Contributions of Gut Bacteria and Diet to Drug Pharmacokinetics in the Treatment of Parkinson’s Disease

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Parkinson’s disease is the second-most common neurodegenerative disorder worldwide. Besides deciphering the mechanisms that underlie the etiology of the disease, it is important to elucidate the factors that influence the efficacy of the treatment therapeutics. Levodopa, which remains the golden treatment of the disease, is absorbed in the proximal small intestine. A reduction in levodopa absorption, leads to reduction in striatal dopamine levels and, in turn, an “off”-episode. In fact, motor fluctuations represent a major problem during the progression of the disease and alteration between “on” (mobility often with dyskinesia) and “off” (immobility, akinesia) episodes contribute to a decreased quality of life. Dietary amino acids can interfere with the absorption of levodopa from the gut lumen and its transport through the blood brain barrier. In addition, higher abundance of specific gut bacteria that restrict levodopa absorption plays a significant role in motor fluctuations in a subset of Parkinson’s disease patients. Here, we review the impact of factors potentially interfering with levodopa absorption, focusing on levodopa transport, diet, and gut bacterial interference with the bioavailability of levodopa.

Keywords: levodopa, transporters, bioavailability, small intestinal bacterial overgrowth, gut motility

INTRODUCTION

Parkinson’s disease (PD) is the second-most common neurodegenerative disorder worldwide (1). In 2015–2016, 6.1–6.2 million individuals were diagnosed with PD all over the globe (1, 2). The prevalence of PD globally increases with age and peaks at 1.5% between 85 and 89 years of age (2). During the progression of PD, patients encounter increasing severity of symptoms, which is associated with rising costs for medical treatment, hospitalizations and nursing home care (3), besides a significant decrease in the quality of life (3–6). The aggregation of α-synuclein in Lewy bodies and loss of dopaminergic neurons (pars compacta) in the substantia nigra is the main feature observed in PD patients (7). Although the exact factors contributing to the etiology of PD are not well understood, the gut microbiota is likely to be a key contributor. This is evident from the alteration in gut microbiota composition detected in fecal samples of PD patients compared to healthy controls (HC) (8–12). Moreover, the production of short-chain fatty acids (SCFAs), the main metabolic products produced by the large intestinal bacteria, is reduced in PD patients (12). The latter has been shown to be involved in α-synuclein pathology in the gut in mouse models (13) supporting the hypothesis that α-synuclein pathology starts in the enteric nervous system (14), which synergizes with the finding of α-synuclein aggregates in colon tissue and appendix prior to the onset of PD (15, 16). Equally important to elucidating the mechanisms involved in the cause...
of PD is to uncover the microbial and dietary interference with the pharmacological treatment of the disease. Previous studies have shown that *Helicobacter pylori* (HP) can interfere with levodopa treatment and can bind to levodopa (3,4-dihydroxyphenylalanine; L-DOPA) (17, 18). Recently, we showed that bacteria can alter the levels of levodopa treatment in the gut (19) resulting in quenching the availability of the drug to be effective in the brain. This bacterial mediated reduction in levodopa absorbed from the small intestine would lead to reduction in striatal dopamine levels and an "off"-episode, especially in patients with advanced stage PD, who have a reduced capacity to store dopamine in the brain (20, 21). Besides, fluctuating levodopa plasma levels could result in increased pulsatile stimulation which is associated with dyskinesia (22). The pharmacological treatment of PD and the gastrointestinal (GI) dysfunction in PD have been extensively reviewed (23, 24), mainly from a clinical perspective. This review focuses on the impact of levodopa transport, gut bacterial degradation of PD medication, and its impact on drug bioavailability. Furthermore, we discuss the potential mediators that could lead to a vicious circle where certain conditions (i.e., proton pump inhibitors and gut motility) would favor the colonization of small-intestinal bacteria, ultimately restricting the absorption of levodopa.

**ADMINISTRATION ROUTES AND TRANSPORT PROCESS OF LEVODOPA**

The most common route for levodopa administration is orally via immediate-release or extended-release formulations of levodopa, where the latter might have potential benefits over other levodopa formulations, reviewed in Mittur et al. (25). Parenteral administration via subcutaneous injections are impossible due to the low solubility of levodopa (26) and continuous intravenous administration, although effective (27), is impractical, as it requires large volumes of daily injections. A promising alternative option to conventional levodopa therapy for advanced PD patients with motor fluctuations and dyskinesia is intestinal infusion of a levodopa/carbidopa gel via a nasoduodenal tube (28) or via gastroscopejuinostomy (22).

When levodopa is administered orally, it is absorbed in the proximal small intestine (29), where it has to be actively transported from the lumen over the intestinal epithelial barrier into the blood stream (30). To prevent peripheral and intestinal levodopa metabolism by DOPA decarboxylase (DDC), peripheral DDC inhibitors, such as carbidopa, are co-administered with levodopa. Levodopa (Figure 1) is a non-proteinogenic large neutral amino acid (LNAA), and is therefore transported by amino acid transporters in the GI-tract and at the blood brain barrier (BBB) (Figure 2). The human body contains at least 11 different epithelial amino acid transport systems expressed in the intestine, 10 of which are also expressed in the renal epithelia, which was thoroughly reviewed before (31). Only two amino acid transporters are expressed on the blood brain barrier (BBB), LAT1 (SLC7A5) and SNAT5/11 (SLC38A5/11) (32). The amino acid transporters, which are most likely responsible for the transport of levodopa from the GI-tract to the blood and over the BBB, based on *in vitro/ex vivo* studies, are discussed below and summarized in Figure 2.

As a model for the BBB, a mouse brain endothelial cell line (MBEC4), was tested for the expression of 4F2hc/LAT1 (SLC3A2/SLC7A5) and [3H]-levodopa transport was evaluated in the presence of other amino acids (1:100 levodopa/amino acids). The study showed that tryptophan, tyrosine, phenylalanine, isoleucine, leucine, histidine, and 2-amino-2-norbornane-carboxylic acid (BCH), which is used as the defining synthetic amino acid for the L-system (consisting of LAT1 to 4) (33), inhibited at least 80% of the [3H]-levodopa uptake independent of Na+ (34). However, the potential contribution of 4F2hc/LAT2 (SLC3A2/SLC7A8) or other transporters were not addressed. Similar results were obtained in Caco2 cells (35–38), renal proximal tubular epithelial cells (39), and opossum kidney cells with either a high (HC) or a low (LC) Na+ influx. Comparing the HC and LC cell lines indicated that there was a minor contribution of Na+ dependent transport. The authors concluded that 4F2hc/LAT2 (apparent from BCH transport) and rBAT/b0,+ (SLC3A1/SLC7A9; apparent from the uptake of the rBAT defining amino acid dimer, cystine) were involved in levodopa transport (40). Although these studies indicate which transporters are involved in levodopa transport in the GI-tract, renal epithelia and the BBB, it remains unclear which specific transporter is involved.

Studies using *Xenopus laevis* oocytes, an ideal single-cell expression system for transporters due to its relatively large size and low background activity (41), showed that 4F2hc/LAT1 (from rat C6 glioma cells) (42), 4Fhc/LAT2 (43), rBAT/b0,+ (from rabbit intestine and human) (43, 44), and LAT1 (SLC16A10) (from rat intestine) (45) are independently responsible for levodopa transport. Only substrates with both positive and negative charges at the α-carbon (the relative positive and negative charges are from the amine-group and carboxyl-group from levodopa, respectively, Figure 1) are being able to be transported via 4F2hc/LAT1 (42). Importantly levodopa analogs (m-O-methylDOPA, α-methylphenylalanine, α-methyltyrosine, α-methylDOPA), gabapentin [γ-aminoobutyric acid (GABA) analog], melphalan (a chemotherapeutic agent), and thyroid hormones (T3, triiodothyronine and T4, thyroxine) were able to inhibit transport of L-[14C]-phenylalanine, and thus levodopa (42), showing the broad range of potential levodopa transport inhibitors. In fact, anti-thyroid treatment in a 70-year-old male subject with PD on levodopa treatment had a beneficial effect on the exaggerated Parkisonian tremor (46). The authors could not explain why the Parkinsonian tremor was aggravated by the presence of hyperthyroidism. However, a plausible explanation, which was not discussed, is the interference of exaggerated thyroid hormone levels with levodopa uptake in the brain. Thus, hyperthyroidism, which is prevalent at higher age, should be considered in PD patients (46).

In *X. laevis* oocytes expressing Tat1, around 80% of L-[14C]-tryptophan uptake was inhibited by tryptophan and tryptophan and about 40% was inhibited by phenylalanine, levodopa, and m-O-methylDOPA, indicating that Tat1 is an aromatic amino acid transporter partly responsible for levodopa uptake. Using N-acetylated amino acids, the authors concluded that
the α-carboxyl group (Figure 1) is essential for substrate recognition by TAT1. Furthermore, it was shown that TAT1 is mainly expressed throughout in the rat GI-tract and in the liver, in particular, on the basolateral side of rat small intestine (45) (Figure 2). Using trans-well culturing and everted murine jejunal sacs, the authors concluded that 4F2hc/LAT2 (LAT1 was not tested) and TAT1 are responsible for the basolateral transport of levodopa (30). In contrast to 4F2hc/LAT1, 4F2hc/LAT2, and TAT1, which are expressed basolaterally, rBAT/b0,+AT is expressed apically and thus is mainly responsible for levodopa absorption from the intestinal lumen. Further characterization of rBAT/b0,+AT showed that the common co-administered...
The transport of levodopa via other apical transporters, PAT1, SIT1/ACE2, ASCT2, and B0AT1/ACE2 (the main other natural amino acid transporter), expressed in X. laevis oocytes was investigated and showed that none of them was able to transport levodopa, indicating that rBAT/b0,+AT is the main apical levodopa transporter (30) (Figure 2).

**EFFECT OF DIET AND AGE ON THE BIOAVAILABILITY OF LEVODOPA**

Early studies in vivo, using radiolabeled levodopa ([14C]-levodopa) showed that ~90% of the total radioactivity is transported into the circulatory system as measured in urine samples after 48 h (47-49). Notably, only ~13% of the total radioactivity in blood plasma after the first hour was from intact levodopa, and decreased further overtime. When carbidopa was used in combination with levodopa the intact levodopa after the first hour increased to ~43% (47). These studies indicate that less than half of the administered levodopa would reach the brain and that approximately 10% of the total levodopa radioactivity is not absorbed and could end up in fecal samples. Moreover, levels of unabsorbed levodopa increase over age. For example, a 10-fold increase (24.6–35.4% vs. 2.7–3.5% recovered radioactivity) in levels of levodopa (including its metabolites) were detected in fecal samples of old rats (0.5–2 years old) when compared with their younger counterparts (5–15 weeks old) after oral administration of [14C]-levodopa (50). This was not related to an increased fecal excretion or decreased jejunal blood flow, suggesting that there is impaired uptake at older age (50). When levels of levodopa were measured over time in plasma (AUC), older animals (1–2 years) had a higher AUC and a longer half-life (T½) of systemic levodopa compared to younger animals (9–26 weeks), suggesting an age-dependent slower total body clearance of levodopa (50). Furthermore the study showed that the intestinal metabolism (mainly by DDC), which prevents levodopa to reach the brain and decreases over age, contributes the most to the increased systemic availability of levodopa at older age (50). The decreased clearance of levodopa at higher age in rats is in agreement with a study performed in healthy human subjects, who were administered levodopa without DDC inhibitors (51). Coherently, a higher AUC and systemic levodopa bioavailability (AUCoral/AUCintravenous) for levodopa was observed in elderly (71.0 years n = 9) compared to young subjects (21.8 years n = 8). Administration of carbidopa diminished the differences in systemic levodopa bioavailability between the two groups, while a higher AUC was still observed in the elderly group. This suggests a lower systemic clearance at higher age because carbidopa abolished the age differences in systemic levodopa bioavailability (51). In PD patients, age correlated significantly with higher levodopa (supplied with DDC inhibitor) AUC and decrease in clearance (52, 53). However, the high scatter in the correlation (r² = 0.15–0.24) from that study implies that other factors besides age contribute to the variation among PD patients in the pharmacokinetics of levodopa (52).

**FIGURE 2** | Bacterial degradation and dietary components restrict levodopa transport. Levodopa is taken up in the small intestine by the apical transporter rBAT/b0,+AT, and is sequentially transported over the basolateral membrane by 4F2hc/LAT2 and TAT1. The uptake from the lumen can be compromised by LNAA apically and by LNAA and AAA basolaterally. Bacterial degradation can interfere with levodopa before it is transported and elevate levels of dopamine in the lumen. Higher levels of luminal dopamine could affect the gut motility, which, in turn, could result in a state of small intestinal bacterial overgrowth, creating a vicious circle. The fraction of levodopa that ends up in the blood has to be transported over the BBB via 4F2hc/LAT1, which can be compromised by high levels of thyroid hormones (T3/T4), or LNAA. Serine left over from a late proteic meal, can trans-stimulate 4F2hc/LAT2 inducing higher efflux of levodopa in the circulation. Finally, the remaining levodopa will be converted to dopamine in the brain by DDC, to compensate the loss of striatal dopamine levels in PD patients.
Indeed, impaired uptake of [14C]-levodopa into the brain was observed when rats were supplied intravenously with the amino acids, phenylalanine, tryptophan, and to a lesser extent histidine (54). The same effects were reported in humans, for example, a clinical study showed that PD patients (n = 9), who received levodopa/carbidopa intravenously directly after a protein rich meal (containing LNAAs) or administration of LNAAs, had increased Parkinsonian symptoms. Similarly, when levodopa/carbidopa was taken orally, levodopa absorption from the intestine was delayed after a protein-rich meal (55). When levodopa/benzerazide (another DDC inhibitor) was infused intraduodenally, motor functions decreased after protein ingestion (56), indicating fluctuation in levodopa uptake in the brain. Nonetheless no decrease in levodopa absorption was observed (56) suggesting that the variability in plasma LNAAs, absorbed from the intestine, could be responsible for the fluctuating levodopa uptake in the brain (57). The authors concluded that during ingestion of regular (hospital) diets, 10% of the levodopa brain uptake variability is explained by LNAAs in plasma and the other 90% by levodopa plasma levels (57). These hospital diets contained 2–3.7-fold less LNAAs compared to other human studies [615 ± 105 µM (57) compared to 1,235–1,973 µM (55), 1,615–2,012 µM (58), 1,624–2,292 µM (56)] indicating that high LNAAs levels do interfere with levodopa absorption in PD patients but are not solely responsible for the “on”–“off” fluctuations observed in PD patients. Notably, cationic (lysine) or small (glycine) amino acids had no effect on the “on”–“off” fluctuations (55). Using regional jejunal perfusion of levodopa in healthy human subjects it was shown that the LNAAs L-leucine interfered with the levodopa absorption from small intestine (59), at least at high concentrations. This finding supports the involvement of the L-transport system for levodopa transport (as described above) from the intestine to the blood circulation, and, ultimately, to the brain (Figure 2).

In vitro data and clinical investigations on the effect of amino acids on the transport and bioavailability of levodopa clearly indicate that amino acids can interfere with the uptake of levodopa from the lumen or the systemic circulation. Therefore, low protein diets (LPD) or protein redistribution diets (PDR), where all dietary protein is ingested only during the evening meal, are proposed for PD patients with motor fluctuations (60). Refined physiologically based pharmacokinetic (PBPK) modeling for GI absorption (WB-ACAT, Whole Body—Advanced Compartmental Absorption and Transit Model) combined with dynamic flux balance analysis (which measures the flow of metabolites through a metabolic network) on an epithelial cell (sIEC) model for small intestine segmented into 7 parts (WB-ACAT-sIEC), was used to investigate the spatiotemporal relationship between amino acids and levodopa uptake kinetics (61). Simulation of levodopa absorption during an aproteic or proteic meal showed that that dietary intervention would be beneficial for PD patients with Hoehn and Yahr scale 3/4 (HY3/4; HY describes the disease progression from (mild = 1) to severe = 5) (61). These findings are in agreement with the guidelines for PD treatment, where dietary interventions are proposed for advanced PD patients (20, 21). Comparing a LPD (in silico administration of 0.8 g/kg amino acids together with 200 mg levodopa) vs. a PDR (assuming a high fraction of amino acids present in the systemic circulation before the morning levodopa dose) in the WB-ACAT-sIEC model showed a cumulative increase in AUC of levodopa during PDR. Furthermore, the AUC after a morning levodopa dose was higher (11.23%) during PDR than during a fasting state, which was attributed to a higher influx of residual systemic NAA from the last protein meal taken the evening before levodopa administration. This higher influx through the basolateral antiporter induced a higher efflux of levodopa (trans-stimulation) into the circulation (61) (Figure 2). Although PDR could provide short-term benefits as evident by the reported response rates of >80% (60), it might not provide a long-term solution as it is undesired by patients and is an imbalanced diet (20, 21) that results in weight loss among patients (60). Extending the WB-ACAT-sIEC model with kidney and brain compartments and setting the objective function (a desired outcome) for optimizing levodopa transport across the BBB revealed that threonine, serine and asparagine resulted in the highest brain bioavailability of levodopa. This led the authors to propose that a serine-rich meal taken after the last levodopa treatment could be beneficial for the levodopa bioavailability (61). Nonetheless, sensitivity analyses (i.e., the variable that contributes most to the dependent outcome) showed that intestinal loss of levodopa was the most influential factor on levodopa bioavailability (61). Indeed, changes in the levels of levodopa in the small intestine are affected by gut bacterial interference (17, 19), as discussed in the next section.

GUT BACTERIAL INTERFERENCE WITH LEVODOPA BIOAVAILABILITY

Levodopa is a non-proteinogenic amino acid produced by the hydroxylation at the meta-position of the phenyl ring of tyrosine. Subsequently, levodopa can be converted to dopamine by DDC or to m-O-methylDOPA by COMT methylation of the m-hydroxyl group in the human body (Figure 1). The microbiota also poses enzymes able to perform similar or additional reactions, which metabolize levodopa. In the early 70s, a study, comparing the metabolic profile of germ-free and conventional rats when fed with levodopa, suggesting that a bacterial interference with levodopa treatment in the proximal small intestine, which was supported by the caecal incubations (62). When rat caecal content was incubated with levodopa or dopamine for 6 days also m-tyramine was found, confirming earlier findings in humans (63). Metabolites were detected over periods of 3 days in the urine indicating that the detected metabolites could originate from in the large intestine, which is supported by the caecal metabolism of levodopa in the large intestine would affect the drug bioavailability. Therefore, it is crucial to investigate potential bacterial interference with levodopa treatment in the proximal small intestine.
Recently, we showed that gut bacteria harboring tyrosine
decarboxylases (TDC), mainly enterococci, can effectively
decarboxylate levodopa to dopamine in the small intestine of
rat. The study concluded that the natural variation of the tdc-
gene negatively correlated with the levodopa levels in the blood
of rats and positively correlated with the daily dose require-
ment of levodopa in PD patients (19). High abundance of these
bacteria in PD patients, which could be caused by small intestinal
overgrowth (SIBO), could have implications on the absorption
of levodopa from the small intestine (Figure 2). To assess the
contribution of those bacteria to the bioavailability of levodopa in
PD patients, we are currently performing further clinical studies.

In healthy conditions, SIBO is prevented by the ileocecal
valve, pancreatic enzyme activity, gut motility and gastric acid
(64). Importantly in PD patients, the prevalence of gut motility
dysfunction (constipation) and proton pump inhibitor (PPI)
usage is relatively high (77.1 and 39.6% respectively, n = 39)
(65) and is associated with SIBO (66). Studies looking at the alteration of the microbiota in subjects using
PPIs showed increased levels of Bacilli (including Lactobacillus, Staphylococcus, and Enterococcus) in fecal samples (67, 68). In
duodenal samples, SIBO was also observed in 56% of patients on
PPIs (n = 25) and included mainly genera from the Bacilli class
(69). Bacterial species from the Bacilli class are of importance
as they harbor TDCs, which are able to interfere with levodopa
levels (19). When SIBO is eradicated in PD patients with
Helicobacter pylori infection using rifaximin, a common non-
absorbable antibiotic used to treat SIBO (70), motor fluctuations
were improved as apparent from the significant decreased
delayed “on” episodes/day and daily “off” time, although no
significant increase in levodopa pharmacokinetics was observed
(71). The underlying explanation of improved motor fluctuations
following SIBO eradication remains to be elucidated. However, a
plausible explanation is that eradication of bacterial degradation
of levodopa in the small intestine altered levels of the levodopa
metabolite, dopamine, in the small intestinal lumen (19), and/or
eliminated SIBO-induced small intestinal inflammation (71).

In 2001, investigators observed a clinical improvement in
PD patients after treatment with antibiotics used to eradicate
Helicobacter pylori in two almost identical reports. When
HP-infections were treated, the mean AUC of levodopa in the
blood significantly increased by ∼1.2-fold. A UPDRS-
III motor examination showed indeed a significant decrease
in motor score (72, 73). A follow-up study confirmed these
findings in a larger cohort (n = 17) and showed that either
2 weeks or 3 months after HP eradication, PD patients had
higher levodopa blood levels (AUC) and lower UPDRS-III
motor scores compared to before the eradication (18). Other
studies did not find a significant difference in pharmacokinetics
(74) or LEDD (levodopa equivalent daily dose) (75, 76) of
levodopa between PD patients tested positive or negative for
HP infection. In addition, no motor improvement (UPDRS-
III) was found after HP eradication in 34 patients (75). Despite
the discrepancy among studies, Helicobacter pylori might still
play a significant role in drug absorption. The mechanism of
Helicobacter pylori affecting the levodopa absorption is unclear,
one possible explanation for altered drug absorption might
be the gastric acidity, which is altered by Helicobacter pylori
infection and therefore interferes with drug pharmacokinetics
of levodopa, delavirdine, and thyroxine (77). Interestingly, an in
vitro study showed that adhesins exposed on the outer membrane
of Helicobacter pylori might bind to levodopa and therefore
might contribute to the lower pharmacokinetics in Helicobacter pylori infected PD patients (17). No follow-up studies were
published and it remains to be elucidated which adhesin(s) are
responsible for binding levodopa. Besides, whether the antibiotic
cocktail used to treat Helicobacter pylori infections (1,000/500 mg
amoxicillin/clarithromycin) could also eradicate other bacterial
species in the small intestine, which might interfere with the
availability of levodopa, and thus could be the actual reason
behind the observed increase in blood levels of levodopa, was
not investigated.

**EFFECT OF DOPAMINE AND DOPAMINE
AGONISTS ON GUT MOTILITY**

Bacterial species from the Bacilli class, especially enterococci, are
able to produce luminal dopamine (19). Importantly, dopamine
and their agonists have been shown to affect the gut motility
(discussed below), which could potentially favor the colonization
of levodopa decarboxylating bacteria (19) (Figure 2). In addition,
the dopamine agonists, which are usually used in combination
with levodopa treatment, could have a similar effect on
influencing gut motility to favor colonization of specific bacterial
species. Therefore, studies investigating the effects of dopamine
on gut motility of rodents, dogs, and humans were reviewed, with
a complete overview in Table 1.

Using electrical field stimulation (EFS) on longitudinal muscle
strips of guinea pig ileum in organ baths, dopamine (1–100µM)
and bromocriptine (0.15–15µM), a dopamine agonist used in PD
treatment, inhibited the cholinergic twitch up to 46 and 82%,
respectively. Neither dopamine antagonists, metoclopramide
nor pimozone prevented the observed inhibition by dopamine
or bromocriptine. When using the α-adrenoceptor antagonist,
phentolamine, only the observed inhibition of dopamine but
not of bromocriptine was rescued, indicating that dopamine acts
through the α-adrenoceptors (78). The same conclusions on the
inhibitory effect of dopamine were shown in an almost
identical study using ileum of guinea pig (79). Notably, tyramine,
a product of bacterial TDC, resulted in similar inhibitions of
cholinergic twitch (79). Dopamine, bromocriptine, and to a
lesser extent tyramine, were also able to relax methacholine-
contracted jejunal tissues from guinea pig (80). In rats, dopamine
initiated directly a short longitudinal contraction followed by
relaxation within 5 min in the duodenum and jejunum. However,
in the ileum, only relaxations were observed (81). In addition,
dopamine had also an inhibitory effect on the spontaneous
contractions of longitudinal muscle strips from rat distal colon
(82). The motility of mouse longitudinal fixed ileum (83), circular
muscle strips of colon (84) and longitudinal fixed colon (85)
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<td>None</td>
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<td>All tested</td>
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<th>Other inhibitors</th>
<th>Effect inhibited by</th>
<th>Conclusion</th>
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</thead>
<tbody>
<tr>
<td>Auteri et al. (84)</td>
<td>Mouse</td>
<td>Organ bath</td>
<td>Colon; circular muscle strips; Carbachol precontracted or electrical field stimulation</td>
<td>Relaxation/Inhibitory</td>
<td>Dopamine (1–300), SKF-38393 (up to 100), bromocriptine (0.3–100), isoproterenol</td>
<td>SCH-23390 (3), domperidone (9)</td>
<td>Prazosin (1), Yohimbine (1), propranolol (1), SR-59230A (0.1)</td>
<td>TTX (1), ω-conotoxin (0.1), SNX-482 (0.1), ω-agatoxin TK (0.1), L-NMMA (100)</td>
<td>Domperidone (during carbochol contraction); SCH-23390 (during electrical field stimulation)</td>
<td>Relaxation induced by DA via a D2-like receptor; Not dependent on NO or P2Y1 receptors; Not affected by adrenergic antagonists; not dependent on enteric neuronal action potential or on modulation of neurotransmitter release; SCH-23390 increased basal tone and the amplitude of the spontaneous contractions; Relaxation of bromocriptine is inhibited by domperidone</td>
</tr>
<tr>
<td>Walker et al. (85)</td>
<td>Mouse</td>
<td>Organ bath</td>
<td>Distal colon (WT and DAT-/-); Longitudinal fixation; Electrical field stimulation</td>
<td>Inhibitory</td>
<td>Dopamine (0.01–300)</td>
<td>SCH-23390 (10), sulpiride (10)</td>
<td>Not tested</td>
<td>None</td>
<td>None</td>
<td>SCH-23390/sulpiride</td>
</tr>
<tr>
<td>Fioramonti et al. (86)</td>
<td>Dog</td>
<td>Implanted Ni/ Cr electrodes</td>
<td>Duodenum and jejunum</td>
<td>Inhibitory</td>
<td>Intracerebroventricularly dopamine (10 µg/kg); Intravenous dopamine (100 µg/kg)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>SCH-23390/sulpiride</td>
<td>Decreased the duration of the migrating motor complex episodes in the small intestine 1 h before a meal compared to controls (from 9.4 to 3.4 h and 7.8 to 2.4 h in duodenum and jejunum), although intravenously (100 µg/kg) this effect was not observed</td>
</tr>
<tr>
<td>Bueno et al. (87)</td>
<td>Dog</td>
<td>Implanted strain gauge transducers</td>
<td>Ascending, traverse, descending colon</td>
<td>Inhibitory and Inducing</td>
<td>IV injections of dopamine at 1 mg/kg/min or bromocriptine 40 µg/kg</td>
<td>Haloperidol (0.2 mg/kg), Phentolamine (0.1 mg/kg), Tolazoline (2 mg/kg), Prazosin (0.2 mg/kg), propranolol (0.5 mg/kg)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Phenolamine, prazosin and haloperidol for dopamine inhibitory effect, Dopamine had a inhibitory effect on the ascending and transverse colon and a inducing effect on the descending colon MMCs; Potentially through adrenergic and dopaminergic action</td>
</tr>
<tr>
<td>Marzio et al. (88)</td>
<td>Human, healthy</td>
<td>Intestinal radiopaque tube consisting of four polyvinyl catheters with 4 side openings equally spread perfused with 1.59 ml/min with distilled water. Closure of the openings gives rise 100 mm hg/sec</td>
<td>Duodenum, proximal jejunum</td>
<td>Inducing</td>
<td>Intravenously dopamine 5 µg/kg/min for 15 min</td>
<td>Domperidone (10 mg) and sulpiride (100 mg)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Domperidone and sulpiride</td>
</tr>
</tbody>
</table>

(Continued)
TABLE 1 | Continued

<table>
<thead>
<tr>
<th>Study</th>
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<th>Tissue</th>
<th>Effect on motility</th>
<th>Tested agonists (µM)</th>
<th>Dopaminergic receptor antagonist (µM)</th>
<th>Adrenergic receptor antagonist (µM)</th>
<th>Other inhibitors</th>
<th>Effect inhibited by</th>
<th>Conclusion</th>
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<tbody>
<tr>
<td>Marzio et al. (89)</td>
<td>Human, healthy</td>
<td>Nasoduodenal probe consisting of 5 polyethylene catheters with evenly spaced openings 20 cm apart continuously perfused with 0.5 ml/min distilled water</td>
<td>Stomach, Duodenum, Proximal Jejunum</td>
<td>Inducing/Inhibitory</td>
<td>Intravenously dopamine 5 µg/kg/min for 15 min</td>
<td>Domperidon (20 mg)</td>
<td>None</td>
<td>None</td>
<td>Domperidon</td>
<td>Dopamine induced phase-III like MMCs during fed state in the small intestine, which was inhibited by domperidone, and decreased the motility of the stomach. After the phase-III MMCs a short period of complete quiescence was observed</td>
</tr>
<tr>
<td>Levein et al. (90)</td>
<td>Human, healthy</td>
<td>Paracetamol AUC; orocecal transit time</td>
<td>Mouth - &gt; Ileum</td>
<td>Inhibitory</td>
<td>Intravenously dopamine 5 µg/kg/min</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NA</td>
<td>Dopamine reduced the AUC(60 min) of paracetamol significantly, associated with a delayed gastric emptying; OCT time was significantly longer then controls indicating a delayed gastric emptying and gut motility</td>
</tr>
<tr>
<td>Dive et al. (91)</td>
<td>Human, critically ill adults under mechanical ventilation without suffering from active gastro-intestinal disease</td>
<td>Multilumen tube consisting of polyvinyl catheters with side openings, 1.5 cm apart for stomach and 10 cm apart for duodenum continuously perfused with 0.2 ml/min distilled water</td>
<td>Stomach, duodenum</td>
<td>Inhibitory/Inducing</td>
<td>Intravenously dopamine 4 µg/kg/min</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NA</td>
<td>Decreased number of contractions in the gastric antrum (only significant during fasting) and induced phase III motor activity in the duodenum (only significant during feeding)</td>
</tr>
</tbody>
</table>

*P2, the concentration that produces a 2-fold shift in the agonist concentration-response curve; **Dopaminergic antagonists**: SCH-23390, D1 receptor antagonist; Domperidone, Haloperidol, Metoclopramide, Pinzoide, Raclopride, Sulpiride, D2 receptor antagonist; cis-flupentixol, D1 and D2 receptor antagonist; **Adrenergic antagonists**: Tolazoline, Phentolamine, Prazosin, α1 adrenergic receptor antagonist; Yohimbine, α2 adrenergic receptor antagonist; Propranolol, β adrenergic receptor antagonist; SR-59230A, β3 adrenergic receptor antagonist; **Other antagonists and inhibitors**: Apamin, SKCa channel blocker; Atropine, Muscarinic receptor blocker; Carbachol, Cholinergic agonist; Charybdotoxin, IKCa channel blocker; DDA, Adenylyl cyclase inhibitor; DMPX, Adenosine A2 receptor antagonist; DPCPX, Adenosine A1 receptor antagonist; Iberiotoxin, BKCa channel blocker; L-NAME, NO synthase inhibitor; Methysergide, 5-HT receptor antagonist; MRS-1220, Adenosine A3 receptor antagonist; MRS-2179, Purinergic P2Y1 receptor antagonist; Reserpine, VMAT inhibitor; SNX-482, P/Q-type Ca2+ channel blocker; TTX, Na+ voltage-gated neural channel blocker; ω-agatoxin TK, R-type Ca2+ channel blocker; ω-conotoxin, N-type Ca2+ channel blocker.
were all inhibited by dopamine and in the latter study also by bromocriptine, attributed to dopaminergic and/or adrenergic receptors. In dogs, the gut motility of the small intestine (86) and the colon (87) was monitored in vivo using implanted electrodes. Injection of dopamine (10 µg/kg) intracerebroventricularly 1 h before a meal decreased the duration of the migrating motor complex (MMC; intestinal motility pattern of the interdigestive state) episodes in the small intestine compared to controls, although this effect was not observed when dopamine was injected intravenously (100 µg/kg) (86). In the colon, a similar inhibition was observed, although with a 10 times higher concentration of dopamine (1 mg/kg/h) injected intravenously (87). Importantly, bromocriptine had an opposite effect, where it induced the colon motility instead (87). In fasted human subjects, intravenous administration of dopamine (75 µg/kg in 15 min) induced phase-III like MMCs (last phase in the MMC cycle which consists of strong contractions to completely occlude the lumen) in the duodenum (88), which is in contrast to the previous studies in rodents (organ bath experiments) and dogs. The MMCs were similar to spontaneous phase-III MMCs, although with a slightly longer period of complete inhibition after phase-III MMCs (88). Similar results were found in terminally ill patients (91). A follow up study in humans during fed state showed that dopamine disrupted the fed state MMCs and induced phase-III like MMCs, followed by a short period of complete quiescence (phase-I like MMCs), which was inhibited by the dopamine receptor D2 blocker (DRD2) domperidone, suggesting the involvement of peripheral D2 receptors (89). Lastly, when the gut motility was investigated using orocecal transit time (OCT) and paracetamol pharmacokinetics as gastric emptying marker during intravenous injection of dopamine (90), a reduction in the AUC_{paracetamol} was observed. This suggests that dopamine causes delayed OCT time, which could be due to delayed gastric emptying and a decrease in gut motility (90). Functional studies investigating the dopamine receptors in the GI-tract of mouse showed that the dopamine receptor D2 (Drd2) is important for gut motility. Mice lacking Drd2, but not Drd3, receptor showed an increased gut transit time compared to the controls (92) suggesting that endogenous dopamine has an inhibitory effect on intestinal motility (92). The findings confirm the earlier organ bath experiments with rodent tissue. In summary, these studies (Table 1) show that in rodents and dogs the GI motility is inhibited by dopamine through dopaminergic and adrenergic receptors.

In contrast, in humans, dopamine seems to inhibit stomach motility and induce phase-III like MMCs followed by a short time of quiescence through dopaminergic receptors. A potential explanation of the discrepancy among the human and the animal studies might be the experimental setup. In rodents, dissected intestinal parts were placed in an organ bath ex vivo and in dogs electrodes were implanted on the basal side of segments of the GI-tract (86, 87). In contrast, in human studies, nasojejunal luminal-tubes consisting of catheters with side openings were fluoroscopically placed in the GI-tract and perfused with 0.2–1.59 mL/min water (88, 89, 91). The latter might induce an altered gut motility per se in a non-physiological manner. More studies should be conducted to test the effects of dopamine on the gut motility in humans, and especially in PD patients, who might already have an altered gut motility (4).

**CONCLUSIONS AND FUTURE PERSPECTIVES**

The “on”/“off” motor fluctuations in PD patients are highly dependent on the pharmacological treatment and factors contributing to its efficacy. Dietary amino acids and gut bacterial interference with levodopa treatment can contribute to the reduction of levodopa dosage absorbed in the small intestine, thereby restrict the effectiveness of the treatment. Especially luminal dopamine, which is produced by gut bacterial degradation of levodopa and is affecting the gut motility, would enhance the overgrowth of these bacteria in the small intestine and result in a vicious circle that enhances SIBO. The effect of dopamine on (small) intestinal motility, urges the investigation of the effect luminal dopamine and dopamine agonists on the gut motility of PD patients. Finally, it is crucial to accurately measure levels of SIBO in PD patients, especially in those who administer PPIs, and to diagnose other possible underlying diseases, such as hyperthyroidism. These precautions will help reduce the factors contributing to compromised levodopa bioavailability and the unwarranted side effects that result from increased frequency of dosage treatment regimen.

**AUTHOR CONTRIBUTIONS**

SK wrote the original manuscript that was reviewed and edited by SE. Funding was acquired by SE.

**FUNDING**

This research was funded by Rosalind Franklin Fellowships, co-funded by the European Union and University of Groningen.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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