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Short communication

Quinolizidine alkaloids isolated from *Lupinus* species enhance insulin secretion

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Abstract

We have analyzed the effect of quinolizidine alkaloids from *Lupinus* species upon insulin secretion. Isolated normal rat islets were incubated with 3.3, 8.3, and 16.7 mM glucose, in the presence or absence of different concentrations of lupanine (0.05, 0.5, and 1.0 mM), 13-α-OH lupanine, 17-oxo-lupanine, and 2-thionosparteine. Insulin release was measured by radioimmunoassay. While 2-thionosparteine enhanced insulin secretion at all glucose concentrations, lupanine did at 8.3 and 16.7 mM, and 13-α-OH lupanine or 17-oxo-lupanine only at 16.7 mM glucose. Diazoxide (0.1 mM) decreased the effect of all alkaloids, without suppressing it completely. Consequently, blockage of β-cell K_{ATP}-sensitive channels is at least one of the mechanisms involved in the enhancing secretagogue effects of quinolizidine alkaloids. The fact that 13-α-OH lupanine and 17-oxo-lupanine only exert their secretagogue effect at high glucose concentrations could be of additional value when considering their potential use in the treatment of type 2 diabetes.

Keywords: Insulin secretion; Quinolizidine alkaloid; Islet; *Lupinus*; K_{ATP} channel

1. Introduction

The *Lupinus* genus (Genistae) is widely distributed. Approximately 300 species are found in the Mediterranean countries, Africa, and North and South America (Glandstones, 1998). In the Mexican territory, more than 80 lupin species have been reported to exist (Dunn, 1979; Mc Vaugh, 1987). More than 150 alkaloids of the quinolizidine group have been identified in different *Lupinus* species (Wink, 1988). Sparteine and lupanine are the major quinolizidine alkaloids present in almost all American *Lupinus* species. Lupanine, 13-α-OH lupanine, and 17-oxo-lupanine have also been identified in the remaining non-American lupins (Wink, 1993); on the other hand, 2-thionosparteine is a recently synthetized quinolizidine alkaloid derivative (Wysocka et al., 1999).

It has been shown that oral administration of *Lupinus termis* and *Lupinus angustifolius* reduces high blood pressure and hyperglycemia in rabbits, rats, and mice (Cabo et al., 1984; Omran, 1996). Indeed, the addition of lupin seeds to the food of diabetic–hypercholesterolemic rabbits decreases cholesterol levels and postprandial hyperglycemia (Ahmed and Esmaiel, 1993). On the other hand, sparteine sulfate administered by intravenous infusion to normal men increases either basal or glucose-induced insulin secretion (Sgambato et al., 1986), simultaneously raising glucagon secretion in insulin-dependent diabetic subjects (Paolisso et al., 1987). Further, sparteine administration to patients with type 2 diabetes stimulates β-cell secretion, causing a fall in

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plasma glucose levels (Paolisso et al., 1988). Moreover, Pereira et al. (2001) have demonstrated that aqueous lupine extract enhances insulin release from isolated rat pancreatic islets, and sparteine has been shown to increase insulin secretion in vitro (Paolisso et al., 1985).

Blockage of the β-cell membrane K_{ATP} channel is a key step in the chain of events leading to the release of insulin by various secretagogues, including sulphonylureas (Mandarino and Gerich, 1985). The stimulatory effect of sparteine and aquous lupine extract upon insulin release by mouse and rat pancreatic islets involves a decrease in the K^+ permeability of β-cells (Paolisso et al., 1985; Pereira et al., 2001).

In an attempt to further characterize the activity and mechanism of action of quinolizidine alkaloids on insulin secretion, we studied the effect of several natural quinolizidine alkaloids and the synthetic 2-thionosparteine on glucose-induced insulin release by isolated normal rat pancreatic islets. Our results showed that all the quinolizidine alkaloids tested enhanced glucose-induced insulin secretion, and that the magnitude of this effect was related to the glucose concentration in the medium; the secretagogue effect of these quinolizidine alkaloids is due, at least partly, to the blockage of β-cell plasma membrane K_{ATP}-sensitive channels.

2. Materials and methods

2.1. Chemicals and drugs

Collagenase was obtained from Serva Feinbiochemica (Heidelberg, Germany), while bovine serum albumin fraction V and other reagents of the purest available grade were purchased from Sigma (St. Louis, MO, USA). Extraction of lupanine, 13-α-OH lupanine, and 17-oxo-lupanine was performed as described by Wink (1993), while 2-thionosparteine was synthesized as described by Wysocka et al. (1999).

2.2. Animals and islet isolation

Pancreatic islets were obtained from male Wistar rats (180–200 g) that were fed ad libitum and kept under controlled temperature and lighting conditions (12 h light and 12 h dark). Nonfasted animals were sacrificed in the morning; pancreata were entirely removed and the islets were isolated by collagenase digestion (Lacy and Kostianovsky, 1967).

2.3. Islet incubation

Groups of five freshly isolated islets were incubated for 60 min at 37 °C in 600 μl of Krebs–Ringer–bicarbonate (KRB) buffer with the following composition (in mM): 118 NaCl, 25.96 $NaHCO_3$, 4.74 KCl, 2.24 $CaCl_2$, 1.19 $MgSO_4 \cdot 7H_2O$, and 0.91 KH_2PO_4 (pH 7.4), previously gassed with a mixture of 5 % CO_2/95 % O_2 (vol/vol) and supplemented with 1% (wt/vol) bovine serum albumin and Trasylol™ (400 IU/ml) (Gagliardino et al., 1974). The KRB medium also contained 3.3, 8.3, or 16.7 mM glucose alone, or different quinolizidine alkaloids (lupanine, 13-α-OH lupanine, 17-oxo-lupanine, and 2-thionosparteine) at three different concentrations (0.05, 0.5, and 1.0 mM). Diazoxide (0.1 mM) was added to the incubation media containing different concentrations of glucose and 0.5 mM of the different quinolizidine alkaloids tested. This fixed concentration of quinolizidine alkaloids was selected because, in preliminary studies, as well as in the current one, quinolizidine alkaloids produced their maximal enhancing effect upon glucose-induced insulin secretion. In all cases, aliquots of the medium were collected at the end of the incubation period and frozen for subsequent insulin determinations by radioimmunoassay (RIA) (Herbert et al., 1965).

2.4. Data analysis

For the statistical evaluation of the data, we used both variance analysis and paired Student's *t* test.

The study protocol was approved by the institutional ethics committee, and experiments were performed following the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, DC, 1996).

3. Results

Table 1 shows the effect of quinolizidine alkaloids upon glucose-induced insulin secretion; only data significantly different from each control (islets incubated with glucose alone) are presented. Even though all quinolizidine alkaloid

Table 1
Enhancing effect of quinolizidine alkaloid compounds upon glucose-induced insulin secretion

Glucose (mM)	Quinolizidine alkaloids	0.05 mM	0.5 mM	1 mM
3.3 (100±9)	2-Thionosparteine	171±29*	408±103*	358±134[a]
8.3 (100±11)	2-Thionosparteine	740±149*	902±134*	152±34*
	Lupanine	136±6*	351±38*	159±15*
16.7 (100±6)	2-Thionosparteine	165±49[a]	168±29*	104±9*
	Lupanine	173±23*	420±124*	486±53*
	13-α-OH lupanine	90±6*	280±12*	283±63*
	17-Oxolupanine	153±18*	202±15*	133±13*

Each value represents the mean±S.E.M. (percentage of increase induced by each compound upon insulin secretion at 3.3, 8.3, and 16.7 mM glucose). Absolute basal values at 3.3 mM glucose, 0.32±0.03 ng/islet/h; 8.3 mM, 1.23±0.14 ng/islet/h; 16.7 mM, 7.42±0.44 ng/islet/h.

[a] NS (n=12).

* P<0.05 (n=12).

Table 2
Insulin secretion from three different experiments in the presence of 0.5 mM lupanine, 13-α-OH lupanine, 17-oxo-lupanine, and 2-thionosparteine, with or without 0.1 mM diazoxide

Glucose	Without diazoxide	With diazoxide
3.3 mM		
Control	100±9	78±16[a]
2-Thionosparteine	100±20	31±7*
8.3 mM		
Control	100±11	30±7*
2-Thionosparteine	100±15	53±8*
Lupanine	100±13	8±1*
16.7 mM		
Control	100±6	12±2*
2-Thionosparteine	100±18	45±3*
Lupanine	100±8	48±10*
13-α-OH lupanine	100±11	8±1*
17-Oxolupanine	100±14	17±3*

Values are mean±S.E.M. (percentage of each control without diazoxide) of each group.
[a] NS (n=12).
* P<0.05 (n=12).

concentrations tested enhanced insulin release, the highest release was achieved at 0.5 mM. Whereas 2-thionosparteine significantly enhanced the release of insulin at any glucose concentration tested, lupanine only enhanced such release in the presence of 8.3 and 16.7 mM glucose, and 13-α-OH lupanine and 17-oxo-lupanine only did at 16.7 mM glucose.

Since quinolizidine alkaloids produced a maximal enhancement of insulin release at 0.5 mM, this concentration was used to test the effect of 0.1 mM diazoxide upon quinolizidine alkaloid-induced insulin secretion.

The addition of diazoxide (0.1 mM) to the incubation media decreased significantly the insulin released in all the conditions studied (Table 2). However, the percentage of inhibition varied according either to the glucose concentration or the quinolizidine alkaloids considered, but never attained 100% values. The highest blocking effect of diazoxide was obtained in the presence of 13-α-OH lupanine or 17-oxo-lupanine. Conversely, the high diazoxide inhibition observed with lupanine at 8 mM glucose (92%) unexpectedly decreased to 52% in the presence of 16.7 mM glucose.

4. Discussion

Our results show that lupanine, 13-α-OH lupanine, and 17-oxo-lupanine, as well as the synthetic 2-thionosparteine, enhanced glucose-induced insulin release from isolated rat islets. However, their effect on insulin secretion was dependent on the glucose concentration in the incubation media. While 2-thionosparteine increased insulin release at every glucose concentration tested, lupanine required higher concentrations of glucose (8.3 and 16.7 mM), and 13-α-OH lupanine and 17-oxo-lupanine only increased insulin secretion at the highest glucose concentration tested (16.7 mM). The report of Pereira et al. (2001) showing that an aqueous lupine extract displayed a pronounced transient stimulatory effect on insulin secretion at 7 mM glucose and minimal effect at 3 mM glucose lends support to our current results.

Oral hypoglycemic agents have been used to control type 2 diabetes, and many of them act by stimulating insulin secretion (Mandarino and Gerich, 1985). Most of these agents stimulate insulin secretion even in the presence of low glucose concentrations, with the consequent risk of producing hypoglycemia—one of the most undesirable side effects of treating diabetes with oral agents such as sulphonylureas (Seltzer, 1972). Consequently, we could speculate that administration of compounds such as 13-α-OH lupanine and 17-oxo-lupanine, which stimulate insulin release only at high glucose concentrations, would decrease the risk of hypoglycemia. Similarly, since these two quinolizidine alkaloids are natural components of wild *Lupinus* species (Wink, 1988, 1993), they could also probably be obtained at a relatively low cost, an important factor when assessing the value of a potential therapeutic agent.

At the level of the β-cell, sulphonylureas and other newer insulin secretagogues such as meglitinides (Plosker and Figgitt, 2004) promote insulin secretion by altering the flux of potassium and calcium ions across the cell membrane. Similarly, sparteine, another quinolizidine alkaloid of lupin extracts, has also been shown to decrease potassium efflux as well as enhance calcium influx through the β-cell membrane (Paolisso et al., 1985). In fact, the effect of sparteine can be blocked by adding diazoxide, a potent opener of K_{ATP}-sensitive channels (Henquin and Meissner, 1982). In the present report, addition of diazoxide to the incubation media significantly decreased—with a different magnitude—the effect of quinolizidine alkaloids upon glucose-induced insulin release. These results support the assumption that at least one of the mechanisms used by quinolizidine alkaloids to enhance insulin secretion is the decrease of K^+ permeability in the β-cell plasma membrane. Since it has been proved that 13-α-OH-lupanine and 17-oxo-lupanine reduce high blood pressure (Kokou et al., 1984), probably other mechanisms are also involved in the secretagogue effect of quinolizidine alkaloids.

5. Conclusion

Our results demonstrate that different quinolizidine alkaloids stimulate insulin secretion in a glucose-dependent manner. This effect can be ascribed, at least partly, to the blockage of β-cell plasma membrane K_{ATP}-sensitive channels. The fact that 13-α-OH lupanine and 17-oxo-lupanine exert their secretagogue effect only in the presence of high glucose could be of additional value when considering these compounds as potential agents for the treatment of type 2 diabetes.

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