

SKELETAL MUSCLE: RESERVOIR FOR EXOGENOUS L-DOPA¹

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ABSTRACT

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In previous studies it has been shown that skeletal muscle concentrates exogenous L-dopa from the blood more effectively than most other tissues. The present study further examines the metabolism of L-dopa in muscle. At all times studied after L-dopa administration to rats, the concentrations of the drug were higher in muscle than in serum or brain. The total amounts of unmetabolized dopa present in muscle were increased by prior insulin administration or carbohydrate consumption; these treatments also increased brain dopa. Of the dopa-derived molecules present in various tissues after administration of the amino acid, muscle contained the largest unmetabolized fraction. This finding is compatible with *in vitro* evidence that muscle contains little catechol-O-methyltransferase or dopa decarboxylase activity and suggests that muscle does not metabolize dopa to a significant degree. Isolated perfused hindquarters taken from rats pretreated with L-dopa released the unmetabolized amino acid into the perfusate; this suggests that when L-dopa concentrations in skeletal muscle exceed those of serum, dopa may also be released into the circulation *in vivo*.

Exogenous L-dopa is rapidly metabolized, primarily by O-methylation and decarboxylation (Wurtman *et al.*, 1970a). The former process lowers the concentrations of the methyl donor

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S-adenosylmethionine in brain (Wurtman *et al.*, 1970b) and other tissues (Chalmers *et al.*, 1971; Ordóñez and Wurtman, 1973) and causes the accumulation of the amino acid 3-O-methyldopa (Kurma *et al.*, 1970; Weiss *et al.*, 1972). The decarboxylation of exogenous L-dopa causes the formation of dopamine within rat tissues, including those that do not normally contain this monoamine (Romero *et al.*, 1973; Lytle *et al.*, 1972).

One hour after rats received an i.p. injection of L-dopa the concentration of this drug in skeletal muscle was found to be greater than in any other tissue examined (Romero *et al.*, 1973). Since muscle constitutes about 45% of the total

mass of the rat (Young, 1970), it could serve as a major, if transient, reservoir for exogenous L-dopa and perhaps for other drugs that also happen to be amino acids. Experiments described in this report show that at all times after L-dopa administration, a major fraction of the unchanged L-dopa remaining in the body is found in skeletal muscle. This fraction is largely unmetabolized and can be released into the circulation.

Methods

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.), weighing 150 g, were housed six per cage, exposed to light (Vita-Lite, Duro-Test Corporation, North Bergen, N.J.) from 9 A.M. to 9 P.M. daily and given free access to food (Big Red Laboratory Rat Chow) and water, except where noted.

Rats were injected i.p. with L-dopa (100 mg/kg, levodopa, Hoffmann-La Roche, Inc., Nutley, N.J.) previously dissolved in 0.05 N HCl (10 mg/ml). Control rats received comparable volumes of the vehicle. At intervals ranging from 15 to 240 minutes after the administration of L-dopa, animals were decapitated. Blood collected from the cervical wound was centrifuged to obtain serum and the various tissues to be studied were removed, frozen on Dry Ice, weighed and homogenized in 10% trichloroacetic acid-0.05 N HCl. The L-dopa present in the 34,000 × g supernatant of the acid extract was separated from its metabolites by column chromatography on Dowex 50W-X4, followed by alumina (Romero *et al.*, 1973). The dopa was then converted to a derivative which was estimated spectrofluorometrically (von Euler and Lishajko, 1959).

When ³H-L-dopa was used the radioactive amino acid was separated by Dowex and alumina chromatography and the radioactivity determined in a Packard scintillation spectrometer after adding 13 ml of naphthalene phosphor; total radioactivity was determined in the 34,000 × g supernatant of the tissue homogenates.

To study L-dopa release from muscle, the isolated hindquarters of rats were continuously perfused in a closed system (Ruderman *et al.*, 1971). The perfusate was 200 ml of Krebs-Henseleit saline solution (containing aged human red cells, 7-8 g of hemoglobin per 100 ml) and bovine serum albumin (4 g/100 ml) flowing at approximately 10 ml/min.

Results

Concentration of exogenous L-dopa by skeletal muscle. L-Dopa concentrations in muscle, brain and blood were all highest at the shortest interval studied after L-dopa administration (15 minutes, table 1) and decreased steadily thereafter (fig. 1). At all times studied, muscle contained the highest concentration of unmetabolized L-dopa; 1 hour after administration of the drug, the ratios of the L-dopa concentration in muscle to those in serum and brain were 6:1 and 30:1, respectively. Four hours after L-dopa only muscle contained detectable amounts of the drug (fig. 1).

When animals were injected with ³H-L-dopa, muscle showed the highest fraction of tissue radioactivity present as unmetabolized L-dopa: the fraction was more than double that of other peripheral tissues (fig. 2).

Effect of insulin on uptake of L-dopa by

TABLE 1

Tissue concentrations in rats after the administration of L-dopa to insulin-treated animals

Animals that received no treatment at all or insulin (2 U/kg i.p.) 1 hour prior to injection of L-dopa (100 mg/kg i.p.) were killed at the times indicated after L-dopa and the tissue concentrations of the amino acid determined. The muscle used for assay in this and all other studies was taken from the thigh (Romero *et al.*, 1973). Results are expressed as microgram per gram or microgram per milliliter of tissue ± S.E.M. (5-6 animals for each determination).

	Time after L-Dopa	Muscle	Brain	Serum	Muscle/Serum Ratio	Brain/Serum Ratio
Insulin-pretreated	15	22.32 ± 1.87	5.69 ± 0.98	16.65 ± 0.81 ^a	1.35 ± 0.13 ^a	0.46 ± 0.04 ^a
Control	15	22.94 ± 2.70	6.48 ± 1.28	23.65 ± 2.42	0.98 ± 0.08	0.27 ± 0.03
Insulin-pretreated	30	30.58 ± 4.76 ^a	5.54 ± 0.72 ^b	9.05 ± 0.81	3.40 ± 0.41 ^b	0.51 ± 0.04 ^a
Control	30	20.08 ± 1.16	3.07 ± 0.29	10.76 ± 0.69	1.95 ± 0.08	0.30 ± 0.02

^a P < .05 differs from control group.

^b P < .01 differs from control group.

skeletal muscle. Insulin influences the uptake by muscle of many amino acids. To determine whether L-dopa uptake is similarly affected, we pretreated fasting rats with a single injection of crystalline insulin (2 U/kg i.p.; Iletin, 40 U/ml; Eli Lilly and Company, Indianapolis, Ind.) 1 hour before the administration of L-dopa. The animals were killed 15 or 30 minutes after the L-dopa injection and the concentrations of unmetabolized L-dopa were measured in serum, muscle and brain. Pretreatment with insulin was associated with a significant increase in the muscle/serum ratio of L-dopa, as well as in the brain/serum ratio, at both times studied (table 1).

To determine whether endogenously released insulin can similarly affect the muscle uptake of L-dopa, rats fasted overnight were allowed to consume a meal containing carbohydrates and fats (Fernstrom and Wurtman, 1971) or left untreated and injected with L-dopa 1 hour later. One hour after L-dopa administration the concentration of the drug in muscle was 47% higher ($P < .05$) in the animals that had eaten the carbohydrate diet than in the comparable group of untreated rats; the ratio of the dopa concentrations of muscle to serum was 24% greater ($P < .05$).

Release of L-dopa from skeletal muscle.

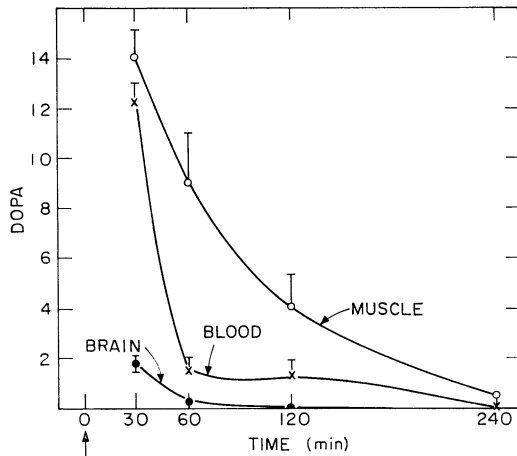


FIG. 1. Tissue dopa concentration in rats at different times after the administration of L-dopa. Groups of six animals received a single injection of L-dopa (100 mg/kg i.p.) and were killed at the times indicated. The concentrations of L-dopa are expressed in micrograms per gram or micrograms per milliliter of tissue. Vertical lines indicate standard errors of the mean. ●, brain; ✕, serum; ○, muscle.

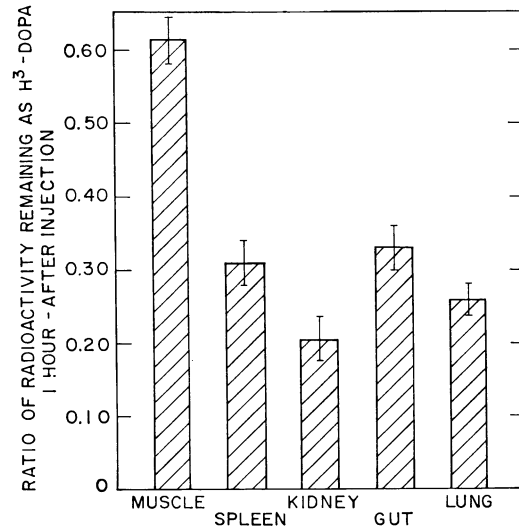


FIG. 2. Fraction of the total radioactivity present as dopa in rat tissues following the administration of L-dopa. Groups of six animals received a single injection of ^3H -L-dopa (100 mg/kg i.p.) and were killed 1 hour later. The results represent the ratio of the radioactivity present as unmetabolized L-dopa to the total radioactivity present in the tissue at the time the animals were killed. Vertical lines indicate S.E.M.

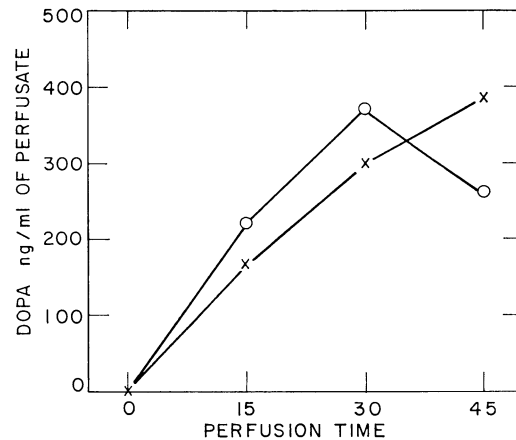


FIG. 3. Dopa concentrations in the medium following perfusion of hindquarters of animals pretreated with L-dopa. Animals were injected with L-dopa (100 mg/kg i.p.) and approximately 1 hour later their isolated hindquarters were perfused in a closed system containing 200 ml of modified Krebs-Henseleit solution (see text). At the times indicated, the concentration of dopa present in the perfusion medium was determined and expressed as nanograms per milliliter of perfusate. Curves for two representative animals are shown; the values are corrected for zero-time equilibration.

To determine whether the exogenous L-dopa present in muscle can be released into the blood stream, the isolated hindquarters of rats treated 1 hour previously with L-dopa (100 mg/kg i.p.) were continuously perfused in a closed system (Ruderman *et al.*, 1971). "Zero-time" samples of the perfusate were assayed for dopa 5 minutes after the perfusion began; the L-dopa concentration in the medium, corrected for "zero-time" equilibration, was subsequently measured at 15-minute intervals for 45 minutes. L-dopa concentrations rose linearly for 30 minutes in the perfusion media (fig. 3), thereby demonstrating that L-dopa present in muscle is available to the circulation without undergoing prior biotransformation.

Discussion

These observations support the hypothesis that skeletal muscle constitutes a reservoir for exogenous L-dopa. Since the uptake of dopa into muscle is dependent upon insulin, the effectiveness and duration of action of any given dose of the amino acid may be influenced by nutritional state.

Previous studies (Romero *et al.*, 1973) demonstrated that 1 hour after the last of 1 or 10 daily L-dopa injections, the concentration of the amino acid in muscle is higher than in any other tissue. The present findings indicate that L-dopa concentrations in muscle are also higher than in brain or serum for up to 4 hours after an injection of the drug (fig. 1). Since muscle accounts for about 40 to 45% of the total body mass in the male rat (Young, 1970) and since circulating L-dopa is metabolized very rapidly (Wurtman *et al.*, 1970a), at least half, and probably much more, of the exogenous L-dopa remaining in the animal 1 hour or more after each dose must be present within muscle.

The relatively high concentration of L-dopa in muscle 4 hours after its administration, at a time when it is no longer detectable in serum or brain (fig. 1), suggests that muscle tissue either contains a mechanism for concentrating L-dopa from the extracellular fluid or lacks the enzymes that metabolize the drug in other tissues, or both. The inability of muscle to metabolize L-dopa significantly is suggested by *in vitro* studies showing that this tissue contains little or no catechol-O-methyltransferase (Axelrod and Tomchick, 1958) or aromatic-L-amino acid decarboxylase activi-

ties (W. Dairman, private communication). Moreover, repeated administration of L-dopa does not lower S-adenosylmethionine concentrations in skeletal muscle (Ordonez and Wurtman, 1973) nor does it cause the accumulation of high concentrations of dopamine in this tissue (Romero *et al.*, 1973). The capacity of muscle to transaminate L-dopa apparently has not been examined; however, transamination does not appear to be a major route for L-dopa metabolism in the whole animal (Wurtman *et al.*, 1970a). These observations, together with the present evidence that 1 hour after the administration of L-dopa 60% of the L-dopa-derived molecules in muscle remain as the unmetabolized amino acid (fig. 2), suggest that one of the mechanisms operating to produce the gradient in L-dopa concentrations between muscle and blood is the lack of significant L-dopa metabolism within muscle. The source of the O-methyl-dopa in muscle may well be circulating molecules synthesized elsewhere in the body.

The uptake of exogenous L-dopa into muscle, and perhaps other organs, is modified by insulin (table 1). This effect of insulin could operate by several mechanisms, *e.g.*, by directly stimulating the transport of L-dopa, by decreasing the blood concentrations of other amino acids that may compete with L-dopa for tissue uptake or by facilitating the simple diffusion of L-dopa into the tissues. Inasmuch as no direct effect of insulin has been demonstrated on the uptake of aromatic amino acids into brain, and since insulin markedly decreases the plasma concentrations of neutral amino acids that presumably share a brain transport system with L-dopa, the increase in brain L-dopa concentration after insulin (table 1) probably represents decreased competition with L-dopa by other amino acids.

The effect of insulin on muscle L-dopa uptake is not easily analyzed because this amino acid is not normally present in the circulation and its flux in and out of the organ during the period studied is not at steady state. The ratios of muscle/blood L-dopa at 15 and 30 minutes after L-dopa administration are higher in insulin-treated animals than in untreated controls (table 1). At 15 minutes, the higher ratio is due to lower serum L-dopa concentrations in the insulin group; at 30 minutes, the higher ratio is due to higher concentrations of the drug being present in muscle (table 1). These observations

suggest that insulin causes L-dopa to be taken up more rapidly from the circulation into peripheral organs soon after L-dopa administration, while the concentrations of the amino acid in blood are rising. Later, when the serum concentration of L-dopa begins to decrease, the higher muscle concentration in insulin-treated animals becomes apparent. That this effect of insulin on L-dopa distribution is operative under physiological conditions is suggested from the evidence that a carbohydrate meal that causes physiological secretion of insulin (Fernstrom and Wurtman, 1971) elevates muscle L-dopa concentrations and the ratio of muscle/serum dopa in a manner similar to exogenous insulin. The ability of insulin to modify the tissue distribution of exogenous dopa suggests that the dose of the amino acid needed by patients with Parkinson's disease may depend upon what and how recently they have eaten.

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