Nitric Oxide Does Not Participate in the Metabolic Effects of Exogenous Bradykinin In Fructose-Fed Rats

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The study was carried out to demonstrate the effects of bradykinin (BK) on hypertension, hyperinsulinemia, and hypertriglyceridemia in fructose-fed rats, and to determine whether these actions are mediated through nitric-oxide (NO) formation.

Eighteen rats, rendered hypertensive, hyperinsulinemic, and hypertriglyceridemic by a fructose-enriched diet, were studied. BK (0.2 mg/day) was infused intravenously using osmotic pumps attached by a catheter to the jugular vein of 12 rats for 12 days. BK was administered either alone (n = 6) or with concomitant inhibition of NO synthase (n = 6). Six untreated rats served as control. Measurements of systolic blood pressure (indirect method) and levels of insulin and triglyceride in serum were taken every second day. BK infused chronically, induced a marked fall in all parameters as early as the second day of infusion: in blood pressure from 152 ± 7 to 126 ± 12 mmHg, in insulin from 8.7 ± 2.9 to 4.6 ± 5.4 pg/mL, and in triglyceride from 308 ± 94 to 76 ± 19 mg/dL. No such reduction was seen in untreated animals. When BK was administered concurrently with NO synthase inhibitor, blood pressure rose significantly, reaching very high values at the end of treatment. However, the reduction in insulin and triglyceride levels induced by BK was not affected.

The capacity of BK to enhance reduction in hyperinsulinemia and hypertriglyceridemia in the fructose-fed rats is not mediated by NO formation. Whether this action of BK is related to a direct effect of this peptide remains to be determined.

Key Words: Bradykinin, nitric oxide, fructose-fed rats.

The benefits of angiotensin-converting enzyme (ACE) inhibition include not only a reduction in blood pressure, which has been potentiated by its vasodilator effect, but also improved insulin responsiveness, and interaction with bradykinin and prostaglandins.

Kininns affect a broad spectrum of physiologic functions. They cause vasodilation, raise vascular permeability, prevent vascular rarefaction, and may alter the responsiveness of glucose metabolism to insulin in skeletal muscle. Thus, the measurement of arterial blood pressure alone cannot provide sufficient information to allow interpretation of the effects of either vasoactive substances or drug therapy.

Nitric oxide (NO) has been suggested to modulate basal vascular tone and to mediate vasodilator responses to bradykinin and acetylcholine in skeletal muscle vasculature of the dog. It may also play a role in bradykinin-induced hypotension, based on evidence of NO’s role as a mediator for bradykinin. Inhibition of NO synthase was found to shorten the hypotensive response in normotensive rats, and, according to Bjornstad-Ostensen et al., part of the hypotensive effect of bradykinin in spontaneously hypertensive rats was mediated through the NO system.

In view of the contribution of both BK and NO to the beneficial effects of ACE inhibitors, the influence of exogenously infused BK on lipid, glucose, and insulin level was examined in the fructose-fed rat with and without an active NO system.

Methods

All procedures were conducted according to the guidelines governing animal studies of the Chaim Sheba Medical Center. Animals were maintained on a 14-h light/10-h dark cycle in a room kept at 23°C and 50% humidity.

Male Sprague-Dawley rats (Anilab, Kiryat Weizmann, Ness Ziona, Israel) purchased at 150 g, were fed standard rat chow until their weight reached 200–300 g, after which they received a fructose-enriched diet ad libitum for 5 weeks. The diet (cat. no. TD 89247, Harlan Teklad, Madison, WI) consisted of fructose 60%, protein 21%, fat 5%,...
cellulose 8%, mineral mix (R-H 170760) 5%, and vitamin mix (Teklad 40060) 1%. The first 3 weeks of fructose feeding established hypertension and associated hyperinsulinemia and hypertriglyceridemia. During the next 2 weeks of fructose feeding, the animals were divided into three groups.

**Group A** This group of six animals received BK (0.2 mg/day, Sigma Chemical Corp., St. Louis, MO) intravenously by osmotic pump (model 2002, Alza, Palo Alto, CA). The osmotic pump was filled with BK (dissolved in saline) containing 3% protease-free serum albumin (Sigma) to enhance protein stability, and was implanted subcutaneously; a polyvinyl catheter was cannulated into the external jugular vein.

**Group B** In Group B, six animals received BK as in Group A, and NO synthase was inhibited by the addition of L-NAME to the drinking water (100 mg/kg/day).

**Group C** This group of six animals received the fructose diet only and served as controls.

Blood pressure, glucose, insulin, and triglyceride (TG) levels were measured before fructose diet, after 3 weeks of fructose feeding, and every 2 days thereafter for 12 days. Systolic blood pressure was measured by indirect tail cuff method using an electrosphygmomanometer and a pneumatic pulse transducer (Narco Biosystems Inc., Houston, TX). The mean of five consecutive blood pressure readings was recorded.

Blood samples for all tests were drawn from the retro-orbital sinus into 1.5-mL Eppendorf tubes, using light ether anesthesia. The samples were centrifuged, aliquoted, frozen, and assayed for insulin (rat insulin 125I RIA kit, DiaSorin, Stillwater, MN) and TG (triglycerides BGPPAP Kit, Boehringer, Mannheim, Germany) concentrations.

Glucose concentration was enzymatically determined in serum using an automatic analyzer (Monarch Chemistry System, Instrumentation Laboratory Inc., Lexington, MA).

**Statistical Analysis** Data were analyzed using SAS software (SAS Corp. Cary, NC). The paired t test and nonparametric sign-rank test were applied to determine paired differences between baseline and postbaseline assessments for quantitative parameters in each study group. Repeated ANOVA measurements determined changes between baseline and postbaseline assessments in the various groups of each experiment. Multiple comparison analysis—the Duncan method—was applied to determine which of the groups differed statistically from the other(s). All tests were two-tailed, and results considered statistically significant when \( P < .05 \). Results are expressed as mean ± SEM.

**Results**

The first 3 weeks of fructose feeding established hypertension and associated hyperinsulinemia and hypertriglyceridemia. Systolic blood pressure (SBP) rose from 136 ± 6 to 151 ± 10 mm Hg, plasma insulin level rose from 2.5 ± 0.8 to 8.5 ± 2.3 ng/mL, and plasma triglyceride level was increased from 83 ± 15 to 309 ± 63 mg/dL.

Fig. 1 shows the response of BP, insulin, TG, and glucose levels in fructose-induced hyperinsulinemic, hypertensive, and hypertriglyceridemic rats to exogenous infusion of BK for 12 days, administered alone (Group A) or together with inhibition of NO synthase (Group B), compared to controls (Group C).

Blood pressure (Fig. 1A) decreased significantly from 152 ± 7 to 126 ± 12 mm Hg \( (P < .05) \) after 2 days of BK infusion in Group A, compared with no change in Group C. BP in Group A subsequently rose slightly, but remained significantly lower than baseline \( (P < .05) \) and than Group C \( (P < .05) \). On the 12th day of BK infusion, BP in Group A reached 146 ± 9 mm Hg, a value not statistically different from baseline.

When BK infusion was given together with L-NAME (Group B), L-NAME not only blunted the hypotensive effect induced by BK on the second day of infusion (BP rose from 148 ± 9 to 151 ± 20; \( P = NS \)), it also caused a gradual elevation in BP. This elevation reached statistical significance \( (P < .05) \) on the 10th and 12th days of concomitant treatment, when BP values were 168 ± 5 and 174 ± 12 mm Hg, respectively. BP values in these rats were significantly higher than those treated with BK alone (Group A) at all postinfusion points \( (P < .05) \) on the 2nd–8th days, \( P < .001 \) on the 10th–12th days.

A steep fall was seen in insulin (Fig. 1B) and TG levels (Fig. 1D) by the second day of BK infusion in Group A. Insulin level fell from 8.7 ± 2.9 to 4.6 ± 5.4 pg/mL \( (P < .05) \) and remained low until the end of treatment, significantly lower \( (P < .05) \) than Group C. TG levels fell from 308 ± 94 to 76 ± 19 mg/dL \( (P < .001) \) and remained lower than baseline \( (P < .05) \) until the end of treatment. TG levels of Group A rats differed from those of Group C animals at all points measured after infusion. L-NAME did not affect the reductions in insulin and TG levels induced by BK. By the second day of BK infusion (Group B), insulin level was reduced from 8.4 ± 1.5 to 4.8 ± 2.1 pg/mL \( (P < .05) \), and remained low until the end of treatment. No significant differences were found between these animals and Group A at any points measured. TG level was reduced from 315 ± 64 to 83 ± 15 mg/dL \( (P < .001) \) by the second day of concomitant treatment (Group B), and remained similar to the values of Group A treated with BK only.

Glucose level did not change in any of the groups (Fig. 1C).

**Discussion**

The marked fall in BP induced by chronic infusion of BK in our study is in agreement with other reports demonstrating BK-induced hypotension both acutely\(^{10,11}\) and chronically\(^2\) in various models of hypertensive rats. However, in another study chronic infusion of exogenous BK
did not reduce BP in rats with angiotensin-II–induced hypertension despite exerting systemic and renal vasodilatory effects. The discrepancy between this report and ours might stem from the different hypertensive mechanisms in these two experimental models.

The low values of BP in the present study were limited to the first 10 days of treatment, after which the reduction in BP was reversed. This might result from a gradual decline in the level of BK, which was infused throughout the treatment period in a vehicle solution that was not peptidase free. This possibility cannot be evaluated, however, because plasma BK levels were not determined in our study. Nevertheless, infusion of BK dramatically and persistently lowered both insulin and TG levels in the treated rats for the entire 12-day treatment period, suggesting that BK can indeed improve the metabolic disorders of the fructose-fed rats. The fact that BK infusion had no effect on glucose level, whereas insulin level was markedly reduced suggests that reduced hyperinsulinemia after BK infusion may reflect an improvement in insulin resistance.

Indeed, a dose of BK comparable to that used here (0.2 mg/day) was previously shown to improve insulin resistance in SHR after 4 days of infusion. Moreover, it was recently demonstrated that chronic administration of BK to obese Zucker rats led to significant reduction in hyperinsulinemia and enhanced insulin action on glucose disposal. Exogenous BK in humans also increased glucose tolerance and insulin sensitivity in a variety of diseases. The beneficial metabolic effect of BK described here might be secondary to vasodilatation and augmented glucose delivery to peripheral tissues. However, at least part of this vasodilatory response to BK may be mediated by NO, as evidenced by the finding of Vallance et al that BK-induced vasodilation is blunted by L-NMMA.

When the effect of exogenous BK was measured in the absence of an active NO system by inhibiting NO synthase with L-NAME given together with BK infusion, BP rose throughout the study, reaching very high values at the end of treatment. These data concur with those of Bjornstad-Ostreens et al, who demonstrated an amplification of kinin-induced hypotension by NO synthase in spontaneous hypertensive rats, and Fulton et al, who showed a reduction in renal vasodilator response to BK by inhibition of NO synthesis in the rat kidney. Thus, like these and other studies, the present results support a role for NO formation in BK-induced hypotension. However, the possible involvement of prostaglandins and endothelium-derived hyperpolarizing factor cannot be excluded.

In the present study, insulin and TG levels were markedly decreased despite the blockade of NO synthase, suggesting that in this setting the metabolic improvement induced by BK was independent of NO, and that the BK-induced metabolic effects are dissociated from the hemodynamic changes. Moreover, the argument that the beneficial effect of BK on glucose metabolism might be purely hemodynamic was weakened in this experiment.

![Graphs showing parameters measured in the three groups at baseline and during treatment. (A) Blood pressure; (B) insulin; (C) glucose; (D) triglycerides.](image)
However, improvement of glucose metabolism and insulin resistance by kinins might also be related to their stimulatory effect on prostaglandin synthesis, mainly on prostacyclin. Moreover, BK has been shown to improve the sensitivity of isolated organs and tissues to insulin.

Thus, our data support the hypothesis regarding the contribution, direct or indirect, of BK to the beneficial metabolic effects of ACE inhibitors in the fructose-fed rat model. Although BK metabolic activity does not seem to be mediated by the NO system when BK is exogenously infused to the fructose-fed rats, one cannot ignore the importance of an active NO system in the metabolic activity of ACE inhibitors. Because the biochemical and physiologic effects of exogenously administered kinins may differ from those of endogenously formed kinins, it is still a matter of debate whether the pharmacologic effect of BK seen in this study fully reflects the physiologic action of endogenous BK.

References