# Serotoninergic modulation of mesolimbic and frontal cortical dopamine neurons

P. C. Waldmeier

*Departement Forschung, Division Pharma, Ciba-Geigy AG, CH-4002 Basel (Switzerland), 17 January 1980*

*Summary.* Potentiation of the effect of haloperidol on dopamine metabolism by the 5-HT uptake inhibitor CGP 6085 A, and antagonism of this effect by the 5-HT antagonist mianserin were observed in the mesolimbic area and the frontal cortex of the rat brain. A similar effect was reported earlier in the corpus striatum. This suggests that serotoninergic modulation of dopamine neurons is a generally-occurring phenomenon in the brain.

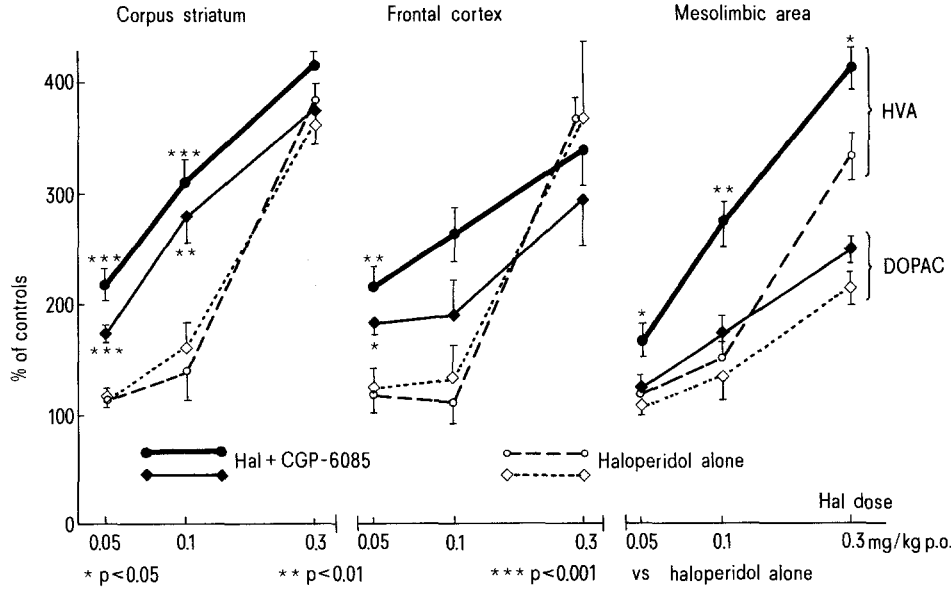
The modulation of nigrostriatal dopamine (DA) neurons by serotonin (5-HT)-containing fibres has been extensively investigated in recent years (for reviews see Kostowski[1] and Samanin et al.[2]). Treatments leading to increased 5-HT transmission were found to potentiate the effects of certain neuroleptics on the striatal levels of deaminated DA metabolites[3,4], on catalepsy[4-6] or on apomorphine stereotypies[4], whereas treatments or procedures reducing it had opposite effects[4,6-8].

A recent study by Carter and Pycock[9] of the effects of 5,7-dihydroxytryptamine lesions in the nucleus accumbens, tuberculum olfactorium, corpus striatum and substantia nigra on locomotor behaviour and amphetamine-induced stereotypies furnished evidence that such DA-5-HT interactions might not be restricted to the nigrostriatal system. To investigate this problem, we extended our earlier study[4] of the interaction of 5-HT-uptake inhibitors and 5-HT antagonists with the effect of haloperidol on striatal levels of deaminated DA metabolites to include the mesolimbic area and the frontal cortex. Changes in the levels of these metabolites are sensitive indicators of the functional state of dopaminergic neurons[10,11].

Haloperidol was obtained from Cilag AG, Schaffhausen, Switzerland, and para-chlorphenylalanine methylester-HCl (PCPA) from Sigma, St. Louis, Missouri. Mianserin was synthesized in our Chemistry Department by A. Storni, and CGP 6085 A (4-(5,6 dimethyl-2-benzofuranyl)-piperidine-HCl) is an experimental compound of Ciba-Geigy. The experiments were performed on female Tif: RAIf (SPF) rats (Tierfarm Sisseln, Switzerland).

Homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were isolated from the corpus striatum, the mesolimbic areas (containing the nucleus accumbens and tuberculum olfactorium as the major components; for dissection procedure see Waldmeier and Maître[12]) and the frontal cortex, by chromatography on Sephadex G 10 columns[13]. Aliquots of 100 µl were then subjected to high-pressure liquid chromatography with electrochemical detection, according to a modification of the method of Felice et al.[14]. The apparatus was supplied by Bioanalytical Systems Inc., West Lafayette, USA, fitted with an LC 4 controller and an Altex 110 pump. A 60 cm Zipax Sax (Dupont) column was used. The mobile phase was a 4:1 mixture of an acetate buffer 0.025 M, pH 4.7, and a citrate buffer, 0.025 M pH 5.3[15], pumped at 0.6 ml/min. A potential of +0.75 V was applied. The sensitivity limit of the method was about 1 ng/ml extract. In some animals 5-HT was determined as described by Curzon and Green[16]. Statistical evaluation was done by means of Student's t-test, or, where necessary, by Dunnett's t-test[17].

In a 1st experiment, rats were pretreated on days 1, 4, 8, 11 and 15 with 300 mg/kg i.p of the tryptophan hydroxylase




Potentiation of the effect of haloperidol on deaminated DA metabolites by CGP 6085 A.

Rats were treated with 10 mg/kg p.o. CGP 6085 A 30 min before graded doses of haloperidol (Hal). HVA and DOPAC were determined 2 h later in the 3 areas indicated. Data represent $\bar{x} \pm$ SEM ($n = 5$) in percent of controls. Absolute control values were (in ng/g):

striatum: HVA = 479 ± 32, DOPAC = 909 ± 67;
mesolimbic area: HVA = 172 ± 13, DOPAC = 702 ± 80;
frontal cortex: HVA = 36 ± 6, DOPAC = 36 ± 6.

Statistical significance of differences between groups receiving the combined treatment and haloperidol alone was calculated by means of Student's t-test.

Table 1. Effect of 5-HT depletion on the increase in DA metabolites produced by haloperidol

| | Striatum | | Mesolimbic area | |
|---|---|---|---|---|
| | HVA (ng/g) | DOPAC (ng/g) | HVA (ng/g) | DOPAC (ng/g) |
| Controls | 307± 34 (5) | 956± 136 (5) | 205± 16 (5) | 640± 47 (5) |
| PCPA* | 229± 22 (5) | 570± 43 (5) | 123± 4 (5) | 490± 26 (5) |
| Haloperidol 0.3 mg/kg p.o. | 1515± 127[b] (5) | 2710± 323[b] (5) | 577± 24 (4) | 1409± 84[b] (4) |
| Haloperidol + PCPA | 668± 109[a,c,d] (5) | 1174± 172[d] (5) | 302± 40[a,c,d] (5) | 758± 64[c,d] (5) |

*PCPA (300 mg/kg i.p.) was given on days 1, 4, 8, 11 and 15. Haloperidol was administered the day after the last PCPA injection and the animals decapitated 2 h thereafter. At the time of the experiment, 5-HT levels in the striata of the PCPA-treated rats were reduced to 14± 1% of controls. Data represent means± SEM. The number of animals per group is given in brackets. Statistical evaluation by Dunnett's t-test. [a] $p < 0.05$, [b] $p < 0.01$ vs controls. [c] $p < 0.01$ vs PCPA. [d] $p < 0.01$ vs haloperidol.

Table 2. Effect of mianserin on the increase in DA metabolites produced by haloperidol

| | Striatum | | Mesolimbic area | | Frontal cortex | |
|---|---|---|---|---|---|---|
| | HVA (ng/g) | DOPAC (ng/g) | HVA (ng/g) | DOPAC (ng/g) | HVA (ng/g) | DOPAC (ng/g) |
| Controls | 417± 33 (5) | 1242± 57 (5) | 163± 16 (5) | 553± 60 (5) | 33± 13 (5) | 29± 3 (4) |
| Mianserin 30 mg/kg i.p. | 417± 41 (5) | 1392± 107 (5) | 165± 8 (4) | 489± 34 (4) | 42± 8 (4) | 47± 14 (4) |
| Haloperidol 0.3 mg/kg p.o. | 1404± 83 (5) | 2945± 124 (5) | 676± 48 (4) | 1694± 175 (4) | 121± 19 (4) | 109± 27 (4) |
| Haloperidol + mianserin | 493± 26[c] (5) | 1396± 81[c] (5) | 181± 30[c] (5) | 567± 140[b] (5) | 64± 5[a] (5) | 77± 17 (5) |

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ vs haloperidol alone. Mianserin was given 30 min before haloperidol and the animals decapitated 2 h thereafter. The data represent means± SEM. The number of animals per group is given in brackets. Statistical evaluation by Student's t-test.

inhibitor PCPA. In 1 group of these animals and a control group, 5-HT was determined in the striatum on the day after the last dose of PCPA. PCPA treatment was found to deplete striatal 5-HT to 14± 1% of controls (absolute values: controls 635± 35 ng/g, n = 5; PCPA 89± 9 ng/g, n = 4). In previous experiments, this treatment did not affect brain catecholamine levels (unpublished).

1 day after the last administration of PCPA, the treated animals and controls received 0.3 mg/kg p.o. haloperidol and were decapitated 2 h later. HVA and DOPAC were determined in the striatum and mesolimbic area. In both areas, PCPA treatment reduced the endogenous content of HVA and DOPAC and largely prevented the increase caused by haloperidol (table 1).

In another experiment, rats were given 30 mg/kg i.p. of the 5-HT antagonist mianserin, 30 min before 0.3 mg/kg p.o. haloperidol. HVA and DOPAC were determined 2 h later in the striatum, the mesolimbic area and the frontal cortex. Whereas mianserin alone did not affect the levels of the 2 DA metabolites in any of these areas, it greatly and significantly diminished the increase induced by haloperidol in the striatum and mesolimbic area. In the frontal cortex, only the reduction of the haloperidol effect on HVA was significant. The increase in DOPAC was also reduced, but not to a statistically significant degree, owing to the larger scatter (table 2).

In a 3rd experiment, rats were pretreated with the 5-HT-uptake inhibitor CGP 6085 A (10 mg/kg p.o.) 30 min before 0.05, 0.1 or 0.3 mg/kg p.o. haloperidol. Again, HVA and DOPAC were determined in the striatum, the mesolimbic area and the frontal cortex 2 h after haloperidol. In all 3 areas, the 5-HT-uptake inhibitor significantly potentiated the effect of 0.05 and 0.1 mg/kg p.o. haloperidol. The effect on HVA of the 0.3 mg/kg p.o. dose of haloperidol alone was already near-maximal, so that a significant potentiation was no longer possible. The effects of the 2 lower doses of haloperidol on DOPAC were also potentiated by CGP 6085 A in the striatum. In the frontal cortex, only the effect of the lowest dose of the neuroleptic was significantly potentiated; no significant effect was observed in the mesolimbic area, although DOPAC levels in the groups receiving combined treatment were consistently higher than in those receiving haloperidol alone (figure).

CGP 6085 A is a highly specific 5-HT uptake blocker with negligible other effects[3,19,20]. Moreover, other 5-HT uptake inhibitors also potentiated the effect of haloperidol on DA metabolism in the striatum[4]. It seems therefore reasonable to attribute this effect of CGP 6085 A to its 5-HT uptake inhibitory properties.

Mianserin is a 5-HT antagonist already at low dosages[18], but possesses also $\alpha_1$- and $\alpha_2$-adrenolytic properties[18,21]. These are probably manifested in vivo in the increase in noradrenaline turnover observed after relatively high doses of this drug[22]. However, in our earlier study[4], we have shown not only that mianserin antagonized haloperidol effects on DA metabolism highly significantly with a dose as low as 1 mg/kg i.p., but also that similar effects are seen with other 5-HT antagonists. Hence, this effect of mianserin is likely to be related to its antiserotoninergic properties.

In this study, we have confirmed the result of Westerink and Korf[23] that the 5-HT synthesis inhibitor PCPA reduced the effect of haloperidol on DA metabolism, both in the striatum and the mesolimbic area. However, PCPA treatment alone reduced the endogenous levels of HVA and DOPAC, an effect not observed with 5-HT antagonists[4]. Thus, the possibility has to be considered that PCPA affected DA synthesis directly. In fact, evidence for an inhibitory effect of this compound on in vivo tyrosine hydroxylation has been presented[24,25]. In view of the recent suggestion that tyrosine hydroxylase might not be fully saturated in the rat brain[26], it is interesting that PCPA interfered with the transport of tyrosine to the brain[25]. Therefore, it is doubtful whether PCPA reduced the effect of haloperidol on DA metabolism because it impaired 5-HT transmission.

This is surprising in view of the fact that precisely this was observed when 5-HT transmission was impaired by means of receptor blockade[4]. It seems conceivable that adaptive phenomena developing during the 2 week's PCPA treatment might be responsible, although experimental evidence for this is lacking.

The principal result of this study, however, was that potentiation of the effects of haloperidol on DA metabolism by drugs increasing 5-HT transmission and their antagonism by drugs reducing it were observed in the projection areas of the 3 main dopaminergic systems of the brain. Although there were some differences in the statistical significance of these effects from one area to another, there is no reason to assume that there are fundamental differences in their extent. If they are, in fact, due to functional interactions between 5-HT and DA, the serotoninergic modulation of dopaminergic transmission must presumably be a general phenomenon in the brain.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## In vivo $^3$H-d-LSD binding in small punched out rat brain regions


K. Kräuchi and H. Feer[1]

*Psychiatrische Universitätsklinik, Wilhelm-Klein-Strasse 27, CH-4025 Basel (Switzerland), 21 December 1979*



*Summary.* We have developed an in vivo $^3$H-d-LSD-binding method in the rat brain to measure the $^3$H-d-LSD concentration in specific brain regions and nuclei. In the cortex, d-LSD binding sites increase from the occipital to the frontal part without a change in their affinity. Compared to the hippocampus, striatum, cortex and raphe nuclei, the substantia nigra contains a d-LSD binding site of lower affinity.


In contrast to in vitro methods, the binding of d-LSD in vivo is not disturbed by a change in the environment or a disruption of membranes. The binding sites remain in a physiological state and in their natural surroundings. As the in vivo method is very sensitive[2] it seemed that it would be possible to compare the affinity for d-LSD of small brain regions and single nuclei and to gain insight into the pattern of specific d-LSD binding within the brain. We therefore developed an in vivo $^3$H-d-LSD binding method with subsequent isolation of small brain regions by a punch-out technique. Neurochemical, electrophysiological and behavioural studies have shown that d-LSD binds preferentially to serotoninergic and dopaminergic receptors[3-6]. We were interested in comparing d-LSD binding (capacity and affinity) in serotoninergic and dopaminergic nuclei with that in some of their projection areas.

*Experimental.* Adult male albino Wistar rats (200–250 g) were kept under controlled conditions of light:dark (12 h:12 h), temperature 24 °C and feeding ad libitum. Tritium-labelled d-LSD (Amersham 15.3 Ci/mmole; 1 µg/kg in 100 µl 0.1% ascorbic acid) was injected i.v. via the tail vein. In displacement experiments increasing doses of cold d-LSD were simultaneously injected i.v. with $^3$H-d-LSD. The rats were decapitated 30 min after the injection and their brains rapidly removed and frozen on dry ice. Transverse parallel slices (1 mm) were prepared on a slicing apparatus at a temperature of −20 °C. Different regions and nuclei were located using König and Kippel's atlas[7] and were punched out with a cooled metallic needle (diameter 1.5 mm). Each region was solubilizied with 0.2 ml Soluene (Packard), acidified with $H_2SO_4$ and counted for 20 min (Instagel, Packard) in a tricarb scintillation counter. In the cerebellum only a negligible amount of the

$^3$H-dLSD binding in brain regions expressed as the ratio of the respective brain part to cerebellum (1 µg/kg $^3$H-d-LSD)

| | Mean values ± SEM | No. of rats |
|---|---|---|
| Frontal cortex | 3.03 ± 0.24 | 11 |
| Striatum | 2.65 ± 0.23 | 5 |
| Parietal cortex | 2.36 ± 0.09 | 11 |
| Hippocampus | 1.83 ± 0.09 | 5 |
| Occipital cortex | 1.81 ± 0.09 | 11 |
| Substantia nigra | 1.72 ± 0.07 | 11 |
| Raphe nuclei d + m | 1.53 ± 0.07 | 11 |
| Cerebellum | 1.00 | |