AMYLIN is a 37-amino acid peptide that is cosecreted with insulin from pancreatic β-cells (3, 13). It inhibits insulin-mediated glucose uptake and disposal into glycogen in skeletal muscle and raises the blood lactate level (2, 6, 18, 22, 24, 28, 29). The mechanism of this decrease in glucose uptake from the blood into muscle is not completely understood (4, 6, 15, 22, 23, 29, 30) but may occur secondarily to an inhibition of glycogen synthesis and stimulation of glycogenolysis, resulting in an increase in intracellular glucose 6-phosphate (G-6-P) concentration (2, 16, 22, 29). An increase in intracellular G-6-P has been postulated to decrease net glucose uptake via feedback inhibition of hexokinase (2, 22, 27).

A glucose analog commonly used to study in vitro glucose metabolism, 3-O-methyl-d-glucose (3-O-MG), is transported across the sarcolemma but not phosphorylated by hexokinase. Therefore, accumulation of this glucose analog under a short duration and a large concentration gradient approximates unidirectional glucose transport across the sarcolemma. Zierath et al. (30) demonstrated an amylin-mediated inhibition of insulin-stimulated 3-O-MG transport in human muscle strips without changes in glycogen and lactate concentration. However, several other investigators have not been able to confirm these observations in isolated muscle preparations (23, 29). Decreased 3-O-MG transport without changes in glycogen or lactate concentrations would suggest that amylin inhibits glucose transport without the involvement of glycolytic intermediates (30). This is an important observation, because the intermediate metabolites G-6-P and products of the glucosamine pathway [uridine diphosphate (UDP)-N-acetylhexosamines] have been implicated in inhibition of insulin-stimulated glucose transport (5, 8, 26). The present study was undertaken to further examine whether amylin inhibits skeletal muscle 3-O-MG transport and whether this inhibition is associated with metabolic intermediates. To this end, we used the nonrecirculating rat hindlimb perfusion model to investigate the effect of amylin on insulin-stimulated 3-O-MG transport. We found that a pharmacological dose of amylin inhibited insulin-stimulated 3-O-MG transport and that inhibition was associated with an increase in intracellular G-6-P but not UDP-N-acetylhexosamines.

METHODS

Animal care. Eighteen Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) 3–4 wk of age were purchased and housed three to a cage and allowed access to food and water ad libitum. The temperature of the animal room was maintained at 23°C with a 12:12-h light-dark cycle. All procedures used in this study were approved by the University of Texas Animal Care and Use Committee.

Perfusion and surgical procedure. Rats weighing 174.5 ± 1.74 g were fasted for 4–6 h, which resulted in muscle and red gastrocnemius 31.3 ± 1.6 µmol/g, respectively. 3-O-MG was weight-matched into control and amylin groups, and each pair was perfused consecutively to minimize the effects of fasting duration on glycogen concentration and glucose transport rates. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg); hindlimbs were surgically isolated, and catheters were placed in the descending aorta and vena cava as previously described (14). Immediately before catheterization, the soleus (slow-twitch oxida-

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Volume of 1 mol/l HCl was then added to the sample and finally incubated for an additional 10 min at 65°C. An equal KOH by incubating it for 20 min at 65°C, then mixed, and muscle or muscle section was first dissolved in 1 ml of 1 mol/l sectioned and weighed frozen. A 60- to 100-mg piece of each

continuously gassed (mixture 95% O2-5% CO2), and its temperature were constantly monitored to ensure uniformity among experimental groups. All perfusates consisted of KHB, pH 7.4, 4% diazyl fatty acid-free bovine serum albumin (ICN 105033), 30% (vol/vol) blood bank time-expired human

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80% (229.2 ± 38.0 vs. 45.3 ± 3.8 nmol G-6-P·g⁻¹·min⁻¹), and the sensitivity declined 44% (5.91 ± 0.32 vs. 3.33 ± 0.35 mmol G-6-P/l) compared with the control. Both the changes in sensitivity and responsiveness were highly significant (P < 0.01).

Muscle lactate increased in response to amylin when all muscles were analyzed collectively (P < 0.001, Fig. 3). Compared with control, amylin increased lactate by 76% in FO muscle and 82% in FG muscle, and this effect did not differ between fiber types.

Amylin significantly increased G-6-P concentration by 547% in FO and 319% in FG muscle (P < 0.001, Fig. 4). No significant difference between FO and FG muscle occurred. Although G-6-P increased three to five times control levels with amylin treatment, no difference was detected in the UDP-N-acetylhexosamines between control and amylin-treated muscles (Fig. 5). The concentration of UDP-hexoses, however, decreased by 36% in FO and 41% in FG muscle with amylin treatment.

DISCUSSION

In the present study we demonstrated that a pharmacological concentration of amylin induced inhibition of insulin-stimulated 3-O-MG transport, stimulation of glycogenolysis, and an increase in the glycolytic inter-

mediates G-6-P and lactate with no change in UDP-N-acetylhexosamines. The relationship between glycogenolysis and the elevation in G-6-P and lactate after amylin exposure has been demonstrated before, both in vivo and in vitro under conditions in which extracellular glucose was provided (2, 4, 16, 22, 28, 29). However, in this study we evaluated the effects of amylin on
Intracellular G-6-P concentration during conditions in which no glucose substrate was present.

Our finding that amylin reduced the rate of insulin-stimulated glucose transport in skeletal muscle is in agreement with the findings of Zierath et al. (30). However, our results extend these previous findings by demonstrating that the effects of amylin are fiber type independent. Furthermore, we observed that the decrease in insulin-stimulated 3-O-MG transport on amylin exposure was coupled with increases in glycogenolysis and lactate production. This finding is in contrast to the findings of Zierath et al. (30), in which neither glycogenolysis nor lactate production was observed. This is an important distinction between studies, because the results of Zierath et al. suggest that amylin must inhibit transport independently of metabolically driven inhibition, although these investigators did not measure intracellular G-6-P levels. One possible explanation for the differences in results between the two studies is the difference in muscle preparations used. Zierath et al. used an isolated human muscle preparation, whereas we used the rat hindlimb perfusion technique. Thus it is possible that the effect of amylin on human muscle differs from that on rat muscle, or that the human isolated muscle preparation lacks the sensitivity to detect small changes in muscle glycogen and lactate concentrations. However, regardless of the mechanism, both studies clearly demonstrated that amylin inhibits skeletal muscle 3-O-MG transport.

An inhibition of muscle glucose transport by amylin has not always been observed (23, 29). The reason for these disparate findings may involve different experimental models and duration of preexposure to amylin before measurements of glucose transport. In demonstrating the inhibition of insulin-stimulated glucose transport by amylin, Zierath et al. (30) exposed muscle to amylin for 45 min before transport was measured. In the present study, glucose transport was determined after a prior amylin exposure of 20 min. In contrast, Pittner et al. (23) could not detect any effect of amylin on glucose transport. They measured the efflux of 3-O-MG from preloaded isolated rat soleus muscles immediately after exposure to amylin. Also, Young et al. (29), using the isolated soleus preparation, did not observe a decrease in glucose transport after amylin exposure. However, it was unclear as to how long their muscles were exposed to amylin before measurement of glucose transport. Thus, it may take an extended period of time for amylin to induce 3-O-MG transport inhibition, and this temporal response may be subject to the experimental model and conditions being used. For example, the temporal response to amylin could be faster when amylin is administered through hyperperfused capillaries, as occurs during hindlimb perfusion, as opposed to administration during an isolated muscle incubation.

Although extended exposure to amylin appears to result in 3-O-MG transport inhibition, Pittner et al. (23) clearly demonstrated that acute 2-deoxyglucose uptake inhibition can occur in the absence of 3-O-MG transport inhibition. They concluded that hexokinase inhibition occurs after amylin exposure and subsequent to a rise in G-6-P. The large rise in G-6-P seen in our study supports their findings in that hexokinase inhibition would likely occur under these conditions. Also, Young et al. (29) demonstrated that intramuscular G-6-P rises after amylin exposure, whereas glucose uptake and disposal into glycogen are reduced. Their findings led them to conclude that amylin inhibits glucose uptake through feedback inhibition of hexokinase (29).

It needs to be considered that transport and uptake mechanisms are not mutually exclusive but complementary. Acute inhibition of hexokinase would protect the cell from excess glycolytic flux, and inhibition of transport could be advantageous in protecting intracellular compartments from accumulating high glucose concent-

Fig. 4. G-6-P concentrations in FO and FG muscle. Amylin increases G-6-P concentration (P < 0.001). *Significant fiber type-specific increases in G-6-P (P < 0.05).

Fig. 5. Concentrations of UDP-N-acetylhexosamines (UDP-N-ACETYLHEX) and UDP-hexoses (UDP-HEX) in FO and FG muscle. Amylin decreases UDP-hexose concentration (P < 0.001). *Significant fiber type-specific decreases in UDP-hexoses (P < 0.05).
chronic hyperglycemia and glucosamine infusion have on the glucosamine pathway, and increases in these metabolites during an indicator of increased flux through the glucosamine pathway and lead to a hormone-induced glucose toxicity similar to what has been observed during hyperglycemia (26).

Amylin caused a sharp rise in intracellular G-6-P that corresponded to an increase in glycogenolysis. The rise in G-6-P was disproportional to the increase in muscle lactate, suggesting that glycolysis may be partially inhibited under these conditions. This is in agreement with earlier findings from our laboratory, which investigated this phenomenon in more depth by use of a selective antagonist (2). In addition, amylin caused a substantial inhibition of glycogen synthase activity by reducing both its sensitivity and responsiveness to G-6-P. These changes in glycogen synthase are likely due to phosphorylation and not changes in enzyme concentration (16, 25). Evaluation of the dose-response curve for glycogen synthase indicates that it would normally prevent large fluxes in intracellular G-6-P. This observation supports the theory of Shulman and Rothman (27), who proposed that glycogen synthase activity controls G-6-P concentration rather than G-6-P controlling the rate of glycogen synthase. Thus our observations suggest that amylin may control the concentration of G-6-P indirectly through an effect on glycogen synthase rather than just a mass action effect from increased glycogenolysis. This coordination of glycogen synthase inhibition and glycogenolysis activation in the presence of amylin is important, because it prevents the development of a futile cycle between glycogenolysis and glycogen synthesis.

It has been proposed that increases in the glucosamine biosynthetic pathway may lead to inhibition of glucose transport (7, 26). Fructose 6-phosphate is the substrate for the rate-limiting enzyme glutamine fructose-6-phosphate aminotransferase of this pathway. Fructose 6-phosphate in turn is formed from G-6-P by an equilibrium reaction. Because we have produced a 433% increase in G-6-P by treating the muscles with amylin, an increase in hexosamine metabolites should occur if this pathway is involved in the amylin-induced inhibition of glucose transport. The increase in concentration of UDP-N-acetylhexosamines has been used as an indicator of increased flux through the glucosamine pathway, and increases in these metabolites during chronic hyperglycemia and glucosamine infusion have been correlated to decreased rates of glucose uptake (26).

We could not detect any change in UDP-N-acetylhexosamines. This suggests that the glucose transport inhibition demonstrated in this study is not due to a buildup of glucosamine pathway metabolites. Several studies have shown that glucose toxicity characterized by increases in UDP-N-acetylhexosamines takes several hours to develop in glucose clamp studies and that G-6-P is actually lower than control levels during inhibition of glucose transport (9, 10), whereas, in our study, the G-6-P concentration was greatly elevated. Interestingly, the UDP-hexoses decreased with amylin treatment. UDP-hexoses have been shown to decrease when UDP-N-acetylhexosamines increase (26). This would suggest that a decrease in UDP-hexoses, or an increase in the ratio of UDP-N-acetylhexosamines to UDP-hexoses, could be involved in amylin-mediated inhibition of glucose transport. Thus amylin-induced glucose transport inhibition appears to work differently from chronic hyperglycemia. In addition, the decrease in UDP-hexoses without increases in UDP-N-acetylhexosamines might suggest that the latter is not directly responsible for glucose transport inhibition when this pathway is activated. Clearly, more studies are needed to determine the role of the glucosamine pathway in both chronic and acute transport inhibition.

In conclusion, three important aspects of amylin action have been revealed in this study. First, amylin can inhibit insulin-stimulated glucose transport, and thus glucose uptake, without the involvement of hexokinase. This brings into question whether decreases in glucose uptake, which occur concurrently with sustained increases in G-6-P, are necessarily the result of hexokinase inhibition. Second, an increase in the proposed end point of the glucosamine pathway is not involved in glucose transport inhibition, although it is possible that other aspects of this pathway may be involved. Third, amylin can elevate intracellular G-6-P in the absence of extracellular glucose. This appears to be due to activation of glycogenolysis accompanied by restrictions in glycolysis and glycogen synthesis. Further research will be required to determine the exact mechanism by which amylin inhibits insulin-stimulated glucose transport in skeletal muscle.

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