PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 345–349 (1987)

RAPID COMMUNICATION

YOHIMBINE INCREASES PLASMA INSULIN CONCENTRATIONS OF DOGS

WALTER H. HSU, DAVID D. SCHAFFER AND MAURICIO H. PINEDA

Department of Veterinary Physiology and Pharmacology
Iowa State University, Ames, Iowa 50011

Abstract. Recent evidence suggests that catecholamines inhibit insulin release by stimulating α_2-adrenoreceptors in β-cells of the pancreatic islets. In the present study, iv injections of 0.1 and 0.3 mg/kg of yohimbine, an α_2-adrenoreceptor antagonist, resulted in increased plasma insulin and decreased plasma glucose concentrations in the dog. The use of α_2-adrenoreceptor antagonists may be of value in non-insulin-dependent diabetic patients by counteracting the inhibitory influence of endogenous catecholamines.

The pancreatic islets of Langerhans are richly supplied with sympathetic and parasympathetic nerves (1) suggesting autonomic regulation of insulin release and secretion. Electrical stimulation of the pancreatic sympathetic nerves inhibited insulin release (2-4). This effect was blocked by phentolamine (3), a nonselective α-adrenoreceptor antagonist. Therefore, activation of α-adrenoreceptors may inhibit insulin release (5).

The inhibitory effects of α-adrenoreceptor agonists such as norepinephrine, epinephrine, clonidine, and xylazine on insulin release were blocked by yohimbine, an α_2-adrenoreceptor antagonist (6), whereas α_1-adrenoreceptor antagonists such as phenoxybenzamine and prazosin produced little or no blockade (7-11). These results suggested that the inhibitory sympathetic influence on insulin release was mediated by α_2-adrenoreceptors. This receptor activity for catecholamines has been shown in β-cells isolated from the pancreatic islets (12). Accordingly, blockade of α_2-adrenoreceptors should result in increased plasma insulin concentrations by preventing the influence of endogenous catecholamines on β-cells of the pancreatic islets. Phentolamine (13-16) and yohimbine (17) caused a transient increase in plasma insulin concentrations; however, to our knowledge, no published reports describe a prolonged stimulatory effect of α_2-adrenoreceptor antagonists on insulin release.

Xylazine, an α_2-adrenoreceptor agonist used as a sedative in veterinary medicine, suppressed the rise in plasma insulin produced by iv injection of glucose in dogs (18). Administration of yohimbine 5 min after the injection of xylazine reversed the effects of xylazine on plasma insulin and glucose concentrations, while yohimbine alone increased plasma insulin and decreased plasma glucose concentrations. These results support the hypothesis that α_2-adrenoreceptors in β-cells of the pancreatic islets are involved in the inhibition of insulin release. Since yohimbine alone induced a marked increase in insulin release, the present study was undertaken to investigate the time course and dose response of yohimbine on plasma insulin and glucose concentrations in the dog.

Materials and Methods

Fifteen male mixed-breed dogs weighing 10 to 22 kg were used. The dogs were allowed free access to water, but food was withheld for 14 to 18 hr prior to treatment. Dogs were randomly assigned to 3 groups of 5 dogs each, and received 1 of 3 treatments: 1) 0.1

0037-9727/87 $1.50

ml/kg of 0.15 M NaCl, 2) 0.1 mg/kg yohimbine, and 3) 0.3 mg/kg yohimbine. Dogs had a resting period of 7 days between experiments. A catheter was implanted into the jugular vein 60 min before the start of the experiment to avoid the stress caused by venipuncture. The dogs were conscious and unrestrained during the experiment. In experiment 1, each treatment was given iv at 0 time, and blood samples were taken from the jugular vein immediately before the treatment and at 5, 15, 30, 60, 120, and 240 min posttreatment. In experiment 2, the dogs were given the same saline or yohimbine treatment as in experiment 1 except that a solution of glucose (50%, wt/vol) was injected iv as a bolus at a dose of 0.6 g/kg immediately after the saline or yohimbine treatment. Yohimbine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water at a concentration of 2 mg yohimbine base per ml. The blood samples were collected from the venous catheter immediately before the injections and at 5, 15, 30, and 60 min after the injections. Plasma glucose was determined by the ferricyanide method (19) using an AutoAnalyzer (Technicon, Chauncy, N.Y.). Plasma insulin was measured by a double antibody radioimmunoassay (20) using a kit from Amersham Co., Arlington Heights, IL. This kit has been validated for evaluation of canine plasma samples (21).

Data on plasma concentrations of insulin were transformed to $\sqrt{x}$ to normalize the variance (22) due to the small number of observations per treatment group. Analysis of variance (ANOVA, 22) was used to determine the effects of dog, treatment, and time on plasma concentrations of glucose and insulin, and to compare the plasma concentrations of glucose and insulin before and after treatment. The dog x time interaction was used as an error term to determine the effect of time. The conservative F value (23) was used to establish significance for the effect of time. Tukey's ω-procedure (22) was used to test for differences between means of end points for which the ANOVA indicated a significant ($P < 0.05$) F ratio.

Results

In experiment 1, both doses of yohimbine increased plasma insulin concentrations within 5 min after yohimbine injection (Fig. 1A). The 0.1 mg/kg dose of yohimbine increased ($P < 0.05$) plasma insulin concentrations for at least 30 min. The increase was maximal (approximately 4 fold) 15 min after treatment. The 0.3 mg/kg dose of yohimbine increased ($P < 0.05$) plasma insulin concentrations for at least 120 min. The increase was maximal (approximately 7 fold) in the blood samples collected between 5 and 30 min after treatment (Fig. 1A). The 0.1

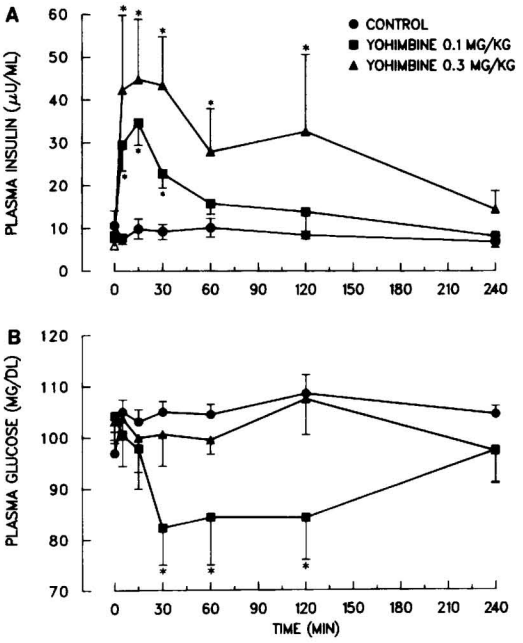

Figure 1. Effect of yohimbine on plasma glucose and insulin concentrations. Yohimbine was given iv at 0 min. For each treatment group, each point represents the mean ± SEM for 5 dogs; *$P < 0.05$ when compared with the 0 time value within each treatment group or with the control group at the corresponding time. No significant differences were observed between the yohimbine-treated groups. The F ratios were as follows: Insulin levels: treatment effect, $P < 0.05$; time effect, $P < 0.0001$; treatment x time interaction, $P < 0.0001$. Glucose levels: treatment effect, $P < 0.025$; time effect, $P > 0.1$; treatment x time interaction, $P < 0.0001$.

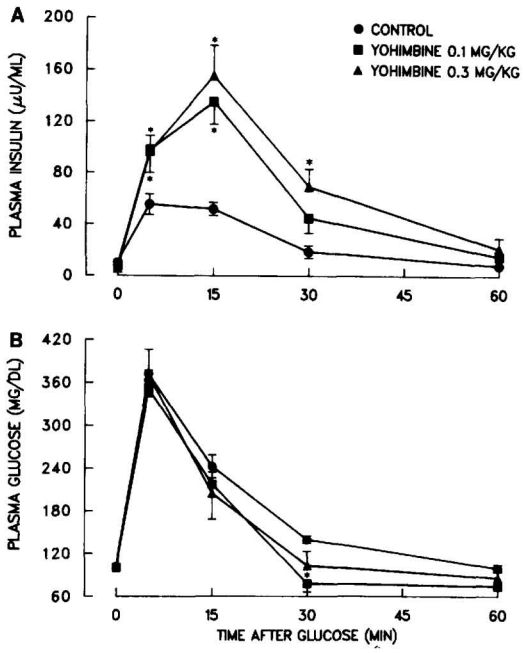

Figure 2. Effect of yohimbine on glucose-stimulated plasma insulin concentrations. Glucose (0.6 g/kg) was injected iv with and without yohimbine. For each treatment group, each point represents the mean ± SEM for 5 dogs; *P < 0.05 when compared at the corresponding time with the glucose control group. No significant differences were observed between the yohimbine-treated groups. The F ratios were as follows: Insulin levels: treatment effect, P = 0.025; time effect, P < 0.0001; treatment x time interaction, P < 0.0001. Glucose levels: treatment effect, P < 0.05; time effect, P < 0.0001; treatment x time interaction; P > 0.1.

mg/kg dose of yohimbine decreased plasma glucose concentrations significantly (P < 0.05) from 30 to 120 min after yohimbine injection as compared to the controls (Fig. 1B). Although the 0.3 mg/kg dose of yohimbine increased plasma insulin concentrations, this dose of yohimbine did not alter (P > 0.05) plasma glucose concentrations with respect to the controls.

In experiment 2, iv injections of glucose increased both insulin and glucose concentrations in the plasma (Fig. 2). Plasma glucose and insulin were increased (P < 0.05) for at least 15 min. Yohimbine and glucose appeared to synergistically increase plasma insulin concentrations. Insulin concentrations were 3 to 4 times higher 15 min after treatment with yohimbine and glucose than when yohimbine was given alone (Figs. 1A and 2A). By 15 min after treatment, the plasma insulin concentrations of dogs treated with yohimbine at the doses of 0.1 mg/kg and 0.3 mg/kg increased 17 and 28 fold, respectively, over the 0 time concentrations. In experiment 2, yohimbine also lowered (P < 0.05) plasma glucose 30 min after the 0.1 mg/kg dose was given, but yohimbine did not change plasma glucose (P > 0.05) when given at the 0.3 mg/kg dose (Fig. 2B).

Discussion

In the present study, iv injections of 0.1 and 0.3 mg/kg of yohimbine resulted in increased plasma insulin concentrations of dogs. Yohimbine at the dose of 0.1 mg/kg also decreased plasma glucose concentrations. Although the higher dose of yohimbine at 0.3 mg/kg elevated plasma insulin concentrations, it did not lower plasma glucose concentrations significantly. Yohimbine at 0.3 mg/kg has been shown to increase plasma norepinephrine concentrations in dogs (24). Thus, yohimbine at 0.3 mg/kg may have caused the release of more norepinephrine from the adrenergic nerve terminal than did the 0.1 mg/kg dose. Perhaps the higher norepinephrine concentrations then raised glucose output by facilitating hepatic glycogenolysis through activation of α_1-adrenoreceptors (25, 26), offsetting the low plasma glucose normally induced by increased concentrations of insulin. Additional studies using α_1-adrenoreceptor antagonists are needed to test this hypothesis. Also, it would be necessary to determine whether yohimbine raises catecholamines, glucagon, or cortisol concentrations in the blood, because these hormones might blunt the hypoglycemic effect of yohimbine.

In addition to α_2-adrenoreceptor blocking activity, yohimbine has antidopaminergic and antiserotonergic

activities (6). The participation of dopamine and serotonin in the regulation of insulin release is obscure (1). Nevertheless, the results of the present study cannot rule out the possibility that these additional activities are involved in the insulin-releasing effect of the drug.

Yohimbine alone does not alter insulin release from β-cells of the pancreatic islets in vitro (7, 8, 10). But in this study, yohimbine increased insulin release in vivo. On the basis that yohimbine is an α_2-adrenoreceptor antagonist (6), the results of the present study suggested that the inhibitory influence of catecholamines on insulin release was mediated by α_2-adrenoreceptors in β-cells of the pancreatic islets.

Excessive sympathetic activity has been suggested as an important factor in the pathogenesis of non-insulin-dependent diabetes mellitus (27, 28). Since α_2-adrenoreceptors mediate the inhibitory effect by the sympathetic nervous system on insulin release (7-11), the use of α_2-adrenoreceptor antagonists, such as yohimbine, may be of value in the treatment of non-insulin-dependent diabetes mellitus. This is supported by the recent findings that a new α_2-adrenoreceptor antagonist (DG-5128) improved insulin release and glucose disposal in human patients (29).

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Received November 5, 1986.
P.S.E.B.M. 1987, Vol. 184.
Accepted December 18, 1986.