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Original

Sildenafil does not enhance but rather attenuates vasorelaxant effects of antidiabetic agents

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Abstract

Type 2 diabetic men commonly experience erectile dysfunction for which phosphodiesterase-5 (PDE5) inhibitors like sildenafil (Viagra) are often recommended. By preventing degradation of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle, these inhibitors also enhance arterial vasorelaxant effects of nitric oxide donors (which stimulate cGMP synthesis). In the present work, we confirmed this enhancing effect after co-administration of sildenafil with nitroprusside to freshly-isolated rat tail arterial tissues. However, in the same tissues we also observed that sildenafil does not enhance but rather attenuates vasorelaxant effects of three commonly-used antidiabetic drugs, i.e. the biguanide metformin and the thiazolidinediones pioglitazone and rosiglitazone. Indeed, sildenafil completely blocked vasorelaxant effects of low concentrations of these drugs. In addition, we found that this same novel anti-vasorelaxant interaction of sildenafil with these agents was abolished by either 1) omitting extracellular glucose or 2) inhibiting specific smooth muscle glycolytic pathways; pathways known to preferentially utilize extracellular glucose to fuel certain adenosine triphosphate (ATP)-dependent ion transporters: e.g. ATP-sensitive K channels, sarcoplasmic reticulum Ca-ATPase, plasma membrane Ca-ATPase and Na/K-ATPase. Accordingly, we suspect that altered activity of one or more of these ion transporters mediates the observed attenuating (anti-vasorelaxant) interaction of sildenafil with the antidiabetic drugs. The present results are relevant because hypertension is so common and difficult to control in Type 2 diabetes. The present data suggest that sildenafil might interfere with the known antihypertensive potential of metformin and the thiazolidinediones. However, they do not suggest that it will interact with them to cause life-threatening episodes of severe hypotension, as can occur when it is co-administered with nitrates.

Key words: metformin, pioglitazone, rosiglitazone, glucose, glycolysis

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Introduction

It is now widely recognized that sildenafil interacts with organic nitrates and other nitric oxide (NO)-donors to markedly enhance their inherent arterial vasodilatory (vasorelaxant) activity (1–10). This interaction can potentially cause life-threatening episodes of severe hypotension (11). While more than one mechanism may be involved, it is generally assumed that this type of interaction is due largely to the ability of sildenafil to inhibit degradation of NO-induced increases in cyclic guanosine monophosphate (cGMP) in arterial smooth muscle (1–10). In addition, this interaction can be demonstrated with relatively low yet therapeutically-effective concentrations of sildenafil that have little or no systemic arterial relaxant activity of their own (1, 5, 7, 10, 12) and it is particularly easy to demonstrate *in vitro* with intact arterial vascular preparations freshly isolated from animal models (2, 4, 5, 7, 8, 10). Accordingly, other studies have been conducted to determine if sildenafil interacts similarly with nonnitrate vasorelaxant agents which are commonly used to treat hypertensive patients (13). However, no attention has been given to whether sildenafil interacts with those particular types of antidiabetic agents that are known to possess direct vasorelaxant properties independent of their antihyperglycemic effects. Thus, the goals of the present study were to determine whether such interactions exist and, if so, to begin exploring mechanisms responsible for them.

The antidiabetic agents in question are the biguanide metformin and the thiazolidinediones pioglitazone and rosiglitazone. At high concentrations, well above levels observed in plasma of diabetic patients (14–16), their relaxation of isolated arterial vascular tissues *in vitro* occurs rapidly (17–21). At lower, more therapeutically-relevant concentrations, the appearance of their relaxant action *in vitro* can be delayed for a number of hours (19, 21–23). We tested for the ability of sildenafil to interact with the latter because it is more clinically relevant. Our results will obviously have important implications for the use of sildenafil in adult diabetic men; a large population in which erectile dysfunction is common (24) and begins early affecting more than half of all patients before the age of 60 (25).

Materials and Methods

Preparation of arterial tissues

Sildenafil's ability to enhance nitrate-induced arterial relaxation has been demonstrated repeatedly *in vitro* with intact vascular rings prepared from animal aorta (1, 7, 8, 10). However, we will employ rings from the ventral tail artery of the laboratory rat since all of our previous experiments with direct vasorelaxant properties of metformin and the thiazolidinediones were successfully performed with that particular preparation (18–23). In addition, while certainly much smaller, the rat tail artery is also more muscular and richer in adrenergic nerve endings and receptors than the aorta and, as such, more closely resembles systemic arterial resistance vessels (26–28). Also, its notable length allows for the preparation of several rings with nearly identical contractile properties from a single vessel. Accordingly, ventral tail arteries were isolated from adult male Sprague-Dawley rats after euthanizing them by procedures approved in advance by the Institutional Animal Care and Use Committee of Midwestern University. On each experimental day, a short uniform portion of a single artery would then be cleaned and carefully sectioned into multiple 3-mm cylindrical rings using a bound set of evenly-spaced scalpel blades. In our experience, individual rings sectioned in this manner exhibit more uniform contractile responses than if sectioned with multiple cuts by a single blade. A maximum of eight rings were selected at random for experimental treatments on each particular day. [Eight was the largest number that our *in vitro* bath system and Grass recorder could accommodate on any given day]. Each ring was mounted

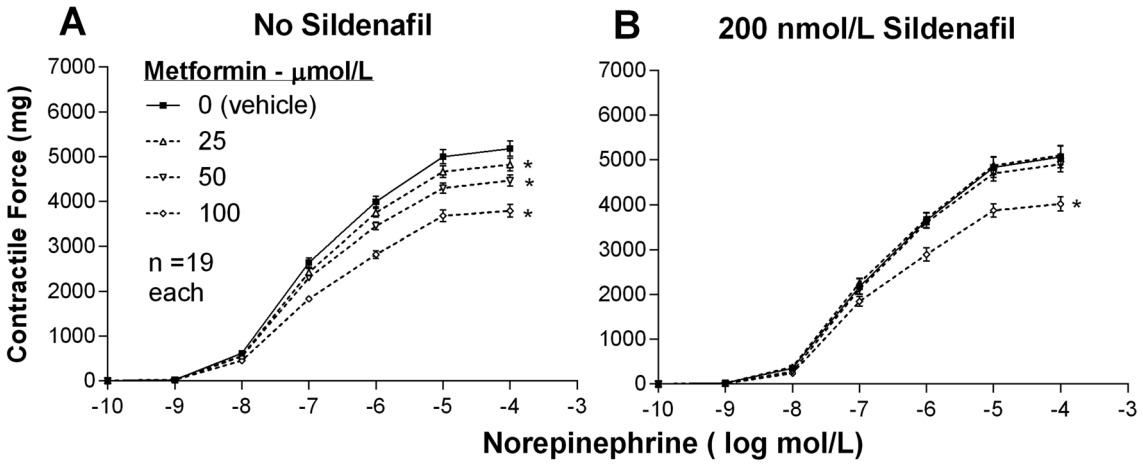

Fig. 1. Effects of 4-h co-administration of sildenafil with the antidiabetic agent metformin on norepinephrine (NE) contractions in vascular rings prepared from 19 rat tail arteries. Metformin significantly reduced (relaxed) the force of NE contractions as determined by 2-factor ANOVA (*P<0.05 versus vehicle at all levels of NE from 10^{-7} to 10^{-4} mol/l). That same ANOVA also revealed a significant interactive effect of sildenafil on these relaxant effects of metformin (P<0.05).

between two tungsten wire stirrups which in our experience were strong enough not to bend during ring contractions yet thin enough not to damage the inner endothelial cell layer (18–23). Then, each ring was suspended in a 40 ml tissue bath and equilibrated for several minutes before experimentation at a resting (baseline) tension of 1000 mg in standard physiological (Krebs) buffer which was warmed to 37°C and gassed to pH 7.4 with regulated delivery of O_2/CO_2.

Experiments with glucose present and glycolysis intact

Initial experiments were performed to find a concentration of sildenafil that would not cause vasorelaxation by itself but yet significantly enhance vasorelaxant effects of nitroprusside in norepinephrine-contracted vascular rings prepared from tail arteries of adult male rats (See *Preparation of arterial tissues*). We found that 200 nmol/l (95 ng/ml) was best suited for that purpose (data not shown). This is well within the range of sildenafil's plasma concentrations typically found in patients after oral administration (29). Then we examined additional rat tail arterial rings in 3 more experiments for the ability of this same level of sildenafil to interact with the abovementioned vasorelaxant effects of 1) metformin, 2) pioglitazone and 3) rosiglitazone (Fig. 1–3). In each of these 3 separate experiments, some rings were incubated for 4 h in the presence of sildenafil's vehicle with either one of these antidiabetic agents or its vehicle, while other rings were incubated similarly but in the presence of the 200 nmol/l sildenafil. All incubations (renewed hourly) were carried out with standard Krebs buffer (with glucose present). After 4 h, all rings were contracted with stepwise cumulative administration of norepinephrine as shown in Figs. 1–3. The various experimental agents remained present during this administration of norepinephrine (which required 15 min to complete).

Experiments with glucose absent and glycolysis inhibited

We then conducted two additional experiments to evaluate the role of extracellular glucose and its glycolytic metabolism in the findings gathered from the experiments described above. In both, we re-administered sildenafil along with metformin at 50 µmol/l, pioglitazone at 5 µmol/l and rosiglitazone at 10 µmol/l; concentrations which after 4 h in Figs. 1–3 had produced relaxations that sildenafil clearly abolished. In the first of

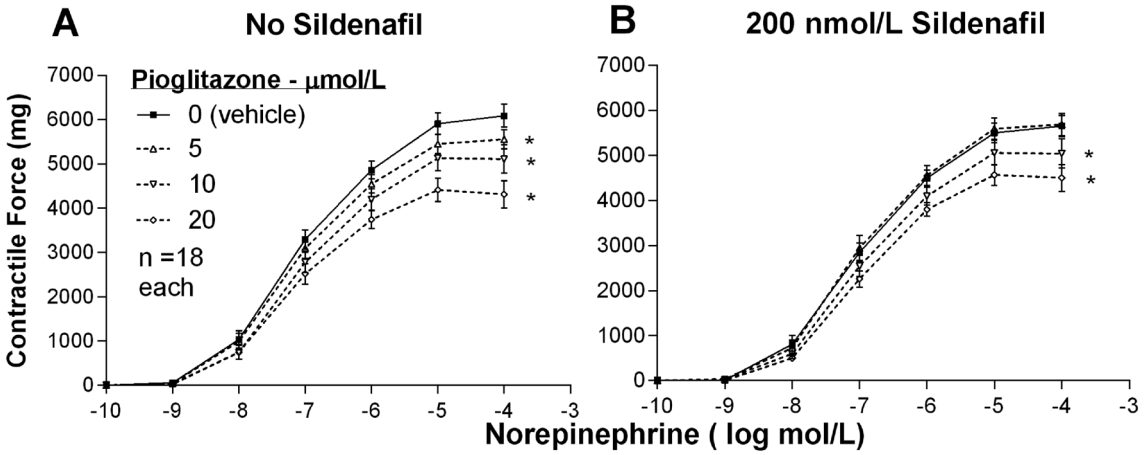

Fig. 2. Effects of 4-h co-administration of sildenafil with the antidiabetic agent pioglitazone on norepinephrine (NE) contractions in vascular rings prepared from 18 rat tail arteries. Pioglitazone significantly reduced (relaxed) the force of NE contractions as determined by 2-factor ANOVA (**P*<0.05 versus vehicle at all levels of NE from 10^{-7} to 10^{-4} mol/l). That same ANOVA also revealed a significant interactive effect of sildenafil on these relaxant effects of pioglitazone (*P*<0.05).

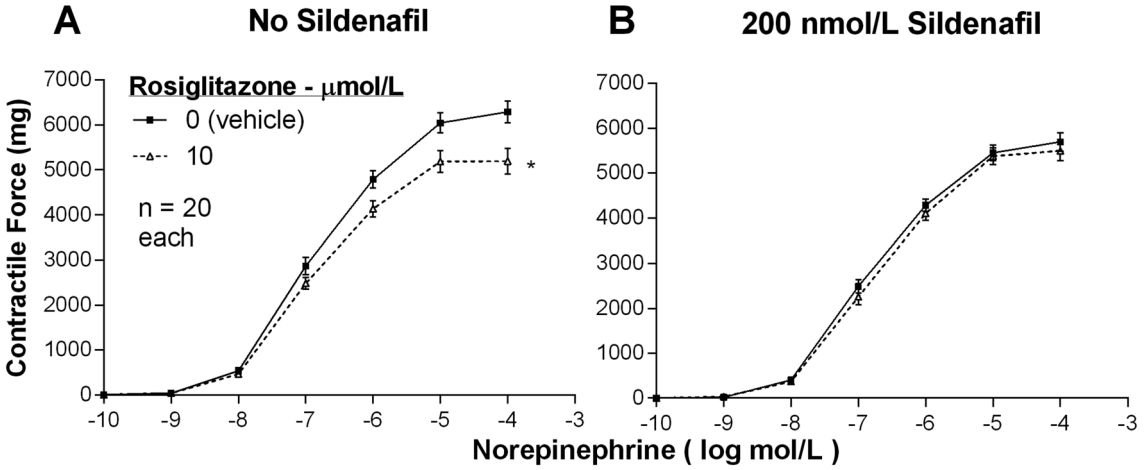

Fig. 3. Effects of 4-h co-administration of sildenafil with the antidiabetic agent rosiglitazone on norepinephrine (NE) contractions in vascular rings prepared from 20 rat tail arteries. Rosiglitazone significantly reduced (relaxed) the force of NE contractions as determined by 2-factor ANOVA (**P*<0.05 versus vehicle at all levels of NE from 10^{-7} to 10^{-4} mol/l). That same ANOVA also revealed a significant interactive effect of sildenafil on these relaxant effects of rosiglitazone (*P*<0.05).

these additional experiments (Fig. 4), we omitted extracellular glucose starting 1.5 h before norepinephrine. We chose to wait 1.5 h to allow transient increases in baseline arterial tensions (which occurred only occasionally in response to the glucose omission) to fully dissipate before administering norepinephrine. Longer periods were avoided because even though arterial smooth muscle is known to retain as much as half its normal content of glycogen ("stored" glucose) after 3 h of glucose omission (30, 31), control norepinephrine contractions in our tissues were compromised too much (after 3 h) for our purposes. In the second additional experiment (Fig. 5), we did not omit extracellular glucose. Rather, at 1.5 h before norepinephrine, we administered the glycolysis inhibitor iodoacetate at a low level (30 μmol/l); thus, similar to the 50 μmol/l which in arterial smooth muscle is reported to specifically inhibit only the lactate-producing glycolytic pathways that

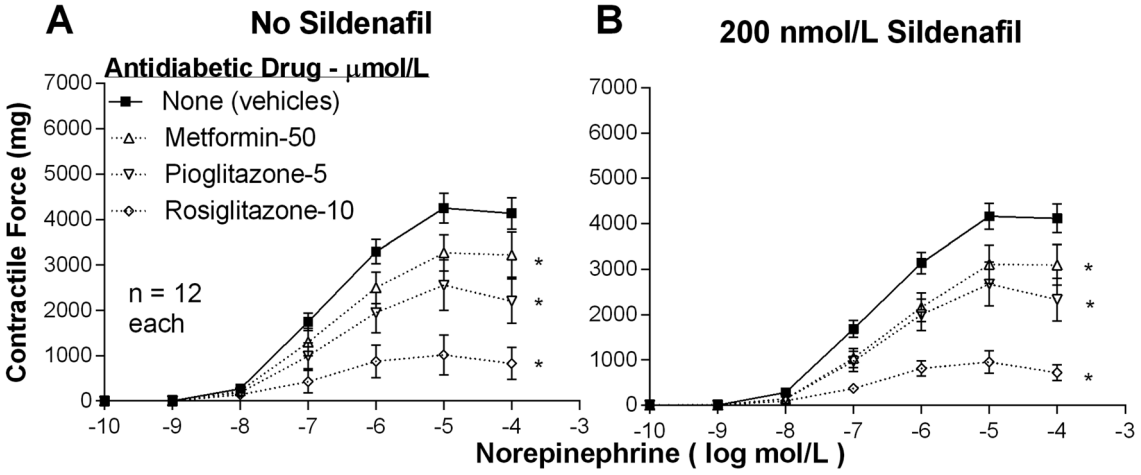

Fig. 4. Effects of 4-h co-administration of sildenafil with antidiabetic agents on norepinephrine (NE) contractions in vascular rings prepared from 12 rat tail arteries. Extracellular D-glucose was omitted from all incubation buffers starting 1.5 h before NE. All agents significantly reduced (relaxed) the force of NE contractions as determined by 2-factor ANOVA (*P<0.05 versus vehicles at all levels of NE from 10^{-7} to 10^{-4} mol/l). That same ANOVA did not reveal interactive effects of sildenafil on these relaxant effects of the antidiabetic agents.

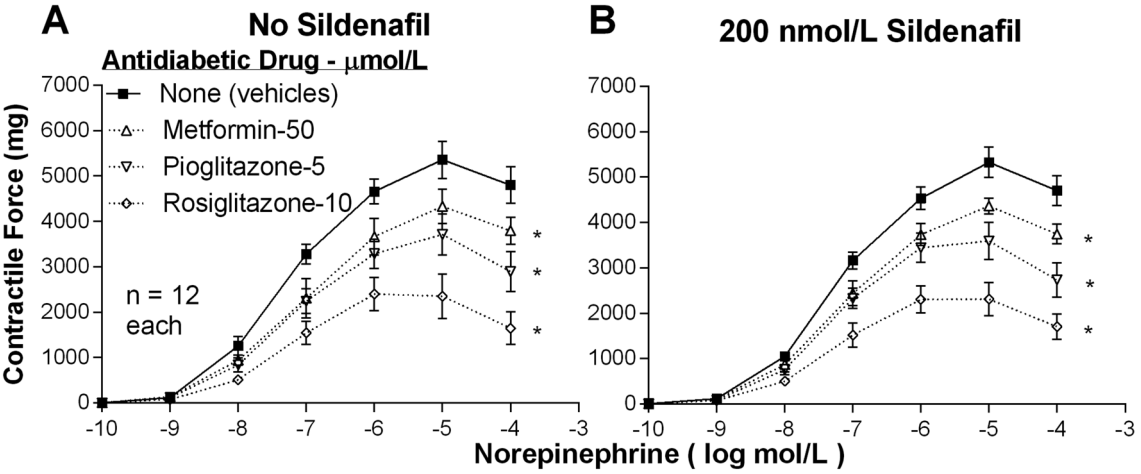

Fig. 5. Effects of 4-h co-administration of sildenafil with antidiabetic agents on norepinephrine (NE) contractions in vascular rings prepared from 12 rat tail arteries. Iodoacetate (30 μmol/l) was administered to all incubation buffers starting 1.5 h before NE. All agents significantly reduced (relaxed) the force of NE contractions as determined by 2-factor ANOVA (*P<0.05 versus vehicles at all levels of NE from 10^{-8} to 10^{-4} mol/l). That same ANOVA did not reveal interactive effects of sildenafil on these relaxant effects of the antidiabetic agents.

preferentially utilize extracellular glucose to fuel membrane-bound ATP-dependent ion transporters, not the cytoplasmic glycolysis that normally metabolizes intracellularly-stored glucose (glycogen) to acetyl-CoA for mitochondrial production of ATP for contractile proteins (32, 33). Complete inhibition of all arterial smooth muscle glycolysis requires 1000 μmol/l iodoacetate (32), which was not used because it abolishes all contractile function in the rat tail artery.

Analysis of data

At each level of norepinephrine administered in the above experiments, the numerical values describing its contractile force (expressed in milligram units) were summarized as mean ± S.E.M. and then subjected (at each level of norepinephrine separately and from both sides A and B of each figure together) to 2-factor analysis-of-variance (ANOVA) to determine statistically significant effects of sildenafil (factor one) on the relaxant effects of each antidiabetic agent (factor two). Effects were considered statistically significant if the standard probability of error (*P* value) was less than 0.05.

Results

Experiments with glucose present and glycolysis intact

There were no changes in baseline arterial tension caused by sildenafil or any of the antidiabetic agents (either separately or combined) during the 4-h co-administration periods preceding administration of norepinephrine to rat tail arterial rings in these experiments. During administration of norepinephrine (10^{-10} to 10^{-4} mol/l) the following results were observed (data in all figures are illustrated as mean ± S.E.M.). As shown in Fig. 1 (A, left side), metformin significantly reduced (relaxed) the force of norepinephrine contractions at all levels of treatment, i.e. 25, 50 and 100 µmol/l metformin. But as also shown in Fig. 1 (B, right side), sildenafil abolished the relaxant effects of the 25 and 50 µmol/l metformin and partially but significantly attenuated the greater relaxant effect of 100 µmol/l metformin (by 36% overall from 10^{-8} to 10^{-4} mol/l norepinephrine). Similarly, pioglitazone significantly relaxed norepinephrine contractions at all levels of treatment, i.e. 5, 10 and 20 µmol/l pioglitazone (Fig. 2A, left side). Sildenafil completely abolished the relaxant effect of the 5 µmol/l pioglitazone and partially attenuated that of the 10 and 20 µmol/l pioglitazone (by 38% and 30%, respectively, overall from 10^{-8} to 10^{-4} mol/l norepinephrine) (Fig. 2B, right side). Similar results were seen with rosiglitazone (Fig. 3 A and B). Sildenafil abolished a statistically significant relaxant effect of 10 µmol/l rosiglitazone (Fig. 3B). Thus, while exerting little if any vasorelaxant effect on its own, a therapeutically-relevant concentration of sildenafil appeared to interact nonspecifically with all three antidiabetic agents, interfering with the ability of each to relax norepinephrine contractions in isolated arterial tissues (the exact opposite of its interaction with nitrovasodilators).

Experiments with glucose absent and glycolysis inhibited

As expected, omission of extracellular glucose reduced control contractions of norepinephrine (see Fig. 4 versus Fig. 1–3), but it also unmasked notably greater relaxant effects of the antidiabetic drugs (particularly the two thiazolidinediones). In addition, with glucose omitted, sildenafil failed to abolish these relaxant effects (Fig. 4B). As seen in Fig. 5, 30 µmol/l iodoacetate did not suppress control norepinephrine contractions nearly as much as the glucose omission in Fig. 4. Yet, it had nearly the same impact on antidiabetic drug and sildenafil effects as seen with glucose omission. Most importantly, sildenafil failed to abolish the relaxant effects of the antidiabetic agents in the presence of the iodoacetate. Finally, in a limited number of additional tests (not shown), neither glucose omission nor iodoacetate influenced vasorelaxations produced by other commonly-used vasodilators, such as the calcium channel blocker nifedipine. Thus, their impact on sildenafil/antidiabetic drug effects may be unique and not a common phenomenon for all vasodilatory agents.

Discussion

New findings

Type 2 diabetic men commonly experience erectile dysfunction (24) for which phosphodiesterase type 5 (PDE5) inhibitors like sildenafil are often recommended (34–36). By preventing degradation of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle, these PDE5 inhibitors also enhance arterial vasodilatory (vasorelaxant) effects of organic nitrates and other nitric oxide (NO) donors which stimulate cGMP synthesis (1–10). In our experiments, we observed this enhancing effect *in vitro* in norepinephrine-contracted vascular tissue rings (prepared from tail arteries of adult male rats) after exposing them for four hours to co-administration of 200 nmol/l sildenafil (a level typical of plasma concentrations in humans after oral administration, 29) and the NO-donor nitroprusside. However, in the same tissues we also observed that 4-h exposure to the same level of sildenafil does not enhance but rather attenuates vasorelaxant effects of three important oral antidiabetic drugs commonly used to improve glucose metabolism in type 2 diabetic patients, i.e. the biguanide metformin and the thiazolidinediones pioglitazone and rosiglitazone. Indeed, sildenafil completely blocked vasorelaxant effects of low concentrations of these drugs. In additional experiments, we found that this same novel anti-vasorelaxant interaction of sildenafil with these antidiabetic drugs was abolished by either 1) omitting extracellular glucose or 2) inhibiting specific vascular smooth muscle glycolytic pathways; pathways known to preferentially utilize extracellular glucose to fuel the following adenosine triphosphate (ATP)-dependent ion transporters: ATP-sensitive K channels, sarcoplasmic reticulum Ca-ATPase, plasma membrane Ca-ATPase and Na/K-ATPase (37–45).

Potential mechanisms

Among the various types of oral antidiabetic agents currently used in the treatment of hyperglycemia in type 2 diabetes, there are some that stimulate insulin secretion (e.g. sulfonylureas) and some that mimic and/or improve insulin action (the biguanide metformin and the thiazolidinediones pioglitazone and rosiglitazone) (46–50). Only agents of the latter type are known to exert direct vasodilatory (vasorelaxant) effects on the arterial vessel wall (17–23, 49, 51–58); effects now recognized as largely responsible for their antihypertensive action (49, 54–58). Also, the full expression of their vasorelaxant effects (at least with low, therapeutically-relevant concentrations) is delayed for a number of hours (19, 21–23). In our laboratory at least 4 h is required for the full effect of each agent. But this does not readily explain why sildenafil would antagonize relaxant effects of these antidiabetic drugs as seen in our initial set of experiments.

Most evidence indicates that metformin and thiazolidinediones dilate arterial vessels by activating relaxant mechanisms within the smooth muscle cell itself; the most studied being voltage-operated ion channels. Both reduce arterial smooth muscle calcium (Ca) influx by inhibiting voltage-operated Ca channels (17, 51–53). Evidence shows that thiazolidinediones do so largely directly (21, 51–53) while metformin does so only indirectly, i.e. by repolarizing arterial smooth muscle cell membranes (17) due to activation of voltage-dependent K channels (20). However, we found it difficult to appreciate how sildenafil, simply by coincidence, would interfere with these distinctly different mechanisms to produce essentially the same net effect. Rather, it seemed more likely that sildenafil would interfere with a singular action that these structurally-unrelated antidiabetic agents might have in common. One action that metformin and thiazolidinediones are known to have in common is stimulation of glucose uptake (59–66), which increases availability of glucose to intracellular storage and utilization. And this occurs in arterial vascular smooth muscle cells as well (59–61, 66) where such increased glucose uptake can potentially support active (ATP-driven) relaxant as well as contraction-enhancing mechanisms.

In vascular smooth muscle, carbohydrate metabolism and the role it plays in relaxation as well as contraction is highly compartmentalized (31, 32, 37–41, 67–69). There are at least two functionally independent Embden-Meyerhof (glycolytic) compartments (or pathways) in vascular smooth muscle (32, 39, 68, 69). One is localized deep in the cytosol (linked to contractile filaments) where it preferentially utilizes glucose-6-phosphate from breakdown of stored glycogen to provide acetyl-CoA for mitochondrial production of ATP to fuel contraction (31, 39, 41, 67–69). The others (also called lactate-producing glycolysis compartments) are associated largely with the cell plasma membrane (PM) and membranes of the sarcoplasmic reticulum (SR) (37–45, 70). These membrane-associated glycolytic pathways preferentially utilize glucose-6-phosphate directly from extracellular glucose to fuel at least the following four ATP-dependent ion transporters: PM-Ca-ATPase, Na/K-ATPase, ATP-sensitive K (K_{ATP}) channels and SR-Ca-ATPase (37–45, 70). Supported by intracellular ATP, the first two of these four transporters can potentially relax (inhibit) contractions exerted by vasoconstrictors such as norepinephrine (by maintaining specific cell membrane cation gradients and contributing to cell membrane polarization, 41, 71–75) while the latter two can potentially enhance (facilitate) norepinephrine contractions (37, 41–43, 45, 76–78). Increased intracellular ATP from glycolysis of extracellular glucose closes K_{ATP} channels (42–44). In rat tail artery, closure of K_{ATP} channels participates in norepinephrine-induced smooth muscle membrane depolarization (76) which in turn can potentially enhance (facilitate) contraction. SR-Ca-ATPase utilizes ATP from glycolysis of extracellular glucose to maintain optimum filling of norepinephrine-releasable intracellular SR Ca stores (37, 41, 45). There is evidence that this filling involves opening of specific store-operated Ca channels in the cell plasma membrane in close proximity to SR-Ca-ATPase in the SR membrane (79). If filling of the SR with Ca in this manner is compromised by omission of extracellular glucose, norepinephrine contraction is reduced (37, 41, 45). Thus, while it is generally accepted that uptake of extracellular glucose supports norepinephrine contraction by maintaining optimal intracellular glycogen levels (80), it is also known to do so by maintaining optimal SR Ca levels (37, 41, 45).

Therefore, given the common ability of the antidiabetic drugs to stimulate glucose uptake in arterial vascular smooth muscle (and thereby potentially affect norepinephrine contractility), we decided to conduct two additional experiments. Results from these experiments are shown in Figs. 4 and 5. Essentially, we found that 1) omission of extracellular glucose and 2) specific inhibition of the vascular smooth muscle lactate-producing glycolytic pathways (described above) completely abolished sildenafil's anti-vasorelaxant interaction with the antidiabetic drugs. In addition, these same two interventions (especially the glucose omission) notably increased the magnitude of the antidiabetic drug-induced vasorelaxations; indeed markedly so for the thiazolidinediones. Accordingly, we strongly suspect that in addition to activating the abovementioned ion channel-based smooth muscle relaxant (inhibitory) mechanisms which inherently do not require extracellular glucose (43, 51), these antidiabetic agents also activate contraction-facilitating mechanisms that 1) do require glucose and 2) mask the full potential of their relaxant (inhibitory) mechanisms. Furthermore, we conclude that sildenafil's anti-vasorelaxant interaction with these antidiabetic agents, as observed in the presence of extracellular glucose and fully intact glycolysis (Figs. 1–3), most likely involves enhancement of whatever glucose/glycolysis-dependent contraction-facilitating mechanisms they possess.

In other words, while the abovementioned effects of metformin and thiazolidinediones on voltage-operated channels (17, 20, 51–53) are the most likely mechanisms for the relaxant (inhibitory) effects we observed in the absence of extracellular glucose (Fig. 4) and in the presence of a specific inhibitor of the lactate-producing glycolysis pathways (Fig. 5), these are not the mechanisms affected by sildenafil. Rather, sildenafil must be interacting with glucose/glycolysis-dependent mechanisms. Accordingly, it is then reasonable to hypothesize that sildenafil may be interacting with the antidiabetic drugs at the level of the four abovementioned glucose/

glycolysis-dependent ion transporters, i.e. Na/K-ATPase, PM-Ca-ATPase, K_{ATP} channels and SR-Ca-ATPase. We hypothesize that altered activity of one or more of these four ATP-dependent ion transporters is involved in mediating the novel attenuating (anti-vasorelaxant) interaction of sildenafil with metformin, pioglitazone and rosiglitazone that we observed. We intend to test this hypothesis in the future beginning by measuring effects of specific inhibitors of those four transporters on the ability of sildenafil to attenuate antidiabetic drug-induced vasorelaxations. Such experiments are beyond the scope and funding of the present study.

Clinical relevance

Hypertension is a highly prevalent difficult-to-control condition in type 2 diabetic patients (81–87) and little attention has been given to whether PDE5 inhibitors interact with any of the known therapeutic actions of the abovementioned antidiabetic agents, particularly their well-documented blood pressure lowering action (49, 56, 81–106). Our data clearly raise a question whether PDE5 inhibitors might compromise that action by interfering with the one mechanism most likely responsible for it (i.e. their arterial vasorelaxant effects). We are unaware of any clinical studies designed specifically to address this concern. But we are aware of one study in which adult men were treated simultaneously with pioglitazone (at a dose known to lower arterial pressure) and sildenafil to determine how their erectile dysfunction was affected (107). After nine weeks, pioglitazone (30 mg daily versus placebo) improved their erectile responsiveness to sildenafil but, interestingly, failed to lower either their systolic or diastolic arterial pressures (107). And according to some of the blood pressure lowering reports cited above (82, 85, 88, 103), in which the same dose of pioglitazone (in the absence of PDE5 inhibition) did indeed lower arterial pressures, nine weeks is more than enough time for it to do so. On the other hand, our data clearly do not provide any evidence that sildenafil will interact with either pioglitazone, rosiglitazone or metformin to cause life-threatening episodes of severe hypotension when used to treat diabetic men. And to our knowledge, there are no reports of such episodes in that population.

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Conflict of Interest

The authors declare they have no conflict of interest.

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