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5-HT-moduline, a 5-$HT_{1B/1D}$ receptor endogenous modulator, interacts with dopamine release measured in vivo by microdialysis

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Abstract

5-Hydroxytryptamine-moduline (5-HT-moduline) is an endogenous tetrapeptide (Leu-Ser-Ala-Leu) recently isolated and characterized from mammalian brain. This compound interacts with 5-HT_{1B} receptors as a non-competitive, high-affinity antagonist and has the properties of an allosteric modulator. 5-HT-moduline could play an important role in the regulation of serotonergic transmission and also, through heteroreceptors, dopaminergic transmission. The aim of this work was to examine the potential ability of 5-HT-moduline to modify the basal extracellular concentration of dopamine and its metabolites (3-methoxytyramine, dihydroxyphenylacetic acid and homovanillic acid), in the rat striatum and to determine its potential interaction with the stimulating activity of a specific 5-HT_{1B} receptor agonist, 3-(1,2,5,6-tetrahydropyrid-4-yl) pyrrolo [3,2-b] pyrid-5-one (CP-93,129), on the release of dopamine. The technique is based on in vivo microdialysis using probes implanted in the striatum of the conscious rat. Results showed that the perfusion of 5-HT-moduline directly into this structure (1.25 mM) increased the striatal level of dopamine by two-fold (104% of the absolute basal release values, $P = 0.0015$) and that of 3-methoxytyramine by 3-fold (293%, $P = 0.0001$) without any change in the terminal metabolite concentrations. The intrastriatal administration of CP-93,129 induced a statistically significant, dose-dependent increase of dopamine levels ($P < 0.0001$). Coperfusion of 5-HT-moduline did not significantly alter the effect of CP-93,129 at 0.1 and 0.5 mM, but appeared to have an additive effect on the lowest dose ($P = 0.0406$). The results obtained show that 5-HT-moduline directly administered into the striatum increases the release of dopamine in this area. Presumably, this effect results from the desensitization of 5-HT_{1B} receptors located on dopamine terminals. However, the fact that a 5-HT_{1B} receptor agonist (CP-93,129) also increased the release of dopamine in the striatum and that 5-HT-moduline exhibited a slight additive effect with that of a low concentration of CP-93,129 suggests that the two substances interact with different mechanisms.

Keywords: 5-HT-moduline; 5-HT (5-hydroxytryptamine, serotonin); Dopamine; CP-93,129; Microdialysis

1. Introduction

5-Hydroxytryptamine-moduline (5-HT-moduline) is an endogenous tetrapeptide (Leu-Ser-Ala-Leu) recently isolated and characterized from mammalian brain (Rousselle et al., 1996). It was shown to specifically interact with 5-HT_{1B} receptors as a non-competitive antagonist with a very high apparent affinity (10^{-11} M) (Fillion et al., 1996; Massot et al., 1996). The interaction likely corresponds to an allosteric mechanism leading to the desensitization of the receptor. Accordingly, it was demonstrated that activation of the 5-HT_{1B} receptor-related transduction system was blocked by the peptide, that the inhibitory effect of a 5-HT_{1B} receptor agonist on the neuronal release of serotonin (5-hydroxytryptamine, 5-HT) was also markedly decreased by 5-HT-moduline, leading to a net release of the amine, and that, in vivo, in an animal model of long-lasting isolation, the behavior of the animal was significantly affected by intracerebral administration of the peptide (Massot et al., 1996). The binding of the radiolabelled peptide was to a site corresponding to that of the 5-HT_{1B} receptor with a matched distribution in rat brain (Cloëz-Tayarani et al., 1997). Furthermore, it was shown using

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immunochemistry techniques that the peptide itself was heterogeneously distributed in the brain and located in cells with the morphological appearance of neurons (Grimaldi et al., 1997). In vivo and ex vivo experiments also have shown in the rat substantia nigra that, after acute stress, 5-HT_{1B} receptors become desensitized, that intracerebroventricular administration of the peptide mimics the effect of stress and that this acute stress increases the concentration of the peptide in various brain areas (Bonnin et al., in preparation).

Thus, 5-HT-moduline not only interacts with 5-HT_{1B} autoreceptors regulating the release of 5-HT itself (Massot et al., 1996; Seguin et al., 1997) but may also interact with 5-HT_{1B} heteroreceptors which regulate the release of other neurotransmitters such as those demonstrated to control the release of acetylcholine (Maura and Raiteri, 1986; Maura et al., 1989; Harel-Dupas et al., 1991; Bolaños-Jiménez et al., 1993) or glutamate (Maura et al., 1998).

The interactions of the serotonergic system with dopaminergic activity, as measured by microdialysis techniques, have recently been reviewed by Saito et al. (1996). The enhancement of dopamine release by serotonergic stimulation was reported in the prefrontal cortex (Chen et al., 1992), striatum (Benloucif and Galloway, 1991; West and Galloway, 1996) and nucleus accumbens (Parsons and Justice, 1993; Boulenguez et al., 1996). Coperfusion of 5-HT receptor antagonists attenuates 5-HT-induced dopamine release (Benloucif et al., 1993; Invernizzi et al., 1995). Although a consensus exists as to the in vivo (and not in vitro) facilitating effect of 5-HT on dopamine release and its reversal by 5-HT receptor antagonists, the 5-HT receptor subtypes mediating this effect and the corresponding intracellular mechanisms remain to be determined. Several receptors, including 5-HT_{1A}, 5-HT_3 and 5-HT_4 receptors, are reported to facilitate the release of 5-HT in in vitro (Galzin and Langer, 1991; Blier and Bouchard, 1993; Blier et al., 1993; Haddjeri and Blier, 1995) and in in vivo assays (Martin et al., 1992). The observed effects suggest the involvement of interneurons (see Göthert and Schlicker, 1997).

The role of 5-HT_{1B} receptors, present in the striatum, in the 5-HT-induced facilitation of dopamine release is supported by the fact that a 5-$HT_{1A/1B}$ receptor agonist, 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl) indole (RU-24,969) (Benloucif et al., 1993), and a 5-HT_{1B} receptor agonist, 3-(1,2,5,6-tetrahydropyrid-4-yl) pyrrolo [3,2-b] pyrid-5-one (CP-93,129) (Galloway et al., 1993), enhance dopamine release. CP-93,129 is more potent than RU-24,969 and almost equipotent to 5-HT. The observed effect of 5-HT_{1B} receptor stimulation on dopamine release in the striatum is in agreement with the high density of these receptors in this brain region (Waeber et al., 1989).

The involvement of the dopamine system in a number of psychiatric diseases, i.e., schizophrenia, Parkinson's disease, and drug abuse suggests that the study of the control of the dopamine system via 5-HT_{1B} receptor interactions might be a promising approach for the discovery of novel therapeutic tools.

The aim of this work using microdialysis was to examine the potential ability of 5-HT-moduline to affect the basal release of dopamine in rat brain by quantifying dopamine and its metabolites: 3-methoxytyramine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Moreover, the effects of the peptide on the increase in dopamine release induced by a 5-HT_{1B} specific receptor agonist (CP-93,129) were also examined.

2. Material and methods

2.1. Animals

This study was conducted with Sprague–Dawley (Charles River) male adult rats with a mean weight of 300–320 g; food and water were provided ad libitum. Animals were housed in an approved animal house and the care provided before, during and after the protocol was in compliance with ethical considerations and good laboratory practice (authorization no. A 35006 of the Veterinary Department of the French Health Ministry). The animals were kept in the animal house at least one week before the experiments started.

2.2. Drugs

CP-93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl) pyrrolo [3,2-b] pyrid-5-one) was a gift from Pfizer laboratories. It was used as a fresh solution diluted in Ringer solution.

5-HT-moduline was dissolved in distilled water, divided into aliquots of 500 μg, and immediately frozen and lyophilized. These samples were kept at −75°C until used. The day of experiment, the lyophilized peptide was dissolved in Ringer solution (1 ml), corresponding to a 1.25 mM concentration.

2.3. Microdialysis experiments

The animals were anesthetized with 350 mg kg^{-1} chloral hydrate injected intraperitoneally and placed in a stereotaxic frame (D. Kopf). A probe guide canula (CMA 10 Carnegie Medicine) was implanted above the right striatum (AP + 1.5, L −2, V −3.4 relative to the bregma and the dura surface) according to stereotaxic coordinates of Paxinos and Watson's atlas, and fixed with dental cement. The day after surgery, a 4-mm long microdialysis probe (CMA 10 Carnegie Medicine) was positioned in the probe guide. The animal was awake. The dialysis probe was perfused with Ringer solution (NaCl 147 mM, KCl 4 mM, $CaCl_2$, 1.2 mM) at a flow rate of 1 μl min^{-1}, using an infusion pump (CMA/100 Carnegie Medicine), or with Ringer solution + drug(s) after switching to another sy-

ringe (three syringes were available). All the drugs were directly administered into the striatum through the dialysis probe. Ringer was always perfused for at least 3 h after probe implantation to allow stabilization of dopamine and metabolite levels. Indicated times of perfusion are corrected for the existence of dead volumes. Dialysates were collected as 20-min samples in microtubes containing 5 μl of a 0.5 M perchloric acid solution with 4-dihydroxyhydrocinnamic acid as internal standard, or containing only 5 μl perchloric acid solution (for 3-methoxytyramine assays). They were frozen and stored at −75°C until analysis.

2.4. Assay of neurotransmitter and metabolites

Each sample, 25 μl, was injected without additional modification. Dopamine, DOPAC and HVA concentrations were quantified simultaneously whereas the 3-methoxytyramine concentration was separately determined.

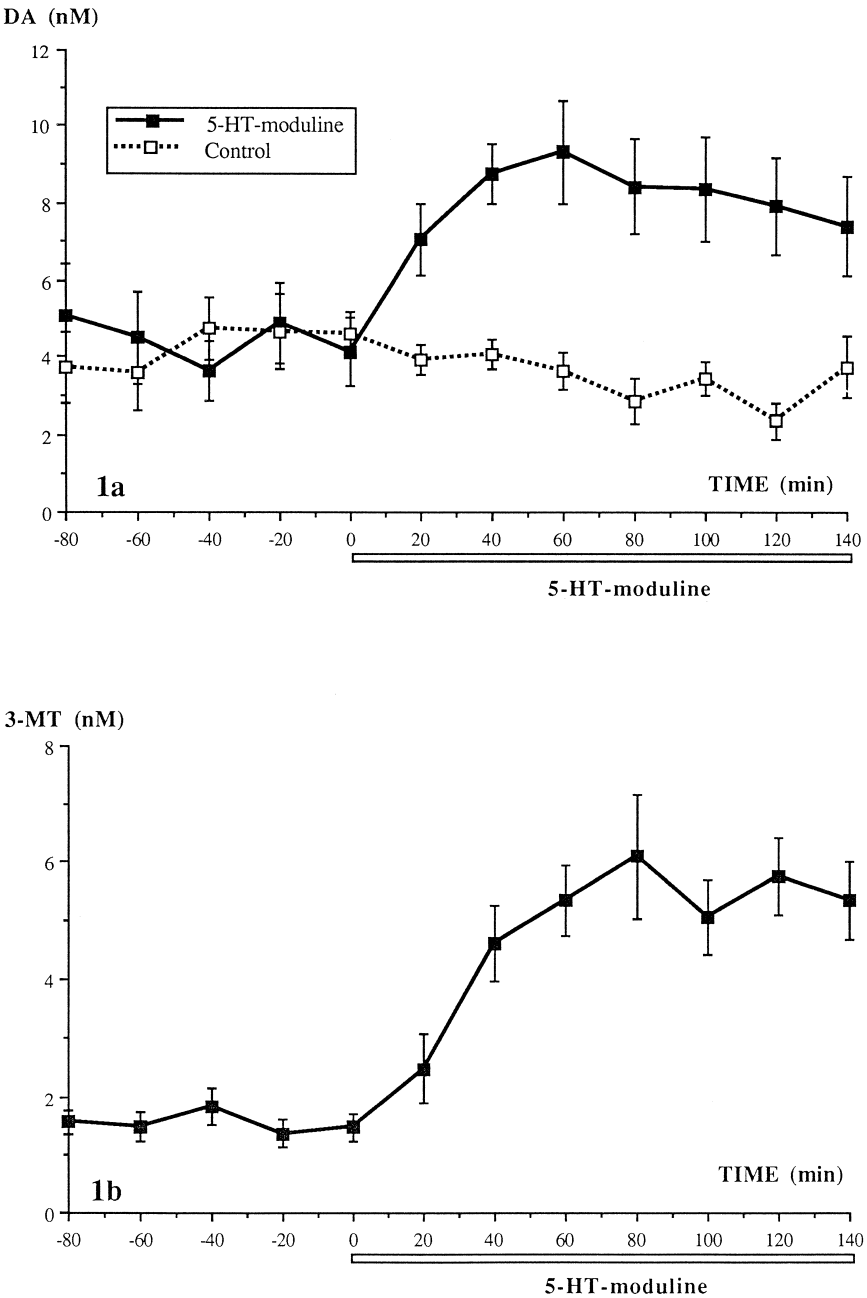

Fig. 1. (a) Effect of a 140-min perfusion of 5-HT-moduline (1.25 mM) on extracellular striatal level of dopamine, vs. Ringer control perfusion. Values (uncorrected for probe recovery) are expressed as means ± S.E.M. Two-way repeated measures ANOVA showed that the peptide significantly increased dopamine concentrations ($P = 0.0015$). (b) Effect of a 140-min perfusion of 5-HT-moduline (1.25 mM) on extracellular striatal level of 3-methoxytyramine (3-MT). Values (uncorrected for probe recovery) are expressed as means ± S.E.M. One-way repeated measures ANOVA showed that the peptide significantly increased 3-methoxytyramine concentrations ($P < 0.0001$).

Assays were performed with an automated and computerized high-performance liquid chromatography (HPLC) processor (SP 8810 isocratic pump, SP 8880 refrigerated autosampler, PC 1000 Ver 3.0 system software, Thermo Separation Products®) fitted with a Coulochem II electrochemical detector and an analytical cell model 5011 (ESA®). The analytical column was maintained at 32°C in an oven. For dopamine, DOPAC and HVA assays, the analytical column used was a 230 × 4.6 mm, ODS-2, 5-μm Spherisorb cartridge (Thermo Separation Products®). The mobile phase was composed of a 0.05 M citrate/0.06 M acetate buffer, 0.5% counterions (Pic B8, Waters®), 17% methanol and 200 mg EGTA. For dopamine, the sensitivity threshold was 0.5 nM. Sensitivity was never a limiting factor for the metabolite assays.

3-Methoxytyramine was assayed using a 80 × 4.6 mm, RCP-18, 3 μm (ESA HR 80) analytical column. The mobile phase consisted of 75 mM NaH_2PO_4, 0.5% Pic B8, 20 μM EDTA, 100 μl l^{-1} triethylamine, 10% acetonitrile, and 8% methanol; The pH was adjusted to 5.6 with NaOH 2 N. It was verified that 5-HT did not coelute with 3-methoxytyramine and that 5-HT-moduline itself, at concentrations up to 1.25 mM, did not interfere with the measurements of dopamine and its metabolites.

2.5. Protocol design and drug administration

2.5.1. Protocol 1—Effect of 5-HT-moduline on the extracellular concentration of dopamine and its metabolites

2.5.1.1. Treated animals. Treated animals received 5-HT-moduline via the dialysis probe. The perfusion of the peptide lasted 140 min, corresponding to 1.25 nmol min^{-1}. The animals received a single dose of the peptide as indicated. The dose chosen had been shown to affect the behavior of animals in an in vivo model (Massot et al., 1996).

Dopamine, DOPAC and HVA ($n = 10$) and 3-methoxytyramine ($n = 8$) were measured in the perfusates from dialyzed animals.

2.5.1.2. Control animals. The control animals ($n = 6$) were submitted to the same experimental protocol but received only Ringer solution during perfusion.

2.5.2. Protocol 2—Effect of 5-HT-moduline on the dopamine release induced by a 5-HT_{1B} receptor agonist

The animals were divided into various groups according to the drug which they received.

Group 1 ($n = 6$), group 2 ($n = 7$) and group 3 ($n = 7$) received CP-93,129 at concentrations of 0.05, 0.1 and 0.5 mM, respectively, for 120 min, and then they were perfused again with Ringer solution. Group 4 ($n = 6$), group 5 ($n = 6$) and group 6 ($n = 6$) also received 0.05, 0.1 and 0.5 mM CP-93,129 as in the animals of group 1, 2 and 3 but in addition received 5-HT-moduline (1.25 mM) for 60 min, 1 h after the beginning of the perfusion with CP-93,129. Then the animals received Ringer solution.

The animals of group 7 received 0.1 mM CP-93,129 and 5-HT-moduline (1.25 mM) simultaneously for 120 min and then the animals were perfused again with Ringer solution. The control group 8 ($n = 5$) was perfused with Ringer solution throughout the whole experimental procedure.

2.6. Statistical analysis

Results are presented as the time course of the mean ± S.E.M. concentrations (uncorrected for probe recovery) of

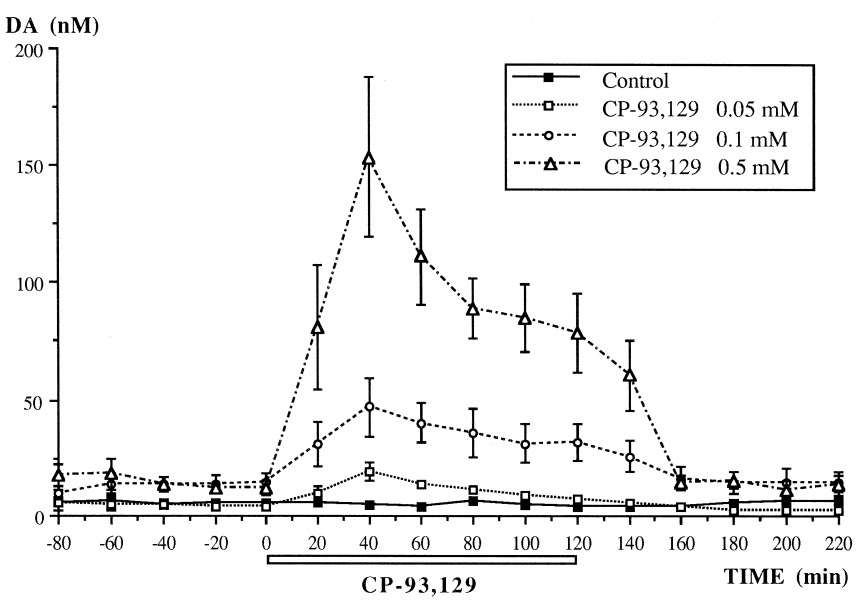

Fig. 2. Effect of a 2-h perfusion of 0.05, 0.1 and 0.5 mM CP-93,129 on striatal extracellular level of dopamine, compared with Ringer control. Values are expressed as means ± S.E.M. Two-way repeated measures ANOVA showed that a significant increase occurred at each concentration: 0.05 mM $P = 0.025$, 0.1 mM $P = 0.0009$ and 0.5 mM $P = 0.0003$.

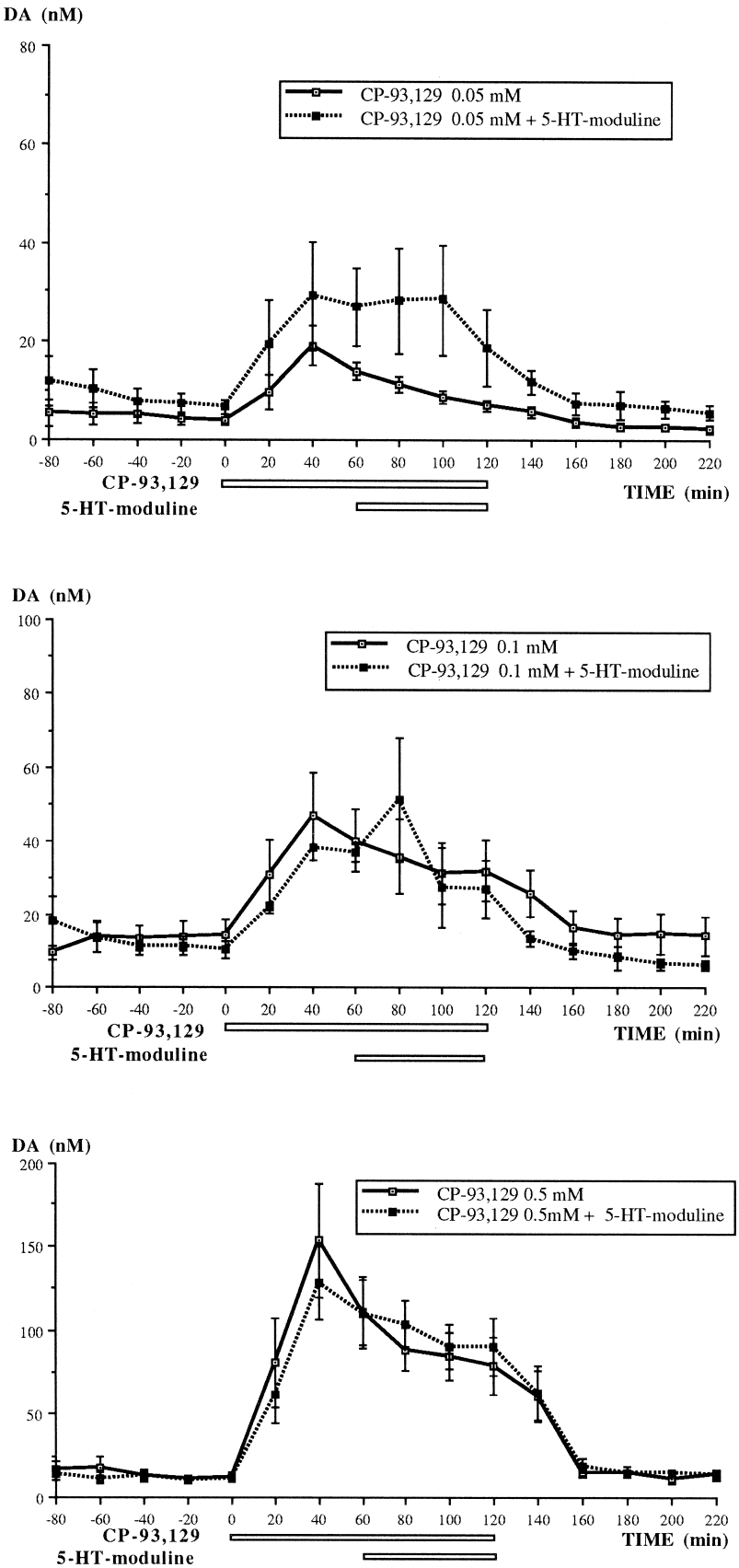

Fig. 3. Effect of 1-h administration of 5-HT-moduline (1.25 mM) on the dopamine release induced by a 2-h perfusion of 0.05, 0.1 or 0.5 mM CP-93,129. Administration of CP-93,129 began 1 h before perfusion of the peptide. Values (uncorrected for probe recovery) are expressed as means ± S.E.M. Two-way repeated measures ANOVA showed a significant difference only for the lowest dose ($P = 0.0406$).

dopamine and its metabolites. Differences in concentrations were compared by using an analysis of variance (ANOVA): two-way ANOVA with repeated measurements with time and drug treatment as the two factors or one-way ANOVA for 3-methoxytyramine (Statview® for the MacIntosh). Statistical significance was set at 0.05. Non-significant results are not presented.

3. Results

3.1. Effect of 5-HT-moduline on the extracellular concentration of dopamine and its metabolites DOPAC, HVA and 3-methoxytyramine

The basal extracellular level of dopamine before administration of the compound was the same in the two groups.

5-HT-moduline (1.25 mM) administered through the dialysis probe induced a significant enhancement of extracellular dopamine ($F(1,14) = 15.37$, $P = 0.0015$). The dopamine increase occurred early after peptide perfusion. There was an increase of 104% of the basal extracellular concentration after 1 h and this increase was maintained throughout the perfusion period (Fig. 1a). 5-HT-moduline also increased the extracellular concentration of 3-methoxytyramine ($F(1,110) = 117.48$, $P < 10^{-4}$) which was 293% of the basal extracellular level 80 min after the beginning of the perfusion. This value remained steady for the rest of the perfusion period (Fig. 1b). In contrast, DOPAC and HVA extracellular concentrations were not significantly affected by 5-HT-moduline; only a time effect was observed: F (6,78) = 2.28, $P = 0.0445$ and $F(6,78) = 5.46$, $P < 10^{-4}$, respectively (not shown).

3.2. Effect of 5-HT-moduline on the dopamine release induced by a 5-HT$_{1B}$ receptor agonist

3.2.1. Effect of CP-93,129 on the release of dopamine

The basal extracellular level of dopamine measured before the perfusion of any drug was similar in the 8 groups, namely, 10.32 ± 0.45 nM.

CP-93,129 administered alone during the 120-min period increased the dopamine extracellular level in a dose-dependent manner ($F(3,21) = 36.05$, $P < 10^{-4}$). The difference in dopamine level in the controls and during perfusion of the lowest concentration of CP-93,129 (0.05 mM) was already significant ($F(1,9) = 7.11$, $P = 0.025$). The observed effect increased further at 0.1 mM ($F(1,11) = 20.42$, $P = 0.0009$), and at 0.5 mM the observed increase was more marked than that elicited by 0.1 mM CP-93,129 ($F(1,12) = 24.72$, $P = 0.0003$). The maximal increase in dopamine extracellular concentration occurred 40 min after the beginning of the perfusion with CP-93,129. Concentrations decreased thereafter to a steady intermediate value up until the end of drug perfusion. Dopamine concentrations returned to basal levels 40 min after the CP-93,129 perfusion stopped (Fig. 2). No effect of CP-93,129 was observed on DOPAC and HVA levels at any time (not shown).

3.2.2. Effect of 5-HT-moduline on CP-93,129-induced dopamine release

The potential interaction between 5-HT-moduline and dopamine release was determined in parallel assays. The

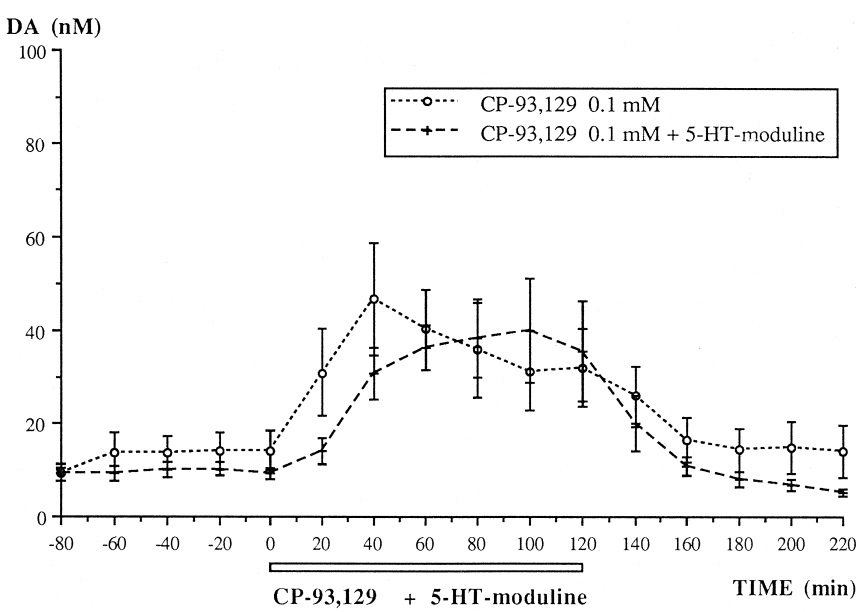

Fig. 4. Effect of coperfusion of CP-93,129 (0.1 mM) and 5-HT-moduline (1.25 mM) vs. perfusion of CP-93,129 alone, on the striatal extracellular level of dopamine. Two-way repeated measures ANOVA did not detect a statistically significant difference.

effects on dopamine release of CP-93,129 at 0.05, 0.1 and 0.5 mM were measured in the presence of 5-HT-moduline (1.25 mM) in the last hour of perfusion. The effects of CP-93,129 at the different doses during the first hour of perfusion were as expected, i.e., not significantly different to those measured in the previous series of assays. The presence of the peptide in the perfusion medium during the second hour of perfusion with CP-93,129 did not significantly alter the effect of the drug at 0.1 and 0.5 mM. 5-HT-moduline appeared to have an additive effect or to potentiate the effect of the lowest dose of CP-93,129, 0.01 mM ($F(1,10) = 5.52$, $P = 0.0406$) (Fig. 3). No significant effect was shown on DOPAC and HVA (not shown).

The effect of 5-HT-moduline was also determined after coperfusion with the agonist at 0.1 mM at the very beginning of the perfusion with the drug. A shift of the curve for the increase of the extracellular dopamine concentration was observed in the first 40 min of perfusion, suggesting a slight initial inhibitory effect of the peptide on CP-93,129-induced dopamine release. However, the maximal effect of CP-93,129 was not affected in the following 60 min, being not significantly different to that observed in the absence of the peptide (Fig. 4).

4. Discussion

The main result of this study was that 5-HT-moduline, administered in the striatum, significantly increased the release of dopamine in the conscious rat. The observed effect was sustained during the whole duration of administration and roughly corresponded to a 2-fold increase in dopamine release. This effect was paralleled by a 2- to 3-fold increase in 3-methoxytyramine, a metabolite of dopamine which is assumed to reflect, to a certain extent, the release of dopamine (Brown et al., 1991). The fact that no concomitant variations in DOPAC and HVA concentrations were observed suggests that the increase in extracellular dopamine concentration measured in these experiments is most likely due to an enhanced release of dopamine and not to a change in dopamine synthesis, because DOPAC has been shown to originate from an intraneural pool of newly synthetized dopamine (Zetterström et al., 1988).

The effect of 5-HT-moduline on dopamine release was measured at a high dose of the peptide (1.25 mM); however, it is known that the delivery of a molecule through a microdialysis probe is generally low, probably 10-20% of the amount contained in the perfusion solution (Benveniste et al., 1989; Ungerstedt, 1984). Moreover, the peptide is rapidly degraded and inactivated in biological medium (Rousselle et al., 1996). An important proportion probably diffuses into the tissue and reaches acceptor sites which are not relevant for peptide activity, hence significantly decreasing the availability of the exogenous molecule relative to the availability of the endogenous molecule. The concentration of 1.25 mM was chosen from previous behavioral studies as being effective (Massot et al., 1996).

The direct effect of a potent and specific 5-HT_{1B} receptor agonist, CP-93,129 (Macor et al., 1990), was determined and the potential interaction of 5-HT-moduline as a 5-HT_{1B} receptor antagonist was examined using the same biological model. CP-93,129 (0.05, 0.1 and 0.5 mM) was tested at the concentrations previously used in the literature ((0.1 and 0.3 mM in the prefrontal cortex) (Iyer and Bradberry, 1996); 0.4–12 nmol/40 μl in the striatum (Galloway et al., 1993)). The effect of the drug observed under the experimental conditions used in the present assays was in agreement with the results previously reported, showing a facilitation of dopamine release by CP-93,129 in the striatum (Macor et al., 1990; Hjorth and Tao, 1991; Galloway et al., 1993) and in the nucleus accumbens (Iyer and Bradberry, 1996; Boulenguez et al., 1996).

The interaction of the serotonergic system on dopamine release is complex since it has also been reported that other serotonergic ligands induce dopamine release, i.e., 5-HT_{1A} partial receptor agonists (ipsapirone 5 and 10 mg kg^{-1}, buspirone 2.5 and 5 mg kg^{-1}) in the rat prefrontal cortex (Wedzony et al., 1996), a 5-$HT_{1A/1B}$ receptor agonist (RU 24969 0.02–2 mg kg^{-1}) in the nucleus accumbens (Boulenguez et al., 1996), a 5-HT_3 receptor agonist (1-phenylbiguanide 0.1–1 mM) in the nucleus accumbens (Chen et al., 1991; 1992) and a 5-HT_4 receptor agonist (endo-*N*-8-methyl-8-azabicyclo [3.2.1.] oct-3-yl-2,3-dihydro-3-iso-propyl-2-oxo-1H-benzimidazol-1-carboxamide or BIMU 8, 0.1 mM) in the striatum (Bonhomme et al., 1995). Accordingly, various 5-HT receptor antagonists (5-HT_1, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3 and 5-HT_4) have been shown to reduce 5-HT-induced dopamine release (Benloucif et al., 1993). However, De Deurwaedère et al. (1996) suggested that while 5-HT has a facilitatory effect on striatal dopamine release, it acts via a dopamine reuptake-dependent process unrelated to receptor activation. Furthermore, Pehek and Bi (1997) have shown that a 5-HT_2 receptor antagonist (ritanserin 5 mg kg^{-1}) increases the basal release of dopamine in the mesocorticolimbic pathway and potentiates amphetamine-induced dopamine release in the prefrontal cortex.

It should be noted that, in in vitro systems, serotonergic inhibition of dopamine release is generally reported (see Kapur and Remington, 1996 for review) and was also observed in our laboratory with a striatal synaptosome preparation (unpublished data). Experimental evidence also exists in support of 5-HT-mediated stimulation of dopamine release in striatal slices (Blandina et al., 1989).

The differences observed between in vitro and in vivo preparations may originate from the fact that in simple preparations (i.e., synaptosomes), the complex functional interactions related to various receptors present on interneurons are suppressed, allowing examination of the sole effects of receptors present on dopamine neuron ter-

minals. Thus, in microdialysis experiments, a more complex system is present, possibly involving various receptors and various neurotransmitters.

Under these conditions, the results presented here show that 5-HT-moduline increased dopamine release. This effect was expected for an antagonist acting at 5-HT_{1B} heteroreceptors located on dopamine neuron terminals and is in good agreement with the fact that 5-HT-moduline has clearly been shown to be a non-competitive antagonist at 5-HT_{1B} receptors in biochemical experiments with in vitro systems (Massot et al., 1996). Moreover, this effect of 5-HT-moduline, on its own, is in accordance with tonic serotonergic activity causing the release of endogenous 5-HT in the conscious animal (Jacobs and Fornals, 1993). In contrast, CP-93,129 activates 5-HT_{1B} receptors and, accordingly, would be expected to decrease the release of dopamine from terminals. The fact that CP-93,129 enhanced the release of dopamine, suggests that the drug did not directly interact with 5-HT_{1B} receptors located on dopamine terminals. Even if the 5-HT_{1B} receptor agonist acts at these receptors, the corresponding inhibitory activity was masked by other interactions, leading to an increase of dopamine release. The latter mechanisms may correspond to the action of CP-93,129 on 5-HT_{1B} autoreceptors, producing a decrease in 5-HT activity which, directly or indirectly, produces an increase in dopamine release. CP-93,129 may also stimulate 5-HT_{1B} heteroreceptors located on non-dopaminergic neuron terminals, decreasing the release of neurotransmitters which inhibit dopamine activity, i.e., GABA (see Pauwels, 1997). The present results suggest that 5-HT-moduline and CP-93,129 do not interact at the same sites because the two drugs exhibited a similar facilitating effect on dopamine release although one is an agonist (CP-93,129) and the other is an antagonist (5-HT-moduline) of 5-HT_{1B} receptors. In agreement with this hypothesis, it has already been shown that a given G-protein-coupled receptor, and particularly 5-HT_{1B} receptors, may exhibit different pharmacological properties depending on the coupling of the receptor to a particular G protein (Clawges et al., 1997) or on the interaction with another G-protein-coupled receptor (Dickenson and Hill, 1998). Thus, CP-93,129 and 5-HT-moduline may interact with distinct 5-HT_{1B} receptors on the basis of their different pharmacological properties. The preliminary results of in vitro experiments being carried out in our laboratory favor this hypothesis. The observation that 5-HT-moduline had an apparent additive effect with that of CP-93,129 at 0.05 mM supports the hypothesis that the releasing effects of the two substances do not involve the same mechanisms. The observation that the additive effect of 5-HT-moduline was observed at a low concentration of CP-93,129, but not at higher concentrations (0.1 and 0.5 mM), can be explained by the relatively modest effect of 5-HT-moduline on dopamine release: at 1.25 mM it was equipotent to 0.05 mM CP-93,129 and had only 10% and 5% of the effect of 0.1 and 0.5 mM of CP-93,129 respectively.

In conclusion, the ability of 5-HT-moduline to participate in the control of dopaminergic activity in the striatum could lead to a better knowledge of various neuropsychiatric situations. Accordingly, 5-HT-moduline may also be involved in dysfunctions of this aminergic system, leading to severe psychiatric diseases. In parallel, these results open new directions of research into the development of novel therapeutic tools for use in psychiatric disorders.

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