# Milrinone, a Selective Phosphodiesterase 3 Inhibitor, Stimulates Lipolysis, Endogenous Glucose Production, and Insulin Secretion

Peter Cheung, Gangyi Yang, and Guenther Boden

**In vivo effects of milrinone, a selective phosphodiesterase 3 (PDE-3) inhibitor, on plasma free fatty acids (FFA), glucose, and insulin levels were examined in alert rats. In dose response studies, intravenous injection of 1, 5 or 25 $\mu$mol/kg of milrinone provoked an immediate increase in plasma concentrations of FFA and insulin, while glucose levels rose only in response to the 5- and 25-$\mu$mol/kg doses. During euglycemic-hyperinsulinemic ($\sim$450 pmol/L) clamps, intravenous injection of milrinone (25 $\mu$mol/kg) completely inhibited insulin suppression of lipolysis and of endogenous glucose production, while having no effect on insulin-stimulated glucose uptake (ISGU). To explore the reason why ISGU was not affected, we performed reverse-transcriptase polymerase chain reaction (RT-PCR) with RNA from skeletal muscle, fat, and liver. The results showed that PDE-3B mRNA was expressed in adipose tissue and liver, but it was not detected in skeletal muscle. We conclude that PDE-3 plays a major role in the inhibitory action of insulin on lipolysis in fat and on glucose production in liver and, in addition, seems to be involved in insulin secretion in pancreatic $\beta$ cells.**


MILRINONE (Primacor, Sanofi-Synthelabo, New York, NY) is a selective phosphodiesterase 3 (PDE-3) inhibitor that has positive inotropic and vasodilatory activities and is used in clinical practice for short-term treatment of patients with acute decompensated heart failure.[1,2]

Milrinone inhibits both members of the PDE-3 family, PDE-3A, which predominates in heart, vascular smooth muscle, and platelets, and PDE-3B, which is prominent in fat, liver, and pancreatic islets.[3]

Activation of PDE-3B (a 135-kd membrane-associated enzyme) plays a major role in insulin's antilipolytic action in adipose tissue.[4] Insulin increases PDE-3B activity in a phosphoinositide 3 kinase (PI3K)-dependent manner, resulting in hydrolysis of cyclic adenosine monophoshate (cAMP) and decreased activities of cAMP-dependent kinase (PKA) and hormone-sensitive lipase (HSL).[5] Inhibition of PDE-3B, therefore, increases lipolysis and plasma free fatty acid (FFA) levels. Since FFA are known to cause peripheral (muscle), as well as hepatic insulin resistance,[6,7] inhibition of PDE-3B could potentially cause problems with insulin action. Surprisingly, however, we have been able to find only 2 studies that have examined in vivo actions of milrinone on carbohydrate metabolism. Parker et al orally administered milrinone to ob/ob and to lean mice and found that it reduced glucose tolerance in the ob/ob but not in the lean mice.[8] Cases et al reported that milrinone, administered intravenously to rats during hyperglycemic clamps, increased insulin levels.[9] The objective of the present study was, therefore, to examine the effects of acute intravenous administration of milrinone in rats on basal plasma FFA, insulin, and glucose levels and on insulin-stimulated glucose uptake (ISGU) and insulin suppression of endogenous glucose production (EGP) during euglycemic-hyperinsulinemic clamping.

*From the Division of Endocrinology/Diabetes/Metabolism and the Clinical Research Center, Temple University School of Medicine, Philadelphia, PA.*

*Submitted February 22, 2003; accepted April 7, 2003.*

*Supported by National Institutes of Health Grants No. R01-AG15363, R01-DK58895, and M01-RR00349 (General Clinical Research Center Branch of the National Center for Research Resources), and by a Mentor Based Training Grant from the American Diabetes Association.*

*P.C. and Y.G. contributed equally to this project.*

*Address reprint requests to Guenther Boden, MD, Temple University Hospital, 3401 N Broad St, Philadelphia, PA 19140.*



*0026-0495/03/5211-0052$30.00/0*

*doi:10.1016/S0026-0495(03)00271-3*

## MATERIALS AND METHODS

### *Preparation of Animals*

Male Sprague-Dawley rats weighing between 250 to 300 g were purchased from Charles River Laboratories (Wilmington, MA) and housed in an environmentally controlled room with a 12-hour light/dark cycle, where they had free access to standard rat chow and water. One week before the studies, the animals were anesthetized with an intraperitoneal injection of phenobarbitol (50 mg/kg bodyweight). A polyvinyl catheter (inner diameter = 0.02 in) was inserted into the right internal jugular vein and extended to the right atrium. Another catheter was advanced through the left carotid artery until its tip reached the aortic arch. The free ends of both catheters were attached to long segments of steel tubing and tunneled subcutaneously to the back of the neck where they were exterialized and secured to the skin with clips. At the end of the procedure, the catheters were flushed with isotonic saline containing heparin (40 U/mL) and ampicillin (5 mg/mL) and then filled with a viscous solution of heparin (300 U/mL) and 80% polyvinyl pyrolidone (PVP-10, Fisher Scientific, Pittsburgh, PA) to prevent refluxing of blood into the catheter lumen. All studies were performed in accordance with the guidelines for the use and care of laboratory animals of the Temple University Institutional Animal Care and Use Committee.

### *Study Design*

The rats were allowed 1 week to recover from the effects of surgery. At that time, they were within 3% of their preoperative weight. All studies were conducted in the morning after a 14-hour overnight fast. Throughout the studies, the animals were allowed to move freely in their cages. All substrates were administered into the arterial catheter and blood samples were obtained from the venous catheters. Two different studies, a dose response and a euglycemic-hyperinsulinemic clamp study, were performed.

### *Dose Response Study*

Four groups of rats were studied. In group 1 (n = 6), a 25% solution in saline of dimethylsulfoxide (DMSO 1 mL/kg) was injected at 0

minutes to serve as control. In the other 3 groups, varying doses of milrinone (purchased from Biomole Research Labs, Plymouth Meeting, PA) in 25% DMSO were administered at 0 minutes. Group 2 received 1 $\mu$mol/kg (n = 4), group 3 received 5 $\mu$mol/kg (n = 5), and group 4 received 25 $\mu$mol/kg (n = 6). Blood samples for determination of glucose, insulin, and FFA concentrations were obtained at −30, −15, 0, 2, 10, 15, 30, and 60 minutes during the study. Each blood sample was replaced by the same volume of fresh whole blood obtained from littermates of the test animals to prevent intravascular volume depletion and anemia.

### *Euglycemic-Hyperinsulinemic Clamp Study*

Euglycemic-hyperinsulinemic clamps were performed as described[10] with some modifications. Insulin (4.8 mU/kg min) was infused through the carotid catheter from 0 to 180 minutes. Glucose concentrations were clamped at euglycemic levels by a variable rate infusion of 25% glucose. Blood glucose levels were monitored with an Elite Glucometer (Bayer, Elkhart, IN) and glucose infusion rates were adjusted every 5 to 10 minutes as needed. At 120 minutes, 25% DMSO or milrinone (25 $\mu$mol/kg) was administered as a bolus injection. Blood samples for determination of glucose, insulin, FFA, and 6,6 $^2H_2$ glucose enrichment were obtained at −90, 0, 120, 130, 140, 150, 160, and 180 minutes. Each blood sample was replaced by the same volume of fresh whole blood.

### *Glucose Rates of Appearance and Disappearance*

Glucose rate of appearance ($G_{Ra}$) was determined with 6,6 $^2H_2$ glucose (Mass Trace, Woburn, MA) as described.[11] 6,6 $^2H_2$ glucose infusion was started at −90 minutes with a bolus of 10 mg followed by a constant infusion of 0.1 mg/min throughout the 270-minute study. To prevent dilution of the isotope during the studies, 6,6 $^2H_2$ glucose was added to the glucose infused to maintain euglycemia.

6,6 $^2H_2$ glucose enrichment in plasma was determined with gas chromatography–mass spectrometry (Hewlett-Packard 5973 MS, 5890 GC, Palo Alto, CA) using select ion monitoring according to Wolfe.[12] Plasma glucose concentrations were measured with a Beckman Glucose Analyzer using the glucose oxidase method. $G_{Ra}$ and glucose rate of disappearance ($G_{Rd}$) were calculated using the non–steady-state equations of Steele et al.[13] The effective distribution volume for glucose was assumed to be 150 mL/kg.[14]

### *Endogenous Glucose Production*

EGP was calculated as the difference between the isotopically determined $G_{Ra}$ and the rate of glucose infused to maintain euglycemia (GIR): EGP = $G_{Ra}$ − GIR.

### *Analytical Procedures*

Plasma insulin was measured in deproteinized serum by radioimmunoassay using rat insulin as standard (Linco, St Charles, MO). Enzymatic colorimetric kits were used to determine plasma concentrations of FFAs (Wako, Richmond, VA).

### *Detection of PDE-3B mRNA in Rat Tissues*

Untreated rat tissues (liver, fat, and skeletal muscle) were pulverized in liquid nitrogen and homogenized in TRIZOL (Invitrogen, Carlsbad, CA). Two micrograms of total RNA was used for polymerase chain reaction (PCR) (50 $\mu$L). First-strand cDNAs were amplified by Taq DNA polymerase (Promega, Madison, WI), labeled with digoxigenin deoxy uridine triphophatase (dUTP) (20 $\mu$mol/L), and primed with rat $\beta$-actin amplimers (accession no. NM 031144; rat $\beta$-actin-sense: 5′-TTCAACACCCCAGCCATGTACGTA-3′; rat $\beta$-actin-antisense: 5′-GAGGAGCAA TGATCTTGATCTTCA-3′; size 618 bp) and rat PDE-3B amplimers (accession no. Z 22867; rat PDE-3B-sense: 5′-CCTGGTGCTGAGCTGC GTGGGCTGCT-3′; and rat PDE-3b-antisense: 5′-TGTTCTCGAGAAATACAAGGCAACGAC -3′; size 381 bp). PCR conditions were 2 minutes at 94°C followed by 25 cycles of amplification for 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C and an extension of 7 minutes at 72°C.

PCR products were separated on a 2% gel of NuSieve agarose: ME agarose (1:1) and transferred to positive charge nylon membrane by 3 mol/L sodium chloride, 0.3 mol/L sodium citrate. Digoxigenin-labeled PCR cDNA was then detected by alkali phosphatase-tagged anti-digoxigenin antibody and CDP-Star chemiluminescent detection method (Roche Applied Science, Indianapolis, IN). The chemiluminescent signals were detected by BioMax x-ray film (Kodak, Rochester, NY). The images were scanned and analyzed by ScanJet ADF (Hewlett Packard, Palo Alto, CA) and Scion Image (www.Scioncorp.com).

## RESULTS

### *Dose Response Studies*

Three different doses of milrinone (1, 5, and 25 $\mu$mol/kg) or an equivalent volume of DMSO (to serve as control) were administered as an intravenous bolus into overnight fasted rats.

Plasma FFA concentrations rose in response to all 3 doses of milrinone within 2 minutes of injection. FFA returned to basal values within 15 minute in the 1- and 5-$\mu$mol/kg groups, while remaining elevated for between 15 and 30 minutes in the 25-$\mu$mol/kg group.

Plasma glucose concentration rose in response to the 5- and 25-$\mu$mol/kg doses and remained significantly elevated for 30 minutes.

Serum insulin levels rose after the 1-, 5-, and 25-$\mu$mol/kg doses. Insulin levels remained significantly elevated until 20 minutes after the 5-$\mu$mol/kg dose and until 30 minutes after the 25-$\mu$mol/kg dose (Fig 1).

### *Euglycemic-Hyperinsulinemic Clamp Studies*

The increase in glucose concentration seen after the 25-$\mu$mol/kg dose of milrinone in the dose response study occurred despite a large simultaneous increase in insulin. This indicated that milrinone had either inhibited insulin stimulated glucose uptake or insulin suppression of EGP or a combination of both.

To explore these possibilities, we investigated effects of the 25-$\mu$mol/kg bolus of milrinone on rates of total body $G_{Rd}$ and EGP under euglycemic-hyperinsulinemic (~450 pmol/L) clamp conditions. Prior to milrinone injection, hyperinsulinemia decreased plasma FFAs from approximately 750 to less than 200 $\mu$mol/l. After milrinone injection, plasma FFA rose immediately and remained elevated for approximately 30 minutes before returning to basal levels (Fig 2).

Before milrinone, GIR, the rate of glucose infused to maintain euglycemia, increased equally in both groups in response to hyperinsulinemia. In the control group, GIR continued to rise after 120 minutes. In the milrinone group, the rise in GIR after 120 minutes was completely suppressed (Fig 3). This suggested that milrinone had either decreased glucose uptake or increased EGP or both.

Before milrinone, hyperinsulinemia completely suppressed EGP in both groups. Between 120 and 180 minutes, EGP rose and returned to pre-injection level in the milrinone group, while remaining suppressed in the control group (Fig 3).

Milrinone had no significant effect on ISGU, which rose

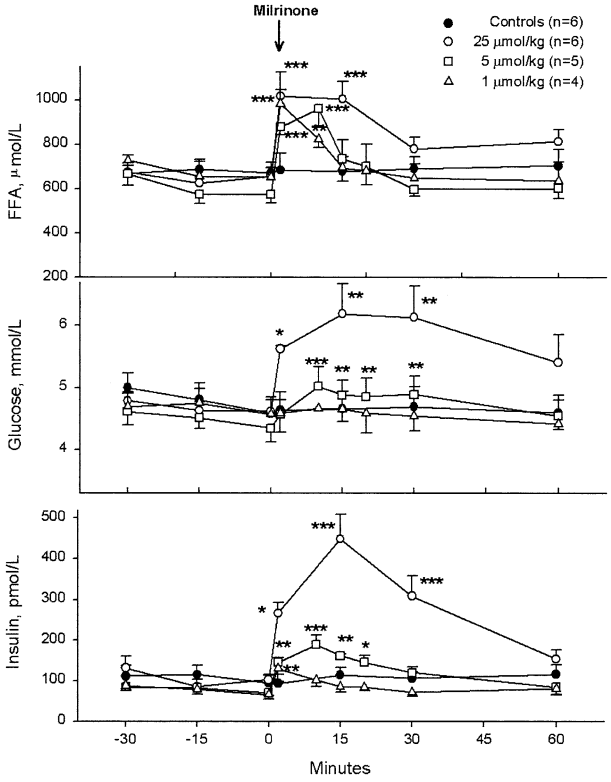


**Fig 1. Milrinone dose responses. Plasma FFA, glucose, and insulin concentrations before and after intravenous injection of milrinone in doses of 1, 5, and 25 μmol/kg or of 25% DMSO (controls) in alert rats. Shown are means ± SE. *$P < .05$; **$P < .01$; ***$P < .001$ compared to 0 minutes.**

approximately 3-fold over basal in response to insulin in both groups (Fig 3). To explore the reason why milrinone had no effect on ISGU (>80% of which occurs in muscle) while strongly inhibiting insulin suppression of lipolysis and hepatic glucose production, we examined PDE-3B expression (by reverse transcriptase [RT]-PCR) in rat skeletal muscle, fat, and liver. The results showed that PDE-3B mRNA was detected in liver and adipose tissue but not in skeletal muscle (Fig 4).

## DISCUSSION

In this study, we found that milrinone given in doses similar to recommended human doses (2.8 to 5.4 μmol/kg/24 h)[15] had strong effects on lipolysis, EGP, and insulin secretion.

### *Lipolysis*

In the dose response study, the lowest dose of milrinone (1 μmol/kg) caused plasma FFA levels to rise within 2 minutes, indicating stimulation of lipolysis (changes in FFA levels correlate closely with changes in lipolysis).[16]

During the hyperinsulinemic clamp study, milrinone completely reversed the insulin-induced suppression of lipolysis. This provided strong in vivo support for the concept, derived from in vitro studies, that activation of PDE-3B is a major mechanism by which insulin inhibits lipolysis.[5] For example, previous studies had shown that specific PDE-3 inhibitors blocked the antilipolytic action of insulin in isolated fat cells[17,18] and that insulin inhibited the lipolytic effects of only those cAMP analogues[19] that were substrates of insulin-sensitive PDE.

### *Glycogenolysis*

Milrinone also raised plasma glucose levels. This effect was only seen with the highest doses (5 and 25 μmol/kg) and persisted for more than 30 minutes. It seems likely, however, that the glucose-elevating effects of milrinone was attenuated by the concurrent increase in serum insulin.

To examine whether the increase in plasma glucose was

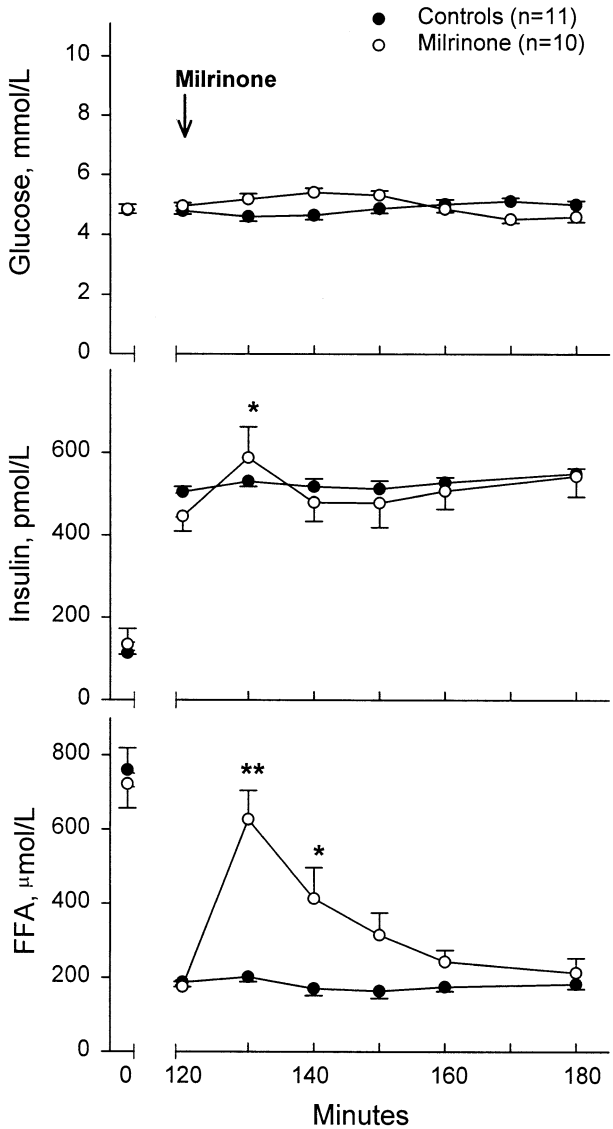


**Fig 2. Milrinone during euglycemic-hyperinsulinemic clamping. Plasma glucose, insulin, and FFA concentrations before and after intravenous milrinone (25 μmol/kg) or DMSO (control) injection (at 120 minutes) during euglycemic-hyperinsulinemic (4.8 mU/kg min) clamping in alert rats. Values at 0 minutes were obtained before insulin infusion. *$P < .03$; **$P < .001$ compared to 120 minutes.**

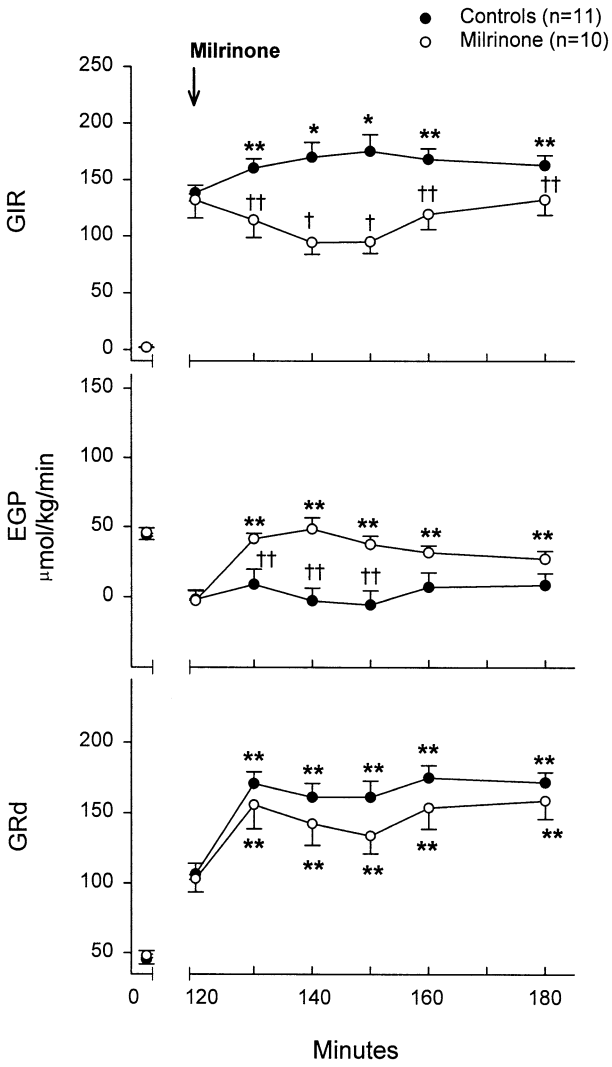


**Fig 3. GIR, $G_{Rd}$, and EGP before and during intravenous milrinone (25 $\mu$mol/kg) or DMSO (control) injection (at 120 minutes) during euglycemic-hyperinsulinemic clamping in alert rats. Values at 0 minutes were obtained before insulin infusion. *$P < .01$; **$P < .001$ compared to 120 minutes. †$P < .03$; ††$P < .01$ comparing milrinone *v* DMSO (control) injections. The differences in $G_{Rd}$ between the milrinone-treated and the control groups were not statistically significant.**

caused by a decrease of peripheral glucose uptake or an increase in EGP or a combination of both, we performed euglycemic-hyperinsulinemic clamps. The results showed that milrinone had no effects on insulin-stimulated glucose uptake but strongly increased EGP. Before milrinone administration, insulin infusions completely suppressed EGP. After milrinone injection, EGP rose rapidly back to basal levels. This was almost certainly due to an acute stimulation of glycogenolysis, since it seems unlikely that gluconeogenesis could have been stimulated to such a degree in such a short time. Moreover, the notion that milrinone inhibited insulin suppression of glycogenolysis is supported by several lines of in vitro evidence: (1) PDE-3B is present in hepatocytes[3] (this study); (2) milrinone has been shown to acutely increase glucose production and to decrease glycogen content in isolated hepatocytes;[20] and (3) activation of PDE-3B has been found to be important in the antiglycogenolytic action of insulin[19] presumably by reduction of a cAMP-dependent protein kinase and of phosphorylase kinase a.

*Glucose Uptake*

The finding that milrinone inhibited insulin action on lipolysis and on glycogenolysis but had no effect on ISGU suggested that PDE-3 might not be present in skeletal muscle (which accounts for > 80% of ISGU). Indeed, RT-PCR revealed that PDE-3B was expressed in rat liver and epididymal fat but could not be detected in rat skeletal muscle (Fig 4).

*Insulin Secretion*

Milrinone infusion was associated with an approximately 4-fold increase in serum insulin levels with the 25-$\mu$mol/kg dose and smaller increases with the 1- and 5-$\mu$mol/kg doses. Since glucose levels rose at the same time, some of the insulin rise seen with the 5- and 25-$\mu$mol/kg doses was probably glucose-induced. However, the fact that insulin rose also with the 1-$\mu$mol/kg dose without a rise in glucose and that insulin increased by greater than 400% (from 100 to >400 pmol/L) with the 25-$\mu$mol/kg dose while glucose only rose approximately 30% (from 80 to 105 mg/dL) indicated that milrinone had direct stimulatory effects on insulin secretion. Moreover, there was also a significant rise in serum insulin immediately after milrinone injection during the hyperinsulinemic clamp studies (Fig 2).

In this context, it is of interest that several studies have shown that PDE-3 is present in rat and human pancreatic $\beta$ cells[21] and that activation of PDE-3B (by insuling-like growth factor 1 [IGF-1] or leptin) decreased cAMP and insulin secretion from cultured neonatal rat $\beta$ cells.[22,23] In addition, milrinone and other specific PDE inhibitors have been shown to potentiate glucose-stimulated insulin secretion in vitro,[24-27] suggesting that PDE-3 may play an important role in the control of insulin secretion.

In summary, we have shown in alert rats that milrinone, a selective PDE-3 inhibitor, increased plasma FFA, glucose, and

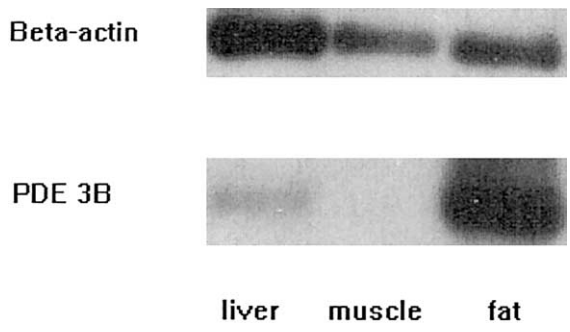


**Fig 4. RT-PCR detection of PDE-3B in rat tissues. Lane 1: liver; lane 2: skeletal muscle; lane 3: epididymal adipose tissue. Upper panel: $\beta$-actin; lower panel: PDE-3B.**

insulin levels and inhibited insulin suppression of lipolysis and glycogenolysis. These data suggested that PDE-3, which is expressed in fat and liver, is important for insulin action on lipolysis in fat cells and on glucose production in liver cells, and it may be involved in insulin secretion from pancreatic $\beta$ cells. On the other hand, PDE-3B was not detected in rat skeletal muscle and milrinone did not affect insulin stimulation of glucose uptake (>80% of which occurs in skeletal muscle).

## REFERENCES


1. Baim DS, McDowell AV, Cherniles J, et al: Evaluation of a new bipyridine inotropic agent—milrinone—in patients with severe congestive heart failure. N Engl J Med 309:748-756, 1983
2. DiBianco R, Shabetai R, Kostuk W, et al: A comparison of oral milrinone, digoxin, and their combination in the treatment of patients with chronic heart failure. N Engl J Med 320:677-683, 1989
3. Reinhardt RR, Chin E, Zhou J, et al: Distinctive anatomical patterns of gene expression for two cGMP-inhibited nucleotide phosphodiesterase sub-families. J Clin Invest 95:1528-1538, 1995
4. Hagstrom-Toft E, Bolinder J, Eriksson S, et al: Role of phosphodiesterase III in the antilipolytic effect of insulin in vivo. Diabetes 44:1170-1175, 1995
5. Degerman E, Landstrom TR, Holst LS, et al: A role for phosphodiesterase 3B in the antilipolytic action of insulin, in LeRoit D, Taylor SI, Olefsky JM (eds): Diabetes Mellitus: A Fundamental and Clinical Text. Philadelphia, PA, Lippincott Williams & Wilkins, 2000, pp 284-291
6. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46:3-10, 1997
7. Boden G, Cheung P, Stein TP, et al: FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. Am J Physiol 283:E12-E19, 2002
8. Parker JC, Van Volkenburg MA, Nardone NA, et al: Modulation of insulin secretion and glycemia by selective inhibition of cyclic AMP phosphodiesterase III. Biochem Biochem Res Commun 236:665-669, 1997
9. Cases JA, Gabriely H, Ma XH, et al: Physiological increase in plasma leptin markedly inhibits insulin secretion in vivo. Diabetes 50:348-352, 2001
10. Rossetti L, Laughlin MR: Correction of chronic hyperglycemia with Vanadate, but not with phlorizin, normalizes in vivo glycogen repletion and in vitro glycogen synthase activity in diabetic skeletal muscle. J Clin Invest 84:892-899, 1989
11. Shah P, Vella A, Basu A, et al: Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. J Clin Endocrinol Metab 85:4053-4059, 2000
12. Wolfe RR: Tracers in metabolic research, in Radioisotope and Stable Isotope/Mass Spectrometry Methods. New York, NY, Liss, 1984, p 261
13. Steele R, Wall JS, DeBodo RC, et al: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 187:15-24, 1956
14. Smith D, Rossetti L, Ferrannini E, et al: In vivo glucose metabolism in the awake rat: Tracer and insulin clamp studies. Metabolism 36:1167-1174, 1987
15. Physicians Desk Reference. Thomson Healthcare 55th edition. Montvale, NJ, Medical Economics Co, 2001, p 2760
16. Nestel PG, Whyte HM: Plasma free fatty acid and triglyceride turnover in obesity. Metabolism 17:1122-1128, 1968
17. Elks ML, Manganiello VC, Vaughan M: Hormone-sensitive particulate cAMP phosphodiesterase activit in 3T3-L1 adipocytes. J Biol Chem 258:8582-8587, 1983
18. Eriksson H, Ridderstrale M, Degerman E, et al: Evidence for the key role of the adipocyte cGMP-inhibited cAMP phosphodiesterase in the antilipolytic action of insulin. Biochim Biophys Acta 1266:101-107, 1995
19. Beebe SJ, Redmon JB, Blackmore PF, et al: Discriminative insulin antagonism of stimulatory effects of various cAMP analogs on adipocyte lipolysis and hepatocytes glycogenolysis. J Biol Chem 260: 15781-15788, 1985
20. Parker JC, Van Volkenburg MA, Nardon NA, et al: Modulation of insulin secretion and glycemia by selective inhibition of cyclic AMP phosphodiesterase III. Biochem Biochem Res Commun 236:665-669, 1997
21. Beavo JA: Cyclic nucleotide phosphodiesterases: Functional implications of multiple isoforms. Physiol Rev 75:725-748, 1995
22. Zhao AZ, Zhao H, Teague J, et al: Attenuation of insulin secretion by insulin-like growth factor 1 is mediated through activation of phosphodiesterase 3B. Proc Natl Acad Sci USA 94:3223-3228, 1997
23. Zhao AZ, Bronfeldt KE, Beavo JA: Leptin inhibits insulin secretion by activation of phosphodiestarase 3B. J Clin Invest 102:869-873, 1998
24. Parker JC, VanVolkenburg MA, Ketchum RJ, et al: Cyclic AMP phosphodiesterases of human and rat islets of Langerhans: Contributions of types III and IV to the modulation of insulin secretion. Biochem Biophys Res Commun 217:916-923, 1995
25. Leibowitz MD, Biswas C, Brady EJ, et al: A novel insulin secretagogue is a phosphodiesterase inhibitor. Diabetes 44:67-74, 1995
26. Ahmad M, Abdel-Wahab YHA, Tate R, et al: Effect of type-selective inhibitors on cyclic nucleotide phosphodiesterase activity and insulin secretion in the clonal insulin secreting cell line BRIN-BD11. Br J Pharmacol 129:1228-1234, 2000
27. Shafiee-Nick N, Pyne NJ, Furman BL: Effects of type-selective phosphodiesterase inhibitors on glucose-induced insulin secretion and islet phosphodiesterase activity. Br J Pharmacol 115:1486-1492, 1995