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AGE DEPENDENCE IN CAPACITY-LIMITED JEJUNAL DECARBOXYLATION OF LEVODOPA IN RATS*

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Abstract—The extent of levodopa decarboxylation in a jejunal preparation permitting continuous *in situ* collection of the mesenteric blood was compared in rats 5 to 104 weeks old. The fraction of the absorbed amount that was metabolized (decarboxylated) in the jejunal segment was relatively high (approximately 0.7 to 0.8) in 9- to 15-week-old rats, whereas the fraction in relatively aged (52- and 104-week-old) and young (5- and 7-week-old) rats was considerably less (approximately 0.4 or less). These fractions were found to correlate better with the extent of relative contribution of the intestine in producing the overall first-pass effect of this drug after oral administration. In addition, kinetic studies on the jejunal metabolism of levodopa *in vitro* showed that a capacity-limited (i.e. saturable) decarboxylation rate was highest in 11-week-old rats. Therefore, the capacity-limited decarboxylation rate in the small intestine may be a determining factor in the age-dependent systemic availability of this drug when administered orally.

Significant variability in the individual systemic availability of levodopa has led to a modified dose requirement in elderly Parkinsonian patients compared with other age-groups [1–3], i.e. a "tailor-made" dose regimen for each individual patient [4]. Our previous report [5] demonstrating rather extensive intestinal first-pass metabolism (i.e. lower systemic availability) of this drug in 9- to 15-week-old rats as compared with aged (52- to 104-week-old) rats was found to be fairly consistent with another report suggesting higher oral systemic availability in elderly (mean age: 77 years) Parkinsonian patients than in young (mean age: 26 years) healthy volunteers [6]. In addition, it has been suggested that intestinal decarboxylation is the predominant factor in the overall first-pass metabolism of levodopa in rats [5].

A few studies on the distribution and metabolism in rats have suggested that levodopa, when administered orally in a relatively low dose, is decarboxylated to dopamine to an appreciable extent at peripheral sites such as the stomach mucosa [7], intestinal tissue, and liver [8], resulting in a relatively low level of the parent drug in the circulating blood [9]. Furthermore, a noticeable inhibition of dopa-decarboxylase by carbidopa was found, especially in the kidney, liver and small intestine [10]. However, there have been no reports clarifying age-dependent change in dopa-decarboxylase activity with respect to the intestinal first-pass metabolism of this drug.

The present work, therefore, was designed to test the effect of age on the extent of *in situ* levodopa decarboxylation in rat upper jejunal preparation (which permits continuous mesenteric blood collection) and on the metabolic kinetics in both *in situ* and *in vitro* systems.

MATERIALS AND METHODS

Animals. Male Wistar rats, 5 (110–135 g), 7 (210–230 g), 9 (300–330 g), 11 (355–390 g), 15 (385–420 g), 26 (455–510 g), 52 (640–730 g) and 104 (790–860 g) weeks old, were used throughout the experiments. Each rat was fasted overnight [5] and anesthetized with urethane (800 mg/kg, i.p.) shortly before the following experiments.

Measurement of jejunal metabolism of levodopa. Immediately after abdominal midline incision (about 5 cm), the second or third jejunal segment with a corresponding mesenteric vein was prepared by a slight modification of the method described by Barr and Riegelman [10] for rabbits. The present experimental procedure was essentially the same as that reported previously [11]. The vein was cannulated with PE-10 or PE-50 tubing while the arterial blood supply was kept intact. The blood lost from the vein was replenished continuously by constant infusion via the femoral vein with an approximately equal volume of heparinized blood, previously collected from donor animals. The segmental lumen was rinsed gently with 10 ml of warm 0.9% NaCl solution. The drug solution (in saline) contained 1.0 mg/ml of levodopa (Sankyo Pharmaceutical Co., Tokyo, Japan) and 10 μCi/ml of [^{14}C]levodopa (sp. act. 10.9 mCi/mmol, radiochemical purity more than 97%; Amersham Intern. Ltd., Buckinghamshire, England). This solution (1 ml/kg) was placed in the closed loop, which was tied at both ends. The mesenteric venous blood samples were collected continuously for 60 min after drug administration, unless otherwise specified. In the preliminary experiments, blood samples were also collected periodically from the femoral artery to determine whether radioactivity had entered the

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systemic circulation after placing [^{14}C]levodopa in the closed loop. In 7-, 11- and 52-week-old rats, six different intraluminal doses were tested (0.5, 1.0, 2.5, 5.0, 7.5 and 10 mg/kg). The cannulated rats were maintained in the same manner as described previously [11].

Unchanged levodopa in the mesenteric venous plasma was determined in the same way as reported previously [5]. The total (i.e. unchanged plus metabolized) about that was absorbed from the lumen was evaluated by measuring the total radioactivity in the mesenteric venous plasma. Radioactivity counts in both mesenteric venous and femoral arterial plasma samples were done in the same manner as previously described for the urine samples [5].

In vitro *metabolism of levodopa by the jenunal mucosa.* Using 7-, 11- and 52-week-old rats, the jejunal mucosal layer was gently scraped off with a glass slide after rinsing the lumen in the same manner as above. The wet weight of the mucosal tissue ranged, approximately, from 1.5 to 3.2 g/rat for 7- to 52-week-old rats respectively. One gram (as wet weight) of the tissue was then suspended in 10 ml of pH 7.4 Krebs–Ringer bicarbonate buffer solution to which levodopa had been added at 0.1 mg/ml, and the resultant mixture was incubated at 37° under anerobic conditions for 30 min. Periodic samples (0.1 ml at 0, 10, 20 and 30 min) of the incubation mixture were assayed for remaining levodopa in the same way as described above.

RESULTS

Time–course of cumulative total radioactivity and unchanged levodopa in mesenteric venous plasma. Figure 1 shows the time–course of cumulative amounts of unchanged levodopa and total (unchanged plus metabolites) radioactivity expressed as percent of the dose which appeared in the mesenteric venous plasma after placing 1 mg/kg into the jenunal segment of 7-week-old rats. Similar time–courses for both unchanged levodopa and total radioactivity were obtained in other age groups (5 to 104 weeks). Total radioactivity (percent of the dose) that entered the mesenteric circulation in the 60 min after the dosing was found to decrease with age, ranging from about 40 to 25% in 5- to 104-week-old rats respectively. Data for the cumulative amount of metabolites (i.e. difference of unchanged levodopa from total radioactivity) in the mesenteric venous plasma after 60 min was used as a plateau value in the following examination.

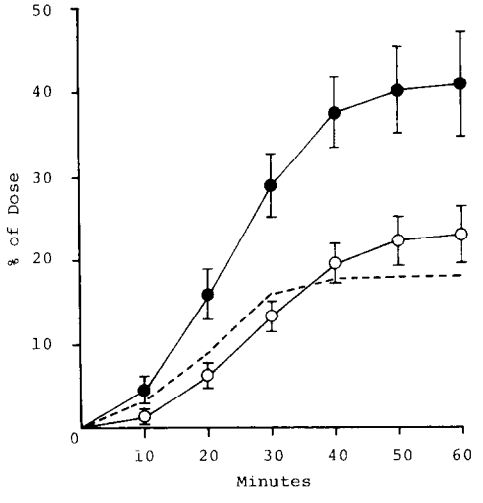

Fig. 1. Time–course of cumulative percent of dose appearing as total radioactivity (●) and unchanged levodopa (○) in the jejunal mesenteric venous plasma after dosing 7-week-old rats with 1.0 mg/kg of levodopa (10 μCi/kg as [^{14}C]levodopa). The broken line indicates the cumulative percent of dose that entered into the mesenteric venous plasma as metabolites (calculated from the difference of unchanged from total amount). Each point is the mean ± SD of four rats.

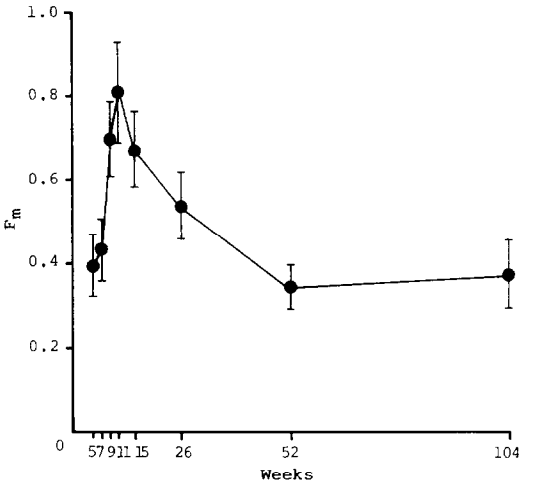

Fig. 2. Effect of age on the fraction of the total absorbed amount that existed as metabolites (F_m) in the cumulative mesenteric venous plasma at 60 min after dosing a rat jejunal segment with 1.0 mg/kg of levodopa (10 μCi/kg as [^{14}C]levodopa). Each point is the mean ± SD of four rats.

Effect of age on the jejunal metabolism of levodopa. The fraction of adsorbed levodopa that was metabolized in the jejunal segment is plotted against the age (weeks) of rats in Fig. 2. The fraction of jejunal levodopa metabolism was relatively high in 9- to 15-week-old rats. In contrast, young (5 and 7 weeks) and aged (52 and 104 weeks) rats metabolized this drug less extensively.

The extent of the relative contribution of the intestine (f_g) in producing the overall first-pass effect of levodopa following the oral administration to 5- to 104-week-old rats has been reported previously [5]. This parameter was estimated from the area under plasma concentration–time curves for levodopa after oral and intraportal administration. Figure 3 shows the mean value for f_g against that for the fraction of jejunal metabolism (F_m) estimated as above in various age groups. A high correlation ($r = 0.973$, $P < 0.001$) was obtained between both parameters over the range of ages.

Effect of dose on the jejunal metabolism of levodopa. Figure 4 shows the effect of dose on the cumulative amounts of total radioactivity, unchanged levodopa and its metabolites (i.e. difference of unchanged levodopa from total radioactivity) that appeared in the mesenteric venous

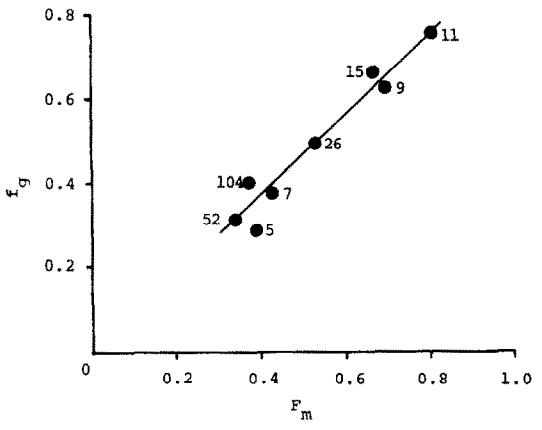

Fig. 3. Correlation between fraction of metabolites (F_m) in the cumulative jejunal mesenteric venous plasma for 60 min and relative contribution of intestine to the overall first-pass effect of levodopa (f_g) after oral administration of 20 mg/kg to variously aged rats. The solid line indicates the regression line for this correlation between both mean values, as Y = 0.972X (where $r = 0.973$, $P < 0.001$), where Y and X represent f_g and F_m respectively. The numbers indicate the age (weeks) of the rats. The values for f_g were obtained from Ref. 5.

plasma over 60 min in 7-, 11- and 52-week-old rats. In each age group, cumulative total amounts absorbed across the jejunal wall increased with intraluminal dose, whereas there was no linearity for cumulative amounts of either levodopa or its metabolites with dose. Cumulative amounts of the metabolites in 60 min were then converted to the mean rate for metabolism. As shown in Fig. 5, a single reciprocal linear transformation (Harnes–Woolf type plots) of the mean rate value (i.e. dose/rate) against the dose yielded a straight line, which enabled us to estimate more precisely the kinetic parameters than when estimating them by conventional double-reciprocal plots. Estimated values for V_{max} from the reciprocal of the slope and K_m from the negative intercept with the abscissa were as follows: in 7-week-old rats, $V_{max} = 1.87$ μg/min and $K_m = 2.46$ mg/kg; in 11-week-old rats, $V_{max} = 5.02$ μg/min and $K_m = 2.16$ mg/kg; in 52-week-old rats, $V_{max} = 2.89$ μg/min and $K_m = 2.05$ mg/kg.

Effect of age on the in vitro *jejunal metabolism of levodopa.* Under the present incubation conditions, the periodical sampling of the incubation mixture did not affect the metabolic kinetics of levodopa. In any age group, the metabolite level in the incubation medium increased almost linearly with time up to the first 20 min and tended to approach a plateau level thereafter. The percentage of metabolism in the first 30 min of the incubation was 18.1 ± 4.2, 46.7 ± 8.1 and 20.1 ± 6.8% in 7-, 11- and 52-week-old rats respectively. However, the fraction of dopa undergoing metabolism in the present *in vitro* experiments (1 mg levodopa/g jejunal mucosal tissue) was about half of that obtained in the *in situ* experiments (1 mg/kg body weight, Fig. 2).

DISCUSSION

It has been reported that levodopa, when administered orally in a relatively low dose, is decarboxylated rapidly to dopamine to a considerable extent at the peripheral sites where dopa-decarboxylase is localized, such as the gastrointestinal tissue [7, 8] and the liver [8]. Rapid and extensive decarboxylation of levodopa in rat peripheral tissues including the gastrointestinal tract results in a relatively low level of this drug in the circulating blood [9, 12]. Furthermore, it has been reported that after oral administration of labeled levodopa, relatively high distribution (uptake) of the radioactivity is observed in the intestine, liver and adrenal gland [13]. Although eighteen metabolites have been detected in urine of rats that were administered levodopa, the main metabolite in the intestinal tissue was identified as conjugated dopamine that was poss-

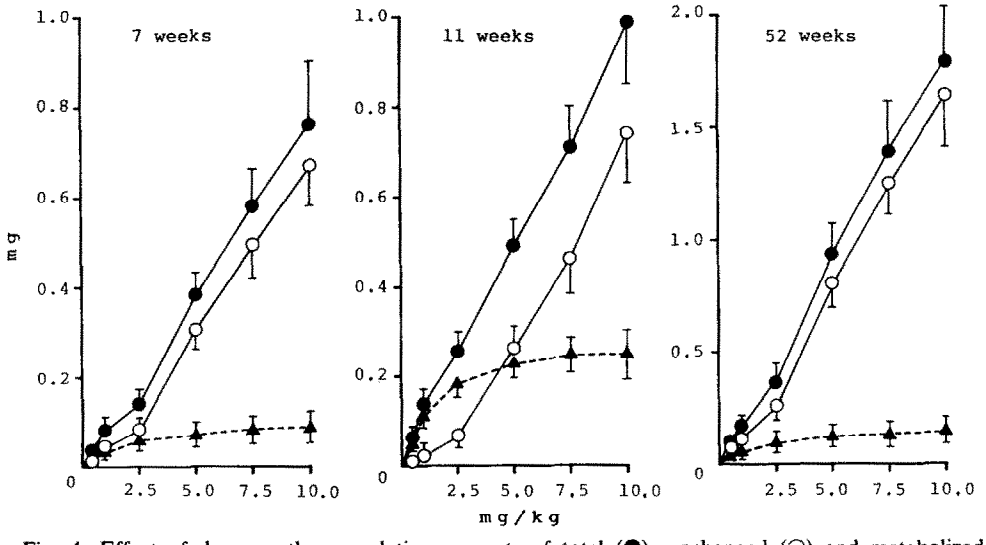

Fig. 4. Effect of dose on the cumulative amounts of total (●), unchanged (○) and metabolized (▲) substrate in the mesenteric venous plasma at 60 min after administering levodopa (10 μCi/kg as [^{14}C]levodopa) to jejunal segments of 7-, 11- and 52-week-old rats. Each point is the mean ± SD of four rats.

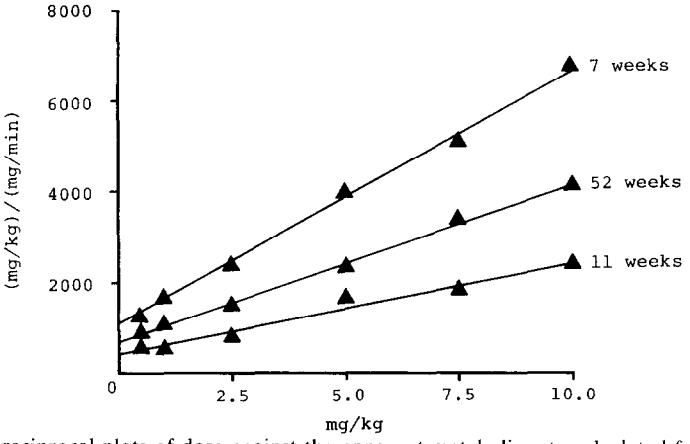

Fig. 5. Single reciprocal plots of dose against the apparent metabolic rate calculated from the data in Fig. 4. The ordinate is expressed as dose (mg/kg) divided by the apparent metabolic rate (mg/min). Each point is the mean value of four rats. Regression lines are expressed as follows: 7 weeks, $Y = 533X + 1320$ (where $r = 0.989$); 11 weeks, $Y = 199X + 431$ (where $r = 0.986$); 52 weeks, $Y = 345X + 705$ (where $r = 0.999$).

ibly derived after rapid decarboxylation [12]. In fact, the small intestine of male Wistar rats (7 to 8 weeks) was found to contain high dopa-decarboxylase activity, about 280 nmol dopamine formed/min/g tissue [14]. However, there have been no reports suggesting age-dependent changes in the rate and extent of intestinal decarboxylation of levodopa, except our previous report [5].

The same method as the present *in situ* approach to collect the mesenteric venous plasma periodically after placing drug solution into a rat jejunal segment has already been applied to rats in order to investigate the capacity-limited (i.e. saturable) conjugation of morphine [15], nalorphine [15], aspirin [11] and salicylamide [11]. This approach enabled us to examine the effect of age on the intestinal metabolism of levodopa in rats. Inasmuch as arteriovenous anastomoses are known to exist in the gastrointestinal circulation [16, 17], the preliminary examination employing the arterial plasma samples was made to establish that the amount of radioactivity reaching the systemic circulation was practically negligible. There have been no reports demonstrating that levodopa is also transported via the lymphatic route. The *in situ* extent of absorption of this drug, however, was diminished considerably (to 40% or less) even after 60 min of intraluminal exposure to the drug solution, compared with the previous *in vivo* extent of oral absorption that ranged from more than 95% in 5- to 15-week-old rats to 60% in 104-week-old rats [5]. A similar discrepancy has been reported with aspirin and salicylamide [11].

Unique but distinct age dependence was observed of the fraction of total absorbed amount that was metabolized when levodopa was given into the *in situ* jejunal segment. The relatively high fraction in 9- to 15-week-old rats corresponded with our previous evidence that the relative contribution of the gut in producing the overall first-pass effect of this drug after oral dosing was rather extensive in these age groups [5]. Consequently, a fairly good correlation was obtained between the relative contribution of the intestine and the fraction of the absorbed dose that was metabolized by the jejunum. The relatively low fraction of jejunal metabolism resulting in a reduced contribution of the intestine to the overall first-pass effect in the aged (52 and 104 weeks) and young (5 and 7 weeks) rats may have been due to the age-related decrease in intestinal decarboxylation activity. Additional *in vitro* experiments employing rats with rapid (11 weeks) and slow (7 and 52 weeks) metabolism of levodopa supported the above *in situ* results. Further *in situ* kinetic studies in 7-, 11- and 52-week-old rats demonstrated a saturable metabolic profile, with the highest V_{max} value being in 11-week-old rats, but almost the same K_m values. This also supports the unique age-related change in jejunal metabolic capacity by levodopa decarboxylase in rats.

In conclusion, the saturable metabolic rate depending on the intrinsic decarboxylase activity in the small intestine may be a determining factor in the age-related change in the systemic availability of levodopa when administered orally.

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