Disparate Effects of Antidiabetic Drugs on Arterial Contraction

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Type II diabetic patients and others with insulin resistance are at risk for development of hypertension characterized by elevated peripheral vascular resistance and loss of insulin's normal vasodilating activity. Oral antidiabetic drugs have recently been recognized to have disparate effects on arterial pressure in such patients and in related rodent models. Sulfonylureas (eg, glyburide), which stimulate insulin secretion, have been reported either to increase or not to affect arterial pressure, whereas nonsulfonylurea agents with insulin-sensitizing properties, the biguanide metformin and various thiazolidinediones (eg, pioglitazone), have been reported to decrease arterial pressure in humans and rodents. To help elucidate these disparate effects, we investigated these agents for direct actions on arterial vascular contractility and its sensitivity to insulin. Preincubation of intact rat tail arterial tissue rings for 2 hours with known therapeutically effective antidiabetic concentrations of metformin and pioglitazone significantly attenuated the force of contractions produced by either potassium (membrane depolarization) or norepinephrine ([NE] adrenergic receptor activation). Glyburide did not influence these contractions. Preincubation with metformin also induced an attenuating (vasodilating-like) action of insulin on arterial tissue rings contracted by potassium. Conversely, glyburide induced an accentuating action of insulin on potassium-mediated contractions. These results are consistent with measures of vascular function obtained in the past after oral administration of the drugs, which suggested but did not prove that they may exert direct effects on arterial vascular contractility. Thus, metformin and thiazolidinediones may decrease arterial pressure partly by direct vasorelaxant mechanisms, with metformin having an additional effect of inducing vasorelaxation by insulin. In contrast, sulfonylureas may directly induce a paradoxical vasoconstrictor response to insulin.

CURRENT ANTIDIABETIC DRUGS have now been examined by various groups for antihypertensive potential following oral administration.[1-21] Generally, the studies indicate disparate effects of sulfonylurea versus nonsulfonylurea drugs on arterial pressure. In both humans and animals, chlorpropamide and glyburide (a first- and a second-generation sulfonylurea, respectively) have been observed either to aggravate or not to affect preexisting hypertension.[1-3] In contrast, thiazolidinediones (pioglitazone, ciglitazone, and troglitazone) have been reported to reduce arterial pressure in humans[4] and attenuate hypertension in most animal models.[5-9] Similarly, the biguanide metformin has been shown to reduce arterial pressure in humans[10-17] and to attenuate hypertension in fructose-fed and spontaneously hypertensive rats.[1,18-21]

The mechanisms responsible for these disparate drug effects on arterial pressure remain to be determined. Some evidence suggests that they may relate to direct actions on arterial vascular contractile tone. For example, pioglitazone has been reported to blunt contractile responses of aortic tissues (isolated from normal rats) to norepinephrine (NE) and to a high extracellular concentration of KCl.[9] (The latter produces contraction by simply depolarizing the vascular smooth muscle cell membrane, thus opening voltage-gated calcium channels permitting rapid calcium influx.[22]) Ciglitazone (the prototype thiazolidinedione) has been reported to attenuate agonist-induced increases in intracellular free calcium levels in cultured vascular smooth muscle cells.[6] Similarly, attenuation of agonist-induced increases in intracellular calcium has been observed in vascular smooth muscle cells treated with metformin.[20,23] Theoretically, sulfonylureas could do the opposite, ie, accentuate agonist-induced increases in intracellular calcium, since they are known to inhibit potassium channels in the vascular smooth muscle cell membrane.[22]

In addition, pioglitazone and troglitazone were recently reported to directly sensitize aortic tissue isolated from normal rats to an attenuating action of insulin on contractions induced by either KCl[24] or adrenergic agonists.[24,25] Such an "insulin-sensitizing" action could be highly relevant in patients with concomitant hypertension and diabetes. Insulin's normal "vasodilating-like" (attenuating) action on contractile tone is impaired in type II diabetes and other insulin-resistant states, and this impairment is thought to contribute not only to hypertension in such states but also to deficient uptake of glucose by peripheral tissues (presumably due to less delivery of glucose).[26] Thus, it is important to determine whether metformin and/or sulfonylureas can also directly modulate this attenuating action of insulin on arterial contractile tone, and whether they and the thiazolidinediones can do so in resistance-like arteries that clearly contribute more to the control of arterial pressure and glucose delivery than the aorta.

Accordingly, the aims of the present study were (1) to compare the effects of glyburide, pioglitazone, and metformin on membrane voltage-related (potassium-induced) and adrenergic receptor-related (NE-induced) contractile tone in intact rat tail artery vascular rings, and (2) to determine whether these drugs can differentially modulate the effects of insulin on contractile tone in the same arterial tissue. We chose the tail artery of the Wistar rat. Like other resistance arteries, the tail artery of the rat is much smaller, more muscular, and much

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richer in adrenergic (sympathetic) nerve endings and adrenergic receptors than the aorta.[27-30] The tail artery from the adult male Wistar rat has already been identified as a vessel in which insulin not only can attenuate normal adrenergic contractile tone[31] but also can modulate the effects of other substances on that tone.[32]

MATERIALS AND METHODS

In each experiment described under studies I and II (described later), a short proximal segment of ventral tail artery was isolated at approximately 8 AM on the day of experimentation from an adult male Wistar rat fed ad libitim under a 16-hour light/8-hour dark cycle. This segment of artery was then cut into four intact rings of similar length (3 mm each[27]). Ring lengths measured with the aid of a magnified micrometer scale did not differ significantly between the various experimental treatment groups in either study. In addition, tail arterial rings prepared in this manner showed relaxation responses to acetylcholine in preliminary studies, demonstrating a functionally intact endothelium.

In each experiment, the four tail arterial rings were mounted on tungsten wire stirrups[27] and suspended from isometric-force transducers into 50-mL tissue baths (one ring per bath) containing physiological buffer as described previously.[2] Each ring was washed repeatedly with fresh insulin-free and drug-free buffer over a 2-hour period to remove endogenous substances. Concurrently, these rings were stretched mechanically to a resting tension of 1,000 mg each. No experimental treatments (drugs or insulin) were begun until all four rings in each experiment were stabilized (with additional mechanical adjustment if needed) at 1,000 mg.

This level of resting tension (1,000 mg) was not influenced by any of the experimental antidiabetic drug or insulin treatments described herein, and it optimized contractions produced by either KCl or NE. These contractions were also highly reproducible when repeated on the same rings after rinsing and reequilibrating them in normal buffer. Thus, in each experiment, each of the four separate arterial rings was assigned a single drug treatment (glyburide, pioglitazone, metformin, or vehicle) and then exposed to that treatment twice, first in the absence of insulin and then again (after rinsing and reequilibrating) in the presence of insulin (regular human insulin). This allowed us to calculate values for the action of insulin on contractions produced by either KCl or NE, and then to evaluate the effect of drugs on the calculated values.

To aid our choice of drug concentrations for testing with insulin, we first conducted several preliminary experiments without insulin but with multiple concentrations of each drug in the 50-mL tissue baths (2.5, 25, and 50 μmol/L glyburide; 5, 25, and 50 μmol/L pioglitazone; and 100 to 16,000 μmol/L metformin). (Higher concentrations of glyburide and pioglitazone greater than 50 μmol/L were not examined, since these drugs are likely insoluble in aqueous media above this level [based on drug chemical data from Upjohn, Kalamazoo, MI].) The following results were obtained from these preliminary experiments. Tail arterial rings precontracted with NE and then, at 10-minute intervals, treated with increasing concentrations of drugs (one drug per ring) showed rapid, marked, and sustained relaxation in response to 25 and 50 μmol/L pioglitazone and to all levels of metformin 500 μmol/L or greater.[33] Precontractions with NE were not altered in this manner (ie, acutely) by (1) the lowest concentrations of pioglitazone (5 μmol/L) and metformin (100 μmol/L), (2) all glyburide concentrations, and (3) the drug vehicles (DMSO for glyburide and pioglitazone and H_2O for metformin). However, other preliminary experiments demonstrated that after arterial rings were first exposed for 2 hours to the low drug levels, both pioglitazone (5 μmol/L) and metformin (100 μmol/L) but not glyburide (2.5 μmol/L) could significantly attenuate contractions produced by NE when compared with the vehicles.[34] Additional experiments of this type showed identical results after 4 hours of exposure to the same drug levels and also when the contractions were produced by KCl. We and others have further found that any action of insulin by itself on arterial contractile tone in vitro also requires prolonged exposure (at least 1 to 2 hours) to the hormone.[2,31,32] Thus, we chose prolonged exposures to these three low drug concentrations to test for interactions with insulin.

Other reasons for choosing these particular drug concentrations were as follows. First, they are not cytotoxic in vitro to vascular smooth muscle and other cells, whereas most of the higher concentrations are.[35-38] Second, they are within the range of levels often found in plasma after oral administration of effective blood glucose–lowering doses; the higher concentrations are not.[37,39-41] Finally, they approximate the levels that have been demonstrated previously as clearly effective in vitro in terms of various other important activities, including effects on glucose metabolism and insulin receptor properties.[6,37,38,40-43]

The concentration of insulin used in all experiments described herein was 1 mU/mL, which is similar to plasma levels in hypertensive, hyperinsulinemic obese male rats[7] and within a range of concentrations known to modulate NE contractions in the rat tail artery.[31]

Study I. Effects of Antidiabetic Drugs and Insulin on KCl-Induced Contractions in Rat Tail Arterial Rings

There were two sets of experiments performed in this study. In each experiment of the first set (14 experiments total), all three antidiabetic drugs at the levels already described (and the vehicles) were added in parallel to the four separate rings in the four separate baths. After 2 hours, all four rings were contracted with KCl administered cumulatively from 10 to 125 mmol/L. After thorough rinsing and reequilibrating each ring in normal buffer, these 2-hour drug treatments were repeated but in the presence of the insulin level described earlier. Then, the cumulative KCl administrations were repeated.

At each concentration of KCl, the contractile force observed in the presence of each drug treatment was compared with that observed with vehicle treatment and the other two drugs, both in terms of absolute tension and calculated insulin-induced changes in tension. Half-maximally effective KCl concentrations (EC_{50} values) were also computed (probit analysis) and compared for treatment effects.

In an additional set of 14 experiments (with arteries from 14 more rats), all procedures were performed in the same manner as for the first set except that insulin was omitted altogether (ie, it was not added during the second 2-hour drug treatment period). This was done to evaluate the influence of added time alone on drug effects, ie, whether 4 hours of drug treatment had a different effect on KCl-induced contractions than 2 hours.

Study II. Effects of Antidiabetic Drugs and Insulin on NE-Induced Contractions in Rat Tail Arterial Rings

In each experiment in this study (two sets, 18 experiments per set), four tail arterial rings like those described in study I (but from different rats) were subjected to the same experimental treatments with antidiabetic drugs and insulin (or simply the additional 2 hours of drug treatment without insulin). However, cumulative administration of NE (10^{-9} to 10^{-4} mol/L) rather than KCl was used to produce contractions.

Forces of contraction at each concentration of NE, EC_{50} values for NE, and calculated changes in these parameters were analyzed as described for KCl contractions in study I.

Statistical Analyses

The results are summarized as the mean ± SE and were subjected to comparisons between means (Scheffé) after repeated-measures ANOVA.

Differences were considered statistically significant if the probability of error was less than .05.

RESULTS

Study I. Effects of Antidiabetic Drugs and Insulin on KCl-Induced Contractions in Rat Tail Arterial Rings

In the absence of insulin, contractions produced by approximately half-maximal to maximal levels of KCl in the first set of experiments were attenuated by 2 hours of treatment of the arterial rings with either pioglitazone or metformin, but not with glyburide (Fig 1). In addition, 2 hours of insulin treatment either by itself (in the presence of drug vehicle) or in the presence of pioglitazone did not influence these KCl-induced contractions. However, in the presence of metformin, this insulin treatment attenuated these KCl contractions. Conversely, in the presence of glyburide, the same insulin treatment accentuated these KCl contractions (Table 1).

These contrasting effects of metformin and glyburide on insulin action were not likely due to the added time the tissues were exposed to the drugs (2 additional hours were used to coadminister insulin with drugs). As in our preliminary study, in a second set of experiments in which insulin was omitted altogether (not shown), the second 2-hour period of drug treatment produced only effects on KCl contractions similar to those seen after the first 2-hour period.

Finally, computed EC_{50} values for KCl were not influenced by either drugs or insulin in any of the experiments from this study.

Study II. Effects of Antidiabetic Drugs and Insulin on NE-Induced Contractions in Rat Tail Arterial Rings

In the absence of insulin, contractions produced by nearly all levels of NE in the first set of experiments were attenuated by 2 hours of treatment of the arterial rings with either pioglitazone or metformin, but not with glyburide (Fig 2). Two hours of insulin also attenuated contractions produced by these levels of NE. At lower NE levels (10^{-8} to 10^{-6} mol/L), this attenuating action of insulin was not influenced by any of the drugs (Table 2). However, at higher NE levels (10^{-5} to 10^{-4} mol/L), this attenuating action of insulin was smaller in the presence of glyburide compared with metformin (as evident from the statistically significant differences between the two drugs shown in Table 2).

These differential effects of metformin and glyburide on insulin action were not likely due to the added time the tissues were exposed to the drugs. As in our preliminary study, in a second set of experiments from this study in which insulin was omitted altogether (not shown), the second 2-hour period of drug treatment produced only effects on NE contractions similar to those seen after the first 2-hour period.

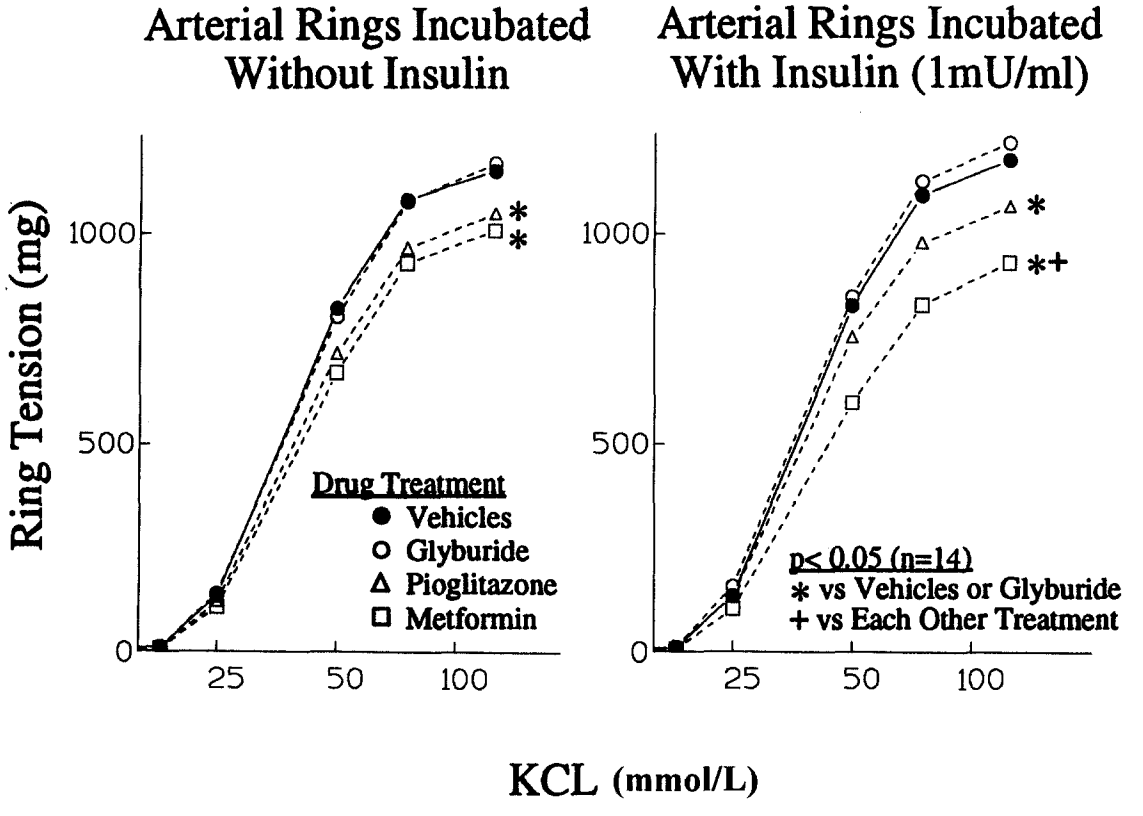

Fig 1. Effects of antidiabetic drugs and insulin on KCl-induced contraction in rat tail arterial rings. Mean values are illustrated without SE values, which were 56 mg tension overall. Calculated effects of insulin on these contractions are shown in Table 1. Statistically significant differences between drugs as derived from Scheffé's multiple mean comparison procedure apply at all levels of KCl from 50 to 125 mmol/L with or without insulin. Preceding Scheffé's test, repeated-measures ANOVA indicated a statistically significant 3-factor interaction (drugs × insulin × KCl, $P < .05$) and 1 statistically significant 2-factor interaction (drugs × insulin, $P < .05$).

Table 1. Effects of Antidiabetic Drugs on the Ability of Insulin to Change the Force of KCl-Induced Contractions in Rat Tail Arterial Rings

KCL	Change in Tension (mean ± SE, mg) Produced by Insulin Administered Concurrently With			
	Drug Vehicle	Glyburide	Pioglitazone	Metformin
18 mmol/L	−2 ± 2	3 ± 4	2 ± 3	−1 ± 2
25 mmol/L	0 ± 8	18 ± 14	15 ± 10	−4 ± 14
50 mmol/L	9 ± 22	48 ± 13*†	34 ± 27	−71 ± 24*§
75 mmol/L	11 ± 14	48 ± 12*†‡	16 ± 25	−99 ± 27*§
125 mmol/L	23 ± 20	49 ± 13*‡	18 ± 19	−78 ± 20*§

NOTE. Calculated effects of insulin are based on data illustrated in Fig 1. There were 14 experiments (rats), as described in study I.

*$P < .05$ *v* 0 change (via paired *t* test).

†$P < .05$ *v* drug vehicle (via Scheffé's test).

‡$P < .05$ *v* pioglitazone (via Scheffé's test).

§$P < .05$ *v* drug vehicle and all other drug treatments (via Scheffé's test).

Finally, EC_{50} values for NE were not influenced by either drugs or insulin in any of the experiments from this study.

DISCUSSION

This is the first investigation of the direct effects of antidiabetic drugs and their interaction with the direct effects of insulin on the rat tail artery, a highly contractile vessel with dense adrenergic innervation and several other characteristics of resistance vessels.[28-30] There are at least two major new findings that emanate from this study. First, only the nonsulfonylurea antidiabetic agents pioglitazone and metformin inhibited contractile responses of isolated intact rat tail arterial rings to KCl and NE; the sulfonylurea glyburide did not. Second, glyburide and metformin at known therapeutically relevant concentrations both interacted with insulin to modulate contractions produced by KCl and NE. Insulin by itself did not alter contractions produced by KCl. However, metformin, which decreased KCl-induced contractions, did so nearly twice as much in the presence of insulin than in its absence. In contrast, glyburide increased KCl-induced contractions in the presence of insulin. Although much less notable, similar contrasting effects of these two drugs on the ability of insulin to attenuate contractions produced by high levels of NE were also detected. Glyburide tended to decrease and metformin tended to increase insulin's attenuating effect. Altogether, these findings indicate disparate direct effects of nonsulfonylurea versus sulfonylurea agents on (1) the force of arterial vascular contractions and (2) the ability of insulin to modulate the contractions in a small muscular artery.

These results are consistent with previously reported vascular changes seen after oral administration of these agents for several weeks or months to both humans and animals. Indeed, the above-mentioned contrasting effects of glyburide and metformin on contractile responsiveness of arterial tissue to insulin (as seen here in vitro) are particularly consistent with their previously reported contrasting effects on systemic vascular resistance (as seen in vivo) in type II diabetic patients.[16] After 4 weeks of oral administration with dosages of each drug titrated

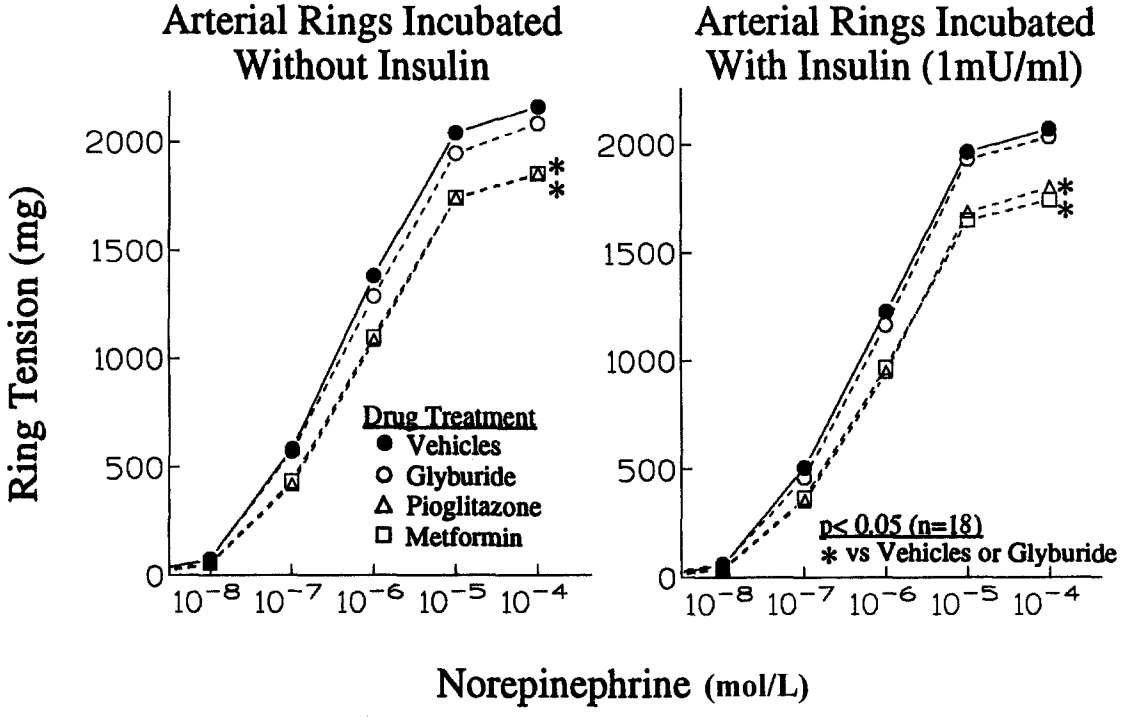

Fig 2. Effects of antidiabetic drugs and insulin on NE-induced contraction in rat tail arterial rings. Mean values are illustrated without SE values, which were 78 mg tension overall. Calculated effects of insulin on these contractions are shown in Table 2. Statistically significant differences between drugs as derived from Scheffé's multiple mean comparison procedure apply at all levels of NE from 10^{-7} to 10^{-4} mol/L with and without insulin.

Table 2. Effects of Antidiabetic Drugs on the Ability of Insulin to Change the Force of NE-Induced Contractions in Rat Tail Arterial Rings

	Change in Tension (mean ± SE, mg) Produced by Insulin Administered Concurrently With			
NE	Drug Vehicle	Glyburide	Pioglitazone	Metformin
10^{-8} mol/L	−20 ± 7*	−13 ± 5*	−15 ± 7*	−14 ± 5*
10^{-7} mol/L	−71 ± 24*	−80 ± 28*	−64 ± 26*	−68 ± 25*
10^{-6} mol/L	−145 ± 34*	−122 ± 33*	−116 ± 34*	−120 ± 33*
10^{-5} mol/L	−73 ± 25*	−24 ± 31	−61 ± 24*	−94 ± 28*†
10^{-4} mol/L	−83 ± 23*	−46 ± 14*	−59 ± 18*	−114 ± 21*†

NOTE. Calculated effects of insulin are based on data illustrated in Fig 2. There were 18 experiments (rats), as described in study II.

*$P < .05$ *v* 0 change (via paired *t* test).

†$P < .05$ *v* glyburide (via Scheffé's test).

to achieve similar glycemic control, systemic vascular resistance in these patients was increased by glyburide and decreased by metformin in the same study.[16] The interesting ability of glyburide to directly induce a "vasoconstrictor-like" response to insulin in the present study also supports our previous evidence of a similar phenomenon seen after oral administration to rats for 15 weeks.[2] Several months of treatment with oral metformin has been shown to increase arterial blood flow to distal extremities in patients with peripheral vascular disease.[44] Ten weeks of oral metformin was recently reported to reduce the intrinsic constrictor responsiveness of the superior mesenteric artery to NE in both normal and fructose-fed rats.[21] Finally, only 8 to 9 days of oral pioglitazone was recently found to inhibit arterial pressor responses to graded intravenous infusions of NE and angiotensin II in conscious Dahl salt-sensitive rats.[25] The results of the present study now allow us to more conclusively attribute these previously reported changes in vascular function in general to direct rather than indirect effects of the drugs on arterial vascular contractile reactivity.

The results of the present study are also consistent with the previously reported effects of these drugs on blood pressure. However, the possibility exists that these drugs could also influence blood pressure by other mechanisms. For example, metformin can decrease renal sympathetic neuronal activity from a central site of action.[45] There is also evidence of a possible nonneuronal pressor action of metformin[46] that could potentially limit the extent to which the drug can decrease arterial pressure. Thus, it is not surprising that in one recent study in which 10 weeks of oral metformin decreased both arterial pressure and arterial vascular contractile reactivity to adrenergic stimulation in one group of rats (fructose-fed) it decreased only the arterial vascular reactivity and not the arterial pressure in another group (normally fed rats).[21] Presumably, more than just a direct vasorelaxant action of metformin may be required in some cases for the drug to decrease arterial pressure.

Our results with direct administration of pioglitazone to tail arterial tissues differ from recently reported results with aortic tissues also isolated from normal rats.[24,25] Both pioglitazone and troglitazone in vitro (at 10 μmol/L each) appeared to sensitize rat aortic tissues to the attenuating actions of insulin on contractions produced by either adrenergic stimulation[24,25] or KCl.[24] Although we found that a 2-hour exposure to pioglitazone at 5 μmol/L in vitro could by itself significantly inhibit adrenergic- and KCl-induced contractions in rat tail arterial rings, the same treatment failed to alter the sensitivity of the same tail arterial rings to any action of insulin on these contractions. It is possible that this difference in results relates to the relatively small difference in drug concentrations applied to the tissues. It could also relate to inherent differences in responsiveness of rat aortic versus tail arterial tissues. Whether insulin-related effects of thiazolidinediones will eventually be found at still lower drug concentrations and with still smaller arteries remains to be determined. In addition, whether any of these antidiabetic agents can alter the insulin action on arterial contractility in insulin-resistant humans with hypertension also now remains to be determined.

The present study was not designed to explore mechanisms responsible for the disparate effects of these drugs on arterial contractions. However, our results would suggest that these drugs (at least at the low therapeutically relevant concentrations tested) do not alter contractions in arterial tissue through the same rapid channel-related mechanisms as, for example, affected by known potassium channel openers and/or calcium channel blockers. Rather, our results indicate that they exert inhibitory effects on contractility after a delay in time. This does not rule out the possibility of delayed actions at the level of the same channels, and it also raises the possibility of other mechanisms. These antidiabetic drugs at these particular concentrations may exert effects at sites of delayed insulin action unrelated to channel activities. Delay in the ability of insulin to alter arterial smooth muscle contractility has been attributed by some to changes in expression of proteins associated with the activity of Na/K-ATPase,[47] Ca-ATPase,[48] and glucose transporters[49] in the cell membrane. Like insulin, these drugs could also alter contractility of the smooth muscle indirectly, ie, by acting on arterial endothelial cells or sympathetic neuronal terminals. The latter are especially abundant in tail artery,[28-30] and there is evidence that insulin alters their release and uptake of NE.[50] Furthermore, a portion of the contractile force produced by KCl in isolated rat tail arteries is thought to be adrenergically mediated.[51] That is, whereas part of the KCl-induced contraction may be caused by depolarization of the smooth muscle cell membrane, another part may be due to depolarization of the neuronal cell membrane in the sympathetic terminal, causing release of NE and possibly inhibiting its reuptake.[51] Thus, it is possible that the modulating effects of metformin and glyburide on insulin's ability to influence KCl-induced contractions in these rat tail arteries actually originate in sympathetic terminals. This and other potential mechanisms remain to be further elucidated in future studies.

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