Effects of Adrenergic, Cholinergic and Ganglionic Blockade on Acute Depressor Responses to Metformin in Spontaneously Hypertensive Rats

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ABSTRACT

Metformin lowers blood pressure in humans and in experimental animal models. To determine the mechanism of acute metformin-induced hypotension, we measured changes in mean arterial pressure (MAP) and heart rate (HR) during metformin alone (0, 10, 50, 100 mg/kg i.v.; n = 10) and during concomitant alpha adrenergic (phentolamine, 5 mg/kg; n = 5), beta adrenergic (propranolol, 3 mg/kg; n = 6), muscarinic (atropine, 200 μg/kg; n = 7), ganglionic (hexamethonium, 30 mg/kg; n = 11), nitric oxide synthase (N^G^-methyl-L-arginine acetate salt, 15 mg/kg; n = 9) and combination ganglionic plus alpha adrenergic plus beta adrenergic (n = 6) blockade in spontaneously hypertensive rats (SHR). Responses to metformin alone were also assessed in normotensive Wistar-Kyoto rats (n = 6). In SHRs, metformin elicited depressor responses accompanied by tachycardia (100 mg/kg; ΔMAP, −26 ± 3 mm Hg; ΔHR, +49 ± 12 bpm). Depressor responses in Wistar-Kyoto rats were significantly attenuated (100 mg/kg; ΔMAP, −9 ± 4 mm Hg; P < .01). Hypotensive actions of metformin in SHRs were abolished and reversed into pressor responses by hexamethonium (100 mg/kg; ΔMAP, +24 ± 6 mm Hg), phentolamine (100 mg/kg; ΔMAP, +62 ± 10 mm Hg) and by combination ganglionic plus adrenergic (100 mg/kg; ΔMAP, +62 ± 10 mm Hg) blockade. Neither propranolol, atropine nor N^G^-methyl-L-arginine acetate salt affected hypotensive responses to metformin. We conclude that acute intravenous metformin administration decreases MAP by causing withdrawal of sympathetic activity. The increase in MAP uncovered by hexamethonium and phentolamine suggests that the original depressor response to metformin is buffered by mechanisms unrelated to the autonomic nervous system.

Antihyperglycemic agents, such as metformin, have recently been used to test the concept that insulin resistance and hyperinsulinemia contribute to the development of arterial hypertension. Consistent with this hypothesis, it was postulated that if insulin resistance causes hypertension, then reducing resistance with metformin should lower BP (Anderson and Mark, 1993). In a series of recent studies with both experimental animal models and humans, metformin did indeed produce concomitant reductions in insulin resistance, plasma insulin and arterial pressure (Giugliano et al., 1993a, b; Landin et al., 1991; Morgan et al., 1992; Velazquez et al., 1994; Verma et al., 1994a, b). Perhaps most striking among these findings was the demonstration in SHRs and fructose-fed rats that BP decreases by metformin could be reversed by restoration of plasma insulin to pretreatment levels by use of subcutaneous insulin implants (Verma et al., 1994a, b).

Although these experiments support the concept that insulin resistance and hyperinsulinemia contribute to the development of hypertension, other studies indicate that metformin may lower arterial pressure, at least in part, through mechanisms unrelated to insulin metabolism. For example, both acute intravenous and intracerebroventricular administration of metformin produce dose-dependent reversible decreases in efferent renal sympathetic nerve activity and BP in SHRs and Sprague-Dawley rats (Liu et al., 1996; Petersen and DiBona, 1996). In agreement with a sympathoinhibitory role for metformin, Giugliano and colleagues (1993a) reported that 12 weeks of metformin treatment evoked decreases in plasma norepinephrine and BP in obese hypertensive females. Although a similar study showed no effect of metformin on either parameter in obese hypertensives, this experiment examined male subjects for only 6 weeks (Gudbjörnsdóttir et al., 1994).

The present study was designed to determine whether

ABBREVIATIONS: BP, blood pressure; HR, heart rate; MAP, mean arterial pressure; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat; L-NMMA, N^G^-methyl-L-arginine acetate salt.
acute BP decreases evoked by metformin treatment are caused by withdrawal of sympathetic nerve activity. In addition, because generation of nitric oxide in the medulla oblongata causes inhibition of sympathetic nerve activity (Togashi et al., 1992; Zanzinger et al., 1994, 1995), we sought to determine whether metformin lowers BP by initiating nitric oxide production. We measured HR and BP in conscious chronically instrumented, unrestrained SHRs during intravenous bolus administration of metformin alone or during concomitant ganglionic, alpha adrenergic, beta adrenergic, cholinergic or nitric oxide synthase blockade. In addition, cardiovascular responses to acute metformin in SHRs were compared with responses in normotensive WKY rats.

Methods

Animals

Experiments were performed in male SHRs and WKYs, weighing 250 to 300 g (Taconic, Germantown, NY). All procedures were performed in accordance with the Lehman College and National Institutes of Health guidelines for the care and use of experimental animals.

Surgical Preparation

For implantation of vascular catheters, rats were anesthetized with ketamine (40 mg/kg i.m.) supplemented with xylazine (5 mg/kg i.m.). Polyethylene catheters were inserted into the left femoral artery for monitoring arterial pressure and HR and into the left femoral vein for drug administration. The free end of the catheters were routed subcutaneously to exit through the dorsal surface of the neck. The tubing was filled with heparinized saline (75 U/ml) and plugged with stainless steel wire. The rats were given 48 h of recovery before cardiovascular testing.

Experimental Procedure

HR and BP were recorded in the home cages of conscious, unrestrained rats. Arterial pressure was measured by a Statham P23 XL pressure transducer and displayed continuously on a Grass model 7E polygraph. HR was recorded by a linear cardiotachometer (Grass model 7P4) triggered by the arterial pressure waveform. The rats were attached to an overhead-tethered catheter connected to the pressure transducer and allowed to stabilize for 40 min before the start of the experiment. Rats participated in three to five protocols. The order of the experimental protocols was randomized with an interval of ≥2 days between tests.

Protocol 1: Metformin alone. Metformin (0 [isotonic saline], 10, 50, 100 mg/kg i.v.) was administered as bolus injections in a random dose-dependent manner. Injection volume was 300 μl at all doses. Successive doses were separated by a period of 15 min, allowing complete recovery to base line of all cardiovascular parameters. This protocol was administered to a group of SHRs (n = 14) and to a group of WKYs (n = 6).

Protocol 2: Metformin administered after hexamethonium pretreatment. After base-line measurements, hexamethonium was administered as a bolus dose (30 mg/kg i.v.). After 15 min, new base-line measurements were taken and metformin was administered as in protocol 1. This protocol was administered to a group of SHRs (n = 11).

Protocols 3–6: Metformin administered after phentolamine, L-NMMA, propranolol or atropine pretreatment. These protocols were identical with protocol 2 except that, instead of hexamethonium, phentolamine (5 mg/kg i.v., n = 5; protocol 3), L-NMMA (15 mg/kg i.v., n = 9; protocol 4), propranolol (3 mg/kg i.v., n = 6; protocol 5) or atropine (200 μg/kg i.v., n = 7; protocol 6) were administered as bolus injections to SHRs.

Protocol 7: Metformin administered after concomitant hexamethonium, phentolamine and propranolol pretreatment. The aim of this protocol was to remove the potential cardiovascular effects of adrenal medullary epinephrine after ganglionic blockade with hexamethonium. In a group of SHRs (n = 6), a protocol identical with protocol 2 was repeated, except that, in addition to hexamethonium, phentolamine and propranolol were administered with the doses described in protocols 3 and 5.

Protocol 8: Assessment of efficacy of cardiovascular ganglionic, alpha adrenergic, beta adrenergic and muscarinic blockade. Fifteen additional SHRs were used in this protocol. To test the efficacy of ganglionic cardiovascular blockade, BP depressor and reflex tachycardia responses to bolus doses of sodium nitroprusside (10 μg/kg i.v., n = 10) were measured before and 60 min after administration of hexamethonium as described in protocol 2. To test the efficacy of alpha adrenergic vascular blockade, BP increases to norepinephrine (5 μg/kg i.v., n = 11) were measured before and 60 min after administration of phentolamine as described in protocol 3. To test the efficacy of beta adrenergic vascular blockade, BP depressor responses to isoproterenol (2 μg/kg i.v., n = 9) were measured before and 60 min after administration of propranolol as described in protocol 5. To test the efficacy of vascular cholinergic blockade, BP decreases to acetylcholine (5 μg/kg i.v., n = 4) were measured before and 60 min after injection of atropine as described in protocol 6.

Drugs

Ketamine (100 mg/ml) was supplied in 10-ml ampoules (Fort Dodge Laboratories, Inc., Fort Dodge, IA) and xylazine (20 mg/ml) was supplied in 20-ml ampoules (Bayer Corporation, Shawnee Mission, KA). Metformin (1,1-dimethylbiguanide; ICN Biomedicals, Inc., Irvine, CA) was dissolved in isotonic saline in concentrations of 10, 50 or 100 mg/ml. Hexamethonium bromide (Sigma Chemical Co., St. Louis, MO) was dissolved in isotonic saline (30 mg/ml). Phentolamine methanesulfonate salt (5 mg/ml; Sigma) was dissolved in isotonic saline. L-NMMA (Sigma) was dissolved in isotonic saline (22.5 mg/ml). DL-Propranolol hydrochloride (Sigma) was dissolved in isotonic saline (3 mg/ml). Atropine sulfate salt (0.2 mg/ml; Sigma) was dissolved in isotonic saline. Sodium nitroprusside (ICN) was dissolved in isotonic saline (1 μg/ml) and stored at 5°C. Norepinephrine bitartrate salt (5 μg/ml; Sigma) was dissolved in isotonic saline and stored at 5°C. Isoproterenol (Sigma) was dissolved in isotonic saline (2 μg/ml). Acetylcholine chloride (Sigma) was dissolved in isotonic saline (5 μg/ml) and stored at 5°C. Solutions with metformin, hexamethonium, phentolamine, L-NMMA, propranolol, atropine and isoproterenol were prepared fresh on each experimental day.

Data Analysis

During intravenous bolus injection of metformin, the peak response was defined as the average BP and HR change during the 5-s period showing maximal deflection from base line. Base-line measurements were taken from the average of the last 10 sec of HR and MAP immediately preceding metformin injection. When no well-defined response was observed (during vehicle injection), readings were performed 25 to 30 sec after intravenous injection. The data were analyzed by appropriate single or repeated measures analysis of variance and presented as means ± S.E.M. Post hoc comparisons were made using Fisher's least significant difference tests. Differences between groups were considered significant at the P < .05 level.

Results

Base-line values. Base-line HR and BP in all experimental groups are shown in table 1. Hexamethonium, phentolamine and the mixture of hexamethonium plus phentolamine with propranolol all decreased MAP in SHRs. In contrast, L-NMMA and propranolol produced elevations in
MAP. HR was decreased by hexamethonium, L-NMMA, propranolol and by the mixture of hexamethonium plus phentolamine plus propranolol in SHRs. Both phentolamine and atropine elevated HR.

**Responses to metformin.** Intravenous bolus injections of metformin in SHRs elicited rapid, reversible decreases in MAP accompanied by increases in HR (fig. 1). Both the decreases in MAP and increases in HR were dose dependent (figs. 2 and 3). Analysis of BP responses to metformin revealed a main effect for strain (SHR vs. WKY; P < .01), reflecting blunted depressor responses in WKYs compared with SHRs at all doses tested (fig. 2; P < .05). The dose-dependent increases in HR in WKYs were not different from the increases observed in SHRs (fig. 3).

The decrease in MAP induced by metformin in SHRs was not affected by prior cholinergic, beta adrenergic or nitric oxide synthase blockade (fig. 4). In contrast, the acute hypotensive action of metformin was abolished and reversed into a pressor response by prior ganglionic and alpha adrenergic blockades, and by combination ganglionic, alpha adrenergic and beta adrenergic blockade.

The metformin-induced elevation in HR was not appreciably affected by ganglionic or by nitric oxide synthase blockade (fig. 5). Blockade of cholinergic and beta adrenergic receptors blunted the increase in HR to metformin. The HR increase to metformin was completely blocked by combination ganglionic, alpha adrenergic and beta adrenergic blockade. Finally, alpha adrenergic blockade alone reversed the tachycardia to metformin into a bradycardia response.

**Assessment of vascular ganglionic, alpha adrenergic, beta adrenergic and cholinergic blockade.** Reflex increases in HR evoked by sodium nitroprusside-induced hypotension were nearly abolished by ganglionic blockade with use of hexamethonium. Before treatment with hexamethonium, sodium nitroprusside produced an increase in HR for a given decrease in MAP (ΔHR/ΔMAP = 1.4 ± 0.2). After ganglionic blockade, sodium nitroprusside evoked similar depressor responses that were unaccompanied by HR increases, which produced a significant decrease in ratio of HR to blood pressure change (ΔHR/ΔMAP = 0.5 ± 0.1; P < .01). Similarly, phentolamine markedly attenuated increases in MAP evoked by intravenous bolus injection of norepinephrine. Before phentolamine, norepinephrine increased MAP by 43 ± 4 mm Hg, whereas after alpha adrenergic blockade with phentolamine, norepinephrine increased MAP by 12 ± 4 mm Hg (P < .01, before vs. after phentolamine). Depressor

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<th>HR Base Line</th>
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*Values are expressed as means ± S.E.M.

†P < .01 vs. SHR base-line groups.

**Fig. 1.** Segments of original records from a representative SHR showing HR (beats per minute, bpm), BP (expressed in mm Hg) and MAP (expressed in mm Hg) during bolus intravenous injection of metformin (100 mg/kg, at arrow). Intravenous metformin elicited a depressor response accompanied by tachycardia.

**Fig. 2.** MAP responses to graded doses of intravenous metformin in SHRs and WKYs. Entries are means ± S.E.M. *P < .05, WKY vs. SHR.

**Table 1.** Base-line values of MAP and HR in experimental groups.

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**Fig. 3.** MAP responses to graded doses of intravenous metformin in SHRs and WKYs. Entries are means ± S.E.M. *P < .05, WKY vs. SHR.
responses elicited by isoproterenol ($\Delta$MAP = $-49 \pm 5$ mm Hg) were essentially abolished by prior treatment with propranolol ($\Delta$MAP to isoproterenol = $-8 \pm 4$ mm Hg; $P < .0001$). Finally, the depressor response to acetylcholine ($\Delta$MAP = $-76 \pm 4$ mm Hg) was greatly attenuated by pretreatment with atropine ($\Delta$MAP to acetylcholine = $-27 \pm 3$ mm Hg; $P < .0001$).

**Discussion**

With the recent demonstration that metformin lowers arterial pressure, new interest has been generated in the cardiovascular actions of this drug traditionally administered for the treatment of hyperglycemia. Although metformin lowers BP in both humans (Chan et al., 1993; Giugliano et al., 1993a,b; Haupt et al., 1991; Landin et al., 1991; Velazquez et al., 1994) and experimental animals (Morgan et al., 1992; Petersen and DiBona, 1996; Verma et al., 1994a,b), the mechanisms of this antihypertensive action remain unknown. To determine whether acute BP decreases are caused by sympathetic withdrawal, we examined BP responses to metformin in the presence and absence of ganglionic blockade with hexamethonium. We found that alpha adrenergic blockade or ganglionic blockade abolished the acute depressor response to metformin, which suggests that the drug may indeed lower BP by sympathoinhibition. However, the present results also indicate that the drug exerts other actions on the cardiovascular system independent of the sympathetic nervous system.

Acute intravenous metformin elicited a decrease in BP accompanied by tachycardia. The depressor response was unaffected by cholinergic-muscarinic, beta adrenergic and nitric oxide synthesis blockade. These results agree with early observations (Sterne, 1969) showing that metformin-induced hypotension in dogs is not altered by pretreatment with atropine. Furthermore, our findings concur with the recent demonstration that depressor responses to metformin are unaffected by pretreatment with L-NMMA in anesthetized rats (Petersen and DiBona, 1996), which indicates that neither central nervous system nor endothelial nitric oxide synthase is necessary for metformin-induced depressor responses.

Because metformin reduces both sympathetic nerve activity and BP in rats (Liu et al., 1996; Petersen and DiBona,
1996), we predicted that blockade of the sympathetic nervous system would abolish metformin-induced hypotension. When the autonomic nervous system was blocked with hexamethonium, injection of metformin elicited an unexpected increase in arterial pressure. Identical pressor responses to metformin were observed following alpha adrenergic receptor blockade and combined alpha adrenergic, beta adrenergic and ganglionic blockade. These findings suggest that acute intravenous metformin administration lowers BP by causing sympathetic withdrawal, because blockade of the sympathetic nervous system abolished metformin-induced depressor responses. Although the mechanisms of metformin-induced sympathoinhibition remain unknown, Petersen and DiBona (1996) demonstrated that metformin injected into the lateral cerebral ventricle decreased renal sympathetic nerve activity, which indicates that the drug exerts its effects primarily in the central nervous system. The current studies, however, do not permit conclusions about the site of action of intravenously administered metformin.

Because metformin raises BP under conditions of autonomic blockade, alpha adrenergic blockade and beta adrenergic blockade, the drug appears to exert pressor actions which are not related to the sympathetic nervous system and are not secondary to the release of epinephrine from the adrenal medulla. Nonneural mechanisms by which metformin might elevate BP include a direct release of renin, and thereby angiotensin II, the release of antidiuretic hormone or direct vasoconstrictor actions of metformin on vascular smooth muscle. The latter possibility, however, is not supported by recent in vitro studies showing metformin-induced vasodilation. In these experiments, metformin inhibited contractile tone in rat tail artery rings precontracted with noradrenaline (Miller and Peuler, 1996). Further supporting direct vasorelaxation, treatment of smooth muscle cells with metformin attenuated angiotensin II-induced elevations in intracellular calcium levels (Sharma and Bhalla, 1995) and suppressed increases in intracellular calcium to thionin (Dominguez et al., 1996).

When metformin was given alone in the present studies, the decrease in BP was accompanied by an elevation in HR. In contrast to our findings with conscious rats, Petersen and DiBona (1996) demonstrated metformin-induced bradycardia in Saffan-anesthetized SHRs. The decreases in HR to metformin in that study may be secondary to a specific interaction with Saffan anesthesia, because pentobarbitaleanesthetized rats in an experiment by Liu and colleagues (1996) exhibited tachycardia responses to metformin that were similar to the present findings. The metformin-induced tachycardia in the current experiment was not secondary to activation of the baroreceptor reflex, because blockade of the autonomic nervous system with hexamethonium did not alter the increase in HR observed during metformin injection. Tachycardia to metformin in rats treated with hexamethonium indicates that the drug may increase HR by a nonneural mechanism, perhaps through direct stimulation of epi
dephrine release and subsequent activation of cardiac beta-1 adrenergic receptors. Indeed, when metformin was given during concomitant ganglionic and beta adrenergic blockade, the increase in HR was abolished. When metformin was given to rats pretreated with the alpha adrenergic blocker, phentolamine, an increase in BP was observed accompanied by bradycardia. Under these conditions of BP increases to metformin, activation of the baroreceptor reflex appears to be the cause of the decrease in HR. In hexamethonium-treated rats and in rats given hexamethionum plus phentolamine plus propranolol, a similar baroreceptor-mediated bradycardia may have been abrogated by the ganglionic blockade with hexamethionum.

Thus, acute intravenous metformin appears to lower BP through sympathetic withdrawal, but exerts pressor actions when sympathetic effects are removed by alpha adrenergic, ganglionic or combination blockade. When metformin is given alone, the drug-induced decrease in BP is accompanied by increases in HR which may be secondary to epinephrine release from the adrenal medulla. It remains to be determined, however, whether the observed cardiovascular effects of these intravenous doses of metformin in rats (10–100 mg/kg) are related to the antihypertensive actions of orally administered metformin given in human clinical trials (15–40 mg/kg/day).

The present results also show that bolus administration of metformin evokes greater depressor responses in SHRs than in WKYs. These findings concur with previous reports showing hypotensive actions of oral or interperitoneal metformin in SHRs but not in WKYs or Sprague-Dawley rats (Morgan et al., 1992; Verma et al., 1994b). Providing further evidence that metformin only lowers BP under specific physiological or genetic conditions, chronic metformin administration prevented fructose-induced hypertension in Sprague-Dawley rats (Verma et al., 1994a), but did not affect pressure in Dahl salt-sensitive rats nor in one-kidney, one-clip hypertensive Sprague-Dawley rats (Zhang et al., 1994).

Whether such disparate findings in rats reflect a similar situation in human studies is unclear. Metformin lowered BP in several human clinical trials but did not in other equally controlled experiments. Of the human subjects in which metformin lowered BP, including lean hypertensives (Landin et al., 1991), obese hypertensives (Giugliano et al., 1993a,b), hypertensive and normotensive individuals with type II diabetes (Chan et al., 1993; Giugliano et al., 1993b; Haupt et al., 1991) and normotensive women with polycystic ovary syndrome (Velazquez et al., 1994), all were characterized by the common denominator of insulin resistance. Because insulin resistance in obesity, essential hypertension and type II diabetes is often associated with elevated sympathetic nerve activity (Anderson and Mark, 1993; Tuck, 1992), we speculate that the antihypertensive effects of metformin require the higher level of basal sympathetic activity that distinguishes these patient subgroups. However, it should be noted that in other studies, subjects with hypertension (Calle-Pascual et al., 1995; Gudbjarnsdottir et al., 1994; Hermann et al., 1994; Semplicini et al., 1993), obesity (Campbell et al., 1987; Gudbjarnsdottir et al., 1994) and type II diabetes (Calle-Pascual et al., 1995; Campbell et al., 1987; Hermann et al., 1994) showed no change in BP during similar metformin administration.

In conclusion, this study indicates that acute intravenous metformin causes a decrease in arterial pressure that is not significantly altered by cholinergic, beta adrenergic or nitric oxide synthase blockade. That the decrease in BP is caused by sympathetic withdrawal is suggested by the finding that both ganglionic and alpha adrenergic blockade abolished metformin-induced depressor responses. The increase in BP induced by metformin, uncovered by hexamethonium and
phenolamine, suggests that the original depressor response is buffered by mechanisms unrelated to the autonomic nervous system.

References


