The Journal of Neuroscience, April 15, 1996, 16(8):2605–2611

The Pharmacology of Mesolimbic Dopamine Neurons: A Dual-Probe Microdialysis Study in the Ventral Tegmental Area and Nucleus Accumbens of the Rat Brain

B. H. C. Westerink, H.-F. Kwint, and J. B. deVries

Department of Medicinal Chemistry, University Center for Pharmacy, University of Groningen, Groningen, The Netherlands

Receptor-specific compounds were applied by retrograde microdialysis to the ventral tegmental area (VTA) of the rat brain. The effect of the intrategmental infusions on extracellular dopamine in the ipsilateral nucleus accumbens were recorded with a second microdialysis probe. Intrategmental infusion of muscimol (10–40 μM) or baclofen (50 μM) decreased extracellular dopamine in the nucleus accumbens. Intrategmental infusion of NMDA (1 mM, 15 min) or kainate (50 μM, 15 min) increased extracellular dopamine in the nucleus accumbens. The effects of the excitatory amino acids were suppressed by co-infusion of MK-801 (1 mM), (+)-3-amino-1-hydroxy-2-pyrrolidone [(+)-HA966; 1 mM], (±)-3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP; 100 μM), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 300 μM). Intrategmental infusion of of carbachol (50 μM) increased extracellular dopamine in the nucleus accumbens. These results provide evidence for localization of $GABA_A$, $GABA_B$, NMDA, non-NMDA, and cholinergic receptors on dopamine neurons in the VTA. Infusions of CPP, (+)-MK-801, (+)-HA966, CNQX, mecamylamine, atropine, or 3-[[(3,4-dichlorophenyl)methyl]propyl](diethoxymethyl) phosphonic acid (CGP 52432) into the VTA did not modify extracellular dopamine in the nucleus accumbens. Infusion of bicuculline (50 μM) and (−)-sulpiride (50 μM) was followed by an increase in extracellular dopamine in the nucleus accumbens. These data suggest that dopamine neurons in the VTA are tonically inhibited by GABA and dopamine by acting on $GABA_A$ and D_2 receptors, respectively. A tonic stimulation by glutamatergic or cholinergic neurons was not detected.

Finally, results on A10 neurons are compared with earlier data on A9 neurons. A striking difference was found in that $GABA_A$–dopamine interactions are indirect in the substantia nigra and direct in the VTA.

Key words: dopamine; VTA; microdialysis; nucleus accumbens; NMDA; CGP 52432

Mesolimbic dopamine neurons originate in the A10 region of the ventral tegmental area (VTA) in the brainstem and innervate the nucleus accumbens (often referred to as the ventral striatum). These neurons have received considerable interest in numerous behavioral, electrophysiological, and neurochemical studies. Various workers have emphasized that mesolimbic dopamine neurons play an important role in the mediation of cognitive, rewarding, and affective functions (Fibiger and Phillips, 1986; Sachs and Meisel, 1988; Le Moal and Simon, 1991; Blackburn et al., 1992). In this respect, it is generally supposed that the antipsychotic effects of dopamine antagonists are associated with mesolimbic dopamine neurons, whereas the Parkinsonian side effects are related to the nigrostriatal dopamine (A9) neurons. This hypothesis is attractive for developers of new antipsychotic drugs, because it implies that the design of a drug with a selective action and with less extrapyramidal side effects is theoretically possible. Important for this concept is the possibility of discriminating pharmacologically between the two dopaminergic systems. For this purpose, a detailed knowledge on the innervation of these neuronal systems is crucial.

Neuroanatomical, neurochemical, and electrophysiological studies have already provided a large amount of data about the presence of receptors on and neuronal afferents to cell bodies and dendrites of dopamine neurons in the VTA (for review, see Kalivas, 1993). However, evidence obtained in conscious animals about the regulation of dopamine neurons in the VTA is relatively scarce. An approach that may provide such evidence is the dual-probe microdialysis technique. Dual-probe microdialysis is a variant of the multiple push–pull cannulae that have been applied successfully to dopamine neurons in anesthetized cats (Nieoullon et al., 1977). With dual-probe microdialysis, we have recently characterized the nigrostriatal dopaminergic pathway (Santiago and Westerink, 1991, 1992; Westerink et al., 1992).

In the present approach, we have studied dopamine neurons that originate in the VTA with dual-probe microdialysis. One probe was implanted in the VTA, and a second probe was placed in the ipsilateral nucleus accumbens. Receptor-specific compounds were infused by retrograde microdialysis into the VTA while extracellular dopamine was recorded from the nucleus accumbens.

Three issues were investigated: (1) identification of receptors that modify the activity of mesolimbic dopamine neurons at the somatodentritic level; (2) the participation of afferents to the tonic regulation of the mesolimbic dopamine neurons—for this aim, a series of receptor-specific antagonists was infused into the VTA; and (3) a comparison between the mesolimbic and nigrostriatal dopaminergic system.

Based on earlier studies (Kalivas, 1993), we have investigated three different neuronal pathways that are well known to send efferents to the VTA. These pathways consists of GABAergic,

Received Oct. 5, 1995; revised Jan. 29, 1996; accepted Jan. 31, 1996.

Correspondence should be addressed to Dr. B. H. C. Westerink, University Center for Pharmacy, University of Groningen, A. Deusinglaan 2, 9713 AW, Groningen, The Netherlands.

glutamatergic, and cholinergic neurons. Six different types of receptor interactions were studied: $GABA_A$, $GABA_B$, NMDA, non-NMDA, D_2, and cholinergic.

MATERIALS AND METHODS

Animals, drug treatment, and doses. Male albino rats of a Wistar-derived strain (275–320 gm; Harlan, Zeist, The Netherlands) were used for the experiments. The rats were housed in plastic cages (35 × 35 × 40 cm^3). During the experiments, the animals had free access to food and water.

The following drugs were used: methyl-atropine, (±)-baclofen, (−)-bicuculline methylchloride, carbachol, (±)-3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), (+)-3-amino-1-hydroxy-2-pyrrolidone [(+)-HA966], kainate, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), mecamylamine-HCl, NMDA, dizocilpine maleate [(+)-MK-801], muscimol (all from Research Biochemicals, Natick, MA), and (−)-sulpiride (Ravizzi, Milano, Italy). 3-[[(3,4-Dichlorophenyl)methyl]propyl](diethoxymethyl) phosphonic acid (CGP 52432) was a generous gift from Ciba Geigy (Basel, Switzerland). All drugs were dissolved in the perfusion fluid and infused via retrograde microdialysis into the VTA.

Infused doses were based on earlier studies on related experiments (Santiago and Westerink, 1991, 1992; Westerink et al., 1992). The experiments were approved by the Animal Care Committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

Surgery and brain dialysis. Microdialysis was performed with two I-shaped cannulas. As dialysis tube, polyacrylonitrile/sodium methalyl sulfonate copolymer (inner diameter 0.22 mm; outer diameter 0.31 mm; AN 69, Hospal, Bologna, Italy) was used. One probe (exposed length 1 mm) was implanted into the VTA, and the second probe (exposed length 1.5 mm) was implanted into the ipsilateral accumbens. Via the VTA probe, drugs were infused into the brain, and the nucleus accumbens probe was used to record extracellular dopamine. Coordinates of the implantation were as follows: A/P 2.5, L/M −1.4, and V/D −7.3 (nucleus accumbens); A/P 3.7, L/M −0.9, and V/D −8.0 (VTA), from bregma point and dura. The probes were implanted during general chloralhydrate anesthesia (400 mg/kg, i.p.) and local lidocaine (6%) anesthesia.

Microdialysis experiments were carried out 24–72 hr after implantation of the probes. An on-line approach was used in which the probes were perfused with a Ringer's solution at a flow rate of 2.8–3.0 μl/min (perfusor VI, Braun, Melsungen, Germany). Fifteen minute fractions were collected. The composition of the Ringer's solution was (in mM): NaCl 140.0, KCl 4.0, $CaCl_2$ 1.2, and $MgCl_2$ 1.0.

Implantation of the cannulas was evaluated functionally. The experiments were finished with infusion of 50 μM baclofen into the VTA probe, and the response in the nucleus accumbens was determined. A decrease in extracellular dopamine in the nucleus accumbens to at least 40% of controls was considered an appropriate implantation. When the experiment was terminated, the rat was given an overdose of chloral hydrate and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 μm thick) were made, and dialysis probe placement was localized according to the atlas of Paxinos and Watson (1982).

Chemical assays. Dopamine was quantified by HPLC with electrochemical detection. A Pharmacia LKB2150 pump (Uppsala, Sweden) was used in conjunction with an electrochemical detector (ESA, potential first cell: +200 mV; potential second cell: −250 mV). A reverse-phase column (150 × 4.7 mm^2; Supelco LC18, Bellefonte, PA) was used. The mobile phase consisted of a mixture of 0.1 M sodium acetate adjusted to pH 4.1 with acetic acid, 1.8 mM octanesulfonic acid, 0.3 mM Na_2-EDTA, and 120 ml/l methanol at a flow of 1.0 ml/min. The detection limit of the assay was ~3 fmol/sample (on-column).

Expression of results and statistics. All values given are expressed as percent of controls. The average concentration of three stable samples (<10% variation) was considered the control and was defined as 100%. Data were analyzed using the statistical program Sigmastat. Nonparametric one-way ANOVA analysis was followed by the Dunnett's multiple-comparisons test when appropriate. The level of significance was set at $p < 0.05$.

RESULTS

Basal values

The average basal values of dopamine in dialysates of the nucleus accumbens for the different experiments did not differ significantly. Therefore, they are grouped here together. Basal values ± SD were 3.2 ± 0.8 fmol/min (n = 72).

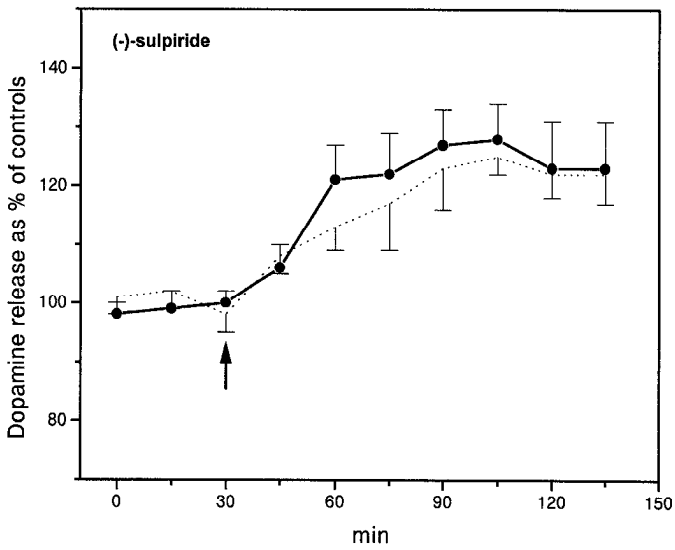

Figure 1. Effect of infusion of (−)-sulpiride (50 μM; *filled circles*) into the VTA (*start arrow*) on the extracellular concentration of dopamine in the ipsilateral nucleus accumbens. Data are given as percent of controls ± SEM and are an average of four to five experiments. Data from a similar experiment carried out in the nigrostriatal pathway (Santiago and Westerink, 1991) are shown by the *dotted line*.

Effect of (−)-sulpiride, infused into the VTA, on the dialysate content of dopamine in the ipsilateral nucleus accumbens

The specific D_2 antagonist (−)-sulpiride, infused in a concentration of 50 μM (a dose that was maximally effective in the nigrostriatal dopamine pathway; Santiago and Westerink, 1991) into the VTA, caused an increase of extracellular dopamine to ~125% of controls in the ipsilateral nucleus accumbens (Fig. 1).

Effect of muscimol and bicuculline, infused into the VTA, on the dialysate content of dopamine in the ipsilateral nucleus accumbens

The $GABA_A$ agonist muscimol was infused via the microdialysis probe into the VTA in concentrations of 10, 20, or 40 μM. The infusions caused a dose-dependent decrease in extracellular dopamine in the ipsilateral nucleus accumbens (Fig. 2). At 40 μM, dopamine decreased to ~40% of controls. The decrease in extracellular dopamine was statistically significant at all three doses 45 min after the start of the infusion.

The $GABA_A$ antagonist bicuculline, infused into the VTA in a concentration of 50 μM, caused an increase of extracellular dopamine in the ipsilateral accumbens to ~180% of controls (Fig. 3). The increase in extracellular dopamine reached statistical significance 15 min after start of the infusion. Higher doses were not studied because of the induction of strong turning behavior.

Effect of baclofen and CGP 52432, infused into the VTA, on the dialysate content of dopamine in the ipsilateral nucleus accumbens and striatum

The $GABA_B$ agonist D,L-baclofen was infused into the VTA in a concentration of 50 μM. This $GABA_B$ agonist caused an decrease of extracellular dopamine in the ipsilateral accumbens to ~35% of controls (Fig. 4; *arrow 1*). The decrease was statistically significant 15 min after start of the infusion. Infusion of 100 μM did not decrease the dopamine output further (data not shown). Infusion

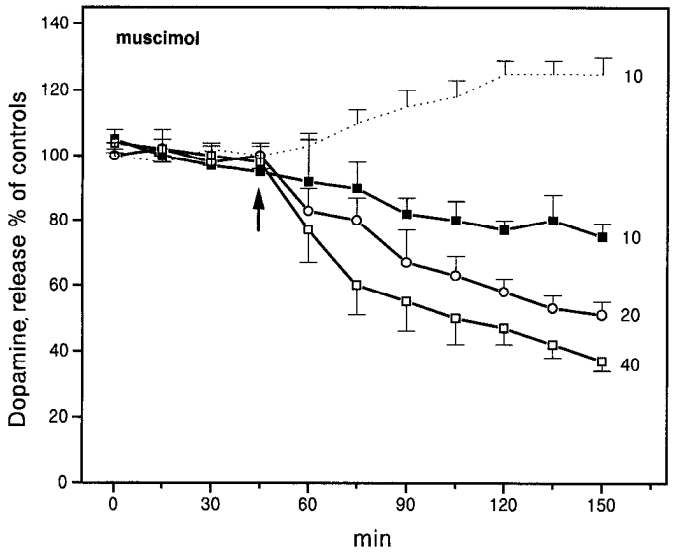

Figure 2. Effect of infusion of muscimol into the VTA (*start arrow*) on the extracellular concentration of dopamine in the ipsilateral nucleus accumbens. *Filled squares*, 10 μM; *open circles*, 20 μM; *open squares*, 40 μM. Data are given as percent of controls ± SEM and are an average of four to five experiments. Data from a similar experiment carried out in the nigrostriatal pathway with 10 μM muscimol (Santiago and Westerink, 1992) are shown by the *dotted line*.

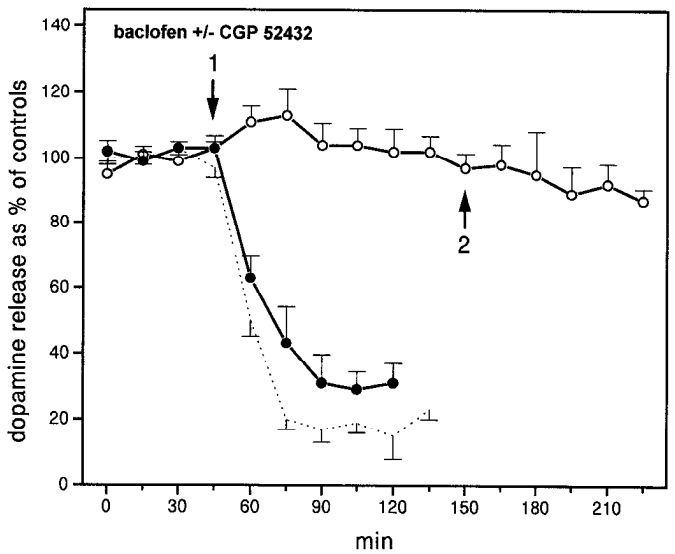

Figure 4. Effect of infusion of (±)-baclofen (50 μM) into the VTA (*start arrow* at *1*; *closed circles*) on the extracellular concentration of dopamine in the ipsilateral nucleus accumbens. Data from a similar experiment carried out in the nigrostriatal pathway (Santiago and Westerink, 1992) are shown by the *dotted line*. The *open circles* represent an experiment in which CGP 52432 (100 μM) was infused (*arrow 1*) followed by 50 μM (±)-baclofen (*arrow 2*). Data are given as percent of controls ± SEM and are an average of four to five experiments.

of the potent and specific $GABA_B$ antagonist CGP 52432 into the VTA (Fig. 4, *arrow 1*) in a concentration of 100 μM did not influence the extracellular levels of dopamine in the ipsilateral nucleus accumbens. When baclofen (50 μM) was added to the CGP 52432 solution (Fig. 4; *arrow 2*), the decrease in extracellular dopamine was completely blocked.

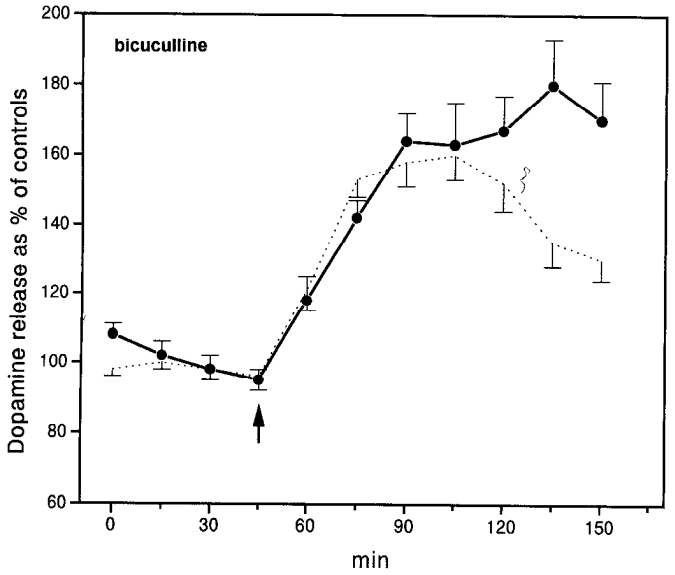

Figure 3. Effect of infusion of (−)-bicuculline (50 μM; *closed circles*) into the VTA (*start arrow*) on the extracellular concentration of dopamine in the ipsilateral nucleus accumbens. Data are given as percent of controls ± SEM and are an average of four to five experiments. Data from a similar experiment carried out in the nigrostriatal pathway (Santiago and Westerink, 1992) in which bicuculline was infused for 60 min are shown by the *dotted line*.

Effect of infusion of NMDA, CPP, (+)-MK-801, and (+)-HA966 into the VTA on the dialysate content of dopamine in the ipsilateral nucleus accumbens

NMDA was infused into the VTA in a concentration of 1 mM. Because of the strong behavioral activation, the NMDA infusion was restricted to 15 min. The extracellular dopamine in the ipsilateral accumbens rose to ~190% of controls (Fig. 5). The increase was statistically significant 15 min after the start of the infusion.

Intrategmental infusion of the competitive NMDA antagonist CPP, the noncompetitive antagonist (+)-MK-801, and the NMDA/glycine antagonist (+)-HA-966 suppressed the increase induced by 15 min infusion of 1 mM NMDA (Fig. 6). To achieve this effect, higher concentrations of (+)-MK-801 and (+)-HA966 (1 mM) were necessary than for CPP (0.1 mM). None of the antagonists modified the basal values of extracellular dopamine in the ipsilateral nucleus accumbens, although there was a tendency to decrease in the case of (+)-MK-801.

The intrategmental infusion of 1 mM NMDA induced a strong behavioral activation, including hyperlocomotion and turning behavior, rearing, and grooming, that lasting for ~20 min, after which the animals returned to their usual resting state. This behavioral effect was completely blocked by co-infusion with CPP and was partly blocked with (+)MK-801 and (+)-HA-966.

Effect of infusion of kainate, CNQX, and kainate + CNQX into the VTA on extracellular levels of dopamine in the ipsilateral nucleus accumbens

Kainate was infused into the VTA in a concentration of 30 μM. Because of the strong behavioral activation, the kainate infusion was restricted to 15 min. The extracellular dopamine in the ipsilateral accumbens rose to ~160% of controls (Fig. 7). The increase was statistically significant 15 min after the start of the infusion.

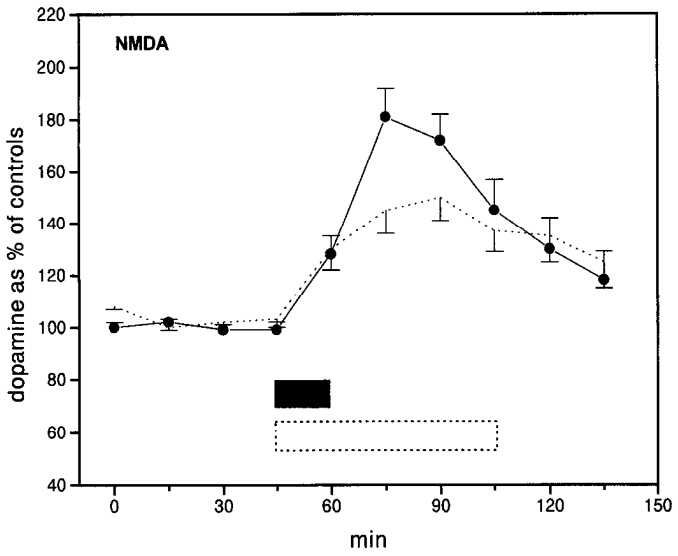

Figure 5. Effect of infusion of NMDA (1 mM) into the VTA (*black bar*) on the extracellular concentration of dopamine in the ipsilateral nucleus accumbens (*filled circles*). Data from a similar experiment carried out in the nigrostriatal pathway (Westerink et al., 1992) are shown by the *dotted line* and *dotted bar*.

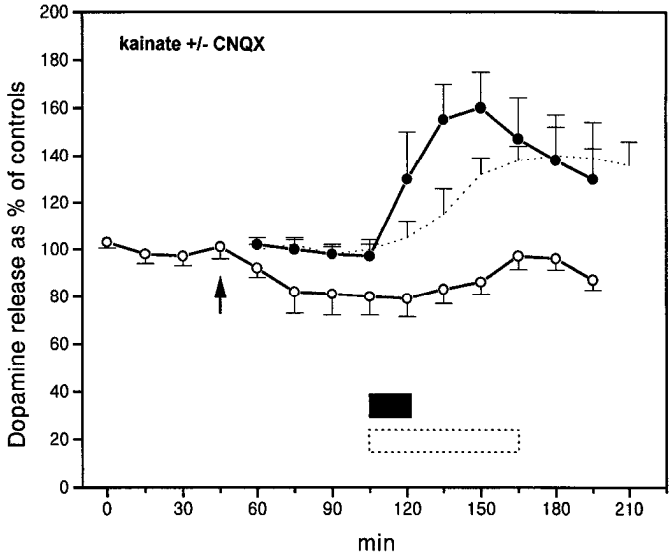

Figure 7. Effect of infusion of kainate (30 μM) into the VTA (*black bar*) on the extracellular concentration of dopamine in the ipsilateral nucleus accumbens (*filled circles*). Data from a similar experiment carried out in the nigrostriatal pathway (Westerink et al., 1992) are shown by the *dotted line* and *dotted bar*. The *open circles* represent an experiment in which CNQX (300 μM) was infused (*start arrow*) followed by kainate (30 μM; *black bar*). Data are given as percent of controls ± SEM and are an average of four to five experiments.

Infusion of the specific non-NMDA receptor antagonist CNQX in a concentration of 300 μM) into the VTA slightly decreased extracellular levels of dopamine in the ipsilateral nucleus accumbens (Fig. 7, *arrow*). However, this decrease did not reach the level of statistical significance. CNQX infusion completely blocked the increase induced by 30 μM kainate.

The intrategmental infusion of 30 μM kainate induced a strong behavioral activation, including hyperlocomotion and turning behavior, rearing, and grooming, that lasted for ~20–30 min, after which the animals returned to their usual resting state. This behavioral effect was completely blocked by co-infusion with CNQX.

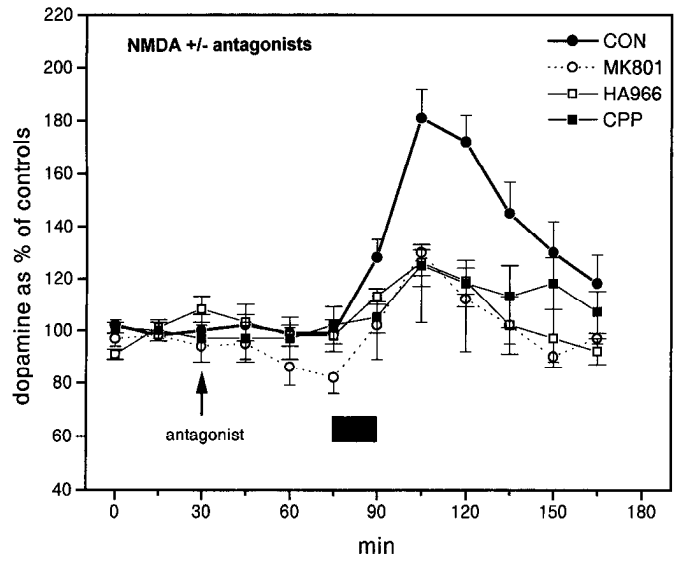

Figure 6. Effect of co-infusion of the glutamate antagonists (*arrow*) CPP (100 μM; *filled squares*), (+)-MK-801 (1 mM; *open circles*), and (+)-HA966 (1 mM; *open squares*) into the VTA on the NMDA-induced (1 mM; *black bar*) increase in extracellular dopamine in the ipsilateral nucleus accumbens. The control NMDA infusion is shown with *filled circles*. Data are given as percent of controls ± SEM and are an average of three to four experiments.

Effect of carbachol, atropine, and mecamylamine, infused into the VTA, on the dialysate content of dopamine in the ipsilateral nucleus accumbens

The aspecific cholinomimetic compound carbachol was continuously infused into the VTA in a concentration of 50 μM. Carbachol caused an increase of extracellular dopamine in the ipsilateral accumbens to ~140% of controls (Fig. 7). The increase in dopamine reached statistical significance 30 min after start of the infusion of carbachol. The muscarinic antagonist atropine and the nicotinic antagonist mecamylamine, both infused in a relatively high concentration of 100 μM, were without effect on the ipsilateral concentration of dopamine in the nucleus accumbens (data not shown).

Comparison of mesolimbic and nigrostriatal neurons

For comparison of the present data with similar experiments carried out on nigrostriatal neurons, we have indicated the results from nigrostriatal experiments with dotted lines in the various figures. The results of intranigral infusion of baclofen, NMDA, kainate, and sulpiride were taken from earlier papers (Santiago and Westerink, 1991, 1992; Westerink et al., 1992). Data on infusion of carbachol were not published previously. Minor differences in the experimental procedure between the mesolimbic and nigrostriatal experiments are indicated below.

Infusion of (−)-sulpiride into the substantia nigra or VTA induced a similar rise in extracellular dopamine in the nerve terminal areas (Fig. 1), suggesting that sulpiride is equally effective in stimulating the two neuronal systems at the level of cell bodies/dendrites.

In contrast to changes in the mesolimbic neurons (Fig. 2),

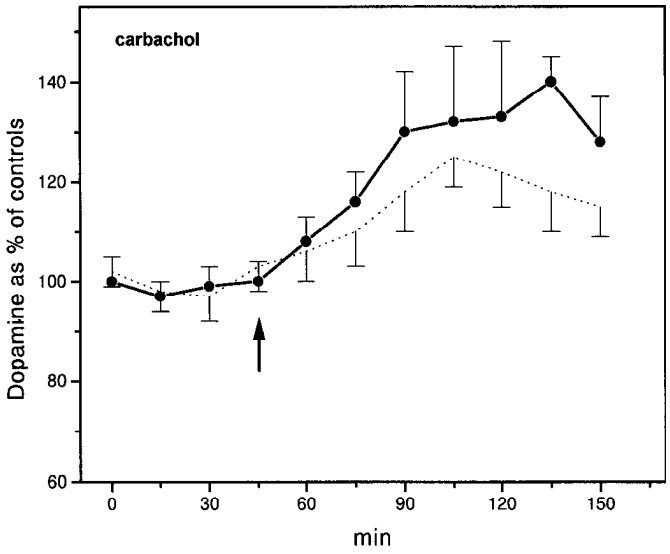

Figure 8. Effect of infusion of carbachol (50 μM; *filled circles*) into the VTA (*start arrow*) on the extracellular concentration of dopamine in the ipsilateral nucleus accumbens. Data are given as percent of controls ± SEM and are an average of four to five experiments. Data from a similar experiment carried out in the nigrostriatal pathway are shown by the *dotted line*.

muscimol increased extracellular dopamine in the ipsilateral striatum to ~125% of controls. Bicuculline was applied to the substantia nigra for 60 min and was applied continuously to the VTA; the infusions induced comparable increases in mesolimbic as well as nigrostriatal neurons (Fig. 3).

Baclofen induced a strong decrease in both the nucleus accumbens and the striatum (Fig. 4). The changes in the striatum were somewhat more pronounced than in the nucleus accumbens.

The increase seen after infusion of 1 mM NMDA in the cell body area was more pronounced in the nucleus accumbens than in the striatum. Although the infusion lasted only 15 min in the VTA, but 60 min in the substantia nigra, the increase in extracellular dopamine in the nucleus accumbens was almost twice as high as in the striatum (Fig. 5). We conclude that NMDA is ~10-fold more effective in stimulating mesolimbic neurons than nigrostriatal neurons.

Kainate was infused in a dose of 30 μM during 15 min in the VTA and in a dose of 10 μM during 60 min in the substantia nigra (Fig. 7). This means that the infused amounts of compound in both experiments are on the same order of magnitude. The maximal increases in extracellular dopamine in the two nerve terminal areas were not statistically different. We conclude that kainate is equally effective in stimulating the two neuronal systems.

Infusion of carbachol into the VTA induced an increase in the nucleus accumbens that was slightly more pronounced than infusion of carbachol into the nigrostriatal system (Fig. 8). The differences did not reach the level of statistical significance.

DISCUSSION

GABA, glutamate, and acetylcholine receptors in the VTA

The first aim of the study was to identify neurotransmitter–receptor interactions that modify the activity of dopamine neurons in the VTA.

Infusion of the $GABA_A$ agonist muscimol as well as the $GABA_B$ agonist baclofen into the VTA caused a pronounced decrease in extracellular dopamine in the ipsilateral nucleus accumbens, whereas infusion of bicuculline stimulated the dopamine neurons. These data suggest that both $GABA_A$ and $GABA_B$ receptors are present on somatodendritic sites of mesolimbic dopamine neurons, a finding that is in good agreement with anatomical data (Bayer and Pickel, 1991) and with data from electrophysiological studies in brain slices (Johnson and North, 1992; Jiang et al., 1993; Seutin et al., 1994). The results with baclofen are comparable with a study in which baclofen was injected into the VTA (Klitenick et al., 1992) and with a recent, similar dual-probe microdialysis study (Yoshida et al., 1994).

Infusion of NMDA or kainate into the VTA induced a pronounced increase in extracellular dopamine in the nucleus accumbens. Kainate was equally effective at ~30 times lower concentrations than NMDA. These results are in line with electrophysiological studies carried out in brain slices on dopamine neurons in the VTA (Seutin et al., 1990; Johnson and North, 1992; Johnson et al., 1992; Wang and French, 1993; Zhang et al., 1994) and a recent dual-probe microdialysis study by Wang et al. (1994), in which NMDA was infused into the VTA of anesthetized rats. Similar conclusions were reached by Suaud-Chagny et al. (1992), who injected NMDA into the VTA and carried out single-unit recording of dopamine neurons in the VTA in combination with electrochemical measurement of extracellular dopamine in the ipsilateral accumbens. The stimulation of NMDA and kainate was suppressed by co-infusion of the competitive antagonists CPP and CNQX. The noncompetitive antagonists were less effective in this respect because a 10-fold higher dose was needed and behavioral effects of NMDA were only partly blocked. Taken together, these data strongly support anatomical evidence (Christie et al., 1989) that NMDA *and* non-NMDA receptors are present on somatodendritic sites of mesolimbic dopamine neurons.

Investigations of the cholinergic–dopamine interactions in the basal ganglia have been focused previously on the striatum. However, recent electrophysiological, anatomical, and behavioral studies have shown that cholinergic neurons innervating midbrain dopamine neurons are also able to increase the activity of nigrostriatal dopamine neurons (Seutin et al., 1990; Nastuk and Graybiel, 1991; Parker and Winn, 1992). The observed increase in extracellular dopamine in the nucleus accumbens, during infusion of carbachol into the VTA, illustrates the ability of the cholinergic projections to stimulate dopamine cells in the VTA. These results are in good agreement with electrophysiological data from Seutin et al. (1990), who reported that VTA neurons were excited during carbachol infusions. Because carbachol is a nonspecific agonist, no specification can be given in terms of muscarinic or nicotinic receptors. Electrophysiological studies on brain slices indicated the presence of muscarinic as well as nicotinic receptors on dopamine cell bodies in the VTA (Calabresi et al., 1989; Lacey et al., 1990). Self-stimulation experiments provided indirect evidence for the presence of muscarinic and nicotinic receptors on A10 neurons in the VTA (Yeomans et al., 1993; Corrigall et al., 1994). Yoshida et al. (1993) and Nissel et al. (1994a,b) have studied recently the mesolimbic dopamine neurons with dual-probe microdialysis. They demonstrated that A10 dopamine neurons are activated by nicotine infusions into the VTA.

Tonic regulation of the activity of A10 dopamine neurons

To investigate the tonic regulation of A10 dopamine neurons, we infused a series of receptor-specific antagonists into the VTA. Midbrain dopamine neurons recorded *in vitro* display a pace-

maker activity. When the neurons are studied *in vivo*, a characteristic burst-firing pattern is observed (Tepper et al., 1995). This suggests that burst-firing is triggered by afferent input. Several authors have suggested that burst-firing of the midbrain dopamine neurons is induced by iontophoretic application of NMDA. Burst-firing of A10 neurons that was induced by NMDA has been observed in brain slices (Seutin et al., 1994; Wang et al., 1994) as well as *in vivo* in anesthetized rats (Overton and Clark, 1992; Cherqui et al., 1993; French et al., 1993; Zhang et al., 1994). These studies suggest a tonic excitation of dopamine neurons by a glutamatergic input. However, results of studies on administration of competitive glutamate antagonists to A10 neurons are controversial. Some authors report an inhibition of burst-firing of midbrain dopamine neurons by glutamate antagonists (Charlety et al., 1991; Overton and Clark, 1992; Chergui et al., 1993), but French et al. (1993) found no effect of systemic administration of competitive glutamate antagonists on the firing rate or the firing pattern of the dopamine neurons. The question of whether a tonic excitation of A9 or A10 dopamine neurons is exerted by glutamatergic afferents is yet unsolved. In this respect, the results of the present experiments carried out on conscious rats are of interest. Because burst-firing is believed to induce a relatively high increase in the synaptic levels of dopamine (Gonon and Buda, 1985), a pronounced decrease in dopamine release is to be expected when the firing pattern of the dopamine neurons is normalized. However, CPP as well as CNQX, infused into the VTA, were unable to decrease extracellular dopamine in the ipsilateral nucleus accumbens. Because the stimulatory effects of infused NMDA and kainate were blocked by co-infusion with CPP and CNQX, respectively, it can be concluded that the infused amounts of antagonists were effective. The present results do not support a direct major role of glutamatergic projections in the induction of burst-firing in midbrain dopamine neurons during control conditions.

The stimulatory effect of the $GABA_A$ antagonist bicuculline demonstrates that dopamine neurons in the A10 are tonically inhibited by $GABA_A$ receptors in conscious rats. In contrast, the absence of any effect of the new, potent $GABA_B$ antagonist CGP 52432 (Lanza et al., 1993) suggests that $GABA_B$ receptors are not activated during normal conditions. The fact that $GABA_A$ receptors are tonically activated, whereas $GABA_B$ receptors are not, may indicate that these receptors are not located on the same cell. That the concentration of the infused $GABA_B$ antagonist was effective was demonstrated by the observation that CGP 52432 completely blocked the effect of baclofen infusion. The finding that the infused GABA agonists muscimol and baclofen were effective in decreasing extracellular dopamine in the ipsilateral accumbens suggests that the capacity of GABAergic neurons to inhibit VTA neurons is much larger than expressed under control conditions. Moreover, the pronounced effects of the GABAergic compounds suggest an important role for GABA in tonic inhibition or burst-firing. A recent electrophysiological study provided evidence that GABAergic projection neurons mediate burst-firing of midbrain dopamine neurons through disinhibition by $GABA_A$ and $GABA_B$ receptors (Tepper et al., 1995). The present data give support to such a mechanism.

Intrategmental infusion of high concentrations of the nicotinic antagonist mecamylamine and the muscarinic agonist atropine was without effect on ipsilateral dopamine. With respect to mecamylamine, similar results were reported recently in a dual-probe study by Nisell et al. (1994b). These findings support the notion that the mesolimbic dopamine system is phasically rather than tonically regulated by cholinergic receptor activation within the VTA.

Finally, we have infused (−)-sulpiride in a dose that is effective in maximally blocking D_2 receptors (Santiago and Westerink, 1991). From the slight increase in extracellular dopamine in the ipsilateral nucleus accumbens, it can be concluded that D_2 autoreceptors in the VTA have a significant but modest contribution to the tonic inhibition of the mesolimbic dopamine neurons.

Summarizing the effects of the infused specific antagonists, we conclude that dopamine neurons in the VTA are tonically inhibited by $GABA_A$ and D_2 receptors. A tonic stimulation by glutamatergic and cholinergic neurons could not be demonstrated.

Comparison between A9 and A10 dopamine neurons

When the present results are compared with previously published data from dual-probe experiments on nigrostriatal neurons (Santiago and Westerink, 1991, 1992; Westerink et al., 1992), some similarities and differences between A9 and A10 dopamine neurons become apparent. A significant difference was noticed with respect to the GABAergic interaction. Although the stimulatory effect of bicuculline is very similar for the two dopamine systems studied, muscimol stimulated the nigrostriatal dopamine neurons when infused into the substantia nigra, whereas it dose-dependently inhibited mesolimbic neurons when infused into the VTA. Stimulation of nigrostriatal neurons after intranigral administration of GABA receptor agonists has been described by various authors, and it is speculated that a second inhibitory interneuron located in the A9 participates in the GABAergic striatonigral pathway (Grace and Bunney, 1979; Leviel et al., 1979). Apparently, such an interneuron is not necessary to hypothesize in the GABAergic regulation of mesolimbic dopamine neurons. The finding that muscimol interacts in an opposite way with the two dopamine systems is of great interest because it opens the possibility of discriminating pharmacologically between mesolimbic and striatal dopamine neurons.

A10 neurons were ~10-fold more sensitive to NMDA than A9 neurons. For stimulation with kainate or carbachol or for blockade by (−)-sulpiride, both neuronal systems seemed equally sensitive.

Conclusions

The present study provides evidence for the presence of D_2, $GABA_A$, $GABA_B$, NMDA, non-NMDA, and cholinergic receptors at somatodentritic sites of A10 neurons. We realize that the VTA is a very complex neuronal structure for which many receptor interactions have been described (Kalivas, 1993). It cannot be excluded that some of the studied interactions are mediated by interneurons. Moreover, other neuronal interactions—especially serotonergic and peptidergic afferents—will need further investigation in this respect.

REFERENCES

Bayer VE, Pickel VM (1991) GABA-labeled terminals form proportionally more synapses with dopaminergic neurons having low densities of tyrosine hydroxylase immunoreactivity in rat ventral tegmental area. Brain Res 559:44–55.

Blackburn JR, Pfaus JG, Phillips AG (1992) Dopamine functions in appetitive and defensive behaviours. Prog Neurobiol 39:247–279.

Charlety PJ, Grenhoff J, Chergru K, De la Chapelle B, Buda M, Svensson TH, Chouvet G (1991) Bursts firing of mesencephalic dopamine neurons is inhibited by somatodendritic application of kynurenate. Acta Physiol Scand 142:105–112.

Calabresi P, Lacey MG, North RA (1989) Nicotinic excitation of rat ventral tegmental neurones in vitro studied by intracellular recording. Br J Pharmacol 98:135–140.

Cherqui K, Charlety PJ, Akaoka H, Saunier CF, Brunet JL, Buda M, Svensson TH, Chouvet G (1993) Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. Eur J Neurosci 5:137–144.

Christie MF, Bridge S, James LB, Beart PM (1989) Excitotoxin lesions suggest an aspartatergic projection from rat medial prefrontal cortex to ventral tegmental area. Acta Physiol Scand 136:135–136.

Corrigall WA, Coen KM, Adamson KL (1994) Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res 653:278–284.

Fibiger HC, Philips AG (1986) Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems. In: Handbook of physiology, Vol 4, Intrinsic regulatory systems of the brain, pp 647–675. Bethesda: American Physiology Society.

French ED, Mura A, Wang T (1993) MK-801, phencyclidine (PCP), and PCP-like drugs increase burst firing in rat A10 dopamine neurons: comparison to competitive NMDA antagonists. Synapse 13:108–116.

Gonon FG, Buda MJ (1985) Regulation of dopamine release by impulse flow and by autoreceptors as studied by in vivo voltammetry in the rat striatum. Neuroscience 41:765–774.

Grace AA, Bunney BS (1979) Paradoxal GABA excitation of nigral dopaminergic cells: indirect mediation through reticulata inhibitory neurons. Eur J Pharmacol 59:211–218.

Jiang ZG, Pessia M, North RA (1993) Dopamine and baclofen inhibit the hyperpolarization-activated cation current in rat ventral tegmental neurones. J Physiol (Lond) 462:753–764.

Johnson SW, North RA (1992) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol (Lond) 450:455–468.

Johnson SW, Seutin V, North RA (1992) Burst firing in dopamine neurons induced by *N*-methyl-D-aspartate: role of electrogenic sodium pump. Science 258:665–657.

Kalivas P (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res Rev 18:75–113.

Klitenick MA, DeWitte P, Kalivas PW (1992) Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an *in vivo* microdialysis study. J Neurosci 12:2623–2632.

Lacey MG, Calabresi P, North RA (1990) Muscarine depolarizes rat substantia nigra zona compacta and ventral tegmental neurons in vitro through M1-like receptors. J Pharmacol Exp Ther 253:395–400.

Lanza M, Fassio A, Gemignani A, Bonanno G, Raiteri M (1993) CGP 52432: a novel and potent and selective $GABA_B$ autoreceptor antagonist in rat cerebral cortex. Eur J Pharmacol 237:191–195.

Le Moal M, Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 155–234.

Leviel V, Chéramy A, Nieoullon A, Glowinski J (1979) Symmetric bilateral changes in dopamine release from the caudate nuclei of the cat induced by unilateral nigral application of glycine and GABA related compounds. Brain Res 174:259–270.

Nastuk MA, Graybiel AM (1991) Pharmacologically defined M1 and M2 muscarinic cholinergic binding sites in the cat's substantia nigra: development and maturity. Brain Res Dev Brain Res 61:1–10.

Nieoullon A, Chéramy A, Glowinski J (1977) Release of dopamine in vivo from cat substantia nigra. Nature 266:375–377.

Nissel M, Nomikos GG, Svensson TH (1994a) Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16:36–44.

Nissel M, Nomikos GG, Svensson TH (1994b) Infusion of nicotine in the ventral tegmental area of the nucleus accumbens of the rat differently affects accumbal dopamine release. Pharmacol Toxicol 75:348–352.

Overton P, Clark D (1992) Iontophoretically administered drugs acting at the *N*-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. Synapse 10:131–140.

Paxinos G, Watson C (1982) The rat brain in stereotaxic coordinates. New York: Academic.

Parker GC, Winn P (1992) Additivity of nicotinic and muscarinic cholinergic stimulation of substantia nigra. NeuroReport 3:1105–1108.

Sachs BD, Meisel RL (1988) The physiology of male sexual behavior. In: The physiology of reproduction (Knobil E, Neill J, eds), pp 1393–1485. New York: Raven.

Santiago M, Westerink BHC (1991) The regulation of dopamine release from nigrostriatal neurons in conscious rats: the role of somatodendritic autoreceptors. Eur J Pharmacol 204:79–85.

Santiago M, Westerink BHC (1992) The role of GABA receptors in the control of nigrostriatal dopaminergic neurons: a microdialysis study in awake rats. Eur J Pharmacol 219:175–181.

Seutin V, Verbanck P, Massotte L, Dresse A (1990) Evidence for the presence of *N*-methyl-D-aspartate receptors in the ventral tegmental area of the rat: an electrophysiological in vitro study. Brain Res 514:147–150.

Seutin V, Johnson SW, North RA (1994) Effect of dopamine and baclofen on *N*-methyl-D-aspartate-induced burst firing in rat ventral tegmental neurons. Neuroscience 58:201–206.

Suaud-Chagny MF, Chergui K, Chouvet G, Gonon F (1992) Relationship between dopamine release in the 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.

Tepper JM, Martin LP, Anderson DR (1995) $GABA_A$ receptor-mediated inhibition of rat substantia nigra dopaminergic neurons by pars reticulata projection neurons. J Neurosci 15:3092–3103.

Wang T, French ED (1993) Electrophysiological evidence for the existence of NMDA and non-NMDA receptors on rat ventral tegmental neurons. Synapse 13:270–277.

Wang T, O'Connor WT, Ungerstedt U, French ED (1994) *N*-methyl-D-aspartic acid biphasically regulates the biochemical and electrophysiological response of A10 dopamine neurons in the ventral tegmental area: in vivo microdialysis and in vitro electrophysiological studies. Brain Res 666:255–262.

Westerink BHC, Santiago M, deVries JB (1992) The release of dopamine from nerve terminals and dendrites of nigrostriatal neurons induced by excitatory amino acids in the conscious rat. Naunyn Schmiedebergs Arch Pharmacol 345:523–529.

Yeomans JS, Mathur A, Tampakeras M (1993) Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons. Behav Neurosci 107:1077–1087.

Yoshida M, Yokoo H, Tanaka T, Mizoguchi K, Emoto H, Ishi H, Tanaka M (1993) Facilitatory modulation of mesolimbic dopamine neuronal activity by a mu-opioid agonist and nicotine as examined with in vivo microdialysis. Brain Res 624:277–280.

Yoshida M, Yokoo H, Tanaka T, Emoto H, Tanaka M (1994) Opposite changes in the mesolimbic dopamine metabolism in the nerve terminal and cell body sites induced by locally infused baclofen in the rat. Brain Res 636:111–114.

Zhang J, Chiodo LA, Freeman AS (1994) Influence of excitatory amino acid receptor subtypes on the electrophysiological activity of dopaminergic and nondopaminergic neurons in rat substantia nigra. J Pharmacol Exp Ther 269:313–321.