

INSULIN SECRETION EFFECT OF 2,17-DIOXOSPARTEINE, 17-THONOSPARTEINE, MULTIFLORINE AND 17-HYDROXY-LUPANINE ON RAT LANGERHAN'S ISLETS

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ABSTRACT

Some lupin species have been used as natural remedy for the treatment of diabetes. It has been also demonstrated that lupin quinolizidine alkaloids (QAs) like multiflorine possess hypoglycemic activity in normal mice and in a streptozotocin-induced diabetes model while lupanine and sparteine increase insulin secretion *in vitro* conditions. Based on this evidence, we evaluated, *in vitro*, the impact of multiflorine (natural QA), sparteine and lupanine derivatives on insulin secretion by pancreatic islets. Intact rat Langerhan's islets were isolated by collagenase digestion. Immediately after, five pancreatic islets were incubated with 3.3, 8.3, or 16.7 mM glucose, in the presence or absence of 0.5 mM of 2,17-dioxosparteine, 17-thionosparteine, multiflorine, or 17-hydroxy-lupanine. After 60 minutes, an aliquot of medium was collected and insulin levels determined by radioimmunoassay. The 2,17-dioxosparteine, 17-hydroxy-lupanine, and multiflorine at 0.5 mM enhanced insulin secretion in pancreatic islets incubated with 16.7 mM glucose. The alkaloid 17-thionosparteine enhanced insulin secretion at 8.3 mM glucose. These results indicate that multiflorine and lupanine and sparteine derivatives only stimulate the insulin secretion at high glucose concentration; one of the most desirable characteristic of the hypoglycemic drugs and that can potentially be used as therapeutic agents.

INTRODUCTION

At present, more than 150 different quinolizidine alkaloids (QAs) have been described for the genus *Lupinus*, including the sweet lupins (alkaloid poor varieties), and other wild species (alkaloid rich varieties), and function as chemical defense against predators (natural enemies). Lupanine and sparteine are the main QAs present in many lupin species from the old and new world (Wink, 1995).

Several studies have demonstrated the biological activity of these compounds, more in particular their hypoglycemic activity. Sparteine has been shown to induce hypoglycemia when administered to Type 2

diabetic subjects (Paolisso *et al.* 1988). On the other hand, chemical structure modification is an approach often used to improve the pharmacological properties of some compounds. In this regard, similar hypoglycemic *in vitro* effects have been observed with the synthetic sparteine derivative, 2-thiono-sparteine. In addition, lupanine and its derivatives enhanced glucose-induced insulin secretion *in vitro* conditions (Garcia Lopez *et al.* 2004). The most plausible action mechanism for the hypoglycemic activity of the QAs is similar to that of sulphonylureas (Paolisso *et al.* 1985, Pereira *et al.* 2001; Garcia Lopez *et al.* 2004). Besides, multiflorine, another lupin alkaloid, and its derivatives exert hypoglycemic activity in normal and streptozotocin induced diabetes mice (Kubo *et al.* 2000; Kubo *et al.* 2006). The objective of this study was to investigate the glucose induced insulin release activity of multiflorine (natural QA) and the sparteine and lupanine derivatives (2,17-dioxosparteine, 17-thionosparteine, and 17-hydroxy-lupanine) in pancreatic islets isolated from normal rats.

MATERIAL AND METHODS

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).

The alkaloids (-)-multiflorine and (+/-)-lupanine were isolated from *Lupinus albus* cv. BAC seeds (Wysocka and Przybył, 1994) and separated by short column chromatography with Kieselgel (Merck 70-230 mesh) and system EtOH:CHCl₃ (3:2) according to a method described previously (Wysocka, 1976; Edwards *et al.* 1954). (+/-)-Lupanine was transformed into 17-hydroxylupanine according to the literature method (Edwards *et al.* 1954). Also 2-thiono-17-oxosparteine was obtained from 2,17-dioxosparteine according to the method already described for 2-thionosparteine (Wysocka *et al.* 1999; Kolanos *et al.* 2001).

ANIMALS AND ISLET ISOLATION

Pancreatic islets were obtained from male Wistar rats (180-200 g) that were fed ad libitum and housed under controlled temperature and lighting conditions (12 h light and 12 h dark). No fasted animals were sacrificed in the morning; pancreata were entirely removed and the islets isolated by collagenase digestion (Lacy and Kostianovsky, 1967).

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 NaHCO₃, 4.74 KCl, 2.24 CaCl₂, 1.19 MgSO₄•7H₂O, and 0.91 KH₂PO₄ (pH 7.4), previously gassed with a mixture of 5% CO₂/95% O₂ (vol/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 and 0 (control) or 0.5 mM of one of three quinolizidine alkaloids (multiflorine, 2,17-dioxosparteine, 17-thionosparteine, and 17-hydroxy-

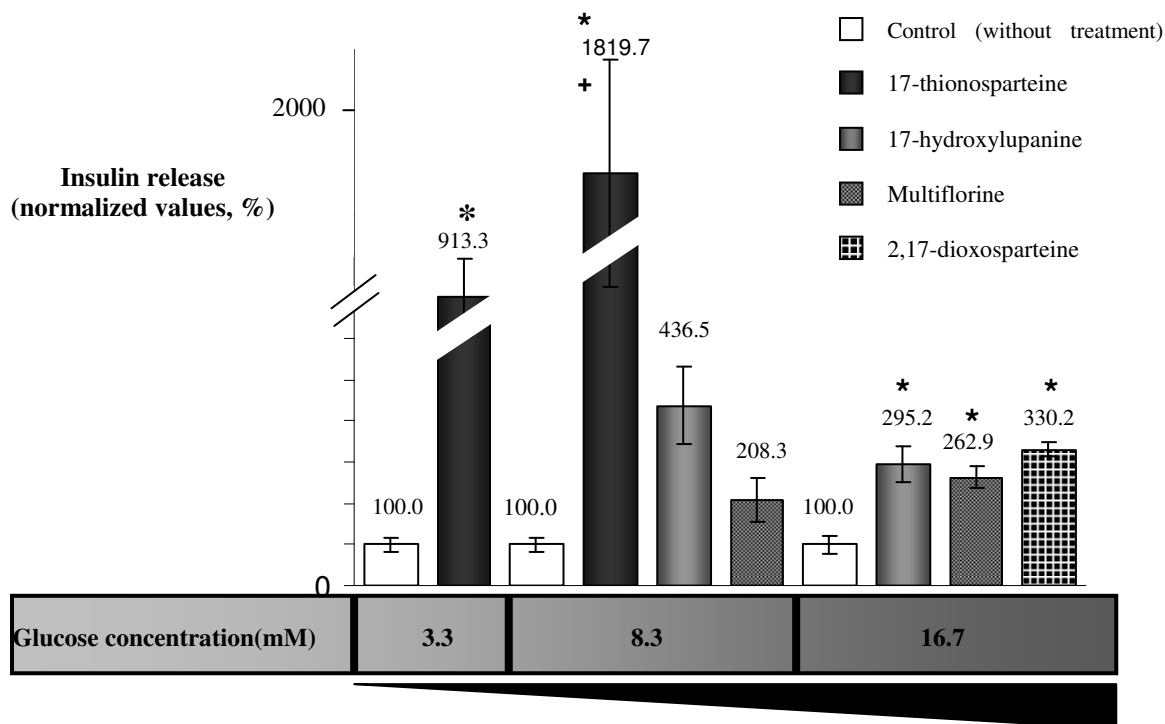
Lupanine) Aliquots of the medium were collected at the end of the incubation period for each compound tested. The insulin concentration was determined by radioimmunoassay (Herbert *et al.* 1965).

STATISTICAL ANALYSIS

Values of released insulin in each tested treatment were normalized with respect to their control (pancreatic islets without QA) at each specific concentration. The results of five independent experiments were averaged and reported as a percentage (±SEM). For the statistical analysis of the crude data, we used analysis of variance and multicomparative *Bonferroni* post-hoc test. P value less than 0.05 was considered as statistically different.

Ethic Considerations

The institutional ethics committee approved the study protocol, 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).



* P < 0.01, difference comparing each treatment vs its respective control.
 + P < 0.00, intertreatment difference at each specific glucose concentration.

RESULTS AND DISCUSSION

The effect of the tested QA upon glucose-induced insulin secretion is shown in Fig. 1; Previous work has shown a dose dependent effect of the QA's, therefore only one concentration (0.5 mM) was evaluated in this study. (Garcia Lopez *et al.* 2004). Only data significantly different from control (islets incubated with glucose alone) are shown.

As can be seen in Fig. 1 insulin release by QA's was glucose dependent. Multiflorine and 17-hydroxy-Lupanine increased insulin release in islets incubated at 8.3 and 16.7 mM glucose concentration. Similarly, 17-thionosparteine enhanced insulin secretion, however, it occurred only in islets incubated in 3.3 to 8.3 mM glucose media. 2,17-dioxoesparteine only increased insulin secretion at high glucose levels (16.7 mM). The highest effect on insulin release was observed with 17-thionosparteine treatment at 8.3 mM glucose (~18 times more than control and ~4 to 8 times higher in comparison to 17-hydroxy-lupanine and multiflorine treatments respectively). However, no significant effect was observed with 17-thionosparteine at 16.7mM glucose (62%, data not shown in the Fig. 1).

Multiflorine has been shown to exert an *in vivo* hypoglycemic effect (Kubo *et al.* 2000 and Kubo *et al.* 2006). The results of this study, as well as other obtained in our laboratory, indicate that the hypoglycemic effect of this QA may be related to its ability to enhance insulin release in pancreatic islets. Interestingly, it has been reported a Mexican wild specie *L. madrensis* rich in multiflorine that could be used as a hypoglycemic remedy (Przybylak *et al.* 2005).

Chemical modification of molecules has been used to enhance their biological activity. In this study and others, we have found that modification of the QA 17-thionosparteine, modified by adding a sulfur atom in the sparteine molecule, enhances the insulin release properties. Although, an increasing glucose-induced insulin secretion effect was expected for 17-thionosparteine treatment, it was not observed stimulation at the 16.7mM glucose concentration. Strikingly, a similar effect was also observed in 2-thiono-esparteine (another sulfur chemically-modified molecule at the position 2, Garcia-Lopez *et al.* 2004). Pharmacological compounds that act by stimulating the insulin secretion belong to the sulphonylureas. The therapeutic disadvantage of this type of compounds is that their use could potentially result in a hypoglycemic stage (Mandarino and Gerich., 1985). These compounds have been shown to enhance insulin secretion even at low glucose concentrations. Interestingly, some QA here studied only act at high glucose concentration representing an important therapeutic approach of these compounds.

CONCLUSION

A novel contribution of this study is the demonstration that other modified QA (lupanine and sparteine derivatives) can be considered as hypoglycemic drugs; however further *in vivo* experiments should be undertaken.

Our results are quite interesting because multiflorine, 17-hydroxy-lupanine and 2,17-dioxoesparteine only release insulin at high glucose concentrations, one of the most desirable effects of the hypoglycemic agents.

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LITERATURE CITED

- Edwards, O.E., F.H. Clarke and B. Douglas. 1954. *J. Can. Chem.*32: 235.
- Gagliardino, J.J., C. Nierle and E.F. Pfeiffer. 1974. The effect of serotonin on *in vitro* insulin secretion and biosynthesis in mice. *Diabetologia* 10: 411-4.
- García López, P.M., P.G. de la Mora, W. Wysocka, B. Maiztegui, M.E. Alzugaray, H. Del Zotto and M.I. Borelli. 2004. Quinolizidine alkaloids isolated from *Lupinus* species enhance insulin secretion. *Eur. J. Pharmacol.* 504(1-2): 139-42.
- Herbert, V., K.S. Lau, C.W. Gottlieb and S.J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* 25: 1375-84.
- Kolanoś, R., W. Wysocka, M. Kwit and J. Gawroński. 2001. *Tetrahedron: Asymmetry* 12: 1337.
- Kubo, H., M. Inoue, J. Kamei and K. Higashiyama. 2006. Hypoglycemic Effects of Multiflorine Derivatives in Normal Mice, *Biol. Pharm. Bull.* 29(10): 2046-50.
- Kubo, H., J. Kobayashi, K. Higashiyama, J. Kamei, Y. Fujii and S. Ohmiya. 2000. The hypoglycemic effect of (7R*,9aS*)-7-phenyl-octahydroquinolizin-2-one in mice. *Biol. Pharm. Bull.* 23(9): 1114-7.
- Lacy, P.E. and M. Kostianovsky. 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16: 35-39.
- Mandarino, L.J. and J.E. Gerich. 1985. Prolonged sulphonylureas administration decrease insulin resistance and increase insulin secretion in non-insulin dependent diabetes mellitus. Evidence for improved insulin action at postreceptor site in hepatic as well as extra-hepatic tissue. *Diabetes Care* 7(Suppl. 1): 89-90.
- Paolisso, G., M. Nenquin, F. Schmeer, H. Mathot, H.P. Meissner and J.C. Henquin. 1985. Sparteine increases insulin release by decreasing the K⁺ permeability of the B-cell membrane. *Biochem. Pharmacol.*34: 2355-61.
- Paolisso, G., S. Sgambato, N. Passariello, G. Pizza, R. Torella, P. Tesauro, M. Varricchio and F. D'Onofrio. 1988. Plasma glucose lowering effect of sparteine sulphate infusion in non-insulin dependent (type 2) diabetic subjects. *Eur. J. Clin. Pharmacol.* 34: 227-232.

- Pereira F., R. Quedraogo, P. Lebrum, R. Barbosa, A. Cunha, R. Santos and L. Rosario. 2001. Insulinotrophic action of white lupine seeds (*Lupinus albus* L.): effects on ion fluxes and insulin secretion from isolated pancreatic islets. *Biomed. Res.* 22: 103-9.
- Przybylak, J.K., D. Ciesioka, W. Wysocka, P.M. García-López, M.A. Ruiz-López, W. Wysocki and K. Gulewicz. 2005. Alkaloid profiles of Mexican wild lupin and an effect of alkaloid preparation from *Lupinus exaltatus* seeds on growth and yield of paprika (*Capsicum annuum* L.) *Industrial Crops and Products* 21(1): 1-7.
- Wink, M., C. Meissner and L. Witte. 1995. Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. *Phytochemistry* 38: 139-53.
- Wysocka, W.J. 1976. *Chromatography* 11: 235.
- Wysocka, W., R. Kolanoś, T. Borowiak and A.J. Korzański. 1999. *Mol. Struct.* 474: 207.
- Wysocka, W. and A., Przybył. 1994. *The Science of Legumes* 1: 37.