INTRODUCTION

The supraoptic nucleus (SON) of the hypothalamus is part of the hypothalamo-neurohypophysial system. It consists of magnocellular neurons that synthesize and secrete either oxytocin (OT) or vasopressin (VP). These neurons project their axons into the neurohypophysial system, where they release hormones directly into the blood stream. Oxytocin is essential for reproductive functions, such as lactation and parturition. Secretion of the two hormones is controlled by the electrical activity of magnocellular neurons (Poulain and Wakerley 1982) that itself is dependent on afferent excitatory and inhibitory synaptic inputs originating from many brain areas (Anderson et al. 1990). γ-amino butyric acid (GABA) and glutamate are the main inhibitory and excitatory transmitters in the hypothalamus (Decavel and Van den Pol 1990; Van den Pol et al. 1990). Electrophysiological recordings have indicated the presence of functional GABA-A receptors on SON neurons responsible for fast inhibitory potentials (Randle et al. 1986). GABA-B receptors have also been reported in the SON on both glutamatergic and GABAergic terminals (Kombian et al. 1996; Mouginot et al. 1998) and on magnocellular neurons (Harayama et al. 1998; Stern et al. 2002). Studies performed at the ultrastructural level have revealed that >40% of all synapses on magnocellular neurons were GABAergic (Gies and Theodosis 1994). Accordingly, GABA has been reported to play an important role in the regulation of firing activity both in OT and VP SON neurons (reviewed in Renaud and Bourque 1991).

In addition to the transmitters GABA and glutamate, several other substances have been described as neuromodulators in the SON (Renaud and Bourque 1991). One of them is dopamine (DA), which acts on a variety of G-protein-coupled receptor subtypes classified in two families. The D1 family includes D1 and D5 receptors, whereas the D2 family consists of D2–D4 receptors. The D1 and D2 families have been reported to be positively and negatively coupled to adenyl cyclase respectively (Missale et al. 1998). The supraoptic nucleus receives a diffuse dopaminergic innervation from cells located in the A14 and A15 regions (Jourdain et al. 1999; Van den Pol et al. 1990). Electrophysiological recordings have indicated the presence of functional GABA-A receptors on SON neurons responsible for fast inhibitory potentials (Randle et al. 1986). GABA-B receptors have also been reported in the SON on both glutamatergic and GABAergic terminals (Kombian et al. 1996; Mouginot et al. 1998) and on magnocellular neurons (Harayama et al. 1998; Stern et al. 2002). Studies performed at the ultrastructural level have revealed that >40% of all synapses on magnocellular neurons were GABAergic (Gies and Theodosis 1994). Accordingly, GABA has been reported to play an important role in the regulation of firing activity both in OT and VP SON neurons (reviewed in Renaud and Bourque 1991).

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Dopamine D4 Receptor-Mediated Presynaptic Inhibition of GABAergic Transmission in the Rat Supraoptic Nucleus

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Azdad, Karima, Richard Piet, Dominique A. Poulain, and Stéphane H. R. Oliet. Dopamine D4 receptor-mediated presynaptic inhibition of GABAergic transmission in the rat supraoptic nucleus. J Neurophysiol 90: 559–565, 2003; 10.1152/jn.00226.2003. The mechanism by which dopamine induces or facilitates neurohypophysial hormone release is not completely understood. Because oxytocin- and vasopressin-secreting supraoptic neurons are under the control of a prominent GABAergic inhibition, we investigated the possibility that dopamine exerts its action by modulating GABA-mediated transmission. Whole cell voltage-clamp recordings of supraoptic neurons were carried out in acute hypothalamic slices to determine the action of dopamine on inhibitory postsynaptic currents. Application of dopamine caused a consistent and reversible reduction in the frequency, but not the amplitude, of miniature synaptic events, indicating that dopamine was acting presynaptically to reduce GABAergic transmission. The subtype of dopamine receptor involved in this response was characterized pharmacologically. Dopamine inhibitory action was greatly reduced by two highly selective D4 receptor antagonists L745,870 and L750,667 and to a lower extent by the antipsychotic drug clozapine but was unaffected by SCH 23390 and sulpiride, D1/D5 and D2/D3 receptor antagonists, respectively. In agreement with these results, the action of dopamine was mimicked by the potent D4 receptor agonist PD168077 but not by SKF81297 and bromocriptine, D1/D5 and D2/D3 receptor agonists, respectively. Dopamine and PD168077 also reduced the amplitude of evoked inhibitory postsynaptic currents, an effect that was accompanied by an increase in paired-pulse facilitation. These data clearly indicate that D4 receptors are located on GABA terminals in the supraoptic nucleus and that their activation reduces GABA release in the supraoptic nucleus. Therefore dopaminergenic facilitation of neurohypophysial hormone release appears to result, at least in part, from disinhibition of magnocellular neurons caused by the depression of GABAergic transmission.
presynaptic D4 receptors has been found recently to inhibit glutamatergic transmission (Price and Pittman 2001).

Because most of the studies performed in vivo points to a general excitatory action of DA on the hypothalamo-neurohypophyseal system, we tested the hypothesis that DA could modulate inhibitory GABAergic inputs in the SON as is the case in other brain regions (Gonzales-Islas and Hablitz 2001; Miyazaki and Lacey 1998; Seams et al. 2001). Whole cell patch-clamp recordings performed in acute hypothalamic slices indicated that application of DA resulted in a consistent and reversible reduction of GABAergic synaptic activity. This inhibitory action appeared to be mediated, at least in part, by the activation of presynaptic D4 receptors in agreement with the presence of these receptors in the SON (Defagot et al. 1997).

METHODS

Preparation of hypothalamic slices

Acute hypothalamic slices were obtained using procedures similar to those described previously (Oliet and Poulaîn 1999). Briefly, female Wistar rats (1–2 mo old) were anesthetized and decapitated. The brain was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O2-5% CO2. Thin coronal slices (300 μm) were cut with a vibratome (Leica) from a block of tissue containing the hypothalamus. Slices including the SON were hemisectioned along the midline and allowed to recover for ≥1 h before recording. A slice was then transferred into a recording chamber where it was submerged and continuously perfused (1–2 ml/min) with ACSF at room temperature. The composition of the ACSF was (in mM) 123 NaCl, 2.5 KCl, 1.2 KH2PO4, 26.2 NaHCO3, 1.3 MgCl2, 2.5 CaCl2, and 10 glucose (pH 7.4; 295–300 mosmol/kg). In experiments in which L745,870 was bath-applied, a different ACSF was made to facilitate solubility of the drug. The composition of this solution was (in mM) 150 NaCl, 2.5 KCl, 1.2 KH2PO4, 10 HEPES, 1.3 MgSO4, 2.5 CaCl2, and 10 glucose (pH 7.4; 295–300 mosmol/kg).

Patch-clamp recording

Magnocellular neurons were visually identified using infrared differential interference contrast microscopy (Olympus). Patch-clamp recording pipettes (3–5 MΩ) were filled with a solution containing (in mM) 141 CsCl, 10 HEPES, 5 QX-314, and 2 Mg-ATP (adjusted to pH 7.1 with CsOH). Membrane currents were recorded using an Axopatch-1D amplifier (Axon Instruments). Signals were filtered at 2 kHz and digitized at 5 kHz via a DigiData 1200 interface (Axon Instruments). Series resistance (6–20 MΩ) was monitored on-line and cells were excluded from data analysis if more than a 15% change occurred during the course of the experiment. All cells were held at −60 mV in voltage-clamp mode. Spontaneous unitary synaptic currents (miniatures) obtained in the presence of tetrodotoxin (TTX) were stored on videotape via a pulse-code modulator (Neurodata), detected, and analyzed off-line using Axograph (Axon Instruments). To evoke synaptic responses, a glass stimulating electrode filled with ACSF and connected to an isolated stimulator (Digitimer) was placed in the hypothalamic region dorsomedial to the SON, as described previously (Kombian et al. 1996). Synaptic responses were evoked at 0.05 Hz, using square pulses of 0.1-ms duration, and analyzed on-line using pClamp (Axon Instruments). To study the paired-pulse facilitation ratio (PPF ratio), two synaptic responses (S1 and S2) were evoked by a couple of stimuli given at 60-ms intervals. PPF ratio was expressed as the ratio of the amplitude of the second synaptic response over the first synaptic response (S2/S1).

Data were compared statistically with either the paired or the unpaired Student’s t-test accordingly. Miniature amplitude and frequency distributions were compared using the nonparametric Kolmogorov-Smirnov test. Significance was assessed at P < 0.05. All data are reported as means ± SE.

Drugs

All drugs were bath-applied. Appropriate stock solutions were made and diluted with ACSF just before application. QX-314 chloride (Alomone labs) was diluted directly in the patch-solution. Drugs used were 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, RBI), bicuculline methobromide, bromocriptine (Tocris), clozapine, dopamine, L750,667, L745,870 (Sigma), PD168077, SCH 23930, SKF 38393 (Tocris), sulpiride, and TTX (Sigma).

RESULTS

Whole cell voltage-clamp recordings were obtained from 74 supraoptic neurons. In the presence of 10 μM CNQX and 0.5 μM TTX in the external solution, inwardly going spontaneous synaptic currents were recorded at a holding potential of −60 mV. These events were completely abolished by bath-application of bicuculline (20 μM; Fig. 1A) and had a reversal potential close to 0 mV (not shown) in agreement with the equilibrium potential for chloride ions in our experimental conditions. Taken together these findings indicated that these unitary currents were entirely mediated by the activation of GABA-A receptors (Randle et al. 1986). The mean frequency of mIPSCs varied greatly between neurons from 0.5 to 7.7 Hz, whereas the mean amplitude ranged from −66.0 to −348.6 pA.
**Dopamine inhibits mIPSCs**

Bath application of dopamine (DA; 300 μM) caused an important and reversible reduction of mIPSC activity (Fig. 1B). This effect was associated with a rightward shift of the event interval distribution (Fig. 1D; P < 0.05), reflecting a decrease in mIPSC frequency. Conversely, DA neither affected the amplitude distribution (P > 0.05) nor the kinetics of the mIPSCs, as illustrated in Fig. 1C. On average, DA significantly inhibited the frequency of mIPSCs by 60.8 ± 5.8% (n = 9; P < 0.05; Fig. 1E), whereas the mean amplitude of these unitary events was not significantly affected (−7.2 ± 4.0%; P > 0.05). This specific modulation of mIPSC frequency is usually considered to reflect a presynaptic modulation of transmission (Redman 1990). Our results, therefore, indicate that dopamine acts presynaptically to inhibit GABA release in the SON. Because DA did not change the amplitude distribution or the kinetics of the unitary synaptic GABAergic currents, we have focused the rest of our study on mIPSC frequency except when otherwise stated.

We next investigated the dose-dependent profile of this inhibition by using varying concentrations of DA (Fig. 2A). The frequency of mIPSCs was reduced, on average, by 4.1 ± 4.8% (n = 5 cells), 2.9 ± 4.3% (n = 5), 53.0 ± 7.0% (n = 5), 69.0 ± 8.2% (n = 3) and 60.8 ± 5.8% (n = 9) for 0.3, 3, 30, 100, and 300 μM dopamine, respectively. As illustrated in Fig. 2B, fitting these data with a Boltzmann function revealed values for half-maximal inhibition (IC_{50}) of 20.8 ± 2.2 μM and a threshold of 3 μM. Maximal inhibitory responses were obtained for concentrations of ≥100 μM.

**Dopamine-mediated inhibition of mIPSCs is affected by D4 receptor antagonist**

To identify the subtype of receptor involved in the modulation of inhibitory GABAergic transmission by DA, we used several dopaminergic antagonists. In this set of experiments, the antagonist was first added to the bathing solution and DA (300 μM) was subsequently applied (Fig. 3, A and B). In the presence of the D2/D3 receptor antagonist sulpiride (10 μM), dopamine retained its full ability to inhibit mIPSC frequency (−57.6 ± 0.7%; n = 4; P > 0.05). Similarly, blockade of D1/D5 receptors with SCH 23390 (100 μM) failed to prevent the dopamine-dependent inhibition (−62.0 ± 0.7%; n = 4; P > 0.05). These findings suggested that D1, D2, D3, and D5 receptors are not implicated in the inhibitory response induced by DA. We next investigated whether D4 dopamine receptors could be involved in the inhibition of GABA release because it has been recently reported that activation of these receptors inhibited glutamate release in the SON (Price and Pittman 2001). Interestingly, in the presence of the atypical antipsychotic drug clozapine (50 μM), an antagonist of dopamine receptors that has a higher affinity than for D4 over D2/D3 receptors (Seeman and Van Tol 1994), dopamine-induced inhibition of mIPSC frequency was partially prevented (−39.9 ± 4.3%; n = 6; P < 0.05). The involvement of D4 receptors was confirmed by the use of L750,667 and L745,870, two highly specific antagonists of these receptors (Kulagowski et al. 1996; Patel et al. 1996). In the presence of L750,667 (50 μM) and L745,870 (50 μM), dopamine reduced mIPSC frequency by 27.7 ± 6.4% (n = 6) and 19.8 ± 5.6% (n = 5) respectively (Fig. 3, A and B). These values were significantly different from those obtained by DA alone in the absence of these antagonists (P < 0.05), indicating that D4 receptors mediate part of, if not all, the action of dopamine on mIPSCs in the SON. Clozapine, L750,667, nor L745,870 affected mIPSC frequency by themselves, suggesting that D4 receptors were not activated tonically by endogenous dopamine.

**Dopamine-mediated inhibition of mIPSCs is mimicked by a D4 receptor agonist**

If D4 receptors mediate the inhibition of GABAergic transmission, a specific agonist of this receptor subtype should mimic this action. On the other hand, agonists of D1, D2, D3, and D5 receptors should be without effect on GABAergic synaptic currents. We first examined the action of PD168077, a potent and selective D4 receptor agonist (Glase et al. 1997). Bath application of PD168077 (30 μM; n = 8) reduced the frequency (−51.6 ± 4.2%; P < 0.05) but not the amplitude (−3.6 ± 1.8%; P > 0.05) of mIPSCs (Fig. 4). This finding confirms the existence of presynaptic D4 receptors on GABAergic terminals in the SON whose activation leads to an inhibition of transmitter release. Conversely, exposure of SON neurons to SKF 38393 (30 μM; n = 4), a specific D1/D5 receptor agonist, and bromocriptine (50 μM; n = 4), a specific D2/D3 receptor agonist, was without effect on mIPSC fre-
quency (−5.2 ± 6.7 and −2.1 ± 4.4%, respectively; \( P > 0.05; \) Fig. 4D). Taken together, these data indicate that, in the SON, indeed dopamine inhibits mIPSCs through the activation of D4 receptors.

Dopamine-mediated inhibition of evoked IPSC amplitude

Although our data indicate that DA inhibits spontaneous GABA-A receptor-mediated currents, it remained to be determined whether GABA release evoked by electrical stimulation was also sensitive to dopamine. As illustrated in Fig. 5, A and B, bath application of DA (300 \( \mu M \)) was found to reversibly reduce the amplitude of evoked IPSCs by 60.5 ± 5.4% \(( n = 9)\). This inhibitory action of DA was accompanied by an increase in paired-pulse facilitation ratio from 1.21 ± 0.15 to 2.22 ± 0.34 \(( n = 9); \) Fig. 5, C and D) as expected from a presynaptic reduction of transmitter release (Zucker and Regehr 2002). To make sure that the action of DA on evoked GABA release was mediated through D4 receptors, we tested the effect of PD168077 (30 \( \mu M \)) on evoked inhibitory postsynaptic currents (IPSCs; Fig. 5, A and B). In the presence of the D4 agonist, the amplitude of the response was inhibited by 54.6 ± 8.6% \(( n = 5)\), an effect similar to that obtained with 300 \( \mu M \) DA. These results are in agreement with those obtained on mIPSCs and confirm the presence of presynaptic D4 receptors whose activation depresses the release of GABA in the SON.

DISCUSSION

Dopamine modulates GABA release in various brain regions such as the cortex (Gonzales-Islas and Hablitz 2001; Seamans et al. 2001) and the substantia nigra (Miyazaki and Lacey 1998). This disinhibitory process represents a potent regulatory mechanism of neuronal excitability. Our data indicate that, in the SON, it is through a similar mechanism that dopamine could contribute to the regulation of neurohypophysial hormone secretion (Bridges et al. 1976; Forsling and Williams 1984; Ivanyi et al. 1986; Moos and Richard 1982; Urano and Kobayashi 1978; Yamagushi and Hama 1989).

Presynaptic inhibition of GABAergic transmission

Suprathreshold applications of dopamine inhibited GABAergic synaptic currents in all SON neurons tested. Because we did not record from a specific area in the SON, it is very likely that DA modulates GABAergic transmission in both OT and VP neurons in agreement with the observation that DA modulates the release of both neurohypophysial hormones (Bridges et al. 1976; Moos and Richard 1982).

FIG. 3. Characterization of the dopamine receptor subtype responsible for the inhibition of mIPSCs. A: example of recordings obtained from 5 different supraoptic neurons where the action of DA (300 \( \mu M \)) was tested in the presence of SCH 23390 (100 \( \mu M \)), sulpiride (10 \( \mu M \)), clozapine (50 \( \mu M \)), L745,870 (50 \( \mu M \)), and L750,667 (50 \( \mu M \)). B: summary histogram of DA-mediated inhibition of mIPSC frequency in the presence of various dopaminergic receptor antagonists. DA-mediated inhibition was significantly affected by clozapine, L750,667, and L745,870 but not by SCH 23390 or sulpiride. Number of cells are indicated above the bars.

FIG. 4. Effect of a D4-dopaminergic receptor agonist on mIPSC activity. A: consecutive traces showing typical mIPSCs before and during application of 30 \( \mu M \) PD168077. B: cumulative plots of amplitude distribution was similar before (thick line) and during (thin line) PD168077 application for cell illustrated in A. C: PD168077 caused the event interval distribution to be shifted toward the right, indicating a decrease in mIPSC frequency. D: summary histogram of the effect of various DA receptor agonists on mIPSC frequency. Application of SKF 38393 (30 \( \mu M \)) and bromocriptine (50 \( \mu M \)) did not affect mIPSC activity, whereas PD168077 (30 \( \mu M \)) significantly reduced the frequency of these events like DA. Number of cells are indicated above the bars.
because transmitter release has been shown to be sensitive to protein kinase C (PKA) (Missale et al. 1998) and dopamine are associated with the modulation of a cAMP-cascade leading to exocytosis. Because most of the effects could affect the proteins involved in the intracellular signaling pathway (Mei et al. 1995).

Alternatively, the activation of presynaptic DA receptors could affect the proteins involved in the intracellular signaling cascade leading to exocytosis. Because most of the effects of dopamine are associated with the modulation of a cAMP-dependent protein kinase (PKA) (Missale et al. 1998) and because transmitter release has been shown to be sensitive to PKA (Kondo et al. 1997; Trudeau et al. 1996), the action of DA observed in our experiments could reflect a change in cAMP level in the terminals. Interestingly, presynaptic inhibition of GABAergic release in the SON appears to be inhibited through the activation of several presynaptic receptors coupled to adenyl cyclase activity. This includes group III mGluRs (Piet et al. 2003; Schrader and Tasker 1997), GABA-B (Mogín et al. 1998), and adenosine A1 (Oliet and Poullain 1999) receptors. It is also possible that other intracellular signaling mediators, like protein kinase C, are involved in this process.

**Receptor identification**

To identify the dopamine receptors involved in the inhibition of GABA release, we used various antagonists and agonists exhibiting different selectivity for DA receptor subtypes. In all likelihood, the receptor responsible for inhibition of mIPSC activity is the D4 receptor. This is based on the sensitivity of DA-induced response to L750,667 and L745,870, which are specific D4 antagonists, and on the observation that the specific D4 agonist PD168077 mimicked DA inhibitory action on both miniature and evoked IPSCs. This finding was strengthened by the lack of effect of sulpiride and SCH 23390, D2/D3 and D1/D5 antagonists respectively, on DA-mediated inhibition of GABAergic transmission. Furthermore, SKF 81297 and bromocriptine, D1/D5 and D2/D3 specific agonists, respectively, did not affect mIPSC activity. Finally, clozapine, an antagonist that has a 10-fold greater affinity for D4 than D2 and D3 receptors (Seeman and Van Tol 1994), was able to partially prevent the inhibitory action of DA. Taken together, these results demonstrate that DA-induced inhibition of GABA release in the SON is mediated by the activation of dopamine D4 receptors. Dopamine D4 receptor activation has also been reported to inhibit GABAergic transmission in the prefrontal cortex through a PKA-dependent mechanism (Wang et al. 2002), but in this structure, D4 receptor appears to have an action on postsynaptic GABA-A receptors rather than on transmitter release.

**Functional implications**

The inhibitory action of DA on GABAergic transmission in the SON neurons should lead to a disinhibition of magnocellular neurons and, as a consequence, to an augmented excitability of the hypothalamo-neurohypophysial system. This is in agreement with the excitatory effects observed in vivo in response to injections of DA in the SON or intracerebroventricularly (Bridges et al. 1976; Moos and Richard 1982; Urano and Kobayashi 1978). However, to understand better the action of DA in the SON, it is important to take into consideration the various actions of DA that have been reported in previous in vitro studies. In particular, it has been shown that DA, at concentrations similar to those used in the present study, inhibits glutamate release via the activation of presynaptic D4 receptors (Price and Pittman 2001), an effect that should reduce cell excitability and hormone secretion. Conversely, DA within the same range of concentrations acts on postsynaptic D2 receptors causing a membrane depolarization due to the activation of a cationic conductance (Yang et al. 1991). In view of these different effects, the overall action of DA on SON

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**Figure 5.** Effect of dopamine on evoked IPSC. A: examples of recordings where applications of 300 μM DA (top) and 30 μM PD168077 (bottom) reversibly reduced the amplitude of evoked IPSC. B: summary histogram illustrating the inhibitory action of DA and PD 168077 on evoked IPSC amplitude. C: example of a recording where pairs of stimulation were applied at 60-ms interval in the presence and absence of DA (top). Superimposition of the 2 traces scaled to the 1st IPSC obtained in absence of DA reveals that paired-pulse facilitation (PPF) ratio is increased (bottom). D: summary histogram illustrating the increased in PPF ratio induced by DA.
neurons and its consequence on hypothalamo-neurohypophyseal hormones secretion remains speculative. In particular, whether one of these dopamine responses is predominant in situ has to be determined. Inhibiting glutamatergic and GABAergic synaptic activity, and therefore reducing background synaptic noise, could have several consequences on the postsynaptic cell. It could make the neuron more electrically compact by increasing input resistance (Paré et al. 1997), reduce shunting inhibition and increase the gain of the input-output neuronal response (Chance et al. 2002; Prescott and De Koninck 2003) as well as render the cell less sensitive to weak signals (Wiesenfeld and Moss 1995). One attractive hypothesis could be that DA-mediated inhibition of both GABA and glutamate synaptic activities in the SON isolates, to some extent, the neurons from excitatory and inhibitory drives mediate through these inputs. This will make SON neurons more responsive to the activation of D2 postsynaptic receptors, for instance. In other words, this process could serve to significantly enhance the signal-to-noise ratio for the information originating from dopaminergic inputs.

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DISCLOSURES

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