Dopamine Modulates Excitability of Basolateral Amygdala Neurons In Vitro

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Kröner, Sven, J. Amiel Rosenkranz, Anthony A. Grace, and German Barrionuevo. Dopamine modulates excitability of basolateral amygdala neurons in vitro. J Neurophysiol 93: 1598–1610, 2005.—The basolateral amygdala complex (BLA; lateral, basal and accessory basal nuclei) is involved in the expression of affective behaviors, including the learned associations between stimuli and the affective responses to those stimuli (Aggleton 2000; Blair et al. 2001; McGaugh 2002). The BLA receives a moderate innervation of dopaminergic fibers (Asan 1997, 1998), and global elevations of dopamine (DA) or systemic administration of DA agonists facilitate amygdala-dependent behaviors (Borowski and Kokkinidis 1996; Harmer and Phillips 1999), whereas DA antagonists diminish them (Greba and Kokkinidis 2000). Furthermore, manipulations of DA directly within the BLA alter amygdala-dependent affective behaviors (Greba and Kokkinidis 2000; Greba et al. 2001; Lamont and Kokkinidis 1998; Nader and LeDoux 1999), and in addition, the concentration of DA in the BLA is increased by the presentation of affective stimuli and during affective learning (Harmer and Phillips 1999; Inglis and Moghaddam 1999; Suzuki et al. 2002). Previous studies indicate that the DAergic modulation of these learned associations is the result of a direct action of DA on BLA neurons during affective learning. Thus during an affective training procedure, neurons of the lateral nucleus of the BLA display increases in apparent input resistance (Rosenkranz and Grace 2002b) and enhanced responses to conditioned stimuli (Maren 2000; Pare and Collins 2000; Quirk et al. 1995; Rosenkranz and Grace 2002b). Both of these changes are blocked by the DA antagonist haloperidol (Rosenkranz and Grace 2002b).

Some of the enhancing effects of DA on affective behaviors may be explained by cellular actions of DA on BLA neurons. Systemic or iontophoretic administration of DA agonists alters the firing rate of BLA projection neurons and interneurons (Bashore et al. 1978; Ben-Ari and Kelly 1976; Rosenkranz and Grace 1999; Spehlmann and Norcross 1984). However, systemic administrations of DA receptor agonists also exert actions on synaptic inputs to the BLA (Rosenkranz and Grace 2001, 2002a). Thus in these previous studies, it was difficult to exclude indirect effects of DA on BLA neurons via actions on afferent regions. Therefore in this study we utilized in vitro whole cell recordings from rat brain slices to test the hypothesis that DA exerts direct postsynaptic actions that alter BLA neuronal excitability, which could account for the effects observed in vivo with systemic administration of DA agonists, and to determine the receptor subtypes involved in these actions of DA.

Our data show that DA increases the excitability of BLA projection neurons via mechanisms involving both D1 and D2 receptors and also increases evoked and spontaneous spike firing in fast-spiking interneurons. In projection neurons, the D1 receptor-dependent increase in excitability was due to the reduction in an outward-rectifying, 4-aminopyridine (4-AP) and α-dendrotoxin (DTX)-sensitive K⁺ current.

Methods

All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Male Sprague-Dawley rats were housed in pairs with 12 h on-off light/dark schedule in a temperature-controlled environment. Food and water were available ad libitum.

Slice preparation and electrophysiology

Brain slices were prepared from rats aged 3–5 wk. Rats were anesthetized with equithesin (200 mg/kg ip) or chloral hydrate (400...
mg/kg ip) and perfused transcardially with high-sucrose artificial cerebrospinal fluid (ACSF), containing (in mM): 229 sucrose, 1.9 KCl, 1.2 NaH2PO4, 33.3 NaCO3, 20 glucose, 0.5 CaCl2, and 6 MgCl2. The brain was removed and sectioned at 350 μm (Leica VT1000S, Leica, Germany) in ice-cold ACSF composed of (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 10 glucose, 2 CaCl2, 1 MgCl2, and 0.075 Na-metabisulfate, an antioxidant used to preserve DAergic terminals. All current-clamp experiments were conducted at the presence of 20 μM 6-cyano-7-nitroquinoline-2,3-dione (CNQX, to block AMPA receptors) and 100 μM (±)-2-amino-5-phosphonopentanoic acid [APV, to block N-methyl-D-aspartate (NMDA) receptors] and 20 μM (−)-bicuculline methiodide or (+)-bicuculline (to block GABA<sub>A</sub> receptors), as indicated. In voltage-clamp experiments, this combination of antagonists completely blocked all synaptic currents. For voltage-clamp recordings of spontaneous IPSCs (sIPSCs), the membrane potential was held at −85 to −90 mV, and GABA-mediated events were pharmacologically isolated by adding 20 μM CNQX and 100 μM APV to the bath. Action-potential independent miniature IPSCs (mIPSCs) were recorded under the same conditions but in the presence of 1 μM tetrodotoxin (TTX) to block sodium channels. The frequency and amplitude of events were measured using MiniAnalysis (Synaptosoft, Decatur, GA). For IPSCs, the total number of events from 8 min of continuous recordings before and after DA application was compared. All data are presented as means ± SE. Most statistical comparisons between groups used two-tailed paired t-test; comparisons after an ANOVA used unpaired t-test, as indicated (differences of alpha ≤0.05 were considered significant). For multiple post hoc comparisons the alpha-level was Bonferroni-adjusted.

**Histology**

After recording, slices were fixed in 4% paraformaldehyde in 0.12 M phosphate-buffered saline (PBS). Slices were sectioned at 70 μm on a freezing microtome and collected in PBS. For histochemical staining of biocytin-filled neurons, slices were incubated in 0.5% H2O2 for 30 min. Slices were washed in PBS, and floating sections were incubated overnight at 4°C in the avidin-biotin complex (ABC Elite, Vector Labs; 1:100 in PBS with 0.3% Triton X-100, Sigma). After washing in PBS, sections were mounted, dehydrated, and coverslipped. Individual cells were reconstructed using Neurolucida software (Microbrightfield, Williston, VT).

**Drugs and chemicals**

All compounds were obtained from Sigma (St. Louis, MO) with the exception of CNQX (Tocris Cookson, Bristol, UK), tetrodotoxin (TTX) and α-DTX (DTX; Alomone, Jerusalem, Israel).

**RESULTS**

**Properties of BLA projection neurons and interneurons**

A total of 122 neurons were recorded in current-clamp mode from the BLA. The majority of data reported here was obtained from neurons in the lateral nucleus of the amygdala. However, no obvious differences were found in firing characteristics or the effect of D1/D5 agonists when compared to the location of neurons within the BLA. Therefore data from cells in the lateral and the basal nucleus of the amygdala were pooled. Principal neurons (n = 107) in the BLA showed a continuum of high- and low-threshold action potentials with the majority of neurons (72%) exhibiting a clear separation between the two thresholds.
of response patterns to a depolarizing pulse that ranged from rapid, full accommodation (1–5 spikes at the beginning of a depolarizing current pulse; Fig. 1A1) to a “regular-”spike firing pattern (repetitive firing with conspicuous frequency adaptation; Fig. 1A2), consistent with features previously reported for BLA projection neurons (Faber et al. 2001; Rainnie et al. 1993; Washburn and Moises 1992). Some BLA neurons that responded with regular firing patterns to somatic current injection displayed a voltage- and time-dependent outward rectification that caused a slow, ramp-like depolarization and delay in the firing of the first action potential (Fig. 1A3). Similar “late-spiking” cells have previously been described in the BLA (Washburn and Moises 1992). Some basic membrane properties and characteristics of single action potentials of three classes of BLA projection neurons are described in Table 1. As can be seen from this overview, the three different cell types that we identified based on their response pattern did not differ significantly in their basic membrane properties such as resting membrane potential and $R_m$ or the properties of a single spike. Morphologically, cells from all three electrophysiological classes were characterized by densely spinous dendrites and polarized dendritic trees typical of pyramidal-like BLA projection neurons (Fig. 1B).

The remaining 15 neurons exhibited morphological and electrophysiological characteristics consistent with BLA interneurons (Fig. 1, A4 and C) (Lang and Pare 1998; Washburn and Moises 1992). Their response pattern showed little or no spike frequency adaptation, typical of the class of “fast-spiking” (FS) interneurons. Accordingly, they had shorter action potential durations, and they displayed short-duration, large-amplitude afterhyperpolarizations. On average, FS cells clearly differed from all three types of projection neurons in their membrane properties at rest and single spike parameters (Table 1). Morphologically, FS BLA interneurons were characterized

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**Fig. 1.** Electrophysiological and morphological characteristics of basolateral amygdala complex (BLA) neurons. A: characteristic of firing patterns for 3 types of projection neurons and a fast-spiking interneuron elicited in response to membrane depolarization. Principal neurons were classified as late-spiking, regular-spiking, or strongly adapting, respectively, based on their membrane response to depolarizing current injections. Late-spiking cells showed ramp-like depolarizations and delayed firing at potentials near spike threshold. Regular-spiking cells were characterized by repetitive firing with strong frequency adaptation. Strongly adapting cells fired 1–5 spikes at the beginning of the current pulse before showing full accommodation. Fast-spiking interneurons showed short-duration action potentials and little adaptation of action potential firing during suprathreshold current injection. B: microphotograph (left) and computer reconstruction (right) of 2 biocytin-filled neurons that displayed morphological features of spiny, pyramidal-like BLA projection neurons. Inset: the location of the reconstructed neuron in the BLA. The scale bar in the microphotograph represents 100 μm. C: computer reconstruction of a neuron that showed fast-spiking firing characteristics. Cells of this type had dendrites with few or no spines and dense local axonal arborizations. In the reconstructed neurons the somata and dendrites are drawn in red and the axonal arborizations in blue. CPu, caudate-putamen.
by smooth stellate dendrites and dense local axonal arborizations (Fig. 1C).

**Effects of DA on projection neurons**

Bath application of DA (10 μM) for 2–3 min (to mimic the in vivo kinetics of DA release and to minimize receptor desensitization) led to an increase in the membrane excitability (Fig. 2) defined here as the number of spikes evoked by a set level of depolarizing current injection (500-ms duration). For statistical analysis, we selected a current intensity that evoked repetitive firing (3–5 spikes) under control conditions regardless of cell type. Thus under baseline conditions the mean number of spikes was 3.4 ± 0.7 action potentials. After application of DA, the number of spikes increased to 4.9 ± 0.8 (P < 0.01; n = 13). In many cases, a small membrane depolarization (2–3 mV) was observed on DA application that was compensated with DC injection before changes in excitability were measured. Application of DA lead to a significant change in the threshold of the first action potential (Fig. 2D; baseline: −40.1 ± 1.2 mV; DA: −41.1 ± 1.2 mV P < 0.01; n = 13), and this was also reflected in a reduction of the rheobase current necessary to evoke the first spike (baseline: 183.1 ± 31.4 pA; DA: 165.5 ± 28.6 pA, P < 0.05; n = 13).

We found no effect of DA receptor activation on spike half-width (control: 1.18 ± 0.1 ms; DA: 1.16 ± 0.09 ms) or the amplitude of the fast afterhyperpolarization (AHP; control: 

### TABLE 1. Basic electrophysiological properties of BLA neurons

<table>
<thead>
<tr>
<th></th>
<th>Late Spiking</th>
<th>Regular Spiking</th>
<th>Strongly Adapting</th>
<th>Fast Spiking</th>
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<tr>
<td>n</td>
<td>32</td>
<td>31</td>
<td>44</td>
<td>15</td>
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<td>Resting membrane potential, mV</td>
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<td>−69.5 ± 1.0</td>
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<td>Input resistance, MΩ</td>
<td>135.8 ± 11.0</td>
<td>167.2 ± 21.2</td>
<td>138.0 ± 12.2</td>
<td>246.5 ± 36.3</td>
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<tr>
<td>Rheobase current, pA</td>
<td>232 ± 21</td>
<td>190 ± 17</td>
<td>212 ± 18</td>
<td>126 ± 24</td>
</tr>
<tr>
<td>Action potential threshold, mV</td>
<td>−38.6 ± 0.5</td>
<td>−38.8 ± 0.5</td>
<td>−38.7 ± 0.4</td>
<td>−40.1 ± 0.6</td>
</tr>
<tr>
<td>Action potential amplitude, mV</td>
<td>69.9 ± 1.3</td>
<td>68.1 ± 1.4</td>
<td>68.3 ± 1.3</td>
<td>58.3 ± 1.4</td>
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<tr>
<td>Action potential half-width, ms</td>
<td>1.20 ± 0.05</td>
<td>1.23 ± 0.03</td>
<td>1.22 ± 0.02</td>
<td>0.80 ± 0.08</td>
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*Significant differences indicated by the use of a two-tailed, unpaired t-test for multiple comparisons after ANOVA (P < 0.05).

**FIG. 2.** Dopamine (DA) increases the excitability of BLA projection neurons. A: bath application (2–3 min.) of 10 μM DA (bottom) increased the number of action potentials evoked by a suprathreshold current step when compared with the response to the same current step under control conditions (top). B: average of the responses of 13 BLA projection neurons. DA increased the number of evoked spikes from 3.4 ± 0.7 to 4.9 ± 0.8 (mean ± SE; **P < 0.01). C: blockade of synaptic transmission by glutamate and GABA receptor antagonists (see METHODS for details) did not prevent the increase in neuronal excitability after bath application of 10 μM DA, indicating a postsynaptic mechanism of DA action (**P < 0.01; n = 7). D: time course of the DA-mediated increase in evoked spikes (●) and change in action potential threshold (●). See RESULTS for details. Note the different scales on the y axes in D. E: DA receptor activation increased Rm only when synaptic inputs were blocked. Data for individual cells (●) and averages (●) illustrating the change in Rm when DA was applied alone (left) or in the presence of 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX) and bicuculline (right). In each panel, values on the left side are baseline measures; values on the right are after DA application (**P < 0.05; n = 7). F: example traces illustrating the lack of DA modulation of subthreshold electrotonic potentials. An effect of DA on Rm only becomes apparent at potentials close to spike threshold (top).
12.8 ± 1.04 mV; DA: 12.9 ± 1.12 mV) and its time-to-peak (control: 3.6 ± 2.0 ms; DA: 3.9 ± 2.0 ms) in traces containing only single spikes. Furthermore, we found no effect of DA on the medium or slow AHP, which is activated after repetitive firing and which has been implicated in spike adaptation (Sah 1996); this AHP is known to be modulated by a variety of neurotransmitters (Faber and Sah 2002). In current-clamp recordings, the amplitude of the AHP that followed a train of five spikes was 13.7 ± 1.47 mV under control conditions and 12.7 ± 2.1 mV in the presence of DA (n = 10, P = 0.7). The kinetics of this medium or slow AHP were similarly unaffected by DA (not shown).

Because it is possible that the change in excitability was due to DAergic actions on synaptic inputs, DA applications were repeated in a separate group of neurons in the presence of glutamate and GABA antagonists (20 μM CNQX, 100 μM APV, 20 μM bicuculline). Nevertheless, with synaptic inputs blocked, application of DA exerted the same effect on excitability (Fig. 2C; baseline: 3.8 ± 0.8; DA: 5.7 ± 0.7, P < 0.01, n = 7), demonstrating that the increased excitability was likely due to a postsynaptic action of DA. This effect was accompanied by an increase in apparent R∞ (Fig. 2E; baseline: 164.2 ± 21.8 MΩ; DA: 179.1 ± 19.8 MΩ, P < 0.05); this change persisted in the absence of DA, returning to baseline values after extended wash-out. In contrast, there was no significant change of R∞ in the absence of synaptic blockade (Fig. 2E; baseline: 179.0 ± 44.4 MΩ; DA: 177.3 ± 42.0 MΩ); As with DA alone, DA in the presence of CNQX and bicuculline significantly reduced the rheobase current (baseline CNQX/bic: 175.8 ± 24.7 pA; CNQX/bic + DA: 152.4 ± 23.1 pA, P < 0.05, n = 7). These findings imply that DA exerts at least two actions: a postsynaptic change in the membrane properties that underlie increased excitability and R∞ and a DA-mediated change in synaptic inputs that may decrease apparent R∞ and thus could offset the DA-mediated postsynaptic increase in R∞. An increase in GABAergic transmission onto BLA projection neurons is a likely mechanism that could increase the conductance and thus decrease apparent R∞ in projection neurons. Results from a previous study (Rosenkranz and Grace 1999) and our data presented in the following text show that DA increases the firing of BLA inhibitory interneurons. To determine whether an increase in GABA release may offset the DA-mediated changes in apparent R∞, the experiments were repeated in the presence of (+)-bicuculline alone (without CNQX or APV). Bicuculline (10 μM) increased the R∞ (baseline: 157.1 ± 18.6 MΩ, bicuculline: 166.2 ± 18.5 MΩ, P < 0.05, n = 6) and excitability of BLA projection neurons (baseline: 3.3 ± 0.8 action potentials, bicuculline: 4.1 ± 0.8, P < 0.05, n = 6). When DA (10 μM) was added to the bath, a further increase in R∞ and excitability was observed (DA: 179.5 ± 19.1 MΩ, P < 0.05, n = 6; pre-DA: 3.6 ± 0.7 action potentials, post-DA: 5.9 ± 0.6 action potentials, P < 0.01, n = 6). These data indicate that there is a tonic GABAergic inhibition that influences the R∞ and excitability of BLA projection neurons. Furthermore, DA may increase this GABAergic tone, which offsets the direct DA-mediated enhancement of R∞ and excitability of BLA projection neurons. We identified a potential mechanism for this hypothesized increase in GABAergic tone in recordings from interneurons and sIPSCs (see following text).

The effects attributed to DA are not simply due to time-dependent changes during prolonged recording or nonspecific (non-DA receptor mediated) drug effects for several reasons: the changes attributed to DA are not seen in the presence of DA antagonists (see following text), after extended washouts (>30 min after DA application), the actions of DA could be at least partly reversed, and when repeating the protocol in the absence of DAergic drugs, excitability, R∞ and action potential threshold remained unchanged (n = 4; data not shown).

Effects of DA agonists on BLA projection neurons

To determine the DA receptor subtypes involved in modulating the excitability of BLA projection neurons, receptor-specific agonists were applied in the same manner as DA. The actions of DA on excitability were mimicked by application of the DA D1 agonist SKF81297 (10 μM; Fig. 3). D1 receptor activation increased the number of spikes (baseline: 3.5 ± 0.7 spikes, SKF81297: 4.9 ± 1.8 spikes, P < 0.01, n = 9) and decreased the rheobase current (baseline: 193.3 ± 27.2 pA, SKF: 171.1 ± 21.5 pA; P < 0.05). This was not accompanied by a significant change of R∞ (baseline: 131.5 ± 25.3 MΩ, SKF: 134.0 ± 25.1 MΩ). In contrast, the DA D2 agonist quinpirole (10 μM) did not significantly increase neuronal excitability (Fig. 3; baseline: 3.9 ± 0.5 spikes, quinpirole: 4.4 ± 1.2 spikes) but did increase the neuronal R∞ (baseline: 164.3 ± 21.3 MΩ, quinpirole: 171.0 ± 21.4 MΩ, P = 0.01,

![Image](http://jn.physiology.org/)

FIG. 3. The actions of DA are mimicked by the D1 receptor agonist SKF81297. A and B: individual sweeps (A) and group data (B) showing that bath application of the DA D1 receptor agonist SKF81297 (10 μM) increased the number of action potentials in response to a control current step (**P < 0.01; n = 9). C and D: individual sweeps (C) and group data (D) demonstrating that activation of D2 receptors via bath application of the DA D2 agonist quinpirole (10 μM) did not significantly increase the number of evoked action potentials. E: activation of D2 receptors by quinpirole, but not of D1 receptors by SKF81297, resulted in a small, but significant increase in apparent input resistance vs. baseline conditions. Data for individual cells (●) and averages (□) illustrating the change in R∞ for SKF81297 (left) or quinpirole (right). In each panel values on the left side are baseline measures; values on the right are after drug application (**P < 0.01; n = 7).
Neither agonist had significant effects on action potential threshold. This indicates that DA may exert complementary actions via D1 and D2 receptor activation working in concert to increase neuronal responses to inputs.

**Blockade of DA-mediated effects by DA antagonists**

To further analyze the contribution of DA receptor subtypes involved in DA-mediated changes of BLA projection neuron excitability, specific antagonists were applied prior to DA administration and throughout the recording. Interestingly, preapplication (>10 min) of either the D1 receptor antagonist SCH23390 (5 μM) or the D2 receptor antagonist sulpiride (5 μM) blocked the effects of DA on excitability (Fig. 4; SCH23390 baseline: 4.2 ± 0.5 spikes, SCH23390 + DA: 4.6 ± 0.5 spikes, n = 8; sulpiride baseline: 4.2 ± 0.4 spikes, sulpiride + DA: 4.6 ± 0.5 spikes, n = 7). Neither sulpiride nor SCH23390 alone had a significant effect on excitability, R_in, or action potential threshold (data not shown). Because excitability is increased by the D1 agonist, but not the D2 agonist, yet both the D1 and D2 antagonists block DAergic effects on excitability, it is possible that the D1-mediated effects of DA depend on, or are enhanced by, the activation of D2 receptors, and a tonic D2 receptor activation may be present in the slice. Such a synergistic action would be similar to effects observed in the dorsal striatum (Hu and White 1994; Keefe and Gerfen 1995) and the nucleus accumbens (Hopf et al. 2003; O’Donnell and Grace 1994). Consistent with this interpretation, preapplication of the DA D2 antagonist sulpiride (5 μM) attenuated the effects of the D1 receptor agonist SKF81297 on projection neuron excitability (n = 7, Fig. 4E). These data indicate that D2 receptor activation increases R_in, and this in theory could augment the D1-mediated actions on excitability. A D2 receptor-specific effect on R_in is further supported by the observation that R_in was increased both by the application of quinpirole (see preceding text), as well as by the application of DA in the presence of the D1 receptor antagonist SCH23390 (5 μM SCH23390 baseline: 147.4 ± 19.6 MΩ, SCH23390 + DA: 167.3 ± 24.0 MΩ, P < 0.01, n = 8). In the presence of the D2 receptor antagonist sulpiride (5 μM), DA application did not result in a similar change of R_in (sulpiride baseline: 132.5 ± 17.8 MΩ, sulpiride + DA: 133.7 ± 17.6 MΩ, n = 7). Thus it appears that activation of the D1 receptor effectively counteracts the D2-mediated increase in R_in. One possible mechanism could be a D1-mediated increase in synaptic conductance in projection neurons. Previous data demonstrate that DA increases the firing of BLA interneurons (Rosenkranz and Grace 1999). To test whether DA and D1 receptor activation results in an increase of GABAergic transmission, we examined the effects of DA on membrane excitability in inhibitory interneurons and on spontaneous GABAergic events recorded from projection neurons.

**Effects of DA on interneurons**

BLA interneurons were identified based on their electrophysiological properties and post hoc morphological examination (see preceding text). As with BLA projection neurons, DA receptor activation increased the excitability of BLA interneurons. Thus bath application of DA (10 μM, as in the preceding

**FIG. 4.** The effects of DA on excitability are blocked by specific antagonists. A and C: individual sweeps (A) and group data (C) showing that bath application of the D1 antagonist SCH23390 (5 μM) blocked the effect of DA (10 μM) on evoked spikes without affecting neuronal excitability on its own (n = 8). B and D: individual sweeps (B) and group data (D) showing that bath application of the D2 antagonist sulpiride (5 μM) similarly blocked the effect of DA (10 μM) on evoked spikes also without having an effect on excitability on its own (n = 8). E: blockade of D2 receptors by sulpiride prevented the effects of the D1 agonist SKF81297 on neuronal excitability. F: effects of the antagonists on input resistance either alone or in the absence of DA. Only co-application of SCH23390 (5 μM) and DA (10 μM) resulted in a significant increase in R_in. This is further evidence that a D2 receptor-mediated mechanism can increase R_in in BLA projection neurons (see RESULTS for details).
FIG. 5. Dopamine increases the excitability of BLA interneurons: A: bath application of 10 μM DA increased the number of action potentials evoked by a suprathreshold current step in a fast-spiking BLA interneuron. B: DA application (but also SKF81297, not shown) decreases the action potential threshold of interneurons (see RESULTS for details). C: both DA and the D1 receptor agonist SKF81297 increased the excitability of BLA interneurons (DA, **P < 0.01, n = 8; SKF81297, *P < 0.05, n = 5). D: both DA and SKF81297 significantly hyperpolarized the threshold for initiation of the first action potential (DA, **P < 0.01; SKF81297, *P < 0.05).

text) increased the number of spikes in response to a fixed amplitude depolarizing current injection (Fig. 5; baseline: 4.8 ± 1.6 spikes, DA: 8.3 ± 2.1 spikes, P < 0.01, n = 8), without significant effects on Rm (baseline: 315.6 ± 49.9 MΩ, DA: 310.8 ± 46.5 MΩ). A depolarization of the membrane potential was seen after DA application that was compensated to baseline values with hyperpolarizing current injection. The effect of DA on spike firing was mimicked by application of 10 μM of the D1 receptor agonist SKF 81297 (control: 6.5 ± 0.6 spikes, SKF81297: 9.8 ± 3.3 spikes, P < 0.05, n = 5). Both DA and the D1 agonist SKF81297 decreased action potential threshold (action potential threshold pre-DA: −42.5 ± 1.1 mV, DA: −45.3 ± 1.3 mV, P < 0.01, n = 8; action potential threshold pre-SKF81297: −40.4 ± 1.9 mV, post-SKF81297: −43.0 ± 1.7 mV, P < 0.05, n = 5). One potential consequence of the increased excitability of BLA interneurons is an increase in the spontaneous firing of interneurons, resulting in an increase in the frequency of GABAAergic events impinging on BLA projection neurons.

DA modulation of IPSCs

Spontaneously-occurring IPSCs (sIPSCs) represent both action-potential-independent and -dependent release of GABA. In contrast, miniature IPSCs (mIPSCs) are recorded in the presence of TTX (1 μM) to eliminate the contribution of action-potential-mediated release events. Therefore a differential effect of DA on sIPSCs and mIPSCs would indicate either a selective effect on intrinsic interneuron excitability or modification of GABA release probability, respectively. We recorded pharmacologically isolated sIPSCs and mIPSCs from large projection neurons with whole cell pipettes containing CsCl and TEA. All IPSCs were recorded at holding potentials of −85 to −90 mV as inward currents. These currents were completely blocked by the addition of 20 μM bicuculline at the end of the experiment, indicating they were mediated by GABA_A receptors (data not shown). Bath application of DA (1 or 10 μM) resulted in a 65.6% increase in the frequency of sIPSCs in eight of eight projection neurons tested (control: 10.8 ± 2.7 Hz; DA: 17.8 ± 3.4 Hz; P < 0.05; n = 8; Fig. 6). However, DA had no significant effect on mean sIPSC amplitude (control: 41.1 ± 3.5 pA; DA: 48.1 ± 6.9 pA) or decay time (control: 7.8 ± 0.8 ms; DA: 7.5 ± 0.8 ms).

In contrast, neither mIPSCs frequency (control: 6.3 ± 0.7 Hz; DA: 7.7 ± 0.9 Hz; n = 6), nor overall average amplitude (control: 18.1 ± 1.5 pA; DA: 18.8 ± 1.4 pA) or decay time

FIG. 6. Dopamine increases the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in BLA projection neurons. A: spontaneous IPSCs (sIPSCs) were measured in the presence of glutamate antagonists (20 μM CNQX, 100 μM APV) in voltage clamp (left). Representative traces showing that bath application of DA (1 μM) increased the frequency of sIPSCs. B: histogram of the amplitude distribution of the events sampled from all cells (n = 8) during 8 min of continuous recording before (black square) and after (gray square) DA application, respectively. Inset: same data replotted as cumulative frequency distribution to show that DA (gray line) did not significantly change the relative number of events of a given amplitude. C: histogram of the average frequency of sIPSCs recorded from all neurons before (black bar) and after (gray bar) DA application.
DAergic modulation of BLA neuronal excitability

Postsynaptic mechanisms of DA actions in BLA projection neurons

DA has been shown to modulate a variety of currents that could be responsible for the increase in intrinsic excitability, including L-type Ca2+ and various K+ channels (Dong and White 2003; Hernandez-Lopez et al. 1997; Surmeier et al. 1995; Yang and Seamans 1996).

Because we found no effect of DA or D1-receptor activation on the amplitude of the fast or medium AHP (see preceding text), it is unlikely that the effects of DA on repetitive firing were due to modulation of a delayed rectifying K+ current or the slow calcium-dependent K+ current (sI_AHP). Another K+ conductance that has a prominent role in action potential initiation and repetitive firing is the slowly inactivating, outward rectifying K+ conductance. This I_K current was originally identified by its ability to delay the firing of action potentials from a depolarizing current step (Storm 1988), but it is now more commonly defined by its slow inactivation kinetics and sensitivity to low concentrations of 4-AP and DTX (Coetzee et al. 1999).

In current-clamp recordings we applied low doses (50 μM) of 4-AP to test whether blockade of a D-type K+ current may mimic the effects of D1 receptor activation on the repetitive firing behavior of BLA projection neurons and to test whether this manipulation occludes the effect of subsequent DA or SKF-81297 application. All recordings were done in the presence of 20 μM CNQX and 20 μM bicuculline to control for the increase in synaptic transmission resulting from 4-AP application.

4-AP significantly increased the number of evoked spikes, lowered the threshold for action potential initiation and decreased rheobase current (Fig. 8A–D; n = 8). Consistent with an effect on outward rectifying K+ currents that activate mainly at depolarized potentials, this increase in excitability occurred without a change in R_in at rest (Fig. 8E). These effects of 4-AP were not limited to late-spiking cells, but could also be observed in strongly adapting and regular-spiking neurons, indicating that these cell types also possess a significant outward K+ current that is sensitive to low doses of 4-AP. Blockade of K+ currents by 4-AP occluded the effects of subsequent SKF-81297 (n = 5) or DA (n = 3) application on excitability, resulting in no further changes in the number of evoked spikes, action potential threshold or rheobase current (Fig. 8, A, right, and B–D).

At a concentration of 50 μM 4-AP blocks potassium channels containing Kv1.1, Kv1.2, Kv1.3, Kv1.5, Kv1.6, Kv3.1, and Kv3.2 subunits (Coetzee et al. 1999) and thus may partially block the A-type potassium current, causing changes in excitability and confounding the effect of I_K blockade (Hoffman et al. 1997). Blockade of the Kv1.1, Kv1.2, and Kv1.6 subunits by the more selective I_K blocker α-DTX also increases the excitability of LA neurons and reduces spike frequency adaptation (Faber and Sah 2004). We therefore sought to replicate these results and to see whether the application of α-DTX also would occlude the effects of subsequent DA application on repetitive spike firing. As with 4-AP, application of α-DTX (100–500 nM) significantly increased the excitability of BLA projection neurons. Figure 8 (F–I) shows that α-DTX increased the number of evoked spikes, lowered the threshold for action potential initiation and decreased the rheobase current (n = 6). Like 4-AP, α-DTX had no effect on the R_in at rest (Fig. 8J). Finally, blockade of K+ currents by α-DTX also occluded the effects of subsequent DA (n = 5) application on excitability, resulting in no further changes in the number of evoked spikes, action potential threshold or rheobase current (Fig. 8, F, right, and G–I).

In voltage-clamp experiments, we isolated a slowly inactivating outward K+ current by adding 1 μM TTX (to block sodium channels), 200 μM cadmium (to block voltage-gated Ca2+ channels), and 50 μM carbachol (to block the M-current) to the bath. Furthermore, synaptic transmission was blocked with 20 μM CNQX and 20 μM bicuculline. Cells were held at a hyperpolarized potential of −100 mV to deactivate inacti-

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**FIG. 7.** Dopamine does not affect the frequency of miniature IPSCs in BLA projection neurons. A: action-potential-independent miniature IPSCs (mIPSCs) were recorded in the presence of 20 μM CNQX, 100 μM APV, and 1 μM TTX in voltage clamp from BLA projection neurons. Representative traces showing that bath application of DA did not change frequency or amplitude of mIPSCs. B: histogram of the average frequency of mIPSCs recorded from all neurons (n = 6) before (black bar) and after (gray bar) DA application during 8 min of continuous recording for each condition. C: histogram of the amplitude distribution of the events sampled from all cells (n = 6) before (black bars) and after (gray bars) DA application. Inset: Same data replotted as cumulative frequency distribution to show that DA (gray line) did not change the relative number of events of a given amplitude.
vated K⁺ channels and whole cell voltage-gated K⁺ currents were elicited by long (1.5 s) depolarizing steps (from −80 to +20 or +30 mV with 10-mV increments) delivered every 10 s. A prominent outward current activated at voltages just hyperpolarized to the threshold for spiking (at about −40 mV) and activated steeply with depolarization (Fig. 9). The current was stable over 10–15 min (3–5 repetitions of the protocol) under control conditions before application of drugs. As shown in Fig. 9, A and C, this outward current was sensitive to low concentrations of 4-AP (50 μM; n = 5) or α-DTX (100–300 nM; n = 5), respectively. The peak amplitude was reduced to 71.2 ± 5.0% by 4-AP and 64.7 ± 9.0% by α-DTX of their respective control value (Figs. 9, B and D). This effect was partly reversible during 30 min of recording. Similarly, application of DA (10 μM) led to a large reduction in the outward current (mean reduction to 69.8 ± 7.3% of control current; P = ...
0.016, n = 7). This effect was mimicked by 10 μM SKF 81297 (4 of 4 cells; mean reduction to 70.7 ± 3.8% of control current; Fig. 9). Furthermore, when either 4-AP (n = 4) or α-DTX (n = 3) was applied 10–15 min after DA application, there was only a small further reduction in the peak outward current (data not shown).

Taken together, these data show that DA via D1-receptor activation can change neuronal excitability and increase repetitive firing by reducing a 4-AP- and α-DTX-sensitive, slowly inactivating outward K⁺ current.

Finally, we tested whether an enhancement of L-type Ca²⁺ channels through D1 receptor activation similar to that described previously in medium-spiny striatal neurons (Hernandez-Lopez et al. 1997) could contribute significantly to the observed increase in neuronal excitability. Therefore we repeated current-clamp recordings similar to those shown in Fig. 8 but in the presence of the L-type Ca²⁺ channel blocker nimodipine (1 μM) to see whether blockade of L-type channels would abolish or reduce the increase in excitability after D1 receptor stimulation. Overall (n = 7), nimodipine by itself had no significant effect on repetitive firing and intrinsic excitability. Furthermore, blockade of L-type Ca²⁺ channels had no effect on the D1 receptor-mediated increase in excitability in any of the cells tested. Application of SKF-81297 in the presence of nimodipine resulted in a significant increase in the number of evoked spikes (baseline nimodipine: 3.1 ± 0.3 spikes; nimodipine + SKF-81297: 6.9 ± 0.9 spikes; P < 0.01, df = 6) and a reduction in the spike threshold (baseline nimodipine: −36.3 ± 1.6; nimodipine + SKF-81297: −40.7 ± 2.1 mV; P < 0.05, df = 6) without changing other measures of intrinsic excitability (not shown). Thus these data suggest that DA regulates spike initiation and repetitive firing via actions on GABAergic interneurons, Rₐ, and potassium conductance but not by modulation of L-type Ca²⁺ channels.

**DISCUSSION**

Behavioral studies have shown that activation of DA receptors in the BLA enhances amygdala-dependent affective behaviors (Borokowski and Kokkinidis 1996; Harmer and Phillips 1999). Our data are the first demonstration that activation of DA receptors results in a direct postsynaptic increase in the excitability of both BLA projection neurons and interneurons; this may account for these effects of DA on behavior. At the network level, DA receptor activation could enhance the BLA output in response to strong inputs that cause spike firing, while at the same time further suppressing weaker inputs via the increased GABAergic tone that results from the activation of interneurons.

**DA D1 versus D2 effects**

Our data indicate that both D1 and D2 receptors contribute to the increased excitability of BLA projection neurons after DA application. Earlier anatomical studies found primarily D1 receptor mRNA and only low levels of D2 receptor mRNA with mostly nonoverlapping distributions (Scabilia et al. 1992; Weiner et al. 1991); however, a recent study demonstrated abundant colocalization of D1 and D2 mRNA in individual neurons (Maltais et al. 2000). We show that in projection neurons D1 receptor stimulation increases the number of evoked action potentials. At the same time, D2 receptor activation results in a moderate increase in Rₐ without significantly affecting the number of spikes. DA D1 receptor stimulation was also shown to increase membrane excitability and spike firing in principal neurons of the neocortex and striatum (Henze et al. 2000; Hernandez-Lopez et al. 1997; Penit-Soria et al. 1987; Yang and Seamans 1996). In contrast, investigations into the effects of DA on Rₐ in the neocortex are variable and include a decrease (Gulledge and Jaffe 2001), no consistent change (Henze et al. 2000; Yang and Seamans 1996), or large increase (Bracci et al. 2002 in striatal interneurons; Penit-Soria et al. 1987) in Rₐ.

In BLA projection neurons, the increase in Rₐ from resting membrane potentials was due to postsynaptic mechanisms, was specific for D2 receptor activation, and was observed only under three conditions: when a selective D2 agonist was applied, when DA was applied in the presence of a D1 antagonist, and when DA was applied while synaptic inputs were blocked. Whereas the first two observations point to an involvement of D2 receptor activation, the third observation implies that D1 receptor activation can counter the D2-mediated increase of Rₐ possibly through an indirect effect on synaptic inputs. Projection neurons in the BLA are under tight inhibitory control by GABAergic interneurons (Chen and Lang 2003; McDonald et al. 2002), and we show that D1 receptor activation augments interneuron excitability and increases the frequency of sIPSCs in BLA projection neurons. Thus the D1-mediated enhancement of synaptic inputs and the resulting increase in conductance could offset the D2-mediated increase in Rₐ of projection neurons. Under our recording conditions, the D2-mediated increase of Rₐ was so small, however, that D2 agonists alone exerted only subtle effects on excitability. In the neocortex, Gulledge and Jaffe (2001) have demonstrated that DA can have independent actions on spike generation and Rₐ. In their study, DA indirectly inhibited pyramidal cells by increasing local GABAergic tone, an effect that in the prefrontal cortex was strong enough to mask the DA-mediated increase in excitability (Gulledge and Jaffe 2001). In our data from the BLA, however, the effect was less pronounced, and the postsynaptic D1-mediated enhancement of membrane excitability always dominated. Finally, modulation of spontaneous EPSCs could potentially also influence input resistance in projection neurons. However, the frequency of spontaneous EPSCs (unpublished results) and the amplitude of evoked EPSPs (Bissiere et al. 2003) are actually decreased by DA application and are thus unlikely to cause the synaptically mediated offset of postsynaptic enhancement of input resistance by DA.

DA D1 and D2 receptors are traditionally believed to exert opposite cellular effects, according to their stimulating or inhibiting effects on adenylyl cyclase activity, respectively (Kebabian and Calne 1979). However, in the BLA, adenylyl cyclase is not the only target of DA modulation (Leonard et al. 2003). Our data indicate that the postsynaptic effects of D1 and D2 receptor activation can have complementary actions on BLA function through the modulation of different cellular properties that contribute to action potential firing. Because excitability was increased by the D1 agonist, but not the D2 agonist yet preapplication of either a D1- or a D2 antagonist blocked the effects of DA on excitability, it appears that the D1-mediated effects of DA depend on, or are enhanced by, a
tonic D2 receptor activation, which may be present in the slice even in the absence of exogenous agonists (cf. O’Donnell and Grace 1994). However, whether the effects of D1 and D2 receptor activation result from the convergence onto a common intracellular pathway remains to be investigated.

**Postsynaptic mechanisms of the DA-mediated increase in excitability in BLA projection neurons**

DA D1 receptor activation can change the excitability of BLA projection neurons and increase repetitive firing by reducing a 4-AP- and α-DTX-sensitive, slowly inactivating outward K⁺ current (Figs. 8 and 9). Blockade of K⁺ currents by both 4-AP and α-DTX strongly increased the firing frequency during current injections (Fig. 8). Both antagonists mimicked the effects of DA and D1 receptor activation and occluded the otherwise observed augmenting effects of subsequent DA application. The slowly inactivating D-type current probably results from various combinations of K⁺ channel subunits, most likely Kv1.2 with other subunits (Coetzee et al. 1999). In the lateral amygdala, the Kv1.2 subunit appears to be critical in regulating spike frequency adaptation (Faber and Sah 2004). In both neocortical and amygdalar neurons, α-DTX-sensitive channels are strategically located at the soma (Bekkers and Delaney 2001) or the primary apical dendrite (Faber and Sah 2004) to influence neuronal excitability. In cortical neurons, I_D requires deinactivation from potentials more negative than the resting membrane potential. Once it is activated by a depolarizing pulse, it takes several seconds to inactivate (Storm 1988). Functionally, I_D opposes membrane depolarization and regulates subthreshold synaptic events and repetitive firing (Hammond and Crépel 1992; Storm 1988; Yang and Seamans 1996). In striatal (Nisenbaum et al. 1994), hippocampal (Storm 1988), and cortical (Hammond and Crépel 1992) neurons, this conductance underlies the outward rectification responsible for the late-spiking behavior in these cells. A subset of neurons in the BLA was shown to exhibit prominent late-spiking behavior (Washburn and Moises 1992), and we found neurons with similar firing characteristics in both the basal and the lateral nucleus of the BLA. However, our own voltage-clamp recordings and recent data by Faber and Sah (2004) show that other cell types in the BLA also are endowed with a slowly inactivating outward K⁺ current, which as we show is regulated in the same manner by DA and D1 receptor stimulation. A selective reduction of the D-like current through D1 receptors similar to our results in BLA projection neurons has also been reported for cortical pyramidal cells (Dong and White 2003; Yang and Seamans 1996) and fast-spiking interneurons (Gorelova et al. 2002). Data from other brain regions suggest that DA may modulate the D-type K⁺ current through activation of protein kinase A (Dong and White 2003).

In addition, in cortical and striatal cells, a variety of other currents, including Na⁺ and Ca²⁺ currents that govern spike initiation and repetitive firing, are also modulated by DA (Hernandez-Lopez et al. 1997; Yang and Seamans 1996). We found that blockade of L-type Ca²⁺ channels by the selective antagonist nimodipine did not prevent the D1-mediated increase in excitability. Although these experiments do not rule out the possibility of a DAergic modulation of L-type Ca²⁺ channels (Hernandez-Lopez et al. 1997; Surmeier et al. 1995), they demonstrate that in BLA neurons, at least under our recording conditions, the activation of L-type channels is not required for the increase in firing. However, it is likely that the actions of DA on BLA pyramidal neurons will depend on the timing and strength of synaptic inputs as well as on the membrane potential range at which the cells are operating, and therefore other currents may still prove to be important targets of DAergic modulation as it is true in other brain regions.

**DA modulation of BLA interneurons**

A modulation of BLA interneurons by DA was suggested by previous anatomical studies (Brinley-Reed and McDonald 1999) and extracellular recordings (Rosenkranz and Grace 1999). Our data show that DA augments the excitability of fast-spiking interneurons and increases both evoked and spontaneous firing. Similar to BLA projection neurons, the increase in the excitability of interneurons was mimicked by activation of D1 receptors. Similar increases in membrane excitability via D1 receptors have also been described for cortical and striatal interneurons (Bracci et al. 2002; Gorelova et al. 2002). The enhanced D1-mediated excitability of interneurons results in increased frequency of sIPSCs recorded from BLA projection neurons. This effect was also recently shown by Bissiere et al. (2003), who observed that sIPSCs were not blocked by a D2 antagonist.

**Comparison with in vivo studies**

The enhanced excitability of BLA neurons after DA application in vitro is similar to the effects of DA agonists observed in vivo (Rosenkranz and Grace 2002a). However, the extent to which specific DA receptor subtypes contribute to this increase in excitability appears to differ between the slice and in vivo conditions. Thus while in both preparations D2 receptor activation increases R_in, D1 receptor activation has less impact on membrane excitability in vivo (Rosenkranz and Grace 2002a,b). Potential explanations for these differences may lie in the degree of spontaneous network synaptic inputs that impinge on the neuron in vivo or in the basal levels of DA present.

**Functional implications of DA modulation of BLA neurons**

DA is well situated to enhance the response of BLA projection neurons to excitatory drive, thereby increasing BLA output and ultimately enhancing amygdala-mediated behaviors. Our data suggest that large inputs are more likely to depolarize the membrane potential above action potential threshold due to a D2-mediated increased R_in. Once threshold is reached, the combined effects of D1 and D2 receptor activation will enhance action potential firing. However, it would be maladaptive if the BLA projection neurons were sensitive to all excitatory inputs. DA D1-mediated excitation of BLA interneurons, resulting in increased GABAergic inhibition of projection neurons, can counter the enhanced response of BLA projection neurons to weaker inputs. The increased GABAergic inhibition may filter smaller inputs by transient hyperpolarizations and a reduction of R_in in BLA projection neurons (Häusser and Clark 1997).

Studies have associated amygdala abnormalities with depression (Drevets 2003), anxiety (Rauch et al. 2003), schizophrenia (Chance et al. 2002), and drug abuse (Robbins and
Everitt (2002). In some of these disorders, there are indications for a DAergic involvement in the cause or treatment (Reynolds 1983; Silvestri et al. 2000; Volkow et al. 2002). The current study shows that DA acts directly on BLA neurons to change their excitability, thereby altering the contribution of the amygdala to behavior.

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