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Effects of Manganese on Extracellular Levels of Dopamine in Rat Striatum: An Analysis *In vivo* by Brain Microdialysis

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The aim of this study is to determine the effects of intrastriatal administration of $MnCl_2$, on the extracellular levels of dopamine (DA) and metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in basal conditions and stimulated by depolarization with KCl and pargyline administration. Also, we studied the effect of $MnCl_2$ on extracellular levels of l-Dopa in the presence of aromatic amino acid decarboxylase (AADC) inhibitor 3-hydroxybencilhydracine-HCl (NSD 1015). This study concluded that $MnCl_2$, reduced the basal and K^+-stimulated DA-release in striatum, without notably affecting the DOPAC and HVA levels. Intraperitoneal injection of pargyline increased striatal DA levels, decreasing DOPAC and HVA levels. The infusion of $MnCl_2$ removed the increase in DA levels, without affecting DOPAC and HVA levels. Perfusion of NSD 1015 increased the extracellular levels of L-DOPA in striatum, and $MnCl_2$ increased the effect of NSD1015 on L-Dopa.

KEY WORDS: Dopamine; HPLC; L-Dopa; manganese; microdialysis; rat striatum.

INTRODUCTION

Manganese (Mn) is an essential nutrient necessary for a variety of physiological processes responsible for regulating reproduction, formation of connective tissue and bone, glucidic and lipidic metabolism and normal brain function. Mn deficiencies during early development can produce skeletal abnormalities and an irreversible ataxia (1). In addition, it is an important cofactor for numerous enzymes involved in DNA and neurotransmitter biosynthesis and signal transduction (2). An example is the astrocytic enzyme glutamine synthetase, involved in the biosynthetic pathway of excitatory amino acid glutamate that contains 80% of Mn in the brain (3). Chronic exposure to high levels of Mn, however, can produce a syndrome known as *manganism* characterized by extrapyramidal dysfunction (bradykinesia, rigidity and dystonia) and neuropsychiatric symptoms that resembles idiopathic Parkinson disease (4).

In *manganism*, the levels of manganese are increased in the brain areas known to be iron-rich: striatum (caudate and putamen nucleus), globus pallidus, substantia nigra and subthalamic nuclei, all included in the basal ganglia (5). Excessive exposure to Mn produces neurological lesions (neuronal loss and gliosis), primarily in the output pathways downstream of the dopaminergic projections (globus pallidus and the substantia nigra pars reticulata) (6). Neuropathological abnormalities are also present in the striatum and subthalamic nucleus in the brains of monkeys receiving $MnCl_2$ via inhalation (7). The neurotoxic effects of Mn probably results from the failure of protective enzymes capable to detoxify

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critical amounts of Mn or of altering its oxidation potential, so that the bivalent form Mn^{2+} can oxidize to Mn^{3+}, a powerful oxidizing species. Moreover, Mn shows a high affinity to neuromelanin-rich areas such as nigrostriatal tract (8) producing the oxidation of dopamine by electron transfer reaction (9).

The aim of this study is to determine the *in vivo* effects of intrastriatal administration of manganese chloride ($MnCl_2$) on dopaminergic system by means of brain microdialysis. In particular, we analyzed the effect of Mn on spontaneous, K^+-stimulated and pargyline-induced dopamine (DA) release in stratum. Extracellular levels of metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were also analyzed. The study also set out to determine the effect of Mn on extracellular levels of L-Dopa, the metabolic precursor of dopamine, in the presence of aromatic amino acid decarboxylase (AADC) inhibitor, 3-hydroxybencilhydracine-HCl (NSD 1015).

EXPERIMENTAL PROCEDURES

Animals, Drugs Treatments and Experimental Groups

Female adult Sprague–Dawley rats (weighing between 240 and 260 g) were used in all the experiments. Animals were housed under monitored conditions of temperature (22 ± 2°C) and photoperiod (light:dark 14:10 h) with free access to food and water. The experiments were performed according to the Guidelines of the European Union Council (86/609/EU) for the use of laboratory animals.

The experimental groups were as follows: (1) 2 mM $MnCl_2$; (2) 100 mM KCl medium; (3) 100 mM KCl medium + 2 mM $MnCl_2$; (4) Pargyline (100 mg/kg) i.p; (5) Pargyline (100 mg/kg) i.p + 2 mM $MnCl_2$; (6) 0.2 mM 3-hydroxybencilhydracine-HCl (NSD 1015); (7) 0.2 mM NSD 1015 + 2 mM $MnCl_2$.

Drugs were supplied by Sigma, St. Louis (USA). All other chemicals were analytical grade.

Microdialysis Procedure

For microdialysis sampling, animals were anesthetized via i.p. with chloral hydrate (400 mg/kg) and placed in a stereotaxic apparatus (Narishige SR-6) for the implantation of the guide-cannula. A microdialysis probe (CMA/12, 3 mm membrane length, CMA/Microdialysis, Stockholm, Sweden) was implanted through the guide-cannula into the left striatum at the following coordinates from Bregma: A/P +2.0 mm; L +3.0 mm; V +6.0 mm.The experiments were carried out 24 h after implantation of the guide cannula. Continous perfusion was performed with a Ringer's solution (147 mM NaCl; 4 mM KCl; 3.4 mM $CaCl_2$; pH 7.4) using a CMA/102 infusion pump (CMA/Microdialysis, Stockholm, Sweden) at a flow rate of 1.5 μl/min. For the analysis of dopamine and metabolites, the experiments were conducted during 3 h, sampling striatal dialysates every 20 min (30 μl). After collection of three basal samples (60 min), $MnCl_2$ was administered for 120 min until the end of the experiment. The KCl medium was administered for 60 min through the microdialysis probe. After this, the medium was switched back to the unmodified Ringer's solution and the sampling was continued for an additional period of 60 min. Pargyline was administered intraperitoneally 60 min after the start of the experiment. In order to measure L-Dopa in dialysates, it was necessary to include an AADC inhibitor in the perfusion fluid; for this reason, we used NSD 1015 (0.2 mM). After collecting three basal samples (60 min), NSD 1015 (0.2 mM) was administered for 140 min; after this, the medium was switched back to the unmodified Ringer's solution and the sampling was continued for an additional period of 60 min. In the case of the treatment with $MnCl_2$, NSD1015 was administered for 80 min; then NSD1015 and $MnCl_2$ (2 mM) were coadministered during 60 min, after this, the sampling was continued for an additional period of 60 min with unmodified Ringer's solution in perfusion medium. All the experiments were made with awake, conscious and freely moving animals.

HPLC-EC Analysis

The samples obtained from the microdialysis procedure (30 μl) were collected by means of a CMA/142 microsampler (CMA/Microdialysis, Stockholm, Sweden) and L-DOPA, DA, DOPAC and HVA levels were quantified by high-performance liquid chromatography (HPLC) with electrochemical detection. The dialysates (20 μl) were injected into a Hewlett–Packard Series 1050 Liquid Chromatograph using a Rheodyne 7125 injection valve. The isocratic separation of L-DOPA, DA, DOPAC and HVA was achieved using Teknokroma Kromasil 100 C18 reversed-phase columns (5 μm particle size). For the analysis of DA, DOPAC, and HVA, the eluent was prepared as follows: 70 mM KH_2PO_4, 1 mM octanesulfonic acid, 1 mM EDTA and 10% methanol, pH 4. Elution was carried out at a flow rate of 1.6 ml/min and the chromatograms obtained allowed the determination of the three substances with a run time of 18 min (10). On the other hand, the eluent used for the analysis of L-DOPA was a solution of 0.1 M trichloroacetic acid with 0.1 mM EDTA, pH 3.2 (11), and the elution in these conditions was carried out at a flow rate of 1.5 ml/min.

Detection of the susbtances was achieved using an ESA Coulochem 5100A electrochemical detector (ESA, USA) at a potential of +400 mV.

Expression of Results and Statistics

Data were corrected using the percentage of *in vitro* recovery for every microdialysis probe. The averages of concentrations of the substances in the three samples before treatment administration were considered as basal levels that were taken as 100% in order to compare the different responses of L-DOPA, DA and metabolites after every treatment. The results are shown as the mean ± SEM expressed as a percentage respect to basal levels.

Statistical analysis of the results was performed by means of ANOVA and Student–Newman–Keuls multiple range test, considering the following significant differences:

$^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ respect to the basal levels.

$^aP < 0.05$, $^bP < 0.01$ and $^cP < 0.001$ respect to control (non-treated) groups.

RESULTS

Effect of $MnCl_2$ on the Basal DA Release

Intrastriatal infusion of 2 mM of $MnCl_2$ produced a progressive decrease in extracellular DA levels. The significant decrease was observed 40 min after Mn infusion and 3 h later, the striatal DA content was 47.6 ± 7.5% respect to basal values ($P < 0.001$). The levels of DOPAC and HVA remained without significant changes respect to basal values (Fig. 1).

Effect of $MnCl_2$ on the KCl-evoked DA Release

To investigate the effect of $MnCl_2$ on KCl-evoked DA release, we infused 100 mM KCl through the dyalisis probe. As shown in Fig. 2, high K^+ medium induced a maximal increase in extracellular DA levels to 1651.5 ± 295.5% respect to basal values ($P < 0.001$), whereas DOPAC and HVA levels decreased to 49.4 ± 5.5% and 75.5 ± 5.6% ($P < 0.01$), respectively. When 2 mM $MnCl_2$ was coadministered with 100 mM KCl medium, striatal DA levels increased only to 607 ± 159% ($P < 0.001$) respect to basal values, without notably affecting the DOPAC and HVA levels. Therefore, $MnCl_2$ decreased the KCl-evoked DA release.

Effect of $MnCl_2$ on the DA Release Induced by Pargyline

After intraperitoneal injection of pargyline (100 mg/kg), the striatal DA levels increased to 169.4 ± 45.6% respect to basal values ($P < 0.05$), whereas DOPAC and HVA levels decreased to 26.7 ± 6.4 and 21.6 ± 5.3% ($P < 0.01$), respectively (Fig. 3). When $MnCl_2$ was administered in the perfusion fluid, the increase of DA levels produced by pargyline was inhibited, whithout affecting the levels of DOPAC and HVA. Therefore, $MnCl_2$ decreased the pargyline-evoked DA release.

Effect of $MnCl_2$ on the Extracellular Levels of L-Dopa

In order to measure L-Dopa in dialysates, it was necessary to include an AADC inhibitor in the perfusion fluid: we used NSD 1015 (0.2 mM). Before its administration, basal L-Dopa levels in dialysates were not detected. NSD 1015 induced a progressive increase in the striatal L-Dopa levels, the maximum increase was reached 1 h after NSD 1015 administration (267.6 ± 32.5% respect to the levels obtained at 20 min of NSD 1015 administration). These levels remained stable, and decreased after removing the NSD 1015 in the perfusion fluid.

The coinfusion of $MnCl_2$ with NSD 1015 increased extracellular L-Dopa levels to 496.5 ± 40.5% ($P < 0.001$) (Fig. 4). Therefore, $MnCl_2$ increased striatal L-Dopa levels in presence of NSD 1015.

DISCUSSION

In this paper, we note that intrastriatal administration of 2 mM $MnCl_2$ to conscious and freely moving rats produces a significant decrease in the extracellular levels of DA, as well as an increase in L-Dopa levels in the presence of the decarboxylase inhibitor NSD 1015.

Several studies have noted that manganese decreased DA striatal levels and suggested different action mechanisms: Mn can affect mitochondrial function inhibiting Ca^{2+} efflux from mitochondria, compromising neuronal ATP production (12,13); Futhermore, striatal neurons showed losses of mitochondrial membrane potential and complex II activity following manganese exposure (14). Mn also produces nerve cell death and injury to dopaminergic nigrostriatal pathway and GABA-ergic nerve cells intrinsic to basal ganglia (15).

Other studies have proposed the oxidative stress produced by the autoxidation of DA as a cause of neurotoxicity (8,16). Striatal DA concentrations were considerably decreased by intranigral injection of $MnCl_2$. DOPAC levels were also decreased by $MnCl_2$, indicating that DA decay was related to either inhibition of the transmitter synthesis or degeneration of DA neurons (17). Subsequent studies have suggested that Mn produced excytotoxic lesions in the striatum by an indirect mechanism, which involves an early impairment of oxidative metabolism causing depletion in DA and DOPAC levels (18).

In this study, we note that Mn reduces DA extracellular levels, as well as KCl^- and pargyline-evoked DA release. KCl induces neurotransmitter release by exocitosis after nerve cell depolarization (19), whereas pargyline (non-selective MAO inhibitor) inhibits DA catabolism increasing its availability for release (20).

When a decrease in the levels of DA in basal conditions and in stimulated conditions by different mechanisms occurs, it seems that Mn would have a metabolic effect on the synthesis of DA instead of an

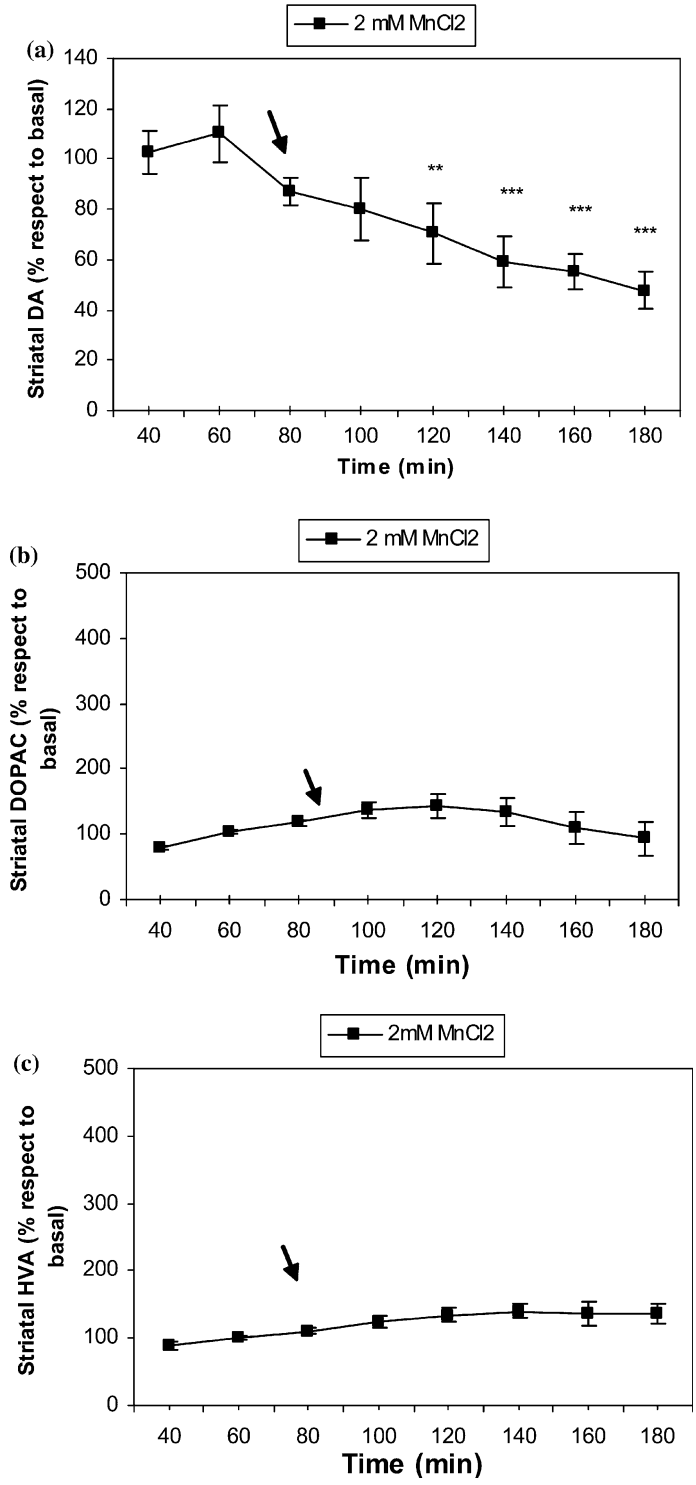

Fig. 1. Effect of intrastriatal administration of $MnCl_2$ (2 mM) on extracellular levels of (a) DA, (b) DOPAC and (c) HVA from rat striatum. After collecting basal samples for 60 min, manganese was infused for 120 min, as indicated by the arrow. The results are shown as the mean ± SEM of five experiments, expressed as a percentage of basal levels (100%). Basal levels were considered as the mean of substance concentration in the three samples before manganese was administered.

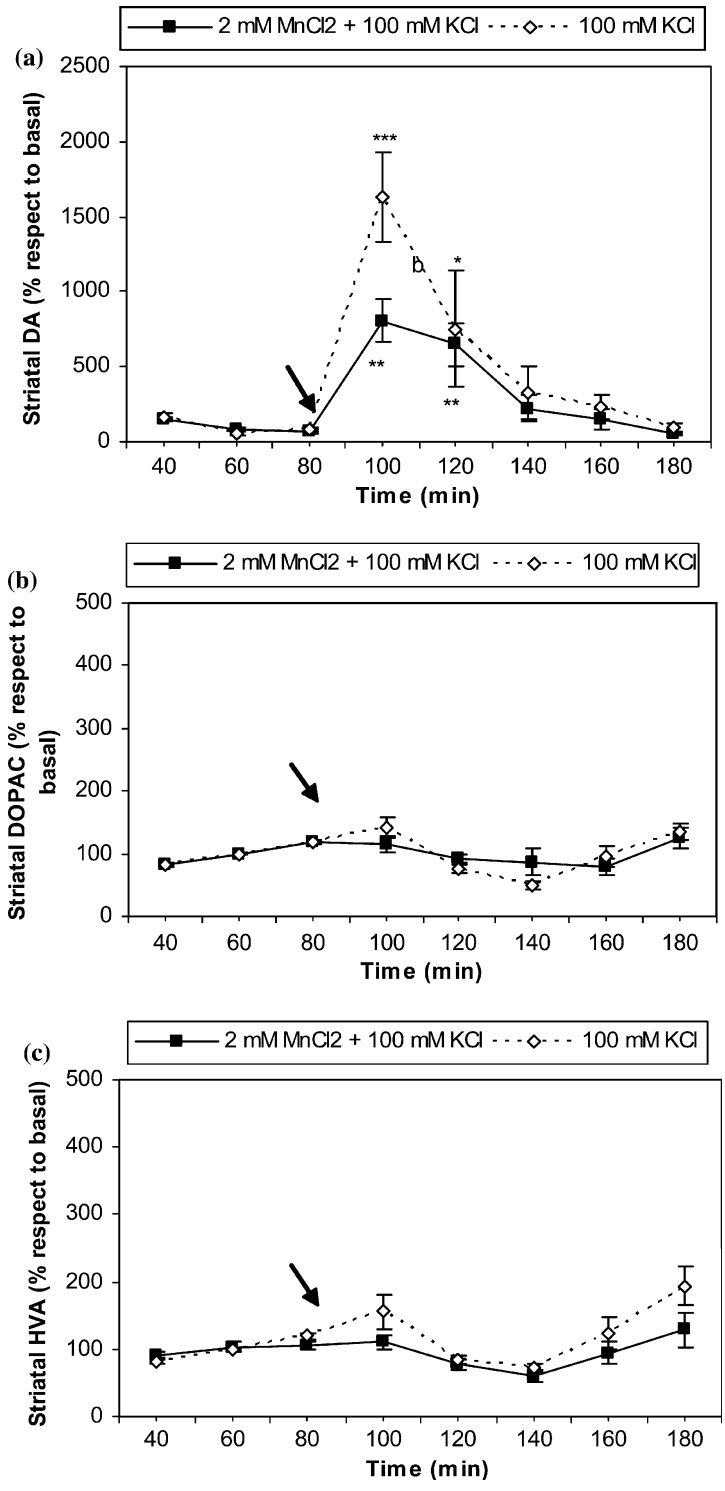

Fig. 2. Effect of $MnCl_2$ (2 mM) on intrastriatal levles of (a) DA, (b) DOPAC and (c) HVA induced by KCl (100 mM). After collecting basal samples for 60 min, KCl and $MnCl_2$ were coinfused for 60 as indicated by the arrow. The results are shown as the mean ± SEM of five experiments, expressed as a percentage of basal levels (100%). Basal levels were considered as the mean of substance concentration in the three samples before manganese was administered. Differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $^{a}P < 0.05$, $^{b}P < 0.01$ comparing the coinfusion of 100mM KCl medium, and $MnCl_2$ respect to basal.

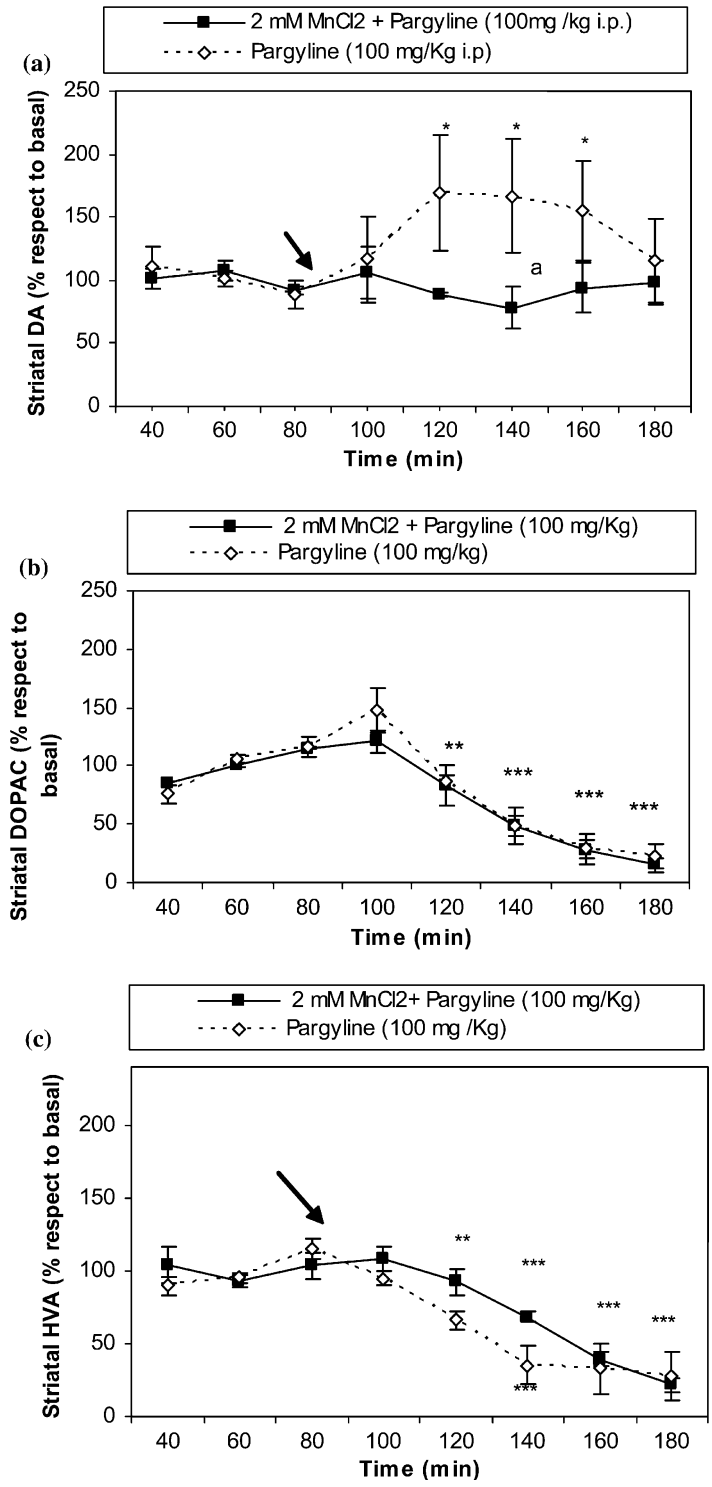

Fig. 3. Effect of $MnCl_2$ on the extracellular levels of DA (a), DOPAC (b) and HVA (c) in striatum of pargyline-pretreated rats. After collecting basal samples for 60 min, animals received an I.P. pargyline injection (100 mg/kg) and then infused with $MnCl_2$ for 60 min. The results are shown as the mean ± SEM of five experiments, expressed as a percentage of basal levels (100%). Basal levels were considered as the mean of substance concentration in the three samples before manganese was administered. Significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $^{a}P < 0.05$, comparing the coinfusion of $MnCl_2$ and pargyline with pargyline pretreatment.

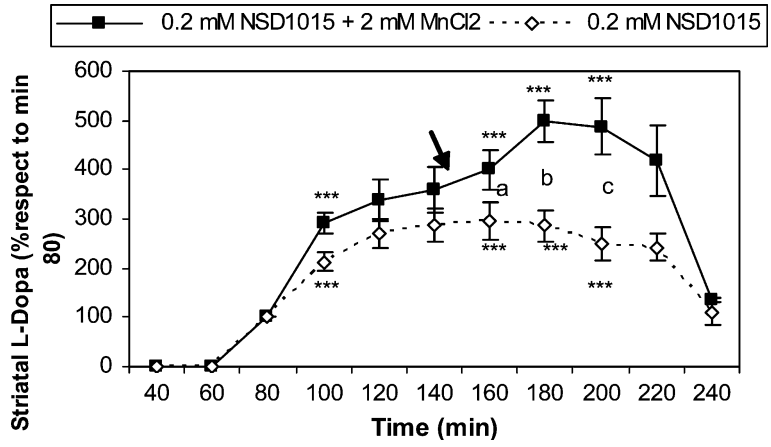

Fig. 4. Effect of $MnCl_2$ on extracellular levels of L-Dopa. After collecting three basal samples (60 min), NSD 1015 (0.2 mM) was administered during 140 min. The coinfusion of NSD 1015 and $MnCl_2$ were for 60 min after the administration of NSD 1015 alone for 80 min. After this, the medium was switched back to the unmodified Ringer's solution and the sampling was continued for an additional period of 60 min. The results are shown as the mean ± SEM of five experiments, expressed as a percentage respect to the first sample with NSD 1015 (100%). Significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ comparing the coinfusion of NSD 1015 and $MnCl_2$ with the administration of NSD 1015 alone.

effect on the release of DA, so that a decrease in this synthesis would also decrease its availability for release (basal or stimulated).

Because of this, we studied the effect of Mn on the levels of L-Dopa in the presence of a decarboxylase inhibitor. There are studies in which the effect of Mn on tyrosine hydroxylase (TH) activity (the key enzyme of DA biosynthesis that converts Tyr in L-Dopa) is analyzed. These studies have suggested that TH is inhibited in dopaminergic neurons soon after exposure to certain amounts of Mn (21). Moreover, Mn induced loss of striatal TH inmunoreactivity after 72 h of intranigral microinjections (22). In this paper, we noted that intrastriatal administration of $MnCl_2$ produced a significant increase in the extracellular levels of L-Dopa, in the presence of NSD 1015. This could point to an inhibiting effect of Mn on L-Dopa decarboxylase or other metabolic effects on striatal DA, or even a possible effect on L-Dopa that could affect its transformation to dopamine. Nevertheless, we may not reject a possible long-term effect on TH activity.

In any case, the effects of Mn on striatal dopaminergic system are apparently metabolic rather than affecting DA release.

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