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GLUTAMATE REGULATES THE SPONTANEOUS AND EVOKED RELEASE OF DOPAMINE IN THE RAT STRIATUM

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Abstract—Resting and evoked extracellular dopamine levels in the striatum of the anesthetized rat were measured by fast-scan cyclic voltammetry in conjunction with carbon fiber microelectrodes. Identification of the substance detected *in vivo* was achieved by inspection of background-subtracted voltammograms. Intrastriatal microinfusion of kynurenate, a broad-spectrum antagonist of ionotropic glutamate receptors, caused a decrease in the resting extracellular level of dopamine. The kynurenate-induced decrease was unaffected by systemic pretreatment with pargyline, an inhibitor of monoamine oxidase, but was significantly attenuated by systemic pretreatment with α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase. Although glutamate by itself did not affect resting extracellular dopamine levels, glutamate did attenuate the kynurenate-induced decrease. Kynurenate decreased dopamine release in response to electrical stimulation of the medial forebrain bundle, an effect that was also attenuated by glutamate.

These results suggest that both spontaneous and evoked dopamine release in the rat striatum are under the local tonic excitatory influence of glutamate. Interactions between central dopamine and glutamate systems that have been implicated in the etiologies of Parkinson's disease, schizophrenia, stress, and substance abuse. The precise nature of those interactions, however, remains a matter of some controversy. © 2001 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: voltammetry, carbon fiber microelectrode, ionotropic glutamate receptors, kynurenate, electrical stimulation, medial forebrain bundle.

The rat striatum receives dopaminergic projections from the substantia nigra and glutamatergic projections from both the cortex and the hippocampus. Ultrastructural studies show that these efferents form synaptic contacts with striatal neurons and dendrites but do not form axo-axonic synapses with each other.[3,10,47,48] This anatomical arrangement implies that dopamine and glutamate may act in concert to regulate the activity of striatal neurons but leaves open the question of to what extent, if any, do dopamine and glutamate interact with each other within the striatum? The study reported herein focused on examining whether striatal dopamine release is locally regulated by glutamate.

Intrastriatal delivery of glutamate agonists elicits dopamine release via a mechanism that appears to be independent of impulse traffic, since it is resistant to the removal of Ca^{2+} and to the co-delivery of tetrodotoxin.[5,13,25,27,31,33] Furthermore, intrastriatal application of glutamate antagonists cause extracellular dopamine levels as measured by microdialysis either to not change or to increase.[25,36,39] Additionally, direct infusion of glutamate into the striatum does not elicit an increase in dopamine-related electrochemical signals, except when the concentration of glutamate is sufficiently high to induce a pathological spreading depression.[40,54] The consensus that emerges from these reports is that glutamate does not excite dopamine release by physiologically relevant mechanisms acting locally within the striatum itself. This consensus is further supported by reports that relatively few glutamatergic receptors are present on dopaminergic terminals.[16]

In contrast, several other studies suggest that dopamine release is under the local control of striatal glutamate receptors.[7,8,19,20,35,45,62] For example, using voltammetry we recently found that the electrically evoked release of dopamine in striatal slices was increased in the presence of the broad-spectrum glutamate antagonist, kynurenate.[59] Although glutamate itself did not affect electrically evoked dopamine release, glutamate did prevent the kynurenate-induced increase. These *in vitro* results suggest that glutamate receptors within the striatum regulate dopamine release. Elsewhere, we examined the hypothesis that this inhibitory action of glutamate is mediated by GABAergic elements of the striatum.[26]

The ability of kynurenate to increase electrically evoked dopamine release in striatal slices[59] implies a tonic occupation of receptors by endogenous glutamate in this *in vitro* preparation. This may explain the apparent lack of an effect of exogenously applied glutamate on evoked dopamine release: exogenous glutamate may simply not be able to stimulate glutamate receptors beyond the level of activation already caused by

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Abbreviations: aCSF, artificial cerebrospinal fluid; αMTP, α-methyl-*p*-tyrosine; MFB, medial forebrain bundle.

endogenous glutamate. Our recent observation of an impulse-dependent (i.e. tetrodotoxin-sensitive) component of basal extracellular glutamate in the rat striatum[29,30] leads to the hypothesis that a similar mechanism may be at work under *in vivo* conditions as well. The goal of the present study, therefore, was to extend the use of voltammetry to the investigation of the effect of kynurenate on striatal dopamine release under *in vivo* conditions.

EXPERIMENTAL PROCEDURES

Animals and surgical techniques

All experiments involving animals were conducted with the approval of the University of Pittsburgh Institutional Animal Care and Use Committee in accordance with appropriate guidelines. Male Sprague–Dawley rats (280–350 g) were anesthetized with an initial dose of chloral hydrate (400 mg/kg i.p.) and given atropine (0.1 mg/kg i.p). The anesthetized rats were wrapped in a homeothermic blanket that maintained body temperature at 37°C. They were then placed in a stereotaxic surgical frame with the incisor bar raised 5 mm above the interaural line.[43] Throughout the experiments, the rats were kept anesthetized with additional doses of chloral hydrate as needed to prevent any detectable motor response to gentle pressure applied to the hind paw. A midline incision was made through the scalp, which was retracted to expose the skull. Appropriately positioned holes were drilled through the skull to permit subsequent placement of voltammetric and stimulating electrodes, as well as a microinfusion pipet.

Voltammetric electrodes and techniques

Voltammetric microelectrodes were prepared from T300 carbon fibers (Amoco Specialty Products, Greenville, SC), which have a radius of 3.5 μm. A single fiber was sealed with epoxy (Spurr Embedding Medium, Polysciences, Warrington, PA) into a pulled glass capillary and the exposed fiber was trimmed to a length of 400 μm. Before use, each electrode was electrochemically pretreated with a 0–2 V vs Ag/AgCl triangular voltage waveform applied at 200 V/s for 1 s. The electrode was allowed to equilibrate and then calibration was performed with dopamine in a flow-stream apparatus equipped with a sample injection valve. Flow through the system was created by gravity feed from an elevated reservoir containing nitrogen-purged artificial cerebrospinal fluid (aCSF) with the following composition: 145 mM Na^+, 1.2 mM Ca^{2+}, 2.7 mM K^+, 1.0 mM Mg^{2+}, 152 mM Cl^-, 2.0 mM phosphate buffer, pH 7.4.[38]

Dopamine was detected by fast scan-cyclic voltammetry[1] performed with a custom-designed, computer-controlled potentiostat (EI 400, Ensman Instruments, Bloomington, IN). The microelectrode was held at a rest potential of 0 V vs Ag/AgCl between voltammetric scans. Each scan consisted of three contiguous potential sweeps, first to +1 V, then to –0.5 V, and back to 0 V vs Ag/AgCl. The sweeps were performed at 300 V/s and were repeated at 200-ms intervals. Changes in extracellular dopamine concentration were quantified by means of the change in voltammetric current flowing in the potential window on the first sweep corresponding to the maximum rate of dopamine oxidation (0.5–0.7 V vs Ag/AgCl). Signals recorded *in vivo* were converted to apparent dopamine concentration changes by post-calibration of each microelectrode immediately after it was removed from the rat brain. Background-subtracted voltammograms were used to identify the substance detected during *in vivo* experiments.

Experimental design

A voltammetric electrode was implanted in the striatum at a point 4.0 mm below dura, 2.5 mm anterior with respect to bregma, and 2.5 mm lateral with respect to midline. The tip of a microinfusion pipet was placed about 250 μm from the voltammetric electrode. Where indicated, the voltammetric electrode and micropipet were implanted instead in the thalamus, at a point 5.5 mm below dura, 0.0 mm anterior with respect to bregma, and 1.5 mm lateral with respect to midline. Contact between brain tissue and an Ag/AgCl reference electrode was established with a salt bridge resting on the cortical surface.

When electrical stimulation was used, the stimulating electrode (MS 303-1, Plastics One, Roanoke, VA, USA) was implanted into the medial forebrain bundle ipsilateral to the voltammetric electrode in striatum. The stimulating electrode was placed 2.2 mm posterior with respect to bregma and 1.6 mm lateral from midline. To set the vertical placement of the electrode, it was slowly lowered from an initial depth of 7.5 mm below dura until evoked dopamine release was detected by voltammetry in the striatum. The stimulus current was optically isolated and delivered according to the following parameters: biphasic square pulses, 2-ms pulse width each phase, 45 Hz, 50 μA rms. The stimulus pulses were synchronized with the voltammetric sweeps to avoid crosstalk.

Microinfusion pipets were prepared from fused silica capillary tubing that had an internal diameter of 27 μm (Polymicro Technologies, Phoenix, AZ, USA). The outlet end the capillary was etched to a fine tip with hydrofluoric acid. The inlet end was attached to a 50-μl gas-tight syringe mounted on a microprocessor-controlled driver (NA-1, Sutter Instruments, Novato, CA, USA). The syringe and the capillary were filled with the desired infusion solution before implantation. The infusion vehicle was aCSF, the infusion volume was 200 nl, and the delivery rate was 7 nl/s.

Experiments were initiated about 2 h after implantation of the voltammetric electrodes to allow for equilibration of the background signal. In one series of experiments, pairs of infusions were performed at an interval of 1 h. In experiments involving electrical stimulation of the medial forebrain bundle (MFB), voltammetric stimulus responses were recorded at 20-min intervals. After at least three reproducible stimulus responses had been collected, the first infusion was performed 3 min before the next stimulus. Where indicated, a systemic dose of pargyline (75 mg/kg i.p) or α(75 m.g/k(of)-2

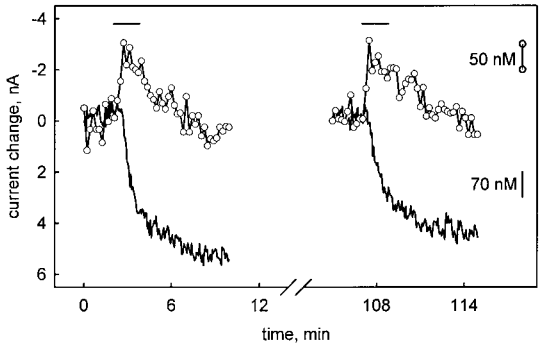

Fig. 1. The effect of intrastriatal infusion of kynurenate (1 mM, 200 nl, solid lines) and aCSF (200 nl, symbols) on the resting voltammetric signal observed in striatum with a carbon fiber microelectrode. The horizontal bars indicate when the infusions took place. The vertical scale bars were determined by post-calibration of each microelectrode immediately after it was removed from the rat.

Effect of intrastriatal infusions on resting voltammetric signals

Fig. 1 shows that the resting voltammetric signal in the striatum decreased noticeably upon the intrastriatal infusion of kynurenate (1 mM, 200 nl). However, the signal was unaffected by intrastriatal infusion of aCSF vehicle (200 nl). Furthermore, a similar response was obtained when a second intrastriatal infusion of kynurenate or aCSF was performed about 100 min after the first infusion. Although this implies that the signal returned to baseline after the first kynurenate infusion, clear observation of the return to baseline was prevented by long-term drift in the background signal. According to post-calibration of the microelectrodes, the decrease in the voltammetric signal over the 5-min interval after the start of the first kynurenate infusion corresponded to a decrease in dopamine concentration of 485 nM (Table 1). In our hands, intrastriatal infusion of glutamate (1 mM or 10 mM, 200 nl) by itself had no effect on the resting voltammetric signal (data not included). Nevertheless,

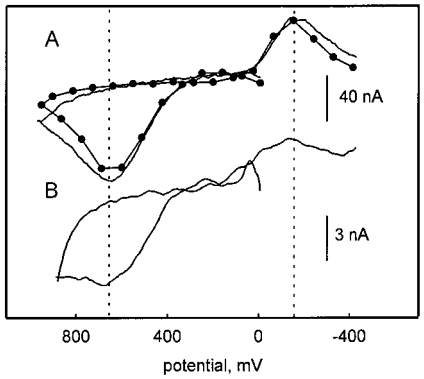

Fig. 2. (A) Background-subtracted voltammograms recorded *in vitro* during post-calibration in authentic dopamine standard (closed symbols) and *in vivo* during electrical stimulation of the medial forebrain bundle (solid line). (B) Background-subtracted voltammogram of the substance that decreased upon intrastriatal infusion of kynurenate (1 mM, 200 nl). The vertical dashed lines show the correspondence of the position of the peaks for the oxidation of dopamine and the reduction of dopamine-*o*-quinone.

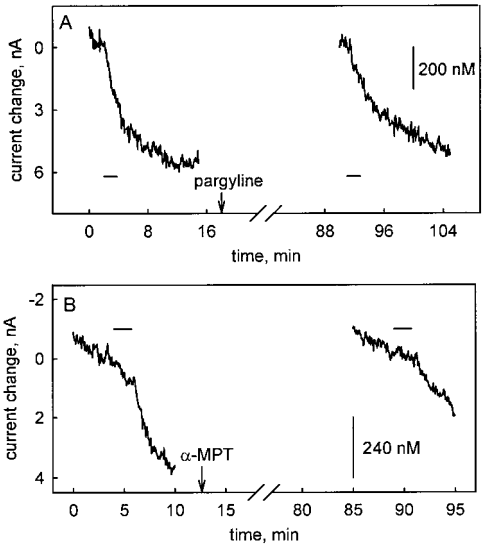

Fig. 3. The effect of pargyline (A) and αMPT (B) on the amplitude of the decrease in the resting voltammetric signal upon intrastriatal infusion of kynurenate (1 mM, 200 nl). The horizontal bars indicate when the infusions took place. The vertical scale bars were determined by post-calibration of each microelectrode immediately after it was removed from the rat.

coinfusion of glutamate (1 or 10 mM) with kynurenate (1 mM) attenuated the apparent decrease in dopamine concentration compared to that caused by infusion of kynurenate alone (Table 1).

Voltammetric identification

Figure 2 provides voltammetric evidence that dopamine is the substance responsible for the decrease in the resting voltammetric signal observed upon kynurenate infusion. Figure 2A compares the background-subtracted voltammogram obtained *in vitro* during post-calibration of a microelectrode in a solution of authentic dopamine with that obtained *in vivo* during electrical stimulation of the MFB. The features of the two voltammograms are similar in terms of the position and relative magnitude of the dopamine oxidation peak and the dopamine-*o*-quinone reduction peak, which show that the qualitative features of the dopamine voltammogram are similar under *in vitro* and *in vivo* conditions. Figure 2B shows the voltammogram of the substance that decreased in concentration upon infusion of kynurenate. The position and relative magnitude of the oxidation and reduction peaks are in excellent agreement with those in Fig. 2A (vertical dashed lines), which provides evidence that that dopamine is the substance observed to decrease following intrastriatal kynurenate infusion. Similar background subtracted voltammograms were obtained in all experiments reported herein.

Pharmacological identification

Figure 3 contains pharmacological evidence that dopamine is the substance responsible for the decrease in the resting voltammetric signal following intrastriatal infusion of kynurenate. In these experiments, rats received a

Table 1. The effect of intrastriatal drug infusion on resting extracellular dopamine

Infused drug(s)*	Concentration (mM)*	(*n*)†	Decrease in‡ dopamine	Significance§	Significance‖
Kynurenate	1	6	485 ± 120		
+ Glutamate	1	3	272 ± 65	$P < 0.01$	
+ Glutamate	10	3	123 ± 64	$P < 0.01$	$P < 0.05$

*Drugs infused in artificial cerebrospinal fluid vehicle solution (200 nl).
†Number of individual rats.
‡Data reported as the mean and S.D. in nM units.
§Significance compared to the effect of kynurenate alone determined by one-way ANOVA followed by Duncan's test.
‖Significance compared to the effect of kynurenate with 1 mM glutamate determined by one-way ANOVA followed by Duncan's test.

Table 2. Effect of systemically delivered drugs on the response to intrastriatal microinfusion of kynurenate

Drug*	Dose (mg/kg)*	(*n*)†	% of pre-drug response‡	Significance§
Control		6	89 ± 8.5	
Pargyline	75	3	86 ± 5.5	n.s
αMPT	250	3	39 ± 13	$P < 0.01$

*Drugs delivered i.p. in phosphate buffered saline vehicle.
†Number of individual rats.
‡Amplitude of the second response relative to first reported as the mean and S.D.
§Significance compared to control response determined by one-way ANOVA followed by Duncan's test (n.s. means not significant).

systemic dose of a drug between the two infusions of kynurenate. Figure 3A shows that administration of the MAO inhibitor, pargyline (75 mg/kg i.p.), had no impact on the decrease in the voltammetric signal upon the subsequent infusion of kynurenate. However, Fig. 3B shows that administration of the tyrosine hydroxylase inhibitor, αMPT (250 mg/kg i.p.), attenuated the decrease in the voltammetric signal upon the subsequent infusion of kynurenate. To quantify these changes, the magnitude of the decrease in the signal over the 5 min following the second kynurenate infusion was converted to a percentage of the magnitude of the decrease in the signal over the 5 min following the first kynurenate infusion (Table 2). Table 2 shows that αMPT, but not pargyline, caused a statistically significant attenuation of the voltammetric response to kynurenate infusion.

Anatomical identification

Anatomical evidence that dopamine is the substance detected following intrastriatal infusion of kynurenate was obtained by carrying out the same procedure in the thalamus, a region that contains very little dopamine.[28,41] Intrathalamic infusion of kynurenate had no effect on the resting voltammetric signal at a nearby carbon fiber electrode ($n = 3$, data not shown).

Correlation with voltammetric stimulus responses

Figure 4 reports the effects of pargyline and αMPT administration on the amplitude of the voltammetric response to electrical stimulation of the MFB. After pargyline administration, the amplitude of the evoked response increased slightly over the course of 1 h.[37,52] This suggests that the releasable pool of dopamine is preserved after pargyline administration, which is consistent with the lack of effect of pargyline on the decrease in resting dopamine caused by kynurenate infusion (Fig. 3, Table 2). However, the amplitude of the evoked response decreased after the administration of αMPT, which suggests that inhibition of tyrosine hydroxylase diminishes the size of the releasable pool of dopamine.[11,37,52] The decrease occurred quite slowly, however, which provides an explanation as to why the response to kynurenate infusion was attenuated rather than abolished after αMPT administration (Fig. 3, Table 2).

Effect of intrastriatal infusions on voltammetric stimulus responses

The impact of intrastriatal infusion of aCSF, kynurenate and glutamate on the amplitude of the voltammetric response to electrical stimulation of the MFB was examined. Table 3 gives the amplitude of the voltammetric stimulus response recorded 3 min after intrastriatal infusion relative to the average amplitude of the three stimulus responses recorded prior to infusion. No infusion was performed in control experiments. The amplitude was not significantly changed by intrastriatal infusion of aCSF (200 nl) or glutamate by itself (1 mM or 10 mM, 200 nl). Intrastriatal infusion of kynurenate (1 mM, 200 nl) caused a small but significant decrease in the response amplitude. As was observed for resting levels,

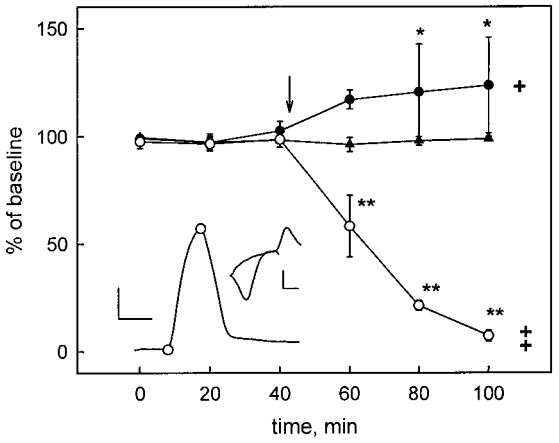

Fig. 4. The amplitude (mean ± S.D., $n=3$) of the voltammetric response to brief electrical stimulation of the MFB in rats that received no drug (triangles), pargyline (closed circles), or αMPT (open circles) after the third stimulus. Statistical analysis of the results for each drug was by one-way ANOVA followed by Duncan's test ($+P<0.05$; $++P<0.001$): stars indicate difference from pre-drug response amplitude ($*P<0.05$; $**P<0.01$). The inset illustrates the voltammetric stimulus response. Scale bars = 20 nA and 10 s. The background-subtracted voltammogram obtained at the peak of the response. Scale bars = 30 nA and 300 mV.

the effect of kynurenate was attenuated by the coinfusion of glutamate (1 or 10 mM).

DISCUSSION

As noted in the introduction, the striatum receives dopaminergic and glutamatergic efferents that apparently do not form axo–axonic synaptic connections but do make appositional contact with one another.[3,10,47,48] This anatomical arrangement leads to the question of whether local interactions between dopamine and glutamate occur within the striatum. Some evidence suggests, for example, that the nigrostriatal and mesolimbic dopamine pathways are regulated by glutamate via mechanisms acting in the vicinity of midbrain dopaminergic soma,[12,22,42,53,55] perhaps exclusively so.[23,24] Several reports that the intrastriatal infusion of glutamate has little effect on apparent extracellular dopamine levels, except by pathological mechanisms, appear to support this conclusion.[25,38,54,62]

In a recent study, we noticed that kynurenate had a significant effect on electrically evoked dopamine release in striatal slices.[59] This observation may indicate that glutamate receptors within the slice are activated by endogenous glutamate. The pre-existing occupation of receptors by endogenous glutamate may mask the effect of exogenously applied glutamate. In the present study, we wished to test the hypothesis that a similar mechanism is at work under *in vivo* conditions. Therefore, we examined the impact of the intrastriatal infusion of kynurenate on resting and evoked voltammetric signals recorded in the chloral hydrate-anesthetized rat.

The results reported herein provide *in vivo* evidence that the resting extracellular level of dopamine in the striatum is regulated by glutamate receptors within the striatum. Intrastriatal infusion of kynurenate caused a marked decrease in the resting voltammetric signal in striatum (Fig. 1, Table 1). Voltammetric, pharmacological, and anatomical evidence combine to confirm that the decrease in signal was due to a decrease in the resting extracellular concentration of dopamine. Background-subtracted voltammograms were similar to those obtained for dopamine during *in vitro* calibration and during *in vivo* stimulation of dopamine pathways (Fig. 2). Inhibition of dopamine synthesis, but not of dopamine metabolism, attenuated the response (Fig. 3, Table 2). Intrathalamic infusion of kynurenate had no effect on the resting voltammetric signal, consistent with the low level of dopaminergic innervation of the thalamus.[28,41] The effect of pargyline and αMPT on the response to kynurenate infusion correlated well with the effects of these drugs on the voltammetric response to electrical stimulation (Fig. 4), which further supports the hypothesis that the response to kynurenate infusion is related to a releasable pool of striatal dopamine.

Consistent with prior reports, this study did not produce evidence that dopamine release is excited by exogenous glutamate.[25,40,54,62] Intrastriatal infusion of glutamate at a concentration of 1 or 10 mM had no impact on the resting or evoked voltammetric signals. Nevertheless, these same concentrations of glutamate significantly attenuated the effect of kynurenate infusion

Table 3. Effect of intrastriatal infusion on the amplitude of the voltammetric response to electrical stimulation of the medial forebrain bundle

Infused drug(s)*	Concentration (mM)*	(*n*)†	% of pre-infusion‡ response	Significance§	Significance‖
Control		3	99 ± 1	n.s.	
ACSF		6	95 ± 4	n.s.	
Kynurenate	1	3	80 ± 5	$P<0.001$	$P<0.01$
+ Glutamate	1	3	91 ± 4	$P<0.05$	$P<0.01$
+ Glutamate	10	3	97 ± 8	n.s.	n.s.
Glutamate	1	3	99 ± 3	n.s.	n.s.
Glutamate	10	4	99 ± 6	n.s.	n.s.

*Drugs infused in artificial cerebrospinal fluid (200 nl).
†Number of individual rats.
‡Data reported as the mean and S.D.
§Significance compared to pre-infusion response amplitude with the *t*-test.
‖Significance compared to aCSF by one-way ANOVA followed by Duncan's test (n.s., not significant)

on both the resting (Table 1) and evoked (Table 3) signals, which is consistent with the ability of glutamate to attenuate the effect of kynurenate on electrically evoked dopamine release in striatal slices.[59] From these observations, we conclude that both the spontaneous and evoked release of dopamine is regulated by glutamate receptors within the striatum that are normally occupied by glutamate under resting conditions.

Evidence that glutamate receptors within the striatum are occupied by endogenous glutamate implies the presence of neuronally derived glutamate in the extracellular space of the striatum under basal conditions. The existence of neuronally derived extracellular glutamate has not been clearly established by microdialysis studies, however, since the basal level of glutamate in striatal microdialysate is reported to be insensitive to reduced calcium and to tetrodotoxin.[17,18,46,49,56] Nevertheless, we recently reported that the resting signal observed at glutamate microsensors in the striatum of the chloral hydrate-anesthetized rat is tetrodotoxin-sensitive.[29,30] This observation suggests the presence of impulse-dependent glutamate in the extracellular space of the striatum and lends support to the interpretation of the results of the present study.

An intriguing aspect of these results is that kynurenate apparently caused the resting extracellular dopamine concentration to decrease by several hundred nanomolar (Table 1). This is surprising in light of several determinations of basal extracellular dopamine concentration by the no-net-flux methods of microdialysis that suggest resting extracellular dopamine levels in striatum are in the low-nanomolar range,[9,14,21,50,51] which is below the magnitude of the decrease we observe in response to kynurenate infusion. However, our recent studies with combined voltammetry and microdialysis suggest that no-net-flux microdialysis may underestimate basal extracellular dopamine.[34,44,61] Consistent with the prior estimates of Lindefors *et al.*[32] and of Benveniste and Hanson,[2] our recent findings suggest that basal extracellular dopamine concentrations are in the low micromolar range.[60] The finding of the present study that intrastriatal infusion of kynurenate causes a several hundred nanomolar decrease in extracellular dopamine is consistent with these alternative, although higher, estimates of resting extracellular dopamine levels.

The findings of the present study are consistent with previous *in vitro* and *in vivo* studies,[6,25,33,59] which indicate that dopamine terminals are regulated by glutamate via mechanisms acting locally within the striatum itself. The present *in vivo* study, however, provides evidence to suggest that endogenous glutamate can exert a tonic excitatory action on dopamine release. In contrast, although we have previously observed excitatory effects of exogenous glutamate agonists, the apparent impact of endogenous glutamate has always been inhibitory. The dual nature of the impact of glutamate on dopamine release, which has been noted before,[7,31] suggests that the regulation of dopamine by glutamate is complex and may involve multiple mechanisms and perhaps different time-courses as well. The exact experimental conditions that cause the excitatory and inhibitory mechanisms to prevail have yet to be fully resolved.

The results of this study clearly reveal that resting and evoked dopamine release in the striatum is regulated by ionotropic glutamate receptors within the striatum. By themselves, however, the results of the present study do not permit the mechanisms underlying that regulation to be described. A simple explanation for the results of this study would be that ionotropic glutamate receptors exist on striatal dopamine terminals. Ultrastructural studies, however, suggest that relatively few glutamate receptors are present on dopamine terminals.[16] This leaves open the possibility that the regulation of dopamine by glutamate is mediated by other elements of the striatum. In ongoing studies, for example, we are examining the hypothesis that GABA may mediate interactions between dopamine and glutamate in the striatum.[26] The possibility also exists that extrastriatal loops may be involved, even though the glutamate receptors themselves reside in the striatum, as revealed by this work.

CONCLUSION

Interactions between central dopaminergic and glutamatergic systems are heavily implicated in several human disease states such as Parkinson's disease,[4] schizophrenia,[4,15,57] and substance abuse.[58] Hence, there has been long-standing interest in understanding the complex nature of these interactions, which may occur at multiple loci and appear to have a dual excitatory–inhibitory nature. Some of the recent literature on the topic of these interactions leads to the consensus that they do not involve mechanisms acting locally within the striatum: intrastriatal delivery of glutamate and glutamate agonists, for example, does not excite physiologically relevant dopamine release. Nevertheless, the findings of the present study, and related recent reports,[29,30,59] suggest that the effects of exogenously applied agonists may be masked by an extensive tonic occupation of glutamate receptors by endogenous glutamate, even under resting conditions in the anesthetized rat. We conclude, therefore, that interactions occurring locally within the striatum are a functional component of the interactions between central dopaminergic and glutamatergic systems.

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REFERENCES

1 Baur J., Kristensen E. W., May L. J., Wiedemann D. J. and Wightman R. M. (1988) Fast-scan voltammetry of biogenic amines. *Analyt. Chem.* **60,** 1268–1272.

2 Benveniste H. and Hansen A. J. (1991) Practical aspects of using microdialysis for determination of brain interstitial concentrations. In *Techniques in the Behavioral and Neural Sciences. Microdialysis in the Neurosciences* (eds Robinson T. E. and Justice J. B.), Vol. 7. Elsevier, Amsterdam.

3 Bouyer J. J., Park D. H., Joh T. H. and Pickel V. M. (1984) Chemical and structural analysis of the relation between cortical inputs and tyrosine hydroxylase-containing terminals in rat neostriatum. *Brain Res.* **302,** 267–275.
4 Carlsson M. and Carlsson A. (1990) Interactions between glutamatergic and monoaminergic systems within the basal ganglia-implications for schizophrenia and Parkinson's disease. *Trends Neurosci.* **13,** 272–276.
5 Carrozza D. P., Ferraro T. N., Golden G. T., Reyes P. F. and Hare T. A. (1992) *In vivo* modulation of excitatory amino acid receptors: microdialysis studies on *N*-methyl-D-aspartate-evoked striatal dopamine release and effects of antagonists. *Brain Res.* **574,** 42–48.
6 Castro S. L., Sved A. F. and Zigmond M. J. (1996) Increased neostriatal tyrosine hydroxylation during stress: role of extracellular dopamine and excitatory amino acids. *J. Neurochem.* **66,** 824–833.
7 Cheramy A., Romo R., Godeheu G., Baruch P. and Glowinski J. (1986) *In vivo* presynaptic control of dopamine release in the cat caudate nucleus-II. Facilitatory or inhibitory influence of L-glutamate. *Neuroscience* **19,** 1081–1090.
8 Clow D. W. and Jhamandas K. (1988) Characterization of L-glutamate action on the release of endogenous dopamine from the rat caudate–putamen. *J. Pharmac. exp. Ther.* **248,** 722–728.
9 Crippens D., Camp D. M. and Robinson T. E. (1993) Basal extracellular dopamine in the nucleus accumbens during amphetamine withdrawal: a 'no-net flux' microdialysis study. *Neurosci. Lett.* **164,** 145–148.
10 Freund T. F., Powell J. F. and Smith A. D. (1984) Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striato-nigral neurons, with particular reference to dendritic spines. *Neuroscience* **13,** 1189–1215.
11 Ewing A. G., Bigelow J. C. and Wightman R. M. (1983) Direct *in vivo* monitoring of dopamine from two striatal compartments in the rat. *Science* **221,** 169–171.
12 Gariano R. F. and Groves P. M. (1988) Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. *Brain Res.* **462,** 194–198.
13 Giorguiff M. F., Kemel M. L. and Glowinski J. (1977) Presynaptic effect of L-glutamic acid on dopamine release in rat striatal slices. *Neurosci. Lett.* **6,** 77–78.
14 Glick S. D., Dong N., Keller R. W. Jr and Carlson J. N. (1994) Estimating extracellular concentrations of dopamine and 3,4-dihydroxyphenylacetic acid in nucleus accumbens and striatum using microdialysis: relationships between *in vitro* and *in vivo* recoveries. *J. Neurochem.* **62,** 2017–2021.
15 Grace A. A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* **41,** 1–24.
16 Gracy K. N. and Pickel V. M. (1996) Ultastructural immunocytochemical localization of the *N*-methyl-D-aspartate receptors and tyrosine hydroxylase in the shell of the rat nucleus accumbens. *Brain Res.* **739,** 169–181.
17 Gray A. M., Rawls S. M., Shippenberg T. S. and McGinty J. F. (1999) The K-opioid agonist, U-69593, decreases acute amphetamine-avoked bahaviors and calcium-dependent dialysate levels of dopamine and glutamate in the ventral striatum. *J. Neurochem.* **73,** 1066–1107.
18 Herrera-Marschitz M., You Z.-B, Goiny M., Meana J. J., Silveira R., Godukhin O. V., Chen Y., Espinoza S., Pettersson E., Loidl C. F., Lubec G., Andersson K., Nylander I., Terenius L. and Ungerstedt U. (1996) On the origin of extracellular glutamate levels monitored in the basal ganglia of the rat by *in vivo* microdialysis. *J. Neurochem.* **66,** 1726–1735.
19 Iravani M. M. and Kruk Z. L. (1996) Real-time effects of *N*-methyl-D-aspartic acid on dopamine release in slices of rat caudate–putamen: a study using fast cyclic voltammetry. *J. Neurochem.* **66,** 1076–1085.
20 Jin S. and Fredholm B. B. (1997) Electrically-evoked dopamine and acetylcholine release from rat striatal slices perfused without magnesium: regulation by glutamate acting on NMDA receptors, *Br. J. Pharmac.* 1269–1276.
21 Kalivas P. W. and Duffy P. (1993) Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. *J. Neurosci.* **13,** 266–275.
22 Kalivas P. W., Duffy P. and Barrow J. (1989) Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. *J. Pharmac. exp. Ther.* **251,** 378–386.

39 Moghaddam B. and Gruen R. J. (1991) Do endogenous excitatory amino acids influence striatal dopamine release? *Brain Res.* **544,** 329–330.
40 Moghaddam B., Gruen R. J., Roth R. H., Bunney B. S. and Adams R. N. (1990) Effect of L-glutamate on the release of striatal dopamine: *in vivo* dialysis and electrochemical studies. *Brain Res.* **518,** 55–60.
41 Moore R. Y. and Bloom F. E. (1978) Central catecholamine neuron systems: anatomy and physiology of the dopamine systems, *Ann. Rev. Neurosci.* 129–169.
42 Murase S., Grenhoff J., Chouvet G., Fonon F. G. and Svensson T. H. (1993) Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studies *in vivo*. *Neurosci. Lett.* **157,** 53–56.
43 Pellegrino L. J., Pellegino A. S. and Cushman A. J. (1979) *A Stereotaxic Atlas of the Rat Brain*, 2nd edn. Plenum, New York.
44 Peters J. L., Yang H. and Michael A. C. (2000) Quantitative aspects of brain microdialysis. *Anal. Chim. Acta* **412,** 1–12.
45 Segovia G., Del Arco A. and Mora F. (1997) Endogenous glutamate increases extracellular concentrations of dopamine, GABA, and taurine through NMDA and AMPA/kainate receptors in striatum of the freely moving rat: a microdialysis study. *J. Neurochem.* **69,** 1476–1483.
46 Semba J., Kito S. and Toru M. (1995) Characterization of extracellular amino acids in striatum of freely moving rats by *in vivo* microdialysis. *J. neural Transm.* **100,** 3–52.
47 Sesack S. R., Deutch A. Y., Roth R. H. and Bunney B. S. (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *Phaseolus vulgaris* leucoagglutinin. *J. comp. Neurol.* **290,** 213–242.
48 Sesack S. R. and Pickel V. M. (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J. comp. Neurol.* **320,** 145–160.
49 Shiraishi M., Kamiyama Y., Huttemeir P. C. and Benveniste H. (1997) Extracellular glutamate and dopamine measured by microdialysis in the rat striatum during blockade of synaptic transmission in anesthetized and awake rats. *Brain Res.* **759,** 221–227.
50 Smith A. D. and Justice J. B. Jr. (1994) The effect of inhibition of synthesis, release, metabolism and uptake on the microdialysis extraction fraction of dopamine. *J. Neurosci. Meth.* **54,** 75–82.
51 Smith A. D. and Weiss F. (1999) Ethanol exposure differentially alters central monoamine neurotransmission in alcohol-preferring versus - nonpreferring rats. *J. Pharmac. exp. Ther.* **288,** 1223–1228.
52 Stamford J. A., Kruk Z. L. and Millar J. (1988) Stimulated limbic and striatal dopamine release measured by fast cyclic voltammetry: anatomical, electrochemical and pharmacological characterization. *Brain Res.* **454,** 282–288.
53 Suaud-Chagny M. F., Chergui K., Chouvet G. and Gonon F. (1992) Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local *in vivo* application of amino acids in the ventral tegmental area. *Neuroscience* **49,** 63–72.
54 Svensson L., Zhang J., Johannessen K. and Engel J. A. (1994) Effect of local infusion of glutamate analogues into the nucleus accumbens of rats: an electrochemical and behavioral study. *Brain Res.* **643,** 155–161.
55 Taber M. T. and Fibiger H. C. (1995) Electrical stimulation of the prefrontal cortex increases dopamine release in the nucleus accumbens of the rat: modulation by metabotropic glutamate receptors. *J. Neurosci.* **15,** 3896–3904.
56 Timmerman W., Cisci G., DeVries J. B. and Westerink B. H. C. (1999) Effects of handling in extracellular levels of glutamate and other amino acids in various areas of the brain measured by microdialysis. *Brain Res.* **833,** 150–160.
57 Weinberger D. R., Berman K. F. and Zec R. F. (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. *Archs gen. Psychiat.* **43,** 114–124.
58 Wolf M. E. (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog. Neurobiol.* **54,** 67–720.
59 Wu Y., Pearl S. M., Zigmond M. J. and Michael A. C. (2000) Inhibitory glutamatergic regulation of evoked dopamine release in striatum. *Neuroscience* **96,** 65–72.
60 Yang H., Qian J. and Michael A.C. (2000) Striatal extracellular dopamine: A study by microdialysis with *in vivo* probe calibration. *J. Neurosci. Meth.* (submitted).
61 Yang H., Peters J. L. and Michael A. C. (1998) Coupled effects of mass transfer and uptake kinetics on *in vivo* microdialysis of dopamine. *J. Neurochem.* **71,** 684–692.
62 Youngren K. D., Daly D. A. and Moghaddam B. (1993) Distinct actions of endogenous excitatory amino acids on the outflow of dopamine in the nucleus accumbens. *J. Pharmac. exp. Ther.* **264,** 289–293.

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