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Changes in dopamine and acetylcholine in striatum of the awake rat after chronic treatment with a dopamine uptake blocker

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Abstract

The effects of chronic treatment with a dopamine uptake blocker on dopamine and acetylcholine extracellular concentrations in striatum of the awake rat was studied. Male Wistar rats received daily injections (i.p.) of the dopamine uptake blocker nomifensine (10 mg/kg) during 22 days. Control group was injected with vehicle (saline). Microdialysis experiments were performed on days 1, 8, 15 and 22 of treatment. Nomifensine injections increased extracellular concentration of dopamine in striatum in all days of treatment without differences among days. In contrast, acetylcholine levels showed no changes in days 1 and 8 but increased in days 15 and 22 of treatment. These results shows that chronic treatment with a dopamine uptake inhibitor, nomifensine, has no effects on dopamine release but it increases acetylcholine release in striatum of the awake rat. These results would help to further understand the effects of chronic dopamine uptake inhibition.

Keywords: Dopamine uptake inhibitor; Acetylcholine; Dopamine; Striatum; Microdialysis

1. Introduction

Converging evidence supports the existence of a functional interaction between dopamine and acetylcholine in striatum. Immunocytochemical studies indicate the existence of synaptic contacts between dopaminergic terminals in both soma and dendrites of striatal cholinergic interneurons [7,24]. The fact that D1 [2,18] and D2 [5,30] dopamine receptors have been localized on striatal cholinergic interneurons further support this interaction. Also the effects of dopamine receptor agonists and antagonists on the in vitro release of acetylcholine [11,35] and in vivo turnover of acetylcholine [14,34] give strong support to the interaction between these two neurotransmitters in striatum. All these data make the striatum an ideal target to study the interactions between dopamine and acetylcholine.

Nomifensine as well as other type of dopamine uptake blockers has long been used to treat depression [22,38]. As a consequence, the study of the changes in the striatal dopaminergic system as the result of chronic blockade of dopamine uptake has been the focus of intensive research [27–29,31]. Chronic treatment with nomifensine has been shown to produce decreases of D2 and D3 dopamine receptors in striatum [29,31] but does not affect the number or affinity of the uptake transporters [27,28]. In contrast, chronic treatment with other dopamine uptake blockers like for instance cocaine produces increases of D1 receptors [4,36] and the number of dopamine uptake transporters [4,36]. However, no studies have so far investigated the effects of chronic blockade of dopamine uptake on other neurotransmitters such as acetylcholine.

The findings reviewed above suggest the possibility that the changes in the dopaminergic system as a consequence of chronic treatment with dopaminergic uptake blockers might change the interaction between dopamine and acetylcholine in striatum. The aim of this study was to investigate the responses of dopamine and acetylcholine to daily injections of a dopamine uptake blocker in striatum of the awake rat. For that purpose, an inhibitor of the uptake of dopamine, nomifensine, was injected daily intraperitoneally and microdialysis experiments were performed. The use of the microdialysis technique allows the simultaneous analysis of different neurotransmitters and, therefore, to evaluate their possible interactions.

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2. Materials and methods

2.1. Animals and surgery

Young (2–4 months) male Wistar rats were housed in individual wire mesh cages, provided with food and water ad libitum, and maintained in a temperature-controlled room under a light/dark cycle (lights on/off at 8:00 p.m./8:00 a.m.). All in vivo experiments, carried out at the Universidad Complutense of Madrid, were conducted during the dark period of the light/dark cycle and following the guidelines of the International Council for Laboratory Animal Science (ICLAS).

Under Equithesin (2 ml/kg i.p.) anesthesia rats were stereotaxically implanted with bilateral guide-cannulae to accommodate microdialysis probes in striatum of the rats. When inserted, the tip of the probe was located in: 0.6 mm rostral and 2.5 mm lateral from Bregma and 7.5 mm ventral from dura mater [23].

2.2. Drug treatment and microdialysis

Ten days after surgery rats received a daily injection (i.p.) of nomifensine (10 mg/kg) or saline, during 22 days. On days 1, 8, 15 and 22 of treatment microdialysis experiments were performed in the freely moving rat. Briefly, microdialysis probes (membrane cut off 5000 Da and length 4 mm) were inserted and perfused (2 μl/min) with artificial CSF (composition in mM: NaCl, 137; $CaCl_2$, 1.2; KCl, 3; $MgSO_4$, 1; NaH_2PO_4, 0.5; Na_2HPO_4, 2; glucose, 3) containing neostigmine 1 μM (pH = 7.3). After basal concentrations of neurotransmitters were established (3 h of perfusion), 20 min samples were collected and separated in two aliquots to analyse them separately for each neurotransmitter. The first three samples were used as control.

At the end of the experiments, the animals were anesthetised with Equithesin and perfused intracardially with 0.9% saline followed by 10% formalin. The brain was removed and the placement of the microdialysis probe was verified with a cryostat microtome and viewing lens.

2.3. Dopamine analysis

The dopamine content of samples was analysed by reverse-phase HPLC and electrochemical detection (Coulochem II model 5200A, ESA). Mobile phase consisted of 0.1 M acetate–citrate buffer (pH = 3.5 adjusted with HCl 1N), 1 mM EDTA, 2.9 mM sodium octyl sulphonate, and 18% methanol. The flow rate was maintained at 1 ml/min [32]. Chromatograms were processed using the Millenium 32 (Waters, Milford, MA) software. The detection limit in our 20 μl samples was 0.15 nM for dopamine.

2.4. Acetylcholine analysis

The acetylcholine contents of samples was analysed by reverse-phase HPLC and electrochemical detection (HP1049A, Agilent, Palo Alto, CA). The mobile phase consisted of 50 mM phosphate buffer, 0.5 mM EDTA, and ProClin® 150 Reagent 5 ml/l (pH = 8.5 adjusted with NaOH 1N). The flow rate was maintained at 0.15 ml/min [20]. The detection limit in our 10 μl samples was 5 nM for acetylcholine.

2.5. Chemicals

Nomifensine was purchased from Sigma–Aldrich (Madrid, Spain). Nomifensine was dissolved in saline before i.p. administration.

2.6. Statistical analysis

For the study of the effects of chronic treatment with nomifensine on the actions of this drug on the extracellular concentration of dopamine and acetylcholine, a three-way ANOVA (treatment × day × perfusate) with repeated measures design was used to perform planned comparisons [33]. For the study of the effects of chronic treatment with nomifensine on the basal extracellular concentration of dopamine and acetylcholine, two-way ANOVA (treatment × days) design was used to perform planned comparisons. For ANOVA analysis, absolute microdialysis data were normalised by subtracting basal concentration (average of the three sample values) to each post-basal sample. Pearson's coefficient and independence test were used for the study of the correlations between basal extracellular concentrations of the neurotransmitters and days of treatment.

3. Results

3.1. Effects of chronic dopamine uptake blocker treatment on dopamine extracellular concentrations in the striatum

Basal dialysate concentrations of dopamine were not affected by the chronic treatment with nomifensine (Table 1).

The injection of nomifensine produced an increase in dialysate concentrations of dopamine at all days studied compared to saline: day 1 ($F_{1,24} = 17.719$; $p = 0.000$); day 8 ($F_{1,24} = 12.396$; $p = 0.001$); day 15 ($F_{1,24} = 15.559$; $p = 0.000$) and day 22 ($F_{1,24} = 15.106$; $p = 0.000$) (Figs. 1 and 2). There were no differences between days of treatment.

3.2. Effects of chronic dopamine uptake blocker treatment on acetylcholine extracellular concentrations in the striatum

Basal dialysate concentrations of acetylcholine were increased with nomifensine chronic treatment. Basal levels on days 15 and 22 were significantly different from day 1:1 versus 15 ($F_{1,14} = 7.42$; $p = 0.016$) and 1 versus 22 ($F_{1,14} = 5.504$, $p = 0.034$) (Table 1). Moreover, there is a positive correlation between acetylcholine basal levels and days of treatment ($r = 0.601$; $p < 0.01$) (data not shown).

The injection of nomifensine produced an increase in dialysate concentration of acetylcholine compared to saline on days 15 and 22: day 15 ($F_{1,26} = 10.962$; $p = 0.002$) and day 22 ($F_{1,26} = 6.055$; $p = 0.020$) (Fig. 2). Acetylcholine concen-

Table 1
Basal dialysate concentrations of dopamine and acetylcholine in striatum on days 1, 8, 15, and 22 of treatment

Day	DA (nM)		ACh (nM)	
	Nomifensine	Saline	Nomifensine	Saline
1	1.887 ± 0.570 ($n = 4$)	1.766 ± 0.339 ($n = 4$)	36.528 ± 3.486 ($n = 4$)	39.549 ± 5.845 ($n = 4$)
8	1.768 ± 0.465 ($n = 5$)	1.933 ± 0.065 ($n = 3$)	24.798 ± 4.924 ($n = 5$)	34.139 ± 5.030 ($n = 3$)
15	1.667 ± 0.347 ($n = 4$)	2.630 ± 0.885 ($n = 4$)	68.862 ± 10.249[a] ($n = 5$)	47.806 ± 4.730 ($n = 4$)
22	2.683 ± 0.610 ($n = 5$)	1.883 ± 0.439 ($n = 3$)	65.874 ± 7.722[a] ($n = 4$)	35.695 ± 12.804 ($n = 3$)

Data (mean ± S.E.M.) are absolute values of the average of the three basal values.
[a] $p < 0.001$ (vs. nomifensine day 1 in a two-way ANOVA).

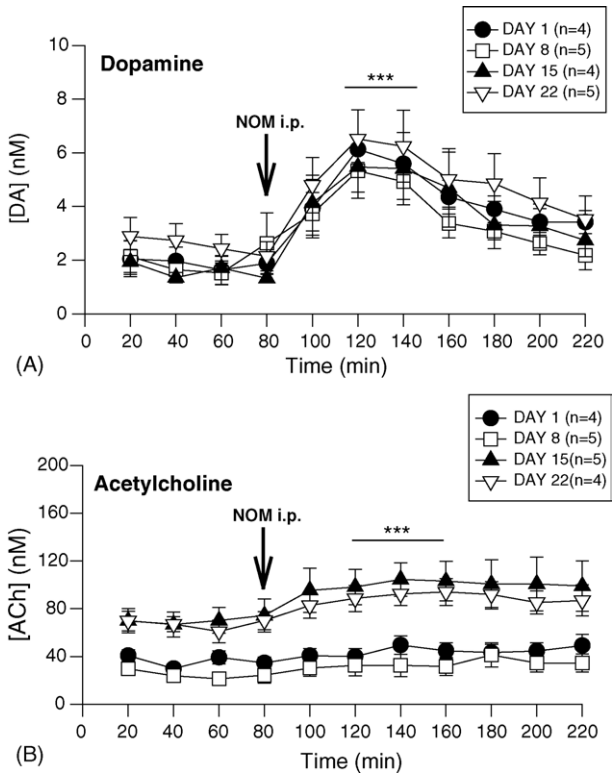

Fig. 1. Effects of nomifensine (NOM 10 mg/kg, i.p.) on dopamine (A) and acetylcholine (B) extracellular concentrations in striatum ($n = 4$–5). Data (mean ± SEM) are expressed as absolute values. ***$p < 0.001$ A (all days); B (days 15 and 22) (vs. average of three basal values; planned comparisons in a three-way ANOVA with repeated measures design).

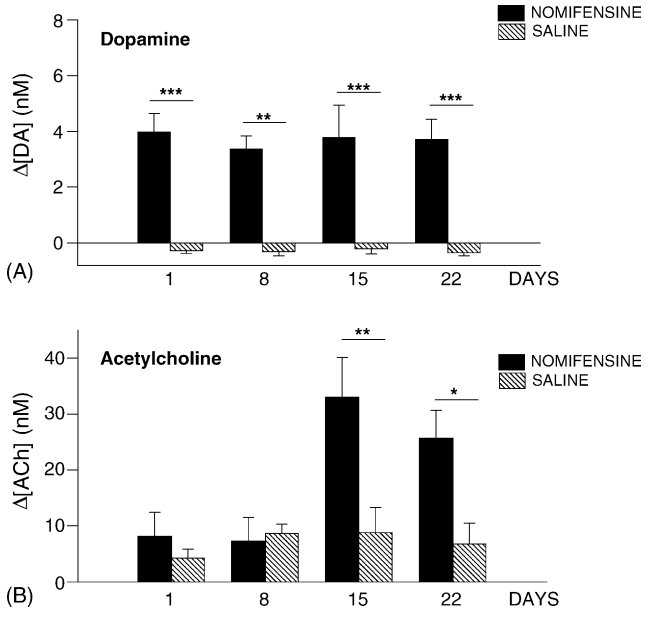

Fig. 2. Effects of nomifensine (NOM 10 mg/kg, i.p.) on dopamine (A) and acetylcholine (B) extracellular concentrations in striatum (nomifensine, $n = 4$–5; saline, n = 3–4). Data (mean ± SEM) are absolute increases (differences between average of maximal increase and average of three basal values) *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (vs. saline group; planned comparisons in a three-way ANOVA with repeated measures design).

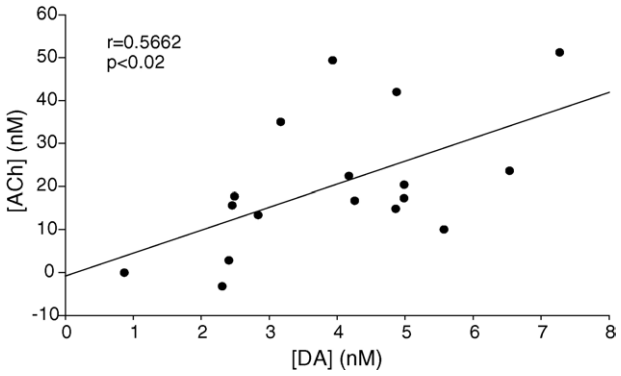

Fig. 3. Correlation between the increases of dialysate concentration of dopamine and acetycholine produced by nomifensine (10 mg/kg) in striatum. Increases of both neurotransmitters were calculated as the average of two samples with the highest increase when nomifensine is injected.

tration on days 1 and 8 were no different from saline: day 1 ($F_{1,26} = 0.252$; $p = 0.619$) and day 8 ($F_{1,26} = 0.230$; $p = 0.635$) (Fig. 2).

There was a correlation between increase in dopamine and increase in acetylcholine extracellular concentrations after nomifensine systemic administration ($r = 0.5662$, $p < 0.02$) (Fig. 3).

4. Discussion

In the present study, we show that nomifensine chronic treatment increased dopamine extracellular concentration in striatum without differences along days of treatment. Chronic administration of this dopamine uptake inhibitor also produced significant increases of acetylcholine on days 15 and 22 of treatment.

Daily intraperitoneal injections of nomifensine increased extracellular concentrations of dopamine in striatum. This is in agreement with previous studies showing that acute injections of nomifensine increase dopamine extracellular concentration in vivo [6,25]. One of the goals of this study was to investigate the possible changes of dopamine in striatum under chronic treatment with a dopamine uptake blocker (nomifensine). We show here that neither the basal dopamine levels nor the increase of the extracellular concentrations of dopamine were changed by the chronic injections of nomifensine. However, several studies have described changes of several other biochemical parameters in the striatal dopaminergic system after chronic treatment with dopamine uptake inhibitors like nomifensine or cocaine such as a decrease in density of the D2 and D3 dopamine receptors [29,31]. Other reports have shown that chronic treatment with nomifensine do not change the number of D1 receptor [8] nor the number of dopamine uptake sites in striatum of the rat [27,28]. Chronic treatment with cocaine increased D1 receptors [4,36], and dopamine uptakes sites [4,36] without changes in D2 receptors [15]. Studies with chronic cocaine administration reported no changes in dopamine extracellular concentration in striatum [3], a similar finding to the one reported here with nomifensine. In spite of all of these findings, we show no changes in the increases of dopamine extracellular concentration during

all days of treatment. Therefore, our results suggest that under chronic blockade of dopamine uptake, and despite the many biochemical changes reported, the presynaptic release of dopamine remains unaltered.

Chronic treatment with nomifensine increased the extracellular concentrations of acetylcholine after 15 days of treatment. These increases of acetylcholine were also detected on day 22 of treatment. A correlation between the increases of acetylcholine and dopamine extracellular concentrations induced by nomifensine was found (Fig. 3), which suggests that the increases of acetylcholine were produced by endogenous dopamine. Moreover, an increase in basal concentrations of acetylcholine and a positive correlation between basal levels of acetylcholine and days of treatment was also found (see Section 3). To our knowledge, this is the first report showing a change in extracellular concentrations of acetylcholine after chronic injections with a dopamine uptake blocker and suggests that a functional interaction between dopamine and acetylcholine may exist in the striatum of the rat.

There are reports showing no effects [1] or an increase in striatal acetylcholine concentration after acute systemic administration of dopamine uptake blockers [14]. On the contrary, some other reports have shown a decrease in acetylcholine extracellular concentration after local perfusion of dopamine uptake blockers [1,13]. It is possible that the effect of systemic administration of dopamine uptake blockers is the result of the activation of both striatal and extrastriatal mechanisms that regulate local acetylcholine release. These mechanisms seem to be differentially mediated by D2 and D1 receptors respectively, since systemic injections of D2 agonists decreased and D1 agonists increased acetylcholine release in striatum [10,12,34]. In agreement with these results, perfusion of D2 agonists in the striatum decreased acetylcholine release [13,35]. Local perfusion of D1 agonist in substantia nigra reticulata increased acetylcholine concentration in striatum [1], which suggest the involvement of a substantia nigra-thalamo-cortico-striatal loop in the release of acetylcholine in the striatum [9,10]. Other pathways such as the cholinergic afferents from pedunculopontine nucleus could also be involved in the release of acetylcholine in striatum [37].

Chronic treatment with dopamine uptake blockers produce an increase in D1 receptors and a decrease in D2 receptors in multiple areas of the brain [4,29,31,36], although some controversy exists [8,15,21]. Therefore, the effects of nomifensine on acetylcholine concentration reported here might be the result of a shift in the balance between inhibitory and excitatory dopamine receptors. Indeed, the potentiation of acetylcholine release shown after 15 days of treatment could be produced by an attenuation of local D2-receptor mediated inhibition and an enhancement of extrastriatal D1-receptor mediated activation of acetylcholine release.

It has been previously reported that nomifensine and other monoamine uptake blockers are able to inhibit nicotinic acetylcholine receptors in the brain, changing therefore the neurotransmitter-release modulating properties of this type of receptors [19]. Such an effect on nicotinic receptors could lead to the increase in acetylcholine release observed in the present study, due to long-term changes in sensitivity. Further studies would be necessary to clarify the exact contribution of nicotinic acetylcholine receptors to the results reported here.

The striatum is an area of the brain involved in motor, cognitive, sensorial and reward processes. Alterations in its function leads to disorders such as Parkinson's disease, schizophrenia and addiction [16,17]. Substances like amphetamine and cocaine increase dopamine extracellular concentration, relying the addictive properties of these substances on the chronic blockade of dopamine uptake [16,26]. Moreover, the treatment with inhibitors of monoamine uptake (nomifensine) has been used to treat depression [22,38]. The change in the interaction between dopamine and acetylcholine reported in the present study would help to further understand the mechanisms that are involved in the effects of chronic dopamine uptake inhibition.

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