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Effect of clonidine on the synthesis of cerebral dopamine

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A possible interaction between dopamine (DA) and noradrenaline (NA) containing neurons in the brain has been suggested by different studies. The stimulation of dopaminergic receptors by apomorphine [1] or ET 495 [2] increases the turnover of cerebral NA. The disappearance of cerebral NA is about two times faster after inhibition of dopamine β-hydroxylase than after inhibition of tyrosine hydroxylase [1]. These observations can be explained if it is assumed that dopaminergic neurons have a stimulating influence on noradrenergic ones.

In the present work, we have studied the influence of clonidine (ST 155, Catapresan), a NA receptor stimulating agent [3], on the synthesis of cerebral DA. The dose of clonidine used (50 μg/kg) produces a significant fall in the blood pressure of the rat [4] and it reduces the synthesis [5] and release [4] of peripheral NA.

Two groups of fourteen male Charles River rats (220–250 g) were injected in the tail vein with 400 μCi/kg of ^{3}H-TY (L-3,5-^{3}H-tyrosine, 49 Ci/mM, Saclay) and were killed by decapitation 30 min later. The first group were treated with clonidine (50 μg/kg, i.p.) 1 hr before the injection of ^{3}H-TY, the second group serving as the control. The brain was immediately removed and divided into the striatum and telencephalon (the remaining brain from which the brain stem and the hypothalamus have been exluded). The brain areas, pooled two by two, were homogenized in 10 ml of 0·4 N $HClO_4$ containing 0·1% sodium metabisulfite and 0·1% disodium EDTA. Endogenous and ^{3}H-TY and DA were estimated in the striatum and telencephalon: endogenous and ^{3}H-NA were estimated in the telencephalon. DA and NA were separated from TY by chromatography on Dowex 50 WX4 according to the technique of Costa *et al.* [6] DA and NA were purified by adsorption on alumina [7] and TY by chromatography on Dowex 50 WX4 pH 1·5 [6]. Endogenous NA [8], DA [9] and TY [10] were determined fluorimetrically and radioactive products by liquid scintillation counting. An estimate of catecholamine (DA or NA) synthesis was made using the following formula [11]:

$$\text{catecholamine synthesis index (nM/g/30 min)} = \frac{^3\text{H-catecholamine (dis/min/g)}}{\text{Sp. act. } ^3\text{H-TY (dis/min/nM)}}$$

The results, summarized in Table 1, show that the amounts of both endogenous TY and ^{3}H-TY were both reduced by clonidine ($P < 0·05$ in the telencephalon, not significant in the striatum) but the ^{3}H-TY specific activities were not significantly altered. Clonidine treatment reduced ($P < 0·05$) the ^{3}H-DA levels in the striatum and the telencephalon and the endogenous DA levels ($P < 0·05$ in the telencephalon, non significant in the striatum). The ^{3}H-DA specific activities were not significantly modified by clonidine. The DA synthesis index was reduced by 25 per cent in the striatum (not significant, $P = 0·2$) and by 44 per cent in the telencephalon ($P < 0·01$). In the latter, clonidine reduced the ^{3}H-NA levels ($P < 0·01$) and the NA synthesis index by 52% ($P < 0·01$).

The fact that DA synthesis is much more reduced in telencephalon than in striatum can be explained by the presence of large amounts of NA in the telencephalon; consequently, the amount of ^{3}H-DA found in this structure represents at the same time ^{3}H-DA which is stored in dopaminergic neurons [12] and ^{3}H-DA which acts as a precursor of NA. The inhibition of NA synthesis in the telencephalon may account for the reduction in DA synthesis which is observed in this structure. Persson's results [13] differ from ours since he reported that the amount of cerebral ^{3}H-DA synthesized from ^{3}H-TY increased following the injection of clonidine (1–3 mg/kg). This difference may be due to the use of differ-

Table 1. Effect of clonidine on dopamine and noradrenaline synthesis

Determination	Telencephalon		Striatum	
	Control	Clonidine	Control	Clonidine
Total radioactivity (dis/min/g)	206,000 ± 7000	210,000 ± 11,200	264,800 ± 13,600	245,500 ± 12,300
^{3}H-TY (dis/min/g)	90,600 ± 8900	60,800 ± 4100*	117,400 ± 10,500	80,100 ± 9500
Endogenous TY (nM/g)	64·91 ± 3·64	48·39 ± 2·15†	91·38 ± 11·04	67·29 ± 11·54
Specific activity ^{3}H-TY (dis/min/nM)	1386 ± 99	1294 ± 164	1468 ± 136	1224 ± 145
^{3}H-DA (dis/min/g)	1024 ± 134	666 ± 48*	19,160 ± 2260	12,548 ± 1330*
Endogenous DA (nM/g)	0·84 ± 0·10	0·50 ± 0·04†	18·76 ± 1·47	13·82 ± 2·28
Specific activity ^{3}H-DA (dis/min/nM)	1223 ± 91	1415 ± 200	1057 ± 114	892 ± 54
DA synthesis index	0·88 ± 0·07	0·49 ± 0·04†	14·01 ± 2·37	10·47 ± 1·23
^{3}H-NA (dis/min/g)	1208 ± 98	530 ± 64†		
Endogenous NA (nM/g)	0·70 ± 0·08	0·83 ± 0·08		
Specific activity ^{3}H-NA (dis/min/nM)	1831 ± 207	663 ± 93†		
NA synthesis index	0·88 ± 0·06	0·42 ± 0·04†		

Clonidine (50 μg/kg, i.p.) was injected 1 hr before ^{3}H-TY (400 μCi/kg, i.v.) and the rats were killed 30 min later. Each value is the mean ± S.E.M. of seven determinations.
* $P < 0·05$; † $P < 0·01$ vs control.

ent doses of clonidine. Thus, at a peripheral level, low doses of clonidine stimulate adrenergic receptors whereas high doses (1 mg/kg) cause adrenolytic effects [14].

Anden *et al.* [3] have shown that clonidine (100 μg/kg) reduced slightly but significantly DA disappearance from the whole brain after inhibition of its synthesis by α-methyltyrosine. Since clonidine had no stimulating effect on dopaminergic receptors [3, 15], it was suggested that NA receptor stimulation directly or indirectly may inhibit the neural activity in the central DA neurons. The present work does not support this hypothesis since our results indicate that clonidine does not alter significantly DA synthesis in the striatal dopaminergic neurons.

L. ROCHETTE and J. BRALET
Laboratoires de Physiologie pharmaceutique et Pharmacodynamie,
Facultés de Médecine et Pharmacie,
7 Bd. Jeanne d'Arc, 21 000 Dijon, France

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Δ^9-Tetrahydrocannabinol—Uptake by rat liver lysosomes*

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Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the main psychoactive constituent of marihuana and hashish [1]. The liver appears to be a major site for the accumulation of Δ^9-THC and its metabolites, which become associated with the particulate subcellular fractions [2]. We have recently shown that Δ^9-THC has a marked disruptive effect on rat liver lysosomes *in vitro* which causes the release of hydrolytic enzymes from this organelle [3]. To determine the distribution *in vivo* of Δ^9-THC and its metabolites within liver cells, we administered ^{14}C-Δ^9-THC to rats and have observed that liver lysosomes become highly labeled with ^{14}C-radioactivity compared to other subcellular fractions.

Female Wistar rats (150–200 g) were each given 1 μCi ^{14}C-Δ^9-THC in 0·05 ml of 95% ethanol by intravenous injection. In addition, each animal received an intraperitoneal injection of Triton WR 1339 (85 mg/100 g body wt) in sterile isotonic saline 4 days prior to sacrifice. At 5 min, 15 min, 1, 3, 18 and 122 hr after the injection of ^{14}C-Δ^9-THC, groups of four rats were sacrificed by decapitation.

Separate fractionations of subcellular particles were carried out on each rat liver by the procedure of Trouet [4] as modified by Leighton [5] *et al.* for the isolation of Triton WR 1339-filled rat liver lysosomes. Subcellular fractions were analyzed for protein [6] and for the lysosomal enzyme β-*N*-acetylglucosaminidase [7]. Samples were digested using NCS solubilizer (Amersham–Searle, Arlington Heights, Ill.) and diluted in Aquasol (New England Nuclear, Boston, Mass.) for determination of ^{14}C-radioactivity by liquid scintillation counting.

The Δ^9-THC radioactivity is taken up rapidly by the liver, so that the whole liver contains at 5 min 8·7% of the dose, at 15 min 15·5% at 1 hr 7·5%, at 3 hr 7·8%, at 18 hr 3·9% and at 122 hr 0·8%. Figure 1 shows that this radioactivity is concentrated in the lysosomes at all time periods studied. All other subcellular fractions show lower uptakes of radioactivity, not greatly differing in specific activity (dis/min/mg of protein) from the homogenate. Lysosomes, however, have a specific activity five times that of the homogenate within 5 min of the injection of ^{14}C-Δ^9-THC. This specific activity for the isotope in lysosomes increases to a maximum of 17-fold compared to the homogenate at 18 hr after the injection, and thereafter the specific activity declines slowly.

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