Long-Term Glucose Infusion Increases Arterial Pressure in Dogs With Cyclooxygenase-2 Inhibition

Michael W. Brands, Allison E. Hailman, Sharyn M. Fitzgerald

Abstract—A series of studies has shown that long-term infusion of insulin and glucose does not increase mean arterial pressure (MAP) in dogs, but we have shown that the same infusion protocol or infusion of glucose alone increases arterial pressure in rats. This study tested the hypothesis that infusing glucose alone in dogs, with all insulin derived from endogenous secretion, would increase arterial pressure. Because fructose feeding in dogs also has been shown not to cause hypertension and because we have shown that prostaglandin production increases during insulin and glucose infusion, this study also tested whether prostaglandins prevent the pressor response in dogs. Dogs were instrumented and assigned in random crossover design to long-term cyclooxygenase-2 (COX-2) inhibition. After baseline measurements, glucose was infused in all dogs for 6 days (=500 g/d IV). Plasma insulin increased 3- to 4-fold and blood glucose increased significantly in both groups. The MAP (measured 24 h/d) response in control dogs was variable but on average tended to increase, although not significantly. In the dogs with COX-2 inhibition, however, MAP increased significantly to a peak of 9±2 mm Hg and an average of 6±1 mm Hg above control. There was significant sodium and volume retention during glucose infusion and a significant increase in glomerular filtration rate, but there were no between-group differences. Plasma renin activity increased only in the control group. This is the first study to report a long-term pressor response with glucose infusion and hyperinsulinemia in dogs, and it suggests that the inability to detect this relationship previously was due to prostaglandins. (Hypertension. 2001;37[part 2]:733-738.)

Key Words: insulin ■ hypertension ■ prostaglandins ■ COX-2 ■ renin-angiotensin

D erangements in glucose homeostasis are associated closely with the excess cardiovascular disease in conditions such as obesity and diabetes, and there has been continued interest in determining whether and how these factors may be related. One early hypothesis was that hyperinsulinemia may mediate the hypertension that accompanies these conditions,1–4 but despite considerable research, the role of insulin remains unclear.

Feeding rats a diet high in simple sugar, such as sucrose or fructose, has been shown to increase plasma insulin levels and blood pressure,5–9 and studies in which we infused insulin and glucose chronically in rats also reported a significant increase in mean arterial pressure (MAP).10–15 On the other hand, long-term insulin and glucose infusion has been shown repeatedly in dogs not to cause hypertension and in most instances to actually decrease arterial pressure significantly.16–20 The explanation for the species differences has not been clear, but because blood glucose increased in all the rats and decreased in the dog studies, a difference in the models may play a role.

We recently tested the hypothesis that infusing glucose in rats, as we had done previously,10–15 but without infusing insulin would produce the same blood pressure, glucose, and insulin responses, and our results showed that was true.21 In the present study, therefore, we infused glucose chronically in dogs, also as done previously,16–20 but now without insulin, to test whether glucose would increase blood pressure in dogs when all insulin was derived solely from endogenous secretion. In addition, because fructose feeding in dogs also has been shown not to cause hypertension22 and because we have shown that prostaglandin production increases during insulin and glucose infusion,13 this study also tested whether prostaglandins prevent the pressor response in dogs.

Methods

Experiments were conducted in 12 conditioned mongrel dogs weighing between 20 and 25 kg, and the experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Under isoflurane anesthesia with aseptic technique, Tygon catheters were implanted in the femoral arteries and veins and advanced to the inferior abdominal aorta and vena cava. The catheters were tunneled subcutaneously to the scapular region, exteriorized, filled with 1000 U/mL heparin solution, and then closed.

After 1 to 2 weeks of recovery, the dogs were placed in individual metabolic cages and fitted with harnesses on which a pressure transducer (Cobe) was mounted. The harnesses were attached to a flexible hose that allowed safe routing of electrical lines and tubing to the cage exterior. Arterial pressure signals were amplified (CB Sciences) and sampled continuously at 100 Hz throughout the experiment with a PowerLab system.

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Sodium intake was maintained constant at ~90 mmol/d by feeding the dogs a low-sodium diet (Hills H/D, 2 cans) coupled with a continuous infusion of ~500 mL/d IV 0.9% saline. In addition, all dogs received ~1000 mL/d sterile water via continuous intravenous infusion. All solutions were pumped through disposable filters (0.22 μm, Cathivex, Millipore Corporation) to prevent contaminants and bacteria from passing into the circulation. Drinking water was available ad libitum, and antibiotics were administered daily for the duration of the study.

Experimental Protocol
Approximately 2 weeks were allowed for acclimation to the metabolic cages before the dogs were assigned randomly to receive the cyclooxygenase-2 (COX-2) inhibitor nimesulide (140 mg/d per dog given orally [capsules] as two 70 mg/d doses) to block prostaglandin production. The nimesulide capsules were administered with the daily antibiotic capsules to provide a placebo control for any possible influence of administering oral capsules. After 2 days of nimesulide, a 4-day control period was begun. Glucose (500 g/d) then was infused in all dogs for 6 days by replacing the 1000-mL sterile water infusion with an equal volume of 50% dextrose solution. After 6 days, the sterile water infusion was restored for a postexperimental control (recovery) period. Blood samples were drawn for hormone and related measurements during the control period, on glucose infusion days 2 and 5, and during the recovery. In the last group of 4 dogs, glomerular filtration rate (GFR) also was measured on those days.

After an additional week of recovery, all dogs then were crossed over to repeat the protocol with the other treatment (ie, control dogs now repeated the experiment receiving nimesulide and vice versa). Not all dogs were able to complete their second protocol because of problems that developed, such as catheter failure. Thus, the final number of dogs per treatment was 8 for control and 9 for nimesulide, with 7 dogs completing both treatments. Data were analyzed to determine whether treatment order affected outcome, and no effect was measured.

Analytical Procedures
GFR was determined from the total plasma clearance of [125I]iothalamate, and the distribution space of [125I]iothalamate was used as an index of extracellular fluid volume. Plasma and urine sodium and potassium concentrations were determined with ion sensitive electrodes (NOVA Biomedical); plasma protein concentration was measured by refractometry; and plasma renin activity (PRA) was measured by radioimmunoassay. Plasma insulin concentration was measured with a Cayman Chemical EIA kit, and blood glucose concentration was measured with an Accucheck III analyzer. To provide an index of the inhibition of prostaglandin synthesis, a bolus infusion of 10 mg IV arachidonic acid was administered in resting dogs with continuous measurement of arterial pressure for 20 minutes. Measurements in 2 control dogs on 4 different days showed that the area over the depressor curve averaged −5297±745 and −4834±1568 mm Hg · min. Similar measurements in 2 nimesulide dogs showed areas of −870±70 and 90±330 mm Hg · min, with the second dog actually having a mild increase in blood pressure on 3 of the 4 days.

Data were analyzed with a repeated-measures ANOVA. Significant F tests for between-group differences were followed by post hoc t tests between the 2 treatment groups for each day, and significant F tests for within-group differences were followed by post hoc Dunnett’s tests for comparisons of experimental to control values. Statistical significance was considered to be P<0.05. All data are expressed as mean±SEM.

Results
Blockade of prostaglandin synthesis with the COX-2 inhibitor nimesulide did not have a significant effect on baseline blood pressure, heart rate, or any other variable related to cardiovascular or renal function. During glucose infusion, however, Figure 1 shows the significant increase in MAP that occurred in the nimesulide group, with the change in pressure reaching a peak difference of 9±2 mm Hg above control and averaging 6±1 mm Hg above control over the last 3 days of glucose. The overall response of the control group to glucose was not significantly different from that in the nimesulide group (P=0.69 for the between-group F test), but none of the within-group changes in the control group reached statistical significance. The tendency for average blood pressure to increase in the control group was due primarily to responses in 2 dogs, with MAP in 1 control dog averaging ~6 mm Hg above control for the 6-day period. However, the other control dogs had either no change or a modest decrease in pressure. In the nimesulide group, on the other hand, blood pressure did not decrease in any of the dogs. Heart rate averaged 65±5 and 69±3 bpm in the control and nimesulide groups, respectively, during the control period and increased progressively and in parallel during glucose infusion to an
average of 86±4 and 92±4 bpm by day 6. Heart rate returned gradually to control levels during the recovery period.

Urinary sodium excretion decreased significantly in both groups with the start of glucose infusion (Figure 2), and there were no differences between groups. This was associated with significant volume retention, and extracellular fluid volume increased from 6840±272 and 6726±125 mL during control to 7876±237 and 8038±426 mL by glucose day 5 in the control and nimesulide groups, respectively. Plasma protein concentration tended to decrease during glucose infusion in both groups and recover afterward (the Table), but the changes were not statistically significant.

PRA averaged 0.43±0.08 and 0.30±0.10 ng AI · mL⁻¹ · h⁻¹ in the control and nimesulide groups, respectively, at baseline, but the difference did not reach statistical significance. PRA increased significantly in the control dogs, averaging 1.30±0.17 and 1.79±0.90 ng AI · mL⁻¹ · h⁻¹ on glucose days 2 and 5, respectively. There was a tendency for PRA to increase in the nimesulide group, but the changes, to 0.70±0.13 and 0.62±0.04 ng AI · mL⁻¹ · h⁻¹ on glucose days 2 and 5, respectively, were not significant.

GFR averaged 84±1 and 90±3 mL/min in the control and nimesulide groups, respectively, during baseline; increased by similar percentages to 96±4 and 102±1 mL/min by day 5 of glucose infusion; and returned to control during recovery.

Blood glucose increased significantly in both groups during the glucose infusion and was associated with significant increases in plasma insulin concentration (the Table). Interestingly, although the percent increase in insulin during glucose infusion was similar for the 2 groups, there was a significantly lower baseline in the nimesulide group that caused lower insulin values at all time points. This probably was not due to interassay variation, however, because all samples from a given dog, ie, from both treatments, were run on the same 96-well plate. The glucose levels at each time point also tended to be lower in that group, but not significantly, and although this is a consistent relationship, it cannot be determined from this study how the control of these 2 variables may have been influenced by nimesulide.

### Plasma Insulin, Glucose, Na⁺, K⁺, Protein Concentrations, and Hematocrit

<table>
<thead>
<tr>
<th>Period/Group</th>
<th>Insulin, µU/mL</th>
<th>Glucose, mmol/L</th>
<th>Na⁺, mmol/L</th>
<th>K⁺, mmol/L</th>
<th>Protein, g/dL</th>
<th>Hematocrit, %</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
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<tr>
<td>Control</td>
<td>18.6±4.2</td>
<td>5.6±0.3</td>
<td>147.1±0.4</td>
<td>5.0±0.2</td>
<td>6.6±0.2</td>
<td>37±1</td>
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<tr>
<td>COX-2 inhibition</td>
<td>5.4±1.6‡</td>
<td>4.8±0.1</td>
<td>147.4±0.6</td>
<td>4.6±0.1</td>
<td>6.3±0.2</td>
<td>39±2</td>
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<td><strong>Glucose day 2</strong></td>
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<tr>
<td>Control</td>
<td>51.3±9.1*</td>
<td>6.6±0.4*</td>
<td>147.2±1.0</td>
<td>4.1±0.1*</td>
<td>6.1±0.2</td>
<td>37±2</td>
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<tr>
<td>COX-2 inhibition</td>
<td>21.7±7.4*‡</td>
<td>5.9±0.4*</td>
<td>148.6±0.5</td>
<td>4.0±0.1*</td>
<td>6.1±0.2</td>
<td>40±3</td>
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<td><strong>Glucose day 5</strong></td>
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<tr>
<td>Control</td>
<td>43.3±8.8*</td>
<td>7.0±0.7*</td>
<td>147.7±0.9</td>
<td>4.5±0.2</td>
<td>6.1±0.2</td>
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<tr>
<td>COX-2 inhibition</td>
<td>31.7±8.1*†</td>
<td>5.8±0.3*</td>
<td>147.3±0.3</td>
<td>4.6±0.1</td>
<td>5.8±0.2</td>
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<tr>
<td><strong>Recovery</strong></td>
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<tr>
<td>Control</td>
<td>18.5±4.1</td>
<td>4.8±0.4</td>
<td>147.5±0.6</td>
<td>4.9±0.1</td>
<td>6.5±0.2</td>
<td>35±1</td>
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<tr>
<td>COX-2 inhibition</td>
<td>8.6±2.3†</td>
<td>5.4±0.2</td>
<td>146.1±0.3</td>
<td>5.2±0.1</td>
<td>6.0±0.4</td>
<td>34±1</td>
</tr>
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</table>

*P<0.05 vs the control period for that group; †P<0.05 vs the control group on that day.
Urinary potassium excretion decreased significantly during glucose infusion in both groups. From a similar baseline of \(\approx 53 \text{ mmol/d, potassium excretion decreased to } 16.0 \pm 3.7\) and \(13.8 \pm 2.9 \text{ mmol/d by day 2 of glucose in the control and nimesulide groups, respectively, before gradually returning to } \approx 30 \text{ mmol/d by day 6 of glucose. A tremendous increase in excretion occurred in both groups after glucose infusion was stopped, reaching } \approx 110 \text{ mmol/d on recovery day 1 before returning toward control levels. The curves for both groups were virtually superimposable. As shown in the Table, these changes in potassium excretion were accompanied by a significant but transient decrease in plasma potassium in both groups on glucose day 2.}

**Discussion**

The results from this study show for the first time that long-term glucose infusion can cause a sustained increase in MAP in dogs. A significant increase in blood pressure occurred only in the dogs with blockade of prostaglandin synthesis (nimesulide), but there was evidence that some normal dogs could have a pressor response as well. Glucose infusion also caused significant sodium and volume retention, as well as significant potassium retention, but the role of those and other factors in mediating the blood pressure responses is not clear.

Because derangements in glucose homeostasis are so closely associated with the excess cardiovascular-related morbidity and mortality in conditions such as obesity and diabetes, there has been continued interest in determining whether and how these factors may be related. One early hypothesis was that hyperinsulinemia may mediate the hypertension that accompanies these conditions,\(^1\) and experiments feeding rats high intakes of simple sugars supported this hypothesis by showing increases in blood pressure that correlated with increases in plasma insulin levels.\(^5\)\(^-\)\(^9\) To test the role of insulin per se, we infused insulin for up to 28 days in dogs and found no evidence of a pressor effect.\(^\text{16 –20}\) In fact, the insulin infusion tended to decrease blood pressure in dogs. On the other hand, we also showed that a similar insulin infusion protocol increased arterial pressure in rats.\(^\text{10 –15}\) It has not been clear why the 2 species differ in their responses, but the question is of central importance regarding the potential application of these results to humans, because the response in dogs appears to more closely resemble the effects of insulin in humans.\(^\text{23,24}\)

Recently, however, our laboratory began to question certain aspects of the model that were used in both the rat and dog insulin infusion studies. In all those studies, intravenous glucose was infused to prevent hypoglycemia during insulin infusion. The dose was constant throughout the experiment, and one major difference between the rat and dog studies was that plasma glucose decreased in the dog studies during insulin infusion but increased in the rat studies. A recent study in rats by Koopmans et al.,\(^\text{25}\) using an insulin infusion protocol similar to ours, required much less glucose than our studies did over a 24-hour period to maintain normoglycemia, because they adjusted their glucose infusion on the basis of multiple daily glucose measurements. Together, these findings suggested that the glucose infusion in our rat studies exceeded the amount required to prevent hypoglycemia, whereas the insulin dose in the dog studies may have been too great for the amount of glucose provided. We tested the former by repeating our rat study, using the same glucose infusion rate but eliminating the insulin.\(^\text{21}\) The result was a similar increase in plasma glucose and insulin levels and the same degree of hypertension that we have reported previously with the combined insulin and glucose infusion protocol in rats. The present study therefore tested whether glucose infusion alone, when the insulin levels would be determined solely by endogenous secretion, would increase blood pressure in dogs.

In addition, this study addressed the possible protective role that prostaglandins may exert, thereby preventing a pressor response. Romero’s laboratory has shown that long-term fructose feeding in dogs caused insulin resistance but not hypertension.\(^\text{22}\) and we have shown that the insulin/glucose infusion protocol in rats caused significant increases in prostaglandin production.\(^\text{15}\) Testing the role of prostaglandins in dogs has not been feasible, however, because of the intense intestinal distress caused by long-term nonselective COX inhibition. Although there is no evidence that long-term glucose infusion or hyperinsulinemia causes a direct, sustained increase in COX-2 per se, the use of a specific COX-2 antagonist allowed us to test our hypothesis on the protective role of prostaglandins based on the chance that COX-2 may be induced and play a role in stimulating prostaglandin production as shown in our rat studies.\(^\text{15}\) Nimesulide was selected because of its degree of selectivity for COX-2, because of its prior use in long-term large-animal and human studies, and because it did not decrease thromboxane synthesis.\(^\text{26 –32}\)

Our results indeed revealed that nimesulide treatment enabled long-term glucose infusion to increase blood pressure significantly in dogs. The assertion that this effect was due to inhibition of prostaglandin synthesis is strengthened by previous studies\(^\text{26 –32}\) and by our finding that the depressor response to bolus intravenous administration of arachidonic acid was prevented by nimesulide (see Methods). The nearly total blockade of that response suggests that at the dose used in these studies, some inhibition of COX-1 may have occurred; however, it was not to a degree that had any observable effect on gastrointestinal function. Moreover, the possible blockade of COX-1 to some degree also does not alter our interpretation of the role of prostaglandins in protecting against glucose-induced increases in blood pressure. Further studies will be required, however, to determine the precise prostaglandin synthetic pathways that are activated and to confirm the specific role of COX-2 and determine whether it is induced by glucose infusion.

The mechanism for the increase in blood pressure is not clear from these results, but it is tempting to suggest a role for sodium and volume retention. However, although they may be involved, there were no differences between the control and nimesulide groups. Moreover, those changes, as well as the significant increase in extracellular fluid volume, are similar to the responses reported previously in dogs during long-term insulin/glucose infusion in which blood pressure decreased significantly.\(^\text{16 –20}\) The increases in GFR and PRA...
in the control dogs also are similar to those in our previous studies\(^\text{16–20}\) and are consistent with the possibility that glucose infusion increased tubular sodium reabsorption at a site prior to the macula densa. However, in the insulin/glucose studies, the decrease in blood pressure likely contributed to that effect via pressure natriuresis, whereas arterial pressure did not decrease in the present study. In addition, whereas the increase in GFR was not different in the nimesulide group, the increase in PRA was prevented. One possibility is that PRA decreased in the nimesulide group in response to the increase in blood pressure, but our studies in rats showed that an increase in thromboxane during insulin/glucose infusion had a suppressing influence on renin levels.\(^\text{15,33}\) Thromboxane production was not evaluated in this study, but stimulation of thromboxane synthesis combined with blockade of prostaglandin production also could provide an explanation for the increase in blood pressure and decrease in PRA in the nimesulide group during glucose infusion. Clearly, there are many new questions remaining to be addressed.

Considering the relationship between these results and the previous insulin/glucose infusion studies, one must presume that insulin was responsible for the decrease in blood pressure that occurred in the dog studies.\(^\text{16–20}\) The glucose infusion rates were nearly identical in the 2 protocols, but in the studies in which insulin was infused, plasma insulin increased more on average, and there also was a consistent tendency for blood glucose to decrease. The mechanism through which insulin may have mediated the decrease in blood pressure in those dog studies is not certain, but its reported effect to stimulate nitric oxide synthesis\(^\text{34}\) may provide a mechanism. In support of this possibility, we showed recently that the hypertensive response to glucose infusion in rats\(^\text{21}\),\(^\text{35}\) was potentiated tremendously in rats with long-term blockade of nitric oxide synthesis.\(^\text{35}\) The role of nitric oxide in the dog model of glucose infusion remains to be tested, but these findings together suggest that the term “insulin hypertension” that has been used to describe the results of the long-term insulin/glucose infusion studies in rats may no longer be appropriate. In other words, long-term glucose infusion or high intake of simple sugars increases blood pressure and insulin levels, but slightly greater increases in insulin, at least as shown in the dog studies, cause a significant decrease in blood pressure. The role that the increase in endogenous insulin plays in the blood pressure response to glucose remains to be determined.

In summary, these results show that long-term glucose administration can increase MAP in dogs. That effect was apparent in control dogs but was statistically significant and more consistent in dogs treated long term with the COX-2 inhibitor nimesulide. The results from this study did not reveal a clear mechanism for that effect of COX-2 inhibition, but an apparent improvement in insulin sensitivity in the nimesulide group warrants further investigation. Further study also is needed to quantify the degree of prostaglandin synthesis inhibition, as well as the changes in COX-2 versus COX-1 expression and activity. Despite these uncertainties, however, these results are consistent with our hypothesis that prostaglandins play an important role in counteracting a glucose-induced pressor response and raise anew the question of whether alterations in glucose homeostasis might directly affect blood pressure control in humans.

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References


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