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# Synergistic effect of dimethyl sulfoxide on glucagon-like peptide 1 (GLP-1)-stimulated insulin secretion and gene transcription in INS-1 cells: characterization and implications

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**Abstract**

Glucagon-like peptide 1 (GLP-1) is an incretin hormone that is secreted from the enteroendocrine L-cells of the gut in response to nutrient ingestion. GLP-1 enhances both insulin secretion and insulin gene expression in a glucose-dependent manner via activation of its putative G-protein-coupled receptor on pancreatic β-cells. In the presence of DMSO (0.5–2.5%), these functional responses were enhanced significantly (2- to 2.5-fold) in a concentration-dependent manner in the β-cell line INS-1, although basal levels were not affected. Rat insulin 1 (rINS1) promoter activity appeared to be augmented in a cAMP-response element (CRE)-dependent manner as the effect of DMSO was abolished following a mutation in the CRE of the rINS1 promoter. Also, expression of a generic cAMP-driven reporter gene was enhanced by 1.5% DMSO in response to GLP-1 (3.5-fold), forskolin (2-fold), and 3-isobutyl-1-methylxanthine (2-fold). Analysis of intracellular signaling components revealed that DMSO did not elevate cAMP levels, protein kinase A activity, or phosphorylated levels of CRE-binding protein (CREB), CRE-modulator (CREM), and activating transcription factor-1 (ATF-1). These data suggest that GLP-1 induces insulin gene transcription in a CREB, CREM, and ATF-1-independent manner in β-cells. The mechanism by which DMSO imparts this amplifying action is unclear but may involve redistribution of intracellular compartments or a direct molecular interaction with a downstream target of the GLP-1 receptor signaling pathway in the β-cell. These effects of DMSO on incretin action may provide novel applications with respect to further characterizing GLP-1 receptor signaling, identifying incretin-like compounds in screening assays, and as a therapeutic treatment in type 2 diabetes.


*Keywords:* Dimethyl sulfoxide; Insulin; INS-1; Pancreatic β-cell; GLP-1; Glucagon-like peptide 1

## 1. Introduction

DMSO is most notably appreciated in cell biology for its solvent properties. However, a variety of applications have also been identified including its use in cryoprotection [1] and the enhancement of stem cell differentiation to cardiac-, smooth-, and skeletal-muscle, blood, and neuronal lineages among others [2–6]. Furthermore, DMSO induces physiological effects such as anti-inflammation [7], muscle relaxation [8], and protection against ischemic injury [8]. Many of these applications were discovered serendipitously, underlined by the fact that the mechanism(s) of action remains poorly understood [9]. To address this issue, it will be necessary to investigate the effects of DMSO at the cellular and subcellular level. Here, we report a significant effect of DMSO on signal transduction in pancreatic β-cells.

The primary function of the β-cell is to synthesize and secrete insulin in order to maintain glucose homeostasis. Thus, insulin is both produced and secreted in a glucose-dependent manner. These functions are facilitated by incretin peptides such as GLP-1 [10], which is released from L-cells in the gut in response to nutrient ingestion and binds to its cognate G-protein-coupled receptor on the surface of the β-cell [11]. Intracellular signal transduction is directed primarily via $G_s$, adenylyl cyclase, and the second messenger cAMP, which mediates a synergistic

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*Abbreviations:* ATF-1, activating transcription factor-1; cAMP, cyclic AMP; CRE, cAMP response element; CREB, CRE-binding protein; CREM, CRE-modulator; GLP-1, glucagon-like peptide 1; IBMX, 3-isobutyl-1-methylxanthine; PKA, protein kinase A; and rINS1, rat insulin 1.

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functional response to glucose [12,13]. The specific downstream determinants of these pathways that mediate GLP-1 receptor-induced insulin secretion and gene expression are not understood completely [14]. A clearer understanding of these pathways may provide new targets for therapeutic treatments of diabetes mellitus. In these studies, we show that DMSO induces a synergistic effect on GLP-1-stimulated insulin secretion and gene expression. The mechanism, properties, and implications are discussed herein.

## 2. Materials and methods

### 2.1. Materials

All cell culture reagents were purchased from Life Technologies, Inc. except for fetal bovine serum (FBS), which was obtained from Omega Scientific. GLP-1 was obtained from BioNebraska Inc. Forskolin and IBMX were purchased from Calbiochem. DMSO was obtained from the Sigma Chemical Co.

### 2.2. Cell culture

INS-1 cells (passage numbers 99–110) were cultured in RPMI 1640 containing 10 mmol/L of HEPES, 11.1 mmol/L of glucose, 10% FBS, 100 μU/mL of penicillin G, 100 μg/mL of streptomycin, 1 mmol/L of sodium pyruvate, and 50 μmol/L of 2-mercaptoethanol, and maintained at 37° in a humidified incubator gassed with 5% $CO_2$. Cultures were passaged by trypsinization and subcultured every 5 days. The cells were provided by C. Wollheim, Geneva, Switzerland [15].

### 2.3. Plasmid DNA constructs

The rat insulin promoter sequence, rINS1, was provided by Dr. M. German (University of California at San Francisco, Medical School). It was fused to the coding sequence of luciferase in the pxp2-basic vector to generate −410rINS1-LUC. The construct containing deletions of the rINS1 CRE were prepared by site-directed mutagenesis. The pCRE-LUC and pAP1-LUC expression plasmids were obtained from Stratagene.

### 2.4. Transfection of INS-1 cells

Adherent INS-1 cells grown to 80–90% confluence in Falcon 100-mm tissue culture dishes were transfected using Lipofectamine 2000 (Gibco BRL). Cells were rinsed twice in serum-free culture medium before the addition of 5.0 mL of transfection mixture containing 1.0 μg of plasmid DNA. Cells were incubated in this mixture for 4 hr, then were trypsinized and transferred in culture medium to 24-well plates (Costar, Corning) at 500 μL cell suspension per well, and incubated for 48 hr.

### 2.5. Luciferase assays

Test substances were dissolved in RPMI culture medium and added to 24-well plates at a final volume of 300 μL/well. Cells were exposed to test substances for 4 hr unless stated otherwise at 37° in a humidified incubator. Inhibitory test substances were added 30 min before the addition of GLP-1. After 4 hr, cells were lysed, and measurements of luciferase activity were performed using a luciferase assay kit (Promega) in conjunction with a dual injection port luminometer (Wallac, Inc.) with automated application of ATP and luciferin assay substrates. All experiments were carried out in triplicate.

### 2.6. Insulin secretion assays

Cells were transferred into 24-well plates (Costar, Corning) and grown to 90% confluence. After washing with fresh medium, 500 μL of test solution (in fresh medium) was added to the wells for 30 min at 37° in a humidified incubator. Medium was then transferred to Eppendorf tubes, spun at 2000 *g*, 4°, for 1 min, and the supernatant collected and placed on ice. Samples were diluted into assay buffer (Linco Research, Inc.) and radioimmunoassayed for insulin concentration according to the instructions of the manufacturer.

### 2.7. cAMP accumulation assays

Cells were transferred to 96-well plates and grown to 90% confluence. After washing with fresh medium, 100 μL test solution (in fresh medium) was added to the wells for 30 min at 37° in a humidified incubator. IBMX was added 5 min prior to the test compounds. The medium was then aspirated, and the cells were lysed. cAMP accumulation was measured using the Biotrak cAMP enzyme immunoassay (EIA) system (Amersham Pharmacia Biotech) according to the instructions of the manufacturer. cAMP levels were determined by optical density at 450 nm.

### 2.8. PKA assays

PKA assays were carried out using the SignaTECT cAMP-dependent protein kinase (PKA) assay system (Promega), according to the instructions of the manufacturer. Briefly, increasing concentrations of PKA catalytic subunit were added to a mixture containing 0.1 mM ATP, trace [$\gamma$-$^{32}$P]ATP, 40 mM Tris–HCl, pH 7.4, 20 mM $MgCl_2$, 0.1 mg/mL of BSA, and 100 μM PKA biotinylated peptide substrate for 10 min in the absence or presence of DMSO at 30°. The reaction was terminated by 2.5 M guanidine hydrochloride. The terminated reaction mixture was spotted onto biotin capture (SAM) membranes and washed rigorously with 2 M NaCl in 1% $H_3PO_4$, followed by deionized water. Membranes were dried, and the specific activity of [$\gamma$-$^{32}$P]ATP was determined by scintillation counting. Enzyme specific activity was then calculated.

### 2.9. Immunoblotting

Immunoblotting for CREB and phospho-CREB was carried out in accordance with the instructions of the manufacturer with modifications (Cell Signaling Technology). After treatment, cells were washed with PBS, and lysed in 70 μL lysis buffer [25 mM Tris-phosphate (pH 7.8), 2 mM dithiothreitol, 1% Triton X-100, 1 mM EGTA, 1 mM sodium orthovanadate, 50 mM β-glycerophosphate, 10% glycerol, and 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid]. Cell lysates were collected into Eppendorf tubes, vortexed, and frozen overnight. After centrifugation at 14,000 *g* for 5 min at room temperature, the resultant supernatant was collected, followed by the addition of SDS–PAGE buffer containing 50 mM Tris–Cl, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and bromophenol blue (pH 6.8). Samples were boiled for 2 min, centrifuged at 14,000 *g* for 2 min at room temperature, then analyzed on 10% SDS–polyacrylamide gels, and transferred to nitrocellulose membranes. The membranes were blocked with 10% non-fat dry milk in Tris-buffered saline with 0.1% Tween-20 (TBS/T) for 1 hr at 22°. The blots were then treated with the primary antibody in TBS/T containing 5% BSA at 4° overnight. After washing, immunolabeling was detected by LumiGlo chemiluminescent reagent (Cell Signaling Technology) according to the instructions of the manufacturer. Then membranes were stripped by incubation for 45 min at 65° in a solution containing 10 mM Tris (pH 6.7), 100 mM β-mercaptoethanol, and 2% SDS. After washing, the efficacy of stripping was determined by re-exposure of the membranes to LumiGlo reagent. Thereafter, blots were re-blocked and immunolabeled as described above.

### 2.10. Statistical analysis

Concentration–response curves were analyzed by non-linear regression with a variable slope, using Graphpad Prism (Graphpad Software Inc.). Other graphs were generated using Microsoft Excel. Data represent means ± SEM. Statistical significance was determined using Student's *t*-test.

## 3. Results

### 3.1. DMSO augumentation of the GLP-1 incretin response in INS-1 cells

In cells transiently expressing a luciferase reporter gene construct under the transcriptional control of the rINS1 promoter, 10 nM GLP-1 and 1 μM forskolin induced a 2.5- and 4.5-fold increase in luciferase gene expression, respectively (Fig. 1A). In the presence of 1.5% DMSO in the medium, the fold increase in luciferase gene expression was augmented to 6.5- and 11-fold, respectively. The basal expression level was not affected, suggesting that the

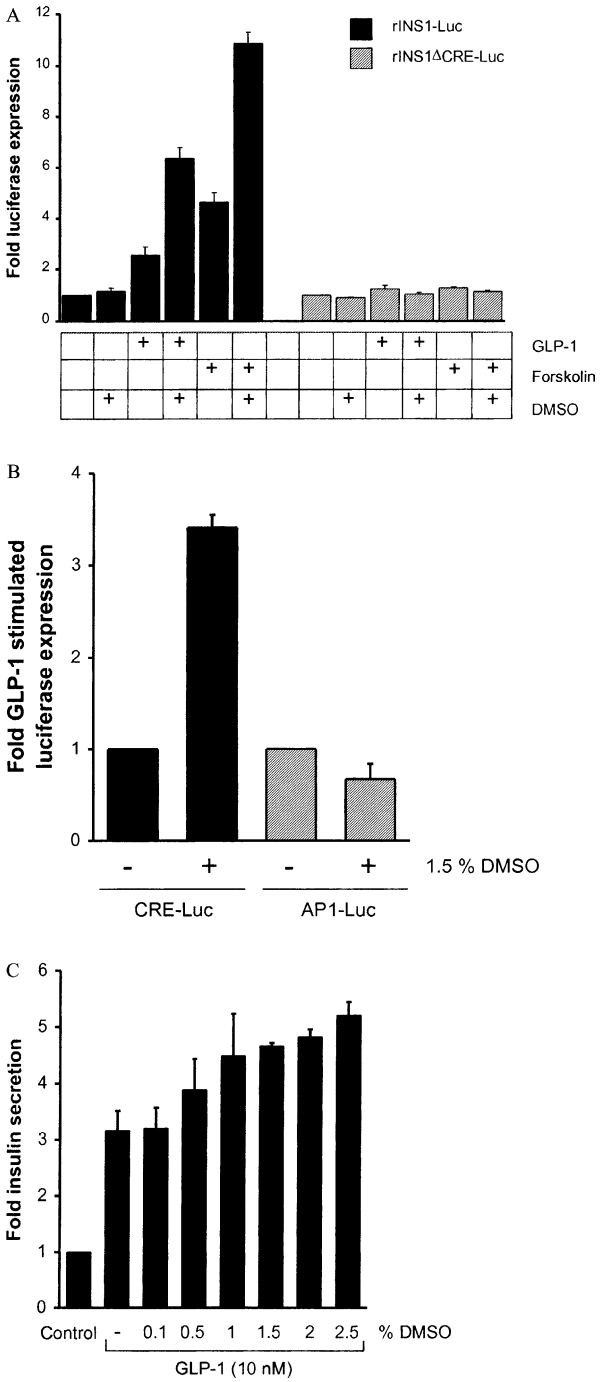


Fig. 1. Augmentation of GLP-1 mediated responses by DMSO in INS-1 cells. (A) INS-1 cells expressing either the rINS1-luciferase reporter gene (*rINS1-Luc*, solid bars) or the CRE-mutated rINS1ΔCRE-luciferase gene (*rINS1ΔCRE-Luc*, shaded bars) were treated for 4 hr with either 10 nM GLP-1 or 1 μM forskolin in the absence or presence of 1.5% DMSO (v/v). Data are normalized to the basal level of gene expression for both reporter genes. (B) INS-1 cells expressing a generic CRE-luciferase gene (*CRE-Luc*, solid bars) or a generic AP1 luciferase gene (*AP1-Luc*, shaded bars) were treated for 4 hr with GLP-1 in the absence or presence of 1.5% DMSO. Data are normalized to GLP-1-stimulated levels for both reporter genes. (C) Insulin secretion was monitored following a 30-min treatment of INS-1 cells with 10 nM GLP-1 in the presence of increasing concentrations of DMSO in the medium. Basal levels of secretion were not augmented by DMSO at any concentration. In all experiments, cells were incubated at 37° in a humidified atmosphere. Data points are mean values (±SEM) of three independent experiments carried out in triplicate.

DMSO-induced response was cAMP-dependent as activation of both the GLP-1 receptor and adenylyl cyclase (direct targets of GLP-1 and forskolin) leads to elevated cAMP levels. In cells expressing the same reporter construct but with a mutated (non-functional) CRE within the promoter sequence (rINS1ΔCRE-Luc), GLP-1 and forskolin induced a greatly attenuated increase in luciferase expression, which was not augmented in the presence of DMSO (Fig. 1A). The induced response in rINS1 promoter activity appeared, therefore, to be mediated through the CRE. This was supported further by the relative response in reporter gene expression of the CRE-reporter gene, whereas no significant effect was observed in AP1-luciferase reporter gene expression (Fig. 1B) (N.B., GLP-1 induced a 2.7-fold increase in AP1-luciferase expression over basal level).

DMSO also enhanced GLP-1-stimulated insulin secretion from INS-1 cells, in a concentration-dependent manner (Fig. 1C). Alone, GLP-1 stimulated a 3-fold increase in secretion, which was augmented to an approximately 5-fold increase in the presence of 2.5% DMSO. Basal levels of insulin secretion were not augmented by DMSO (data not shown).

### 3.2. Characterization of the DMSO effect on CRE-mediated gene transcription

As GLP-1 induces both insulin secretion and gene expression in a cAMP-dependent manner, we employed the CRE-luciferase reporter construct as a robust readout of cAMP-mediated gene expression to further characterize the effects of DMSO. Ten nanomolar GLP-1 induced a 6-fold increase in luciferase gene expression, which was augmented concentration-dependently to a 25-fold increase at 2.5% DMSO (Fig. 2A). Basal levels were not increased. In a time–course

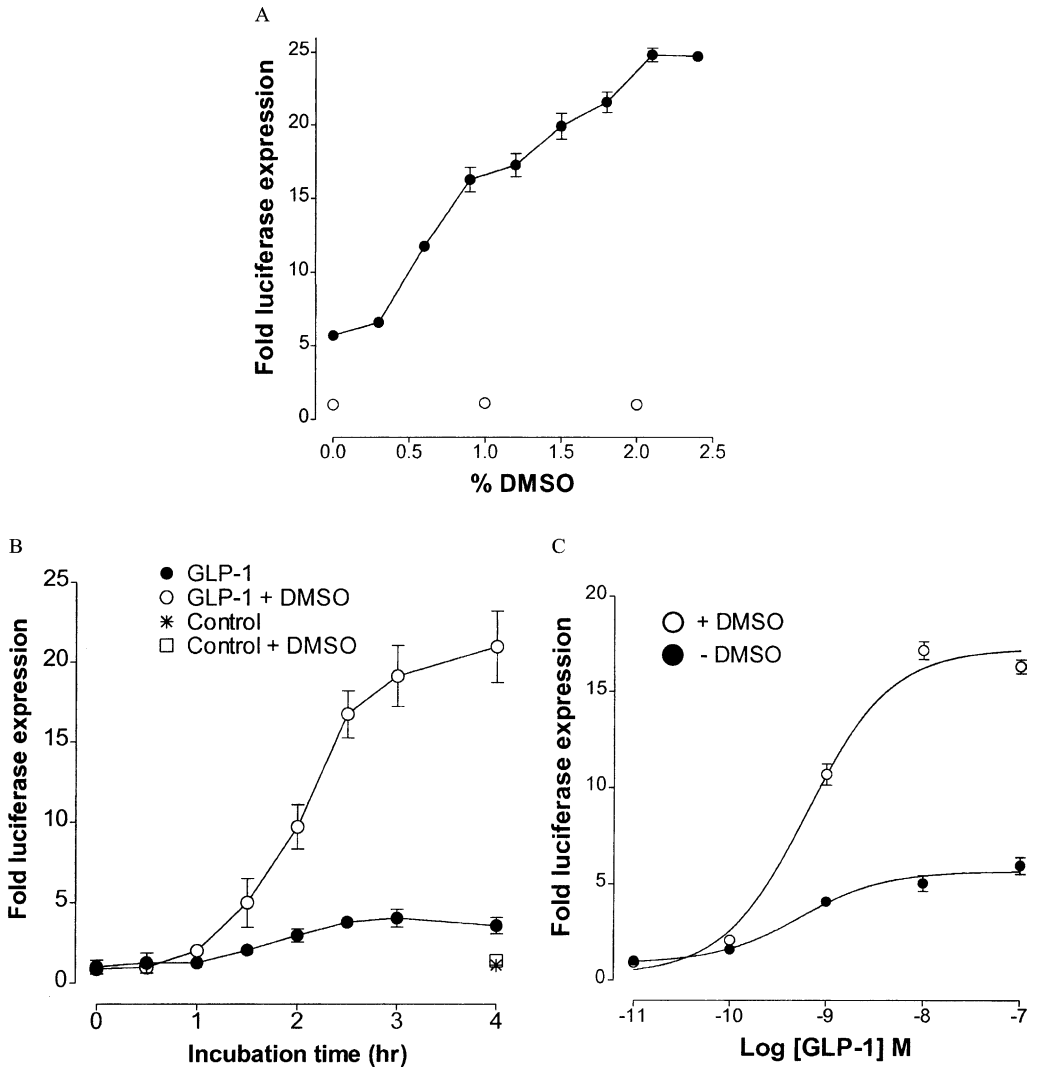


Fig. 2. Concentration- and time-dependence of the DMSO effect. INS-1 cells expressing the generic CRE-luciferase reporter gene (*CRE-Luc*) were incubated at 37° in a humidified atmosphere. (A) Treatment with 10 nM GLP-1 and increasing concentrations of DMSO for 4 hr (open circles represent basal levels in the presence of increasing DMSO concentrations). (B) Treatment with 10 nM GLP-1 in the presence and absence of 1.5% DMSO for increasing incubation times. (C) Treatment with increasing concentrations of GLP-1 in the presence (open circles) and absence (closed circles) of 1.5% DMSO for 4 hr. Data points are mean values (±SEM) of three independent experiments carried out in triplicate.

experiment, augmented responses were observed after a 1-hr incubation (Fig. 2B), which reflected an almost instantaneous effect, as increased luciferase levels were not observed until after a 1-hr incubation in the absence of DMSO, presumably due to the time required for transcriptional/translational mechanisms to be completed. The potency of the GLP-1 response ($pEC_{50} = 9.27 \pm 0.2$) was not altered by DMSO ($pEC_{50} = 9.20 \pm 0.1$) (Fig. 2C).

To determine whether enhancement was induced in a GLP-1 receptor-specific manner or was mediated by a downstream point in the signaling pathway, we assessed the effect of DMSO on CRE-luciferase activity induced by forskolin and IBMX (Fig. 3A and B, respectively). Both cAMP-elevating agents induced a concentration-dependent increase in luciferase expression, which was augmented by 1.5% DMSO. Again, no alterations in the $EC_{50}$ values were observed.

### 3.3. Lack of effect of DMSO on intracellular cAMP levels

In the presence of 100 μM IBMX (a non-specific inhibitor of cAMP-dependent phosphodiesterases), neither GLP-1- nor forskolin-stimulated cAMP levels were enhanced by DMSO (Fig. 4A and B). To address the possibility that the mechanism of action was targeted to phosphodiesterase activity (which could not be determined in the presence of IBMX), forskolin-stimulated cAMP levels were monitored in the absence of IBMX. A more modest increase in cAMP accumulation was detected, which could only be achieved at 10 μM forskolin (Fig. 4C). Again, DMSO had no enhancing effect on cAMP levels.

Further evidence to suggest that DMSO is acting at a downstream target relative to cAMP resulted from CRE-luciferase reporter gene studies. In the presence of 100 μM IBMX, the forskolin concentration response was shifted to the left (from $pEC_{50} = 6.18 \pm 0.07$ [−IBMX] to $7.3 \pm 0.15$ [+IBMX], Fig. 4D) and the basal luciferase expression level was elevated, both as a result of increased cAMP levels in the cytoplasm. However, the peak luciferase expression level was the same in the absence or presence of IBMX. The transcriptional rate-limiting factor appears to be downstream of cAMP because the peak cAMP level in response to forskolin was clearly augmented in the presence of IBMX (see Fig. 4B and C). However, in the presence of DMSO, the responses of the reporter gene to forskolin ± IBMX were clearly augmented (Fig. 4E), suggesting that DMSO imparts its effect at a target downstream of cAMP.

### 3.4. Effects of DMSO on PKA activity and phosphorylation levels of CREB, CREM, and ATF-1

Because insulin secretion and CRE-dependent transcription are mediated at least in part by PKA, we investigated whether DMSO augments PKA activity directly. Using an *in vitro* method to determine PKA-dependent phosphorylation of a synthetic peptide (Kemptide), increasing concentrations of the PKA-catalytic subunit induced a linear increase in phosphorylation (Fig. 5A). However, in the presence of DMSO, the catalytic activity of PKA was attenuated, suggesting that the augmentation effect of DMSO is not mediated directly by PKA activity. Because CREB is a direct downstream target of PKA, resulting in phosphorylation at Ser 133, we confirmed the above result by determining pCREB levels in response to GLP-1 treat-

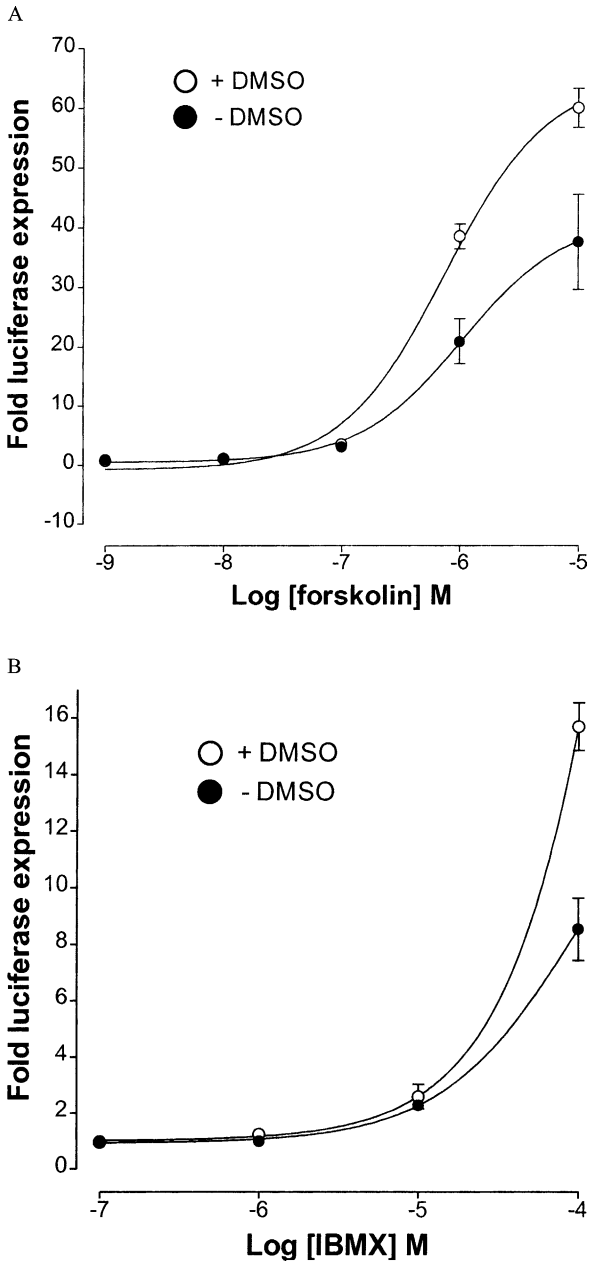


Fig. 3. DMSO enhancement of cAMP-mediated transcription. INS-1 cells expressing the generic CRE-luciferase reporter gene (*CRE-Luc*) were incubated at 37° in a humidified atmosphere for 4 hr. (A) Treatment with increasing concentrations of forskolin in the presence (open circles) and absence (closed circles) of 1.5% DMSO. (B) Treatment with increasing concentrations of IBMX in the presence (open circles) and absence (closed circles) of 1.5% DMSO. Data points are mean values (±SEM) of three independent experiments carried out in triplicate.

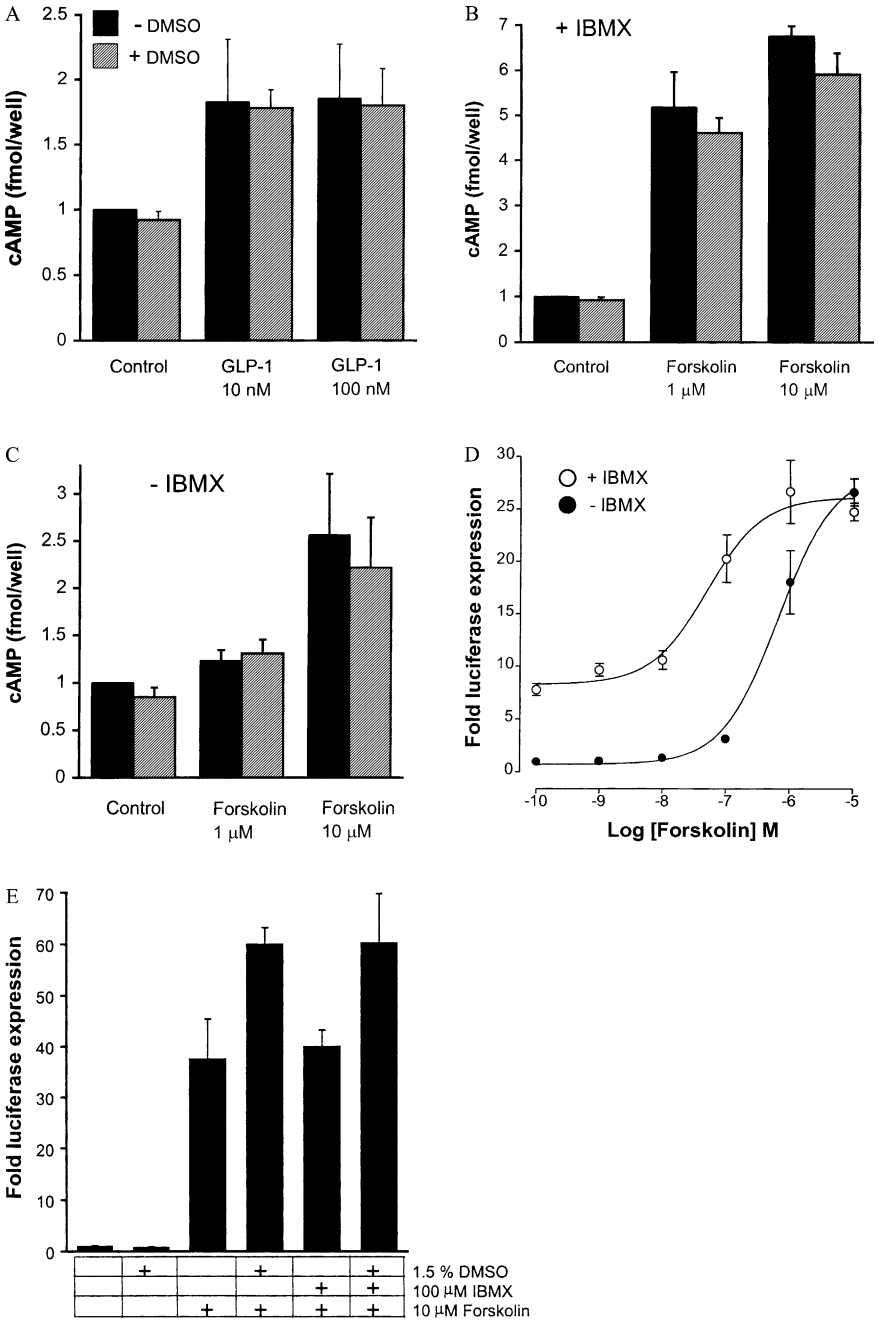


Fig. 4. Effects of DMSO on intracellular cAMP levels. Intracellular cAMP concentrations were determined in INS-1 cells following 30-min incubations with GLP-1 (A) and forskolin (B) in the presence of 100 μM IBMX. Shaded bars represent cAMP levels in the presence of 1.5% DMSO. (C) cAMP concentrations determined in the absence of IBMX. (D) Cells expressing the generic CRE-luciferase reporter gene (*CRE-Luc*) were treated at 37° in a humidified atmosphere for 4 hr with increasing concentrations of forskolin in the presence (open circles) and absence (closed circles) of 100 μM IBMX. (E) Cells expressing the generic CRE-luciferase reporter gene were treated at 37° in a humidified atmosphere for 4 hr with the indicated reagents. Data points are mean values (±SEM) of three independent experiments carried out in triplicate.

ment in the absence and presence of DMSO over an extended time–course by western blot analysis. CREB was phosphorylated rapidly and transiently in response to GLP-1 (Fig. 5B and C). In the presence of 1.5% DMSO, the peak level of phosphorylation was attenuated to a degree similar to that seen by direct PKA activity analysis. As the phospho-CREB antibody also detected pCREM and pATF1, we were able to determine the phosphorylation profile of these transcription factors simultaneously. A similar effect of DMSO was observed in both CREM and ATF1 phosphorylation levels in response to GLP-1 (Fig. 5D and E).

## 4. Discussion

The ability of DMSO to synergistically enhance the incretin effect of GLP-1 (and forskolin) in β-cells at

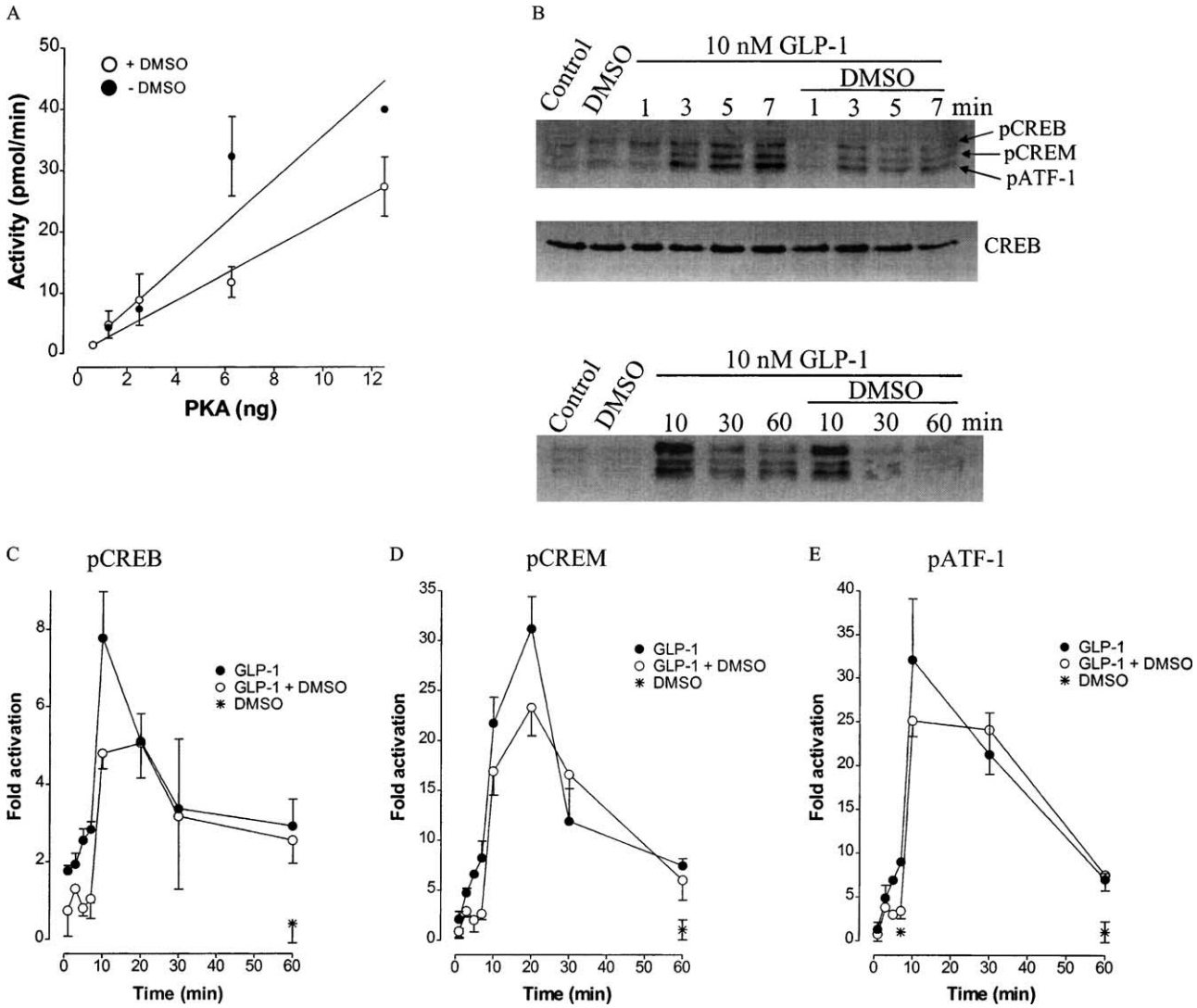


Fig. 5. Effects of DMSO on PKA activity and GLP-1-stimulated CREB phosphorylation. (A) *In vitro* PKA activity was determined at increasing concentrations of PKA catalytic subunit in the presence (open circles) and absence (closed circles) of 1.5% DMSO. Data points are mean values (±SEM) of three independent experiments carried out in triplicate. (B) Representative western blot analyses of time-dependent phosphorylated CREB, CREM, and ATF-1 in response to 10 nM GLP-1 in the presence and absence of 1.5% DMSO. Immunoblots were quantitated by scanning densitometry, and results are expressed as fold stimulation over the control values for (C) pCREB, (D) pCREM, and (E) pATF-1. Data points are mean values (±SEM) of three independent experiments.

concentrations above 0.5% (v/v) was an unexpected phenomenon. Although DMSO has been reported to impart diverse effects in several cell types by various mechanisms [8,9], the observations presented here suggest that DMSO directs a specific mechanistic effect in β-cells. Because no effects on the basal level of insulin secretion or reporter gene expression were observed, it appears that DMSO imparts its functional effect at a specific point on the GLP-1 signaling pathway. As the GLP-1 receptor primarily (but not exclusively) functions via cAMP [12,14], it was notable that forskolin-induced rINS1-Luc activity also was augmented by DMSO, suggesting that the site of action is situated at a point along the cAMP signaling cascade. Enhancement of cAMP-dependent responses by DMSO has been reported previously [8]. Wieser *et al.* [16] described an increase in propanalol-induced cAMP accumulation in the presence of 1.1 M DMSO in white fat cells, which was shown to be caused by the inhibition of phosphodiesterase activity. In our study, however, cAMP levels were not elevated directly by DMSO, in either the absence or the presence of the phosphodiesterase inhibitor IBMX. Panek *et al.* [17] showed in *Saccharomyces cerevisiae* that the effects of cAMP were enhanced by DMSO. Interestingly, in that model, cAMP levels were not increased, but DMSO was able to augment the action of cAMP. A possible explanation may be that permeabilization, membrane penetration, and solvency properties of DMSO altered the intracellular compartmentation structure of the cell to enhance molecular interaction.

The downstream signaling determinants of GLP-1-stimulated insulin promoter activity remain elusive, and recent evidence suggests an independence of signaling

on PKA activity [18] (which characteristically mediates CRE activity in response to cAMP elevation in many cell types). In our studies, we found that DMSO did not increase PKA activity or GLP-1-induced phosphorylation of CREB, supporting the notion that GLP-1-stimulated insulin promoter activity is independent of PKA. Furthermore, in nuclear extracts of HIT cells, protein complex binding to the insulin CRE displayed little or no proteins with CREB-like immunoreactivity [19,20], suggesting that an alternate transcription factor is responsible for insulin promoter induction. This was also implied here since CREB, CREM, and ATF-1 phosphorylation levels were not augmented by DMSO, but were inhibited. Interestingly, in rat primary cultured islets, transfection of a CREB expression plasmid resulted in a repression in human insulin promoter activity [21]. Thus, the attenuation in GLP-1-induced CREB phosphorylation by DMSO may also reflect an inhibitory action of CREB on insulin gene expression, resulting in enhanced promoter activity.

In cultures of insulinoma cells, such as RIN, only a small percentage of cells present a β-cell phenotype (i.e. express insulin) [22,23]. However, incubation with the differentiation agent sodium butyrate increased glucagon and insulin expression by recruiting immunocytochemically negative cells to produce hormone [23,24]. Thus, DMSO, also an established inducer of differentiation, may recruit cells to express insulin, resulting in the enhanced secretion and transcription levels observed in these studies. This model is unlikely, however, as enhanced levels of secretion were detected less than 30 min after the addition of DMSO, and increases in transcription were equally immediate, whereas sodium butyrate was required for at least 12 hr to affect a detectable increase in insulin mRNA in RIN cells [24]. Also, no increase in basal levels was noted following DMSO treatment in our experiments, and elevated levels of cAMP appeared to be a prerequisite for enhanced functional responses. This circumstantial evidence does not, however, preclude this model, and further investigation is warranted.

Recently, differentiation of exocrine AR42J cells into insulin-producing cells was shown to be induced by GLP-1 [25]. It may be hypothesized from these present data that DMSO could augment the differentiation process, by enhancing GLP-1-induced intracellular signaling. A role may also be envisioned for DMSO in the differentiation of stem cell-derived β-cells [26]. This concept is substantiated by reports that erythroid cell differentiation is induced by DMSO and cAMP-stimulating ligands (e.g. erythropoietin, forskolin, and IBMX) in erythroid progenitor cells [27]. Furthermore, co-incubation of erythropoietin and DMSO synergistically increased the differentiation rate of these cells. However, the mechanism by which DMSO acted was not resolved, and was shown not to affect cAMP levels [27,28]. Thus, it is tempting to suggest that DMSO may augment or induce a downstream target of cAMP in erythroid progenitor cells to facilitate differentiation, in a similar mechanistic event as described here.

As no discernable effect was observed on basal activities in our studies, DMSO may be an attractive candidate for treatment of type 2 diabetes, whereby pancreatic function would be enhanced without the caveat of a hypoglycemic response. However, this approach may not be an appropriate avenue due to the multitude of unrelated effects of DMSO on other tissues.

Finally, as a functional tool, DMSO may be useful in identifying novel cAMP-mediated incretin-like compounds. By augmenting functional responses in insulin secretion and gene expression, compounds that effect a small but significant induction may be distinguished more easily. This may be beneficial for identifying ligands imparting longer acting or sustained effects on β-cell function, which may otherwise be overlooked in screening regimens. The use of this interaction between DMSO and GLP-1 signaling may also add insight into further characterizing the signaling pathway(s) induced by GLP-1 receptor activity.

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