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PRELIMINARY REPORT

Effect of Masoprocol on Glucose Transport and Lipolysis by Isolated Rat Adipocytes

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Masoprocol (nordihydroguaiaretic acid) is a lipoxygenase inhibitor isolated from the creosote bush and used by native healers to treat type 2 diabetes. It has been recently shown to decrease serum glucose, free fatty acid (FFA), and triglyceride (TG) concentrations in rodent models of type 2 diabetes. The current study was initiated to quantify the effects of masoprocol incubation of adipocytes isolated from normal rats. The results indicate that masoprocol significantly increased glucose uptake by adipocytes in both the absence and presence of insulin. In addition, the maximal rate of insulin-stimulated glucose transport was increased in adipocytes incubated with masoprocol and the insulin concentration resulting in a half-maximal glucose transport rate (ED_{50}) decreased. Finally, isoproterenol-stimulated increases in FFA and glycerol release were significantly decreased in the presence of masoprocol. These results provide an explanation at the cellular level for the observation that masoprocol decreases serum glucose, insulin, and FFA concentrations in rodent models of type 2 diabetes.

HISTORICALLY, extracts of the creosote bush (*Larrea tridentata*) have been extensively used by native healers throughout the Southwest region of North America for the treatment of type 2 diabetes.[1] Masoprocol, also known as nordihydroguaiaretic acid, is a lipoxygenase inhibitor[2,3] isolated from the creosote bush by a process of in vivo fractionation[4] that has recently been shown to decrease serum glucose, triglyceride (TG), and free fatty acid (FFA) concentrations in rodent models of type 2 diabetes.[4,5] These results suggested that masoprocol may act both to enhance glucose disposal and to inhibit lipolysis. The current study was initiated in an effort to extend these observations, and to evaluate the possibility that glucose disposal increases and lipolysis decreases when masoprocol is incubated with adipocytes isolated from normal rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats initially weighing 200 g were used for all experiments. They were fed conventional rat chow and water ad libitum and maintained on a 12-hour (6 AM to 6 PM) light-dark cycle. On the day of the experiment, food was removed in the morning and the rats were decapitated, the epididymal fat pads were isolated, and adipocytes were prepared as described previously.[6,7] In brief, fat pads were minced with scissors and placed in plastic flasks in Krebs bicarbonate buffer with 3.5% bovine serum albumin, 3 mmol/L glucose, and 1 mg collagenase/mL. Following collagenase digestion at 37°C in a gyratory water-bath shaker for 1 hour, the cells were washed three times in fresh Krebs buffer with 2% albumin and allowed to separate from the infranatant by flotation, and aliquots were taken for measurement of lipolysis. A 100-μL aliquot of diluted cells was fixed in a solution of 2% osmium tetroxide in collidine buffer and counted in a Coulter Counter (Hialeah, FL) to determine the cell number.

Glucose transport was determined by a method based on the observation that glucose uptake is a measurement of glucose transport when studies are performed at a trace glucose concentration.[8,9] Briefly, isolated adipocytes (20% lipocrit) were preincubated in the presence of 0, 5, 10, and 30 μmol/L masoprocol in 500 μL Krebs buffer containing 2% albumin at 37°C for 90 minutes with continuous shaking at 40 cycles per minute (cpm). The period of preincubation was shown in preliminary studies as being necessary to demonstrate a consistent effect of masoprocol. Following the period of preincubation, cells were incubated with different concentrations of insulin (0 to 800 pmol/L) for 30 minutes, followed by 60 minutes of incubation with trace (300 nmol/L) amounts of D-[U-^{14}C]-glucose. The incubation was terminated by centrifuging a 200-μL aliquot along with 100 μL silicone oil in a 500-μL microfuge tube, and the amount of activity associated with the adipocytes (and total radioactivity in the incubation medium) was determined by liquid-scintillation counting. The concentration of insulin resulting in a half-maximal glucose transport rate (ED_{50}) was determined from linear regression of the transport rate versus the

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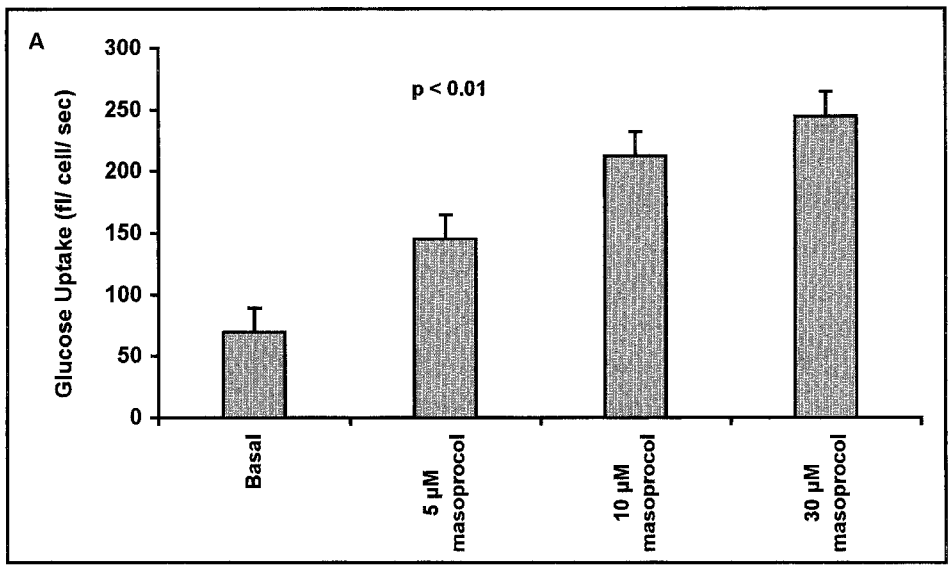

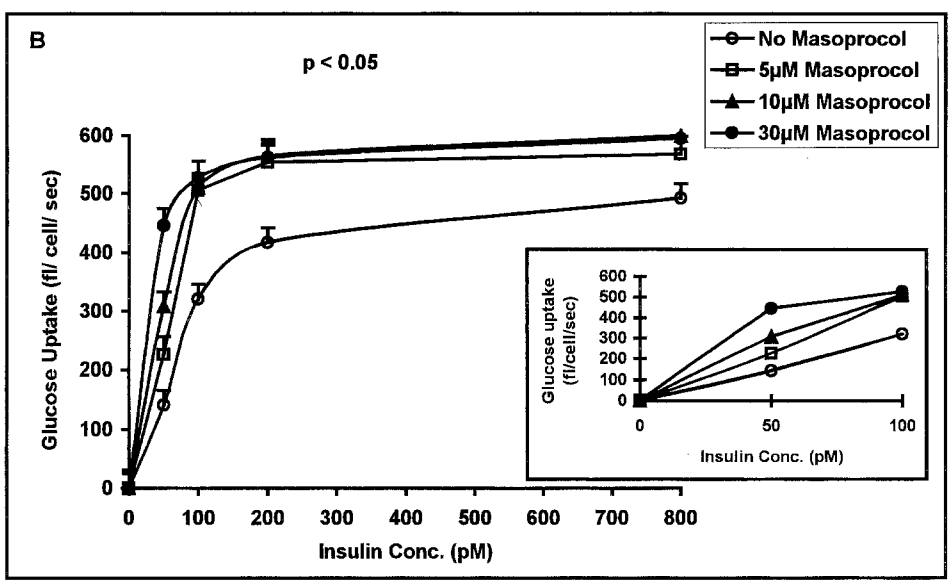

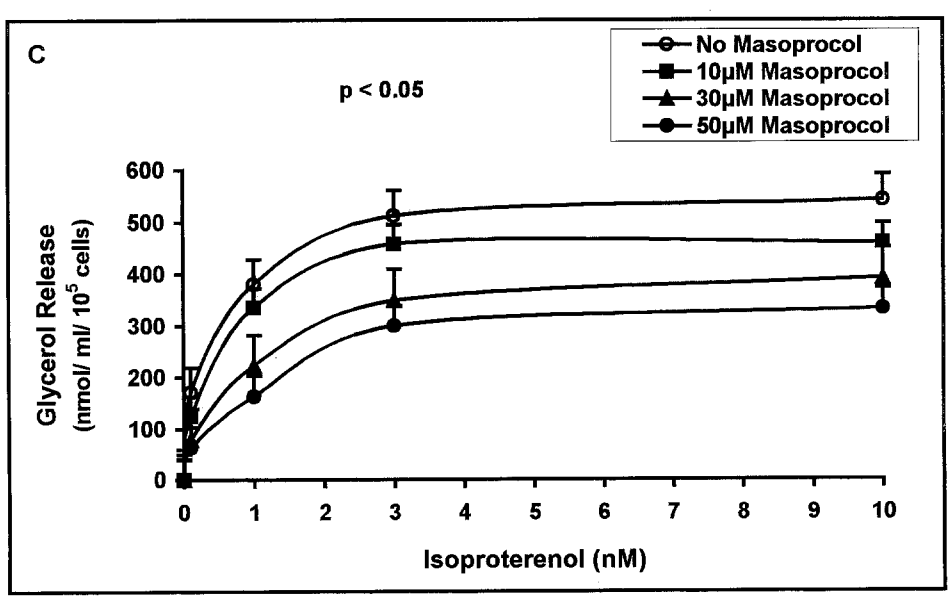

Fig 1. (A) Dose-effect of increasing doses of masoprocol on glucose uptake in the absence of insulin. (B) Effect of 5, 10, and 30 µmol/L masoprocol on insulin-stimulated glucose uptake. (C) Effect of 10, 30, and 50 µmol/L masoprocol on isoproterenol-induced lipolysis. Results are means ± SEM of 6 incubations.

logarithm of the insulin concentration at 50, 100, 200, and 800 pmol/L insulin as previously described.[8]

Lipolysis was determined as previously described by our group,[7] with minor modifications. Briefly, adipocytes were diluted in Krebs buffer with 2% albumin, pH 7.6, and aliquots of diluted cells ($\sim 1 \times 10^5$/mL) were placed in plastic vials and preincubated in the presence of 0, 5, 10, and 30 μmol/L masoprocol in 1 mL Krebs buffer containing 2% albumin at 37°C for 60 minutes with continuous shaking at 40 cpm. The cells were then incubated with different concentrations of isoproterenol for 60 minutes at 37°C. At the end of the incubation, the cells were centrifuged and the infranatant was collected to measure the glycerol concentration by an enzymatic method[10] using a TG kit (Sigma Chemical, St Louis, MO).

Results are expressed as the mean ± SEM of six experiments, and the significance of differences between adipocytes treated with and without masoprocol was estimated by ANOVA.

RESULTS

The ability of masoprocol to stimulate glucose uptake by isolated adipocytes in the absence of insulin is shown in Fig 1A. Glucose uptake increased significantly in response to masoprocol in a dose-related fashion, an approximately threefold increase following incubation with 30 μmol/L masoprocol (244 ± 24 *v* 69 ± 8 fL/cell/s, $P < .001$).

The effect of increasing the amount of masoprocol on glucose uptake by adipocytes in the presence of different doses of insulin is illustrated in Fig 1B. These results indicate a significant ($P < .05$) dose-related increase in glucose uptake above basal by adipocytes incubated with masoprocol at every concentration of insulin versus adipocytes incubated with insulin alone. The insert in Fig 1B more clearly demonstrates the effect of an increasing dose of masoprocol on glucose uptake as the insulin concentration was increased from 0 to 100 pmol/L. The maximal rate also was higher ($P < .01$) at every dose of masoprocol (592 ± 36 *v* 459 ± 24 fL/cell/s) in comparison to its absence. Furthermore, the ED_{50} decreased progressively from 82 pmol/L for no masoprocol to 59, 43, and 29 pmol/L, respectively, as masoprocol was increased from 5 to 10 to 30 μmol/L.

The inhibitory effect of an increasing amount of masoprocol on isoproterenol-induced lipolysis is illustrated in Fig 1C. The results show that isoproterenol caused a dose-dependent increase in isoproterenol-stimulated lipolysis by adipocytes, and masoprocol significantly decreased ($P < .05$) the amount of glycerol released by adipocytes at every concentration of isoproterenol in a dose-dependent manner. Furthermore, there was a progressive increase in the ED_{50} for glycerol release from a level of 0.41 nmol/L as the masoprocol concentration was increased to 10 μmol/L (0.52 nmol/L), 30 μmol/L (0.65 nmol/L), and 50 μmol/L (0.95 nmol/L). Parenthetically, the antilipolytic effect of 50 μmol/L masoprocol was approximately equal to the effect of 100 pmol/L insulin. Finally, to verify the conclusion that the decrease in glycerol release was due to decreased lipolysis, we also measured FFA release by the adipocytes. Adipocytes incubated with isoproterenol plus 30 μmol/L masoprocol showed a significant reduction in both glycerol (83 ± 4 *v* 165 ± 14 nmol/mL, $P < .005$) and FFA (0.18 ± 0.03 *v* 0.44 ± 0.05 μEq/mL, $P < .02$) release compared with adipocytes incubated with isoproterenol alone.

DISCUSSION

These results show that glucose uptake increased and isoproterenol-stimulated lipolysis decreased when adipocytes isolated from normal rats were incubated with masoprocol. As such, they provide evidence at the cellular level of relevance to the physiological effects on glucose and lipid metabolism of masoprocol treatment in rodent models of type 2 diabetes.[4,5] If we first focus on glucose metabolism, the results show that the decrease in glycemia in masoprocol-treated rats was associated with enhanced insulin-mediated glucose uptake independent of any increase in insulin secretion. The demonstration that masoprocol increased insulin-stimulated glucose uptake, as well as decreasing the ED_{50} of this process, adds further support to the conclusion that the antihyperglycemic effect of this compound is due at least partly to an increase in insulin action.

The fact that both FFA and TG concentrations decreased in masoprocol-treated rats with type 2 diabetes led to the hypothesis that masoprocol was antilipolytic,[5] and that hepatic TG secretion and serum TG concentrations were lower as a consequence of the resultant decrease in FFA flux to the liver. The current results provide direct support at the cellular level for the view that masoprocol is antilipolytic. In addition, since it has recently been shown in normal subjects that inhibition of lipolysis enhances whole-body glucose disposal,[11] it is possible that the antilipolytic effect of masoprocol contributes to its ability to decrease plasma glucose in rodent models of type 2 diabetes.

In conclusion, masoprocol stimulates glucose uptake and inhibits isoproterenol-induced lipolysis when incubated with isolated rat adipocytes. These findings provide a mechanistic explanation at the cellular level to account for the decrease in serum glucose, TG, and FFA concentrations observed in masoprocol-treated rodent models of type 2 diabetes.[4,5] Although masoprocol is a well-recognized lipoxygenase inhibitor,[2,3] we are not aware of any previously published data showing that masoprocol would be of clinical utility in the treatment of type 2 diabetes. Furthermore, it is not clear if similar results on adipocyte glucose uptake and antilipolysis would be produced by other lipoxygenase inhibitors. The fact that incubation of 3T3-L1 cells with masoprocol neither increased the expression of GLUT4 mRNA nor affected the inhibition of GLUT4 mRNA by arachidonic acid[12] suggests that the insulin-like effects of masoprocol are unrelated to its role as a lipoxygenase inhibitor.

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