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Milrinone efficiently potentiates insulin secretion induced by orally but not intravenously administered glucose in C57BL6J mice

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Abstract

To study the effect of phosphodiesterase (PDE) 3 inhibition on plasma insulin and glucose levels, the selective PDE 3 inhibitor milrinone (0.25, 1.0, and 2.5 mg/kg) was given orally to anesthetized CL57Bl/6J mice 10 min before a gastric glucose gavage (150 mg/mouse). It was found that milrinone augmented the glucose-mediated increase in plasma insulin at 1.0 and 2.5 mg/kg without, however, any improvement in glucose elimination. In contrast, when given 10 min before intravenous glucose (1 g/kg), milrinone (1 mg/kg) did not affect the insulin response to glucose. The increase in glucagon-like peptide-1 (GLP-1) levels after gastric glucose was not altered by milrinone. However, the PDE3 inhibitor augmented the insulin response to intravenous GLP-1 (2.8 nmol/kg). We therefore conclude that PDE3 inhibition by milrinone augments insulin secretion in vivo in mice after oral but not after intravenous glucose, which may be explained by enhanced response to the cAMP-dependent insulinotropic action of endogenously released GLP-1.

Keywords: Phosphodiesterase; Type 2 diabetes; Milrinone; Glucagon-like peptide-1; Insulin secretion

1. Introduction

The second messenger cAMP plays a critical role in pancreatic β cells to potentiate glucose-mediated insulin secretion. In particular islet neuropeptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP) and gut incretins, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), mediate their potentiating effects on glucose-mediated insulin secretion mainly by increasing β cell cAMP (Ahrén, 1998; Ahrén, 2003; Meier et al., 2002; Filipsson et al., 2001). The cellular level of cAMP is determined by the net actions of cAMP forming adenylate cyclases and cAMP degrading cyclic nucleotide phosphodiesterases (PDEs). Eleven gene families of PDEs (PDE1-11) have been identified, which have distinct properties, and are regulated by different mechanisms and show different expression patterns in different cells (Manganiello and Degerman, 1999). The PDE3 family (Shakur et al., 2001; Degerman et al., 2004) consists of two subfamilies, PDE3A and PDE3B. PDE3B is expressed in tissues of importance for energy homeostasis, such as white and brown adipocytes, hepatocytes and in pancreatic β cells. The adipocyte PDE3B has been extensively studied and has been shown to have a key role in the antilipolytic action of insulin (Eriksson et al., 1995; Hagström-Toft et al., 1995). The hepatocyte PDE3B is believed to be involved in insulin-mediated inhibition of glycogenolysis (Beebe et al., 1985). In pancreatic β cells, PDE3B has been suggested to have an important role in glucose- as well as cAMP-mediated insulin secretion. Thus, PDE3 inhibitors potentiate glucose-mediated and GLP-1-mediated insulin secretion using different cell models including isolated islets (Pyne and Furman, 2003; Parker

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et al., 1995; Shafiee-Nick et al., 1995; Zhao et al., 1997). Furthermore, overexpression of PDE3B using the adenovirus system inhibits glucose-mediated insulin secretion as well as GLP-1-mediated potentiation of glucose-mediated insulin secretion (Härndahl et al., 2002).

Because a defective insulin secretion from pancreatic β cells is an important pathophysiologic event underlying type 2 diabetes, inhibition of the β cell PDE3 is a potential target for the treatment of the disease. A problem is, however, that the concomitant inhibition of PDE3 in other tissues of importance for energy homeostasis may cause negative effects following in vivo administration. Some in vivo studies indicate that PDE3 inhibitors increase plasma insulin (El-Metwally et al., 1997; Parker et al., 1997; Cases et al., 2001; Cheung et al., 2003; Yang and Li, 2003), although this is not always the case (Nakaya et al., 1999). In addition, the effects of PDE3 inhibitors on glucose elimination and insulin sensitivity vary in different studies using different models and different inhibitors. In particular, different effects may be explained by different degree of cAMP formation in the β cells, possibly being higher after oral than after intravenous glucose due to the action of the incretin hormones.

In this study, we have tested this hypothesis by studying the effect of oral administration of the PDE inhibitor, milrinone, on the insulin response to oral or intravenous glucose and to GLP-1 in mice.

2. Material and Methods

2.1. Animals

Female mice of the C57BL/6J strain weighing 20–30 g (Taconic AB, Ry, Denmark) received a standard rodent chow diet (Lactamine, Stockholm, Sweden). The mice had free acess to food and water. Lund University Ethic Committee approved the study.

2.2. Gastric glucose tolerance test

The studies were performed in late morning after removal of food from the cages 4 h earlier. The animals were anesthetized with an intraperitoneal injection of midazolam (Dormicum®, Hoffman-La-Roche, Basel, Switzerland; 0.2 mg/mouse) as well as a combination of fluanison (0.4 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm®, Janssen, Beerse, Belgium). After induction of anesthesia, milrinone (Corotrop, Sanofi-Synthelabo; 0.25, 1.0, or 2.5 mg/kg body weight, 10 μl/g body weight) was administrated through a gavage tube placed in the stomach. After 10 min, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes, after which D-glucose (150 mg/mouse) was administrated through the gavage tube. Additional blood samples were taken after 10, 30, 60, and 120 min. The samples were taken in heparinized tubes and stored on ice. Following centrifugation, plasma was separated and stored at −20 °C until analysis.

2.3. Intravenous glucose tolerance tests

The mice were anesthetized as above, whereafter milrinone (1 mg/kg body weight) was administered through a gavage tube placed in the stomach. At 10 min later, D-glucose (1 g/kg body weight) was injected intravenously. Blood samples were taken at 0, 5, 10, 20, and 50 min from the retrobulbar, intraorbital, capillary plexus in heparinized tubes. Following centrifugation at 4 °C, plasma was separated and stored at −20 °C until analysis. In one series of experiments, synthetic GLP-1 (Peninsula Merseyside, UK; 2.8 nmol/kg) was injected intravenously together with glucose.

2.4. Analysis

Plasma insulin was determined radioimmunochemically with the use of a guinea pig antirat insulin antibody, ^{125}I-labelled porcine insulin as a tracer and rat insulin as standard (Linco Res., St Charles, MO, http://www.lincoresearch.com). Plasma GLP-1 was measured by a radioimmunoassay after extraction of plasma samples with ethanol; 400 μl 0.05 mol/l sodium phosphate buffer, pH 7.5, containing 6% albumin, and 0.1 mol/l NaCl was added to 100 μl mouse plasma on ice and was mixed well. The mixture was then extracted with 70% ethanol (v/v; final dilution), and after vacuum centrifugation, the residue was reconstituted in assay buffer and assayed as previously described (Ørskov et al., 1994). The antiserum (code no. 89390) raised in rabbits against synthetic GLP-1 is highly specific for C-terminal intestinal GLP-1 and recognizes mouse GLP-1. The sensitivity using this procedure is 5 pmol/l, and the intra-assay coefficient of variation is 10%. The recovery of GLP-1 added to mouse plasma is within ±20% of expected values. Plasma glucose was determined with the glucose oxidase method.

2.5. Calculations and statistics

Data are reported as means±S.E.M. Incremental insulin levels during the oral glucose tolerance test were estimated as the suprabasal area under the 0–120 min insulin curve (AUC_{insu}) as calculated by the trapezoid rule. From the intravenous glucose tolerance test, the acute insulin response to intravenous glucose was calculated as the mean of suprabasal 1- and 5-min values. The glucose tolerance was quantified from the glucose elimination constant (K_G; expressed as percent elimination of glucose per minute) during the intravenous glucose tolerance test as the reduction in circulating glucose between 1 and 20 min after intravenous administration following logarithmic transformation of the individual plasma glucose values. Student's

unpaired *t*-tests were performed for estimating differences between the groups.

3. Results

3.1. Effect of milrinone on insulin levels and plasma glucose; comparison between gastric and intravenous glucose administration

Fig. 1 shows that milrinone (1.0 and 2.5 mg/kg) given orally 10 min before a gastric glucose gavage to mice augmented the plasma insulin when compared to mice given the glucose gavage without milrinone. Thus, at 2.5 mg/kg, AUC_{ins} was 1.29±0.23 μmol/l×min in the group given milrinone versus only 0.18±0.04 μmol/l×min in the controls given glucose alone ($P<0.001$), and the corresponding figures in the experimental series with 1.0 mg/kg were 1.01±0.19 and 0.16±0.02 μmol/l×min, respectively ($P<0.001$). At the lowest examined dose, 0.25 mg/kg, milrinone did not significantly affect insulin levels (data not shown). In spite of the marked increase in plasma insulin, no improvement of glucose elimination was observed because glucose levels showed a tendency of being higher in the milrinone-treated mice than in controls, both at 2.5 and 1 mg/kg (Fig. 1). In contrast, as seen in Fig. 2, when milrinone (1 mg/kg) was administered orally at 10 min before an intravenous glucose injection, the insulin response to glucose was not altered; the acute insulin response being 618±56 pmol/l in the milrinone-treated group versus 598±65 pmol/l in the control group (NS). Furthermore, milrinone did not significantly alter glucose elimination, although there was a trend of higher glucose level at 20 min in the milrinone group (left panel of Fig. 2).

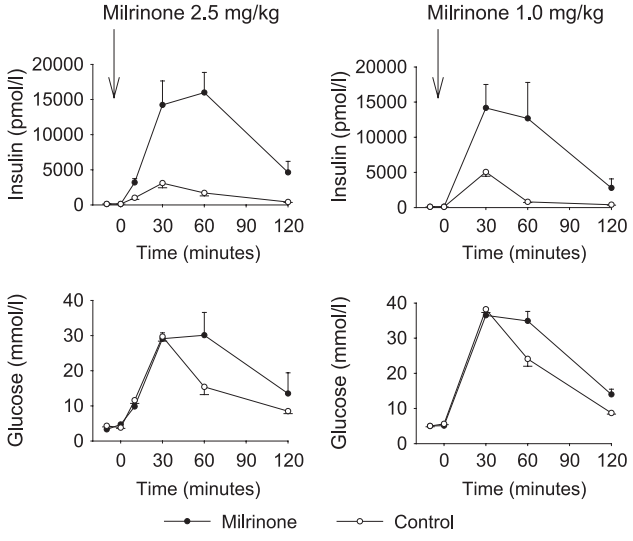

Fig. 1. Plasma levels of insulin and glucose before and after administration of milrinone (1.0 and 2.5 mg/kg body weight) through a gavage tube placed in the stomach in anesthetized mice. At time 0, glucose (150 mg) was given through the gavage tube. Means±S.E.M. are shown. There were 8–12 animals in each group.

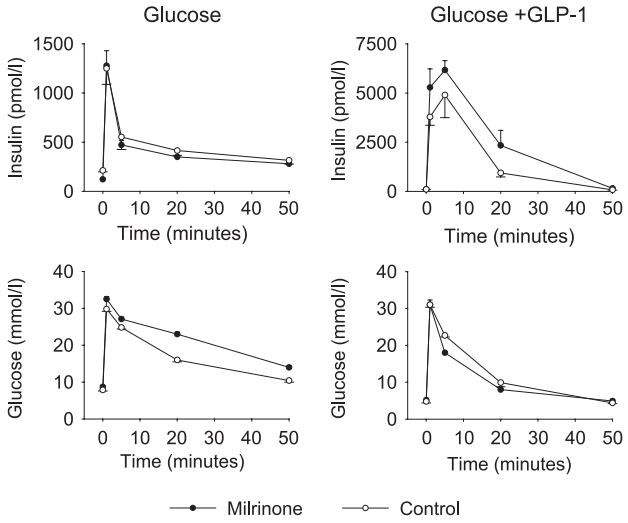

Fig. 2. Plasma levels of insulin and glucose before and after administration of milrinone (1.0 mg/kg body weight) through a gavage tube placed in the stomach in anesthetized mice. At time 0, glucose (1 g/kg) was given intravenously alone (left panels) or together with GLP-1 (2.8 nmol/kg; right panels). Means±S.E.M. are shown. There were 12 animals in each group.

3.2. The role of GLP-1 in mediating milrinone-induced increases in insulin levels

To examine whether the gut hormone, GLP-1, is involved in the milrinone-induced potentiation of insulin secretion induced by oral glucose, GLP-1 levels were determined after gastric glucose with and without milrinone (1 mg/kg) administration 10 min before the glucose challenge. As shown in Fig. 3, GLP-1 levels were increased by gastric glucose at min 15. This effect was, however, not altered by milrinone, suggesting that increased GLP-1 levels could not explain the effect of milrinone on insulin levels. As a next step, the possibility that milrinone could potentiate the effect of GLP-1 to increase insulin secretion was tested. As shown in Fig. 2 (right panel), following milrinone, the

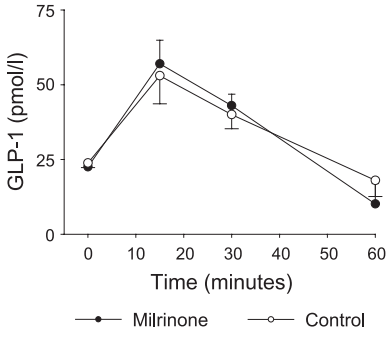

Fig. 3. Plasma levels of GLP-1 before and after administration of milrinone (1.0 mg/kg body weight) through a gavage tube placed in the stomach in anesthetized mice. At time 0, glucose (150 mg) was given through the gavage tube. Means±S.E.M are shown. There were six animals in each group.

increase in plasma insulin in response to GLP-1 was augmented; the acute insulin response being 6.1±0.6 nmol/l in the milrinone-treated group versus only 4.9±0.9 nmol/l in the control group (P=0.036). This was followed by normalization of the glucose elimination rate, because there was no significant difference in glucose levels between the two groups.

4. Discussion

This study shows that milrinone augments insulin secretion after gastric glucose in C57BL/6J mice but that glucose elimination nevertheless was impaired. This is consistent with a key role for PDE3B in insulin-induced antiglycogenolysis in the liver (Beebe et al., 1985) and therefore suggests that the increase in glucose levels is due to release of glucose from the liver. Furthermore, inhibition of PDE3B in adipocytes would counteract the insulin-induced antilipolysis, which would increase fatty acid release resulting in insulin resistance (Arner, 2002; Boden, 2003). Insulin resistance in adipocytes and hepatocytes could further accentuate the negative effects of PDE3 inhibitors in these cells, because insulin utilizes PDE3B to antagonize the effects of catecholamines/cAMP (Hagström-Toft et al., 1995; Eriksson et al., 1995; Beebe et al., 1985). Indeed, in recent studies using male Sprague–Dawley rats, intravenously administered milrinone was shown to impair the ability of insulin to suppress lipolysis and insulin-mediated glucose utilization in peripheral tissues although increases in plasma insulin were observed (Cheung et al., 2003; Yang and Li, 2003). Therefore, milrinone is under in vivo conditions rather diabetogenic than antidiabetogenic, in spite of its insulinotropic action. However, also a glucose lowering effect of milrinone has been reported. Parker et al. (1997) showed that the ability of oral milrinone to potentiate increases in plasma insulin by oral glucose translated into an improvement in glucose tolerance in lean but not in *ob*/*ob* mic. This indicates that in some situations/contexts, the ability of milrinone to increase insulin indeed overrides its ability to generate insulin resistance, leading to, in the end, improved glucose tolerance.

In this study, we compared the influence of milrinone on glucose elimination after gastric glucose versus after intravenous glucose. We administered different amount of glucose in the two experimental conditions in order to achieve similar peak levels of glucose. An intriguing and novel observation in our study was that milrinone enhanced insulin levels after gastric but not after intravenous administration of glucose. This could be explained by an augmentation of milrinone on the GLP-1 release after gastric glucose or by an augmentation of the insulin response to GLP-1 released by gastric glucose. Thus, a major factor explaining the marked increase in insulin levels seen after gastric glucose, as compared to intravenous glucose in spite of lower glucose after gastric glucose, is the incretin hormone GLP-1 (Ahrén, 1998, 2003). This hormone is released by enteral glucose and augments gucose-stimulated insulin secretion, and evidence for its importance includes findings that mice with genetic deletion of the GLP-1 receptor are glucose intolerant (Preitner et al., 2004). However, we found that the GLP-1 response to gastric glucose was not augmented by milrinone. In contrast, the insulinotropic action of GLP-1 was augmented by milrinone. This is somewhat expected because cAMP is a major determinant for GLP-1-induced insulin secretion (Ahrén, 1998, 2003), and this could contribute to the difference in insulin-releasing effect after milrinone between routes of glucose administration.

In summary, PDE3 inhibition by milrinone augments the insulin response to glucose in C57BL/6J mice, but this augmentation is not associated with improved glucose elimination. The augmentation of insulin secretion by milrinone was only observed after gastric administration of glucose, but not during an intravenous glucose tolerance test, which we attribute to be due to augmentation of the insulin response to GLP-1. We conclude that milrinone is unlikely to be developed as an efficient treatment of diabetes when given alone. However, in view of recent interest in developing GLP-1 in the treatment of type 2 diabetes (Holst, 2002; Ahrén, 2003; Drucker, 2003), it may be suggested that PDE3B inhibition is a strategy of enhancing GLP-1-based treatment of the disease.

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