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Further contribution to the study of corticostriatal glutamatergic and nigrostriatal dopaminergic interactions within the striatal network: an in vivo voltammetric investigation

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Summary. In vivo voltammetry was used in freely moving rats to study the processes whereby striatal dopamine (DA) release is regulated by corticostriatal glutamatergic neurons. Electrical stimulation of the cerebral cortex was found to markedly increase the striatal DA-related voltammetric signal amplitude. Similar enhancements have been observed after intracerebroventricular administration of 10nmoles glutamate, quisqualate and AMPA, whereas NMDA was found to decrease the amplitude of the striatal signals. The NMDA receptor antagonist APV did not significantly affect the voltammetric signal but prevented the NMDA-induced depression of the DA-related signals. These data are in agreement with those obtained in numerous previous studies suggesting that the glutamatergic corticostriatal neurons exert activatory effects on the striatal DA release via non-NMDA receptors. The mechanism involved might be of a presynaptic nature. The role of the NMDA receptors may however consist of modulating the dopaminergic transmission phasically and in a depressive way, which would be consistent with behavioural data suggesting the existence of a functional antagonism between the activity of the corticostriatal glutamatergic and nigrostriatal dopaminergic systems.

Keywords: Amino acids – Excitatory amino-acid – NMDA – Dopamine – Striatum – In vivo voltammetry

Abbreviations: Glu: glutamate; DA: dopamine; NMDA: N-methyl-D-aspartate; CPP: 3-(2-carboxypiperazin-4μl)propyl-1-phosphonic acid; AMPA: α-amino-3-hydroxy-5-metylisoxazole-4-propionic acid; APV: aminophosphonovaleric acid; DOPAC: dihydroxyphenylacetic acid; HVA: homovanillic acid; DARPP 32: dopamine-cAMP-regulated phosphoprotein 32; CSF: cerebrospinal fluid.

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Introduction

Although actual axo-axonic contacts between corticostriatal and nigrostriatal nerve terminals rarely occur in the striatum, several lines of evidence have shown that reciprocal functional interactions at both presynaptic and postsynaptic levels may link the activity of these two striatal afferent systems. The possibility that a facilitatory presynaptic control might be exerted by corticostriatal glutamatergic terminals on nigrostriatal dopaminergic transmission was suggested by the finding that glutamate (Glu) markedly stimulates striatal dopamine (DA) release in vitro, mimicking the effect of the in vivo activation of the corticostriatal pathway (see Nieoullon et al., 1983). The activatory effects of Glu on DA release measured in vitro were found to be insensitive to tetrodotoxin (TTX) and were initially thought to possibly involve a direct presynaptic action exerted by Glu on dopaminergic nerve terminals (Giorguieff et al., 1977; Roberts and Shariff, 1978). Although biochemical data were initially obtained showing that a large decrease in the number of ^{3}H-Glu striatal binding sites occurred after lesion of the nigrostriatal dopaminergic system (Roberts et al., 1982), autoradiographic studies and in vitro binding studies using selective ligands of amino acid receptor subtypes failed to confirm the presence of NMDA (CPP) or AMPA ligand-binding sites on nigrostriatal dopaminergic nerve terminals (Errami and Nieoullon, 1988; Samuel et al., 1990). The discrepancy between the data from pharmacological and binding experiments may be due however to the fact that the highly preferential localization of both AMPA and NMDA receptors on intrinsic striatal neurons complicates the interpretation of data from lesion experiments.

Recent investigations have shown the great complexity of these mechanisms involved in the control of striatal DA release exerted by the glutamatergic corticostriatal pathway. Indeed, Glu was initially found to have biphasic dose-dependent effects on striatal DA release in vivo (Chéramy et al., 1986; Leviel et al., 1990). Interestingly, the activatory effects observed with low concentrations of Glu were found to be TTX-resistant, whereas the inhibitory effects of Glu on ^{3}H-DA release occurring with higher Glu concentrations were suppressed by TTX or bicuculline. Glu released from corticostriatal nerve terminals may therefore be able to both directly and indirectly influence the release of DA through presynaptic receptors. In these in vivo studies, where ^{3}H-DA release was measured using the push-pull cannula method, the presynaptic activatory effects of Glu on striatal DA release were found to primarily involve AMPA-kainate non-NMDA receptors thought to be located directly on the dopaminergic nerve terminals. In vivo studies have further shown using striatal microdialysis that NMDA increased the DOPAC and HVA outflow (Kabuto et al., 1989), whereas Glu at high concentrations (10^{-3} M) increased the DA release (Shimizu et al., 1990). Other data from dialysis experiments showed that quisqualate and kainate but not NMDA had activatory effects on DA striatal outflow (Imperato et al., 1990). Using in vivo voltammetry, however, Glu was also found to increase DA release through an APV sensitive mechanism (Moghaddam et al., 1990), which suggests that NMDA receptors may also be involved. Data obtained in vitro have furthermore suggested that Glu may exert

an activatory influence on striatal DA release via NMDA receptors (Roberts and Shariff, 1978; Snell and Johnson, 1986; Jhamandas and Marien, 1987; Clow and Jhamandas, 1989; Krebs et al., 1991a) by means of a mechanism sensitive to Mg^{++} and MK 801 and potentiated by glycine (Krebs et al., 1989; Wang, 1991). The actual contribution of the NMDA receptors to the glutamatergic control of striatal DA release is still a matter of controversy, however, and recent data from in vivo dialysis experiments have even suggested that endogenous excitatory amino acids may not have a tonic excitatory effect at all on striatal DA release, since very high concentrations of NMDA and non-NMDA antagonists are required to elicit changes in the neurotransmitter release (Moghaddam and Gruen, 1991). This result is nevertheless in disagreement with data obtained in a similar manner using MK 801 as a NMDA antagonist, where on the contrary a decrease in DA outflow was found to occur after systemic injections (Kashihara et al., 1990). It is worth noting however that the NMDA-related stimulations of DA release were mainly observed without Mg^{++}. In agreement with the absence of any major corticostriatal influence on the basal striatal dopaminergic transmission, it has been established for a long time that cortical lesions in the rat have no effect on the striatal DA, DOPAC or HVA levels (Hassler et al., 1982; Scatton et al., 1982; Jones et al., 1988). We recently observed however that lesions of the cerebral cortex which only negligibly altered the basal striatal DA parameters, actually influenced the responsiveness of the dopaminergic neurons to pharmacological stimulations. Indeed, the effects of haloperidol on striatal DOPAC concentrations were reduced by about 50% in animals subjected to the cortical lesions, suggesting that corticostriatal glutamatergic system actually contributes to modulating the activity evoked in the nigrostriatal dopaminergic system (Dusticier and Nieoullon, in preparation). Data from behavioural experiments further reinforce this latter proposal, since it was reported some years ago that lesion of the cerebral cortex in the rat potentiates the effects of amphetamine (Glick, 1972) and decreases the catalepsy induced by haloperidol, (Carter and Pycock, 1980; Scatton et al., 1982; Worms et al., 1985). Moreover, NMDA was found on the contrary to potentiate the cataleptogenic effects of haloperidol whereas MK 801 reversed the catalepsy (Mehta et al., 1990; Elliott et al., 1990). Likewise, MK 801 has been recently shown to stimulate locomotor activity in mice pretreated with α-methyl paratyrosine or reserpine and to potentiate the effects of apomorphine and those of D1 agonists (see Carlsson and Carlsson, 1990). The enhancement of the glutamatergic corticostriatal transmission may therefore contribute to reducing the effects of the striatal dopaminergic transmission. This gave rise to the idea that antiglutamatergic compounds may be potent antiparkinsonian agents, which case the dopaminergic nigrostriatal and glutamatergic corticostriatal transmissions may have opposite functional role. Interestingly, at the postsynaptic level, receptor studies have provided evidence that synaptic activation of NMDA receptors may decrease the synaptic action of DA through the D1 receptor subtype by dephosphorylating DARPP 32 at the cAMP-dependent site (see Girault et al., 1990).

The aim of the present study was to further examine in vivo at cellular level the basic functional interactions which may occur between the glutamatergic corticostriatal and dopaminergic nigrostriatal transmissions. We investigated in

the freely moving rat the effects of the direct activation of corticostriatal transmission and those of pharmacological activation of excitatory amino acid receptor subtypes on the extracellular striatal DA levels estimated by means of a voltammetric implanted device with which long-term electrochemical recordings can be carried out in vivo. The electrode we have developed can be used to record DA-related voltammetric signals after priming the carbon electrodes, which make them highly sensitive to DA when tested in vitro (see Forni and Nieoullon, 1984; El Ganouni et al., 1987). This electrode is approximately 1000 times less sensitive to DOPAC than DA. Although DOPAC was found in vitro to decrease the sensitivity of the electrode to DA, numerous in vivo pharmacological experiments have yielded data which all strongly support the view that the striatal signal probably reflects dopaminergic nerve terminal activity and that a method of this kind might therefore be a useful tool for monitoring the response of dopaminergic neurons to induced changes in the glutamatergic corticostriatal transmission. In parallel experiments, we also studied the effects of cortical stimulation or excitatory amino acid agonist administration into the cerebral ventricles on the striatal DA, DOPAC and HVA levels. These data are discussed along with the data obtained from the voltammetric experiments.

Materials and methods

Experiments were performed in successive series of at least 6 female adult Wistar rats. Differential pulse voltammetry was used to measure the electrochemical current. The experimental apparatus, which has been described in detail previously (Forni and Nieoullon, 1984) consisted of a reference electrode and a working carbon electrode. For the in vitro calibration of each electrode prior to implantation into the rat brains, the reference and auxiliary electrodes consisting of Ag/AgCl and platinium wire, respectively, were used. In the in vivo experiments, the reference and auxiliary electrodes were simply small stainless steel screws fixed to the skull. The working electrode was made with a rigid rod of 10,000 carbon fibers (Carbon Lorraine, France, ref. AGT 4F 10,000) bonded with epoxy resin and sharpened at one extremity to reduce the diameter of the tip from 1.5mm to 50μm. The entire electrode was encased in insulating resin and the tip was then exposed under a microscope using an abrasive disk to shape the active surface of the electrode. A linear potential scan from 0mV to +500mV was applied through a PRG 5 polarograph (Tacussel, France) to this electrode versus the reference electrode. A pulse modulation was superimposed on the ascending ramp potential. The difference between the currents measured just before and just after the pulse application gives the differential oxidative current. All electrodes were calibrated in vitro prior to implantation in standard electrolytic solutions containing DA at concentrations varying between 10^{-8} M and 10^{-2} M; the linear concentration-current was found to range from about 10^{-7} to 10^{-4} M. Voltammetric recordings were conducted in series of rats with calibrated carbon electrodes implanted unilaterally into the left striatum using a stereotaxic approach in anesthetized animals (A 8.6; L 2; H +1.4 according to the atlas by König and Klippel, 1963); and the other two electrodes were fixed to the bone.

In some of these animals ($N = 6$), two monopolar electrodes were inserted into the cortex on the left side of the brain and fixed to the bone. One of the electrodes (cathodic stimulation) was placed into the medial part of the frontal cortex and the other electrode (anodic stimulation) into the sensorimotor area to induce a large cortical activation, as previously described (Dusticier and Nieoullon, 1987). All the electrodes were connected to a plug. In the other animals, a permanent guide-cannula (diameter: 0.8mm) was stereotaxically implanted into the left lateral ventricle (A 6.0; L 1.4; H +2 according to König and Klippel, 1963), and intracerebro-ventricular (i.c.v.) injections could then be performed into the

non-anesthetized rats with a cannula (diameter: 0.5mm) attached by polyethylene tube to a 20μl Hamilton syringe; a volume of 10μl was injected in 3 min and the cannula was left in place for 1 min to allow diffusion of compounds within the CSF.

Unilateral cortical stimulations were performed at least a week after the electrode implantation. Electrical stimulation consisting of monophasic square pulses (train duration 50 msec; each shock 0.5 msec; frequency 300Hz; 2 to 7V) was applied at a frequency of 0.5Hz for 5 sec to 1 min through a stimulus isolation unit. In fact, the intensity of the stimulation was adjusted in each case to the threshold for contralateral forelimb flexion to be induced by the cortical activation as determined at the beginning of the session. Voltammetric recordings were conducted in sessions where sampling of the signal was carried out at 5-min intervals. The amplitude of the oxidative current expressed in nA was then plotted against time in experiments with cortical stimulation and some actual recordings have been given in the Figures. In pharmacological experiments, the mean value of the amplitudes of all the signals measured during the 2h prior to drug injection was taken as the control value (100%). The amplitude of each individual signal was then calculated against this control value, before and after drug administration. The results are therefore expressed in these experiments as a percentage of the control value. Mean values corresponding to successive 15-min periods were then calculated and the results were plotted against time for each recording session. With this procedure it was possible to compare the effects obtained during the various sessions at which a given compound was injected, even if the absolute mean signal amplitude was different due to slight variations in the sensitivity of the electrodes. The results were compared with data obtained in recording sessions with a similar duration (about 6 hours) with animals injected with the vehicle of pharmacological compounds. Differences between experimental and control recordings were statistically evaluated using Student's two tailed *t*-test.

Pharmacological experiments consisted of i.c.v. administration of 10nmoles of Glu and of the excitatory amino acid receptor agonists AMPA, quisqualate and NMDA. In some experiments, APV, an excitatory amino acid receptor antagonist acting at NMDA sites, was also administered either alone or to test its activity against NMDA. All compounds were purchased from RBI (U.K.) and solubilized within a sterile phosphate buffered solution (DUBELCO, France). Each experiment was reproduced 2 to 8 times altogether, and at least twice in each implanted animal, in sessions with a minimal interval of one week between any two successive pharmacological treatments.

For the biochemical experiments, the striatum ipsilateral either to the cortex stimulated with the same parameters for 10 min or to the injected lateral ventricle was dissected out 20 min after cessation of the stimulation and 20, 45 and 180 min after the drug injection, in order to determine time course of the changes induced in the striatal voltammetric signal. The endogenous DA, DOPAC and HVA contents were determined by high performance reverse phase liquid chromatography (HPLC) coupled with electrochemical detection of DA and its metabolites, using a procedure which has been described in detail previously (Dusticier and Nieoullon, 1987). The oxidative potential was set at 0.8 V and the sensitivity of the detection at 0.2nA/V. Briefly, tissue samples were homogenized immediately after dissection by weak sonication in perchloric acid 0.1 M (1/10 W/V); the crude supernatant was diluted 20 times in the mobile phase after centrifugation (10 min; 15,000g) and 20μl of the homogenate were injected directly into a C 18 column (5μm). Identification of compounds and quantifications were performed against standard solutions containing 50pg of the products, using a computer. The results are expressed in μg/g tissue as means $\pm$ S.D. of the values obtained in series of at least 6 experiments and compared to those obtained either in control, non stimulated animals or in animals injected with the drug-vehicle alone. Student's *t*-test was used to make statistical comparisons between the means.

Results

1. Effects of cortical stimulation on the striatal voltammetric signal amplitude

Differential pulse voltammograms obtained in vivo on the striatum of freely moving rats show, as described in detail previously, very similar properties to those obtained in vitro with the same electrodes in standard DA solutions before implantation in the brain. The oxidative current recorded showed a single peak, as illustrated in Fig. 1A, and the maximal amplitude of the signal was obtained with a potential of +160mV. The amplitude of the signal stabilized 1–2 weeks after implantation of the electrode, and, the oxidation current thereafter re-

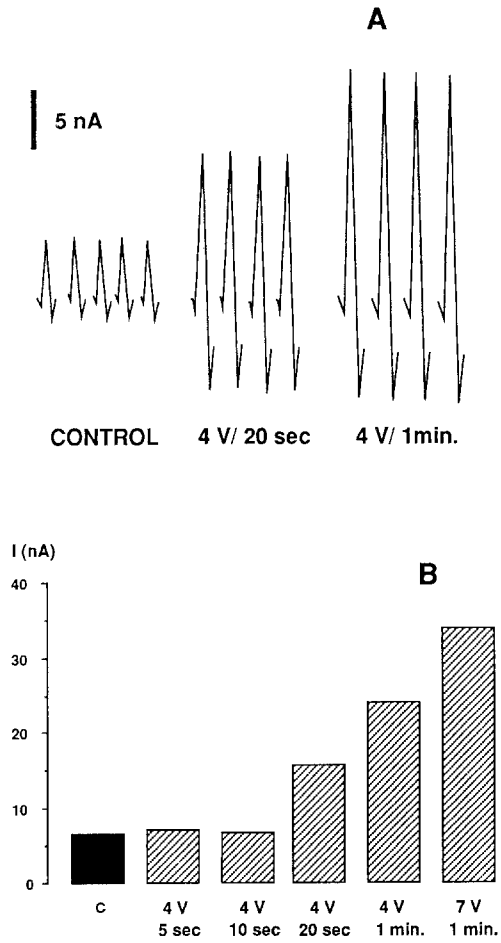

Fig. 1. Effects of the electrical stimulation of the cerebral cortex on the amplitude of the voltammetric signals recorded in the ipsilateral striatum in freely moving rats. The cerebral cortex was stimulated through electrodes implanted in series of 6 animals. The results are those from a typical experiment and were sampled before (*control*) and 2 min after the cessation of cortical stimulations. **A** Actual recording of the striatal voltammetric signals in control situation and with the same electrode after stimulation with 4V (threshold for flexion movement to be evoked in the contralateral forelimb) for 20 sec and 1 min, successively. **B** Changes in the amplitude of the striatal voltammetric signals expressed in nA as a function of the duration in the case of threshold cortical stimulation (from 5 sec to 1 min) and as a function of stimulus intensity in the case of a given duration (from 4V to 7V)

mained remarkably stable in each animal and with each electrode for post-implantation periods as long as 12 months. During the recording sessions, the spontaneous variations in the amplitude of the signal recorded continuously for at least 6 hours never exceeded 6% of the mean signal amplitude during this period of time.

Electrical stimulation of the cerebral cortex was immediately followed by a marked increase in the striatal voltammetric signal amplitude (see Fig. 1). The response developed during the first measurement performed immediately after the cessation of the stimulation and was found to depend upon the intensity and the duration of the stimulus. Fig. 1 illustrates data from a typical experiment where the threshold of the striatal evoked response corresponded to a 4V cortical stimulation (Fig. 1A). Interestingly, with this low current, an increase in the striatal voltammetric signal amplitude was found to occur when the duration of the cortical stimulation was increased from 5 sec to 20 sec and from 20 sec to 1 min (Fig. 1A and 1B). Moreover, in the same experiment, a further increase in the amplitude of the signal was observed with cortical stimulations of the same duration but with current intensities increased from 4V to 7V. The maximal increases in the amplitude of the striatal voltammetric signals following cortical stimulation reached in the most pronounced cases more than 400% of the basal values ($N = 6$ experiments). The return to control values was variable, however, depending on the length and the intensity of the stimulation: with a stimulation lasting 1 min and with 7-V current, significant increases in the voltammetric responses were still observed 20 min after the offset of the stimulation. When the stimulations had shorter durations weaker intensities, however, the activating effects were most transient and the amplitude of the signals returned to control values within 5 to 10 min.

2. Effects of intracerebroventricular administration of glutamate and agonists of excitatory amino acid receptor subtypes on the striatal voltammetric signal amplitude

The i.c.v. injection of 10nmoles Glu into freely moving rats led to an immediate increase in the voltammetric signal amplitude recorded from the striatum ipsilateral to the injected lateral ventricle. Fig. 2A shows the results of 5 experiments, illustrating the increase in the signal amplitude recorded in individual cases, ranging from 25 to 40% at the best ($P < 0.05$ in each individual case) as compared to the data obtained before the drug injection. These responses were established within 10 to 20 min of the drug injection. A return to the control value then occurred progressively within 30 to more than 120 min, depending on the animals. It is noteworthy however that in some cases, a long lasting effect was still detectable as long as 4 hours after the drug administration. Extensive dose-response studies have not been performed but in preliminary experiments, low doses were occasionally used (5nmoles), which led to less marked increases in the striatal voltammetric signal amplitude.

These responses obtained with Glu administration were very similar to those recorded after quisqualate injection (10nmoles) in the same experimental situation. Fig. 2B shows results from 4 experiments using quisqualate where the

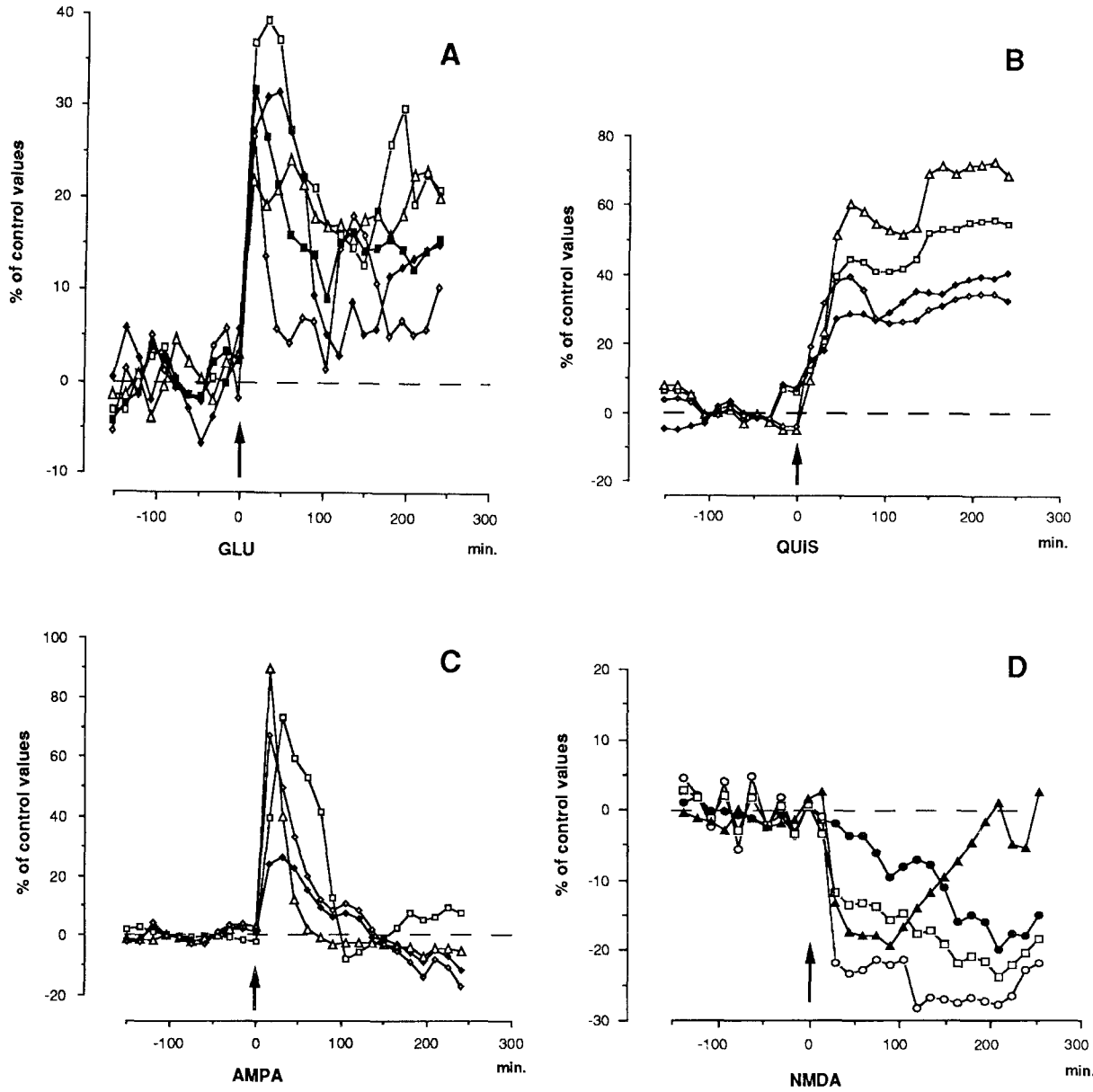

Fig. 2. Effects of intracerebroventricular administration of glutamate (*Glu*), quisqualate (*Quis*), *AMPA* and *NMDA* on the amplitude of the striatal voltammetric signals recorded in freely moving rats. Ten nanomoles of compounds were administered within 10μl into the left lateral ventricle. Recordings were performed on the left striatum. Arrows indicate the drug injection time. The data are individual cases and are expressed as a percentage of an average spontaneous signal calculated in each animal on the basis of the signal amplitude measured during the 2 hours prior to drug injection

striatal signal amplitude was found to have increased considerably by 25 to more than 60% ($P < 0.05$ in each individual case). Here the responses developed more progressively than the effects of Glu, always taking at least 30 min to reach their maximal value after the drug injection. As in the case of Glu, a marked post-effect

was observed, since the amplitude of the voltammetric signal never returned to the control values within the first 4 hours subsequent to the drug administration.

Interestingly, after AMPA administration (10nmoles) under conditions similar to those used for Glu and quisqualate, an almost immediate increase in the striatal signals developed within 10 min, which was comparable in amplitude to that obtained with quisqualate but differed in that the increase was much more transient. Fig. 2C shows that the increase reached at the best about 25 to 90% ($P < 0.05$ in each case) but a complete return to control values was seen in each case as early as 30 to 90 min after the injection ($N = 4$).

Lastly, a completely different response was observed after NMDA i.c.v. administration (10nmoles). The data given in Fig. 2D show that the NMDA injection was followed by an immediate decrease in the striatal response amplitude, amounting to about 25% to 30% within a few minutes ($P < 0.05$ in each experimental case). This decrease in the response amplitude developing within the first 10 min following NMDA injection in 3 cases out of the 4 shown here ($N = 4$) in fact extended into a plateau for a further 2 to 3 hours. In this situation, no return to the control value was usually observed within the 4 hours following the drug injection.

3. Effects of the competitive NMDA antagonist amino-phosphono valeric acid (APV) on the basic and NMDA-induced decrease in the striatal voltammetric signals

APV (10nmoles) injected alone i.c.v. did not significantly affect the amplitude of the striatal voltammetric signal (Fig. 3A) although a slight and progressive tendency for the signal amplitude to increase was consistently observed during the 4 hours following the drug injection ($N = 4$ experiments).

In preliminary experiments, NMDA (10nmoles) was administered i.c.v. 60 min after APV (10nmoles). The data given in Fig. 3B ($N = 2$ experiments) show that in the presence of APV, NMDA no longer significantly depressed the amplitude of the striatal voltammetric signals.

4. Effects of cortical stimulation and excitatory amino acid agonist i.c.v. administration on the striatal levels of DA, DOPAC and HVA (Table 1)

In animals subjected to cortical stimulation, as previously reported in anesthetized animals (Dusticier and Nieoullon, 1987), large decreases in the DOPAC concentrations were observed 20 min after the stimulus offset in the corresponding striatum as compared with the control values (-23.6%; $P < 0.05$). This decrease in DOPAC was concomitant with a reproducible reduction in the striatal HVA content, which was not however statistically significant (-18.5%). The DA striatal levels were found to be practically unaffected by the cortical stimulation, contrary to what was observed in chloral hydrate anesthetized rats. The DOPAC:DA ratio, which is sometimes used as an index to DA utilization, was thus found here to have decreased significantly (-36.4%; $P < 0.05$) in response to cortical stimulation.

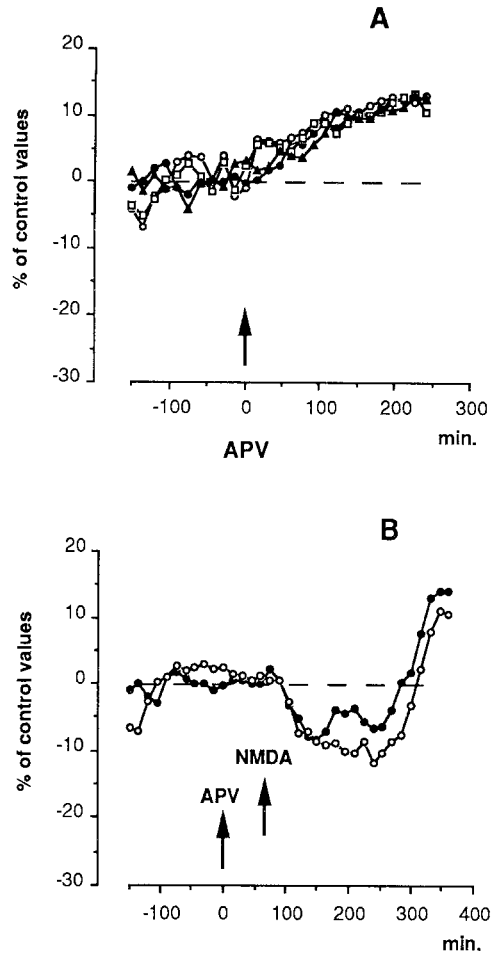

Fig. 3. Effects of intracerebroventricular administration of the excitatory amino acid receptor antagonist APV on the amplitude of the striatal voltammetric signals (**A**). Experiments performed and data expressed as in Fig. 2. **B** Effects of APV injected 60 min before NMDA on the amplitude of the striatal voltammetric signal. These data should be compared with the data obtained with NMDA injected alone (Fig. 2D)

Glu, quisqualate, AMPA and NMDA did not altered DA striatal levels. Only NMDA significantly decreased the DA levels 180 min following its i.c.v. administration (−21.2%; $P < 0.05$). The DOPAC levels showed frequent but transient changes, which were detected only 20 min after the i.c.v. injection of quisqualate, AMPA and NMDA (+20.9%; +39.5%; +33.9%, respectively; $P < 0.05$). Interestingly, Glu did not apparently influence the striatal DOPAC levels. Increased levels of HVA were also detected in the striatum corresponding to the injected lateral ventricle. Glu was found to induce an early but transient increase in the HVA content after 20 min (+33.8%; $P < 0.05$); quisqualate induced later effects, since the striatal HVA concentration showed an increase only 45 and 180 min after the injection (+31.4% and +25.5%; $P < 0.05$ respectively); AMPA effects were detectable only 45 min post-injection (+20.4%; $P < 0.05$); and NMDA effects were observed 20 and 180 min after the drug injection. Interestingly, in the latter case, a biphasic influence was visible: the early effect was found to be a

Table 1. Effects of electrical stimulation of the cerebral cortex and intracerebroventricular administration of glutamate, quisqualate, AMPA and NMDA (10nmoles) on dopamine, DOPAC and HVA (μg/g tissue) measured 20 min after cortical stimulation and 20, 45 and 180 min after the drug injections in the rat. Data are the mean ± S.D. values obtained in series of 6 aminals

					DOPAC
		Dopamine	DOPAC	HVA	Dopamine
Control		19.95 ± 1.8	2.15 ± 0.2	1.37 ± 0.2	0.11
Cortical stimulation	20 min	23.46 ± 2.1	1.64 ± 0.4*	1.12 ± 0.4	0.07*
L-glutamate	20 min	17.37 ± 1.9	2.46 ± 0.3	1.83 ± 0.3*	0.14*
	45 min	18.17 ± 1.9	1.91 ± 0.3	1.34 ± 0.3	0.11
	180 min	17.33 ± 2.1	1.93 ± 0.5	1.20 ± 0.4	0.11
quisqualate	20 min	17.35 ± 1.8	1.91 ± 0.4	1.20 ± 0.3	0.11
	45 min	21.10 ± 2.0	2.60 ± 0.2*	1.80 ± 0.3*	0.12
	180 min	19.67 ± 2.0	1.98 ± 0.4	1.72 ± 0.3*	0.12
AMPA	20 min	18.94 ± 1.6	1.74 ± 0.5	1.31 ± 0.4	0.09
	45 min	19.36 ± 1.8	3.00 ± 0.4*	1.65 ± 0.3*	0.15*
	180 min	17.91 ± 1.8	1.99 ± 0.2	1.08 ± 0.5	0.11
NMDA	20 min	17.66 ± 1.7	2.36 ± 0.2	1.77 ± 0.4*	0.13
	45 min	23.21 ± 1.9	2.88 ± 0.4*	1.55 ± 0.3	0.12
	180 min	15.73 ± 1.3*	1.95 ± 0.3	0.83 ± 0.3**	0.12

* $P < 0.05$; ** $P < 0.01$, Student's t-test.

significant increase in the HVA levels similar to that observed in the case of the other excitatory amino acid agonists (+29.2%; $P < 0.05$), whereas the later effect was found to be a marked decrease in the HVA striatal concentrations (−39.4%; $P < 0.01$). The DOPAC:DA ratio was significantly affected by Glu only 20 min after the injection (+27.2%; $P < 0.05$) and by AMPA, 45 min after the injection (+36.4%; $P < 0.05$).

Discussion

The results of the present study show that direct activation of the corticostriatal glutamatergic pathway as well as the indirect activation of cerebral excitatory amino acid receptor subtypes effected by means of i.c.v. administrations in freely moving rats increased the amplitude of the striatal voltammetric signals recorded from our multifiber carbon electrode, NMDA being the only compound to induce an inhibitory action. These data suggest that the extracellular levels of striatal DA are actually influenced by changes in the glutamatergic corticostriatal transmission acting through the various subtypes of excitatory amino acid receptors, as previously proposed in numerous in vitro and in vivo studies (see Introduction). It indeed seems likely that quisqualate metabotropic and AMPA receptor subtypes may be directly involved through presynaptic mechanisms in the activation of DA release, whereas the NMDA receptor may contribute to reducing the DA release from nigrostriatal nerve terminals.

Our data obtained using cortical stimulation are comparable on the whole to those obtained many years ago in the anesthetized cat using the push-pull

cannula method to measure the release of ^{3}H-DA at caudate nucleus level (Nieoullon et al., 1978), and agree with our initial hypothesis that the nigrostriatal dopaminergic activity undergoes a strong activatory cortical influence. Interestingly, these data have been further extended by recent studies using dialysis in the rat in which it has been reported that pharmacological activation of corticostriatal pathway using kainic acid intracortical administration enhanced the striatal DA release (Herrera-Marschitz, 1991). Our data also further confirm the validity of our voltammetric method for monitoring extracellular DA levels within the striatum. In previous physiological and pharmacological studies, it was established that the amplitude of the striatal voltammetric signal in our hands decreased markedly after injection of 6-hydroxydopamine into the ipsilateral substantia nigra, whereas electrical stimulation of the medial forebrain bundle enhanced the striatal signals. Moreover, a marked decrease in the striatal signal amplitude has been observed after 6-hydroxydopamine, γ-butyrolactone, α-methyl paratyrosine, reserpine and apomorphine treatments. Amphetamine, pargyline, haloperidol and sulpiride on the contrary induced an increase in the striatal signal (Forni and Nieoullon, 1984; El Ganouni et al., 1987; Forni et al., 1989) which is in agreement with the possibility that DA release increased in the striatum. Furthermore, we recently reported that nomifensine, a selective DA uptake inhibitor, also significantly increased the striatal signal amplitude (Forni and Nieoullon, unpublished data), which is consistent with recent data based on dialysis experiments showing that nomifensine increased the DA outflow without affecting the extracellular DOPAC or HVA levels (Butcher et al., 1991). It can therefore be concluded that an increased corticostriatal glutamatergic transmission actually results in an increased DA release in the striatum. Comparisons between our data and those from a recent study, involving dialysis experiments in the rat where it was shown that excitatory amino acid antagonists did not significantly affect the basal DA release (Mogadham and Gruen, 1991), suggest however that the corticostriatal glutamatergic transmission actually exerts a modulatory influence on evoked DA transmission. Our recent finding on the effects of cortical lesions on the striatal DA and DOPAC levels (Dusticier and Nieoullon, in preparation; see Introduction) confirm the view that the corticostriatal system does not tonically influence striatal DA release. This conclusion is also reinforced by the fact that APV alone did not influence the striatal voltammetric-DA related signal, as previously observed in the case of MK 801 on the DA outflow measured by means of dialysis experiments (Weithmuller et al., 1991). In the latter case however, MK 801 was interestingly shown to attenuate the amphetamine-induced striatal DA overflow thus providing further evidence in favour of the idea that the glutamatergic corticostriatal transmission has modulatory effects on the dopaminergic nigrostriatal transmission via NMDA receptors. The idea of this phasic role of NMDA receptor activation was previously suggested by the effects of i.c.v. NMDA receptor agonist and antagonist administration on 3-methoxytyramine striatal levels taken as an index to striatal DA release (Rao et al., 1991).

One major problem raised by studies of this kind concerns the cellular mechanism underlying the glutamatergic control of striatal DA release. The data from in vitro experiments all obviously favour the possibility that Glu has a

direct presynaptic action on glutamatergic receptors located on the dopaminergic nerve terminals. From the anatomical point of view, one might object that direct axo-axonic synaptic contacts between corticostriatal and dopaminergic nerve terminals are rarely found to occur at the ultrastructural level (Bouyer et al., 1984), but this synaptic support is not in fact absolutely necessary. The cellular interactions between these two neuronal systems may involve volume transmission, i.e. a diffusion of Glu acting on receptors located some distance from the sites of release. Mechanisms of this type are nowadays commonly thought to possibly explain putative presynaptic interactions in the brain (see Fuxe and Agnati, 1991).

The second part of our study was designed to further characterize the type of excitatory amino acid receptor involved in the putative activatory effects of corticostriatal transmission on DA striatal release. Although the methodology we have developed has obvious limitations, since the compounds were administered i.c.v. and not directly within the striatal area in these experiments, it is quite striking that Glu, quisqualate and AMPA both induced an increase in the amplitude of the voltammetric DA-related signal recorded in the striatal area close to the injected lateral ventricle. These data are actually in agreement with most of the existing in vitro and in vivo data (see Introduction) indicating that non-NMDA receptors are located directly on the striatal dopaminergic nerve terminals. The case of the NMDA receptors is more puzzling, since some confusion exists in the literature about the status of these receptors in the control of striatal DA release. Our data showing a depressive-APV sensitive effect of NMDA on the striatal voltammetric DA-related signal is comparable with previous in vivo data showing that Glu has an inhibitory effect at high concentration on ^{3}H-DA release in the cat (Chéramy et al., 1986; Barbeito et al., 1990; Leviel et al., 1990), which may actually be mediated by striatal interneurons. However, these data largely disagree with those from in vitro studies showing on the contrary that NMDA has activatory effects on DA release, which are thought to be mainly presynaptic (see Krebs et al., 1989) but are also partly TTX-sensitive, depending on the striatal area concerned (Krebs et al., 1991b). In vivo experiments using microdialysis have shown however that NMDA has no major influence on DA striatal release (see Imperato et al., 1990; Herrera-Marschitz, 1990; 1991). Perhaps the NMDA influence on striatal DA release may be partly transynaptic as suggested by pharmacological data (Krebs et al., 1991a) and by the fact that ibotenic acid-induced striatal lesions abolished the NMDA effect (Carter et al., 1988). We are at present carrying out experiments with a view to elucidating the effects of Glu, AMPA, quisqualate and NMDA under similar experimental conditions in rats previously subjected to ibotenic acid-induced lesions of the striatal neurons preserving the striatal dopaminergic and glutamatergic afferent fibers.

The HPLC data showing the effects of activation of the corticostriatal system on the excitatory amino acid receptor subtypes further showed that the possibility that DOPAC:DA ratio can be used as an index to the DA striatal turnover may need to be reconsidered as previously suggested (see Westerink, 1985; Dusticier and Nieoullon, 1987). In the present experiments no change were detected at the level of the striatal DA content whatever treat-

ment was applied to the animals except for NMDA at 180 min, and only transient changes were noted in the DOPAC and HVA tissue levels. Nor was any direct correlation observed with the changes detected in the voltammetric experiments. In fact, the DOPAC levels were found to increase whatever the pharmacological treatment applied, whereas electrical activation of the corticostriatal system induced a decrease in the striatal DOPAC content. It should be noted however that cortical stimulation may induce global changes in striatal activity, possibly associated with direct activation of nigrostriatal dopaminergic neurons through a putative corticonigral pathway. Moreover, the origin of DOPAC is now being seriously challenged in the literature, and recent data suggest that DOPAC may primarily originate from an intraneuronal pool of newly synthesized DA (see Zetterstrom et al., 1988). Interestingly, it was recently suggested that the Glu influence in the striatum may involve a marked increase in DA synthesis and have only moderate effects on DA release (Leviel et al., 1990). Whatever the mechanism involved, it is worth noting that NMDA, which was the only excitatory amino acid receptor agonist found to decrease the striatal voltammetric DA-related signal, was also the only compound found here to have a long lasting depressive effects on the DA and HVA striatal levels.

Our final point concerns the functional aspects of corticostriatal and dopaminergic nigrostriatal interactions within the striatum. Data from behavioural investigations actually support the idea that a functional antagonism may exist between the activity of these two afferent pathways to the striatum (see Carlsson and Carlsson, 1990). The data from biochemical and pharmacological experiments do not actually support this hypothesis, since corticostriatal activation leads mainly to an increase in the striatal DA release. It is worth noting however, that the NMDA receptors may reflect a functional antagonism of this kind as long as activation of NMDA striatal receptors can be assumed to reduce DA striatal release in vivo. On this connection, if NMDA receptor activation can be taken at molecular level to reduce the activatory D1 receptor effects on DARPP 32 phosphorylation through calcineurin (see Girault et al., 1990), NMDA antagonists can then be said to contribute to facilitating striatal DA transmission.

In conclusion, the data obtained in the present study reinforce the idea that the corticostriatal glutamatergic transmission has a bimodal influence on nigrostriatal dopaminergic transmission. Through direct presynaptic mechanisms mainly involving non-NMDA receptors, and to a lesser degree NMDA receptors putatively located on the dopaminergic nerve terminals as strongly suggested from in vitro studies, the corticostriatal glutamatergic system may actually facilitate the striatal dopaminergic transmission. This direct presynaptic regulation would not have tonic characteristics, since non-NMDA antagonists apparently did not influence the in vivo basal striatal DA release (Moghaddam and Gruen, 1991). This mechanism is therefore probably of a phasic nature. Moreover, the corticostriatal glutamatergic pathway may also be able to reduce the dopaminergic nigrostriatal transmission through NMDA receptor activation. If so, a mechanism of this kind, also apparently phasic in nature, would involve striatal interneurons acting on the dopaminergic nerve terminals. The corticostriatal glutamatergic system therefore appears to be in a position to actually

modulate the nigrostriatal dopaminergic transmission. Interestingly, there seems to exist molecular support for the latter mechanism from the point of view of the interactions possibly occuring between NMDA and D1 striatal receptors (see Girault et al., 1990), and this mechanism seems to be the only one detectable at functional level from the results of behavioural experiments (see Carlsson and Carlsson, 1990).

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