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Neuronal dependence of extracellular dopamine, acetylcholine, glutamate, aspartate and gamma-aminobutyric acid (GABA) measured simultaneously from rat neostriatum using in vivo microdialysis: reciprocal interactions

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Summary. The neuronal origin of extracellular levels of dopamine (DA), acetylcholine (ACh), glutamate (Glu), aspartate (Asp) and gamma-aminobutyric acid (GABA) simultaneously collected from the neostriatum of halothane anaesthetized rats with in vivo microdialysis was studied. The following criteria were applied (1) sensitivity to K^+-depolarization; (2) sensitivity to inhibition of synaptic inactivation mechanisms; (3) sensitivity to extracellular Ca^{2+}; (4) neuroanatomical regionality; sensitivity to selective lesions and (5) sensitivity to chemical stimulation of the characterized pathways.

It was found that: (1) Extracellular DA levels found in perfusates collected from the neostriatum fulfills all the above criteria and therefore the changes in extracellular DA levels measured with microdialysis reflect actual release from functionally active nerve terminals, and so reflect ongoing synaptic transmission. (2) Changes in neostriatal ACh levels reflect neuronal activity, provided that a ACh-esterase inhibitor is present in the perfusion medium. (3) Extracellular Glu, Asp and GABA could be measured in different perfusion media in the rat neostriatum and probably reflect metabolic as well as synaptic release. However, (4) the majority of the extracellular GABA levels found in perfusates collected from the neostriatum may reflect neuronal release, since GABA levels were increased, in a Ca^{2+}-dependent manner, by K^+-depolarization, and could be selectively decreased by an intrinsic neostriatal lesion. (5) It was not possible to clearly distinguish between the neuronal and the metabolic pools of Glu and Asp, since neostriatal Glu and Asp levels were only slightly increased by K^+-depolarization, and no changes were seen after decortication. A blocker of Glu re-uptake, DHKA, had to be included in the perfusion medium in order to monitor the effect of K^+-depolarization on Glu and Asp levels. Under this condition, it was found (6) that neostriatal Glu and Asp levels were significantly increased by K^+-depolarization, although only increases in the Glu levels were

sensitive to Ca^{2+} in the perfusion medium, suggesting that Glu but not Asp is released from vesicular pools. (7) Evidence is provided that selective stimulations of nigral DA cell bodies may lead to changes in release patterns from DA terminals in the ipsilateral neostriatum, which are in turn followed by discrete changes in extracellular levels of GABA and Glu in the same region. Finally, some methodological considerations are presented to clarify the contribution of neuronal release to extracellular levels of amino acid neurotransmitters in the rat neostriatum.

Keywords: Amino acids – Dopamine – Acetylcholine – Glutamate – Aspartate – Gamma-aminobutyric acid (GABA) – Striatum – Microdialysis

Introduction

The nigrostriatal dopamine (DA) pathway constitutes a pivotal system in the basal ganglia of mammals and is involved in a number of neuropsychiatric disorders (Hornykiewicz, 1973; Klawans et al. 1972; Carlsson and Carlsson, 1990). In the neostriatum, DA terminals are linked to several extrinsic and intrinsic neuronal systems (Kobota et al., 1987a,b; Bouyer et al., 1984) making possible complex reciprocal interactions. The neocortex provides a major afferent projection to the neostriatum, with glutamate (Glu) as a main putative neurotransmitter (McGeorge and Faull, 1989; see Tsumoto, 1990). Thus, Glu and DA afferent systems converge onto intrinsic and efferent neuronal systems, such as neurons containing acetylcholine (ACh) and gamma-aminobutyric acid (GABA) (see Smith and Bolam 1990), respectively. In addition, it is probable that DA and Glu terminals can directly interact with each other via axoaxonic links (Roberts et al., 1982; Bouyer et al., 1984). While all these possible circuitries have obtained support from neuroanatomical (see Smith and Bolam, 1990) and immunocytochemical (see Graybiel, 1990) studies, little is known about the actions of neurotransmitters once they are released into the extracellular space surrounding neuronal and glia elements in the neostriatum.

In our laboratory we have developed the in vivo microdialysis technique to monitor neurotransmitters and metabolites present in the extracellular space of the CNS (Ungerstedt et al., 1982). The idea is that changes in extracellular levels reflect actual neurotransmitter release. However, this is still a matter of controversy (Ungerstedt 1984; Westerink et al., 1987, Di Chiara, 1990). Several criteria have been proposed to assert that changes in the levels of substances detected in perfusates collected from the CNS with microdialysis reflect "release" occurring at the synaptic cleft, or elsewhere along neuronal, glia and/or microvascular complexes in the CNS. These criteria have been succesfully applied in the characterization of DA levels recovered from the extracellular space of the neostriatum using in vivo microdialysis (the issue has been extensively discussed in several dissertations at the Karolinska Institute, Stockholm e.g. Zetterström, 1986, Herrera-Marschitz, 1986; Tossman, 1986; Lindefors, 1987; Ståhle, 1987; Hurd, 1989; Reid, 1990; and in reviews by Ungerstedt, 1984; Westerink et al., 1987 and Di Chiara, 1990). These criteria are: (1) sensitivity to K^+ depolarisation; (2) sensitivity to selective inhibition of synaptic inactivation mechanisms (inhibi-

tion of metabolic degradation and/or of active re-uptake); (3) sensitivity to extracellular Ca^{2+}; (4) sensitivity to selective lesions of neuroanatomically characterized pathways and dependence upon regional distribution of neurotransmitter systems; (5) sensitivity to chemical and/or electrical stimulation of the characterized pathways; (6) sensitivity to tetrodotoxin (TTX) (dependency upon voltage-dependent depolarizing mechanisms); (7) sensitivity to specific pharmacological treatments.

In the present paper, we have applied some of the above criteria to analyse the neuronal dependence of extracellular levels of DA, ACh, Glu, aspartate (Asp) and GABA simultaneously collected from the neostriatum of halothane anaesthetised rats with microdialysis. The aim of these studies is twofold: (1) to determine how extracellular levels of these neurotransmitter substances reflect ongoing synaptic transmission, and (2) to monitor *in vivo* some of the reciprocal interactions which may occur between DA, ACh, Glu, Asp and GABA systems in the neostriatum of the rat.

Material and methods

Sprague-Dawley male rats (Alab, Stockholm, Sweden), with access to food and water *ad libitum*, were used in the experiments. The rats were maintained in a temperature-controlled environment on a 12 h light/dark cycle.

In vivo microdialysis

Rats were anaesthetized with a mixture of air and halothane and placed in a Kopf stereotaxic frame. With the skull oriented according to König and Klippel Atlas (1963), two microdialysis probes (CMA 12, Carnegie Medicin AB, Stockholm, Sweden) (dialysis membrane length = 4 mm; diameter = 0.5 mm) were simultaneously implanted into the left and into the right neostriata (coordinates: A 7.9, L $\pm$3.5, V $-$2.0). The microdialysis probes were perfused with either (a) a Ringer solution (NaCl = 148 mM, KCl = 4 mM, $CaCl_2$ = 2.3 mM, pH 6) (Karolinska Apoteket, Stockholm, Sweden), (b) a modified CSF solution (NaCl = 148 mM, KCl = 2.7 mM, $CaCl_2$ = 1.2 mM, $MgCl_2$ = 0.85 mM, pH 6) (Karolinska Apoteket) or (c) a Krebs Ringer solution (NaCl = 138 mM, $NaHCO_3$ = 11 mM, KCl = 5 mM, $CaCl_2$ = 1 mM, $MgCl_2$ = 1 mM, pH 7.4) including 2 g/l glucose, 2 g/l bovine serum albumin (BSA) and 0.3 g/l Bacitracin. Neostigmine (0.1–10 μM) (Sigma), dihydrokainic acid (DHKA) (10–1000 μM) (Diagnostika, Falkenberg, Sweden) and/or KCl (100 mM) (Merck & Co., Inc., Rahway, NJ, USA) were included in the perfusion medium, as indicated for each experiment. A constant flow of 2 μl/min was maintained with a microdialysis pump (CMA 100, Carnegie Medicin AB). Changes in the perfusion medium were performed with a syringe selector coupled to a microfraction collector (CMA 111 and 140, Carnegie Medicin AB). Halothane anaesthesia was maintained throughout the microdialysis experiment by free breathing into a mask fitted over the nose of the rat (1.0–1.5% halothane in an air flow of 1.5 l/min). Body temperature was kept at 37°C using a temperature control system (CMA 150, Carnegie Medicin AB). Samples were collected and injected onto high performance liquid chromatography (HPLC) systems for the detection of catecholamines, ACh and choline (Ch), GABA, Glu and Asp. A 2–3 weeks post-operation period elapsed for 6OHDA-, IBA- and KA-lesioned rats before the microdialysis studies were performed. The microdialysis probes used in this study showed an approximately 20% *in vitro* recovery for DA, ACh, Glu, Asp and GABA (medium = Ringer + 10 μM neostigmine; at room temperature).

Lesions

DA deafferentation: 6-hydroxydopamine (60HDA) lesions

Rats weighing 150–190 g were anaesthetized as above and placed in a Kopf stereotaxic frame. With the skull oriented according to König and Klippel Atlas (1963), 4 μl of 60HDA (2 μg/μl) (Sigma, St Louis, Mo, USA) was injected into the left area ventralis tegmenti containing the bundle of axons leaving the mesencephalic DA cell bodies (coordinates: A 3.0, L −1.2, V −2.2). This lesion extensively denervates forebrain DA-innervated areas unilaterally (Herrera-Marschitz and Ungerstedt, 1984a,b; Christensson-Nylander et al., 1986; Herrera-Marschitz, 1986).

Neostriatal lesions with ibotenic acid (IBA)

Two injections of 1 μl of IBA (5 μg/μl) (Sigma) were stereotaxically administered into the left neostriatum (coordinates: A 8.6, L −2.6, V −0.2; and A 6.6, L −3.2, V 0.2, respectively) of rats weighing 400–450 g. IBA produces a relatively restricted lesion mainly affecting cell bodies located in the neostriatum, while sparing afferent and en passage axons (Schwarcz et al. 1979; Christensson-Nylander et al. 1986).

Decortication

10 × 0.6 μl kainic acid (KA) (0.2 μg/μl) (Sigma) injections were administered into a wide frontoparietal area of the left cortex of rats weighing 400–450 g. Like IBA, KA produces a lesion mainly restricted to cell bodies located in the injected cortical region (Schwarcz and Coyle, 1977; Herrera-Marschitz et al., 1989).

Chemical stimulation of the nigrostriatal DA pathway

In a series of experiments, normal rats were implanted with a microdialysis probe (perfused with a Ringer solution) into the left neostriatum. Twenty min perfusion samples were collected, split and analysed for DA, Glu and GABA. Two hours later an injection cannula was implanted into the pars reticulata of the left substantia nigra (coordinates: A 2.0 L −2.0, V −3.0). Following another 20 min perfusion period, 0.2 μl of either saline or drug (GABA = 300 nmol, Dynorphin A (DYN A) = 0.5 nmol, substance P (SP) = 0.07 nmol, neurokinin A (NKA) = 0.09 nmol) was administered into the substantia nigra, and perfusates were collected for a further period of two hours.

Biochemical analysis

Catecholamines analysis

DA, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined in 20 μl aliquots using a reverse-phase ion pair HPLC system coupled to an electrochemical detector (BAS, West Lafayette, In, USA). The detection limit for DA was ≈0.01 nM (for details see Reid et al., 1988).

ACh and choline analysis

ACh and choline (Ch) were determined in 10 μl aliquots using reverse-phase HPLC with electrochemical detection. ACh and Ch were first separated on a polymeric column using phosphate buffer containing 1 mM sodium 1-octanesulfonate (Sigma) as ion-pairing reagent. An enzymatic post column reactor with immobilized acetylcholinesterase and choline-oxidase transformed ACh and Ch to hydrogen peroxide and betaine. Hydrogen peroxide was then electrochemically detected at a platinum electrode, which was set at 500 mV (vs Ag/AgCl) (in this report, only ACh values are presented, although Ch values are always

detectable in the assay). The limit of detection for ACh was ≈1 nM (for details see Maysinger et al., 1988).

GABA analysis

GABA was determined in 10 μl aliquots using a HPLC system. After precolumn derivatisation with o-phthaldialdehyde/t-butylthiol reagents, the aliquots were separated in a reverse-phase HPLC system under isocratic conditions and the derivatised GABA was electrochemically detected at a carbon electrode set at 750 mV. The limit of detection was ≈1 nM (Kehr and Ungerstedt, 1988).

Glu and Asp analysis

Glu and Asp were determined in 10 μl aliquots using a HPLC system with precolumn derivatisation with o-phthaldialdehyde/mercaptoethanol reagents followed by fluorimetric detection. Briefly, 10 μl of the reagents (0.4 M borate, 0.04 M o-phthaldialdehyde, 0.4 M 2-mercaptoethanol, pH 10.4) was added to the 10 μl aliquot, and after a 60 s reaction period at 4°C in a CMA 200 microsampler (Carnegie Medicin AB), 15 μl of the derivatised aliquot was injected onto a column prepacked with Biophase ODS 5-μm particles. The elution with 0.1 M sodium acetate, 8% methanol, adjusted to pH 6.95, and 1.5% of tetrahydrofurane, was performed with a SP 8800-020 pump (Spectra-Physics, San Jose, CA, USA) equipped with a two-ways valve at a flow of 1 ml/min. A linear gradient (100% eluent changing to 100% methanol over 2 min and returning to 100% eluent 2 min later) was used to rapidly clean the column after the elution of Asp and Glu. The fluorimetric detector was a F1000 (Hitachi, Tokyo, Japan) with excitation wavelength set at 370 nm and emission cut-off filter set at 450 nm. The detection limits for Glu and Asp were ≈1 nM.

Histology

On completion of microdialysis experiments, the brain of the rat was rapidly dissected and stored in 10% paraformaldehyde to confirm, at low microscope magnification, the location of the microdialysis probes and injection cannulae, and to detect any obvious morphological modifications in the surrounding neostriatal tissue, produced by the implantation of the microdialysis probes.

Statistics

DA, ACh, GABA, Glu and Asp are expressed as the concentrations found in the perfusates. In the experiments with intranigral chemical stimulation, changes in neostriatal DA, Glu and GABA levels are expressed as percent of their absolute values prior to the intranigral injection. Means and standard errors of the means (SEM) were calculated and differences were tested with F-ANOVA followed by Student's t-test and Newman-Keuls post hoc comparisons. A level of $P < 0.05$ for two tailed test was considered critical for statistical significance.

Results

Simultaneous measurement of extracellular DA, ACh, Glu, Asp and GABA from neostriatal perfusates

Effects of K^+ depolarisation (Table 1)

Table 1 shows extracellular levels of DA, Glu, Asp and GABA detected in 40 min perfusion periods, prior to and under KCl stimulation. Basal levels generally

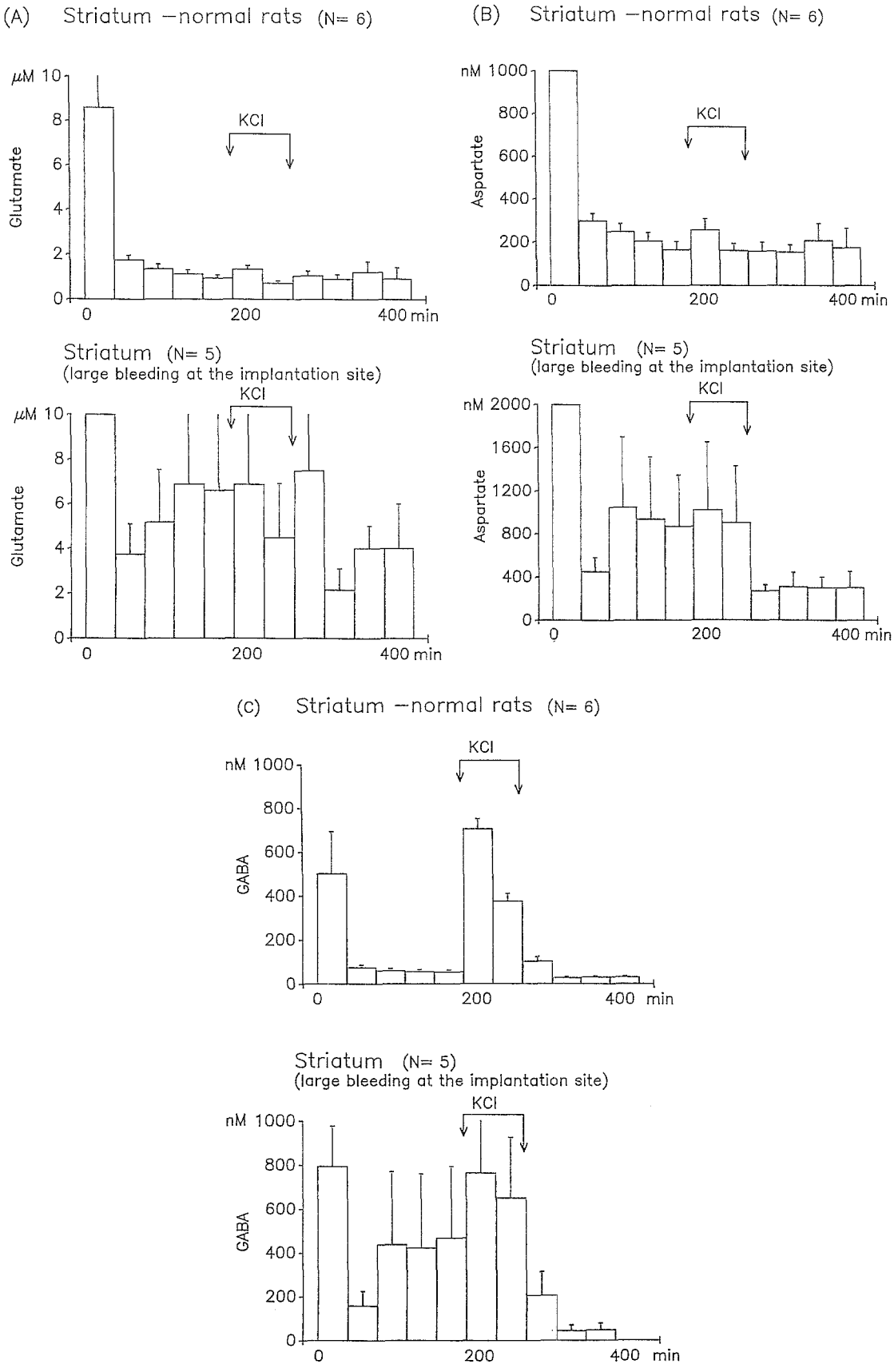
Table 1. Striatal extracellular levels of dopamine (*DA*), acetylcholine (*ACh*), glutamate (*Glu*), aspartate (*Asp*) and gamma-aminobutyric acid (*GABA*) measured by microdialysis in normal rats

Treatment	Basal (nM)	Stimulated (nM)	
[1] Ringer alone ($N = 4$)			
DA	7 ± 2	665 ± 228	(9500%)
ACh	nd	nd	
Glu	3231 ± 715	nd	
Asp	522 ± 128	nd	
GABA	52 ± 16	1600 ± 300	(3077%)
[2] CSF alone ($N = 5$)[1]			
DA	9 ± 0.4	290 ± 40	(3200%)
ACh	nd	nd	
Glu	2400 ± 280	nd	
Asp	244 ± 8	nd	
GABA	42 ± 3	nd	
[3] Krebs alone ($N = 7$)			
DA	3 ± 0.4	261 ± 53	(8700%)
ACh	nd	nd	
Glu	1849 ± 557	4360 ± 859	(236%)
Asp	603 ± 137	807 ± 137	(133%)
GABA	nd	nd	

Means ± SEM of concentrations (nM) measured in 40 min perfusates collected by microdialysis under basal (160–200 min period) and elevated KCl (100 mM) (200– 240 min period) conditions. The KCl-induced changes, expressed as the percentage of the respective basal values are shown within brackets. F-ANOVA was used to analyse differences between groups. Whenever statistically significant differences were obtained, comparisons between individual groups were performed with Student's *t*-test [*t*-test]) and Newman Keuls [N-K] post hoc tests

[1] Estimated from experiments using 2 mm probes (CMA 12; Carnegie Medicin AB). *nd* no detected

Fig. 1A–C. Means of glutamate **(A)**, aspartate **(B)** and GABA **(C)** concentrations detected simultaneously in perfusate samples collected by microdialysis from the left neostriatum of adult rats. Microdialysis probes were perfused with a modified CSF solution including 1 μM neostigmine. At the 200–280 min period following probe implantation 100 mM KCl was included in the perfusion medium. Figures in the upper panels show experiments where, following post microdialysis microscope analysis, no obvious tissue impairement was observed surrounding the tract of the implanted microdialysis probe. Figures in the lower panels show experiments where extensive local bleeding was observed surrounding the microdialysis tract over more than 1 mm^3, and extending along the corpus callosum. Vertical lines show SEM


(A) Striatum –normal rats (N= 6)
μM 10
8
6
4
2
0
Glutamate
KCl
0
200
400 min
(B) Striatum –normal rats (N= 6)
nM 1000
800
600
400
200
0
Aspartate
KCl
0
200
400 min
Striatum (N= 5)
(large bleeding at the implantation site)
μM 10
8
6
4
2
0
Glutamate
KCl
0
200
400 min
Striatum (N= 5)
(large bleeding at the implantation site)
nM 2000
1600
1200
800
400
0
Aspartate
KCl
0
200
400 min
(C) Striatum –normal rats (N= 6)
nM 1000
800
600
400
200
0
GABA
KCl
0
200
400 min
Striatum (N= 5)
(large bleeding at the implantation site)
nM 1000
800
600
400
200
0
GABA
KCl
0
200
400 min

refer to those levels detected in the perfusion fractions (160–200 min period following the microdialysis probe implantation) immediately preceding the period in which 100 mM KCl was included in the perfusion medium (200–240 min). As previously shown with similar experimental protocols, DA (Herrera-Marschitz et al., 1986; Reid et al., 1988; Herrera-Marschitz et al., 1989), as well as Glu, Asp and GABA levels (Fig. 1a–c) were stable two hours after the implantation of the microdialysis probes.

Under basal conditions, extracellular neostriatal DA levels were ≈ 10 nM, irrespective of whether Ringer or a modified CSF solution (both at pH 6) was used as the perfusion medium. However, when a Krebs solution (adjusted to pH 7.4) was used as the perfusion medium, DA levels were ≈ 3 nM. Ch levels (0.5–1 μM) could be detected with Ringer or CSF alone, but ACh could not be detected, either under basal or KCl stimulated conditions. Basal Glu and Asp levels were detected over a wide μM range, when Ringer, CSF or Krebs solutions were used as perfusion media (1–3 μM and 0.2–0.5 μM, for Glu and Asp, respectively). Neostriatal GABA levels were approximately 50 nM when Ringer or CSF was used as the perfusion medium. Basal GABA levels could not be analysed when Krebs was used as a perfusion medium, since the medium itself affected the detection of GABA. When stimulated with KCl, DA and GABA were increased >30 fold. Glu and Asp levels were only slightly increased (≈ 2 fold).

As previously reported for DA (Reid, 1990), reliable measurements of extracellular levels of amino acids were obtained, provided that no obvious tissue impairment was produced during probe implantation. Whenever, microdialysis probes were smoothly implanted, Glu, Asp and GABA levels rapidly decreased after the first 40 min perfusion sample (upper panels of Fig. 1a–c) and reached stable and reproducable levels. However, whenever the implantation procedure was disruptive and local bleeding could be observed (i.e. bleeding signs surrounding the microdialysis tract on more that 1 mm^3, and bleeding extending along the corpus callosum), basal levels of Glu, Asp and GABA were significantly higher than those observed under normal conditions (Fig. 1a–c), and showed large intra- and inter-individual variations. Furthermore, as shown in the case of GABA (Fig. 1c), the effect of KCl could not be clearly observed.

Inhibition of ACh-esterase: effect of neostigmine (Table 2)

As shown in Table 2, ACh could be detected only if the ACh-esterase inhibitor, neostigmine, was included in the perfusion medium. The effect of neostigmine (0.1–10 μM) was dose-dependent. When 1 μM neostigmine was included in a CSF medium, mean basal levels of ACh were ≈ 100 nM, while with 10 μM of neostigmine ACh levels were ≈ 200 nM. When 10 μM neostigmine was included in a Ringer medium, ACh levels were ≈ 300 nM. In all cases, ACh was strongly increased by K^+-depolarisation, with a maximum effect being observed with 10 μM neostigmine included in a CSF medium (>10 fold) (see Table 2). When 0.1 μM neostigmine was included in a CSF perfusion medium, ACh levels were detected in the 10–30 nM range. Interestingly, at this dose of neostigmine, ACh

levels were only slightly increased by K^+-depolarisation (30–60%) (data not shown).

Table 2. Striatal extracellular levels of DA, ACh, Glu, Asp and GABA measured by microdialysis in normal rats: effect of neostigmine

Treatment	Basal (nM)	Stimulated (nM)	
[1] Ringer + 10 μM neostigmine ($N = 10$)			
DA	9 ± 1	465 ± 105	(5167%)
ACh	316 ± 64	2583 ± 320	(817%)
Glu	2691 ± 906	4482 ± 873	(167%)
Asp	254 ± 39	516 ± 55	(203%)
GABA	nd	nd	
[2] CSF + 1 μM neostigmine ($N = 10$)			
DA	7 ± 1	153 ± 26	(2186%)
ACh	97 ± 18	693 ± 137	(714%)
Glu	1411 ± 385	1820 ± 404	(129%)
Asp	211 ± 37	299 ± 49	(142%)
GABA	68 ± 20	605 ± 50	(890%)
[3] CSF + 10 μM neostigmine ($N = 4$)			
DA	7 ± 1	331 ± 15	(4279%)
ACh	219 ± 30	2301 ± 651	(1051%)
Glu	665 ± 197	1364 ± 704	(205%)
Asp	165 ± 5	285 ± 66	(173%)
GABA	nd	nd	

Mean ± SEM of concentrations (nM) measured in 40 min perfusates collected by microdialysis under basal (160–200 min period) and elevated KCl (100 mM) (200–240 min period) conditions (see legend of Table 1)

Inhibition of Glu uptake: effect of DHKA (Table 3)

The effects of the Glu uptake blocker, DHKA (0.01–1 mM) (Johnston et al. 1979), on basal and KCl-stimulated extracellular levels of DA, ACh, Glu, Asp and GABA were studied. The lowest dose of DHKA (0.01 mM) was without any significant effect. After 0.1 mM of DHKA, basal Glu and Asp were increased by approximately 50%, but KCl-stimulated Glu and Asp levels were not affected by this dose of DHKA. When 1 mM DHKA was included in a CSF medium (containing 1 μM neostigmine), two hours after the implantation of the microdialysis probes, basal Glu and Asp levels were increased >2 fold. However, when 1 mM DHKA was present in the CSF medium (+1 μM neostigmine) at the time of the microdialysis implantation, Glu and Asp levels at the 160–200 min period were not significantly different from those found in same medium without DHKA (c.f [2] in Table 2 vs [1] in Table 3). While Glu and Asp levels were only slightly increased by K^+-depolarisation in a CSF medium including 1 μM neostigmine ([2] in Table 2), they were increased >3 and >2 fold, respectively, when 1 mM DHKA was included in the perfusion medium ([1] in

Table 3). The effect of K^+-depolarisation on Glu and Asp was even stronger if DHKA was included at the same time as KCl (Fig. 2).

Table 3. Striatal extracellular levels of DA, ACh, Glu, Asp and GABA measured by microdialysis in normal rats: effect of DHKA and Ca^{2+} dependence

Treatment	Basal (nM)	Stimulated (nM)	
[1] CSF + 1 μM neostigmine + 1 mM DHKA ($N = 10$)			
DA	10 ± 1	157 ± 32	(1570%)
ACh	163 ± 23	414 ± 111	(254%)
Glu	1061 ± 179	3794 ± 46	(358%)
Asp	211 ± 35	441 ± 69	(209%)
GABA	129 ± 34	1512 ± 183	(1172%)
[2] Ca^{2+} free CSF + 2 mM EDTA + 1 μM neostigmine + 1 mM DHKA ($N = 4$)			
DA	6 ± 0.6	32 ± 13	(533%)
ACh	62 ± 11	147 ± 8	(237%)
Glu	1448 ± 26	2186 ± 109	(151%)
Asp	232 ± 32	481 ± 68	(207%)
GABA	99 ± 29	817 ± 93	(825%)

Means ± SEM of concentrations (nM) measured in 40 min perfusates collected by microdialysis under basal (160–200 period) and elevated KCl (100 mM) (200–240 min period) conditions (see legend of Table 1)

Ca^{2+}-dependence of K^+ depolarisation (Table 3)

Two hours after the implantation of the microdialysis probe, the CSF medium was replaced by a Ca^{2+}-free medium, which included 2 mM EDTA (1 μM neostigmine and 1 mM DHKA were also present in the medium). After an additional 2 × 40 min sampling period, 100 mM KCl was included in this perfusion medium. Switching to Ca^{2+}-free medium (including EDTA) produced a significant decrease in basal neostriatal DA (40%), ACh (62%) and GABA (23%) levels, whereas neostriatal Glu and Asp levels were not significantly modified (although a trend for a slight increase was observed). Furthermore, the increases in DA, Glu and GABA levels produced by K^+depolarisation were largely diminished in the Ca^{2+}-free medium. In terms of percent of pre-stimulation levels, ACh levels were similarly increased by KCl in both media. However, both basal and KCl-stimulated levels were significantly diminished in a Ca^{2+}-free medium. Increases in neostriatal Asp levels elicited by KCl were not significantly affected by the absence of Ca^{2+} in the perfusion medium, either when expressed in absolute, or as a percent of pre-stimulation levels (c.f. [1] vs [2] in Table 3).

Dependence upon neuronal pathways: effects of selective lesions

DA deafferentation (Table 4, [1])

As compared to control values (c.f. [1] in Table 2 vs [1] in Table 4), extracellular DA levels were almost totally depleted in the neostriatum ipsilateral to a 6OHDA lesion, while no significant effect was observed on neostriatal ACh or GABA

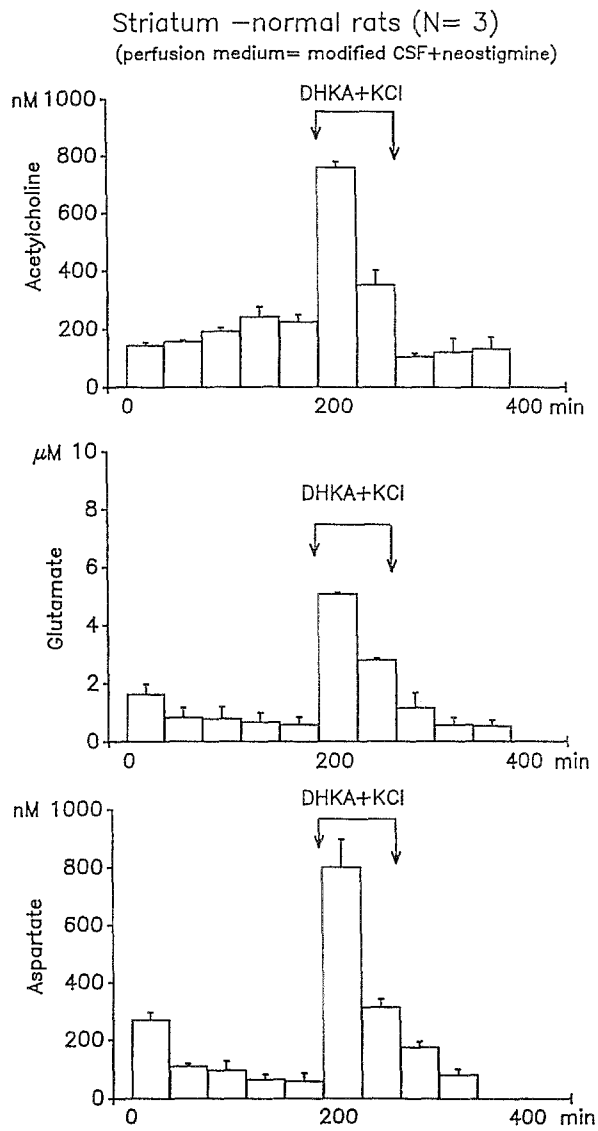


Fig. 2. Means of acetylcholine, glutamate and aspartate concentrations detected simultaneously in perfusate samples collected by microdialysis from the left neostriatum of adult rats. Microdialysis probes were perfused with a modified CSF solution including 1 μM neostigmine. At the 200–280 min period following microdialysis implantation 100 mM KCl + 1 mM dihydrokainic acid (DHKA) was included in the perfusion medium. Vertical lines show SEM

levels (c.f. [1] in Table 2 vs [1] in Table 4). Extracellular Glu levels were, however, significantly diminished (>80%) (Asp could not be reliably measured in the same experiments).

Neostriatal lesion (Table 4, [2])

After two injections of IBA into the left neostriatum, ACh and GABA levels were significantly decreased (ACh by >99% and GABA by >80%) (c.f. [1] in Table

Table 4. Striatal extracellular levels of DA, ACh, Glu, Asp and GABA measured by microdialysis in lesioned rats: effects of lesions

Treatment	Basal (nM)	Stimulated (nM)	
[1] DA deafferentation ($N = 6$)[1]			
DA	>0.1	>0.1	
ACh	270 ± 70	2380 ± 520	(881%)
Glu	503 ± 180	nd	
Asp	nd	nd	
GABA	50 ± 16	2850 ± 600	(5700%)
[2] Striatal lesion ($N = 4$)[2]			
DA	9 ± 0.6	76 ± 6	(844%)
ACh	0.07 ± 0.04	0.16 ± 0.15	(229%)
Glu	3915 ± 2114	7647 ± 3080	(195%)
Asp	177 ± 54	519 ± 195	(293%)
GABA	20 ± 2	249 ± 82	(1470%)
[3] Decortication ($N = 3$)[1]			
DA	6 ± 2	302 ± 83	(5033%)
ACh	218 ± 83	1968 ± 436	(903%)
Glu	4580 ± 2449	5896 ± 3404	(129%)
Asp	307 ± 270	522 ± 150	(170%)
GABA	nd	nd	

Means ± SEM of concentrations (nM) measured in 40 min perfusates collected by microdialysis under basal and elevated KCl conditions. F-ANOVA was used to analyse differences between groups performed with the same perfusion medium (see legend of Table 1)

[1] Perfusion medium = Ringer including 10 μM neostigmine

[2] perfusion medium = CSF including 1 μM neostigmine and 1 mM DHKA

3 vs [2] in Table 4), while DA, Glu and Asp levels were not significantly changed. A tendency for increased Glu levels was observed, although in IBA-lesioned animals Glu values showed large interindividual variations (see [2] in Table 4).

Decortication (Table 4, [3])

An extensive decortication induced by multiple injections of KA produced slight decreases in neostriatal DA and ACh levels (>30%) (c.f. [3] in Table 4 vs [1] in Table 2). Neostriatal Glu and Asp levels were not significantly affected by the decortication, but a tendency for an increase in Glu levels was observed in the decorticated group. However, as in the case of rats with a neostriatal lesion, a large interindividual variation in Glu levels was observed.

Chemical stimulation of nigrostriatal da pathway

Table 5 shows the effects of an intranigral injection of either 0.2 μl of saline, GABA, DYN A, SP or NKA on extracellular levels of DA, Glu and GABA

detected in perfusates collected from the ipsilateral neostriatum. GABA and DYN A injections into the substantia nigra produced a significant decrease in neostriatal DA levels, followed by an increase in neostriatal GABA levels, but without a change in Glu levels. In contrast, intranigral injections of SP and NKA produced a significant increase in neostriatal DA, without a significant change in GABA levels. Furthermore, intranigral injection of SP, but not NKA, produced a significant increase in striatal levels of Glu.

Table 5. Changes in striatal extracellular levels of DA, Glu and GABA following chemical stimulations of the ipsilateral substantia nigra of normal rats

Nigral injection	N	Maximal Change (%)[1] in neostriatum DA	Glu	GABA
Saline (0.2 μl)	4	108 ± 10	86 ± 10	95 ± 4
GABA 300 nmol[2]	5	65 ± 4*	80 ± 11	140 ± 15*
Dyn A 0.5 nmol	5	64 ± 4*	101 ± 4	148 ± 13*
SP 0.07 nmol	8	153 ± 17*	143 ± 11*	110 ± 8
NKA 0.09 nmol	4	135 ± 3*	79 ± 8	108 ± 5

[1] Maximum change detected within five perfusate samples collected in the ipsilateral neostriatum following intranigral injections of the corresponding substance; expressed as the percentage (%) of levels prior to the nigral injection of drug or saline

[2] Volume of injection = 0.2 μl

* $P < 0.05$ for a two-tailed test

Discussion

In the present paper, we studied the neuronal dependence of extracellular levels of DA, ACh, Glu, Asp and GABA in perfusates collected by in vivo microdialysis from the neostriatum of halothane anaesthetised rats. The major concern in this paper was whether the extracellular levels of these neurotransmitter substances recovered with microdialysis are due to passive overflow, i.e. leaking from neuronal and non-neuronal elements surrounding the microdialysis probes, or whether changes in these substances reflect actual release processes from functionally active nerve terminals and so reflect ongoing synaptic transmission. If the latter is the case, then *in vivo* microdialysis would be a valuable tool to monitor reciprocal interactions between several neurotransmitter systems in the basal ganglia of the rat.

Several criteria have been proposed to confirm the neuronal origin of substances monitored with *in vivo* microdialysis. Some of these have already been successfully applied to study the case of extracellular DA levels detected in the CNS of the rat (c.f. Introduction. See also Ungerstedt, 1984; Westerink et al., 1987 and Di Chiara, 1990, for reviews). In this paper we present evidence to further support the idea of a neuronal origin of extracellular DA monitored by microdialysis. Evidence is also presented to support the idea that changes in neostriatal ACh monitored by microdialysis reflect neuronal activity, provided that a ACh-esterase inhibitor is used to prevent the rapid hydrolysis of ACh to Ch and

acetate. Changes in neostriatal GABA, Glu and Asp levels reflect metabolic non-neuronal pools, as well as those derived from synaptic release. Some methodological considerations are presented here which may help to elucidate in which proportion amino acid changes seen in microdialysis perfusates preferentially reflect one or the other releasable neuronal or metabolic pools. Finally, we provide evidence that selective stimulation of nigral DA cell bodies leads to changes in release patterns from DA terminals in the ipsilateral neostriatum. These changes in neostriatal DA release are in turn followed by discrete changes in extracellular levels of GABA and Glu within the neostriatum.

Extracellular levels of DA in the neostriatum were consistent, regardless of whether Ringer or a modified CSF solution was used as perfusion medium (Table 1). The use of Ringer (containing 2.3 mM $CaCl_2$ and 4 mM KCl) or modified CSF solutions (containing 1.2 mM $CaCl_2$, 2.7 mM KCl and 0.85 mM $MgCl_2$) as perfusion medium has been the issue of controversy (Moghaddam and Bunney, 1989; Osborne et al., 1991a). It has been suggested that, since the ionic composition of Ringer solutions is at variance with that found in the extracellular space of the CNS, the extracellular levels of neurotransmitters recovered in a Ringer solution may reflect a non-physiological release. Although this issue cannot be directly answered at present, it appears that regarding basal levels, there is no obvious difference in extracellular DA levels in the neostriatum, whether Ringer or a modified CSF solution is used as a perfusion medium, provided that the same medium is used throughout the experiment. Interestingly, basal DA levels were lower in experiments using a Krebs solution (1 mM $CaCl_2$, 5 mM KCl and 1 mM $MgCl_2$) as the perfusion medium. It is possible that these differences are related to the lower pH of the Ringer and CSF media (pH 6 vs pH 7.4 in the Krebs solution), which may protect the catecholamines from breakdown during collection. However, regardless of the levels of DA found under basal conditions in the neostriatum, K^+-depolarisation strongly increased DA levels. As previously shown, basal (Westerink et al., 1988) as well as K^+-stimulated (Table 3) DA levels were decreased when perfusing with a Ca^{2+}-free medium. Neostriatal Glu and Asp levels were detected in all media (Table 1), however, K^+-depolarization induced a much lower increase in Glu and Asp than in DA. GABA levels were consistent in both Ringer and CSF solutions and were strongly increased by K^+-depolarization. In studies using Krebs as the perfusion medium, GABA could not be analyzed, due to the presence of BSA and Bacitracin in the Krebs medium.

In contrast to DA, extracellular Glu, Asp and GABA levels showed large inter-individual variability in the neostriatum, regardless of the perfusion medium employed (Table 1). This variability seems to depend upon the physiological conditions under which the microdialysis study was performed (disturbances in breathing being the more obvious factor [Goiny et al., in preparation]), and as reported here, upon the extent of tissue disturbances induced by the implantation procedure. Thus, whenever signs of bleeding surpassed 1 mm^3 around the microdialysis tract, and signs of bleeding along the corpus callosum were observed, neostriatal Glu, Asp and GABA levels showed large inter- and intra-individual variations and were several times higher than basal levels found in experiments where no obvious sign of tissue disturbance was observed (Fig.

1a–c). In the case of GABA, it was evident that the effect of K^+-depolarisation was masked by the abnormally high basal levels (Fig. 1c), indicating that the elevated GABA levels are probably derived from a metabolic pool. Thus, it is clear that with respect to levels of amino acids, consideration has to be taken of probable tissue disturbances when evaluating levels obtained by microdialysis.

While Ch was reliably measured with Ringer or CSF in the neostriatum, ACh could not be detected either under basal or elevated KCl conditions. In order to be able to measure ACh, an inhibitor of ACh-esterase had to be included in the perfusion medium. We chose neostigmine as an inhibitor since physostigmine introduces disturbances in catecholamine assays (Maysinger et al., 1988). The levels at which ACh was detected under basal and elevated KCl conditions appear to depend upon the neostigmine concentration included in the perfusion medium (Table 2). While DA levels did not appear to be affected by the presence of neostigmine, Glu and Asp levels showed large variations when 10 μM neostigmine was included in the medium. Basal GABA levels were only slightly elevated in the presence of 1 μM neostigmine (c.f. [2] in Table 2 vs [2] in Table 1). We also observed that with 1 μM of neostigmine in the medium, ACh levels were more reliable and stable than when 10 μM neostigmine was used (unpublished observation). Thus, the inclusion of 1 μM of neostigmine in the perfusion medium permits reliable measurements of extracellular levels of ACh in the neostriatum, without significantly affecting the simultaneous measurement of DA, GABA, Glu and Asp. As previously shown, basal (Westerink et al. 1988), as well as KCl-stimulated ACh (Table 3) levels were decreased when perfusing with a Ca^{2+}-free medium.

Interpreting changes in the extracellular levels of excitatory amino acids, Glu and Asp with in vivo microdialysis is a controversial issue, probably due to the fact these amino acids are widely distributed and involved in general cellular metabolism. In addition, while the lifetime of extracellular ACh in the CNS is largely limited by the potent action of ACh-esterase, the lifetime of the amino acid neurotransmitters depends upon rapid re-uptake by surrounding neuronal and non-neuronal elements. Indeed, there is evidence suggesting that the Glu re-uptake site is mainly located on glia rather than on the neuronal membrane (Currie and Kelly 1981, Paulsen and Fonum 1989). In the present experiments, neostriatal Glu and Asp levels were consistent in all the perfusion media (Glu $\approx 2\,\mu$M; Asp $\approx 0.2\,\mu$M), but were largely unaffected by K^+-depolarization, while simultaneously detected DA, ACh and GABA levels were increased >10 fold. Since re-uptake by both glial cells and neuronal terminals seems to be the main inactivation mechanism for Glu, the selective Glu uptake blocker DHKA (Johnston et al., 1979) was included in the perfusion medium, prior to or together with 100 mM KCl. When DHKA (0.01–1 mM) was included in the perfusion medium, 120 min after probe implantion, extracellular Glu and Asp levels in the neostriatum increased in a dose-dependent manner. In addition, K^+-depolarization induced clear increases in Glu and Asp levels (≈ 3 fold) in the presence of DHKA. Furthermore, when DHKA was included together with 100 mM KCl a strong increase in Glu and Asp levels was also observed (see Fig. 2). Thus, inclusion of a Glu uptake blocker into the perfusion medium amplifies the expression of the neuronal pool in the extracellular space and allows one to

distinguish between neuronal and metabolic pools of Glu and Asp. While neostriatal DA levels were not affected by DHKA levels, GABA levels were significantly increased, both under basal and high KCl conditions, suggesting that DHKA may affect GABA as well as Glu and Asp. Basal ACh levels were increased by 68% in the presence of DHKA, but the effect of K^+-depolarization was reduced (254% increase in the presence of 1 μM neostigmine + 1 mM DHKA vs 714% increase in the presence of 1 μM neostigmine alone) (c.f. [1] in Table 3 vs [2] in Table 2). While the experiments with DHKA support the existence of neuronal and metabolic pools for Glu and Asp in the neostriatum of the rat, they do not answer the question whether neuronal Glu and Asp are released by vesicular or by non-vesicular mechanisms from neurons or glial cells (Szatkowski et al. 1990). Thus, it was necessary to study the Ca^{2+}-dependency of the increases in Glu and Asp levels elicited by K^+-depolarization. Basal and KCl-stimulated levels of neostriatal DA, ACh and GABA were significantly decreased when perfusing with a Ca^{2+}-free medium, which included 2 mM of EDTA (Table 3). Basal Glu levels were slightly increased by the Ca^{2+}-free medium. However, during K^+-depolarization, Glu levels were increased by only 151% in a Ca^{2+}-free medium compared with 358% in the presence of Ca^{2+} (Table 3). Asp levels were not affected by the presence or absence of Ca^{2+}. Thus, these results support the idea that, in the neostriatum, there is a Ca^{2+}-dependent-vesicular pool of releasable Glu and a non-vesicular pool of Asp. These results are in agreement with observations suggesting that Glu but not Asp is found in synaptic vesicles (Storm-Mathisen et al., 1983; Burger et al., 1989).

Whenever releasing mechanisms of a putative neurotransmitter are monitored by microdialysis, it is necessary to demonstrate that the detected levels can be decreased by selective lesions of that particular pathway. For instance, it is well established that extracellular DA levels in the neotriatum are decreased following DA deafferentation induced by a unilateral 60HDA injection into the area ventralis tegmenti, which contains the bundle of axons leaving the mesencephalic DA cell bodies (Zetterström et al., 1986; Herrera-Marschitz et al., 1989, 1990c). We also show here (Table 4) that DA deafferentation is associated with a decrease in neostriatal extracellular DA levels detected under basal and KCl-stimulated conditions (c.f. [1] in Table 4 vs [1] in Table 2), and by a decrease in basal Glu levels (Reid et al., 1990e), but not GABA and ACh levels (Herrera-Marschitz et al., 1990b). The decrease in neostriatal Glu following DA-deafferentation may indicate that midbrain DA neurons exert a tonic-facilitatory modulation on neostriatal Glu terminals (Reid et al., 1990e). Furthermore, the effect of K^+-depolarization on GABA levels was >2 fold stronger on the denervated than on the control-intact side (O'Connor et al., 1991a), suggesting a tonic-inhibitory modulation of neostriatal GABA neurons by DA terminals.

IBA was used to produce a neostriatal lesion affecting intrinsic neurons, while sparing afferent and en passage axons (Schwarcz et al., 1979; Christensson-Nylander et al., 1986). After this lesion, basal and KCl-stimulated ACh and GABA levels were significantly decreased (by $>99\%$ and $>80\%$, respectively) (c.f. [2] in Table 4 vs [1] in Table 3), which is in agreement with evidence showing that, in the neostriatum, cholinergic transmission is derived from intrinsic neurons (Satoh et al., 1983), while GABA is found both in interneurons and pro-

jecting systems (Kim et al., 1971; Brownstein et al., 1977; McGeer and McGeer, 1975). After the neostriatal lesion, a tendency for an increase in Glu, but not Asp levels was observed, although Glu levels showed large intra- and inter-group variations. Basal DA levels were not significantly changed by the neostriatal lesion.

Multiple KA injections into the frontoparietal cortex induced a cortical lesion characterized by a loss of intrinsic and projecting neuronal systems, while, like after IBA, sparing afferent and en passage axons (Schwarcz and Coyle, 1977, Herrera-Marschitz et al., 1989). It was found that both DA and ACh levels were slightly decreased in the striatum ipsilateral to the cortical lesion (both, under basal and KCl-stimulated conditions) ([3] in Table 4 vs [1] in Table 2). However, neostriatal Glu levels were increased after the decortication, although the values showed large variations. The effect of DHKA on Glu levels was not investigated in this study therefore it is not clear if the neuronal pool of neostriatal Glu was selectively affected by the decortication. In recent experiments, we have observed that KCl-stimulated neostriatal levels are significantly decreased when a unilateral decortication is combined with a callosum-tomy, which impairs the Glu innervation provided by the contralateral cortex (Herrera-Marschitz et al., in preparation). Interestingly, Young and Bradford (1986) reported a microdialysis study showing that a unilateral frontal decortication performed by aspiration produced a decrease in both basal and stimulated levels of Glu in the neostriatum ipsilateral to the lesion. It is probable that the cortical aspiration also impaired en passage fibers through the cortex and the corpus callosum.

The striatonigral pathway is the most massive of the striatal outputs, consisting mainly of medium sized spiny cells projecting to the ipsilateral substantia nigra, where striatonigral fibers make monosynaptic contacts with nigrostriatal DA neurons and/or with nigrothalamic and nigrotectal neurons (see Smith and Bolam, 1990). The striatonigral pathway is neurochemically heterogeneous, and fibers containing GABA, DYN, SP and NKA have been described (see McGeer et al., 1984; Herrera-Marschitz, 1986; Lindefors 1987). Several studies indicate that SP and NKA may represent excitatory components, while GABA and DYN A may represent inhibitory components of a striatonigral feedback loop modulating the nigrostriatal DA pathway (see Herrera-Marschitz, 1986; Reid, 1990). Thus, it was found (Table 5) that intranigral injections of SP and NKA produced a significant increase, while GABA and DYN A produced a significant decrease in neostriatal DA levels. (The dose- and receptor-dependences of these effects have been extensively studied elsewhere [Reid et al., 1988, 1990a,b,c]). In addition, changes in neostriatal extracellular DA produced by intranigral injections were followed by discrete changes in neostriatal GABA or Glu levels. The increase in GABA levels, following the decrease in neostriatal DA produced by nigral injection of GABA or DYN A, suggests that DA terminals exert a tonic-inhibitory modulation on neostriatal GABA-ergic terminals. Furthermore, as shown above, an increase in KCl-stimulated, but not in basal GABA levels, was observed in DA deafferentated animals, which indicates that an inhibitory influence of DA on GABA release depends upon the degree of activation of neostriatal neurons (O'Connor et al., 1991a). It has been previ-

ously reported that DA exerts an inhibitory action on neostriatal GABAergic neurons via D-2 receptors, while stimulation of D-1 receptors may produce the opposite effect (Tossman and Ungerstedt, 1986; Reid et al., 1990c; O'Connor et al., 1991b).

Nigral injections of SP, but not NKA, produced an increase in extracellular levels of both DA and Glu in the ipsilateral neostriatum. The increase in neostriatal Glu levels produced by intranigral SP injections is in agreement with the finding that Glu levels are decreased after DA deafferentation ([1] in Table 4) and with earlier studies showing that intranigral electrical stimulation produces an increase in neostriatal Glu release (Godukhin et al., 1984; Girault et al., 1986). Furthermore, it was previously found (Reid et al., 1990d) that neostriatal Glu levels were also increased following stimulation with both D-1 and D-2 DA agonists via a microdialysis probe implanted in the neostriatum of anaesthetized rats. These results suggest that DA exerts a tonic facilitatory modulation of neostriatal Glu release. The neuroanatomical background for such interaction is not yet well established, although it has been shown that corticostriatal terminals possess binding sites for DA ligands (Schwarcz et al., 1978).

There is evidence that neostriatal DA release can be modulated by corticostriatal Glu-ergic inputs (Giorguieff et al., 1977; Roberts et al., 1982; Cheramy et al., 1986; Romo et al., 1986a,b), via mono- and/or polysynaptic loops (Somogyi et al., 1981), and involving different Glu receptors (Herrling 1985). Indeed, studies with in vivo microdialysis have shown that neostriatal DA levels are increased following local stimulation with KA (Carter et al., 1988; Herrera-Marschitz et al., 1990a), supporting the hypothesis of a direct axonal interaction between cortical Glu and striatal DA terminals. However, it has been suggested that Glu terminals may act primarily via neostriatal ACh neurons, since neostriatal ACh, but not DA, levels are increased following cortical stimulation (Herrera-Marschitz, 1991). This is in agreement with evidence showing that the majority of the neostriatal afferents from the cortex make axodendritic synaptic contacts with neostriatal neurons (Somogyi et al., 1981; Bolam et al., 1985). However, in studies where microdialysis probes were implanted simultaneously into the frontoparietal cortex and into the ipsilateral neostriatum, we observed that while neostriatal ACh levels were increased following cortical stimulation with the Glu agonist, N-methyl-D-aspartate (NMDA), neither neostriatal Glu nor Asp levels were significantly changed. Thus, while changes are seen in the targets for the corticostriatal inputs, no changes are observed in their putative links. It may be possible that changes in neostriatal Glu levels could be observed following blockade of Glu re-uptake with DHKA. The possibility also exists that a different neurotransmitter system (e.g. CCK) is the link between cortex and neostriatal ACh (Burgunder and Young, 1990; Meana et al., 1991). There is also the possibility that since corticostriatal inputs are arranged in a very strict topographic-columnar manner (McGeorge and Faull, 1989), changes in the levels of striatal neurotransmitters may be mediated by microcircuits in the neostriatum, which are beyond the present spacial resolution of the available microdialysis probes. However, we are now studying cortico-cortical and corticostriatal interactions in the rat with a columnar approach, using a new series

of very thin microdialysis probes (CMA 11, Carnegie Medicin AB), with a diameter of 0.24 mm and a length of 1 or 2 mm length.

In summary, the present results support the increasing amount of evidence showing that changes in extracellular DA levels, monitored with in vivo microdialysis in the neostriatum of the rat, correlate with changes in the activity of mesencephalic DA neurons, Thus, this and previous papers demonstrate that: [1] Neostriatal DA levels are increased several fold over basal levels by K^+-depolarization (see Ungerstedt, 1984; Zetterström, 1986). [2] Basal DA levels are significantly diminished by perfusing with a Ca^{2+}-free medium, and in this medium, the effect of K^+-depolarization is decreased, suggesting a vesicular releasing mechanism. [3] Unilateral DA deafferentation, induced with 60HDA injected into the mesencephalon, decreases extracellular DA in the neostriatum and other telencephalic regions ipsilateral to the side of the lesion (Zetterström et al., 1986; Herrera-Marschitz et al., 1989). Furthermore, it has been found that the amount of extracellular DA remaining after DA deafferentation closely correlates with functional parameters evaluating the extent of the lesion (Herrera-Marschitz et al., 1990c). In addition, the highest concentration of DA is found in the neostriatum, which is the main target of mesencephalic DA projections (Björklund and Lindvall, 1984; Hökfelt et al., 1984), lower concentrations are found in regions receiving sparse DA innervation, such as the frontoparietal cortex (Descarries et al., 1987; Herrera-Marschitz et al., 1989), while intermediate amounts are found (Zetterström 1986) in those regions receiving intermediate DA innervation, such as accumbens or prefrontal cortex (Björklund and Lindvall, 1984; Hökfelt et al., 1984). [4] Electrical stimulation of the nigrostriatal pathway leads to an increase in extracellular DA levels in the ipsilateral neostriatum (Imperato and Di Chiara, 1984), and releasing patterns of neostriatal DA are changed by selective nigral stimulations with exogenous neurotransmitters (e.g. GABA, DYN A, SP and NKA) found in the striatonigral pathway (Herrera-Marschitz, 1986; Reid, 1990). [5] The TTX dependence of extracellular DA levels measured in the neostriatum of rat has been demonstrated (Westerink et al., 1987; Drew et al., 1990; Osborne et al., 1990, 1991b), indicating that extracellular DA levels monitored by microdialysis are released by voltage-dependent mechanisms. [6] The sensitivity of neostriatal DA levels to specific pharmacological treatments has been extensively reported (see Zetterström, 1986; Lindefors, 1987; Ståhle, 1987; Hurd, 1989; Reid, 1990).

However, the main issue of this paper addresses the simultaneous monitoring of several putative neurotransmitters in the neostriatum of anaesthetized rats. This is also related to the optimal conditions under which putative neurotransmitters can be reliably monitored.

It was found that, *whenever ACh should be analysed*, an ACh-esterase inhibitor had to be included in the perfusion medium. Thus, the effects of different concentrations of neostigmine on neostriatal ACh levels, but also on DA, Glu, Asp and GABA were studied. It was found that at low doses, neostigmine included in the perfusion medium permitted reliable measurements of ACh, without significantly affecting other neurotransmitters. Under these conditions, the sensitivity of neostriatal extracellular levels of ACh to K^+-depolarization and Ca^{2+}-dependence could be clearly demonstrated. Furthermore, it was found

that a lesion of the intrinsic neuronal systems in the neostriatum abolished extracellular ACh levels, without affecting DA, which is released from extrinsic DA neurons. The neuroanatomical dependency of extracellular ACh levels measured with microdialysis has been further demonstrated in experiments where cortical and neostriatal ACh levels were simultaneously monitored. It was found that a IBA lesion of the ipsilateral nucleus basalis magnocellularis significantly decreased ACh levels in the frontoparietal cortex, but not in the ipsilateral neostriatum (Herrera-Marschitz et al., 1990b).

Extracellular neostriatal GABA levels could be measured in Ringer or CSF. It was found that extracellular GABA levels were increased, in a Ca^{2+}-dependent manner, by K^+-depolarization, and could be decreased by an intrinsic neostriatal lesion, supporting the idea that, in the neostriatum, GABA is used as neurotransmiter by intrinsic neurons.

While *extracellular neostriatal Glu and Asp levels* could be detected at high concentrations with different perfusion media, it was not possible to definitively demonstrate their neuronal or metabolic origin. Glu and Asp levels were only slightly increased by K^+-depolarization, and no changes in neostriatal Glu and Asp levels were seen after a lesion damaging a putative corticostriatal Glu pathway. Indeed, neostriatal Glu levels were slightly increased, while no apparent effect on Asp levels was observed. A blocker of Glu re-uptake, DHKA, had to be included in the perfusion medium, in order to monitor the effect of K^+-depolarization on Glu and Asp levels. Under this condition Glu and Asp levels were significantly increased by K^+-depolarisation, although only the increase in Glu was dependent upon the presence or absence of Ca^{2+} in the perfusion medium, suggesting that Glu, but not Asp, is released from vesicular pools in the neostriatum.

Chemical stimulations of discrete neuronal pathways have been used to test the neuronal dependence of extracellular levels of putative neurotransmitters monitored with microdialysis (see Reid, 1990; Herrera-Marschitz et al., 1990a). This approach may be useful to further study neuronal interactions in restricted brain regions.

In conclusion, we provide evidence that *in vivo* microdialysis is a useful technique to monitor discrete neurotransmitter release at the synaptic cleft, or elsewhere along neuronal, glia and/or microvascular complexes in the CNS. However, several methodological aspects have to be considered before interpretation about changes in extracellular levels of putative neurotransmitters can be made. Considerations of the characteristics of the perfusion medium have to be taken into account, as well as the physiological condition of the experimental preparation. Particular attention has to be paid to the impact that the implantation procedure may have on the target tissue to be analysed. However, it is clear that in vivo microdialysis is a valuable neuroanatomical tool to study reciprocal interactions between neurotransmitter substances released in the extracellular space surrounding neuronal and glia elements in the CNS.

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