Effects of oxotremorine and physostigmine on the turnover of dopamine in the corpus striatum and the limbic system

Administration of neuroleptic drugs causes an increase in the concentration of the main dopamine metabolite homovanillic acid (HVA) in the brain (Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965). The percentage increase is of about the same magnitude in the two major dopamine-containing regions, i.e., the corpus striatum and the limbic system (Andén, 1972; Lloyd, Stadler & Bartholini, 1973). Treatment with anti-acetylcholine drugs may not change the neuroleptic-induced antipsychotic effect and the HVA increase in the limbic system, but can counteract both the extrapyramidal manifestations and the HVA increase in the corpus striatum (Andén, 1972). Extrapyramidal signs similar to those produced by neuroleptic drugs can also be seen after administration of drugs facilitating central acetylcholine mechanisms, such as the muscarinic receptor stimulating agent oxotremorine or the acetylcholinesterase inhibitor physostigmine (George, Haslett & Jenden, 1962; Duvoisin, 1967). Therefore the effects of oxotremorine and physostigmine on the HVA concentrations in the corpus striatum and the limbic system have been studied in the present investigation.

Adult white rabbits, 1·3–2·4 kg, were injected with drugs at doses referring to the forms indicated in Table 1. The animals were killed by an intravenous injection of air. The brains were rapidly dissected on ice-cold Petri dishes. The corpora striata were first excised bilaterally. Then, incisions were made in front of the optic chiasm and through the rhinal fissures and, in this way, a large piece of the limbic system (amygdala, pyriform cortex, preoptic area, olfactory tubercle, nucleus accumbens, nucleus interstitialis striae terminalis, septum) was removed including most of the dopamine-rich areas. The parts from two rabbits were pooled. The HVA was determined after tissue freezing on dry ice, extraction with 0·1 N HCl, anion exchange chromatography and oxidation (Andén, Roos & Werdinius, 1963; Korf, Roos & Werdinius, 1971). The homogenate of the corpus striatum was divided into two aliquots and 3 μg HVA was added to one of them. The recovery through the whole procedure was $91 \pm 1{\cdot}0\%$ (mean $\pm$ s.e., $n = 46$) and no correction was made for the recovery.

The results are presented in Table 1. The values obtained from the rabbits not drug-treated are in agreement with those reported previously (Andén, 1972). Injection of the peripheral muscarinic receptor blocking agent *N*-methylscopolamine did not produce any change in the concentrations of HVA in the brain. This drug was given before oxotremorine or physostigmine in order to prevent the otherwise very prominent peripheral cholinergic effects, such as salivation, diarrhoea and hypotension, which can cause deterioration and death of the rabbits. Treatment with oxotremorine (0·1 mg kg^{-1} or 0·5 mg kg^{-1} at 3, 2 and 1 h before death) or physostigmine (0·5 and 0·25 mg kg^{-1} at 3 and 1·5 h before death) for 3 h resulted in an increase in brain HVA of the methylscopolamine-pretreated rabbits. The physostigmine-induced rises were between those seen after the two doses of oxotremorine. In all the three cases, the percentage increases were about the same in the corpus striatum and in the limbic system. Previously it has been reported that the HVA in the corpus striatum can be elevated by oxotremorine or physostigmine (Laverty & Sharman, 1965; Perez-Cruet, Gessa & others, 1971). These drugs can also accelerate the α-methyltyrosine-induced disappearance of brain dopamine (Corrodi, Fuxe & others, 1967; Bhatnagar, 1973).

After blockade of both peripheral and central muscarinic receptors by trihexyphenidyl (benzhexol), the effects of oxotremorine and physostigmine on the con-

Table 1. *Homovanillic acid concentration in the corpus striatum and in the limbic system of rabbits untreated or treated with* N-*methylscopolamine nitrate* (5 mg kg^{-1} i.p., 3·25 h before death; **Pharmacia, Uppsala*), *oxotremorine* (0·1 or 0·5 mg kg^{-1} i.p., at 3, 2, and 1 h before death; *Aldrich-Europe, Beerse*), *trihexyphenidyl HCl* (40 mg kg^{-1} i.p., 3·25 h; **Kabi, Stockholm*), *physostigmine salicylate* (0·5 and 0·25 mg kg^{-1} i.v., 3 and 1·5 h before death; *Sigma, St. Louis*). *The values are in % of the untreated controls.*

Brain region	Treatment		
	No drug treatment	Methylscopolamine	Methylscopolamine + oxotremorine 0·1 × 3
Corpus striatum	100 (11)† (3·34 μg g^{-1})	105 (5)	148 (5)
Limbic system	100 (11) (1·01 μg g^{-1})	94 (5)	145 (5)

Brain region	Treatment			
	Methylscopolamine + oxotremorine 0·5 × 3	Methylscopolamine + physostigmine	Trihexyphenidyl + oxotremorine 0·5 × 3	Trihexyphenidyl + physostigmine
Corpus striatum	190 (5)	163 (8)	112 (7)	103 (7)
Limbic system	208 (5)	159 (8)	143 (7)	122 (7)

† Mean with number of experiments in parentheses. Statistical significances by one-way analysis of variance followed by *t*-test (d.f. within groups 81, F = 35·528, variance within groups 226.922).

centration of HVA in the corpus striatum were almost completely inhibited and the differences from the untreated controls were not significant (Table 1). Also the increases in the limbic system were significantly reduced. The effect of trihexyphenidyl appeared, however, to be smaller in the limbic system than in the corpus striatum; the concentration of HVA as a per cent of untreated controls was significantly higher in the limbic system than in the corpus striatum both after trihexyphenidyl plus oxotremorine ($P<0·001$) and after trihexyphenidyl plus physostigmine ($P<0·025$).

Besides dopamine, the neostriatum and the dopamine-rich areas of the limbic system seem to contain high levels of acetylcholine to judge from the activities of choline acetylase (Hebb & Silver, 1956) and the density of nerve terminals containing acetylcholinesterase (Shute & Lewis, 1967). An increase in acetylcholine in these regions can result in a stimulation of the dopamine turnover judging from the changes produced by physostigmine in the present work. The acetylcholine in the corpus striatum can also be released after treatment with neuroleptic drugs but such an effect is not observed in the nucleus accumbens of the limbic system (Lloyd & others, 1973). The lack of effect of trihexyphenidyl on the HVA increase in the limbic system and on the antipsychotic actions seen after treatment with neuroleptic drugs (Andén, 1972) may thus be due to the absence of an inhibitory influence of the dopamine neurons on the acetylcholine neurons in the limbic system in contrast to the neostriatum. Alternatively, the weaker effect of trihexyphenidyl on the effects of physostigmine and oxotremorine in the limbic system than in the corpus striatum may indicate that there are differences between the acetylcholine receptors in the two regions. Needless to say, the interactions between the dopamine and the acetylcholine mechanisms in the various brain nuclei must be further investigated.

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Further evidence for the stimulation of rat brain dopamine receptors by a cyclic analogue of dopamine

The cyclic analogue of dopamine, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) was suggested as a likely stimulant of dopamine receptors (Woodruff, 1971). The results of recent studies suggest that ADTN is indeed a potent agonist at dopamine receptors in the mammalian brain (Woodruff, Elkhawad & Pinder, 1974; Munday, Poat & Woodruff, 1974) and on specific snail neurons (Pinder, Buxton & Woodruff, 1972).

In the present study, experiments were performed on female Wistar Albino rats (about 150 g), anaesthetized with urethane (1·2 to 1·5 g kg^{-1}). Extracellular recordings of action potentials were obtained from single neurons in the caudate nucleus using standard stereotaxic techniques. The six-barrelled "parallel" electrodes used were similar to those described by Crossman, Walker & Woodruff (1974). The recording barrel was filled with 5M NaCl; other barrels were filled with 1·5M NaCl, DL-homocysteic acid (0·05M, pH 8 to 9), dopamine HCl (0·1M, pH 4 to 5) and ADTN HBr (0·1M, pH 4 to 5). Using the barrel containing 1·5M NaCl the technique of current balancing (Salmoiraghi & Weight, 1967) was used for the iontophoretic ejection of drugs.

All of the neurons were stimulated to fire by the continuous application of homocysteic acid (2 nA). Recordings were made from a total of 15 dopamine sensitive neurons in the caudate nucleus of 3 rats. Dopamine (30 to 60 nA, applied for 15 to 60 s) caused complete inhibition of firing of the cells. Every neuron inhibited by dopamine was also completely inhibited by ADTN (30 to 60 nA applied for 15 to 60 s). ADTN was approximately equipotent with dopamine. Neurons not affected by dopamine were similarly insensitive to ADTN.

Additional behavioural experiments were performed on 6 adult male rats with unilateral lesions of the nigro-striatal pathway produced by the injection of 6-hydroxydopamine HBr (8 μg in 4μl of 0·9% NaCl containing ascorbic acid 0·2 mg ml^{-1})