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Modulation of dendritic release of dopamine by metabotropic glutamate receptors in rat substantia nigra

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Abstract

A superfusion system was used to study the effects of metabotropic glutamate receptor (mGluR) ligands upon the release of [^{3}H]dopamine ([^{3}H]DA) previously taken up by rat substantia nigra (SN) slices. *trans*-(±)-1-Amino-(1*S*,3*R*)-cyclopentane dicarboxylic acid (*trans*-ACPD; 100 and 600 μM), a group I and II mGluR agonist, evoked the release of [^{3}H]DA from nigral slices. This last effect was reduced significantly by (2*S*,3*S*,4*S*)-2-methyl-2-(carboxycyclopropyl)-glycine (MCCG; 300 μM), an antagonist of group II mGluR, or by the addition of tetrodotoxin (D-APV; 1 μM) to the superfusion medium. D-(−)-2-Amino-5-phosphono-valeric acid (100 μM), an *N*-methyl-D-aspartate receptor antagonist, or the presence of Mg^{2+} (1.2 mM) in the superfusion medium did not modify *trans*-ACPD-induced [^{3}H]DA release. In addition, a group II mGluR agonist such as (2*S*,1′*R*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)-glycine (DCG-IV; 100 μM) significantly induced the release of [^{3}H]DA from nigral slices, whereas a group I mGluR agonist such as (*RS*)-3,5-dihydroxyphenylglycine (DHPG; 50 and 100 μM) did not modify the release of the [^{3}H]-amine. Further experiments showed that the NMDA (100 μM)-evoked release of [^{3}H]DA was decreased significantly by prior exposure of SN slices to *trans*-ACPD. Finally, partial denervation of the DA nigro-striatal pathway with 6-hydroxydopamine (6-OH-DA) increased *trans*-ACPD-induced release of [^{3}H]DA, whereas it decreased *trans*-ACPD inhibitory effects on NMDA-evoked release of [^{3}H]DA from nigral slices. The present results suggest that the dendritic release of DA in the SN is regulated by mGluR activation. Such nigral mGluR activation may produce opposite effects upon basal and NMDA-evoked release of DA in the SN. In addition, such mGluR-induced effects in the SN are modified in response to partial denervation of the DA nigro-striatal pathway.

Keywords: Metabotropic; Glutamate; Receptors; Dendrites; Dopamine release

1. Introduction

The SN is one of the main output nuclei of basal ganglia structures within the brain and as such plays an important role in the coordination of motor activity. DA neuronal systems, originating in the SN pars compacta, constitute one of the main actors of such an important role. Indeed, degeneration of ventral midbrain DAergic neurons and subsequent depletion of striatal DA characterize Parkinson's disease. Therefore, understanding the regulatory mechanisms governing DA neuronal activity within the SN may contribute to the search of potential therapies for Parkinson's disease.

The existence of GLU-releasing nerve terminals in the SN [1–3], probably arising from cell bodies located in the subthalamic nucleus and frontal cortex [2,4,5], raises the possibility that these afferent pathways may influence DA neuronal activity in this basal ganglia nuclei. Indeed, iGluRs activated by NMDA or AMPA produce membrane depolarization and increase fast excitatory postsynaptic currents in midbrain DA neurons [6]. In addition, activation of mGluRs has been found to induce inward currents and to mediate inhibition and excitation of the firing rate in DA mesencephalic neurons [7–10]. These data agree with binding, immunohistochemical, and *in situ* hybridization

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Abbreviations: SN, substantia nigra; DA, dopamine; GLU, glutamate; iGluR, ionotropic GLU receptor; mGluR, metabotropic GLU receptor; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole; KRP, Krebs–Ringer-phosphate; 6-OH-DA, 6-hydroxydopamine; GABA, γ-aminobutyric acid; TTX, tetrodotoxin; *trans*-ACPD, *trans*-(±)-1-amino-(1*S*,3*R*)-cyclopentane dicarboxylic acid; KA, kainate; DHPG, (*RS*)-3,5-dihydroxyphenylglycine; DCG-IV, (2*S*,1′*R*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)-glycine; MCCG, (2*S*,3*S*,4*S*)-2-methyl-2-(carboxycyclopropyl)-glycine; D-APV, D-(−)-2-amino-5-phosphono-valeric acid.

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studies showing that iGluRs and mGluRs are expressed in midbrain DA neurons [11–15].

Anatomical, electrophysiological and biochemical evidence indicates that DA is stored and released from dendrites of nigral DA neurons and may contribute to the processing of information within the SN [16,17]. We and others [18–20] have reported that activation of ionotropic NMDA receptors in the SN may trigger a Ca^{2+}-dependent release of DA from dendrites of nigro-striatal DA-containing neurons. DA released within the SN may exert autoregulatory effects on the firing rate of DA cells themselves [17,21]. In addition, dendritically released DA also may regulate the activity of non-DAergic neuronal structures within the SN [22]. In support of this view, nigro-thalamic neurons were found to be activated by iontophoretically applied DA [22], and the inhibition of SN pars reticulata neurons evoked by GABA iontophoresis was diminished by DA [23]. In addition, *in vitro* studies with nigral slices and *in vivo* studies with microdialysis methodology suggest that dendritically released DA may facilitate Ca^{2+}-dependent release of excitatory amino acid transmitters within the SN pars reticulata [24–26]. These findings have led to the proposition that DA, released from nigral dendrites, may affect the function of input and output neurons in the SN. Therefore, NMDA receptor-mediated activation of DA release in the SN may play a pivotal role in determining the outflow of information from the SN to caudal as well as rostral areas in the brain. Currently, no information is available as to whether other excitatory amino acid receptors, such as ionotropic AMPA or metabotropic GLU receptors, modulate dendritic DA release in the SN.

In view of the above, the aim of the present work was to study in rat SN the dendritic release of DA in the presence of chemical agents known to modify the activation of mGluRs. The efflux of [^{3}H]DA from superfused slices of SN was used as an experimental index of DA dendritically released [18]. Studies also were performed taking into account the fact that mGluRs may interact with ionotropic NMDA receptors [27,28], and this could have functional consequences in relation to dendritic release of DA in the SN. In addition, the effect of a partial lesion of the nigro-striatal DA pathway was assessed as regards the efflux of [^{3}H]DA from the SN in the presence of mGluRs and NMDA receptor ligands. A preliminary account of this work has been reported elsewhere [29].

2. Materials and methods

2.1. Preparation and incubation of rat SN slices for release experiments

Adult male Sprague–Dawley rats were decapitated, the brains were removed quickly, and both SN were dissected at 4°, as described previously [18] from 0.9-mm thick coronal sections of the mesencephalon. SN slices (6–8 mg) were prepared using a Sorvall tissue slicer by chopping at 0.2-mm intervals in two directions. The slices were then incubated for 30 min at 37° in 2 mL KRP at pH 7.4, saturated with 95% O_2 and containing [^{3}H]DA (sp. act. 50 Ci/mmol, final concentration, 5.0×10^{-8} M). At the end of the incubation period, the slices were rapidly separated from the medium through a 5.0-mL Lucite chamber with a nylon mesh bottom, prior to conducting the [^{3}H]DA release experiments described below. All the animal experiments performed in the present study were conducted in accordance with guidelines of the Catholic University of Chile and FONDECYT.

2.2. [^{3}H]DA release from SN slices

The spontaneous and ligand-induced release of preaccumulated [^{3}H]DA was followed as described previously by Araneda and Bustos [18]. In brief, nigral slices, previously incubated with [^{3}H]DA for 30 min as described above, were transferred to Lucite chambers, washed with 10 mL of KRP, and then superfused, unless stated differently, with KRP–Mg^{2+}-free solution that was being oxygenated continuously and prewarmed to 37°. A constant flow rate of 2 mL/min was maintained by means of a peristaltic pump, and a two-way system was set up to switch to different superfusing solutions without disrupting the flow. Slices were superfused for 45 min to establish a steady basal release prior to stimulation. Four samples of 1 min each were collected to determine the basal release of the labeled catecholamines. Thereafter, stimulated release from nigral slices was induced by changing the superfusing solution for 2–6 min to KRP–Mg^{2+}-free solution containing mGluR or iGluR agonists. Following that, the superfusion fluid was changed back to KRP–Mg^{2+}-free solution, and five additional samples of 1 min each were collected. Antagonists of amino acid receptors were added to the superfusion system 2 min prior to and during the stimulation period, carried out with the iGluR and mGluR agonists. When used, TTX was added to the superfusion system as described for the antagonists of amino acid receptors. At the end of the experiments, the slices were recovered, and the remaining radioactivity was extracted from the tissue with trichloroacetic acid (15%, w/v) and assessed by liquid scintillation counting as described below.

2.3. Chemical lesions of the medial forebrain bundle

Male Sprague–Dawley rats (220–240 g) were administered desipramine (25 mg/kg) prior to being anesthesized by intraperitoneal injection of chloral hydrate (400 mg/kg). Thereafter, 8 μg of 6-OH-DA in ascorbic acid, 0.01% (w/v), in a total volume of 4 μL (0.5 μL/min), was injected stereotaxically into the right medial forebrain bundle. The syringe (10 μL, Hamilton) was left in place for 5 min

following 6-OH-DA injection. Coordinates for the placement of the syringe tip were: antero–posterior, 4.0 mm; dorso–ventral, 7.7 mm; and lateral, 1.4 mm, according to the rat brain stereotaxic atlas of Paxinos and Watson [30]. The animals were allowed to recover for 7 days prior to conducting the experiments. In sham-operated animals, the stereotaxic injections were made with ascorbic acid solution (0.01%, w/v). Endogenous levels of striatal DA were quantitated in these lesioned rats as described below.

2.4. Chromatographic procedures and radioactivity determinations

Separation and analysis of the tissue and superfusion medium for labeled catechols were carried out by absorption as described previously [31]. Alumina columns were used to concentrate the catecholamines and deaminated metabolites. Eluates from chromatography columns containing labeled catechols were analyzed for [^{3}H] in a Nuclear Chicago Scintillation counter. Reported values represent the amount of both DA and deaminated metabolites collected during the superfusion experiments. HPLC–electrochemical analysis followed by radioactivity measurements indicated that under the experimental conditions reported, labeled DA accounted for 75–80% of the labeled catechols released.

Endogenous levels of DA in the striatum were quantitated by HPLC and electrochemical detection [32]. In brief, each striatum was homogenized in 0.1 M HCl, and aliquots of the homogenate were precipitated with perchloric acid (0.1 M, final concentration). The resulting clear supernatant was analyzed for DA by reversed-phase ion-pairing HPLC (C_{18}, 5 μm, Rainin) and electrochemical detection (BAS). The mobile phase consisted of 100 mM sodium phosphate, 0.2 mM sodium octyl sulfate, 0.01% EDTA (w/v), and 4% CH_3CN, pH 3.5. The oxidation potential was set at 0.8 V.

2.5. Calculation of release data

Overflow of [^{3}H]DA was calculated and expressed as the percentage of total radioactivity present in the nigral slices at the onset of the superfusion period. Total radioactivity was calculated as the sum of the [^{3}H]catechols released during the superfusion and the amount remaining in the tissue at the end of the experiment. The spontaneous level of released catechols corresponded to the average percent of tritium released during 4 min before the stimulation

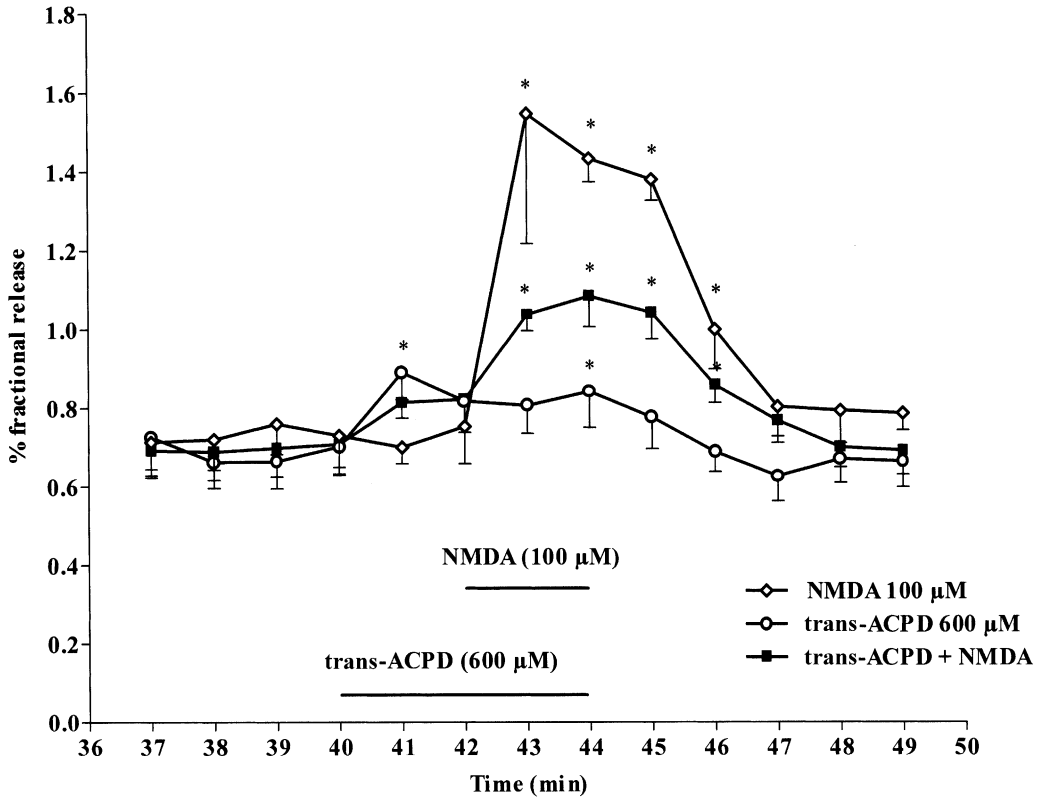

Fig. 1. Time course of *trans*-ACPD effects on NMDA-induced [^{3}H]DA release from nigral slices. The slices were exposed to *trans*-ACPD (600 μM) and NMDA (100 μM) stimulation for 4 and 2 min, respectively. When indicated, *trans*-ACPD was added to the superfusion system 2 min prior to NMDA stimulation. The superfusion study was conducted in the presence of *trans*-ACPD alone or NMDA alone or *trans*-ACPD plus NMDA. The slices took up 32,666 ± 4,455 (*trans*-ACPD), 33,371 ± 1,942 (NMDA) and 33,266 ± 2,969 (*trans*-ACPD plus NMDA) dpm of [^{3}H]DA/μg of protein, respectively. Each value represents the mean ± SEM of three individual experiments. Key: (∗) $P < 0.05$, compared with the respective baseline values as determined by one-way ANOVA for repeated measures followed by the Fisher PLSD test.

period and once steady basal levels of release were established. The stimulus-induced release evoked by the different amino acid receptor ligands was calculated by considering the whole area above the spontaneous levels of release during and 5 min after the period of stimulation. Results are expressed as mean ± SEM. Unless stated differently, statistical analysis were performed by using a one-way ANOVA followed by a Newman–Keuls post-hoc analysis. Statistical analysis of the data shown in Fig. 1 was performed by one-way ANOVA for repeated measures followed by the Fisher PLSD test. Values of $P < 0.05$ were considered significant.

2.6. Solutions and chemicals

The KRP–Mg^{2+}-free solution used in these experiments had the following composition: 128 mM NaCl, 4.8 mM KCl, 0.75 mM $CaCl_2$, 16 mM glucose, and 16 mM Na_2HPO_4 (pH 7.4). When added, $MgSO_4$ was used at a concentration of 1.2 mM. All amino acid receptor ligands were purchased from RBI Inc. and from Tocris Cookson Inc. The following amino acid receptor ligands were used: *trans*-ACPD and KA, obtained from RBI Inc.; DHPG, DCG-IV and MCCG, purchased from Tocris. NMDA and D-APV were obtained from the Sigma Chemical Co.

All other reagents were obtained from Sigma. [^{3}H]DA (50 Ci/mmol) was purchased from Amersham, Life Science.

3. Results

3.1. Effect of mGluR and iGluR ligands on spontaneous release of [^{3}H]DA from nigral slices

Previous results from us have indicated that under our experimental conditions [^{3}H]DA labels and is released mainly from somato-dendritic regions of DA neurons in the SN [18]. Spontaneous levels of [^{3}H]DA release were relatively low and amounted to approximately 0.65%/min of superfusion of the total [^{3}H]catechols accumulated into the slices (Table 1). The introduction of *trans*-ACPD, a group I and group II mGluR agonist, for 4 min into the KRP–Mg^{2+}-free superfusion medium produced a concentration-dependent stimulatory effect on the basal release of [^{3}H]DA from nigral slices (Table 1). *trans*-ACPD, at 100 and 600 μM, significantly increased the basal release of [^{3}H]DA from nigral slices (Table 1). In contrast, 300 μM *trans*-ACPD produced no statistical changes in the basal release of [^{3}H]DA. Concentrations of *trans*-ACPD lower than 100 μM were not used under the present experimental conditions.

The basal release of [^{3}H]DA from nigral slices was also studied in the presence of other GLU receptor agonists (Table 1). A group II mGluR agonist, such as DCG-IV (100 μM), significantly stimulated the release of [^{3}H]DA.

Table 1
Effect of different GLU receptor agonists on basal [^{3}H]DA release from nigral slices

	Release of [^{3}H]DA taken up by nigral slices (%)
Basal	2.65 ± 0.19
trans-ACPD (100 μM)	3.34 ± 0.45*
trans-ACPD (300 μM)	2.89 ± 0.48
trans-ACPD (600 μM)	3.33 ± 0.18*
DCG-IV (100 μM)	3.35 ± 0.22*
DHPG (50 μM)	2.37 ± 0.38
DHPG (100 μM)	2.69 ± 0.14
NMDA (100 μM	5.12 ± 0.24**
KA (100 μM)	3.62 ± 0.45*

Nigral slices were exposed to the different GLU receptor agonists for 4 min. All experiments were carried out in Mg^{2+}-free KRP, except for those conducted with DCG-IV (100 μM) in which Mg^{2+} (1.2 mM) was added to the superfusion medium. Nigral slices previously loaded with [^{3}H]DA were allowed to reach a steady level of release of radioactivity in the superfusion medium before stimulation with the different GLU receptor 1igands. Data shown correspond to the percent of total radioactivity taken up by the nigral slices, which was released during the 4 min exposure to the GLU receptor agonists. The nigral slices took up an average of 32,252 ± 1,863 dpm of [^{3}H]DA/μg of protein. Each value represents the mean ± SEM of at least three individual experiments.

* Compared with basal release ($P < 0.05$).

** Compared with basal release ($P < 0.005$).

In the latter case, the experiments were conducted in the presence of Mg^{2+} (1.2 mM) in the superfusion medium, as some reports suggest that DCG-IV may also act as an agonist upon NMDA receptors [33]. In other experiments, DHPG, a group I mGluR agonist, produced no significant changes on [^{3}H]DA release from nigral slices, at either 50 or 100 μM (Table 1). As documented previously by us [18], the basal release of [^{3}H]DA in the SN was augmented markedly by the presence of iGluR agonists such as NMDA ($P < 0.005$), and slightly but still significantly by KA ($P < 0.05$) (Table 1).

The stimulatory effect of the group I and group II mGluR agonist *trans*-ACPD (600 μM) on basal release of [^{3}H]DA was not modified significantly by the presence of Mg^{2+} (1.2 mM) or by the addition of D-APV (100 μM), an NMDA receptor antagonist, to the superfusion medium (Table 2). In contrast, MCCG (300 μM), an antagonist of group II mGluRs, significantly diminished [^{3}H]DA release induced by *trans*-ACPD (600 μM) (Table 2). Such *trans*-ACPD-induced [^{3}H]DA release was also reduced by the introduction of TTX (1 μM), a blocker of voltage-dependent Na^+ channels (Table 2). MCCG (300 μM) alone or TTX (1 μM) alone produced no changes in the basal release of [^{3}H]DA from nigral slices (data not shown).

3.2. Interactions between mGluRs and NMDA receptors in relation to release of [^{3}H]DA from nigral slices

We proceeded to evaluate the possibility that an interaction between mGluRs and NMDA receptors might get established and produce functional consequences in

Table 2
Pharmacological characteristics of *trans*-ACPD-induced [^{3}H]DA release from nigral slices

	[^{3}H]DA released per stimulus (%)
trans-ACPD (600 μM)	1.06 ± 0.20
trans-ACPD + D-APV (100 μM)	1.13 ± 0.15
trans-ACPD + MCCG (300 μM)	0.34 ± 0.20*
trans-ACPD + Mg^{2+} (1.2 μM)	0.61 ± 0.23
trans-ACPD + TTX (1 μM)	0.42 ± 0.15*

Nigral slices previously loaded with [^{3}H]DA were allowed to reach a constant basal level of release in the superfusing solution. Thereafter, the slices were subjected to 4 min of *trans*-ACPD (600 μM) stimulation in Mg^{2+}-free KRP. D-APV (100 μM), MCCG (300 μM), Mg^{2+} (1.2 mM) and TTX (1 μM), respectively, were added 2 min before and during the 4 min of *trans*-ACPD stimulation. [^{3}H]DA released per stimulus was calculated by considering the whole area above spontaneous release during and 5 min after the period of stimulation with *trans*-ACPD. It was expressed as a percentage (%) of the total radioactivity taken up by the nigral slices. The slices took up an average of 29,386 ± 1,224 dpm of [^{3}H]DA/μg protein Each value represents the mean ± SEM of at least three individual experiments.

* $P < 0.05$ Compared with *trans*-ACPD-induced [^{3}H]DA release.

relation to [^{3}H]DA release from nigral slices. Therefore, the NMDA-induced release of [^{3}H]DA was studied in nigral slices previously superfused during 2 min with *trans*-ACPD (Figs. 1 and 2). NMDA (100 μM) alone produced an increase in the time course of [^{3}H]DA release (Fig. 1), which was diminished significantly when the same NMDA stimulus was performed in the presence of the mGluR agonist *trans*-ACPD (600 μM) (Figs. 1 and 2). *trans*-ACPD alone also evoked a slight but significant ($P < 0.05$) increase of [^{3}H]DA release from nigral slices under the experimental conditions used. The inhibitory effect of *trans*-ACPD on NMDA-induced [^{3}H]DA release was concentration-dependent as it was not observed at lower concentrations of *trans*-ACPD (100 and 300 μM) (Fig. 2). Nevertheless, even in the presence of *trans*-ACPD at 100 and 300 μM, the NMDA-induced effect on DA release was not additive to that induced by *trans*-ACPD and indeed it was much lower than that expected by the addition of the effects of NMDA and *trans*-ACPD alone (Fig. 2). In the presence of D-APV (100 μM), an NMDA receptor antagonist, the effects of *trans*-ACPD on NMDA-induced release disappeared, and the remaining effects on [^{3}H]DA release coincided well with those of *trans*-ACPD alone (Table 3).

To evaluate if the inhibitory effect of *trans*-ACPD on NMDA-induced release of [^{3}H]DA was dependent upon the prior exposure of the nigral slices to the mGluR agonist, we reversed the order of addition of the GLU receptor ligands to the superfusion medium. When the nigral slices were exposed first to NMDA (100 μM) and 2 min later to *trans*-ACPD (600 μM), no inhibitory effect of the mGluR

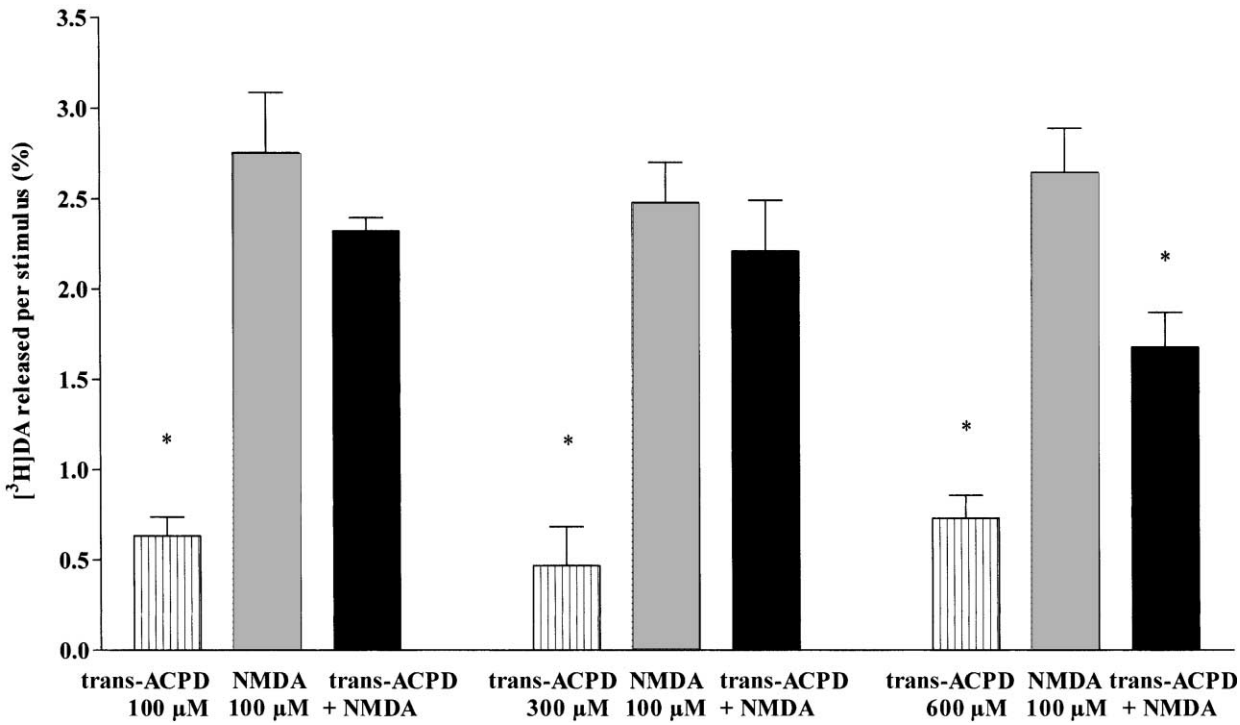

Fig. 2. Effect of increasing concentrations of *trans*-ACPD on basal and NMDA-induced [^{3}H]DA release from nigral slices. The slices were exposed to *trans*-ACPD (100, 300, and 600 μM) 2 min prior to and during the 2 min of NMDA (100 μM) stimulation. The figure also shows the evoked release of [^{3}H]DA when the slices were superfused with *trans*-ACPD alone (100, 300, and 600 μM, 4 min in each case), and with NMDA alone (100 μM, 2 min). [^{3}H]DA released per stimulus was calculated by considering the whole area above spontaneous release during and 5 min after the period of stimulation with *trans*-ACPD alone, NMDA alone, or *trans*-ACPD plus NMDA and it was expressed as a percentage (%) of total radioactivity taken up by the nigral slices. Values are the mean ± SEM of three individual experiments. Key: (*) $P < 0.05$, compared with the respective NMDA experimental group.

Table 3
Effect of D-APV on *trans*-ACPD plus NMDA-induced [^{3}H]DA release from nigral slices

	[^{3}H]DA released per stimulus (%)
trans-ACPD + NMDA	1.38 ± 0.22
trans-ACPD + NMDA + D-APV	0.76 ± 0.15*
trans-ACPD	0.73 ± 0.10*

Nigral slices previously loaded with [^{3}H]DA were allowed to reach a constant basal level of release in the superfusing solution. Thereafter, the slices were exposed for 2 min to NMDA (100 μM) stimulation. *trans*-ACPD (600 μM) was added 2 min before and during the 2 min of NMDA sumulation. D-APV (100 μM) was added to the superfusion solution 2 min prior to *trans*-ACPD stimulation and during the rest of the superfusion period. All the experiments were performed in Mg^{2+}-free KRP. [^{3}H]DA released per stimulus was calculated by considering the whole area above spontaneous release during and 5 min after the period of stimulation with *trans*-ACPD alone or *trans*-ACPD plus NMDA. It was expressed as a percentage (%) of the total radioactivity taken up by the nigral slices. Each value represents the mean ± SEM of at least three individual experiments.

* $P < 0.05$ Compared with *trans*-ACPD plus NMDA effects on [^{3}H]DA release.

agonist was detected upon the NMDA-induced release of [^{3}H]DA (Fig. 3). Instead, under these last experimental conditions, a slight but non-significant stimulatory effect of *trans*-ACPD on NMDA-induced release was observed (Fig. 3). Therefore, the inhibitory effect of *trans*-ACPD on NMDA-induced [^{3}H]DA release depends upon the prior exposure of the nigral slices to the mGluR agonist.

Finally, the prior exposure of the nigral slices to *trans*-ACPD (600 μM) did not modify the release of [^{3}H]DA induced by other iGluR agonists, such as KA (100 μM) (data not shown).

3.3. Effect of partial lesions of the DAergic nigro-striatal pathway upon NMDA- and trans-ACPD-induced release of [^{3}H]DA from nigral slices

To evaluate if lesions of the nigro-striatal DA pathway could modify interactions between mGluRs and NMDA receptors in the SN, we studied [^{3}H]DA release in superfused nigral slices from rats treated with 6-OH-DA. Unilateral lesions of the medial forebrain bundle were produced with 6-OH-DA to induce partial degeneration of nigro-striatal DAergic neurons. Seven days following the lesion there was a 47% reduction in [^{3}H]DA accumulated by slices prepared from ipsilateral SN when compared with sham ipsilateral nigral slices ($P < 0.05$). Parallel to these changes, a significant reduction (89%) was observed in the endogenous levels of DA from the ipsilateral striatum of 6-OH-DA-treated rats compared

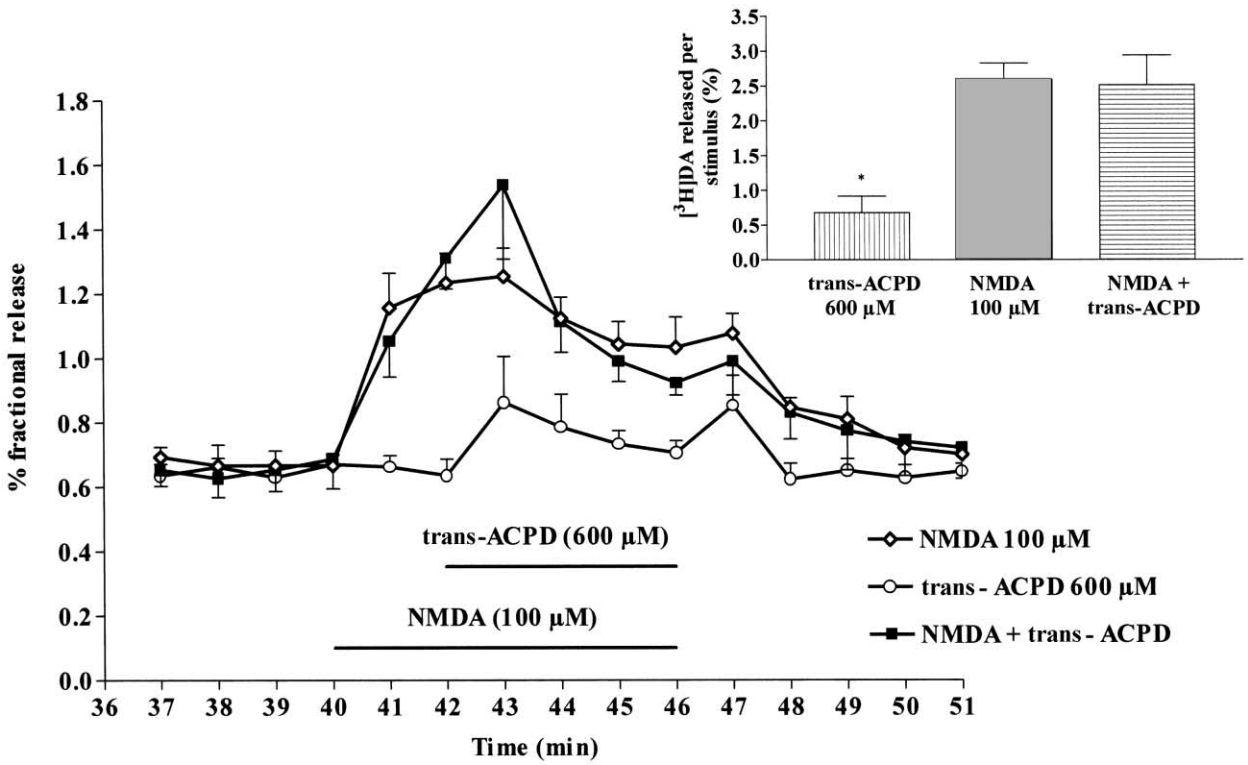

Fig. 3. Time course of the effects of NMDA on *trans*-ACPD-induced release of [^{3}H]DA from nigral slices. The slices were exposed to NMDA (100 μM) and *trans*-ACPD (600 μM) stimulation during 6 and 4 min, respectively. When indicated, NMDA was added to the superfusion system 2 min prior to *trans*-ACPD stimulation. The superfusion study was conducted in the presence of NMDA alone, *trans*-ACPD alone, or *trans*-ACPD plus NMDA. The inset at the upper right shows the evoked release of [^{3}H]DA under different experimental conditions. The slices took up 27,803 ± 1,266 (NMDA), 31,716 ± 1,088 (*trans*-ACPD), and 25,571 ± 2,850 (NMDA plus *trans*-ACPD) dpm of [^{3}H]DA/μg of protein, respectively. Each value represents the mean ± SEM of three individual experiments. Key: (*) $P < 0.05$, compared with the NMDA and the *trans*-ACPD plus NMDA experimental groups.

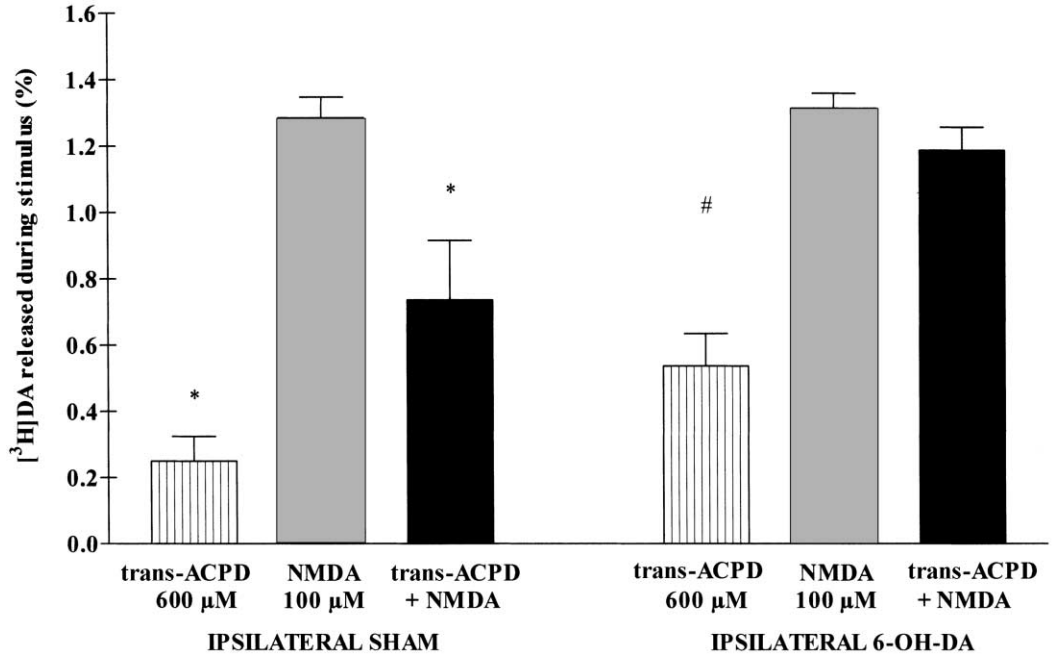

Fig. 4. Effect of partial lesions of the DAergic nigro-striatal pathway on the NMDA- and the *trans*-ACPD-induced release of [^{3}H]DA from nigral slices. Slices from sham- and 6-OH-DA-treated animals were exposed to *trans*-ACPD (600 μM; 4 min), or to NMDA (100 μM; 2 min), or to *trans*-ACPD (600 μM) 2 min prior to and during the 2 min of NMDA (100 μM) stimulation. [^{3}H]DA release per stimulus was calculated by considering the whole area above spontaneous release during and 5 min after the period of stimulation with *trans*-ACPD alone, NMDA alone, or *trans*-ACPD plus NMDA, and it was expressed as a percentage (%) of the total radioactivity taken up by the nigral slices. The slices took up 28,200 ± 994 (sham-treated animals) and 14,877 ± 728 (6-OH-DA-treated animals) dpm of [^{3}H]DA/μg of protein, respectively. Results represent the mean ± SEM of at least three individual experiments. Key: (*) $P < 0.05$ compared with the respective NMDA experimental group; and (#) $P < 0.05$ compared with the ipsilateral sham *trans*-ACPD and with the respective ipsilateral NMDA plus *trans*-ACPD group.

with sham-treated animals (4.2 ± 1.1 vs 38.5 ± 2.6 pmol/mg wet weight; $P < 0.002$).

Partial lesions of the DAergic nigro-striatal pathway modified [^{3}H]DA release in response to stimulations induced by *trans*-ACPD alone and by the combination of *trans*-ACPD plus NMDA. Thus, *trans*-ACPD (600 μM)-induced [^{3}H]DA release in ipsilateral nigral slices from 6-OH-DA-treated animals was almost 2-fold higher than in ipsilateral nigral slices from sham animals (Fig. 4). On the other hand, the inhibitory effect of *trans*-ACPD reported above on NMDA-induced release of [^{3}H]DA from nigral slices disappeared after partial lesioning of the DA nigro-striatal pathway (Fig. 4). Finally, NMDA-induced release of nigral [^{3}H]DA was not modified by 6-OH-DA-produced lesions of the DA pathway (Fig. 4).

4. Discussion

4.1. Effect of local mGluR activation on basal DA release in the SN

Previous studies from us, conducted in nigral slices, have shown that activation of NMDA receptors in rat SN may trigger a release of DA, possibly arising from dendrites of nigro-striatal DA-containing neurons [18]. In the present study, we have provided evidence indicating that activation of mGluRs in the SN may also evoke a release of DA in this brain region. Thus, *trans*-ACPD, a group I and II mGluR agonist, was found to induce an NMDA-independent release of [^{3}H]DA from superfused nigral slices. Such a *trans*-ACPD induced release was not blocked significantly by the addition of Mg^{2+} (1.2 mM) or D-APV to the superfusion medium, two experimental procedures known to antagonize the NMDA receptor–ionophore complex in brain tissue [34,35].

The pharmacological analysis conducted in the present study suggests that group II mGluRs mediate part of the *trans*-ACPD effect on basal DA release in the SN. Thus, DCG-IV, a group II mGluR agonist, stimulated the release of [^{3}H]DA whereas MCCG, an antagonist of these receptors, significantly diminished *trans*-ACPD-induced release of DA from nigral slices. In addition, DHPG, an agonist of group I mGluRs, produced no effects upon the basal release of [^{3}H]DA in SN slices.

It is interesting that electrophysiological studies have shown that *trans*-ACPD, interacting with mGluRs, increases the excitability of rat mesencephalic neurons. The *trans*-ACPD-induced excitation seems to be associated with the combined effect of a reduction in potassium conductance and an increase in sodium conductance [7,9]. Although a thorough pharmacological analysis was not performed in the previous electrophysiological studies, Shen and Johnson [9] have suggested that mGluR1 or mGluR2 may participate in the *trans*-ACPD-mediated currents in mesencephalic DA neurons. Interestingly,

immunocytochemical studies have revealed the existence of subtypes 2/3 mGluR immunoreactivity in neurons of SN zona compacta and in perikarya and proximal dendrites of SN zona reticulata [14]. It is possible that the *trans*-ACPD-induced currents described in DA neurons [7,9] may underlie the increased [^{3}H]DA release occurring in the SN in response to *trans*-ACPD stimulation.

Another observation from the present study is the fact that [^{3}H]DA release evoked by *trans*-ACPD was partially but significantly depressed by TTX, a potent and specific blocker of voltage-dependent Na^+ channels. These results may imply that activation of the mGluR by *trans*-ACPD could somehow activate voltage-dependent Na^+ channels, which, in turn, might trigger the release of DA from nigral dendrites by activating voltage-mediated Ca^{2+}-conductances present in DAergic cells [18,36]. Other explanations exist, including the participation of nigral intrinsic interneurons in mediating the activation mGluRs with the release of nigral DA. For instance, glycine-containing interneurons may be acting in such a way as supported by the fact that glycine through strychnine-sensitive receptors markedly evokes [^{3}H]DA release from nigral slices superfused in a Mg^{2+}-free medium [18]. However, there is still a matter of debate as to whether glycine acts as an endogenous transmitter in the SN. Intrinsic GABA-containing neurons in the SN may not be involved in the *trans*-ACPD-induced effects, as GABA was found to produce only modest effects on [^{3}H]DA release in the SN, both in normal and in Mg^{2+}-free medium [18]. Further research is needed to assess whether mGluRs modulating nigral DA release are located in DA cells themselves, as well as in other intrinsic neurons in the SN.

Basal DA release in the SN exhibited a non-linear response in the presence of increasing concentrations of *trans*-ACPD. Low (100 μM) and high (600 μM) concentrations of *trans*-ACPD stimulated basal release of DA, whereas an intermediate concentration (300 μM) produced no changes upon this release. Indeed, this is not surprising in view of the fact that *trans*-ACPD is an agonist that does not discriminate well among the different types of mGluRs. In addition, *trans*-ACPD has been found to produce a combination of excitatory and inhibitory effects in mesencephalic DA neurons [8,10], which may vary depending on the dose of agonist used and on the desensitization process that follows the activation of the mGluRs. Thus, *trans*-ACPD produces a biphasic effect on the firing rate of DA cells: as indicated above, *trans*-ACPD may induce excitatory effects in SN DA neurons by a G protein-mediated blockade of K^+ channels and by an increase in a TTX-insensitive Na^+ current [7,9]. On the other hand, *trans*-ACPD may produce inhibitory effects on ventral midbrain DA neurons by mobilization of intracellular Ca^{2+} and activation of apamin-sensitive potassium conductances [8,10]. Furthermore, prolonged activation of mGluRs in midbrain DA neurons desensitized the hyperpolarizing response and caused the more commonly reported depolarization responses induced by *trans*-ACPD [10]. It is interesting that Taber and Fibiger [37] have demonstrated in nucleus accumbens that the effect of *trans*-ACPD on DA release is biphasic with respect to dose and that lower doses reduce extracellular DA content, whereas high doses increase it.

The present study showed that partial denervation of the nigro-striatal DAergic pathway was associated with an increase in *trans*-ACPD-induced effects on DA release, whereas no changes in NMDA-induced DA release were observed. In this sense, it is possible that this specific change in *trans*-ACPD-induced DA release could be related to adaptative or compensatory responses to injury in order to maintain adequate levels of extracellular DA in the SN. Interestingly, these results are in accord with rotational studies in rodents conducted by Kearney *et al.* [38], suggesting that under conditions of chronic dopamine denervation there could exist compensatory changes in the sensitivity of striatal mGluRs, which permit mGluR agonist-induced rotation to occur.

A recent report indicates that (+)-2-aminobicyclo[3 1 0]-hexane-2,6-dicarboxylate monohydrate (LY354740), a selective agonist of group II mGluRs, induces a dramatic reversal of catalepsy in a rat model of parkinsonism [39]. These results were accounted for, in part, by the fact that group II mGluRs are presynaptically localized on subthalamic nuclei nerve terminals in SN pars reticulata and that activation of these receptors by LY354740 inhibits excitatory transmission in SN pars reticulata [39,40]. These results could also be partially accounted for by a stimulatory effect of group II mGluR agonists on DA release, helping to maintain adequate extracellular DA in the SN of rats with partial DA-denervation. This is especially relevant considering that the sensitivity of nigral DA release towards mGluR activation is increased after the removal of nigrostriatal DA (Fig. 4). All of these results and those of Kearney *et al.* [38] raise the possibility that selective agonists of group II mGluRs could provide a new pharmacological approach to the treatment of Parkinson's disease [39,40].

4.2. Effect of local mGluR activation on NMDA-evoked release of DA in the SN

We performed further studies analyzing the effect of mGluR activation on NMDA-induced DA release from nigral slices. The results obtained show that *trans*-ACPD produces a significant decrease of NMDA-induced release of DA in the SN. The negative regulation of mGluR activation on NMDA-induced-DA release was opposite to the positive effect exerted by this mGluR upon basal DA release. The inhibitory effects of *trans*-ACPD on NMDA-induced release were very evident at 600 μM, but they also were observed at 100 and 300 μM concentrations of this agonist. Interestingly, these effects depended upon the prior exposure of the nigral slices to

trans-ACPD, and were not observed when the order of exposure to the GLU receptor agonists was reversed, that is NMDA added first and *trans*-ACPD later to the superfusing medium of the nigral slices.

It has been reported in mesencephalic cultures that NMDA may induce cyclic GMP formation, which is prevented in a concentration-dependent fashion by *trans*-ACPD [41]. The pharmacological profile described in this last work suggests that mGluRs negatively coupled to adenylate cyclase are responsible for this effect, i.e. it seems that group II mGluR activation is involved [41]. It is possible to propose then that under the present experimental conditions, *trans*-ACPD may decrease intracellular cyclic AMP levels, which, in turn, could reduce NMDA receptor phosphorylation and subsequently decrease NMDA-induced DA release in the SN.

Our present results and those of Ambrosini *et al.* [41] provide evidence for a mGluR–iGluR interaction at the level of the SN, specifically between mGluR and NMDA receptors. Interestingly, mGluR activation (group I and II) has been found recently to depress AMPA-mediated excitatory postsynaptic potential in rat brain DA neurons [42]. Therefore, both firing rate and dendritic release may be affected at the level of DA neurons by an interaction between iGluRs and mGluRs.

We and others have previously reported a DA–Glu ''positive feedback'' in the SN, which may contribute to chemical transmission in this nuclei [25,26,43]. Under stimulated conditions, mGluRs may negatively regulate this positive feedback by acting upon NMDA receptors functionally coupled to dendritic release of DA in the SN. Furthermore, an additional result found in the present work was that this mGluR effect upon NMDA-evoked release is attenuated following partial DA-denervation, suggesting that this could be a compensatory response in the face of increasing death of the DA cell population.

In summary, the present results indicate that DA release from DA dendrites in the SN may be subjected to regulation exerted by mGluR activation. Such nigral mGluR activation may produce opposite effects upon basal and NMDA-evoked release of DA in the SN. In addition, such mGluR-induced effects in the SN are modified in response to partial denervation of the DA nigro-striatal pathway.

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