Excitatory Actions of Dopamine Via D1-Like Receptors in the Rat Lateral Geniculate Nucleus

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Govindaiah, G. and Charles L. Cox. Excitatory actions of dopamine via D1-like receptors in the rat lateral geniculate nucleus. J Neurophysiol 94: 3708–3718, 2005. First published August 17, 2005; doi:10.1152/jn.00583.2005. The excitability of relay neurons in the dorsal geniculate nucleus (dLGN) can be altered by a variety of neuromodulators. The dLGN receives substantial dopaminergic input from the brain stem, and this innervation may play a crucial role in the gating of visual information from the retina to visual neocortex. In this study, we investigated the action of dopamine on identified dLGN neurons using whole cell recording techniques. Dopamine (2–200 μM) produced a membrane depolarization in >95% of relay neurons tested but did not alter excitability of dLGN interneurons. The D1-like dopamine receptor agonist SKF38393 (2–50 μM) produced a similar depolarization in dLGN relay neurons. However, the D2-like receptor agonists, bromocriptine (25–50 μM) and PPHT (1–50 μM), did not alter the membrane potential of relay neurons. SCH23390 (5–10 μM), a D1-like receptor antagonist, attenuated the depolarizing actions of both dopamine and SKF38393. Furthermore, the excitatory actions of dopamine and SKF38393 were attenuated by ZD7288, a specific antagonist for the hyperpolarization activated mixed cation current, Ih. Our data suggest that dopamine, acting via D1-like receptors, activates Ih, leading to a membrane depolarization. Through the modulation of dLGN neuronal excitability, ascending and descending activating systems may not only control the state of the thalamus such as the transition from slow-wave sleep to waking but also regulate the efficacy of information transfer during waking states.

INTRODUCTION

The transfer of visual information from the retina to visual neocortex is dynamically gated by the dorsal lateral geniculate nucleus (dLGN). It involves a complex interaction between intrinsic membrane properties of these thalamic neurons and an extensive complement of nonretinal inputs to dLGN that arise from cortical and subcortical regions (Jones 1985; Sherman and Guillery 1996, 2001; Steriade et al. 1997). Input from the retina in the visual system accounts for <10% of the total synaptic inputs onto individual dLGN relay neurons (Van Horn et al. 2000). The majority of synaptic inputs (>90%) onto thalamic relay neurons originates from inhibitory neurons (local interneurons and thalamic reticular neurons), deep layer neocortex, and various brain stem nuclei (Sherman and Guillery 2001). Similar proportions of inputs have also been described in the somatosensory system (Liu et al. 1995). The extensive complement of nonretinal inputs to the dLGN combine to modulate the responsiveness of relay cells to their retinal inputs and thereby influences the transfer of visual information to cortex.

The innervation of thalamic nuclei by various brain stem nuclei involves several different neuromodulators: cholinergic innervation from the pedunculopontine region, noradrenergic innervation from the locus coeruleus, serotonergic innervation from the dorsal raphe nucleus, and dopaminergic innervation from the mesencephalic reticular formation (De Lima and Singer 1987; De Lima et al. 1985; Hallanger et al. 1987; Hughes and Mullikin 1984; Jones 1985; McCormick 1992b; Morrison and Foote 1986; Papadopoulos and Parnavelas 1990a,b). One proposed function of these brain stem afferents is the regulation of action potential discharge mode, which is related to levels of arousal as well as sleep/wake cycles (McCormick 1992a,b; Sherman and Guillery 2001; Steriade et al. 1993). Another possible functional role of these neuromodulators is to alter the transfer properties of thalamic relay in the attentive state (e.g., Albrecht et al. 1996; Kayama 1985; Zhao et al. 2002). Characterizing how these neuromodulators affect the properties of thalamic neurons is therefore critical in understanding the ascending modulation of thalamocortical function.

Various in vivo studies suggest the potentially important role of the neuromodulators in modifying the activity of thalamic relay neurons. Dopamine has been found to modify visual responses; however, the mechanisms underlying these actions remain unknown. In general, dopamine inhibits visually evoked unit activity in the majority of dLGN neurons in rats in vivo (Albrecht et al. 1996), suggesting that dopamine may modify contrast gain in dLGN. In addition, recent studies suggest that dopamine produces a concentration-dependent, biphasic response: suppression of visually evoked responses at high concentrations and facilitation of visually evoked responses at lower concentrations (Zhao et al. 2002). To help resolve these issues, we used intracellular recording techniques to investigate the actions of dopamine on individual dLGN neurons.

METHODS

All experimental procedures were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the University of Illinois Animal Care and Use committee. Thalamic slices were prepared from young Sprague Dawley rats (postnatal age 10–17 days) similar to that previously described (Cox and Sherman 2000; Govindaiah and Cox

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The animals were deeply anesthetized with pentobarbital sodium (50 mg/kg) and decapitated. The brains were quickly removed and placed into chilled (4°C), oxygenated (5% CO₂-95% O₂) slicing medium containing (in mM) 2.5 KCl, 1.25 NaH₂PO₄, 10.0 MgCl₂, 2.0 CaCl₂, 26.0 NaHCO₃, 11.0 glucose, and 234.0 sucrose. Parasagittal or coronal slices (300- to 350-μm thick) containing the dLGN were cut using a vibrating tissue slicer, transferred to a holding chamber containing oxygenated, warm physiological saline (32°C), and incubated in physiological saline for ≥1 h prior to recording. Individual slices were transferred to a submersion type recording chamber that was maintained at 30 ± 1°C and continuously perfused (3 ml/min) with oxygenated physiological saline. The physiological solution contained (in mM) 117.0 K-gluconate, 13.0 KCl, 1.0 MgCl₂, 0.07 CaCl₂, 0.1 EGTA, 10.0 HEPES, 2.0 Na-ATP, 0.4 Na-GTP, and 0.3% biocytin. The pH and osmolality of internal solution were adjusted to 7.3 and 290 mosM, respectively. The internal solution resulted in a 10 mV junction potential that has been corrected in the voltage measures. All recordings were obtained using either an Axoclamp 2B or a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA). For current-clamp recordings, an active bridge circuit was continuously monitored and if necessary, adjusted to balance the drop in potential produced by passing current through the recording electrode. Recordings included in this study had initial access resistances ranging from 7 to 15 MΩ and typically remained stable throughout the recording.

Drug application

Concentrated stock solutions of various pharmacological agents were originally prepared in appropriate diluents and diluted in physiological saline to a final concentration prior to use. Agonists were typically applied via a short bolus using a syringe pump (Cox and Sherman 2000; Govindaiah and Cox 2004). Dopamine and SKF38393 were prepared fresh just before application. Dopamine was prepared with 0.08% ascorbic acid to prevent oxidation. Stock solutions of SKF38393, forskolin, and KT5720 were initially made with dimethyl sulfoxide. All antagonists were bath applied 7–10 min prior to application of agonists. Dopamine and phenylephrine were purchased from Sigma (St. Louis, MO), whereas all remaining compounds were purchased from Tocris (Ellisville, MO).

Data analysis

The acquisition and analyses of data were accomplished using pClamp software (Axon Instruments). The apparent input resistance of neurons was calculated from the linear slope of the voltage-current relationship obtained by applying constant current pulses ranging from −100 to +40 pA (800-ms duration). A change in the apparent input resistance of the neuron during agonist application was determined by alterations in the membrane response to single-intensity hyperpolarizing current pulses (10–20 pA, 500 ms, 0.2 Hz). To reduce voltage-dependent changes in apparent input resistance, we would manually adjust the membrane potential using current injection at the peak of the agonist-induced depolarization to the preagonist level and then obtain our measures of the response to the hyperpolarizing current pulses. Quantitative analyses are expressed as means ± SD, and statistical significance was tested using Mann-Whitney U test, Wilcoxon paired, paired Student’s t-test, or one-way ANOVA unless otherwise noted. When an ANOVA test was conducted, Tukey-Kramer multiple comparisons tests were used to test differences between specific groups. P values <0.05 were considered statistically significant.

RESULTS

Dopamine depolarizes dLGN relay neurons

Whole cell current- and voltage-clamp recordings were obtained from a total of 167 relay neurons and 17 interneurons in dLGN. The relay and interneurons were identified by their morphological and electrophysiological properties (Fig. 2, A, ii and iii, and C, ii and iii) and were similar to those described previously (Govindaiah and Cox 2004; Pape and McCormick 1995; Williams et al. 1996; Zhu et al. 1999). All recorded cells were filled with biocytin for post hoc reconstruction, and among these, the morphology of 113 relay neurons and 9 interneurons were recovered. The membrane potential and input resistances of relay neurons were −65.2 ± 3.2 mV and 334 ± 106 MΩ, respectively. Interneurons had a lower average resting membrane potential of −56.6 ± 6.9 mV (n = 17; P < 0.01, Mann-Whitney test) and a greater apparent input resistance (565 ± 224 MΩ; n = 8; P < 0.01, Mann-Whitney test).

Short-duration application (15–20 s) of dopamine (2–200 μM) produced a membrane depolarization with average peak amplitude of 6.1 ± 3.4 mV in almost all relay neurons tested (90/93; Fig. 1). The depolarizing action of dopamine persisted in the presence of TTX (1 μM), suggesting that dopamine is directly activating postsynaptic dopamine receptors on dLGN relay neurons and not indirectly exciting the relay neurons via suprathreshold activation of neurons presynaptic to the relay neurons (Fig. 1A). The depolarization was associated with a small but statistically nonsignificant increase in input resistance (334 ± 96 vs. 380 ± 114 MΩ, n = 34). The dLGN relay neurons responded to dopamine in a dose-dependent manner over the range of concentrations tested (2–200 μM; Fig. 1, B–D). The peak amplitude of the depolarizations produced by increasing concentrations of dopamine averaged 1.8 ± 1.3 (n = 10), 4 ± 1.5 (n = 15), 6.3 ± 2.2 (n = 13), 7.5 ± 3.1 (n = 24), and 8.9 ± 3.3 mV (n = 10) at 2, 10, 50, 100, and 200 μM, respectively (Fig. 1C). Overall, the dopamine-mediated depolarization significantly differed across concentrations (P < 0.001, ANOVA with Tukey-Kramer multiple comparisons). The amplitude of the depolarizations obtained with lower concentrations was not systematically different from those obtained with the higher concentrations.
dopamine concentrations (2 and 10 μM) significantly differed from each of the depolarizations produced by higher dopamine concentrations (50, 100, and 200 μM, P < 0.05). However, these higher concentrations (50, 100, and 200 μM) did not significantly differ from each other. In a subset of cells (n = 12), multiple doses (2–200 μM) of dopamine were applied in the same cells at 10-min intervals. The peak amplitude of the dopamine depolarizations typically showed dose dependence (Fig. 1D).

To determine whether the excitatory actions of dopamine were restricted to dLGN relay neurons, we also tested these different agonists on 17 dLGN interneurons. Dopamine (50–200 μM, n = 13), SKF38393 (50 μM, n = 4), or bromocriptine (50 μM, n = 3) did not alter the membrane potential or input resistance in all interneurons tested (Fig. 2C). The resting membrane potential prior to dopamine agonist application in the interneurons averaged −63.5 ± 4.5 mV and was not significantly altered after agonist application (−62.8 ± 5.0 mV, P > 0.05, Wilcoxon test).

### Depolarizing actions of dopamine are mediated through dopamine D1-like receptors

To evaluate the receptor subtype underlying the dopamine-mediated depolarization in relay neurons, we tested the actions of specific D1- and D2-like dopamine receptor agonists on the relay neurons. The D1-like receptor agonist, SKF38393 (2–50 μM), depolarized the majority of neurons tested (84%; 27/32; Fig. 2Ai). In contrast, the D2-like agonists, (±)-2-(N-phenethyl-N-propyl)amino-5-hydroxytetralin hydrochloride (PPHT, 10–50 μM; n = 10) and bromocriptine (25–50 μM; n = 6), did not alter the membrane potential or apparent input resistance of the relay neurons (Fig. 2Aii). Similar to dopamine, the depolarizations produced by SKF38393 persisted in TTX (1 μM), and the peak amplitude of the depolarization was dose dependent: 1.3 ± 0.5 (n = 3), 3.8 ± 1.0 (n = 5), 3.8 ± 1.5 (n = 10), 4.5 ± 1.8 (n = 10), and 4.5 ± 1.9 mV (n = 4) at 2, 5, 10, 25, and 50 μM, respectively (Fig. 2B). Associated with the depolarizing action of SKF38393 (10–50 μM) was a significant decrease in the apparent input resistance of the relay neurons (pre-SKF38393: 364 ± 112 MΩ vs. post-SKF38393: 329 ± 106 MΩ; P < 0.05, paired t-test, n = 12).

To further determine the specific subtype of dopamine receptor underlying the postsynaptic depolarization in relay neurons, we tested the sensitivity of the dopamine agonist-mediated responses to the D1-like dopamine receptor antagonist SCH23390. The depolarization produced by dopamine (50–100 μM) was significantly attenuated by SCH23390 (5–10 μM; P < 0.01, n = 9; Fig. 3, A and B). The average attenuation of the dopamine-mediated depolarization was 59 ± 6% with 5 μM (n = 4) and 70 ± 11% with 10 μM SCH23390 (n = 5); however, there was no significant difference between the different SCH23390 concentrations (P > 0.05, Fig. 3B). Considering reports that SCH23390 may also act as a serotonergic receptor agonist in certain brain regions (Bourne 2001; Millan

**FIG. 1.** Dopamine depolarizes dorsal geniculate nucleus (dLGN) relay neurons. A: current-clamp recording from a dLGN relay neuron. Bath application of dopamine (DA, 50 μM) depolarizes the relay neuron in a reversible manner. In TTX (1 μM), the dopamine-mediated depolarization persists suggesting a postsynaptic site of action. The downward deflections reflect membrane responses to 10-pA hyperpolarizing current pulses. At the peak of the dopamine-mediated depolarization, the membrane potential was manually adjusted to predopamine levels with current injection to test for alterations in apparent input resistance. B: current-clamp recording from a different relay neuron indicating the dose-dependent increase in the amplitude of depolarization when different concentrations of dopamine (2–200 μM) were applied at 10-min intervals. C: population data illustrating the concentration dependence of the dopamine-mediated depolarization. The numbers in the graph represent the number of cells tested for each dose. D: dose-dependent increase in the amplitude of the dopamine-mediated depolarization when multiple concentrations were tested in individual cells. Each line represents a single neuron. Note the amplitude of the depolarization is positively correlated with the dopamine concentration.
et al., 2001), it is important to note that application of SCH23390 did not significantly alter the membrane potential or input resistance of these neurons. Prior to SCH23390 application, the resting membrane potential and apparent input resistance averaged $-70.4 \pm 3.8$ mV and $356.2 \pm 72.3$ M$\Omega$ ($n = 9$), respectively. After wash in of SCH23390, the membrane potential averaged $-70.5 \pm 3.9$ and input resistance averaged $349.3 \pm 57.7$ M$\Omega$ ($n = 9$), neither of these were significantly different from the pre-SCH23390 condition ($P > 0.5$; Wilcoxon).

We next tested the sensitivity of the SKF38393-mediated depolarization to the D1-like receptor antagonist SCH23390. The depolarization produced by SKF38393 was significantly attenuated by SCH23390 (2–10 $\mu$M; Fig. 3, C and D). In control conditions, SKF38393 (5–25 $\mu$M) produced a depolarization that averaged $3.7 \pm 1.1$ mV ($n = 10$). In the presence of SCH23390 (2 $\mu$M; $n = 4$; 10 $\mu$M, $n = 6$), the depolarization was significantly attenuated to $16 \pm 15\%$ of control values ($P < 0.01$; Fig. 3D). These data clearly demonstrate that the depolarizing actions of dopamine and
SKF38393 involve the activation of D1-like dopamine receptors.

Our data using SCH23390 clearly demonstrated that the SKF38393-mediated depolarization was nearly eliminated in the presence of the antagonist; however, the blockade of the dopamine-mediated depolarization was not as complete. To test the potential cross-talk of dopamine at the concentrations tested (50–100 μM) with other catecholamine receptors, we tested the α1-adrenergic antagonist prazosin and SCH23390 on catecholamine-mediated depolarizations in relay neurons. The α1-adrenergic agonist phenylephrine (100 μM) depolarized dLGN relay neurons with average peak amplitude of 6.5 ± 2.7 mV (n = 11; Fig. 4Ai). In contrast to the actions of phenylephrine, the α2 receptor agonist clonidine (20–100 μM, n = 5; Fig. 4Aii) and the β-adrenergic receptor agonist isoproterenol (50–100 μM, n = 9; Fig. 4Aiii) failed to alter the membrane potential of dLGN relay neurons. The phenylephrine-mediated depolarization was significantly reduced by 91 ± 9% in the presence of the α1-adrenergic antagonist prazosin (5–25 μM; n = 5, P < 0.01; Fig. 4, Aii and B). In contrast, the phenylephrine-mediated depolarization was not significantly altered by SCH23390 (91 ± 18% of control, n = 6, P > 0.1; Fig. 4B). The α1-adrenergic antagonist, prazosin, produced a smaller, but statistically significant reduction of the dopamine (50–100 μM)-mediated depolarization by 25 ± 8% (n = 5, P < 0.05; Fig. 4B).

We further tested the potential cross talk of dopamine by using different dopamine concentrations (10 and 200 μM) in the presence of the D1-dopaminergic (SCH23390) and α1-adrenergic (prazosin) antagonists. Dopamine (200 μM) produced a depolarization that was significantly attenuated to 44 ± 19% of control by 10 μM SCH23390 (Fig. 4, C and D; P < 0.05, n = 6). Subsequent addition of prazosin (5 μM) further reduced the dopamine-mediated depolarization to 10 ± 5% of control (Fig. 4, C and D; P < 0.05, n = 6). In contrast, the depolarization produced by a lower dopamine concentration (10 μM), was reduced by 93 ± 4% (n = 4) in the presence of SCH23390 alone. The actions of these antagonists were partially reversible after a 15- to 25-min wash. Our data suggest that at lower concentrations, dopamine selectively activates D1-like receptors to depolarize thalamic relay neurons; however, at higher concentrations, dopamine also activates α1-adrenergic receptors to further depolarize the thalamic neurons.

**Activation of D1-like receptors increases I_{h} in dLGN relay neurons**

The ionic nature of the dopamine-mediated depolarization has not yet been identified in thalamic neurons. Given that the depolarization is associated with a weak increase in apparent input resistance, we initially tested the effect of the K^{+} channel blocker, Cs^{+}. Using voltage-clamp recording techniques with a holding potential of −60 mV, dopamine (50 μM) produced an inward current (40.4 ± 21.2 pA, n = 15) consistent with the membrane depolarization observed using current-clamp recording techniques. Bath application of Cs^{+} (3 mM) significantly reduced the dopamine depolarization to 28.0 ± 9.0% of control (data not shown, n = 6, P < 0.05, Wilcoxon). However, extracellular Cs^{+} has been shown to not only block K^{+} currents, but also the hyperpolarization activated mixed cation current, I_{h} (Halliwell and Adams 1982; McCormick and Pape 1990b). We next used voltage-clamp recording techniques in conjunction with ramped voltage commands (−60 to −120 mV) to quantify alterations in input conductance. In TTX (1 μM), dopamine produced an inward current with an average peak of 47.6 ± 23.0 pA (n = 9). During the dopamine-mediated inward current, the slope of the current response was reduced in most cells (69%, Fig. 5, A and B). Given the significant contribution of I_{h} to hyperpolarizing regions of the ramped voltage commands, we used the selective I_{h} blocker, ZD7288 (Fig. 5, A and B; +ZD7288). In ZD7288 (100 μM), the dopamine-mediated inward current was significantly reduced to 27.8 ± 18.1% control (n = 9, P < 0.01, Wilcoxon).
increase in the dopamine-mediated inward current results only from an increase in $I_h$ compared with 50% blocked (pre-ZD7288: 13.6 ± 7.3 pA, ZD7288: 0.5 ± 0.6 pA; $n = 4$, $P < 0.05$, paired $t$-test) indicating at low concentrations, the dopamine-mediated inward current results only from an increase in $I_h$ (Fig. 5, C and D).

indicating that dopamine produces an increase in $I_h$ that likely contributes to the membrane depolarization. In the presence of ZD7288, the remaining dopamine-sensitive current is linear (Fig. 5B; +ZD7288) and has an average reversal potential of $-106 ± 31$ mV ($n = 9$), indicative of $K_{leak}$. Thus our data suggest that the dopamine-mediated depolarization is composed of two components: an increase in $I_h$ and a reduction in $K_{leak}$.

Considering our earlier data that higher dopamine concentrations (>50 μM) can also activate α1-adrenergic receptors, we carried out the voltage-clamp experiments with lower dopamine concentrations. At 10 μM, dopamine produced a smaller but consistent inward current (12.7 ± 6.5 pA, $n = 5$) compared with 50 μM dopamine (Fig. 5C). In ZD7288 (100 μM), the dopamine mediated inward current was completely blocked (pre-ZD7288: 13.6 ± 7.3 pA, ZD7288: 0.5 ± 0.6 pA; $n = 4$, $P < 0.05$, paired $t$-test) indicating at low concentrations, the dopamine-mediated inward current results only from an increase in $I_h$ (Fig. 5, C and D).

To specifically address the mechanism underlying the depolarizing response following activation of D1-like dopaminergic receptors, we repeated the voltage-clamp experiments using the selective agonist, SKF38393. With a holding potential of $-60$ mV in TTX (1 μM), SKF38393 produced an inward current that averaged 13.8 ± 8.2 pA ($n = 7$) associated with an apparent increase in conductance (Fig. 6A). Using slow ramped voltage commands, we analyzed the conductance change produced by SKF38393 by calculating the “resting” conductance of the cell prior to and after SKF38393 application in the linear response range of the neurons ($V_{hold}$: −60 to −80 mV, Fig. 6B). SKF38393 produced a significant increase in the neurons’ conductance from 3.1 ± 0.9 nS to 3.6 ± 1.0 (n = 7, $P < 0.01$, paired $t$-test). We next tested the affect of the $I_h$ blocker, ZD7288, on the SKF38393-mediated excitation. In ZD7288 (50–100 μM), the inward current produced by SKF38393 was strongly attenuated (Fig. 6A). In all four cells tested, the response to SKF38393 in the presence of ZD7288 averaged 0.9 nS to 3.6 nS.
0.1 ± 1.4 pA, a significant reduction of the inward current ($P < 0.01$, paired t-test). In addition, SKF38393 did not alter the conductance of the neurons in ZD7288 (2.02 ± 0.5 vs. 2.1 ± 0.5 nS, $P > 0.3$, $n = 4$). These data suggest that activation of D1-like dopaminergic receptors leads to an increase in $I_h$, which in turn accounts for the excitatory actions (inward current, membrane depolarization) of dopamine in thalamic relay neurons.

**Role of cAMP pathway in the depolarizing actions of dopamine**

The D1-like dopamine receptors are positively coupled to the activation of adenyl cyclase/cAMP/protein kinase A signal transduction pathway (Stoof and Kebabian 1984). We next tested whether the excitatory actions of dopamine on thalamic neurons involved the cAMP pathway. Bath appli-
The peak amplitude of the depolarization averaged 7 mV; mediated depolarizations were not significantly altered (Fig. 7F). In ZD7288, after SKF38393 application. In TTX (1 mM), produces an inward current associated with increases in membrane conductance. After application of ZD7288, the overall amplitude of the membrane responses to the voltage commands decreases as expected by the attenuation of $I_h$. The subsequent application of SKF38393 does not alter the holding current or the conductance of the neuron. B: expanded traces of the membrane response to the ramped voltage commands reveal not only the inward current but also the conductance increase by SKF38393 (gray trace). Each trace consists of an average of 6 subsequent responses prior to and at the peak of the SKF38393-mediated inward current in control (left) and in the presence of ZD7288 (right). The difference between the SKF38393 (gray trace) and predrug (black trace) is indicative of the SKF38393-sensitive current ($I_{sam}$). In control conditions, SKF38393 produces a nonlinear change in conductance with an extrapolated reversal potential near −40 mV. In ZD7288, the conductance change (Δ$I_{sam}$) is completely blocked, indicating that SKF38393 leads to an activation of $I_h$.

We next tested the sensitivity of the dopamine-mediated depolarizations to inhibition of cAMP and protein kinase A (PKA). The cAMP analogue and PKA inhibitor, Rp-cAMP (500 μM), was included in the recording pipette. In control conditions (i.e., absence of Rp-cAMP in the pipette), dopamine (10–50 μM) was applied at 8–15 min intervals to test if the agonist could produce repeatable, consistent amplitude depolarizations (Fig. 7C). The responses to the first and second application did not differ significantly (7.7 ± 3.0 vs. 6.9 ± 2.0 mV; n = 6; P > 0.2, paired t-test). With the inclusion of Rp-cAMP in the recording pipette, we first applied dopamine within 2 min of forming the whole cell recording configuration and depolarization to this initial application averaged 4.8 ± 0.6 mV (n = 5; Fig. 7D and H). After a 9- to 11-min interval, the subsequent application of dopamine produced a significantly smaller depolarization (1.6 ± 0.9 mV; n = 5; P < 0.1, paired t-test).

To test whether the dopamine-mediated depolarization involves cAMP-dependent PKA activation, we tested the sensitivity of the dopamine response to the selective PKA inhibitor, KT5720 (Huang and Hsu 2003; Kase et al. 1987). In control conditions, dopamine (10 μM) produced an average depolarization of 3.4 ± 1.1 mV (n = 5). The antagonist, KT5720 (1–5 μM), was applied for 8–15 min, and the subsequent dopamine application produced a similar depolarization (3.3 ± 1 mV) that did not significantly differ from the control response (Fig. 7E, Ei and G; P > 0.1, paired t-test, n = 5). In a different series of experiments, KT5720 (10 μM) was included in recording pipettes. Using a similar experimental protocol as with Rp-cAMP, the initial dopamine-mediated depolarization (within 2 min after whole cell recording configuration) averaged 5.1 ± 0.9 mV (n = 4). The second dopamine application (13 min later) averaged 5.0 ± 0.5 mV and did not differ significantly from the first response (Fig. 7Eii, P > 0.1, paired t-test, n = 4).

In some neurons, continuous bath application of cAMP agonist forskolin (25 μM, 5–10 min) produced a membrane depolarization that plateaued after a few minutes (Fig. 7F). The membrane potential was manually adjusted to the preforskolin level, and the subsequent dopamine application produced a significantly reduced depolarization (0.9 ± 0.2 mV; n = 4, Fig. 7Eiii). The population data clearly indicate that the dopamine-mediated depolarization was significantly occluded following activation of cAMP by forskolin (Fig. 7I, P < 0.01, paired t-test, n = 4). These data suggest that the depolarizing action of dopamine via $I_h$ is mediated through a cAMP-dependent, PKA-independent mechanism.

**DISCUSSION**

The major finding of our present study is that dopamine plays an important role in modulating the excitability of relay neurons in the dLGN. Although there are limited reports indicating that dopamine may alter sensory processing at the thalamic level, the underlying cellular mechanisms remain unknown. We have shown that dopamine and the specific D1-like receptor agonist, SKF38393, produce excitatory responses in dLGN relay neurons but not in dLGN interneurons. The D1-like receptor antagonist, SCH23390, attenuated the excitatory actions of dopamine. In contrast, D2-like receptor agonists did not alter the excitability of either relay neurons or interneurons. The activation of D1-like receptors led to an increase in $I_h$ that is dependent on the activation of adenylyl cyclase and increased cAMP activity but independent of PKA activation. Interestingly, we found that dopamine, at lower concentrations, produced this specific action via D1-like receptors; however, at higher concentrations, dopamine also activated $a_1$-adrenergic receptors, and via a decrease in $K_{Na1}$ led to further depolarization of relay neurons (McCormick 1992b; McCormick and Prince 1988). Overall, our study indicates a
potentially important role of D₁-like receptors in influencing the excitability of thalamic relay neurons and ultimately modulating the gating properties of thalamic information transfer to the neocortex.

The h current ($I_h$) is a hyperpolarization-activated mixed cation current that was originally described in cardiac cells as $I_f$ but has since been identified as $I_h$ in a widespread variety of neural as well as nonneural cells (Brown and DiFrancesco 1980; Halliwell and Adams 1982; Pape 1996). In the thalamus, $I_h$ has been well characterized in relay neurons and plays an integral role in the rhythmic activity of these neurons as well as an important contributor to the resting membrane potential (Lüthi and McCormick 1998; McCormick and Pape 1990a,b; Pape 1996). An interesting aspect of $I_h$ is that this current can be activated by dopamine and forskolin, as shown in Figure 7A.

FIG. 7. Dopamine depolarizes dLGN relay neurons through a cAMP-dependent, protein kinase A (PKA)-independent signal transduction mechanism. A: in TTX, D₁-like receptor agonist SKF38393 (10 μM) depolarizes a dLGN relay neuron. Subsequent application of the cAMP activator, forskolin (0.5 μM, 15 s), also produces a membrane depolarization that recovers after a few minutes. Application of a higher concentration of forskolin (20 μM) produces a larger depolarization. B: summary of dose-dependent action of forskolin on dLGN relay neurons.

The number of cells tested at each concentration is listed. C: repeated application of dopamine (10 μM) produces a similar amplitude membrane depolarization in relay neurons. D: cAMP antagonist/PKA inhibitor, Rp-cAMP (500 μM), is included in the recording pipette. Within 2 min of forming the whole cell recording configuration, the initial application of dopamine (10 μM) produces a membrane depolarization; however, after 10 min, the subsequent application of dopamine produces a significantly smaller depolarization. Ei: in a different neuron, dopamine (10 μM) depolarizes the neuron. Bath application of the PKA inhibitor, KT5720 (2 μM), for 13 min did not alter the amplitude of the subsequent dopamine-mediated depolarization. Eii: in a different neuron, inclusion of KT5720 (10 μM) in the recording pipette did not affect the depolarization produced by dopamine (10 μM) within 2 min of break-in for whole cell recording configuration and 13 min later. In the same neuron, bath application of forskolin (25 μM) produced a large membrane depolarization. During the peak depolarization, the membrane potential is manually adjusted to pretreatment level (arrow), and the subsequent application of dopamine failed to depolarize the neuron. F: phospholipase C inhibitor, U-71322 (0.2 mM in pipette), did not attenuate dopamine-mediated depolarizations when applied at similar intervals as D and Eii. G: population data illustrating that the PKA inhibitor KT5720 did not significantly alter the depolarizing actions of dopamine (10 μM). H: population data illustrating that the antagonistRp-cAMP (500 μM in recording pipette), significantly attenuated the dopamine mediated (10 μM) depolarizations. I: population data indicating that the dopamine-mediated depolarization is occluded in the presence of forskolin. The numbers in the plots indicate number of cells tested. A paired Student’s t-test was used for statistical analyses (G–I).
be directly modulated by alterations in cAMP activity (Frere and Luthi 2004; Kaupp and Seifert 2001; Pape 1996; Santoro and Tibbs 1999). Our data further support the modulatory role of D$_h$ by cAMP in a PKA-independent manner. Considering activation of dopamine receptors is associated with changes in cAMP levels, it is not entirely surprising that dopamine may alter D$_h$ activity. In other tissues, dopamine, via D$_2$ receptors, has been found to decrease I$_h$ (Akopian and Witkovsky 1996; Vargas and Lucero 1999). The novelty of our findings is the increase in I$_h$ that we have observed in thalamic neurons is associated with activation of D$_1$-like receptors.

Within the thalamus, a variety of neuromodulators, that arise from brain stem nuclei, have been found to alter I$_h$ activity in relay neurons, including serotonin, norepinephrine, and histamine (McCormick and Pape 1990a; McCormick and Williamson 1991). The increase in I$_h$ has been associated with increases in cAMP activity, and this may serve as a final common pathway for these neuromodulators. Our data add a newcomer to this list: dopamine. The activity of dopaminergic neurons in the mesencephalic reticular formation have been associated with a variety of arousing events such as novel stimuli and changes in the conditions of a salient environment (Horvitz 2000; Horvitz et al. 1997). The activity of noradrenergic neurons in the locus coeruleus are correlated with an organism state of arousal (Aston-Jones et al. 1991; Hirsch et al. 1983; Livingstone and Hubel 1981; McCarley et al. 1983; Nakai and Takaori 1974; Rasmussen et al. 1986). Considering the release of these neuromodulators are associated with arousal levels, attention, and stimulus novelty, one hypothesis regarding a common function may be that these modulators tend to produce a heightened level of sensitivity, although further investigation regarding this idea is required.

Visual information is transferred from the retina to primary visual cortex via dLGN relay neurons. A variety of neurotransmitters/modulators that are released by intrathalamic neurons, corticothalamic neurons, as well as brain-stem-projecting neurons can modify neuronal excitability and thereby influence visual information that passes through the dLGN (Steriade et al. 1997). The predominant source of synaptic innervation of dLGN relay neurons is from retinal sources with only a small minority of synapses (<10%) that arise from retinal ganglion cells (Van Horn et al. 2000). These nonretinal, modulatory inputs can influence the excitability of thalamic circuits thereby altering the input/output function of the thalamus. In addition, these modulatory inputs may alter the action potential discharge mode of these neurons, which is related to the arousal state of the organism or novelty of the stimulus (Sherman and Guillery 1996, 2002; Steriade et al. 1993). For example, many neurotransmitters including acetylcholine, histamine, glutamate, serotonin, and norepinephrine clearly alter the excitability of relay neurons and transition the firing mode from a burst to tonic discharge mode (Cox and Sherman 2000; McCormick 1992b; Monckton and McCormick 2002; Turner and Salt 2000).

Dopaminergic innervation of the thalamus is relatively sparse compared with other catecholamines such as norepinephrine or serotonin, but nonetheless dopamine receptors are also present within the dLGN (Camps et al. 1990; Khan et al. 1998; Mijnster et al. 1999; Papadopoulos and Parnavelas 1990a). However, the functional role of dopamine in thalamic functioning is less established than that of the other catecholamines. Of the limited number of in vivo studies, dopamine tends to produce primarily inhibitory actions, that is, activation of dopaminergic receptors decreases the light-evoked as well as baseline activity of presumed relay neurons (Albrecht et al. 1996; Zhao et al. 2002). Dopamine has been shown to inhibit flash-evoked activity in the majority of rat dLGN neurons but can also facilitate the activity of other dLGN neurons (Albrecht et al. 1996). Dopamine has also been shown to produce concentration-dependent effects on visually evoked discharge of dLGN relay neurons: at high concentrations, dopamine suppresses the visual response but may facilitate the relay neuron activity at lower concentrations (Zhao et al. 2002). These previous in vivo studies have also suggested that dopamine may alter the activity of dLGN interneurons thereby decreasing relay neuron activity. In addition, the excitatory actions of dopamine have been associated with the activation of D$_2$-like receptors, whereas the inhibitory actions may be due to activation of D$_1$-like receptors (Albrecht et al. 1996; Zhao et al. 2002). Despite these generalizations of dopamine-mediated activity within the dLGN, it is clear that there are as many exceptions to these generalizations regarding the inhibitory and excitatory responses produced by dopamine in in vivo experiments.

Our study provides the first data demonstrating dopamine-mediated actions in dLGN thalamic relay neurons using intracellular recording techniques. We found that both dopamine and the D$_1$ receptor agonist, SKF38393, produced robust membrane depolarizations in relay neurons only, whereas D$_2$ receptor agonists, PPHT and bromocriptine, did not alter the membrane potential of relay neurons. Furthermore, we found no evidence that dopaminergic agonists alter the membrane potential of dLGN interneurons. Clearly, the excitatory actions of dopamine that we have observed using the in vitro slice preparation cannot easily account for the general findings reported in vivo. The inhibitory actions observed in vivo could arise from excitation of adjacent thalamic reticular nucleus neurons but do not appear to involve dLGN interneurons as originally suggested. Future studies should also focus on the possible actions of dopamine on synaptic activity. It is clear that further investigation at both the reduced level (in vitro preparation) and intact level (in vivo) are required to resolve these differences and ultimately understand the cellular mechanisms and functional significance regarding the role of dopamine in visual processing.

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