Levodopa with carbidopa diminishes glycogen concentration, glycogen synthase activity, and insulin-stimulated glucose transport in rat skeletal muscle

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Levodopa is the most commonly prescribed and most effective medication for controlling the symptoms of PD (4).

Several investigators have reported high rates of glucose intolerance among patients with PD (2, 3, 27). For example, in two separate studies of 30 and 57 patients with PD, respectively, ~50% of the patients displayed abnormal oral glucose tolerance (2, 27). Similarly, abnormal intravenous glucose tolerance was found in four of eight patients with PD (3). Notably, hyperglycemic effects of levodopa and dopamine have been documented in humans and laboratory animals (3, 17, 18, 33). The decarboxylase inhibitor carbidopa is given with levodopa to prevent the conversion of levodopa to dopamine in peripheral tissues, because dopamine does not cross the blood-brain barrier (4). However, carbidopa does not prevent accumulation of dopamine in skeletal muscle in animals treated with levodopa (9, 30).

Levodopa transport is stimulated by insulin in skeletal muscle through activation of the insulin receptor tyrosine kinase and phosphorylation of members of the insulin receptor substrate (IRS) family (reviewed in Ref. 35). Tyrosine-phosphorylated IRS proteins provide scaffolding sites for activation of signaling cascades, leading to increased glucose transport. One signaling pathway essential to insulin-stimulated glucose transport is dependent on docking of the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) on tyrosine-phosphorylated IRS, which activates the PI3K p110 catalytic subunit. Downstream effectors of PI3K include Akt, which is thought to participate in the stimulation of glucose transport by insulin (1) and promotes glycogen synthesis through inactivation of glycogen synthase (GS) kinase-3β (8).

Skeletal muscle, the predominant insulin-responsive tissue, responds to dopamine by elevating endogenous cAMP, an effect that is blocked by propranolol, a β-adrenergic antagonist (31). β-Adrenergic action and cAMP have been implicated as negative regulators of insulin action (20, 23, 26, 38, 43). GS, the rate-limiting enzyme for the storage of intracellular glucose as glycogen in skeletal muscle (25), is activated in the presence of insulin and inhibited by agents that increase intracellular cAMP concentrations ([cAMP]) (7, 25, 38). GS is unresponsive to insulin under various conditions of insulin resistance,
including diabetes (14, 19, 21). Under conditions in which GS activity is low and/or insulin does not increase GS activity, nonoxidative glucose disposal and glycogen accumulation in skeletal muscle are severely limited (14, 21, 38), and it has been suggested that impaired glycogen metabolism is a primary defect in diabetes (32). Thus GS activity and glycogen accumulation are key markers of insulin action in skeletal muscle and are susceptible to β-adrenergically mediated negative regulation.

We hypothesized that levodopa-carbidopa would inhibit insulin effects in skeletal muscle and that some of the levodopa-carbidopa effects would be dependent on β-adrenergic action. We used an in vitro skeletal muscle preparation to assess the role of levodopa-carbidopa in glycogen metabolism and glucose transport and examine possible mechanisms for levodopa-mediated insulin resistance. We also evaluated the acute effects of a single dose of levodopa-carbidopa on plasma glucose levels, muscle [cAMP], IRS-1 activation, and GS activity. To determine chronic in vivo effects of levodopa-carbidopa, additional rats were treated with levodopa-carbidopa for 4 wk, after which an oral glucose tolerance test (OGTT) and assays for muscle glycogen content and GS activity were performed.

METHODS AND MATERIALS

Materials. Purified porcine insulin was from Eli Lilly (Indianapolis, IN). Levodopa and carbidopa were from Sigma Chemical (St. Louis, MO). 3H-labeled 2-deoxyglucose (2-DG) and 14C-labeled mannitol were from American Radiolabeled Chemicals (St. Louis, MO). Enzymes and other chemicals were purchased from Roche Diagnostics (Indianapolis, IN) and Sigma Chemical.

Animals. Male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing ~130 g were used for the study. The temperature of the animal room was maintained at 21°C, and a 12:12-h light/dark cycle was set. Rats were allowed free access to food and water. The Saint Louis University Animal Care Committee approved all procedures.

Muscle incubations. The acute effects of 30 μM levodopa with 100 ng/ml carbidopa on insulin-stimulated glycogen accumulation were examined in isolated epitrochlearis muscles. Levodopa and carbidopa concentrations were chosen to approximate one to two times the plasma concentrations of the drugs found in humans who have taken a single dose of levodopa-carbidopa (200 mg-20 mg or 200 mg-50 mg) (5, 37). Rats were anesthetized after an overnight fast via an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), and epitrochlearis muscles were removed. Each epitrochlearis was incubated (14, 26) with shaking in 25-mm-diameter vials containing 2 ml of incubation medium with a gas phase of 95% O2-5% CO2 for 3 h at 35°C in one of five groups. The basal medium contained oxygenated Krebs-Henseleit buffer (KHB), 28 mM mannitol, 0.1% BSA, and 12 mM glucose. This glucose concentration is in the range of blood glucose levels for rats undergoing oral glucose tolerance tests (15), and plasma glucose concentrations in fed Wistar rats (9±0.5 mM, mean±SE, n=8; 13) have been reported to approach this value. Muscles in the insulin group were incubated in the basal medium containing 60 μU/ml insulin, a concentration of insulin that produces half-maximal insulin-stimulated glucose transport (14) and is consistent with plasma insulin concentrations of fed Wistar rats (13). The insulin-levodopa-carbidopa medium contained 60 μU/ml insulin, 30 μM levodopa, and 10 mg/ml carbidopa. The levodopa-carbidopa medium contained 30 μM levodopa and 100 mg/ml carbidopa. The insulin-levodopa-carbidopa-propanolol medium contained 60 μU/ml insulin, 30 μM levodopa, 100 mg/ml carbidopa, and 10 μM propranolol. Vials were wrapped in aluminum foil before and during muscle incubations to prevent breakdown of levodopa, a light-sensitive compound. After the incubations, muscles were quickly blotted and trimmed at 4°C, clamp-frozen with tongs cooled in liquid nitrogen, and stored at −80°C before analysis of glycogen accumulation and GS activity as described below.

Muscle glucose transport determination. Rats were anesthetized after an overnight fast as described above, and the epitrochlearis, soleus, and flexor digitorum brevis muscles were dissected out. Soleus muscles were split longitudinally into thin strips to enhance diffusion of oxygen and nutrients into the tissue. Muscles were incubated as described above for 1 h at 35°C in a basal medium containing oxygenating KHB, 32 mM mannitol, 0.1% BSA, and 8 mM glucose. Muscles were then preincubated for 30 min at 35°C in fresh medium containing 0.1% BSA, 8 mM glucose, and 32 mM mannitol with no insulin, 60 μU/ml insulin, or 60 μU/ml insulin with 30 μM levodopa and 100 ng/ml carbidopa. Muscles were then washed for 10 min at 30°C in glucose-free medium containing 0.1% BSA, 40 mM mannitol, and levodopa-carbidopa and/or insulin if they had been present in the previous step. Next, muscles were incubated for 20 min in medium containing 0.1% BSA, 8 mM 2-DG, 2 μCi/ml 3H-labeled 2-DG, 32 mM mannitol, 0.3 μCi/ml 14C-mannitol, and insulin or levodopa-carbidopa if they had been present in previous incubations. To assess the potential involvement of β-adrenergic signaling in levodopa-carbidopa actions, 10 μM propranolol was included in incubation media for some epitrochlearis muscles that were incubated with levodopa-carbidopa. Muscles were blotted on filter paper, clamp-frozen as described above, and stored at −80°C. Frozen muscle samples were weighed and homogenized in Kontes ground-glass tubes in 1 ml ice-cold 0.3 M perchloric acid, and the homogenate was centrifuged (14,000 g for 10 min at 4°C). For determination of glucose transport, 200 μl of the supernatant was added to 1 ml of scintillation solution (Packard Biosciences, Meriden, CT). Samples were counted for 3H and 14C in an LS-6000 liquid scintillation spectrophotometer (Beckman, Fullerton, CA). 14C-labeled mannitol was used to correct for extracellular space.

Acute in vivo effects of levodopa-carbidopa. For in vivo levodopa-carbidopa treatments, levodopa and carbidopa were dissolved in NaCl at a 10:1 ratio of levodopa to carbidopa to mimic a therapeutic dose. The doses of levodopa and carbidopa used in this study are equivalent to 116 mg in a 70-kg human, a common therapeutic dose of levodopa in a patient with early PD. Carbidopa was in complex with 2-hydroxypropyl-β-cyclodextrin (HBC), which renders the carbidopa water soluble. Vehicle solutions for control animals contained HBC at the same concentration as in the drug solution. After an overnight fast, animals were treated intragastrically with a single dose of either levodopa-carbidopa (0.165 mg levodopa and 0.0165 mg carbidopa per 100 g body wt) or vehicle. Rats were anesthetized 15 min later as described above before they were fed 1 ml/100 g body wt of either 20% glucose in 0.9% NaCl or 0.9% NaCl without glucose. Fifteen minutes after the glucose or NaCl feeding, the epitrochlearis and triceps muscles were excised, clamp-frozen with tongs cooled in liquid nitrogen, and stored at −80°C along with plasma samples from each animal. Plasma glucose and insulin concentrations, muscle cAMP levels, muscle GS activity, and IRS-1 activation were later assayed.

Chronic in vivo levodopa-carbidopa administration and OGTT. Animals were randomly assigned to either a control group or a levodopa-carbidopa treatment group (n=6/group). The two groups were treated intragastrically twice a day (8:00 AM and 5:00 PM), 7 days/wk, for 4 wk with levodopa-carbidopa (as described above for the single drug treatment) or vehicle. Subsequent to 4 wk of drug treatment, rats were fasted overnight before administration of an OGTT as previously described (15). Blood samples were collected from small tail vein incisions using heparinized capillary tubes that were then spun for separation of plasma. Immediately after collection of the first plasma samples (designated −30 min), rats were given a single intragastric dose of levodopa-carbidopa or vehicle (as described above) and allowed free access to food and tap water for 1 h prior to determination of plasma glucose and insulin concentrations. Muscles were excised, clamp-frozen as described above, and analyzed for glycogen content and GS activity as described above.
Thirty minutes later (designated time 0), after collection of another set of plasma samples, rats were fed intragastrically with 1 ml/100 g body wt of 20% glucose in 0.9% NaCl. Additional plasma samples were taken 15, 30, 60, 90, and 120 min after the glucose feeding. Plasma samples were stored at −80°C until analysis of glucose and insulin concentrations.

The twice-daily treatments with levodopa-carbidopa resumed the morning after OGTTS. Three days after the OGT, rats were again fasted overnight and then anesthetized as described above before collection of epitrochlearis, plantaris, extensor digitorum longus, and anterior tibialis muscles. Muscles were clamp-frozen with tongs cooled in liquid nitrogen and stored at −80°C until analysis of glycogen concentration and GS activity.

**Results**

**Effects of levodopa-carbidopa on insulin action in isolated skeletal muscle.** A physiological concentration (60 µU/ml) of insulin increased glycogen accumulation by ~40% above baseline in epitrochlearis muscle (P < 0.05; Fig. 1). Levodopa-carbidopa prevented insulin-stimulated glycogen accumulation, and this inhibition was prevented by propranolol, a β-adrenergic antagonist. Insulin stimulated an increase in GS activity above the basal level in the epitrochlearis by ~30% (P < 0.05; Fig. 2A), and levodopa-carbidopa prevented the insulin-stimulated increase in GS activity (P < 0.05). The decrease in insulin-stimulated GS activity in the presence of levodopa-carbidopa was partially prevented by propranolol. Levodopa-carbidopa treatment tended to increase phosphorylase activity (Fig. 2B), although this effect was not statistically significant for the small sample size. Levodopa-carbidopa prevented the insulin-stimulated increase in glucose transport (P < 0.05; Fig. 3, A–C), and this effect was blocked by propranolol in the epitrochlearis (P < 0.05; Fig. 3C). As shown in Fig. 4, A and B, levodopa-carbidopa had no effect on insulin-stimulated phosphorylation of Akt on Thr308 or Ser473. However, levodopa-carbidopa prevented insulin-stimulated IRS-1 tyrosine phosphorylation (Fig. 4C). It appears that inhibition of GS activity by levodopa-carbidopa occurs independent of changes in Akt activity, which is consistent with muscles were averaged to yield an n = 1 for each animal, except for anterior tibialis, for which values for right and left muscles were not averaged together.
possible protein kinase A-mediated downregulation of GS activity.

**Acute in vivo effects of levodopa-carbidopa.** At the early time point assessed (15 min after glucose feeding), levodopa-carbidopa treatment did not cause a significant increase in plasma glucose concentrations compared with plasma glucose levels in animals given vehicle alone (Fig. 5A). However, the single dose of levodopa-carbidopa caused a significant increase in [cAMP] in triceps muscle compared with muscles from rats treated with vehicle (P < 0.05; Fig. 5B). Levodopa-carbidopa prevented an increase in epitrochlearis GS activity in rats that were fed glucose (P < 0.01; Fig. 5C). Figure 5D shows that levodopa-carbidopa caused a significant decrease in phosphorylation of IRS-1 on Tyr632 (P < 0.05) in epitrochlearis.

**Effects of chronic levodopa-carbidopa treatment.** Four weeks of treatment with levodopa-carbidopa resulted in a slightly lower body weight (P < 0.05) in the treated group compared with the control group. Body weights were similar before treatment began (control 145 ± 9 g, drug 136 ± 15 g), although control animals gained more weight than animals treated with levodopa-carbidopa (2 wk: control 260 ± 8 g, drug 238 ± 14 g; 4 wk: control 352 ± 14 g, drug 324 ± 10 g). Food intakes were not monitored, so it is possible that drug-treated animals ate less than controls. In an OGTT after the 4 wk of levodopa-carbidopa treatments, blood glucose concentrations were higher (P < 0.05) in rats treated with levodopa-carbidopa compared with controls at 60, 90, and 120 min after ingestion of glucose (Fig. 6A). There were no significant differences in plasma insulin concentrations in rats treated with levodopa-carbidopa compared with the control group except at 120 min, when insulin levels were higher in drug-treated rats than in controls (Fig 6B). In the animals treated chronically with levodopa-carbidopa, there was at least 15% less muscle glycogen content compared with controls (P < 0.05; Table 1).
Fig. 4. Effects of levodopa-carbidopa treatment on insulin-stimulated phosphorylation of Akt and insulin receptor substrate (IRS)-1. Isolated epitrochlearis muscles were incubated without insulin (control) or with 60 μU/ml insulin in the absence or presence of 30 μM levodopa + 100 ng/ml carbidopa (L/C). Muscles samples were subjected to SDS-PAGE and immunoblotted using antibodies specific for phosphorylated Akt and IRS-1. Panels contain representative photographs and quantitation for Akt phosphorylated on Ser 473 (A), Akt phosphorylated on Thr 308 (B), and IRS-1 phosphorylated on Tyr 632 (C). Bars represent means ± SE. *Significantly higher than control, P < 0.05; n = 8 muscles/group.

Fig. 5. Acute in vivo effects of levodopa-carbidopa. Rats were given a single intragastric dose of levodopa-carbidopa or vehicle (NaCl). Fifteen minutes later, rats received glucose in NaCl or NaCl alone as described in METHODS AND MATERIALS. After an addition 15 min, muscle and plasma samples were taken. A, plasma glucose concentrations; B, cAMP content of triceps (Tri) muscle; C, GS activity in Epi muscle; and D, representative photograph and quantitation for Epi muscle IRS-1 tyrosine phosphorylation. Bars represent means ± SE. *Significantly higher than control, P < 0.05; n = 4 rats/group.
Furthermore, GS activity in muscles taken from rats after 4 wk of treatment with levodopa-carbidopa was ~50% lower compared with GS activity in muscles taken from control animals (P < 0.05; Table 1).

**DISCUSSION**

The new information provided by this study is that levodopa-carbidopa decreases insulin-stimulated glucose transport, glycogen accumulation, and GS activity in skeletal muscle. These effects of levodopa could be blocked with propranolol, a β-adrenergic antagonist. A single in vivo dose of levodopa-carbidopa increased muscle cAMP concentrations, decreased GS activity, and reduced tyrosine phosphorylation of IRS-1. Chronic levodopa-carbidopa treatment decreased oral glucose tolerance and led to lower muscle glycogen concentration and GS activity. These findings are consistent with previous reports of hyperglycemic effects of levodopa (3, 17, 18, 33). The levodopa-related peripheral insulin resistance we found (i.e., inhibition of insulin-stimulated glucose transport into and storage by muscle) could contribute to hyperglycemia in animals treated with levodopa. It appears that levodopa impinges on muscle glucose metabolism despite the presence of carbidopa, a decarboxylase inhibitor.

Levodopa and its metabolite dopamine have been shown to cause hyperglycemia in humans in a number of studies (17, 18). In one study, a 1.0-g dose of levodopa given orally to seven patients with PD caused an increase in fasting plasma glucose level from 87 to 99 mg/dl within 30 min (3). Four and five hours after a 100-g glucose load, plasma glucose concentrations were still elevated (133 and 122 mg/dl) in subjects who had ingested levodopa before consuming glucose compared with plasma glucose concentrations (83 and 78 mg/dl) in subjects for whom the oral glucose load was administered without levodopa (3). In a separate study, 1.0 g of levodopa caused hyperglycemia in patients with PD who had been treated for 3 mo with levodopa (33). Furthermore, 12 mo of chronic levodopa treatment reduced oral glucose tolerance in these patients, such that mean peak plasma glucose concentrations during oral glucose tolerance tests increased from ~165 to ~190 mg/dl (33). The year of chronic levodopa treatment was associated with a threefold increase in peak circulating insulin concentration and a twofold increase in insulin area under the curve during an OGTT (33). Thus in the absence of a decarboxylase inhibitor, acute and chronic levodopa treatment both appear to cause hyperglycemia (3, 17, 18, 33). The current study indicates that even in the presence of a decarboxylase inhibitor, levodopa can inhibit insulin action and impair glycogen metabolism in skeletal muscle. However, similar studies need to be done in humans.

Dopamine, a metabolite of levodopa, functions through β-adrenergic receptors in skeletal muscle (31), although it is unclear whether dopamine at the concentration (~1 ng/g) at which it is found in skeletal muscle in the absence of exogenous levodopa treatment (11) has a true physiological function. Muscle cells respond to 0.1 μM dopamine with increases in cAMP concentrations, effects that are blocked by the β-adrenergic antagonist propranolol, but not by haloperidol, a dopamine receptor antagonist, or phentolamine, an α-adrenergic receptor antagonist (31). Carbidopa prevents the conversion of levodopa to its metabolite dopamine (6, 39). However, despite the action of carbidopa to decrease plasma dopamine concentration in levodopa-treated animals, carbidopa does not prevent accumulation of dopamine in skeletal muscle (9, 30). Thus it seems possible that levodopa-carbidopa would cause an increase in [cAMP] in muscle secondary to β-adrenergic action stimulated by a levodopa metabolite.

Table 1. *Chronic levodopa/carbidopa treatment reduces muscle glycogen levels and glycogen synthase activity*

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Glycogen, μmol Glucosyl units/g</th>
<th>GS Activity, Fraction in I Form</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Levodopa/carbidopa</td>
</tr>
<tr>
<td>Epithrochlearis</td>
<td>16.8±2.7</td>
<td>8.6±1.0*</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>17.2±0.7</td>
<td>14.8±0.5*</td>
</tr>
<tr>
<td>Plantaris</td>
<td>19.2±0.9</td>
<td>16.4±0.7*</td>
</tr>
<tr>
<td>EDL</td>
<td>19.1±1.0</td>
<td>15.9±1.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals were treated intragastrically twice daily for 4 wk with levodopa-carbidopa or vehicle. GS, glycogen synthase; EDL, extensor digitorum longus. *Different from control, P < 0.05 (n = 6 rats/ group).
When intracellular [cAMP] increases, protein kinase A activity increases, indirectly or directly increasing phosphorylation of GS and phosphorylase kinase. These actions result in decreased glycogen synthesis and activation of glycogen breakdown, respectively (7, 16). Hormone-regulated phosphatases such as PP1G have also been implicated in control of GS activity (10, 28). PP1G is a muscle-specific glycogen-associated protein phosphatase that is regulated by phosphorylation of its targeting subunit, RGL (10, 28). There is some evidence from a knockout mouse model that PP1G/RGL is not required for insulin activation of GS in skeletal muscle and that another GS-specific phosphatase might substitute for PP1G in the transgenic animals (36). It seems likely that multiple phosphatases, including PP1G/RGL, contribute to hormonal regulation of glycogen metabolism and could play roles in the levodopa effects on muscle.

Deficient activation of GS by insulin is a characteristic of diabetes and other models of impaired insulin action (14, 19, 21). Low GS activity can prevent glycogen accumulation in skeletal muscle (14), and defective insulin-stimulated glycogen synthesis in diabetics may play a critical role in insulin resistance (32). Diminished GS activity could cause an intracellular buildup of glucose-6-phosphate (G6P) (24) that could indirectly prevent glucose transport. For example, in skeletal muscle treated with epinephrine, increased intracellular accumulation of G6P appears to mediate decreased insulin-stimulated glucose transport into muscle (26).

Insulin-stimulated activation of Akt promotes glycogen synthesis by phosphorylating GS kinase 3 (GSK3), converting it from its active to inactive form (41). As a result, GSK3-mediated inhibition of GS is relieved and glycogen synthesis is promoted. In our hands, there was no effect of levodopa-carbidopa on insulin-stimulated Akt phosphorylation. Thus it appears that the effects of levodopa with carbidopa on GS activity and glucose transport are mediated by an Akt-independent pathway. In this respect, levodopa-carbidopa effects resemble those of caffeine, which increases [cAMP] in skeletal muscle and inhibits insulin-stimulated glucose transport and GS activity without alteration of Akt activation (38). In the current study, levodopa caused a significant decrease in phosphorylation of IRS-1 on Tyr632. Phosphorylation of this residue has been shown to be necessary for full activation of insulin-stimulated Akt and GLUT-4 localization at the plasma membrane (12).

We found that levodopa-carbidopa impairs insulin-stimulated glucose transport and glycogen accumulation in muscle. Because normally ~90% of plasma glucose that is absorbed in response to insulin is stored as glycogen in skeletal muscle (22, 32), it appears possible that disruption of insulin action by levodopa may contribute to glucose intolerance reported for patients with PD. The current data suggest that similar studies on levodopa effects on glycogen metabolism in humans may be justified.

REFERENCES

15. Foianini KR, Steen MS, Kinnick TR, Schmit MB, Youngblood EB, and Henriksen EJ. Increased intracellular accumulation of G6P appears to mediate decreased insulin-stimulated glucose transport into muscle (26).


