect was sustained and relatively independent of glucose concentration. Efendić *et al.* (1979) found that stimulation of somatostatin release by glibenclamide was maximum in the presence of 0–3.3 mM glucose, and maximally attenuated in 6.7 mM glucose, and intermediate in 16.7 and 33.3 mM glucose. Although our results were similar, precise interpretation of these phenomena remains uncertain. However, enhancement of somatostatin release by KAD-1229 was demonstrated despite its non-sulphonylurea structure.

Glucagon release stimulated with 2.8 mM glucose was completely inhibited by KAD-1229. It is widely accepted that sulphonylureas inhibit glucagon release induced by low glucose or by arginine (Efendić *et al.*, 1979; 1980). KAD-1229 also exerted a potent inhibitory effect on glucagon release induced by 2.8 mM glucose. It is known that insulin and somatostatin released from β- and δ-cells respectively inhibit glucagon release from α-cells via intra-islet interaction. Therefore, the inhibitory effect of KAD-1229 on glucagon release induced by 2.8 mM glucose in the normal rat pancreas may be due, at least in part, to the intra-islet interaction of hormones. When the rats were pretreated with 60 mg kg^{-1} STZ, the basal glucagon secretion was markedly elevated. This STZ-treated pancreas showed little glucagon response to hypoglycaemic stimulation. KAD-1229 stimulates glucagon release transiently in the STZ-pretreated rat prancreas, and reveals subsequent concentration-dependent inhibition of basal enhanced glucagon release. In the pancreas from the STZ-treated rat, somatostatin response to KAD-1229 was also attenuated. Therefore, it is suggested that KAD-1229 acts on α-cells directly in the preparation as reported for a sulphonylurea (Östenson *et al.*, 1986).

The report by Grodsky and co-workers is relevant to the transient increase of glucagon release by KAD-1229 in the STZ-treated pancreas (Grodsky *et al.*, 1977). They found that a low dose of tolbutamide which did not induce insulin release, stimulated glucagon release transiently, while high doses of the drug inhibited it with the induction of insulin secretion. Our present results in the STZ-treated pancreas showing transient stimulation of glucagon release that was not dose-related are consistent with the report. In the absence of insulin release, KAD-1229 was thought to exert transient stimulation followed by the sustained inhibition of glucagon release via a direct action on α-cells.

In conclusion, we investigated the insulinotropic effect of KAD-1229 in the presence of various concentrations of glucose, and clarified the involvement of ATP-sensitive K^+ current with the first and the second phase of insulin release. Further, this non-sulphonylurea hypoglycaemic agent was demonstrated to stimulate somatostatin release, and to inhibit glucagon release following transient stimulation in the absence of insulin release. These effects of KAD-1229 on somatostatin and glucagon coincide with the effect of sulphonylureas which are structurally unrelated to KAD-1229, and suggest that the effects of these agents on glucagon and somatostatin release are related to the inhibition of ATP-sensitive K^+ current in the pancreatic α-and δ-cells.

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