Br. J. Pharmac. (1972), **46**, 708–713.

Effect of chronic drug treatment on intestinal membrane transport of ^{14}C-L-DOPA

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Summary

1. By the use of the everted jejunal sac it was shown that chronic oral treatment of rats with various drugs can either increase or decrease the mucosal transport of ^{14}C-L-DOPA or alter its serosal/tissue ratio.

2. ^{14}C-L-DOPA transport was significantly increased in rats that were chronically treated with L-DOPA and diminished in those that were treated with chlorpromazine and phenobarbitone.

3. Chronic treatment with amantadine and neomycin did not affect ^{14}C-L-DOPA intestinal transport, although direct addition of amantadine to the medium, significantly increased ^{14}C-L-DOPA transport in everted sacs of non-treated rats. Addition of neomycin directly to the medium did not affect ^{14}C-L-DOPA transport.

4. The possible mechanisms of these findings and their clinical significance are discussed.

Introduction

Chronic treatment with certain drugs, such as neomycin (Small, Folen, Abrams, Bledshoe, Norris & Spring, 1966), colchicine (Herbst, Hurwitz, Sunshine & Kretchmer, 1970), diphenylhydantoin (Dahike & Mortens-Roesler, 1967), and imipramine (Consolo & Garattini, 1969) may produce impaired absorption of nutrients or other drugs. The underlying mechanisms vary from effects on gut secretion and motility to structural and biochemical alterations in the gut mucosa. Such drug–gut interaction may be of special importance to patients who require several medications for prolonged periods. Since Parkinson's disease affects primarily the aged, such patients may have other, coexistent diseases for which several medications are given continuously and chronically. L-DOPA ((−)-3-(3,4-dihydroxyphenyl)-L-alanine), a significant advance in the treatment of Parkinsonism, entails administration for the life-time of the patient. Since drugs that may be given chronically for some co-existent disease may affect the absorption of L-DOPA, the effect of chronic treatment with chlorpromazine, neomycin, phenobarbital, amantadine and L-DOPA on the intestinal membrane transport of ^{14}C-L-DOPA in rats was investigated by the use of the everted sac technique.

Methods

Groups of six to eight male Sprague-Dawley rats (160–180 g), housed in individual cages and maintained on the regular Purina chow laboratory diet and water, were each treated orally with a drug for periods ranging from a few weeks to

several months. The drugs, daily dosage, and duration of treatment were as follows:

(a) Levodopa (Nutritional Biochemical Corp.) 100 mg/kg for 3 months.

(b) Amantadine (DuPont Chemicals), 16 mg/kg for 3 months.

(c) Chlorpromazine (Smith, Kline & French), 20 mg/kg for 2–6 weeks.

(d) Phenobarbitone sodium, 60 mg/kg for 1–2 months.

(e) Neomycin sulphate (Sigma Chemical Co.), 100 mg/kg for 4 weeks.

The drugs were dissolved in 1·6 ml of 0·01 N HCl or tap water and were administered orally once a day by means of a metallic gastric tube. Experiments were performed on the day after suspension of treatment and after the rats had been fasted for 12 hours. Rats were anaesthetized with ether, a longitudinal abdominal incision was made, and 5 cm of proximal jejunum was excised, tied at one end and everted, as described by Wilson & Wiseman (1954). The everted sac was then filled with 0·5 ml of modified Krebs bicarbonate buffer (pH 7·4) and securely closed by ligation. The ionic composition of the medium was as follows (mM): K, 5 ; Ca, 1 ; Mg. 0·5 ; Na_1, 146 ; Cl, 129 ; HCO_3, 25. The tissues were separately incubated in 10^{-4}M (0·2 μCi) ^{14}C-L-DOPA labelled at the β carbon (Amersham Searle Laboratories ; specific activity 21 mCi/mmol) in 5 ml modified Krebs bicarbonate buffer. The L-DOPA solution was always added after the tissues had been placed in the medium to avoid its degradation (Rivera-Calimlim, Dujovne, Morgan, Lasagna & Bianchine, 1971). Incubation for 60 min was carried out under 95% O_2 and 5% CO_2 at 37° C in a Dubnoff metabolic incubator at 100 oscillations/minute. After incubation the everted sacs were blotted on tissue paper and weighed. The serosal fluid was recovered by allowing the fluid to drain out into a tube for 10 min through a slit made at one end of the sac. The sacs were then opened and washed three times with 0·1 N HCl. After washing, the tissues were blotted on tissue paper, weighed, and homogenized in 3 ml of 1 N HCl. The homogenate was centrifuged and the supernatant deproteinized with 0·5 ml of 70% perchloric acid. Aliquots from the deproteinized supernatant fluid (0·5 ml), mucosal fluid (1 ml), or serosal fluid (0·2 ml) were added to 10 ml of Triton X-100 toluene scintillation liquid (Turner, 1968) and their radioactivity was counted in a Packard Tricarb scintillation spectrometer. Counting efficiency for ^{14}C was 85–88%. All values were corrected for quenching and background. L-DOPA serosal transfer and tissue content were calculated as (micromoles/g of tissue)/hour.

The effect of 6 mg/100 ml of neomycin and 0·4 mg/100 ml of amantadine in the medium on the mucosal transport of ^{14}C-L-DOPA in everted sacs taken from non-treated rats was investigated by the same method.

Results

The effect of chronic treatment with various drugs on the intestinal transport of ^{14}C-L-DOPA by the everted sac was evaluated by measuring the serosal transfer and tissue content as shown in Table 1 and by calculating the total mucosal transport and the serosal/tissue ratio as shown in Table 3.

Mucosal transport is reflected by the drug content in the serosal fluid and in the tissue. The ratio of the serosal value to that for the tissue is a measure of the release of ^{14}C-L-DOPA from the epithelial cells. This study showed that the

TABLE 1. *Effect of chronic treatment on membrane transport of ^{14}C-L-DOPA in everted sacs*

Drug	Duration of treatment prior to time of experiment	Serosal transfer			Tissue uptake		
		Control	Treated	*P*	Control	Treated	*P*
Phenobarbitone (*n*=6)	3 months	0·82±0·05	0·63±0·04	<0·01	0·64±0·05	0·23±0·01	<0·01
Chlorpromazine (*n*=8)	2 weeks	0·63±0·01	1·13±0·04	<0·01	0·33±0·01	0·51±0·03	<0·01
	6 weeks	0·68±0·01	0·44±0·02	<0·01	0·31±0·01	0·37±0·04	>0·5
L-DOPA (*n*=8)	3 months	0·73±0·05	1·11±0·1	<0·01	0·63±0·05	0·47±0·06	<0·025
Neomycin (*n*=6)	6 weeks	0·62±0·03	0·61±0·22	>0·5	0·32±0·01	0·31±0·24	>0·5
Amantadine (*n*=8)	3 months	1·26±0·08	1·29±0·17	>0·5	0·80±0·04	0·84±0·04	>0·5

Values are expressed as mean±S.E.M. (micromoles of ^{14}C-L-DOPA/g of tissue)/hour. Controls were treated for the same length of time with the diluent used for the particular drug. *n*=number of animals treated.

TABLE 2. *Effect of acute treatment on membrane transport of ^{14}C-L-DOPA*

Drug	Serosal			Tissue		
	Control	Treated	*P*	Control	Treated	*P*
Neomycin (*n*=6)	0·64±0·06	0·75±0·05	>0·5	0·39±0·04	0·42±0·03	>0·5
Amantadine (*n*=12)	1·69±0·22	2·79±0·31	<0·01	0·94±0·13	0·83±0·01	>0·1

Values are expressed as mean±S.E.M. (micromoles/g of tissue)/hour.

TABLE 3. *Effect of treatment on mucosal transport and serosal/tissue ratio of ^{14}C-L-DOPA in everted sacs*

Drug	Duration of treatment prior to time of experiment	Total mucosal transport			Serosal/tissue ratio		
		Control	Treated	*P*	Control	Treated	*P*
Chronic treatment							
Phenobarbitone (*n*=6)	3 months	1·46±0·04	0·86±0·03	<0·01	1·20±0·10	2·82±0·29	<0·01
Chlorpromazine (*n*=8)	2 weeks	0·96±0·008	1·63±0·04	<0·01	1·86±0·05	2·06±0·12	>0·5
	6 weeks	0·98±0·01	0·81±0·03	<0·25	2·28±0·11	1·31±0·10	<0·01
L-DOPA (*n*=8)	3 months	1·34±0·05	1·57±0·08	>0·5	1·32±0·15	2·86±0·50	<0·01
Neomycin (*n*=6)	6 weeks	0·94±0·02	0·92±0·03	>0·5	1·94±0·12	1·99±0·27	>0·5
Amantadine (*n*=8)	3 months	2·06±0·06	2·13±0·08	>0·5	1·56±0·05	1·57±0·6	>0·5
Acute treatment							
Neomycin (*n*=6)		1·03±0·05	1·17±0·06	>0·5	1·67±0·18	1·85±0·19	>0·5
Amantadine		2·50±0·4	3·62±0·3	<0·01	1·88±0·13	3·94±0·6	<0·01

Values for the mucosal transport are expressed as means±S.E.M. (micromoles/g of tissue)/hour. The serosal/tissue ratios are the means±S.E.M.

different parameters (serosal content, tissue content, total mucosal transport and serosal/tissue ratio) can be affected independently of each other by chronic treatment with various drugs. ^{14}C-L-DOPA transport in the everted intestine appears to be significantly impaired in the rats chronically treated with phenobarbitone and chlorpromazine. After three months' treatment with phenobarbitone, both serosal and tissue content of ^{14}C-L-DOPA were significantly lower than in the controls, but the ratio of serosal to tissue content was higher in the treated preparations than in the controls. After two weeks' treatment with chlorpromazine there was a significant parallel increase in the serosal and tissue content of ^{14}C-L-DOPA, resulting in an increase in total mucosal transport without much increase in the serosal/tissue ratio as compared to controls. However, in rats treated with chlorpromazine for six weeks, serosal transfer of ^{14}C-L-DOPA was significantly diminished, although tissue concentration was not altered. The serosal/tissue ratio was diminished.

With rats that were chronically treated with L-DOPA for three months, the total mucosal transport of ^{14}C-L-DOPA was not different from the controls, but the serosal/tissue ratio was significantly higher (by 50%) in the treated rats than in the controls, resulting in an increase in the serosal transfer in the treated rats. It was observed that the everted sacs from treated rats retained less ^{14}C-L-DOPA in the tissues. There was no change in ^{14}C-L-DOPA transport in the everted sacs from rats treated with neomycin for two months or amantadine for three months.

However, Table 2 shows that when amantadine was added to the medium at a concentration of 0·4 mg/100 ml, the serosal transfer of ^{14}C-L-DOPA in the everted sacs taken from non-treated rats was significantly increased with just a slight diminution in tissue content. The serosal/tissue ratio was increased over two-fold, as shown in Table 3. Addition of neomycin to the medium did not significantly change the mucosal transport of ^{14}C-L-DOPA.

Discussion

The therapeutic value of L-DOPA in Parkinson's disease presumably depends upon its adequate absorption from the gastrointestinal tract. Absorption from the gastrointestinal tract is subject to large variations induced by anatomical and physiological factors in the gut, by the chemical structure of the substance ingested, and by disease. In recent years chronic ingestion of certain drugs has been shown to impair absorption of nutrients and drugs as reflected by the D-xylose absorption test, steatorrhoea, and plasma drug levels. Direct investigation of membrane transport activity of the intestine taken from chronically treated animals has not been reported. Changes in the membrane transport for a particular drug could occur despite absence of gross or microscopic structural changes in the intestinal mucosa. Effects of chronic treatment with drugs could possibly affect some biochemical structure of the absorptive mucosa, that could specifically affect absorption of a drug and not of other substances. Such occurrence would not be detected by routine standard absorption tests.

In this study attention was focused on tissue and serosal content which together reflect mucosal transport. The serosal/tissue ratio would seem to have more significance than total mucosal transport, since mucosal transport of two different drugs in everted sacs might be the same, but the drug with the greater serosal/tissue ratio could presumably produce higher plasma values *in vivo*. A low

serosal/tissue ratio would suggest that even if mucosal uptake is adequate, the drug may be stored in the gut tissue in some form and may produce low serum levels *in vivo*. L-DOPA when administered orally has been shown to be metabolized by the gut mucosa (Rivera-Calimlim, *et al.*, 1971) and, together with its metabolites, is taken up and stored in the enterochromaffin granules (Håkanson & Owman, 1966). It is likely that drugs that alter adrenergic uptake, storage and release may also enhance or inhibit L-DOPA transport.

If L-DOPA, being a derivative of phenylalanine, followed the membrane transport kinetics of amino acids, there are indications that membrane transport might be both passive and active (Matthews & Laster, 1965). Therefore, drugs that reduce tissue respiration, like phenobarbitone and chlorpromazine (Mutsubara & Hagikara, 1968), may impair L-DOPA absorption. In *in vitro* models acute treatment with chlorpromazine has been shown to enhance membrane permeability (Forrest & Forrest, 1963), a property which may explain the increased mucosal transport seen after the first two weeks of treatment in our study. Subacute or chronic treatment, however, produces tissue accumulation of chlorpromazine because of the drug's relatively slow tissue respiration. There is evidence that chlorpromazine uncouples oxidative phosphorylation (Dawkins, Judah & Rees, 1959). The above mechanism may explain the significant reduction in membrane transport of ^{14}C-L-DOPA in rats chronically treated with chlorpromazine, since L-DOPA appears to be transported by both active and passive mechanisms.

The present study shows that chronic treatment with L-DOPA produces a significant increase in the serosal/tissue ratio of ^{14}C-L-DOPA without much change in its total mucosal transport. The above finding is compatible with evidence that L-DOPA is taken up by enterochromaffin-like cells in the stomach and intestines and metabolized and stored in granules (Håkanson & Owman, 1966). Continuous chronic treatment may saturate these granules so that succeeding doses of L-DOPA after chronic treatment are better absorbed. They may explain the observations by Muenter & Tyce (1971) that patients who had been treated with L-DOPA for a period of time attained higher plasma levels than patients who had just started treatment with L-DOPA.

Amantidine, originally introduced as an anti-influenza drug, has recently been shown to have antiparkinsonian activity (Schwab, England, Poskanser & Young, 1969 ; Godwin-Austen, Frears, Parkes & Knill-Jones, 1970). Indeed some patients have shown a worsening of the symptoms on combined treatment (Weith, Shealy & Mercíer, 1969). The results described here show no significant effect on ^{14}-C-L-DOPA transport in everted sacs in rats chronically treated with amantadine. Yet when amantadine was added to the medium with ^{14}C-L-DOPA, the serosal radioactivity was markedly increased in previously untreated rats. Amantadine was shown to release catecholamines from the central and peripheral nervous tissue (Farnebo, Fuxe, Goldstein, Hamberger & Ungerstedt, 1971) so that L-DOPA and its metabolites are not 'captured' by the granules and pass through membranes without interference. The marked increase in the serosal/tissue ratio in the acute experiments suggests that ^{14}C-L-DOPA does not accumulate in the tissue in the presence of amantadine. Whether administration of amantadine with L-DOPA *in vivo* will increase plasma levels of L-DOPA warrants investigation. Neomycin, which can produce a malabsorption syndrome, did not affect the intestinal membrane transport of ^{14}C-L-DOPA in these experiments.

This work was supported in part by grant No. GM-15190 from the U.S. Public Health Service and a grant from the Pharmaceutical Manufacturers' Association. I am grateful to Professor Louis Lasagna for his interest and advice in this work and to Alan Siracusa for his technical assistance.

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(*Received May* 29, 1972)