Involvement of the serotonin system in L-dopa-induced dyskinesias

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Abstract

The ability of L-dopa to relieve the motor impairments in Parkinson’s disease patients declines over time and side-effects, such as dyskinesias, appear — limiting the use of the drug in the advanced stage of the disease. Serotonergic neurons are able to convert L-dopa to dopamine and to store this neurotransmitter in synaptic vesicles. This peculiarity might be very important in the advanced disease, when most of the dopaminergic neurons have degenerated. Indeed, an increasing body of evidence points to dopamine released as a false neurotransmitter from the serotonin terminals as the main pre-synaptic determinant of L-dopa-induced dyskinesias in animal models of Parkinson’s disease. These findings make the serotonin system an intriguing target for anti-dyskinetic therapies.

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1. Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compacta. This, in turn, causes a loss of dopamine (DA) release in the corpus striatum, the brain area that receives the projections from the nigral dopaminergic neurons. The loss of neurotransmitter causes severe motor symptoms, of which resting tremor, rigidity and bradykinesia are the main features [1].

Lеводопа (L-dopa) was the first drug to be introduced for the treatment of PD and is still the most effective medication for relief of motor impairments. However, this medication is, in most cases, effective only for a few years and eventually, with the progression of the neurodegeneration, the majority of patients experience motor fluctuation, shortening of the therapeutic window and dyskinesias, which limit the use of the drug in the late stage of the disease [2—5]. Currently, the only anti-dyskinetic drug available for patients is the glutamate antagonist amantadine [6]. However, this treatment is only moderately effective, has side-effects and its efficacy declines over time [7]. It is generally accepted that L-dopa acts in the early stages of the disease by being taken up into the spared DA terminals, where it is converted to DA, stored in synaptic vesicles and released in an activity-dependent manner. However, as the degeneration of nigral neurons progresses, fewer and fewer DA terminals are available for this conversion, and other cell types are believed to play a role in the decarboxylation of L-dopa in the advanced disease. Serotonergic neurons have been suggested to play an important role as (1) they express the aromatic amino-acid decarboxylase (AADC) and the vesicular monoamine transporter 2 (VMAT-2) [8—10], which are responsible for the conversion of L-dopa and storage of DA in vesicles, respectively; and (2) the serotonergic neurons of the dorsal and median raphe nuclei provide extensive innervation of the forebrain, including the striatum [11,12]. Serotonergic neurons have indeed been shown to be able to convert exogenous L-dopa to DA, and store and release DA in an activity-dependent manner in a number of experimental conditions, both in vitro and in vivo [8—17].

In addition to the serotonin system, glial cells also express AADC and have been suggested to play a part in the production of DA after exogenous administration of L-dopa [18—20].
However, in these studies, very high doses of L-dopa were used and the physiological relevance of DA formed in glial cells remains uncertain, given that these doses far exceed those used in humans.

2. The serotonin system in L-dopa-induced dyskinesias: Evidence in rats

In PD patients, the propensity of L-dopa to induce dyskinesias increases over time as the disease progresses, raising the possibility that DA is released from other cell types in an un-physiological, dysregulated manner. The presence of AADC and VMAT-2 in serotonegenic neurons makes it possible for L-dopa-derived DA to be formed, stored and released along with serotonin, thus acting as a “false transmitter” in serotonegenic terminals. Serotonegenic neurons, however, are unable to regulate DA release in a normal way. In dopaminergic synapses, the extracellular DA level is kept within a narrow physiological range through a combination of autoreceptor-mediated feedback control and re-uptake via the DA transporter. Transmitter re-uptake provides an effective mechanism for eliminating excess DA from the synaptic cleft, and the D2 autoreceptor is capable of fine-tuning release from DA terminals in response to changes in extracellular DA levels [21,22]. In the absence of these autoregulatory mechanisms, DA released from serotonin terminals is likely to generate excessive swings in extracellular DA in response to a systemic L-dopa injection [23]. Indeed, de la Fuente-Fernandez et al. [24] have shown in a recent PET imaging study that peak-dose dyskinesias in advanced PD patients are associated with excessive swings in synaptic DA after oral L-dopa administration. These synaptic swings, in turn, would cause a “pulsatile” stimulation of DA receptors on the dopaminceptive striatal neurons, which has been suggested to play an important part in the emergence of motor complications. In fact, more continuous administration of L-dopa and DA agonists — such as duodenal or subcutaneous infusion by pumps — is less likely to induce these side-effects [25–27]. The oral, intermittent administration of L-dopa and the lack of feedback control for the release of DA in serotoninergic neurons might therefore contribute to the emergence of the motor complications. In particular, they might be the triggering element of changes — at the post-synaptic side on the dopaminceptive striatal neurons — that have been associated with dyskinesias [28].

We have recently demonstrated that DA released as a false neurotransmitter from serotonegenic neurons is responsible for L-dopa-induced dyskinesias in 6-hydroxydopamine (6-OHDA)-lesioned rats [23]. Thus, toxic lesion of the serotonergic neurons by intraventricular injection of 5,7-dihydroxytryptamine or pharmacological blockade of DA release from the same neurons by selective 5-HT1A and 5-HT1B autoreceptor agonists produced a near-complete suppression of the abnormal movements induced by chronic treatment with L-dopa. In line with these results, Tanaka et al. [17] have shown that serotonin lesions can reduce L-dopa-derived extracellular DA in the striatum by up to 80%, as measured by microdialysis. This group [29] has also reported a similar decrease in extracellular DA levels after co-administration of L-dopa with the 5-HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetratin (8-OHDPAT), as well as inhibition of rotational behavior [30]. More recently, using the same animal model as we use in our group [31,32], Eskow and co-workers [33] have shown that the partial 5-HT1A agonist buspirone reduces the expression and development of L-dopa-induced dyskinesias.

Overall, these results provide evidence that serotonegenic neurons have a central role in the induction of L-dopa-induced dyskinesias in the rat model of PD. In addition, we have recently shown that the content of serotonin in striatal tissue is reduced by about 50% 1 h after administration of L-dopa (at a dose of 6 mg/kg) as measured by HPLC [23]. This finding confirms earlier observations in mice injected with a high dose of L-dopa (100 mg/kg) [34]. The ability of L-dopa-derived DA to displace the endogenous serotonin from the serotonin terminals suggests that L-dopa-derived DA and serotonin compete for storage in synaptic vesicles in the serotonegenic neurons. This depletion in serotonin content is likely to induce an over-activation of serotonin terminals in order to compensate for the reduced binding of neurotransmitter to the presynaptic serotonin autoreceptors. This, in turn, would add further to the excessive release of DA from these neurons, triggering the abnormal movements.

This view has gained further support by our recent finding that transplants of fetal serotonegenic neurons into the striatum of 6-OHDA-lesioned rats exacerbate L-dopa-induced dyskinesias by up to 70% compared with pre-transplantation scores [35]. The serotonegenic neurons express at least three sub-types of autoreceptors, among which 5-HT1A and 5-HT1B are the most abundant. 5-HT1A receptors are present at the cell body level, as well as on the dendrites, in the dorsal and median raphe nuclei, where they regulate the firing of the serotonegenic neurons [36,37]. 5-HT1B receptors, by contrast, are more abundant at the terminal level in the areas innervated by the serotonin system, including the striatum, where they serve to control the terminal release of the neurotransmitter [38–40]. Together, these two main classes of autoreceptor are able to fine-tune the synaptic release of serotonin in the forebrain target regions [38,39,41].

It is intuitive that drugs acting as serotonin autoreceptor agonists, dampening the release of serotonin, should also decrease the release of L-dopa-derived DA from the serotonin terminals, since the two transmitters co-localize in the same synaptic vesicles after peripheral administration of L-dopa [8–10]. Interestingly, we have discovered a potent synergistic action between the 5-HT1A agonist (±)-8-OHDPAT and the 5-HT1B agonist CP-94253 in blocking L-dopa-induced dyskinesias in our parkinsonian rats. Thus, sub-threshold doses of the two compounds, which individually produced either no effect or only a mild effect, completely suppressed dyskinesias when administered together. We can speculate that the differential location of the two autoreceptors on the serotonegenic neurons plays an important role in this synergistic effect. Regardless of the underlying mechanism, this synergistic action might have interesting clinical applications and deserves further investigation. As the next step, we are now planning...
to test the feasibility of this approach in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys.

3. The serotonin system in l-dopa-induced dyskinesias: Evidence in primates

The MPTP-lesioned monkey model of PD shares several features with the human disease and is considered to be the best animal model to test the ability of drugs to reduce l-dopa-induced dyskinesias. Whether the serotonin system plays the same role in determining l-dopa-induced dyskinesias in primates as in rodents remains an open question. However, Irvani et al. [42] have recently shown a decrease in dyskinesias in MPTP-treated marmosets administered the 5-HT1A agonist (+)-8-OHDPAT. In addition, the same group previously found a similar effect using a 5-HT1B/1D receptor agonist [43]. In these reports, however, the anti-dyskinetic effect was accompanied by a reduction in the therapeutic effect of l-dopa. This raises the possibility that, in MPTP-treated primates, the therapeutic effect of l-dopa is dependent on DA release from serotonergic neurons, and that the increased parkinsonism, as well as the reduction in dyskinesias, observed after 5-HT1A agonist treatment might be due to the reduced DA release from the serotonergic neurons. This, however, cannot be the only explanation as Iravani and co-workers [42] have found a similar reduction in the motor response induced by the D2/3 direct agonist pramipexole after co-administration with (+)-8-OHDPAT. The decreased anti-parkinsonian effect of l-dopa might therefore be due, at least in part, to the high doses of 5-HT1A agonists that are necessary to obtain a significant anti-dyskinetic effect when the agonists are given alone. Indeed, high doses of these compounds are known to activate not only the pre-synaptic receptors, but also the same receptor located post-synaptically on other neurons such as the cortical glutamatergic cells [44–48]. Activation of these post-synaptic receptors has been linked in rodents to the so-called serotonin syndrome, characterized by flat body posture, reciprocal forepaw treading and head weaving [49–53]. Irvani et al. [42] observed a similar behavior after administering 8-OHDPAT in MPTP-lesioned marmosets, which may have contributed to the appearance of dystonia in their animals. In contrast, BIBBIANI et al. [54] have shown that the 5-HT1A partial agonist sarizotan reduced dyskinesias without any significant worsening of the parkinsonian symptoms. This finding might point to partial, rather than full, 5-HT1A agonists as a better choice for this type of pharmacological approach. Alternatively, these discrepancies could also be explained by a different magnitude of DA depletion in the two experimental paradigms. Indeed, different MPTP lesion protocols can result in different degrees of DA depletion. Thus, preservation of partial DA innervation can have profound consequences on the therapeutic effect of l-dopa when DA release from serotonergic neurons is dampened. The 5-HT1A agonist sarizotan has also been tested in recent clinical trials in PD patients. At doses that significantly reduced dyskinesias, this drug also induced side-effects, including worsening of parkinsonism [55], whereas at the low doses employed in the phase III trial the treatment was uneffective (NCT00105521; see Merck website at http://media.merck.de). Sarizotan has, in addition to its action on 5-HT1A receptors, antagonistic properties on DA2/3 receptors, which may account in part for the side-effects observed [56]. It should also be noted that the patients included in the trial by Olanow et al. were at an advanced stage of the disease. It is therefore possible that few DA terminals were available for the conversion of l-dopa to DA in these patients and that, at this stage, the main source of DA might have been derived from serotonergic neurons. If so, a complete silencing of the release from serotonin terminals would be expected to reduce not only l-dopa-induced dyskinesias, but also the therapeutic effect of the drug as observed in MPTP-treated monkeys. This suggests that patients with spared DA innervation in the striatum might benefit more from this approach, as the therapeutic effect of l-dopa would be sustained by DA released from the spared DA terminals.

The advantage of targeting both types of serotonin autoreceptors by a combination of 5-HT1A and 5-HT1B agonists relies on the possibility of being an effective treatment for dyskinesias at very low doses of the drugs, which mainly act on the pre-synaptic serotonin receptors (see [23] for further details), thus avoiding side-effects due to the activation of heteroreceptors on the post-synaptic side (i.e. induction of components of the serotonin syndrome).

4. Conclusions

In conclusion, an increasing body of evidence points to the role of DA released from serotonergic neurons as the main pre-synaptic determinant of l-dopa-induced dyskinesias in the rat PD model. Dysregulated DA release, giving rise to excessive swings in synaptic levels of DA, would in turn trigger the post-synaptic maladaptive changes on the dopaminergic neurons that have been associated with l-dopa-induced dyskinesias. Such post-synaptic modifications, altered D1 and glutamate signalling in particular, would eventually be responsible for the cascade of changes in gene expression that is associated with the appearance of the abnormal movements in 6-OHDA-lesioned rodents [57]. 5-HT1A and 5-HT1B receptor agonists, particularly in combination, have been shown to be highly effective in counteracting l-dopa-induced dyskinesias in rats. With further evidence from MPTP-treated monkeys, it would be intriguing to investigate the efficacy of this treatment in PD patients.

These findings also have implications for DA replacement therapy using cell transplantation. Indeed, fetal midbrain tissue preparations used for grafting are known to contain serotonergic neurons to varying degrees, depending on the landmarks used for dissection and the age of the donor fetus. Accordingly, with the described role of DA released from the serotonin terminals, serotonergic neuron transplants exacerbate l-dopa-induced dyskinesias in the rat PD model and 5-HT1A/1B agonists suppress the abnormal movements in these animals [35]. The present observations suggest that in future clinical trials more attention should be paid to avoid inclusion of serotonergic neurons in the graft cell preparation in order to
provide the maximal functional efficacy with the absence of detrimental effects such as l-dopa-induced dyskinesias.

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References


