CHANGES IN STRIATAL DA METABOLISM INDUCED BY CHOLINERGIC AND ANTI-CHOLINERGIC NIGRAL STIMULATION IN THE RAT

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INTRODUCTION

NUMEROUS reports have demonstrated the existence of an ascending dopamine (DA) nigro-neostriatal pathway (see review of HEDREEN and CHALMERS, 1972). On the other hand, high levels of choline acetyl transferase were found in the neostriatum and could also be detected in the substantia nigra (SN) (FAHN and COTÉ, 1968). Furthermore, confirming the anatomical existence of a descending caudato-nigral pathway (SZABO, 1962) with the help of electro-physiological procedures, FRIGYEZIE and PURPURA (1967) reported that caudate cells influence nigral cells activity. Since a histochemical study revealed the presence of cholinesterasic striato-nigral efferents (OLIVIER *et al.*, 1970), a cholinergic system was presumed to act on the DA nigral cells and to regulate the activity of the DA neurons (CORRODI *et al.*, 1972). The finding of SMELIK and ERNST (1966) support such a mechanism. These authors reported that cholinergic stimulation of the SN activated the DA nigro-neostriatal neurons and concluded to the existence of cholinergic fibers ending on the DA nigral cells.

Therefore, besides the striatal acetylcholine (ACh) and DA balance involved in extrapyramidal motor control (HORNYKIEWICZ, 1971) there seem to exist interactions between DA and ACh neurons at the level of the SN. In view of these data, it was interesting to investigate if chemical stimulation of the compacta layer of the SN by muscarinic (carbachol) or antimuscarinic (atropine) drugs rapidly affected DA metabolism in DA nerve endings of the NCP (caudate nucleus + putamen).

RESULTS

Experiments were performed on male Charles Rivers rats (250 g) positioned in a stereotaxic apparatus, and anaesthetised with a mixture of Halothane, oxygen and nitrousoxide. The pars compacta of the SN was infused bilaterally during 10 min with a physiological solution (2 μl of NaCl 9%). The solution administered in the right SN (treated side) contained either carbachol or atropine at $5 \cdot 10^{-4}$ M. The left side (sham operated side) was used as a control nigro-neostriatal system. Five-min after the onset of the nigral infusions a pulse injection 164 μCi of L-3·5-^{3}H-tyrosine (49 Ci/mM, Amersham) was performed intravenously. The animals were killed 10 min later (15 min after the beginning of the drug infusion). The NCP of the treated and control systems were analysed separately (JAVOY *et al.*, 1972). The values are the mean of groups of eight animals.

The administration of carbachol induced a small but significant increase in DA levels in the ipsilateral NCP when compared to the control side whereas atropine

injection was ineffective (DA μg/g: control: 7·0 $\pm$ 0·2; carbachol: 7·9 $\pm$ 0·3, $P < 0{\cdot}05$; atropine: 7·5 $\pm$ 0·2). In addition, both drugs increased the initial accumulation of newly synthesised ^{3}H-DA in the NCP corresponding to the treated nigro-neostriatal system (^{3}H-DA mμCi/g: control: 35 $\pm$ 1; carbachol: 57 $\pm$ 3 $P < 0{\cdot}001$; atropine: 48 $\pm$ 5 $P < 0{\cdot}05$). The effect was more pronounced after carbachol (60%) than after atropine (35%). Specific activity of tyrosine was not changed. Therefore, both drug treatments affected striatal DA metabolism although the mechanisms involved seemed to differ.

After either pharmacological treatment, DA synthesis was estimated with a method adapted from the experimental approach originally described by CARLSSON *et al.* (1972). The initial rate of accumulation of ^{3}H-DOPA(L-3,4-dihydroxyphenylalanine) synthesised from L-3·5-^{3}H-tyrosine was estimated after inhibition of central DOPA-decarboxylase (aromatic amino acid decarboxylase) with RO 4-4602 [N-(DL-seryl-*N*-2,3,4-trihydroxybenzyl)hydrazine] (800 mg/kg) intraperitoneally. Rats were injected with RO 4-4602, 15 min before the beginning of the nigral infusion with carbachol or atropine. The injection of L-3·5-^{3}H-tyrosine was performed as in previous experiments, 5 min after the onset of the 10 min nigral infusions. Rats were sacrificed 10 min after the injection of the labelled amino-acid. RO 4-4602 pretreatment inhibited the formation of ^{3}H-DA. The accumulation of ^{3}H-DOPA in the NCP was unaffected by carbachol application on the contrary atropine treatment increased the levels of ^{3}H-DOPA in the corresponding NCP when compared to normal (^{3}H-DOPA nCi/g: control: 26·5 $\pm$ 2·4; carbachol: 30 $\pm$ 3; atropine: 33·2 $\pm$ 1·7 $P < 0{\cdot}05$). Thus the anticholinergic drug, through its action at the SN level, stimulates the first step of DA synthesis in the ipsilateral NCP. The cholinomimetic drug is uneffective on this process.

Simultaneously in these experiments, the rate of DA disappearance after inhibition of the amine synthesis with RO 4-4602 was used as an index of the rate of striatal utilization. Such a treatment, induces a (25%) decrease in striatal DA levels (5·9 $\pm$ 0·3 μg/g $P < 0{\cdot}01$) within 30 min when compared to untreated rats (7·8 $\pm$ 0·4 μg/g). When carbachol was applied in the SN 15 min after the injection of RO 4-4602 striatal DA levels in the treated side were 15% higher (6·8 $\pm$ 0·2 μg/g $P < 0{\cdot}05$) than those of the sham operated side. In the contrary after atropine treatment DA disappearance was enhanced, since DA levels in the ipsilateral side (5·3 $\pm$ 0·08 μg/g $P < 0{\cdot}02$) were significantly lower than those of the contralateral NCP. These effects observed in a short-time interval (15 min) suggest that DA utilisation in the NCP was counteracted by carbachol and stimulated by the atropine nigral infusion. Therefore, the increased DA and ^{3}H-DA levels seen in the NCP after carbachol nigral infusion in animals untreated with the synthesis inhibitor, most likely reveal a reduced rate of DA utilisation. On the other hand, the unchanged DA levels associated with an increased accumulation of ^{3}H-DA observed in the NCP of normal rats treated with atropine may be explained by the simultaneous activation of DA utilisation and synthesis induced by the anticholinergic agent.

CONCLUSION

Nigral applications of cholinergic or anticholinergic drugs, induced immediate changes in the metabolism of striatal DA. However, they seem to have antagonistic effects: (1) carbachol did not affect DA synthesis but inhibited DA release. These

results disagree with the conclusions of SMELIK and ERNST (1966): on the basis of behavioural observations these authors provided evidence for the activation of DA nigro-neostriatal neurons after physostigmine implantation in the SN; (2) atropine on the contrary, stimulated both synthesis and utilisation of striatal DA.

Our data strongly suggest the presence of cholinergic receptors in the SN, and consequently an input of cholinergic fibers. These cholinergic neurons innervating the SN seem to be involved in the regulation of DA transmission. The origin of these cholinergic neurons is not known. It has been postulated that striato-nigral cholinergic fibers may contribute to the maintenance of normal DA levels in the NCP (OLIVIER *et al.*, 1970; CORRODI *et al.*, 1972). However, MCGEER *et al.* (1973) failed to demonstrate a cholinergic striato-nigral pathway by lesions studies made in the cat. Therefore, the occurrence of cholinergic interneurons in the SN or the presence of cholinergic terminals originating from other brain structures cannot be excluded. On the other hand MCGEER *et al.* (1973) described a descending striato-nigral gabaminergic pathway in agreement with previous reports of PRECHT and YOSCHIDA (1971) and KIM *et al.* (1971). Studies are in progress to examine the respective contribution of gabaminergic and cholinergic pathways in the regulation of nigro-neostriatal DA neurons in normal and pharmacological states.

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