Somatodendritic dopamine in the substnatia nigra of the rat. Biochemical charachterization and effects of dopaminergic agents

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Dopaminergic neurons of the substantia nigra do not only release dopamine from nerve terminals in the striatum, but also from soma and dendrites in substantia nigra. Although the concept of release of somatodendritic dopamine in the substantia nigra arose nearly twenty five years ago, many questions regarding its biochemistry and its role within normal and pathological basal ganglia remain (Geffen et al., 1976; Korf et al., 1976). This relative lack of knowledge is, at least partly, related to technical difficulties; the extracellular level of dopamine in somatodendritic areas is about 6 times lower as compared to the striatum, a nerve terminal area. In this thesis, an attempt has been made to characterize the storage and release of somatodendritic dopamine in the substantia nigra, and to elucidate its role within the basal ganglia circuitry. The role of somatodendritic dopamine release in the pathophysiology of disabling motor syndromes, like Parkinson's disease and neuroleptic induced tardive dyskinesias, was investigated by using animal models. These studies will increase our understanding of basal ganglia pathology and might lead to more effective (pharmaco)therapeutic strategies in the future.

Chapter 2 of this thesis describes an extensive study on the nature of storage and release of somatodendritic dopamine. At the dopaminergic nerve terminal in the striatum, the physiological release of dopamine generally is accepted as occurring via depolarization-induced exocytosis (Westerink and DeVries, 1988). Based exclusively on indirect indices, the storage and release of somatodendritic dopamine has been suggested to be non-vesicular in nature (Elverfors and Nissbrand, 1991). We directly examined, by using in vivo microdialysis, whether the storage and/or release of somatodendritic dopamine is dependent upon a vesicular pool of transmitter. The storage of dopamine appeared to be vesicular in nature in all brain areas examined, since reserpine, a compound that disrupts vesicular sequestration of monoamines, significantly decreased the tissue levels of dopamine in substantia nigra reticulata, substantia nigra compacta and striatum. Administration of reserpine also resulted in a rapid decrease of extracellular dopamine in substantia nigra as well as in the striatum, both to non-detectable levels. In order to further characterize and compare the intracellular compartmentalization of somatodendritic dopamine versus nerve terminal dopamine, we also employed microdialysis to determine the effects of reserpine pretreatment on amphetamine-stimulated dopamine in substantia nigra and striatum. In contrast to the effects of a low dose of amphetamine, dopamine efflux in response to a high dose of amphetamine was significantly attenuated by reserpine pretreatment both in substantia nigra and in the striatum. Previous studies have shown that a low dose of amphetamine only induces release of the cytoplasmic store, while a high dose also releases from vesicular stores. Thus, our data support the idea of vesicular storage of somatodendritic dopamine, similar to nerve terminal dopamine.

In chapter 3 and 4, we examined both regulation and effects of the release of somatodendritic dopamine within the intact basal ganglia circuitry. Chapter 3 utilizes the microdialysis technique in order to examine whether somatodendritic autoreceptors in the substantia nigra are involved in the regulation of the release of somatodendritic dopamine. Contrary to the expectations, local blockade of nigral dopamine D2 receptors by haloperidol failed to increase the extracellular levels of nigral dopamine. This haloperidol treatment abolished the decrease in extracellular levels of dopamine induced by local perfusion of the D2 agonist quinpirole, thus demonstrating effective blockade of D2 receptors. These observations suggest that the release of somatodendritic dopamine is not under tonic inhibitory control, at least under basal conditions. The results were verified by systemic administration of haloperidol. This treatment, however, resulted in a dose dependent increase of extracellular dopamine in substantia nigra. A similar increase was observed after microinjection of haloperidol in the striatum, suggesting that blockade of striatal dopamine D2 receptors, rather than D2 receptors in substantia nigra, could underlie the effect caused by systemic administration of the drug. Our observation that the effect of intrastriatal microinjection of haloperidol was not altered by simultaneous administration of haloperidol via the systemic route, is in agreement with this hypothesis. Besides these direct conclusions, the studies could also shed light on the relationship between neuronal firing and release of somatodendritic dopamine. Gonon and coworkers have shown that the release of *nerve terminal* dopamine depends, among others, on changes in the firing pattern of dopaminergic neurons. Whether a similar mechanism also underlies the release of somatodendritic dopamine is questionable, at least under the current experimental conditions. Administration of 0.05 mg/kg haloperidol did not alter the release of somatodendritic dopamine in substantia nigra. However, other studies using similar experimental conditions have shown that this dose causes a maximal increase in striatal dopamine release, an effect generally accepted to reflect autoreceptor blockade. A ten times higher dose, 0.5 mg/kg, was necessary to cause an increase in somatodendritic dopamine release. This effect is likely to be explained by blockade of heteroreceptors; Skirboll and collegues demonstrated that heteroreceptors might be ten times less sensitive as compared to autoreceptors. Thus, under the present experimental conditions, the basal release of somatodendritic dopamine may not be under influence of substantia nigra neuronal firing, but instead under influence of nigral afferent projections. Evidence for functional compartmentalization of dendritic and axonal spiking regions on dopaminergic neurons, which may differentially utilize Na^{+} and Ca^{++} as charge carriers, may support such a differential release mechanism for the release of somatodendritic dopamine (Llinás et al., 1984; Nedergaard et al., 1988; Grace, 1990).

Because a "dopamine-acetylcholine imbalance" is likely to play a role in basal ganglia pathology, the dopaminergic regulation of striatal acetylcholine has been studied extensively. Chapter 4 addresses the question whether striatal or nigral D1 receptors, the latter being under influence of somatodendritic dopamine, are involved in the inhibitory effect of direct

dopamine agonists on striatal acetylcholine release. Therefore, the dose-related effect of the direct dopamine D2 agonist quinpirole on the extracellular concentration of dopamine in substantia nigra and striatum *and* of acetylcholine in the striatum were measured using *in vivo* microdialysis. If the inhibitory effect of systemic administration of quinpirole would be due to a diminished stimulation of D1 receptors, a low, autoreceptor sensitive dose of quinpirole would be expected to cause an inhibition of the release of dopamine and of acetylcholine. In contrast, we observed an increase of the extracellular levels of acetylcholine in the striatum by a low dose of quinpirole. These data argue strongly in favor of a directly mediated inhibition of striatal acetylcholine by dopamine D2 receptor agonists. The data also elucidate some issues concerning the autoregulation of somatodendritic dopamine in comparison with nerve terminal dopamine. Perhaps one of the most remarkable differences is the maximum decrease of dopamine release in substantia nigra and striatum after administration of systemic quinpirole; dopamine levels in substantia nigra decrease to 50% of basal levels, whereas striatal dopamine decreases to 20% of basal levels. Thus, autoregulation of somatodendritic dopamine release might be less important when compared to release of dopamine from nerve terminals. The lack of endogenous tone at D2 receptors in the substantia nigra, as suggested by the failure to increase local dopamine levels by intranigral administration of D2 antagonists in chapter 3, supports this hypothesis.

The concept of a dopaminergic involvement in motor behavior arose from two important clinical observations nearly three decades ago. First, Birkmayer and Hornykiewicz observed a dopamine deficiency in the striatum of parkinsonian brains. This finding led to treatment with the dopamine precursor levodopa, which appeared to diminish the symptoms of this motor disease. Secondly, the discovery that (typical) antipsychotics, which treatment is accompanied by extrapyramidal motor symptoms, are in fact dopaminergic antagonists also suggested that an intact dopaminergic neurotransmission is essential for normal motor behavior (Burt et al., 1977). Since these key observations, many studies have been performed in an attempt to elucidate the role of dopaminergic neurotransmission in normal motor behavior and in particular in motor disease. In chapter 5 and 6, the dopaminergic system is examined in *animal models* of Parkinson's disease and antipsychotics-induced tardive dyskinesias, respectively. Levodopa-induced contralateral turning in 6-OHDA-lesioned rats, the animal model used in chapter 5, has been widely used as a behavioral testing paradigm for the potency of anti-parkinsonian agents (Ungerstedt and Arbuthnott, 1970). In order to know whether enzyme inhibition may potentiate levodopa-induced contralateral turning, and thus possibly increase the therapeutic benefit of levodopa in humans, enzyme inhibitors involved in the metabolic inactivation of levodopa or dopamine were co-administered. Inhibition of MAO-A with Ro 41-1049 potentiated levodopa-induced contralateral turning, in contrast to MAO-B-inhibition which only increased the duration of turning but not the total number of turns. Indeed, MAO-A appears to be, at least in the rat, the predominant subtype for oxidative deamination of

endogenous dopamine and levodopa derived dopamine (Kato et al., 1986; Butcher et al., 1990; Colzi et al., 1990; Mannisto and Toumainen, 1991; Wachtel and Abercrombie, 1994). COMT-inhibition with Ro 40-7592 resulted in the largest potentiation of levodopa-induced turning when compared to inhibition of MAO, which suggests a potential role for COMT-inhibitors as adjuvants to the levodopa therapy in the treatment of Parkinson's disease. The site of action of the COMT-inhibitor is not yet known. Earlier studies have shown that Ro 40-7592 might not only inhibit the metabolic inactivation of levodopa in the periphery, but also of dopamine in the striatum (Acquas et al., 1992). However, the observation that turning behavior can be elicited by an increase in the amount of dopamine in the substantia nigra, together with a major role of COMT in the catabolism of nigral dopamine, suggests a contributary role of somatodendritic dopamine to be likely (Westerink and Korf, 1976; Robertson et al., 1991; Robertson and Robertson, 1988, 1989; Kelly et al., 1999). Ro 40-7592, known as tolcapone, was registered in 1997 for the treatment of Parkinson's disease.

The potential role of somatodendritic dopamine in the mediation of antipsychotics-induced tardive dyskinesias is examined in chapter 6, using the vacuous chewing movement (VCM) animal model. Long term treatment with antipsychotics results in tardive dyskinesia in some, but not all individuals. Such an individual susceptibility is also observed in rats, a characteristic exploited in the VCM-model. After long-term treatment with the antipsychotic haloperidol, some rats exhibit vacuous chewing movements (VCM-positive), while others fail to show these dyskinesias (VCM-negative). Since previous neuroanatomical studies have shown that the occurrence of dyskinesias correlates with specific decreases in dopamine D1 receptors in the substantia nigra, we hypothesized a potentiation of the release of somatodendritic dopamine in the VCM-positive group. However, our results showed that both basal release and the release induced by an acute haloperidol challenge or tail-pinch stress were unaltered in the VCM-positive group. In contrast, the effect of these challenges on the release of striatal acetylcholine appeared to be significantly potentiated in the VCM-positive group. The basal levels of striatal acetylcholine showed a tendency to increase in VCM-positive rats, which did not reach significance. The exact way in which these changes in cholinergic transmission are brought about is not exactly known. Several lines of evidence suggest that an enhanced glutamatergic drive by cortical efferents might play a role (Yamamoto and Cooperman, 1994, Roberts et al., 1995). Since previous studies have shown neurochemical correlates of the occurrence of VCMs in both substantia nigra and thalamus, these brain areas are also likely to be involved (Shirakawa and Tamminga, 1994). Recently, it was shown that striatal acetylcholine is under excitatory control of somatodendritic dopamine, a proces depending on striatal glutamatergic afferents. In this way, alterations in the nigrothalamocortical circuitry might underlie the enhanced release of striatal acetylcholine rather than a potentiated release of somatodendritic dopamine in the substantia nigra (Abercrombie and DeBoer, 1997).

Taken together, the present thesis characterizes the release of somatodendritic dopamine in the substantia nigra, and elucidates the mechanisms underlying the effects of dopaminergic agents

in both normal basal ganglia circuitry and animal models of basal ganglia diseases. The main conclusion that can be drawn from the results of this thesis, is that the pharmacological profiles of antiparkinsonian and antipsychotic agents are not only determined by dopaminergic receptors in nerve terminal areas like the striatum, but also by dopamine receptors in the substantia nigra: the targets of somatodendritic dopamine.

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