Prolonged and Extrasynaptic Excitatory Action of Dopamine Mediated by D1 Receptors in the Rat Striatum *In Vivo*

François Gonon

Centre National de la Recherche Scientifique UMR 5541, Laboratoire d'Histologie-Embryologie, UFR 2, Université Bordeaux II, 33076 Bordeaux, France

The spatiotemporal characteristics of the dopaminergic transmission mediated by D1 receptors were investigated *in vivo*. For this purpose dopamine (DA) release was evoked in the striatum of anesthetized rats by train electrical stimulations of the medial forebrain bundle (one to four pulses at 15 Hz), which mimicked the spontaneous activity of dopaminergic neurons. The resulting dopamine overflow was electrochemically monitored in real time in the extracellular space. This evoked DA release induced a delayed increase in discharge activity in a subpopulation of single striatal neurons. This excitation was attributable to stimulation of D1 receptors by released DA because it was abolished by acute 6-hydroxydopamine lesion and strongly reduced by the D1 antagonist SCH 23390. Striatal neurons exhibiting this delayed response were also strongly excited by intravenous administration of the D1 agonist SKF

82958. Whereas the DA overflow was closely time-correlated with stimulation, the excitatory response mediated by DA started 200 msec after release and lasted for up to 1 sec. Moreover, functional evidence presented here combined with previous morphological data show that D1 receptors are stimulated by DA diffusing up to 12 μ m away from release sites in the extrasynaptic extracellular space. In conclusion, DA released by bursts of action potentials exerts, via D1 receptors, a delayed and prolonged excitatory influence on target neurons. This phasic transmission occurs outside synaptic clefts but still exhibits a high degree of spatial specificity.

Key words: dopamine; release; D1 receptor; G-protein-coupled receptor; striatum; *in vivo* electrochemistry; electrophysiology; synaptic cleft; diffusion in the extracellular space

Fast neurotransmission is achieved within a few milliseconds by the release of a neurotransmitter and its action inside synaptic clefts on receptors coupled to ligand-gated ion channels. *In vitro* studies in mammalian brain show that neurotransmission involving G-protein-coupled receptors is much slower (Cole and Nicoll, 1984; Surprenant and Williams, 1987; Hille, 1992; Isaacson et al., 1993; Batchelor et al., 1994) and might occur outside synaptic clefts (Hille, 1992; Isaacson et al., 1993; Batchelor et al., 1994). To my knowledge, these temporal characteristics have never been investigated *in vivo* and have not yet been described regarding dopaminergic transmission. Dopaminergic receptors of the D1 type are coupled with G-proteins (Hille, 1992) and are involved in the excitatory influence of endogenous dopamine (DA) on the *in vivo* activity of target neurons in the rat striatal complex (Young et al., 1991; Haracz et al., 1993; Chergui et al., 1996; Gonon and Sundström, 1996; Moratalla et al., 1996). Here I describe a new experimental model that shows in real time the excitatory effect of DA released by impulse flow on the discharge activity of target neurons. This model was used to describe the spatiotemporal characteristics of the dopaminergic transmission mediated by D1 receptors.

Dopaminergic neuronal cell bodies exhibit two kinds of discharge activity: single spikes and bursts of two to six action potentials (mean, 2.9) (Grace and Bunney, 1984; Freeman and Bunney, 1987).

Individual dopaminergic neurons can switch from one pattern to another, and sensory stimuli favor the bursting pattern in unrestrained rats (Freeman and Bunney, 1987). In monkeys dopaminergic neurons respond to an appetitive stimulus by a burst (Mireniewicz and Schultz, 1996). In a previous *in vivo* study we recorded in target areas the DA overflow (i.e., the changes in the extracellular DA concentration) evoked by electrical stimulation mimicking both discharge patterns. We showed that bursting stimulations (four pulses at 15 Hz) induced a larger DA overflow than stimulations with the same number of pulses at a lower frequency (Chergui et al., 1994). Therefore, these train stimulations (one to four pulses at 15 Hz) were used here to investigate the effect of synaptic DA release on the activity of target neurons in the striatum.

To delineate *in vivo* the temporal characteristics of the dopaminergic transmission, its pre- and postsynaptic sides, i.e., the DA release and the discharge response of target neurons, were recorded sequentially in every experiment with a millisecond time resolution. The electrically evoked DA overflow in the extracellular space was electrochemically monitored with a carbon fiber microelectrode, and the discharge activity of single striatal neurons was extracellularly monitored. Regarding the spatial characteristics of this transmission, morphological studies have shown that most D1 receptors are located outside synaptic contact formed by dopaminergic terminal fibers on target neurons (Levey et al., 1993; Caillé et al., 1996). *In vivo* experiments were conducted here to determine whether these extrasynaptic receptors play a functional role.

MATERIALS AND METHODS

Male Wistar rats (300–350 gm) were anesthetized with urethane (1.15 gm/kg) and fixed on a stereotaxic frame. Electrical stimulations of the

Received April 22, 1997; accepted May 14, 1997.

This work was supported by funds from "La région Aquitaine." I thank B. Bloch, K. Chergui, D. Choquet, M. Jaber, C. Mülle, and P. Svenningsson for helpful discussions.

Correspondence should be addressed to Dr. F. Gonon, Centre National de la Recherche Scientifique UMR 5541, Laboratoire d'Histologie-Embryologie, UFR 2, Université Bordeaux II, 33076 Bordeaux, France.

Copyright © 1997 Society for Neuroscience 0270-6474/97/175972-07\$05.00/0

Figure 1. Excitatory effects of MFB electrical stimulations on the discharge activity of single striatal neurons. Electrical stimulation consisting of four pulses at 15 Hz induced in distinct neuronal populations either (*a*) delayed excitations or (*b*) spike discharges closely linked to every stimulation pulse. The figure shows direct extracellular recordings with representative spike discharges.

medial forebrain bundle (MFB) were applied as described (Chergui et al., 1994; Dugast et al., 1994). The depth of the stimulating electrode was adjusted for every experiment to maximize the DA overflow (Dugast et al., 1994); the highest and lowest effective placements were found at 8.1 and 8.9 mm, respectively, below the cortical surface. This overflow was monitored continuously with amperometry by a carbon fiber electrode, the active part of which is the surface of one carbon fiber 8 μm in diameter and 250 μm long (Chergui et al., 1994; Dugast et al., 1994). Despite the poor selectivity of continuous amperometry to DA versus other oxidizable compounds, we showed that the rapid changes in the oxidation signal evoked by MFB electrical stimulation were caused entirely by the evoked DA overflow (Chergui et al., 1994; Dugast et al., 1994). This electrode was implanted in the striatum with the stereotaxic coordinates 2.7 to 3.1 mm lateral to medial line, 0.9 to 1.3 mm rostral to bregma, and 3.5 to 5.5 mm below the cortical surface. Electrical artifacts evoked by the stimulation pulses were subtracted (Chergui et al., 1994; Dugast et al., 1994). To improve the signal-to-noise ratio, a series of 16 or 20 individual DA overflows were averaged, because when they were evoked every 15 or 20 sec their amplitude remained stable (Chergui et al., 1994; Dugast et al., 1994). DA overflow was estimated as changes in DA concentration on the basis of *in vitro* calibration of the carbon fiber electrode performed after *in vivo* measurement (Dugast et al., 1994).

The discharge activity of single striatal neurons was recorded extracellularly as described (Gonon and Sundström, 1996) with carbon fiber electrodes, the active surface of which was one carbon fiber with a conic shape 8 μm in diameter and 20 μm long (impedance, 6–10 MΩ). The recording electrode was implanted in the striatum at the same coordinates as above. Voltage signals were amplified, bandpass-filtered (300 Hz to 10 kHz), and digitized at 20 kHz as shown in Figure 1. Single spike discharges were isolated by a window discriminator (World Precision Instruments, Sarasota, FL) and recorded using a data acquisition system (CED) running "Spike2" software. Whereas most striatal neurons exhibited a low and very irregular basal activity, some others were tonically active and more regular; these were not considered in this study. Striatal

a: Spikes per bin

b: DA overflow

Figure 2. Effect of acute chemical lesioning of the dopaminergic axons on the evoked DA overflow and the delayed response of striatal neurons to MFB electrical stimulation. Striatal neurons responding to MFB electrical stimulations (four pulses at 15 Hz) with an obvious delayed excitation were identified according to criteria defined in Materials and Methods. In *a*, the responses of three distinct neurons to 20 consecutive stimulations applied every 20 sec are shown as peristimulus time histograms (bin width, 0.1 sec). These three neurons were recorded in a control animal during the same electrode penetration in the striatum. The effect of 6-OHDA injections in the vicinity of the stimulating electrode is shown in *b*. Stimulation with 10 pulses at 40 Hz induced a large DA overflow in the striatum. One hour after 6-OHDA injection the overflow induced by the same stimulation was reduced by >90%. After such an acute chemical lesioning of the dopaminergic axons, the number of striatal neurons exhibiting a delayed excitatory response to MFB stimulation was reduced drastically (see Results).

neurons were identified as excited by DA overflow on the basis of their peristimulus time histograms (bin width, 0.1 sec) constructed from their spike responses to 20 consecutive train stimulations (four pulses at 15 Hz) applied every 20 sec (Fig. 2*a*). These histograms must fulfill the following criteria: the largest bin must be observed between +0.2 and +0.7 sec after the first stimulus, and its amplitude must be at least twofold higher than that of the largest bin recorded during the 10 sec before stimulation and at least five times larger than the averaged bin size recorded during the same control period.

To lesion the dopaminergic axons, a glass pipette (external tip diameter, 35 μm) filled with 6-hydroxydopamine (6-OHDA) (Sigma, St. Louis, MO) (2 μg/μl in a saline solution containing ascorbic acid 1 mM) was implanted in the vicinity of the stimulating electrode. Three injections (0.5 μl, 90 sec each) were made 0.3 mm above, at the level of, and 0.3 mm below the tip of the stimulating electrode. The effectiveness of this lesion was assessed in each experiment by monitoring every 5 min the DA overflow evoked by a strong stimulation (10 pulses at 40 Hz) before and for 1 hr after injections.

Figure 3. Effect of intravenous administration of a D1 agonist on the discharge activity of selected striatal neurons. Fourteen single striatal neurons were selected for their obvious delayed excitatory response to MFB stimulation (see Fig. 2). Their discharge activity was recorded for 7 min, and the D1 agonist SKF 82958 was administered (50 μ g/kg, i.v.) after a control period lasting for 200 sec. The figure shows a representative recording. In 13 neurons, this drug treatment increased the number of spike discharges recorded during the 200 sec after administration from +89% to +4483% (mean +1142%) compared with that recorded during the control period. One neuron was not affected.

The full D1 dopaminergic agonist SKF 82958 (Andersen and Jansen, 1990) (RBI, Natick, MA) can be detected electrochemically by a carbon fiber electrode because it oxidizes at +100 mV versus the Ag/AgCl reference electrode. Following the same procedure as described previously regarding apomorphine (Marcenac and Gonon, 1985), I monitored the extracellular SKF 82958 concentration resulting from its intravenous administration in the frontal cortex by means of an electrochemically treated carbon fiber electrode combined with differential normal pulse voltametry. This SKF 82958 oxidation peak cannot be recorded in the striatum because it is obscured by the large endogenous oxidation peak corresponding to the main DA metabolite 3,4-dihydroxyphenyl-acetic acid (Marcenac and Gonon, 1985). The D1 antagonist SCH 23390 (RBI) was dissolved as described previously (Gonon and Sundström, 1996) and administered subcutaneously, whereas SKF 82958 was dissolved in physiological saline solution and administered via the jugular vein.

RESULTS

Striatal neurons considered in this study exhibited a low and very irregular discharge activity. Electrical stimulation of the MFB with four pulses at 15 Hz evoked, in distinct neuronal populations, two types of excitatory effects: either a delayed excitation (Fig. 1*a*) or an early response consisting of single spikes closely time-correlated with every stimulation pulse (Fig. 1*b*). The delayed excitation started after the last stimulation pulse and lasted for up to 1 sec (Fig. 1*a*). Similar reproducible delayed excitations that fulfilled criteria defined in Materials and Methods were observed in 125 neurons. Their basal activity was low and irregular (mean, 0.90 Hz; range, from 0 to 6.8 Hz; $n = 125$). During vertical penetrations in the striatum by steps of 15 μ m for 2 mm, the number of neurons exhibiting this delayed response was found to be 2.86 ± 0.90 (mean $\pm$ SD; 24 determinations in 12 animals) (Fig. 2*a*); however, when the DA overflow was almost completely abolished (>90% decrease) by acute injection of 6-OHDA in the MFB (Fig. 2*b*), only 0.27 ± 0.20 (mean $\pm$ SD; 14 determinations in seven animals) neurons per track were found. The difference between these numbers is statistically significant (unpaired t test; $p < 0.0001$).

Striatal neurons exhibiting this delayed excitation were also strongly excited by intravenous administration of the full D1 agonist SKF 82958 (50 μ g/kg) (Fig. 3). This delayed excitation was strongly

Figure 4. Kinetics of the DA overflow and of the delayed excitatory response evoked by MFB electrical stimulations before and after administration of a D1 antagonist. The DA overflow evoked by MFB electrical stimulations (four pulses at 15 Hz every 20 sec) was electrochemically monitored in 15 distinct rats. The figure shows a representative recording. Notice that DA was quickly eliminated after the last stimulus with a half-life of 74 ± 13 msec (mean $\pm$ SD). The delayed excitatory response of 15 single striatal neurons to 20 consecutive stimulations (four pulses at 15 Hz every 20 sec) was then recorded in these animals before and 20 min after administration of the D1 dopaminergic antagonist SCH 23390 (0.5 mg/kg, s.c.). The two peristimulus time histograms shown in *b* were calculated by averaging the 15 individual histograms in both groups. Error bars represent SEM. Stars indicate statistically significant differences between bin sizes observed before and after SCH 23390 administration (paired t test; $p \leq 0.0004$).

reduced when the D1 dopaminergic antagonist SCH 23390 (0.5 mg/kg, s.c.) was administered to the animal (Fig. 4), whereas this treatment did not affect the DA overflow evoked by four pulse stimulation (three experiments). Accordingly, with use of another electrochemical approach we showed that SCH 23390 administration does not affect the evoked DA overflow (Suaud-Chagny et al., 1991).

In contrast, the early excitatory response (Fig. 1*b*) was still often observed after 6-OHDA lesion and was not affected by SCH 23390 administration (four neurons tested). Moreover, neurons exhibiting this early excitation were not excited by intravenous administration of the D1 agonist SKF 82958 at the same dose (five neurons tested). Apart from spike responses evoked by MFB stimulation, these neurons were usually completely silent. Tonic active neurons exhibiting a regular discharge activity were not considered in this study because MFB stimulation evoked in these neurons a

Figure 5. Correlation between the amplitude of the DA overflow and that of the delayed excitatory response. DA overflow evoked by MFB stimulations (one to four pulses at 15 Hz applied every 15 sec) was electrochemically monitored. The four curves shown on the *left* show one representative experiment and were obtained by averaging the DA overflow evoked by 16 stimulations of each type. Thereafter, 20 series of four stimulations (one to four pulses every 15 sec) were applied every 1 min, and the delayed excitatory responses of a single striatal neuron were recorded. Four peristimulus time histograms obtained from the same experiment are shown on the *right*. The *bottom* part of the figure summarizes the results obtained from 21 experiments conducted in distinct animals (mean $\pm$ SD). The DA overflow was quantified by measuring the maximal amplitude and the area below the curve in each individual experiment. The amplitude of the spike response corresponded for each experiment to the number of spikes recorded from +0.2 to +0.7 sec after the beginning of each stimulation minus the basal activity for 0.5 sec calculated from the mean discharge activity observed during the 5 sec preceding each stimulation. Both DA overflow and spike response are expressed in percentages of those observed after four pulse stimulations.

complex response (excitation followed by inhibition) that was not altered by 6-OHDA lesion or by SCH 23390 administration.

Train MFB stimulations consisting of one to four pulses at 15 Hz evoked a DA overflow that was closely time-correlated with the stimulation because DA is rapidly cleared with a half-life of 74 msec (Figs. 4 and 5). In contrast the response of target neurons

mediated by D1 receptors was delayed and prolonged. It reached its maximum 394 ± 85 msec (mean $\pm$ SD; 125 neurons) after the first stimulus and lasted for up to 1 sec (see Figs. 1, 2, 4, and 5). Both DA overflow and delayed excitation were enhanced by increasing the number of stimulation pulses from one to four (Fig. 5). More precisely, the amplitude of the DA excitatory response and that of the DA overflow, when it is estimated in terms of area (concentration multiplied by time), were closely correlated and grew linearly with the number of pulses (Fig. 5); however, the maximal amplitude of the DA overflow (i.e., maximal extracellular DA concentration) was not linearly related to the number of pulses (Fig. 5).

The DA overflow was estimated as changes in DA concentration on the basis of *in vitro* calibration of the carbon fiber electrode after *in vivo* measurement. In the experiments illustrated in Figures 4 and 5, the DA overflow evoked by four pulses at 15 Hz corresponded to a maximal increase in DA concentration of 0.57 ± 0.21 μ M (mean $\pm$ SD; 21 determinations). The extracellular concentration of SKF 82958 resulting from its intravenous administration was electrochemically monitored in the frontal cortex. Doses of 1 and 2 mg/kg were necessary to observe a detectable oxidation peak caused by SKF 82958, the amplitude of which was linearly dose dependent (data not shown). *In vitro* calibration of the electrode after *in vivo* measurement for increasing SKF 82958 concentrations was used to estimate the *in vivo* concentration. The maximal concentration observed after an intravenous injection of 1 mg/kg was found to be 56 ± 13 nM (mean $\pm$ SD; four experiments). Assuming that the extracellular concentration of SKF 82958 was also linearly related to the dose administered from 0 to 1 mg/kg, it can be calculated that a dose of 50 μ g/kg induced a maximal SKF 82958 concentration of 2.8 nM in the brain extracellular fluid.

The DA overflow evoked by a single pulse stimulation is dramatically prolonged by pharmacological inhibition of the DA uptake (Fig. 6*a*). The DA half-life, i.e., the time for 50% decrease from the maximum, was 55 ± 28 msec (mean $\pm$ SD; five experiments) before nomifensine administration; however, 20 min after this drug treatment, the DA half-life was maximally enhanced up to 192 ± 62 msec (mean $\pm$ SD; $n = 5$). The differences in half-life were statistically significant (paired *t* test; $p < 0.003$). The maximal amplitude of the DA overflow was enhanced to a smaller extent by nomifensine ($+92 \pm 62\%$; mean $\pm$ SD; $n = 5$).

At a distance r from a release site, the DA concentration resulting from DA diffusion in the extracellular space after exocytosis of a single vesicle has been calculated (Fig. 6*b,c*) according to equation 13 of Nicholson's study (Nicholson, 1985), with the additional assumption that the vesicle content corresponds to 1800 DA molecules (Pothos et al., 1996). Values concerning DA diffusion coefficient, extracellular volume fraction, and tortuosity were as reported (Nicholson, 1995). This concentration reaches a maximum at a time t_m (Fig. 6*c*), which depends on the square of the distance r (Nicholson, 1985). According to this estimate, DA diffusion at 4 and 12 μ m lasts 9 and 74 msec, respectively (Fig. 6*c*).

DISCUSSION

Excitatory effects induced by released DA via D1 receptors

The effect of DA on target neurons is still a matter of debate. Several recent studies using various *in vivo* approaches suggest that the release of endogenous DA exerts, via D1 dopaminergic receptors, an excitatory influence on target neurons in the striatal

Figure 6. Kinetics of DA diffusion in the extracellular space. *a*, Single pulse stimulation of the MFB was applied every 15 sec for 45 min. The DA uptake inhibitor nomifensine (20 mg/kg, s.c.) was administered 15 min after the first stimulation. The resulting DA overflow was electrochemically recorded and averaged by groups of 20 consecutive stimulations. The figure shows the averaged DA overflow recorded before drug administration and that recorded 20 min after, i.e., when the drug effect is maximal (Suaud-Chagny et al., 1995). *b*, This drawing summarizes morphological data concerning the localization of D1 dopaminergic receptors (Levey et al., 1993; Caillé et al., 1996). Notice that synaptic clefts represent a minute fraction of the whole extracellular space that occupies 21% of the brain volume (Nicholson, 1985, 1995). *c*, The left curve represents the DA concentration at a receptor site resulting from the exocytosis of only one DA vesicle in a release site located at a distance r . The DA concentration reaches its maximum at a time t_m , which depends on the square of the distance (right curve).

complex (Young et al., 1991; Haracz et al., 1993; Chergui et al., 1996; Gonon and Sundström, 1996; Moratalla et al., 1996). According to studies using application of exogenous DA and D1 agonists, however, stimulation of D1 receptors either increases (Pierce and Rebec, 1995; Levine et al., 1996a,b; Wickens et al., 1996) or inhibits (Calabresi et al., 1987; Xu et al., 1994; Surmeier

et al., 1995; Nicola et al., 1996) the excitability of target neurons. Opposite effects have also been reported, depending on the DA concentration (Akaike et al., 1987; Hu and Wang, 1988). These contrasting results might be reconciled when the DA concentration and procedure of application are reconsidered (Williams and Millar, 1991; Gonon and Sundström, 1996; Wickens et al., 1996).

The present study further supports the view that stimulation of D1 receptors by released DA enhances the discharge probability of target neurons. In fact, three observations strongly suggest that the delayed excitatory response observed here was actually caused by the evoked DA overflow via D1 receptors. First, dopaminergic fibers were involved in this delayed response because it was almost totally abolished when these fibers were specifically lesioned; second, this delayed response was strongly reduced by administration of the D1 antagonist SCH 23390; and third, striatal neurons exhibiting this delayed response were also strongly excited by administration of the full D1 agonist SKF 82958. The specificity of these approaches toward the dopaminergic transmission mediated by D1 receptors is further documented here. In fact, the early excitatory response evoked in another population of striatal neurons by MFB stimulation was not altered by 6-OHDA lesions and administration of the D1 antagonist. Moreover, these neurons were not excited by administration of the D1 agonist. The present conclusion that stimulation of D1 receptors by released DA excites target neurons does not imply that DA is capable of triggering discharge activity on its own. As suggested by several *in vivo* and *in vitro* studies, DA instead may facilitate the activity of striatal neurons receiving other excitatory inputs (Haracz et al., 1993; Pierce and Rebec, 1995; Gonon and Sundström, 1996; Levine et al., 1996a,b).

Kinetics of the dopaminergic transmission mediated by D1 receptors

Since 1967, many *in vivo* electrophysiological studies have investigated the effects of electrical stimulation of the dopaminergic nigrostriatal pathway on striatal neurons (Frigyesi and Purpura, 1967; Siggins, 1978; Wilson et al., 1982). None of the observed effects, however, have been unequivocally attributed to the evoked DA release (Siggins, 1978; Wilson et al., 1982). In 1978 Siggins pointed out the main difficulty concerning these studies: "selective activation of the DA system should reveal long response latencies." These expected latencies should be attributable to the slow conduction velocity of the dopaminergic axons and to "the additional time required to activate adenylate cyclase" after stimulation of the dopaminergic receptors (Siggins, 1978; Hille, 1992).

Here the latency of the postsynaptic response to DA was shown for the first time. In fact, the DA overflow evoked by MFB stimulation was closely time-correlated with the stimulation, because released DA is rapidly cleared from the extracellular fluid by neuronal reuptake (Suaud-Chagny et al., 1995). In contrast, the response of target neurons to this DA overflow was delayed and prolonged. It could be hypothesized that this latency might be the consequence of intercellular delays. Released DA might activate presynaptic D1 heteroreceptors located on terminals using other neurotransmitters. Morphological studies, however, rule out this presynaptic hypothesis. In the striatum, "D1 immunoreactive axon terminals were exceedingly rare" (Hersch et al., 1995; Caillé et al., 1996). Moreover, more complex intercellular delays involving tonically active striatal neurons cannot be responsible for this latency because MFB stimulation evoked in these neurons a complex response that was not altered by blockage of D1 receptors.

The kinetics of the delayed excitation observed here is very similar to that observed *in vitro* concerning effects mediated by GABA_B, metabotropic glutamatergic, muscarinic, and adrenergic receptors, which are also coupled with G-proteins (Cole and Nicoll, 1984; Surprenant and Williams, 1987; Isaacson et al., 1993; Batchelor et al., 1994). Altogether it is highly likely that the kinetics of the delayed excitation observed here is governed mainly by the kinetics of the intracellular G-protein-mediated messenger systems into target neurons resulting from stimulation of D1 receptors.

Importance of bursts of action potentials

Dopaminergic neurons exhibit two kinds of discharge activity: single spikes and bursts of action potentials. Electrical stimulations of the MFB consisting of one to four pulses at 15 Hz were used here to mimic both discharge patterns. It was observed that train pulse stimulations evoked larger DA overflow and larger DA excitatory responses than single pulse stimulations. Similar observations concerning neurotransmissions involving other G-protein-coupled receptors have already been reported (Cole and Nicoll, 1984; Surprenant and Williams, 1987; Hille, 1992; Isaacson et al., 1993; Batchelor et al., 1994).

More precisely, the amplitude of the DA excitatory response was more closely correlated with the amplitude of the DA overflow when it is estimated in terms of area (concentration multiplied by time) than when the maximal DA overflow (i.e., maximal extracellular DA concentration) was considered. This suggests that a full response develops when D1 receptors are stimulated by DA for a sufficient duration (i.e., for up to 300 msec, as observed with the longest stimulation). This observation supports Hille's hypothesis of a "temporal summation" at G-protein-coupled receptors (Hille, 1992).

At rest, dopaminergic neurons discharge at a low frequency and mainly with the single spike mode, but sensory stimuli, and especially appetitive ones, often evoke a burst of two to six action potentials (Freeman and Bunney, 1987; Mireniewicz and Schultz, 1996). Rewarding stimuli induce sharp changes in extracellular DA (Richardson and Gratton, 1996). At rest the DA extracellular concentration is in the 10 nM range, but it is transiently enhanced by one order of magnitude when dopaminergic neurons are forced to discharge in bursts by chemical stimulation (Suaud-Chagny et al., 1992). In agreement with a previous study using another approach (Chergui et al., 1996), I show here that electrical stimulations mimicking bursts of action potentials are much more potent than single pulse stimulations in triggering DA overflow and evoking the D1-mediated response.

Geometry of the dopaminergic transmission mediated by D1 receptors

Most D1 dopaminergic receptors are located on striatal dendrites outside symmetric contacts formed by dopaminergic terminal fibers (Levey et al., 1993; Hersch et al., 1995; Caillé et al., 1996). These receptors are often located in the vicinity of asymmetric synapses formed by glutamatergic terminals on the head of dendritic spines (Levey et al., 1993; Caillé et al., 1996), whereas most dopaminergic terminal fibers form symmetric synapses on the neck of these spines (Groves et al., 1994). Three lines of evidence presented here show that these extrasynaptic receptors actually play a functional role.

First, considering the size of the carbon fiber electrode, it is obvious that it monitored the DA overflow in the extrasynaptic extracellular space. Therefore, the extrasynaptic hypothesis pre-

dicts that an excellent correlation between the measured DA overflow and the amplitude of the excitatory response must be observed. This expected correlation is shown here.

Second, this extrasynaptic hypothesis implies that D1 receptors can be stimulated by DA at the concentration range actually measured by the carbon fiber electrode during evoked DA overflow: 0.2 to 1 μ M. This estimate is in excellent agreement with *in vitro* observations concerning the affinity of D1 receptors for DA (Andersen and Jansen, 1990) and the efficacy of released DA for stimulating adenylate cyclase activity (Kelly and Nahorski, 1987). Moreover, excitatory effects were also induced in the same neurons by intravenous administration of the D1 agonist SKF 82958. Measurement of the SKF 82958 extracellular concentration shows that this drug exhibits a nanomolar *in vivo* efficacy. This is consistent with the fact that *in vitro* it exhibits a much better affinity than DA for D1 receptors (Andersen and Jansen, 1990).

Third, this hypothesis implies that DA can diffuse a few micrometers away from its release sites before elimination by reuptake. Morphological studies show that most DA reuptake sites are located on dopaminergic fibers outside synaptic contacts formed by dopaminergic terminals (Nirenberg et al., 1996). Functional evidence reported here and in a previous study (Garris et al., 1994a) actually shows that DA can diffuse outside synaptic cleft. In fact, inhibition of DA reuptake dramatically slowed down DA elimination but only moderately enhanced the maximal amplitude of the DA overflow evoked in the striatum by a single pulse stimulation (present study) or in the nucleus accumbens by a "pseudo-one-pulse" stimulation (Garris et al., 1994a). These observations show that released DA can invade the whole extracellular space before significant elimination. This is attributable to the fact that DA diffusion for 4 μ m in the extracellular fluid is much faster than DA clearance. Because dopaminergic terminal fibers densely innervate the striatum, the average distance between release sites is $\sim$ 4 μ m (Doucet et al., 1986; Groves et al., 1994). Moreover, at DA release sites the probability of release per action potential seems very high (Garris et al., 1994a). Therefore, this diffusion step, leading to homogenous DA concentration in the extrasynaptic extracellular space, might be achieved within 9 msec.

Functional evidence and morphological data consistently show that the dopaminergic transmission mediated by D1 receptors occurs outside synaptic clefts formed by dopaminergic terminals; however, this transmission still exhibits a high degree of spatial specificity. In fact, in normal conditions, the time for DA diffusion is limited by reuptake. From the measured DA half-life (74 msec) it can be calculated that the maximal distance for DA diffusion is $\sim$ 12 μ m. Therefore, regional variations regarding the dopaminergic innervation (e.g., a lower probability of exocytosis per action potential) would result in heterogeneous DA overflow with well defined regional boundaries. Sharp regional variations in DA overflow have actually been observed (Garris et al., 1994b).

Conclusion

The present study underlines the importance of bursting impulse flow regarding the phasic dopaminergic transmission. In fact, train stimulations mimicking bursts of action potentials evoked in dopaminergic neurons by rewarding stimuli are more potent than single pulse in triggering DA overflow and DA excitatory responses mediated by D1 receptors. This phasic transmission exerts a delayed and prolonged excitatory influence on target neurons and occurs outside synaptic clefts. It is restricted, however,

inside well defined spatiotemporal domains: it lasts for up to 1 sec, and the distance for DA diffusion is $<12\ \mu\text{m}$.

REFERENCES

- Akaike A, Ohno Y, Sasa M, Takaori S (1987) Excitatory and inhibitory effects of dopamine on neuronal activity of the caudate nucleus neurons in vitro. *Brain Res* 418:262-272.
- Andersen PH, Jansen JA (1990) Dopamine receptor agonists: selectivity and dopamine D1 receptor efficacy. *Eur J Pharmacol* 188:335-347.
- Batchelor AM, Madge DJ, Garthwaite J (1994) Synaptic activation of metabotropic glutamate receptors in the parallel fibre-Purkinje cell pathway in rat cerebellar slices. *Neuroscience* 63:911-915.
- Caillé I, Dumartin B, Bloch B (1996) Ultrastructural localization of D1 dopamine receptor immunoreactivity in the rat striatonigral neurons and its relation with dopaminergic innervation. *Brain Res* 730:17-31.
- Calabresi P, Mercuri N, Stanzione P, Stefani A, Bernardi G (1987) Intracellular studies on the dopamine-induced firing inhibition of neostriatal neurons in vitro: evidence for D1 receptor involvement. *Neuroscience* 20:757-771.
- Chergui K, Suaud-Chagny MF, Gonon F (1994) Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. *Neuroscience* 62:641-645.
- Chergui K, Nomikos GG, Mathé JM, Gonon F, Svensson TH (1996) Burst stimulation of the medial forebrain bundle selectively increases Fos-like immunoreactivity in the limbic forebrain of the rat. *Neuroscience* 72:141-156.
- Cole AE, Nicoll RA (1984) Characterization of a slow cholinergic postsynaptic potential recorded in vitro from rat hippocampal pyramidal cells. *J Physiol (Lond)* 352:173-188.
- Doucet G, Descarries L, Garcia S (1986) Quantification of the dopamine innervation in adult rat neostriatum. *Neuroscience* 19:427-445.
- Dugast C, Suaud-Chagny MF, Gonon F (1994) Continuous *in vivo* monitoring of evoked dopamine release in the rat nucleus accumbens by amperometry. *Neuroscience* 62:647-654.
- Freeman AS, Bunney BS (1987) Activity of A9 and A10 dopaminergic neurons in unrestrained rats: further characterization and effects of apomorphine and cholecystokinin. *Brain Res* 405:46-55.
- Frigyesi TL, Purpura DP (1967) Electrophysiological analysis of reciprocal caudato-nigral relations. *Brain Res* 6:440-456.
- Garris PA, Ciolkowski EL, Pastore P, Wightman RM (1994a) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. *J Neurosci* 14:6084-6093.
- Garris PA, Ciolkowski EL, Wightman RM (1994b) Heterogeneity of evoked dopamine overflow within the striatal and striatoamygdaloid regions. *Neuroscience* 59:417-427.
- Gonon F, Sundström L (1996) Excitatory effects of dopamine released by impulse flow in the rat nucleus accumbens in vivo. *Neuroscience* 75:13-18.
- Grace AA, Bunney BS (1984) The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci* 4:2877-2890.
- Groves PM, Linder JC, Young SJ (1994) 5-Hydroxydopamine-labeled dopaminergic axons: three-dimensional reconstructions of axons, synapses and postsynaptic targets in rat neostriatum. *Neuroscience* 58:593-604.
- Haracz JL, Tschanz JT, Wang ZR, White IM, Rebec GV (1993) Striatal single-unit responses to amphetamine and neuroleptics in freely moving rats. *Neurosci Biobehav Rev* 17:1-12.
- Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J Neurosci* 15:5222-5237.
- Hille B (1992) G-protein-coupled mechanisms and nervous signaling. *Neuron* 9:187-195.
- Hu XT, Wang RY (1988) Comparison of effects of D-1 and D-2 dopamine receptor agonists on neurons in the rat caudate putamen: an electrophysiological study. *J Neurosci* 8:4340-4348.
- Isaacson JS, Solis JM, Nicoll RA (1993) Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron* 10:165-175.
- Kelly E, Nahorski SR (1987) Endogenous dopamine functionally activates D-1 and D-2 receptors in striatum. *J Neurochem* 49:115-120.
- Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA, Price DL, Maggio R, Brann MR, Ciliax BJ (1993) Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Proc Natl Acad Sci USA* 90:8861-8865.
- Levine MS, Altemus KL, Cepeda C, Cromwell HC, Crawford C, Ariano MA, Drago J, Sibley DR, Westphal H (1996a) Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D_{1A}-deficient mutant mice. *J Neurosci* 16:5870-5882.
- Levine MS, Li ZW, Cepeda C, Cromwell HC, Altemus KL (1996b) Neuromodulatory actions of dopamine on synaptically-evoked neostriatal responses in slices. *Synapse* 24:65-78.
- Marcenac FM, Gonon F (1985) In vivo electrochemical monitoring of apomorphine penetration into the rat brain. *Neurosci Lett* 55:305-310.
- Mirenzowicz J, Schultz W (1996) Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* 379:449-451.
- Moratalla R, Xu M, Tonegawa S, Graybiel AM (1996) Cellular responses to psychomotor stimulant and neuroleptic drugs are abnormal in mice lacking the D1 dopamine receptor. *Proc Natl Acad Sci USA* 93:14928-14933.
- Nicholson C (1985) Diffusion from an injected volume of a substance in brain tissue with arbitrary volume fraction and tortuosity. *Brain Res* 333:325-329.
- Nicholson C (1995) Interaction between diffusion and Michaelis-Menten uptake of dopamine after iontophoresis in striatum. *Biophys J* 68:1699-1715.
- Nicola SM, Kambian SB, Malenka RC (1996) Psychostimulants depress excitatory synaptic transmission in the nucleus accumbens via presynaptic D1-like dopamine receptors. *J Neurosci* 16:1591-1604.
- Nirenberg MJ, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM (1996) The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *J Neurosci* 16:436-447.
- Pierce RC, Rebec GV (1995) Iontophoresis in the neostriatum of awake, unrestrained rats: differential effects of dopamine, glutamate and ascorbate on motor- and nonmotor-related neurons. *Neuroscience* 67:313-324.
- Pothos EN, Ryjak I, Sulzer D (1996) Amperometric measurement of synaptic vesicle quantal release in midbrain dopamine neurons: modulation by L-DOPA. *Soc Neurosci Abstr* 22:1990.
- Richardson NR, Gratton A (1996) Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: an electrochemical study in rat. *J Neurosci* 16:8160-8169.
- Siggins GR (1978) Electrophysiological role of dopamine in striatum: excitatory or inhibitory? In: *Psychopharmacology: a generation of progress* (Lipton MA, Di Mascio A, Killam KF, eds), pp. 143-157. New York: Raven.
- Suaud-Chagny MF, Poncet J, Gonon F (1991) Presynaptic autoinhibition of the electrically evoked dopamine release studied in the rat olfactory tubercle by *in vivo* electrochemistry. *Neuroscience* 45:641-652.
- Suaud-Chagny MF, Chergui K, Chouvet G, 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.
- Suaud-Chagny MF, Dugast C, Chergui K, Msghina M, Gonon F (1995) Uptake of dopamine released by impulse flow in the rat mesolimbic and striatal systems in vivo. *J Neurochem* 65:2603-2611.
- Surmeier DJ, Bargas J, Hemmings HJ, Nairn AC, Greengard P (1995) Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14:385-397.
- Surprenant A, Williams JT (1987) Inhibitory synaptic potentials recorded from mammalian neurones prolonged by blockade of noradrenaline uptake. *J Physiol (Lond)* 382:87-103.
- Wickens JR, Begg AJ, Arbutnot GW (1996) Dopamine reverses the depression of rat corticostriatal synapses which normally follows high-frequency stimulation of cortex in vitro. *Neuroscience* 70:1-5.
- Williams GV, Millar J (1991) Differential actions of endogenous and iontophoretic dopamine in rat striatum. *Eur J Neurosci* 2:658-661.
- Wilson CJ, Chang HT, Kitai ST (1982) Origins of postsynaptic potentials evoked in identified rat neostriatal neurons by stimulation in substantia nigra. *Exp Brain Res* 45:157-167.
- Xu M, Hu XT, Cooper DC, Moratalla R, Graybiel AM, White FJ, Tonegawa S (1994) Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. *Cell* 79:945-955.
- Young ST, Porrino LJ, Iadarola MJ (1991) Cocaine induces striatal c-fos immunoreactive proteins via dopaminergic D1 receptors. *Proc Natl Acad Sci USA* 88:1291-1295.