Dopamine D4 Receptor Activation Inhibits Presynaptically Glutamatergic Neurotransmission in the Rat Supraoptic Nucleus

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Price, Christopher J. and Quentin J. Pittman. Dopamine D4 receptor activation inhibits presynaptically glutamatergic neurotransmission in the rat supraoptic nucleus. J Neurophysiol 86: 1149–1155, 2001. Oxytocin and vasopressin release from magnocellular neurons of the supraoptic nucleus is under the control of glutamate-dependent excitation. The supraoptic nucleus also receives a generalized dopaminergic input from hypothalamic sources. To determine if dopamine can influence this excitatory drive onto the magnocellular neurons, we used whole-cell patch clamp to record the effect of dopamine on evoked and miniature excitatory postsynaptic currents in rat hypothalamic slices. Dopamine exposure (30 μM to 1 mM) induced a large and reversible reduction in the amplitude of evoked excitatory postsynaptic current in nearly all magnocellular cells tested. D4 receptors appeared to mediate dopamine’s activity, based on inhibition of the response with 50 μM clozapine, but not by SCH 23390 or sulpiride, and mimicry of dopamine’s action with the D4 specific agonist, PD 168077. Analysis of paired-pulse experiments and miniature postsynaptic currents indicated that dopamine’s action involved a presynaptic mechanism, since the frequency of miniature postsynaptic currents was reduced with dopamine exposure without any change in current kinetics or amplitude, while the paired-pulse ratio increased. We therefore have demonstrated for the first time a role for dopamine D4 receptors in the supraoptic nucleus in the presynaptic inhibition of glutamatergic neurotransmission onto magnocellular neurons.

INTRODUCTION

Dopamine is one of the major modulatory neurotransmitters in the CNS (Jaber et al. 1996). To achieve this function, dopamine binds to one of two families of G-protein-coupled receptors (Missale et al. 1998; Seeman and Van Tol 1994). The D1 family consists of D1 and D5 receptors and, in general, is positively coupled to adenylate cyclase activity. The D2 family consists of D2, D3, and D4 receptors and is negatively coupled to adenylate cyclase activity. D1 and D2 receptors have a widespread distribution throughout the nervous system, while D3, D4, and D5 receptors tend to have a more restricted distribution (Defagot et al. 1997; Jaber et al. 1996; Levant 1997). In regards to cellular localization of the receptor, all subtypes can be found either presynaptically or postsynaptically, allowing dopamine to exert its neuromodulatory effects on both sides of the synapse (Behr et al. 2000; Jaber et al. 1996; Koga and Momiyama 2000; Levant 1997; Nicola et al. 2000; Svingos et al. 2000).

The supraoptic nucleus (SON) of the hypothalamus contains oxytocinergic and vasopressinergic magnocellular neurons. The magnocellular neurons receive glutamatergic inputs resulting in AMPA and NMDA receptor-dependent excitation of the cells and subsequent oxytocin or vasopressin release from the posterior lobe of the pituitary (Armstrong 1995; Stern et al. 1999). Among a variety of other inputs, the SON receives a diffuse and generalized dopaminergic input from neurons from the A14 and A15 cell groups (van Vulpen et al. 1999). In the SON, dopaminergic fibers form synaptic terminals near both the dendrites and the cell bodies of magnocellular neurons (Buijs et al. 1984; Decavel et al. 1987). While the presence of dopamine in the SON is clear, less straightforward is the role that dopamine plays in the modulation of magnocellular neuron function. Previous reports have described the effect of dopamine on the release of oxytocin in lactating rats to be either excitatory, via a D1-like receptor-dependent mechanism, or inhibitory, likely via a D2-like receptor-dependent mechanism (Crowley et al. 1987; Parker and Crowley 1992). Furthermore, dopamine has also been shown to depolarize magnocellular neurons in SON explants from male rats in a D2-like receptor-dependent manner (Yang et al. 1991).

Most of the studies examining dopamine actions on SON magnocellular neurons describe general effects on SON output, while little is known about the effects of dopamine on specific ionic conductances. To look for a specific action of dopamine on the modulation of magnocellular neuron excitation, we directed our attention to examining a specific effect of dopamine on excitatory synaptic responses. Using hypothalamic slices from male rats, whole-cell patch-clamp recordings demonstrated that dopamine exposure resulted in a reduction in the amplitude of evoked excitatory postsynaptic currents (EPSCs) in virtually all neurons tested. Furthermore, this action was pharmacologically attributed to the activation of dopamine D4 receptors, which is consistent with immunocytochemical studies showing D4 receptors to be highly expressed in the SON.

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METHODOLOGY

Preparation of hypothalamic slices

Coronal hypothalamic slices (400 μm) containing the SON were obtained from male Sprague-Dawley rats (130–230 g, U. Calgary Biosciences and Charles River). Briefly, rats were anesthetized with halothane and decapitated, and the brain quickly removed, trimmed, and mounted on a vibratome in ice-cold dissecting solution (in mM: 25 NaCl, 2.5 KCl, 1 CaCl₂, 4 MgCl₂, 1.2 NaH₂PO₄, 18 NaHCO₃, and 200 sucrose), which was constantly bubbled with 95% O₂-5% CO₂. Slices were then incubated for at least 2 h at room temperature before being hemisected and transferred into a recording chamber where the slice was constantly perfused with artificial cerebrospinal fluid (ACSF) (in mM: 126 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 2.4 CaCl₂, 18 NaHCO₃, and 17 glucose). ACSF was heated to 28–32°C to obtain a constant temperature and constantly bubbled with 95% O₂-5% CO₂. To prevent contamination of EPSCs with GABA-mediated currents, 50 μM picrotoxin was added to the perfusing ACSF.

Electrophysiology and analysis

Blind whole-cell patch recordings were then made from electrophysiologically identified SON magnocellular neurons. Neurons were identified as being magnocellular neurons by the presence of a delay in the onset of action potential initiation with depolarizing current pulses during current clamp recordings (Hoffman et al. 1991; Kombian et al. 2000). Further, in several of the recordings, Lucifer yellow (Polysciences) was included in the pipette solution. Subsequent viewing of the slices revealed that all of the filled cells resided within the supraoptic nucleus (data not shown). Electrodes used for whole-cell recordings were pulled from thin-walled borosilicate capillary tubes and had resistances between 2 and 7 MΩ when filled with recording solution (130 mM K-glucuronate, 10 mM KCl, 10 mM NaCl, 1 mM MgCl₂, 10 mM EGTA, 2 mM Mg-ATP, 0.3 mM Li-GTP, 1 μM Leupeptin, and 10 mM HEPES; pH 7.3 with KOH). Leupeptin was included in this solution to improve recording stability (Kay 1992). EPSCs were evoked using a tungsten bipolar electrode placed dorsomedial to the SON, using voltage pulses that typically were between 3 and 30 V and 600 μs in duration. Following each evoked event, a hyperpolarizing voltage pulse was applied to the cell to assess access resistance. EPSCs were recorded at a holding potential of ~70 mV, using an Axon 200A amplifier and Clampex 6.0 software (Axon Instruments). For evoked EPSCs, stimuli-eliciting responses of 50–60% maximum were delivered once every 15 s and four consecutive responses were averaged. Data were acquired at a sampling rate of 10 kHz and filtered at 5 kHz. Data from evoked EPSC experiments were measured using Clampfit 6.0 (Axon Instruments) and exported to Prism 3.0 (GraphPad) where plots were made and statistical analysis performed. Experiments examining miniature excitatory postsynaptic currents (mEPSC) were performed in standard ACSF. As several laboratories (Kabashima et al. 1997; Kombian et al. 2000) have found no difference in the frequency or amplitude of spontaneous EPSCs recorded in the presence or absence of tetrodotoxin, these can be considered to be the activity of glutamatergic afferents. Exposure of slices to 100 μM dopamine for 5 min resulted in a marked reduction in the amplitude of evoked CNQX-sensitive EPSCs to 45 ± 5% of control values (n = 17). The response could often be seen to start within approximately 2 min of starting the flow of dopamine-containing ACSF and peaked at between 4 and 5 min. This reduction in EPSC amplitude was observed in all but two neurons tested, was completely reversible on washing, and was repeatable with reapplication of dopamine (Fig. 1). Furthermore, immunostaining with antibodies against oxytocin-neurophysin of neurons that had been labeled during recording with Lucifer yellow indicated that both stained and unstained neurons, presumably vasopressinergic neurons, responded to dopamine (data not shown). On scaling the EPSC recorded in dopamine back to its original amplitude, it was apparent that the kinetic properties of the EPSC were not altered (Fig. 1A). Measurement of the decay time constant before and in the presence of dopamine revealed no significant difference (paired t-test, P = 0.8795).

Dose response experiments revealed that the receptor responsible for dopamine-dependent reduction in the evoked EPSC had an EC₅₀ of approximately 29 μM, a threshold concentration above 10 μM, and maximal responses at concentrations above 100 μM (Fig. 1B). In addition to the changes in EPSC amplitude brought on by dopamine, in most cells dopamine application resulted in a small inward shift in holding current (~13.7 ± 2.5 pA; n = 24). However, the action of dopamine on the EPSC was not correlated to the magnitude of the dopamine-induced inward current (R² = 0.1933).

D4 receptors mediate dopamine-dependent inhibition of evoked EPSCs

To determine the pharmacological identity of the receptor responsible for dopamine-dependent reduction in EPSC amplitude, the activities of various dopamine receptor agonists and antagonists were tested. In the presence of the D1/D5 receptor antagonist SCH 23390 (50–100 μM), at concentrations over those needed to inhibit D1/D5-mediated actions of dopamine in

RESULTS

Dopamine inhibits evoked glutamatergic EPSCs

During whole-cell recordings from SON magnocellular neurons, EPSCs could reliably be evoked by stimulation of glutamatergic afferents. Exposure of slices to 100 μM dopamine for 5 min resulted in a marked reduction in the amplitude of evoked CNQX-sensitive EPSCs to 45 ± 5% of control values (n = 17). The response could often be seen to start within approximately 2 min of starting the flow of dopamine-containing ACSF and peaked at between 4 and 5 min. This reduction in EPSC amplitude was observed in all but two neurons tested, was completely reversible on washing, and was repeatable with reapplication of dopamine (Fig. 1). Furthermore, immunostaining with antibodies against oxytocin-neurophysin of neurons that had been labeled during recording with Lucifer yellow indicated that both stained and unstained neurons, presumably vasopressinergic neurons, responded to dopamine (data not shown). On scaling the EPSC recorded in dopamine back to its original amplitude, it was apparent that the kinetic properties of the EPSC were not altered (Fig. 1A). Measurement of the decay time constant before and in the presence of dopamine revealed no significant difference (paired t-test, P = 0.8795).

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DOPAMINE INHIBITION OF EPSCs IN THE SUPRAOPTIC NUCLEUS

Dopamine reversibly and repeatedly inhibits evoked EPSCs. A: average EPSCs plotted (4/min) showing the time course of dopamine-dependent inhibition of evoked EPSC amplitude following perfusion of the slice chamber for 5 min with 100 μM dopamine. Perfusion of the slice with 20 μM CNQX confirmed that the EPSCs were non-NMDA glutamate currents. Insert: on the left are averaged EPSC traces taken before (1) and after 5 min of exposure to dopamine (2). On the right are these two traces scaled to the same amplitude, revealing that no change in the kinetic properties of EPSCs was observed following dopamine exposure. B: dose-response relationship for dopamine-dependent reduction in evoked EPSC amplitude. Fraction of current reduction was calculated according to 1 - (EPSC_D / EPSC_control). Fitting of the data with the sigmoidal dose-response equation from Prism gave an EC50 of 29 μM. Data for each concentration are the mean ± SE from 3 to 17 neurons.

Dopamine is known to inhibit the release of glutamate at presynaptic sites in the supraoptic nucleus (SON). This inhibition is mediated through the activation of D4 receptors, which are predominantly found in SON magnocellular neurons. Dopamine reduces the size of the second EPSC, thereby increasing the paired-pulse ratio (pulse2/pulse1) and reducing the amount of transmitter released with the second pulse. This effect is specific to D4 receptors, as it is not observed with D1 or D2 receptors.

To further understand the mechanism of dopamine inhibition, experiments were conducted using dopamine-receptor antagonists. The experiments using dopamine-receptor antagonists suggested that the pharmacological identity of the dopamine receptors involved in dopamine-dependent reduction in EPSC amplitude were D4 receptors. To address this possibility further, dopamine-receptor agonists with specificity for the different receptor subtypes were tested for their ability to mimic dopamine’s actions. The D1 receptor full-agonist SKF 81297 (10–30 μM) failed to mimic dopamine at reducing EPSC amplitude, consistent with the inactivity of the D1 antagonist, SCH 23390 (Fig. 3A). The general D2 receptor agonist quinpirole (50–200 μM), while mimicking dopamine’s action on the inward current (6.5 ± 3.423 pA; n = 7), was ineffective at mimicking the action of dopamine on the evoked EPSC (Fig. 3B). However, exposure of magnocellular neurons to the recently developed specific D4 agonist PD 168077 (Glase et al. 1997; 30 μM) resulted in the reduction of EPSC amplitude to a similar extent to that seen with 100 μM dopamine (Fig. 3C). ANOVA confirmed that the only agonist whose performance was not significantly different from that of dopamine’s was PD 168077 (Fig. 3D). Therefore, from the results of experiments with both dopamine receptor antagonists and agonists, the identity of the dopamine receptor responsible for reduction in EPSC amplitude is consistent with it being a D4 receptor.

Dopamine presynaptically inhibits glutamate release

Dopamine receptors have been demonstrated to occur on both presynaptic and postsynaptic membranes. To determine if the locus of dopamine-dependent inhibition of EPSC amplitude in SON magnocellular neurons is pre- or postsynaptic, experiments examining paired-pulse facilitation and mEPSCs were conducted. The results of paired-pulse experiments, where two voltage pulses separated by 50 ms were used to evoke pairs of EPSCs in the cell, showed that the paired-pulse ratio (pulse2/pulse1) was significantly increased (P < 0.05, n = 9 cells) when neurons were exposed to 100 μM dopamine due to a relatively greater reduction in the amplitude of the first EPSC (Fig. 4). Since paired-pulse facilitation is a result of residual calcium in the presynaptic terminal enhancing the amount of transmitter released with the second pulse and therefore the size of the second EPSC, changes in the paired-pulse ratio suggest a presynaptic locus for dopamine’s action.

A second method to identify the locus of dopamine-depen-
dent inhibition of evoked EPSCs involved examination of mEPSC activity. The frequency of mEPSC events showed a significant and reversible reduction in the presence of 100–200 μM dopamine (Fig. 5A; \(P < 0.05, n = 5\) cells). This change in frequency can be graphically demonstrated as a significant increase in the inter-event interval (585.1 ± 122.6 vs. 1,524 ± 270.4 ms; \(n = 5\) cells; \(P < 0.01\) ) (Fig. 5B). This effect on mEPSC frequency was not accompanied by a significant change in the mean mEPSC amplitude (11.86 ± 0.47 vs. 12.27 ± 0.87 pA, \(n = 5\) cells; \(P > 0.05\) ) (Fig. 5B), further strengthening the case for a presynaptic locus. In addition, analysis of mEPSC kinetic properties indicated that there was no significant difference in either the time to peak or the decay time constant of the events (Fig. 5C; \(P > 0.05\) in both instances). The frequency of mEPSCs was also reduced by 45 ± 11% (\(n = 4\) ) following treatment of slices with the D4-specific agonist PD 168077 (50 μM), again without a significant change in mEPSC amplitude (paired \(t\)-test, \(P = 0.4179\) ).

**FIG. 2.** Dopamine receptor antagonist characterization of dopamine-dependent reduction in evoked EPSC amplitude in representative experiments. A: perfusion with 50 μM of the D1/D5 receptor antagonist SCH 23390 did not block the inhibition of evoked EPSC amplitude induced by 50 μM dopamine. B: the D2/D3 receptor antagonist (+)-sulpiride (10 μM) was also ineffective at inhibiting the reduction of evoked EPSC amplitude induced by 50 μM dopamine. C: 50 μM clozapine blocks dopamine’s actions on the evoked EPSC. D: summary histogram of dopamine antagonist experiments. Fraction of current remaining was calculated according to (EPSC_{control} / EPSC_{DA}). * Only for clozapine and L750667 was the action of dopamine significantly inhibited; \(n\) is located over each column.

**FIG. 3.** Activity of dopamine receptor-specific agonists in representative experiments. A: the D1/D5-specific agonist SKF 81297 (30 μM) did not mimic, but dopamine (100 μM) was effective at reducing EPSC amplitude. B: the stereotypical D2 family agonist quinpirole (50 μM) was a poor mimic of dopamine (100 μM). C: the D4 receptor agonist PD168077 (30 μM) induced a large reduction in the amplitude of evoked EPSCs. D: summary histogram for dopamine receptor agonist activities revealed that only PD 168077 was equivalent to dopamine in the ability to reduce evoked EPSC amplitude as compared with 100 μM dopamine. Fraction of current remaining was calculated according to (EPSC_{control} / EPSC_{DA}). * Significantly different from dopamine; \(n\) is located over each column.
Therefore, these results, together with the results from paired-pulse experiments, indicate that dopamine exerts its effects in the SON by inhibiting glutamate release from excitatory inputs onto the magnocellular neurons.

**DISCUSSION**

In the supraoptic nucleus, the frequency and pattern of magnocellular cell firing is a major determinant of how much vasopressin and oxytocin are released at the posterior pituitary into the systemic circulation (Armstrong 1995; Bourque and Renaud 1991). Individual magnocellular neurons receive both excitatory glutamatergic and inhibitory GABAergic inputs to regulate their excitability. On top of this is another layer of control influencing the fidelity and magnitude of glutamatergic or GABAergic neurotransmission. Adenosine, GABA, opioids, oxytocin, and vasopressin are among the transmitters which inhibit glutamatergic neurotransmission onto magnocellular neurons via presynaptic mechanisms (Kombian et al. 1996, 1997; Liu et al. 1999b; Oliet and Poullain 1999). Adenosine and GABA also presynaptically inhibit the release of GABA onto magnocellular neurons (Mouginot et al. 1998; Oliet and Poullain 1999). The overall result is that the activity of vasopressin and oxytocin neurons is highly regulated via modulation of excitatory and inhibitory transmitter release from afferent fibers contacting these neurons (Pittman 1999).

Here we show that exogenously applied dopamine also inhibits excitatory neurotransmission onto the SON magnocellular neurons. This occurred in nearly all neurons tested. This finding is consistent with immunocytochemical evidence showing dopaminergic fibers having a diffuse, generalized distribution throughout the SON (van Vulpen et al. 1999). We also show that the receptor subtype responsible for inhibition of excitatory neurotransmission is the D4 receptor, based on the sensitivity of the response to clozapine and the D4 specific antagonist L750,667 as well as the ability of the D4 receptor-specific agonist PD 168077 to mimic dopamine’s activity. This is also consistent with immunocytochemical evidence, which shows D4 receptors to be highly expressed in the SON (Defagot et al. 1997). However, the labeling observed by Defagot and colleagues suggests that the magnocellular neurons themselves are labeled with the D4 antibody, which would argue

**FIG. 4.** Effect of dopamine on paired-pulse facilitation. A: for paired-pulse experiments, pairs of electrical stimuli, separated by 50 ms, were delivered at 15 s intervals. The mean (±SE) paired-pulse ratio (pulse2/pulse1) was calculated for 9 cells. Exposure of SON magnocellular neurons to 100 μM dopamine for 5 min resulted in an increase in the paired-pulse ratio. *Inset*: sample traces showing paired-pulse experiment before and 5 min after dopamine application. B: histogram summarizing the results from paired-pulse experiments. The 5 data points just before dopamine application, 5 min after start of application, and the last 5 data points, after 15 min of washing, for each neuron were averaged to give the mean paired-pulse ratios used in the histogram. One-way ANOVA showed that this increase in the paired-pulse ratio to be significant (P < 0.05; n = 9).

**FIG. 5.** Analysis of miniature excitatory postsynaptic currents. A: traces showing 4 consecutive seconds of mEPSC activity before and after 5 min of perfusion of the slice with 100 μM dopamine. Traces are the first 4 s taken from a larger 60 s recording of miniature activity. B: plots of cumulative mEPSC amplitude (left) and inter-event interval (right) distributions reveal identical amplitude but increased inter-event interval in 100 μM dopamine. C: comparison of the kinetic variables of mEPSCs indicated there was no significant difference in the time to peak or the decay time constant before or in the presence of 100 μM dopamine (n = 5).
against our assertion that dopamine-dependent inhibition of excitatory neurotransmission had a presynaptic locus. However, without electron microscopic detail to argue otherwise, D4 receptors could still be located on glutamatergic terminals.

We have demonstrated for the first time a role for D4 receptors in the SON. Surprisingly very little information has been gathered regarding the physiology of D4 receptors in the CNS. In cerebellar granule cells, D4 receptors have been shown to inhibit L-type calcium channels (Mei et al. 1995). Experiments on D4 receptors in expression systems have found receptor activation to inhibit voltage-gated potassium channels and activate inwardly rectifying potassium channels (Liu et al. 1999a; Werner et al. 1996). D4 receptors also have been shown to occur presynaptically in the rat nucleus accumens shell where they may mediate the action of dopamine (Svingos et al. 2000). With the recent availability of D4 receptor-specific agonists, it is likely that this paucity in understanding the physiological role for D4 receptors in the CNS should change.

The mechanism through which D4 receptor activation leads to presynaptic inhibition of evoked EPSCs remains unknown. Modulation of voltage-gated conductances, to alter either action potential kinetics or calcium channel properties could, by either mechanism, cause presynaptic inhibition by reducing calcium entry into the presynaptic terminal. However, because mEPSC activity has been shown to be largely independent of calcium entry, it is unlikely that this mechanism is responsible for dopamine’s actions on EPSCs in magnocellular neurons (Ienaga et al. 1998). There are, however, several studies that have shown the protein machinery directly responsible for synaptic vesicle release can also be subject to modulation (Morishita and Alger 1997; Trudeau et al. 1996). Interestingly, there are studies that have reported that activation of cAMP-dependent kinase can enhance the release of neurotransmitter (Chen and Regehr 1997; Kondo and Marty 1997). As the classical mechanism of D4 receptor activation is inhibition of cAMP-dependent kinase, it is tempting to speculate that this may be the mechanism for inhibition of glutamate release seen here.

In determining the pharmacological identity of the dopamine receptor, we took advantage of the relative selectivity of various agonists and antagonists for the different receptor subtypes. (±)-Sulpiride and SCH 23390 both have relatively high Kᵢ values for D4 receptors and both were ineffective as antagonists, unlike clozapine which shows an approximately 10-fold greater sensitivity for D4 receptors than either D2 or D3 receptors (Seeman and Van Tol 1994). However, less predictable was the situation for quinpirole, which has a Kᵢ for D4 receptors similar to that of dopamine but was a poor mimic at inhibiting evoked EPSCs. Recent experiments using a cloned rat D4 receptor expressed in hamster CCL39 cells have demonstrated that, whereas quinpirole had an EC₅₀ similar to that of dopamine for inhibiting forskolin-dependent cAMP accumulation, quinpirole was deficient compared with dopamine in its G-protein activation based on stimulation of [³⁵S]GTPγS binding in CCL39 cells (Gazi et al. 2000). Therefore, if the G-protein-mediating dopamine inhibition of evoked EPSCs were limiting, then quinpirole would be expected to be less effective than dopamine at initiating the response.

In several studies to date, dopamine has had a generally excitatory influence on the release of oxytocin and vasopressin from the SON. In two reports, one from work in lactating rats and one from experiments on rat hypothalamic slices, exogenously applied dopamine increased oxytocin release and the spiking frequency of oxytocinergic neurons (Mason 1983; Parker and Crowley 1992). Both of these effects were attributed to a D1-like receptor, which is also abundantly expressed in the SON (Rivkees and Lachowicz 1997), but no specific mechanism was found to account for this action. As our experiments were conducted in voltage clamp and examined specifically the evoked EPSC, we would not detect a parallel effect on voltage-gated currents, which would influence excitability in the absence of effects on excitatory neurotransmission. However, in another study, dopamine depolarized magnocellular neurons via activation of a nonspecific cation current through a mechanism dependent on D2 receptor activation (Yang et al. 1991). We found a small inward shift in holding current that would have depolarized the magnocellular neurons to a similar extent to that seen by Yang and colleagues (~4 mV). Moreover, this small inward shift in holding current was inhibited in the presence of the D2 antagonist sulpiride, supporting the observation that this was D2-dependent. This leads to the question of which dopamine response would predominate under physiological conditions. Unfortunately very little is known about the dynamics of dopamine release in the SON. Information examining the nature and control of the dopaminergic input to the SON would go a long way toward clarifying this issue.

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