The Antilipolytic Agent 3,5-Dimethylpyrazole Inhibits Insulin Release in Response to Both Nutrient Secretagogues and Cyclic Adenosine Monophosphate Agonists in Isolated Rat Islets

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This study intended to test the hypothesis that intracellular lipolysis in the pancreatic β cells is implicated in the regulation of insulin secretion stimulated by nutrient secretagogues or cyclic adenosine monophosphate (cAMP) agonists. Indeed, although lipid signaling molecules were repeatedly reported to influence β-cell function, the contribution of intracellular triglycerides to the generation of these molecules has remained elusive. Thus, we have studied insulin secretion of isolated rat pancreatic islets in response to various secretagogues in the presence or absence of 3,5-dimethylpyrazole (DMP), a water-soluble and highly effective antilipolytic agent, as previously shown in vivo. In vitro exposure of islets to DMP resulted in an inhibition (by approximately 50%) of the insulin release stimulated not only by high glucose, but also by another nutrient secretagogue, 2-ketoisocaproate, as well as the cAMP agonists 3-isobutyl-1-methylxanthine and glucagon. The inhibitory effect of DMP, which was not due to alteration of islet glucose oxidation, could be reversed upon addition of sn-1,2-dioctanoylglycerol, a synthetic diglyceride, which activates protein kinase C. The results provide direct pharmacologic evidence supporting the concept that endogenous β-cell lipolysis plays an important role in the generation of lipid signaling molecules involved in the control of insulin secretion in response to both fuel stimuli and cAMP agonists.
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A SINGLE INTRAPERITONEAL administration to rats of the potent antilipolytic drug, 3,5-dimethylpyrazole (DMP),[1-3] causes a rapid and prolonged decrease in circulating free fatty acids (FFA), lasting at least 3 hours.[4] Low-plasma levels of FFA were maintained as long as 12 hours by multiple DMP injections (every 3 hours) and were associated with a marked stimulation of hepatic proteolytic and autophagic processes, probably secondary to hormonal changes.[4,5] Basal insulinemia in 18-hour fasted rats was also rapidly decreased upon DMP treatment, concomitantly with the reduction in plasma FFA.[4] These findings are compatible with the concept that both basal and glucose-stimulated insulin secretion relies upon circulating FFA in the fasting state.[6,7] Alternatively and not exclusively, DMP, which is considered as an inhibitor of adipose tissue triacylglycerol lipase,[8] might, in fact, inhibit insulin secretion through a possible direct antilipolytic activity in the β cells. The latter possibility is in agreement with the view that lipid signaling molecules and the control of lipid partitioning play a crucial role in the regulation of insulin secretion in response to nutrient secretagogues[9] and possibly cyclic adenosine monophosphate (cAMP) agonists.[10] Interestingly, hormone-sensitive lipase has been documented to be highly expressed in the β cell,[11] yet its function, as well as that of endogenous triglyceride stores, remains to be defined. To gain insight into the role of lipolysis in β-cell function, we studied insulin secretion of isolated rat islets in response to various secretagogues in the presence or absence of DMP. An

inhibition of the insulin release stimulated by high glucose was consistently obtained upon exposure of islets to DMP. Because this inhibitory effect is relieved by the addition of a synthetic diglyceride, the results suggest that the antilipolytic drug interferes with β-cell metabolic signaling, possibly by affecting intracellular lipid turnover.

MATERIALS AND METHODS

Isolation and Incubation of Islets

Pancreatic islets were isolated from fed male Sprague-Dawley rats weighing 300 to 400 g by a modification of the method of Lacy and Kostianovsky.[12] After a 60-minute preincubation period in modified Krebs-Ringer bicarbonate (KRB) buffer containing 0.5% bovine serum albumin, 10 mmol/L Hepes (pH 7.4) and 2.8 mmol/L glucose, groups of 7 to 10 islets were incubated for 60 minutes at 37°C in humidified atmosphere of 5% CO_2/95% air, in 1 mL KRB-Hepes buffer containing the secretagogues and test substances.

DMP (300 mmol/L) was dissolved in KRB/Hepes buffer (pH 7.4) by stirring for 30 minutes at room temperature and subsequently diluted with the incubation buffer to obtain the work concentration (usually 3 mmol/L).

The synthetic diglyceride, sn-1,2-dioctanoylglycerol (DOCG), was dissolved in 50% ethanol at a concentration of 25 mmol/L; 20 μL of this stock solution was added to 1 mL of incubation buffer (final concentration 0.5 mmol/L) and sonicated before use. A total of 20 μL of the ethanol vehicle was added to the incubation buffer of islets not exposed to DOCG. At the end of the incubation, incubation media were collected for insulin determinations. A total of 1 mL of cold acidified ethanol (ethanol:H_2O:concentrated HCl, 150:47:3, vol/vol) was added to the islets to extract their insulin content.

Glucose Oxidation Measurements

Glucose oxidation was measured by determining the amount of CO_2 released from [U-^{14}C] glucose, as described by Malaisse et al.[13] In brief, groups of 20 islets were placed in small plastic tubes with 200 μL of KRB-Hepes buffer containing 2.8 mmol/L [U-^{14}C]glucose (30 μCi/mL) either without or with nonlabeled glucose at a final concentration of 16.7 mmol/L. The tubes were inserted into counting vials, gassed with CO_2:O_2 (5:95), sealed with rubber stoppers and incubated for 120

minutes with gentle shaking in a 37°C water bath. At the end of the incubation, 100 μL of HCl 0.2 N and 0.5 mL of hyamine hydroxide were injected through the rubber stopper in the small tube and in the outer vial, respectively. After a further 90-minute incubation at room temperature, radioactivity in hyamine was analyzed after addition of 1 mL triton-toluene scintillation mixture. Mean blank values found in the absence of islets under the same experimental conditions were subtracted from the experimental values. The rate of glucose oxidation is expressed as pmoles/islet/120 minutes.

Insulin Determination

Insulin was measured by radioimmunoassay according to Herbert et al[14] using rat insulin as a standard. The sensitivity and the coefficients of variation of the radioimmunoassay were as follows: detection limit, 0.15 ng/mL; intra-assay and interassay variations, 3.5% and 10.5%, respectively.

Biochemicals and Reagents

DOCG, 3-isobutyl-1-methylxanthine, and 2-ketoisocaproate were purchased from Sigma, St Louis, MO; 3,5-dimethylpyrazole from Fluka AG, Buchs, Switzerland; and glucagon from Novo Industri, Bagsvaerd, Denmark. Collagenase used for islet digestion was obtained from Serva Feinbiochemica GMBH, Heidelberg, Germany. ^{125}I-insulin was kindly provided by Professor R. Navalesi, Cattedra di Malattie del Ricambio, University of Pisa. All reagents were of analytical grade.

Statistical Analysis

Statistical evaluation of the data was performed by analysis of variance (ANOVA) followed by Tukey test or Student's t test, as appropriate, to assess 2-by-2 differences.

RESULTS

The effect of 3 mmol/L DMP on insulin secretion from isolated islets was studied at various glucose concentrations (Fig 1). While not affecting basal insulin secretion at 2.8 mmol/L glucose, the antilipolytic agent partially inhibited glucose-stimulated insulin release. Indeed, DMP caused a 27% decrease of insulin secretion stimulated by 5.6 mmol/L glucose and a 50% decrease at higher glucose concentrations. When
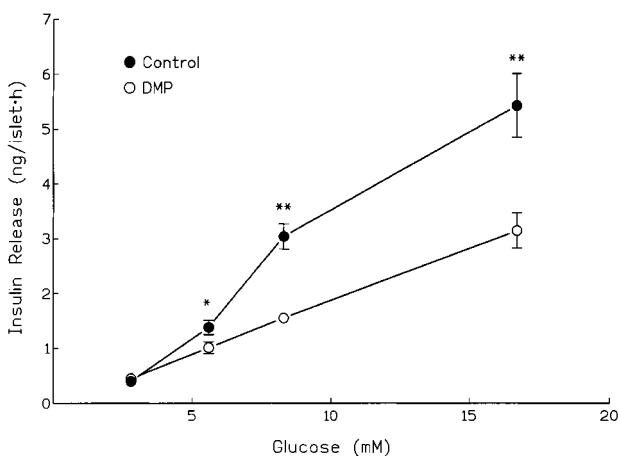
Table 1. Lack of Effect of DMP on [U-^{14}C] Glucose Oxidation in Isolated Rat Islets

Incubation Condition	Glucose Oxidation (pmol/islet/120 min)
Glucose 2.8 mmol/L	62 ± 6.6
Glucose 16.7 mmol/L	196 ± 35.3*
Glucose 16.7 mmol/L + DMP 3 mmol/L	200 ± 35.2*

NOTE. Mean ± SEM of 8 determinations.
*$P < .01$ v 2.8 mmol/L glucose.

used at 1 mmol/L or less, DMP did not alter secretion, whereas at 5 or 10 mmol/L, it did not further inhibit secretion (not shown). Therefore, DMP at 3 mmol/L was used also in subsequent experiments.

As indicated in Table 1, the inhibitory effect of DMP was not due to an alteration of islet glucose oxidation. Indeed, the glucose oxidation rate of islets was similarly increased by raising the glucose concentration from 2.8 mmol/L to 16.7 mmol/L, both in the absence and in the presence of 3 mmol/L DMP.

The possibility that DMP could act via an interference with the turnover of some lipid signaling molecules, such as diacylglycerol (DAG), various phospholipids, or long chain acyl-CoA esters,[10] was investigated through the use of the cell-permeant synthetic diglyceride DOCG. Thus, we tested whether DOCG might reverse the action of DMP. Table 2 shows that 0.5 mmol/L DOCG caused a 100% increase in basal islet insulin release, a significant 60% enhancement of glucose-stimulated insulin secretion at 5.6 mmol/L glucose, but no significant potentiation in the presence of 8.3 mmol/L glucose. The consequences of the addition of 0.5 mmol/L DOCG to isolated islets exposed to DMP in the presence of stimulating glucose concentrations are shown in Fig 2. DOCG in the presence of DMP totally restored glucose-induced insulin secretion to control value at both 8.3 and 16.7 mmol/L glucose. DOCG, in the absence of DMP, did not modify the hormone release elicited by 8.3 mmol/L or 16.7 mmol/L glucose.

Figure 3 shows the results of experiments aimed at exploring the effects of DMP and DOCG on the potentiation of glucose-stimulated insulin release by the cAMP raising agent and phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX). DMP also inhibited the potentiating effect of IBMX on insulin secretion. This inhibitory effect of DMP was par-

Fig 1. Inhibitory effect of DMP on glucose-stimulated insulin release from isolated rat islets. DMP concentration was 3 mmol/L. Mean ± SEM of 7 to 10 determinations. *$P < .05$, **$P < .01$ v the corresponding value with glucose alone.

Table 2. Effects of the Synthetic Diglyceride DOCG, on Insulin Release From Isolated Rat Islets in the Presence of Various Glucose Concentrations

Incubation Condition	Insulin Release (ng/islet/h)
Glucose 2.8 mmol/L	0.53 ± 0.14
Glucose 2.8 mmol/L + DOCG 0.5 mmol/L	1.06 ± 0.11*
Glucose 5.6 mmol/L	1.04 ± 0.10
Glucose 5.6 mmol/L + DOCG 0.5 mmol/L	1.61 ± 0.20*
Glucose 8.3 mmol/L	2.41 ± 0.22
Glucose 8.3 mmol/L + DOCG 0.5 mmol/L	3.22 ± 0.33

NOTE. Mean ± SEM of 5 determinations.
*$P < .05$ v corresponding incubation with glucose alone.
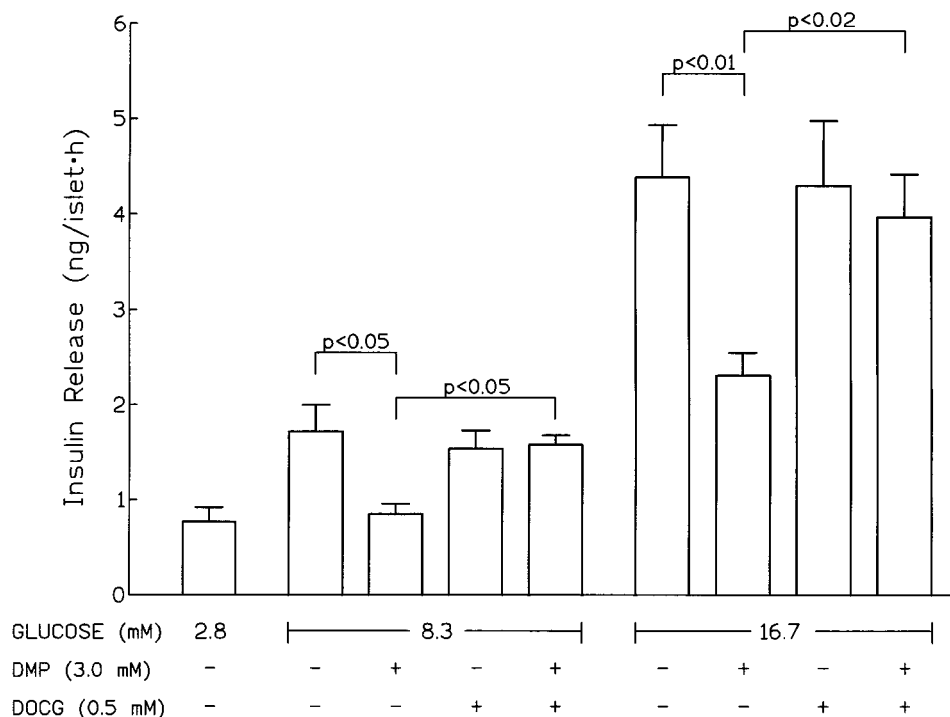
Fig 2. Prevention by the synthetic diglyceride, DOCG, of the inhibitory effect of DMP on glucose-stimulated insulin release from isolated rat islets. Mean ± SEM of 5 determinations.

tially counteracted by DOCG. A similar observation was made with 10 mmol/L glucagon (data not shown).

Finally, experiments were performed to test the effect of DMP on the insulinotropic action of 2-ketoisocaproate (2-KIC), another fuel stimulus that is directly metabolized in the mito-
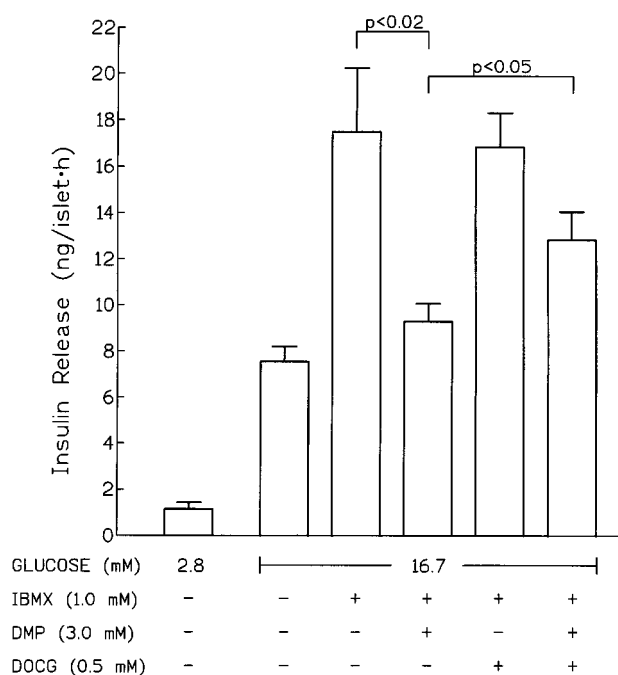

Fig 3. Effects of DMP and DOCG on the insulin release potentiated by IBMX in isolated rat islets. Mean ± SEM of 5 determinations.

chondria.[15] Figure 4 shows that the marked increase in insulin output caused by 20 mmol/L 2-KIC was also significantly inhibited by DMP at both low and high glucose.

DISCUSSION

The results indicate that the antilipolytic agent, DMP, does not alter basal insulin secretion, but inhibits glucose-stimulated insulin release (by approximately 30% at 5.6 mmol/L glucose and 50% at 8.3 or 16.7 mmol/L). Because the inhibitory effect of DMP on insulin release is not due to alteration of islet glucose oxidation, it is attractive to hypothesize that it results from an interference with islet lipid metabolism. Elevated glucose has been reported to increase malonyl-CoA in islets with a resulting diversion of fatty acids from oxidation to glyceride formation, including DAG.[16,17] This latter compound is considered a coupling factor in glucose-stimulated insulin release[18] due to its ability to activate protein kinase C[19] and thereby modulate Ca^{2+}-dependent cellular processes, including insulin secretion.

The total mass of DAG is increased in islets following glucose stimulation,[20] and DAG may be generated by multiple mechanisms.[10] Besides its production through phospholipid hydrolysis, DAG may also derive from fatty acid esterification of α-glycerophosphate, from phosphatidic acid, which also increases in glucose-stimulated islets,[21] and finally from triglycerides (TG) through activation of lipolysis.

Actually, the results of the present study may give support to the hypothesis that lipolysis contributes to the modulation of insulin secretion via the production of DAG and/or other lipid signaling molecules, such as some phospholipids and long chain acyl-CoA esters,[10] even under anaplerotic conditions when citrate and malonyl CoA are elevated and esterification processes promoted. This putative role for intracellular TG
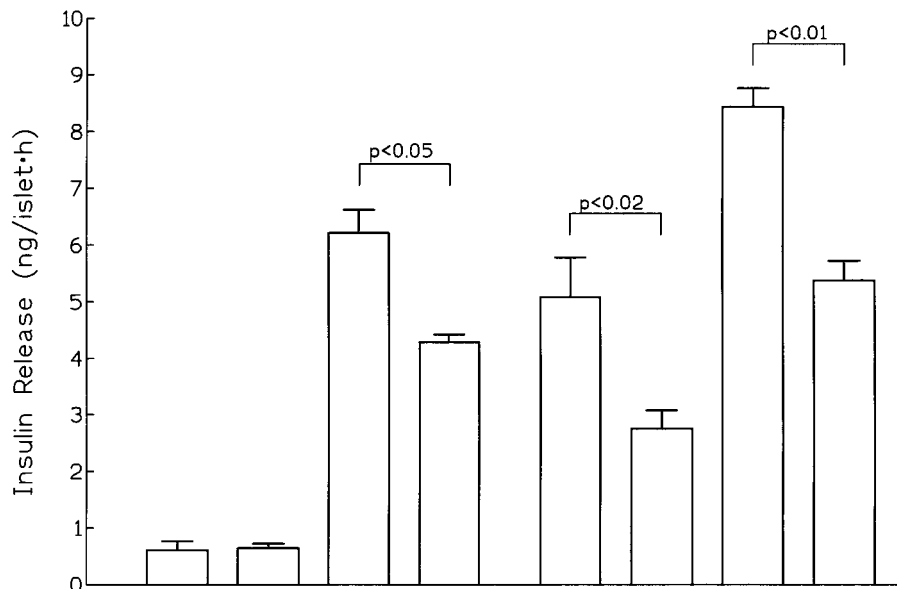
Fig 4. Effects of DMP on 2-KIC–stimulated insulin release from isolated rat islets. Mean ± SEM of 5 determinations.

stores to provide, via lipolysis, not only FFA or FA-CoA,[22] but also DAG and phospholipid signaling compounds, has possible pathophysiologic implications. Thus, it might constitute a major mechanism by which β cells adaptively enhance insulin secretion in obesity[23] or other insulin-resistant states, such as that induced by prolonged dexamethasone administration.[24] In this respect, it is noteworthy that DAG molecules transiently produced from TG by hormone-sensitive lipase are able to activate protein kinase C.[25] Our data are compatible with this view, as the inhibitory effect of DMP on glucose or glucose plus IBMX-stimulated insulin release was reversed in the presence of the synthetic compound, DOCG, a C-kinase activator.[25,26] Incidentally, we should mention that DOCG, by itself, enhances insulin secretion under our experimental conditions only at low glucose concentrations (ie, at 2.8 or 5.6 mmol/L), similar to the results obtained by Malaisse et al[27] with a different synthetic diacylglycerol.

Taking into account that cAMP acts as a competence factor required for normal β-cell glucose responsiveness[28] and also

that the lipase inhibitor, orlistat, was very recently reported to interfere with glucagon-like peptide 1-induced insulin release in clonal β cells,[29] it may be speculated that cAMP-dependent activation of endogenous hormone-sensitive lipase is implicated in this permissive role of cAMP for glucose-induced secretion. The finding that hormone-sensitive lipase is expressed and active in β cells[11] further reinforces the idea that TG turnover may play an important role in the physiologic regulation of insulin secretion.

In conclusion, the results of the present study indicate that in isolated pancreatic islets, an antilipolytic agent, such as DMP, may exert a direct inhibitory effect on insulin release induced by nutrient stimuli and cAMP agonists, which could be relieved by exposure of islets to a synthetic DAG. Such a finding supports the hypothesis that in β cells exposed to elevated concentrations of glucose and cAMP agonists, TG turnover and hydrolysis might contribute to the generation of diacylglycerols and/or other lipid signaling molecules involved in the activation of the insulin secretory machinery.

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