RESEARCH COMMUNICATION

Acyl phosphatase activity of NO-inhibited glyceraldehyde-3-phosphate dehydrogenase (GAPDH): a potential mechanism for uncoupling glycolysis from ATP generation in NO-producing cells

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Treatment of glyceraldehyde-3-phosphate-dehydrogenase (GA(GAPDH) with the NO donors *S*-nitrosoglutathione, 3-morpholinolinosydnonimine or diethylamine NONOate (diethylamine diazeniumdiolate) *in vitro*, inhibited its dehydrogenase activity and induced its acyl phosphatase activity. NO-producing cells, in turn, exhibited reduced GAPDH activity, increased glycolysis,

and decreased ATP content, synthesis and turnover. These cellular alterations could be explained by the uncoupling of glycolytic flux from substrate level phosphorylation by the acyl phosphatase activity of NO-modified GAPDH.

Key words: glucose, macrophage, metabolism.

INTRODUCTION

ATP production in glycolytic cells, such as macrophages, results mainly from substrate level phosphorylation reactions catalysed by glyceraldehyde-3-phosphate-dehydrogenase (GAPDH; D-glyceraldehyde-3-phosphate:NAD⁺ oxidoreductase, EC 1.2.1.12) and pyruvate kinase (ATP:pyruvate 2-*O*-phosphotransferase, EC 2.7.1.40).

Recent work has shown that NO, a product of the metabolism of L-arginine through nitric oxide synthases (NOS), inhibits GAPDH [1–6]. It has been shown, in this regard, that NO inhibits GAPDH reversibly through the *S*-nitrosation of its active-site Cys¹⁴⁹, and irreversibly through the subsequent covalent attachment of NADPH to the enzyme [7]. The suppressive effects of NO on GAPDH led to the proposal that NO should reduce glycolytic flux in NO-producing cells [1–4]. Paradoxically, previous results from this [8,9] and other laboratories [6], and the results presented here, indicate that, while NO indeed decreases cellular GAPDH activity, it simultaneously increases glycolytic flux.

Harary demonstrated in 1957 that an acyl phosphatase isolated from muscle catalyses the hydrolysis of 1,3-bisphosphoglycerate, the product of the GAPDH reaction, into 3-phosphoglycerate with the liberation of P_i [10]. Acyl phosphatase thus effectively uncouples glycolytic flux from the synthesis of ATP at the enzyme reaction that follows that catalysed by GAPDH, 3-phosphoglycerate kinase (ATP:3-phospho-D-glycerate-1-phosphotransferase, EC 2.7.2.3). Because of the ability of acyl phosphatase to allow continued flux of three-carbon fragments through glycolysis without the production of ATP, Harary originally proposed that this enzyme activity could play a regulatory role in cellular energetics [10].

Work by Park and Koshland [11], Allison and Connors [12], and others demonstrated acyl phosphatase to be an alternative activity of GAPDH itself, resulting from the oxidation of a thiol at its active-site Cys¹⁴⁹ to a sulphenic acid derivative. Most recently, Becker et al. [13] reported that NO, as delivered by the

NO donor *S*-nitrosoglutathione, could oxidize an active-site Cys in glutathione reductase [NAD(P)H:oxidized-glutathione oxidoreductase, EC 1.6.4.2] to a sulphenic acid derivative. The hypothesis that NO can oxidize the active site of GAPDH in a similar manner and thereby elicit this enzyme's acyl phosphatase activity while inhibiting its dehydrogenase activity was tested. Results presented in this paper demonstrate inhibition of the dehydrogenase activity of GAPDH and the appearance of acyl phosphatase activity in *S*-nitrosoglutathione-, 3-morpholinolinosydnonimine- or diethylamine NONOate-treated GAPDH *in vitro*.

Metabolic studies on macrophages cultured in conditions modulating NO production, in turn, indicated that cells generating NO exhibited increased glycolytic flux, decreased glucose oxidation, and decreased ATP content and turnover. These findings, together with the present *in vitro* results and previous observations confirming the inhibition of GAPDH in NO-producing cells [9], are consistent with the proposal that NO could uncouple glycolytic flux from ATP generation in cells by endowing GAPDH with acyl phosphatase activity.

MATERIALS AND METHODS

Treatment of GAPDH with NO donors

Rabbit muscle GAPDH (1 mg/ml, Boehringer Mannheim, Indianapolis, IN, U.S.A.) was incubated for 0–24 h with 0–10 mM *S*-nitrosoglutathione in the presence of NAD⁺ or NADP⁺ (0–10 mM) and 5 mM EDTA in 0.1 M triethanolamine buffer, pH 7.6 [14]. *S*-Nitrosoglutathione-treated or untreated GAPDH (150 µg protein/ml) were assayed for acyl phosphatase and GAPDH activities. In additional experiments, GAPDH was treated with 3-morpholinolinosydnonimine, *S*-nitroso-*N*-acetyl-D,L-penicillamine or diethylamine NONOate in the concentrations indicated in the text for 3 h in 0.1 M triethanolamine buffer containing 10 mM NAD⁺, and then assayed for acyl phosphatase and GAPDH activities. Unless indicated, reagents were from Sigma Chemical Co., St Louis, MO, U.S.A.

Abbreviations used: ANOVA, analysis of variance; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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Acyl phosphatase activity assays

Unless indicated otherwise, acyl phosphatase activity was determined enzymically as described in [14]. For this assay, a 1,3-bisphosphoglycerate generating system composed of 6 mM 3-phosphoglycerate, 5 mM ATP and 3-phosphoglycerate kinase (18 units/ml) was added to 0.05 M imidazole buffer, pH 7.0, containing 5 mM EDTA, 5 mM MgCl_2 and 0.5 mM NAD^+ . The sample was added after a 5 min equilibration and the acyl phosphatase activity was calculated from the appearance of ADP, as detected by HPLC [15]. Identical results were obtained when $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (NEN, Cambridge, MA, U.S.A.) was included in the assay, and the acyl phosphatase activity was calculated from the liberation of $[\text{}^{32}\text{P}]\text{P}_i$ (results not shown). That the ADP generated in the assay did not result from non-specific ATPase activity of the preparations was ascertained in each experiment by controls where components of the 1,3-bisphosphoglycerate generating system, 3-phosphoglycerate kinase or 3-phosphoglycerate, were excluded from the assay. No ADP generation was detected in the control reactions. Preliminary results demonstrated ADP production to be linear with reaction time up to 15 min. Assays were performed thereafter using this reaction time. Results are reported as nmol of ADP generated/min per μg of protein.

Acyl phosphatase activity was also measured using acetyl phosphate as a substrate. For this assay, *S*-nitrosoglutathione-treated or untreated GAPDH, or GAPDH that was incubated previously with iodosobenzoate (20 mM) in 0.1 M triethanolamine buffer, pH 7.6, containing 5 mM EDTA and 10 mM NAD^+ , for 1 h on ice, was added at 300 μg per assay to 0.1 M triethanolamine buffer containing 4 mM acetyl phosphate. The concentration of acetyl phosphate remaining in the cuvettes after 20, 40, 60 and 120 min was determined spectrophotometrically at 510 nm using the method of Lipmann and Tuttle [16].

GAPDH activity assay

GAPDH activity was measured and is reported exactly as indicated previously [9].

Glucose and energy metabolism in rat peritoneal macrophages

Resident peritoneal macrophages were harvested from Fischer rats (Charles River, Wilmington, MA, U.S.A.) by peritoneal lavage after CO_2 killing and cultured at 2×10^6 cells/ml in culture medium where the L-arginine concentration was adjusted to either 6 μM (low L-arginine medium) or 1 mM (high L-arginine medium) as reported previously [17]. After 20 h of culture, $[1\text{-}^{14}\text{C}]\text{glucose}$ (0.25 $\mu\text{Ci}/\text{ml}$) or $[6\text{-}^{14}\text{C}]\text{glucose}$ (0.5 $\mu\text{Ci}/\text{ml}$; Dupont NEN, Cambridge, MA, U.S.A.) was added to the cultures and then incubated for an additional 4 h. Glycolysis was calculated from the conversion of $[1\text{-}^{14}\text{C}]\text{glucose}$ to $[^{14}\text{C}]\text{lactate}$ and the initial glucose specific radioactivity [8]. Glucose oxidation through the tricarboxylic acid cycle was calculated from the appearance of $^{14}\text{CO}_2$ in cultures containing $[6\text{-}^{14}\text{C}]\text{glucose}$ and the initial glucose specific radioactivity [8]. Theoretical ATP yields were calculated using 2 mol of ATP/mol of glucose metabolized through glycolysis and 36 mol of ATP/mol of fully oxidized glucose.

Macrophages obtained as described above were also cultured for 20 h in low or high L-arginine media, or in high L-arginine medium containing *N*^G-monomethyl-L-arginine (0.5 mM). $[\text{}^{32}\text{P}]\text{P}_i$ (5 $\mu\text{Ci}/\text{ml}$; DuPont NEN, Boston, MA, U.S.A.) was added to the cultures and then incubated for an additional 2 h. ATP, ADP and AMP contents of the cells, as well as the incorporation of $[\text{}^{32}\text{P}]\text{P}_i$ into the adenylate pool, were measured by HPLC of the

cell lysates obtained at the end of labelling [15]. Adenylate energy charge was calculated using the formula of Atkinson and Walton [18]. Additional cells were washed with PBS at the end of the labelling period and cultured for 30, 60 or 120 min in PBS containing 5 mM glucose and the corresponding L-arginine or *N*^G-monomethyl-L-arginine concentrations. At the indicated times, cells were lysed and the concentrations of ATP, ADP and AMP, as well as the radioactive label contained in these fractions, were analysed by HPLC. Radioactive compounds separated by HPLC were detected using an in-line radioactivity detector (Radiomatic flo-one, Packard, Meriden, CT, U.S.A.).

Data presentation and statistical analysis

Unless specifically indicated, data reported are means $\pm$ S.E.M. from at least 3 independent experiments. When appropriate, statistical analysis of results was performed using non-parametric tests or analysis of variance (ANOVA)–Bonferroni/Dunn. Significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

Treatment of GAPDH with *S*-nitrosoglutathione induces its acyl phosphatase activity

In initial experiments, rabbit muscle GAPDH was incubated with 1 mM *S*-nitrosoglutathione and 10 mM NAD^+ for up to 24 h. Results from these experiments, depicted in Figure 1, demonstrated the appearance of acyl phosphatase activity and the inhibition of GAPDH activity in the *S*-nitrosoglutathione-treated enzyme. As shown in the Figure, maximal acyl phosphatase activity was detected after 3 h of exposure of the enzyme to the NO donor and then it declined. In contrast, the inhibition of GAPDH activity increased and persisted during the exposure of the enzyme to *S*-nitrosoglutathione for up to 24 h.

The dose–response relationship between *S*-nitrosoglutathione concentration and acyl phosphatase activity was determined in experiments where the *S*-nitrosoglutathione concentration was varied between 0 and 10 mM. Data in Figure 2 demonstrate that maximal acyl phosphatase activity was achieved with 1 mM *S*-nitrosoglutathione, and that higher concentrations of the NO donor resulted in increased inhibition of GAPDH activity without augmenting acyl phosphatase activity.

Acquisition of acyl phosphatase activity by *S*-nitrosoglu-

Figure 1 *S*-nitrosoglutathione suppresses GAPDH activity and induces acyl phosphatase activity in rabbit muscle GAPDH

Rabbit muscle GAPDH was treated, or not treated, with *S*-nitrosoglutathione (1 mM) for up to 24 h as described in the Materials and methods section. The incubation mixture contained NAD^+ (10 mM). Acyl phosphatase and GAPDH activities were measured as indicated in the Materials and methods section.

Figure 2 Dose-response relationship between *S*-nitrosoglutathione concentration and acyl phosphatase and GAPDH activities of GAPDH

GAPDH was incubated for 3 h in the presence of 0–10 mM *S*-nitrosoglutathione and 10 mM NAD^+ . Acyl phosphatase and GAPDH activities were measured at the end of incubation as indicated in the Materials and methods section. GSNO, *S*-nitrosoglutathione.

Figure 3 NAD^+ and NADP^+ enhance the induction of the acyl phosphatase activity of GAPDH exposed to *S*-nitrosoglutathione

GAPDH was incubated with 1 mM *S*-nitrosoglutathione for 3 h in buffer containing 0–10 mM NAD^+ or NADP^+ . Acyl phosphatase and GAPDH activities were measured at the end of incubation as indicated in the Materials and methods section. Neither NAD^+ nor NADP^+ altered GAPDH activity when added to the enzyme in the absence of *S*-nitrosoglutathione. Incubation with *S*-nitrosoglutathione with or without NAD^+ or NADP^+ suppressed GAPDH activity by greater than 75% (results not shown).

tathione-treated GAPDH was increased by adding NAD^+ or NADP^+ during the incubation of the enzyme with *S*-nitrosoglutathione. Figure 3 indicates, in this regard, that maximal acyl phosphatase activity was detected in preparations incubated with 1 mM NAD^+ or 10 mM NADP^+ .

Additional experiments determined acyl phosphatase activity in *S*-nitrosoglutathione-treated enzyme by measuring the disappearance of acetyl phosphate. These experiments also included GAPDH treated with iodosobenzoate, a compound known to oxidize the active-site Cys in the enzyme to a stabilized sulphenic group, and resulted in acyl phosphatase activity [12,19,20]. Results shown in Figure 4 demonstrate the consumption of acetyl phosphate by GAPDH treated with either *S*-nitrosoglutathione or iodosobenzoate. While exact kinetic comparisons were not made, these results appear to agree with those of Ehring and Colowick [14] indicating that hydrolysis of 1,3-bisphosphoglycerate by the acyl phosphatase activity of GAPDH proceeds more rapidly than that of acetyl phosphate. Moreover, results from these experiments confirmed that the detected phosphatase activity was not an ATPase since these could hydrolyse C–O bonds [12].

Figure 4 Acyl phosphatase activity of *S*-nitrosoglutathione or iodosobenzoate treated GAPDH

GAPDH was incubated with (○) or without (□) 1 mM *S*-nitrosoglutathione in the presence of NAD^+ (10 mM), or with iodosobenzoate (△), as described in the Materials and methods section, and then added to 0.1 M triethanolamine buffer (pH 7.6) containing 4 mM acetyl phosphate. The disappearance of acetyl phosphate over time was determined spectrophotometrically as described in the Materials and methods section.

Rabbit muscle GAPDH was also exposed to 3-morpholino-sydnonimine (0–10 mM), *S*-nitroso-*N*-acetyl-D,L-penicillamine (0–1 mM) or diethylamine NONOate (0–10 mM). As shown in Table 1, all NO donors suppressed GAPDH activity, but only 3-morpholinosydnonimine and diethylamine NONOate resulted in acyl phosphatase activity. It has been shown that thiols can react with NO to form diverse oxidation products, and that *S*-nitrosoglutathione, but not dinitrosyl-diethylthionyl-iron ($\text{DNIC}[\text{GSH}]_2$), oxidizes the active site Cys in glutathione reductase to a stable Cys sulphenic acid [13,21]. Present findings suggest that *S*-nitrosoglutathione, 3-morpholinosydnonimine, and diethylamine NONOate directly or indirectly oxidize the active site Cys¹⁴⁹ in GAPDH to a sulphenic derivative capable of endowing the enzyme with acyl phosphatase activity. *S*-nitroso-*N*-acetyl-D,L-penicillamine, in turn, probably results in the formation of higher oxidation products at Cys¹⁴⁹ (Cys-sulphenic or Cys-sulphonic acid) which inhibit GAPDH activity without eliciting acyl phosphatase activity.

Impact of NO production on macrophage glucose metabolism and energetics

The preceding results may provide a basis for explaining previous [8,9,17] and present findings on the impact of NO on macrophage metabolism and energetics. In this regard, cells producing NO exhibit profound alterations in substrate and energy metabolism [8]. These alterations include a reduced capacity to oxidize glucose and fatty acids, probably brought about by the inhibition of aconitase and components of the electron transport chain by NO [8,22]. Maintenance of energy equilibrium in cells whose oxidative metabolism is suppressed by NO should result in a quantitatively proportional increase in glycolytic flux. Prior [8,9] and current results (Table 2) obtained from macrophage cultures, where NO synthesis was modulated by the concentration of its metabolic precursor L-arginine [8], demonstrate that the theoretical energy production from glycolysis by cells cultured in conditions allowing the production of NO is many-fold higher than what would be expected were the increase in glycolytic flux solely compensatory for suppressed substrate oxidation. These cells, nonetheless, exhibit a reduced ATP content (–28%), a minimally reduced adenylate energy charge (–4%) and in-

Table 1 Acyl phosphatase and GAPDH activities of rabbit muscle GAPDH following treatment with different NO donors

Rabbit muscle GAPDH was exposed to the NO donors indicated, as described in the Materials and methods section, and then analysed for acyl phosphatase and GAPDH activities. Data are the mean $\pm$ S.E.M. for at least three independent experiments. Enzyme activities are expressed as nmol of product produced/min per μ g of protein. N.D. = none detectable. For each different NO donor and determination, * $P < 0.05$ versus no treatment, † $P < 0.05$ versus other NO donor concentrations (ANOVA, Bonferroni/Dunn). The results shown were obtained using a batch of rabbit GAPDH having a lower specific activity than that used in the experiments reported in Figures 1–4. For this reason, experiments where the enzyme was exposed to *S*-nitrosoglutathione were repeated using the new batch of enzyme and are reported in the Table.

Additives	Acyl phosphatase activity	GAPDH activity
None	0.02 $\pm$ 0.01	27.3 $\pm$ 1.3
3-Morpholinosydnonimine		
1 mM	0.03 $\pm$ 0.01 [†]	22.2 $\pm$ 1.1*
5 mM	0.05 $\pm$ 0.01* [†]	17.7 $\pm$ 0.7*
10 mM	0.13 $\pm$ 0.02* [†]	8.6 $\pm$ 0.2* [†]
<i>S</i> -Nitroso- <i>N</i> -acetyl-D,L-penicillamine		
0.01 mM	N.D.	27.5 $\pm$ 0.1 [†]
0.1 mM	N.D.	7.1 $\pm$ 0.2* [†]
1 mM	N.D.	1.1 $\pm$ 0.1* [†]
Diethylamine NONOate		
1 mM	0.22 $\pm$ 0.01* [†]	20.5 $\pm$ 1.2* [†]
5 mM	0.15 $\pm$ 0.01* [†]	10.4 $\pm$ 0.5* [†]
10 mM	0.02 $\pm$ 0.01 [†]	5.9 $\pm$ 0.1* [†]
<i>S</i> -Nitrosoglutathione		
0.1 mM	0.14 $\pm$ 0.01*	16.7 $\pm$ 0.6* [†]
1 mM	0.30 $\pm$ 0.01* [†]	1.9 $\pm$ 0.1*
5 mM	0.22 $\pm$ 0.01* [†]	1.2 $\pm$ 0.1*
10 mM	0.11 $\pm$ 0.01*	1.2 $\pm$ 0.1*

Table 2 Impact of NO production on glucose metabolism by macrophages

Resident rat peritoneal macrophages were cultured as described in the Materials and methods section. Glucose metabolism in low (6 μ M) or in high (1 mM) L-arginine media was measured during the last 4 h of a 24 h culture. Theoretical ATP yields were calculated as described in the Materials and methods section. Results, expressed as nmol/h per 10^6 cells, are the means $\pm$ S.D. for quadruplicate samples from a representative experiment. NO production was estimated from the concentration of its catabolite, NO_2^- , in culture supernatants. Culture in low L-arginine medium resulted in the accumulation of $0.2 \pm 0.1 \mu\text{M NO}_2^-$, while culture in high L-arginine medium resulted in $17.4 \pm 0.1 \mu\text{M NO}_2^-$. * $P < 0.05$ versus low L-arginine medium, Mann-Whitney's U.

	Low L-arginine medium		High L-arginine medium	
	Glucose metabolism	Theoretical ATP yield	Glucose metabolism	Theoretical ATP yield
Glycolysis	5.09 $\pm$ 0.27	10.17 $\pm$ 0.50	16.77 $\pm$ 1.10*	33.54 $\pm$ 2.18*
Oxidation	0.03 $\pm$ 0.01	1.68 $\pm$ 0.42	0.01 $\pm$ 0.01*	0.36 $\pm$ 0.01*
Total	5.12 $\pm$ 0.28	11.43 $\pm$ 0.82	16.78 $\pm$ 1.08*	33.81 $\pm$ 2.01*

Table 3 ATP content, adenylate energy charge and ^{32}P incorporation into ATP by macrophages cultured in conditions that modulate NO production

Results are from the same experiments shown in Figure 5. Rat resident peritoneal macrophages were obtained, cultured and analysed as described in the Materials and methods section. Radiolabelling of the adenylate pool was performed as detailed in the Materials and methods section and in the legend to Figure 5. Data are the means $\pm$ S.D. for triplicate samples from a representative experiment. Within each determination, * $P < 0.05$ versus other culture conditions ($P < 0.05$, ANOVA, Bonferroni/Dunn).

	Culture conditions		
	Low L-arginine	High L-arginine	High L-arginine + <i>N</i> ^G -monoethyl-L-arginine
ATP (pmol/ 10^6 cells)	692 $\pm$ 43	464 $\pm$ 21*	704 $\pm$ 42
Adenylate energy charge	0.901 $\pm$ 0.002*	0.865 $\pm$ 0.002*	0.894 $\pm$ 0.001*
$10^{-4} \times [^{32}\text{P}]\text{P}_i$ to $[^{32}\text{P}]\text{ATP}$ (c.p.m.)	20.7 $\pm$ 0.7	2.9 $\pm$ 0.2*	18.8 $\pm$ 1.6

corporate less ^{32}P into ATP (–85 %; Table 3). The metabolic alterations found in cells cultured in high L-arginine medium result from the production of NO. This is because the addition

of the nitric oxide synthases inhibitor, *N*^G-monomethyl-L-arginine (0.5 mM), to cultures containing 1 mM L-arginine abrogated all metabolic differences with cultures in low L-arginine

Figure 5 Decreased ATP turnover in macrophages cultured in conditions allowing the production of NO

Resident rat peritoneal macrophages were cultured as indicated in the Materials and methods section for 20 h in low (□) or high (△) L-arginine medium or in high L-arginine medium containing 0.5 mM *N*^G-monomethyl-L-arginine (○), and then pulsed with [³²P]_i (5 µCi/well) for 2 h as described in the Materials and methods section. Following flooding with unlabelled P_i, the radioactivity present in ATP, ADP and AMP in cells lysates obtained every 30 min for 2 h was determined by HPLC. ATP content of the cells, their adenylate energy charge, and the incorporation of ³²P into ATP by the end of the labelling period (corresponding to time 0 in the Figure) are shown in Table 3. Neither the ATP content of the cells nor their adenylate energy charge changed through the course of the chase period (results not shown). Data are means ± S.D. for triplicate samples in a representative experiment. When not shown in the Figure, the bars indicating the magnitude of the S.D. were smaller than the symbols indicating the value of the means.

media, with the exception of that in the adenylate energy charge, which remained marginally (−3%) but significantly ($P < 0.05$) lower in these cells than in those cultured in low L-arginine medium.

Metabolic findings just described could be explained by an increased rate of ATP utilization or by a decreased rate of ATP generation in NO-producing cells. The former explanation does not fit available evidence. Published observations indicate that NO inhibits metabolic pathways responsible for a substantial fraction of ATP utilization in cells, including protein synthesis [23], Na⁺/K⁺-ATPase [24], and other ATPases [25]. Moreover, results shown in Table 3 and those depicted in Figure 5 give evidence for a markedly reduced rate of ATP turnover in cells producing NO, a finding not consistent with an increased rate of ATP utilization.

In contrast, results at hand demonstrate that NO-producing cells have a decreased rate of ATP production and turnover, despite their accelerated rate of glycolysis. Moreover, the reduced rate of ATP production in these cells cannot be quantitatively explained by their decreased ability to fully oxidize glucose through the Krebs cycle (Table 2). These findings are compatible with the proposal that NO uncouples glycolysis from substrate based phosphorylation by endowing GAPDH with acyl phosphatase activity.

Current observations incontrovertibly demonstrate the ability of NO donors to elicit acyl phosphatase activity in purified GAPDH. They do not, however, provide definitive evidence of a physiological role for this activity in explaining metabolic alterations found in macrophages. In this regard, acyl phosphatase and phosphoglycerate kinase will compete in cells for the same

substrate, 1,3-bisphosphoglycerate. Until the affinity of both enzymes for this shared substrate is determined, the outcome of this competition cannot be predicted.

Interestingly, *S*-nitrosoglutathione, which was the NO donor tested that resulted in most acyl phosphatase activity in rabbit muscle GAPDH, has been proposed to be a prominent physiological depot for, and donor of, NO [26–28]. The possibility exists, then, for intracellular *S*-nitrosoglutathione, formed from the reaction of NO and reduced glutathione, to appropriately oxidize the active site of GAPDH and induce its acyl phosphatase activity. Obtaining direct evidence to support this hypothesis in living cells or in cell lysates is, however, likely to be hindered by the known instability of sulphenic derivatives. Indeed, efforts to date to obtain such evidence through measurements of acetyl phosphate hydrolysis in digitonin-permeabilized cells have failed to provide positive results.

Present results, at any rate, demonstrate the ability of NO donors to elicit acyl phosphatase activity in GAPDH *in vitro*. In doing so, they provide a theoretical basis to explain the coincident and conflicting observations of suppressed GAPDH activity and enhanced glycolytic flux in cells producing NO.

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