

## LECTURES

### INSIDIOUS DOPAMINE: PROVOCATEUR OR PROTECTIVE AGENT IN PARKINSON'S DISEASE?

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As the most efficacious drug for treating Parkinson's disease, levodopa (L-DOPA), is oft viewed as a generator of reactive oxygen species (ROS), there is a question as to whether L-DOPA might accelerate progression of Parkinson's disease. In this context we first review the potential of both L-DOPA and dopamine (DA) to generate ROS (including catecholquinones) in the presence of iron and melanin in the substantia nigra. Next, we summarize some of the actions of DA and DA agonists in animals that we have used to model Parkinson's disease. Finally, we present our findings in rats which demonstrate neuroprotective roles for DA and L-DOPA as suppressors or sequestrators of hydroxyl radical (HO<sup>•</sup>) in DA-denervated neostriatum, the target tissue for nigrostriatal DA neurons.

Within the substantia nigra, L-DOPA undergoes autoxidation to *o*-quinones in the process of formation of neuromelanin, a marker for these DA neurons in primates. Neuromelanin has been proposed to be both an inactivator of *o*-quinones and a sink for ROS. In the presence of iron, known to occur at high concentration in the substantia nigra, a battery of ROS are spontaneously formed: hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide (O<sub>2</sub><sup>•-</sup>), and HO<sup>•</sup>. These, along with iron, promote formation of catechol semiquinones (<sup>•</sup>QH) and quinones (Q) from endogenous DA or administered L-DOPA. Survival

of the substantia nigra DA neurons is thus determined by the effectiveness of neuronal cytoprotective enzymes (catalase, superoxide dismutase, glutathione peroxidase and DT-diaphorase), reduced glutathione (GSH) levels, and sequestration of ROS by neuromelanin.

In the studies in rats in which the substantia nigra is largely destroyed and in which the neostriatum is almost totally DA-denervated by 6-hydroxydopamine (6-OHDA) treatment, we have found that some DA receptors become overtly supersensitized while other DA receptors display latent supersensitivity which is unmasked by repeated agonist treatment (priming). Overt supersensitization is notable for DA D<sub>1</sub> receptors but not for D<sub>2</sub> receptors. However, if the balance between DA and serotonin (5-HT) neurons is altered, then D<sub>2</sub> receptors also become overtly supersensitized. Despite their common origin in the brainstem, 5-HT and DA neuronal terminals substantially modulate sensitivity status of the other transmitter receptors. In fact, much of the effect of DA receptor supersensitivity is mediated by the 5-HT system. These findings indicate the intricacies of coordinate neuronal systems, particularly when one of the neuronal systems (DA neurons) is compromised, and demonstrate the inherent safeguards in the brain, allowing one neuronal system (5-HT) to replace a destroyed (DA) system in an attempt to preserve function.

Finally, we focus attention specifically on DA- and L-DOPA-actions in DA-denervated neostriatum of rats. By analyzing HO<sup>•</sup>, the most reactive of the ROS, we have determined that HO<sup>•</sup> levels increase up to 5-fold in the neostriatum in which DA content has been reduced by 99% by 6-OHDA treatment. This finding would seem to indicate that DA terminals normally exert a neuroprotective effect on this target field. Moreover, L-DOPA treatment further reduces HO<sup>•</sup> level in DA-denervated neostriatum, indicating that this antiparkinsonian drug also subserves a neuroprotective function in the target field of nigrostriatal neurons.

In conclusion, the series of studies appear to suggest that DA and L-DOPA have a neurotoxic potential in the substantia nigra, and that this is counterbalanced by the cytoprotective status of these neurons at any particular time. In contrast, in

the target field of the substantia nigra, namely the neostriatum, DA has a neuroprotective role, and exogenously administered L-DOPA is able to subserve this function in the neostriatum that is DA-denervated. The major adaptive response to the substantia nigra degeneration and concomitant DA-denervation of basal ganglia, entails supersensitization of receptors to DA *per se* and alternation of neurochemical systems (e.g. serotonergic) which have the capacity to preserve function. Further studies into these processes and mechanisms are expected to lead to a better understanding of the complex role played by dopaminergic neurons, in relation to Parkinson's disease.

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## COLOSTRININ AND COLOSTRININ-DERIVED NONAPEPTIDE (COLOSTRAL-VAL NONAPEPTIDE, CVNP) FACILITATE LEARNING AND MEMORY IN RATS

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Colostrum, the early milk produced during puerperal period, plays an essential role in surviving of the newborns, and is the main source of immunological defense in the first period of life. One of immunologically active agents from ovine colostrum was isolated by Janusz et al. [9–11] and was characterized as a 18 kD polypeptide, built of three 6 kD subunits, rich in proline (22%) and non-polar amino acids. The preparation was subsequently found to be rather a complex of proline-rich polypeptides (PRP). The polypeptide complex, named colostrinin, was characterized as a new cytokine [8]. Recently, the immunoactive properties of the human analog of ovine colostrinin were described [5, 14]. Colostrinin induces maturation and differentiation of murine thymocytes and affects humoral

and cellular immune reactions, both *in vivo* and *in vitro* [9, 21].

Among the most prominent consequences of the normal and pathological aging in humans are cognitive deficits including learning impairment and delayed amnesia. Perhaps the most severe form of the senile dementia is Alzheimer's disease, which is associated with numerous pathophysiological alterations of the CNS. Pathophysiological changes in Alzheimer's disease patients include, among other things, accumulation of beta-amyloid plaques and neurofibrillary tangles [6]. In addition to the profound impairment in cholinergic transmission, alterations in the function of cytokines and other factors associated with immune reactions have been reported [1–3], (see [4] for the recent review).

The possibility that immunological factors play a key role in Alzheimer's disease was investigated in the double blind, placebo-controlled clinical trial. As measured by the Mini Mental State score and the recent memory test, 11 out of 16 patients undergoing 3–6 cycles of therapy consisting of oral administration of colostrinin (100 g every second day in one cycle lasting for 3 weeks) significantly improved their cognitive functioning [13].

The experiments conducted in our laboratory evaluated the ability of colostrinin to affect spatial learning (Morris water maze) and incidental memory (habituation test) in male Wistar rats of two age groups [15]. Colostrinin, at a dose of 4 g/rat *ip*, facilitated acquisition of spatial learning of 13- (aged) but not 3-month old (young) rats. At the same dose, colostrinin improved incidental learning in aged rats, while the dose of 20 g/rat attenuated it. Colostrinin did not change locomotor activity of rats.

After digestion of colostrinin with chymotrypsin, a nonapeptide fragment of mol. wt. 1000, with a sequence of Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro was isolated [20]. This Colostral-Val nonapeptide (CVNP), both isolated from chymotryptic digest as well as obtained by the chemical synthesis [22], showed the full spectrum of immunotropic activities of colostrinin. CVNP showed a strong effect on the primary and secondary immune response against SRBC (T-cell dependent antigen) in mice [12]. Whereas colostrinin at concentrations of 1–100 g/ml induced production of interferon and tumor necrosis factor alpha in human peripheral blood leukocytes as well as in the whole blood cell cultures, CVNP was considerably less active as a cytokine inducer. Thus, significant levels of interferon and tumor necrosis factor were induced by 100 g/ml of CVNP but lower concentrations were found to be ineffective [7]. Similarly, it was also reported that cytokine-inducing activity of CVNP in cultures of human whole blood cells was considerably lower than that of colostrinin [23].

The cognitive effects of CVNP (100 g/kg) were also studied in aged male Wistar rats that, *per se*, demonstrated learning deficits.

Administered for 14 days, CVNP did not affect the acquisition of spatial learning or memory retrieval in the Morris water maze, suggesting initially that it produced no cognitive effects [16]. In the second part of the experiment, as a result of reversal learning, placebo-treated rats shifted search-

ing behavior and swam less in the area of original, and more in the area of recent platform position, suggesting formation of the new spatial map. Surprisingly, CVNP-treated rats did not change the searching pattern and still investigated the area that contained "original" escape platform, suggesting that CVNP treatment delays the extinction of spatial memory. In another experiment, CVNP administered for 8 days did not influence the acquisition of the active avoidance task, but significantly delayed its extinction.

Altogether, the present findings indicate that colostrinin facilitates the acquisition of spatial and incidental memories of aged rats and that colostrinin-derived nonapeptide delays the extinction of long-term memories, both effects being observed in aged subjects. These beneficial effects on cognitive functioning are consistent with early clinical observations in patients suffering from Alzheimer's disease.

At present, it is difficult to propose the mechanism of action of colostrinin or CVNP. It cannot be excluded that its immunomodulatory effects [7, 12, 23] may be of importance. For instance, in a rodent model of AIDS, Sei et al. [19] demonstrated that spatial learning is impaired in mice suffering profound deficits in immunological responses. Converging lines of evidence indicate the importance of immunological factors in the pathogenesis of Alzheimer's disease [4]. Since CVNP induces interferon gamma and is regarded as a cytokine, these factors may play a role in the mechanism of its promnestic effects. Findings demonstrating that interferon gamma inhibits production of the Alzheimer's amyloid beta precursor protein [17, 18] are in favor of this idea. However, whether or not the immunomodulatory effects of colostrinin-derived nonapeptide are of importance for the improvement of cognitive functions in rats, remains to be established.

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## ANTIDEPRESSANTS WITH DUAL ACTION – PROGRESS IN THE TREATMENT OF DEPRESSION?

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Classic tricyclic antidepressants (TCAs), which remain still a standard treatment of depression, are called by pharmacologists “dirty drugs” because of their complex (“unclean”) mechanism of action, related with several neurotransmission pathways. This complex mechanism of action results in the appearance of numerous side effects. Basic TCAs are highly effective and imipramine remains still a “golden standard” in evaluation of clinical efficacy of new drugs.

One of the main directions in the progress of modern psychopharmacology of depression was development of selective drugs acting on neurotransmission, and particularly on one specific neurotransmitter system (NA or 5-HT). Introduction of such drugs in clinical practice is linked to the question on basic mechanisms of pathogenesis of depression and the mechanisms of antidepressants ac-

tion (which neurotransmission is disturbed: NA or 5-HT?), and to the question on efficacy of selectively acting antidepressants. Those problems remain still to be solved, and data from bibliography are contradictory. It is obvious that selective antidepressants (especially SSRI) are safer than TCAs, but do they have the same efficacy?

The new solution for the abovementioned question can be offered by the implementation of new group of antidepressants with double action on both NA and 5-HT systems, but without influence on other types of neurotransmission (especially on DA, ACh, H<sub>1</sub> and H<sub>2</sub> receptors). This interesting and promising group of drugs needs further clinical trials and evaluation in broad clinical practice, especially in comparison with selectively acting antidepressants.