Dopamine Modulation of Honey Bee (Apis mellifera) Antennal-Lobe Neurons

Christopher G. Perk and Alison R. Mercer
Department of Zoology, University of Otago, Dunedin, New Zealand

Submitted 30 November 2005; accepted in final form 30 October 2005

Perk, Christopher G. and Alison R. Mercer. Dopamine modulation of honey bee (Apis mellifera) antennal-lobe neurons. J Neurophysiol 95: 1147–1157, 2006. First published November 9, 2005; doi:10.1152/jn.01220.2004. Primary olfactory centers [antennal lobes (ALs)] of the honey bee brain are invaded by dopamine (DA)-immunoreactive neurons early in development (pupal stage 3), immediately before a period of rapid growth and compartmentalization of the AL neuropil. Here we examine the modulatory actions of DA on honey bee AL neurons during this period. Voltage-clamp recordings in whole cell configuration were used to determine the effects of DA on ionic currents in AL neurons in vitro from pupal bees at stages 4–6 of the nine stages of metamorphic adult development. In ~45% of the neurons tested, DA (5–50 × 10⁻⁵ M) reduced the amplitude of outward currents in the cells. In addition to a slowly activating, sustained outward current, DA reduced the amplitude of a rapidly activating, transient outward conductance in some cells. Both of the currents modulated by DA could be abolished by the removal of Ca²⁺ from the external medium or by treatment of cells with charybdotoxin (2 × 10⁻⁶ M), a blocker of Ca²⁺-dependent K⁺ currents in the cells. Ca²⁺ currents were not affected by DA, nor were A-type K⁺ currents (I_K). Results suggest that the delayed rectifier-like current (I_K) also remains intact in the presence of DA. Taken together, our data indicate that Ca²⁺-dependent K⁺ currents are targets of DA modulation in honey bee AL neurons. This study lends support to the hypothesis that DA plays a role in the developing brain of the bee.

INTRODUCTION

During metamorphosis, the CNS of the honey bee, Apis mellifera, undergoes dramatic growth and reorganization. Nowhere are the changes more striking than in the primary olfactory centers [antennal lobes (ALs)] of the brain. Around pupal stage 2 of the nine stages of metamorphic adult development, antennal sensory afferent neurons enter the ALs (Masson and Arnold 1984). Their arrival triggers the formation of prominent subunits of synaptic neuropil called glomeruli (Mobbs 1982; Pareto 1972; Suzuki 1975; see also, Boeckh and Tolbert 1993; Hansson and Anton 2000; Oland and Tolbert 1987, 1996; Rospars 1988; Salecker and Boeckh 1996), which are the functional subunits of the AL neuropil (Joerges et al. 1997; Sachse et al. 1999; Stopfer et al. 1997; see also Chistensen and Hildebrand 1987; Hansson and Anton 2000). Each glomerulus contains the terminal arbors of antennal sensory afferent neurons, processes of local interneurons, dendrites of projection (output) neurons, and ramifications of centrifugal neurons that project to the ALs from other sites in the brain (Abel et al. 2001; Flanagan and Mercer 1989; Fonta et al. 1993; Homberg 1984; Müller et al. 2002; Sun et al. 1993).

Immediately before glomerulus formation (pupal stage 3), developing ALs are invaded by dopamine (DA)-immunoreactive processes that ramify extensively in the central neuropil of the lobes (Kirchhof et al. 1999). These processes originate from cell bodies located in the lateral deutocerebral soma rind, posterior to each AL (Kirchhof et al. 1999; Schäfer and Rehder 1989). The same cells extend processes into the dorsal lobe of the deutocerebrum, as well as to the protocerebrum and suboesophageal ganglion (Schäfer and Rehder 1989). Around pupal stage 4, there is a surge in DA levels in the ALs, and rapid neurite outgrowth apparent in stage 5 AL neurons in vitro is enhanced by exposure to DA (Kirchhof et al. 1999). While the identity of the receptors that mediate the effects of DA has yet to be determined, mRNA for three DA receptor genes, Amdop1 (Blenau et al. 1998), Amdop2 (Humphries et al. 2003), and Amdop3 (Beggs et al. 2005), has been detected in cells that surround the developing AL neuropil of the bee (Beggs et al. 2005; Kurshan et al. 2003). The expression of these genes, in particular Amdop2, is strongly developmentally regulated (Kurshan et al. 2003), suggesting that DA plays a central role in the developing brain of the bee (Kirchhof and Mercer 1997; Kirchhof et al. 1999; Kokay et al. 1999; Kurshan et al. 2003).

In adult worker bees, DA levels in the brain (Kokay and Mercer 1997; Schulz and Robinson 1999; Taylor et al. 1992), and levels of dopamine receptor gene expression (Humphries et al. 2003; Kokay and Mercer 1997) change markedly during the lifetime of the bee. Intriguingly, regardless of age, DA levels in the ALs of foragers are higher than in the ALs of bees performing nursing duties, suggesting that DA in ALs is linked to behavioral state (Schulz and Robinson 1999). While it has been suggested that biogenic amines such as DA might influence response thresholds for task-related stimuli (Bicker and Menzel 1989; Hammer 1993; Macmillan and Mercer 1987; Mercer and Menzel 1982; Scheiner et al. 2002; Schulz and Robinson 1999), relatively little is known about the mechanisms through which this amine operates in the brain of the bee.

In this study, whole cell voltage-clamp recordings were used to examine the modulatory actions of DA on ionic currents expressed by honey bee AL neurons in vitro. Our results reveal that Ca²⁺-activated K⁺ currents are targets of DA modulation in these cells.

METHODS

Apis mellifera

Frames of honey bee brood collected from hives at the Department of Zoology, University of Otago, were kept for periods of ≤1 wk in a humidified incubator at 35°C. Metamorphic adult development in the honey bee occurs over an 8- to 9-day period. Pupal honey bees at
stages 4–6 (P4–P6) of the nine stages of metamorphic adult development were collected from the brood frames. The stage of development was ascertained by using a method similar to that described by Jay (1962) based on external cues, such as eye color and head pigmentation. The experiments described in this work comply with the laws of New Zealand regulating scientific research.

**Cell cultures**

Primary cell cultures were prepared from the ALs of pupal bees as described elsewhere (Kirchhof and Mercer 1997; Kreissl and Bicker 1992). The heads of 8–10 pupal bees were removed from their bodies, placed in a dish lined with sylgard (Dow Corning, Midland, MI), and secured with insect mounting pins. The front of the head capsule and glandular tissue surrounding the brain were removed. The exposed brain was rinsed with modified Leibovitz L-15 culture medium (BL15, pH 7.2, 500 mOsm) supplemented with 4.0 g glucose, 2.5 g fructose, 24.0 g sucrose, and 3.3 g proline per liter (all purchased from Sigma, St. Louis, MO). ALs were removed with fine forceps and placed in fresh BL-15 medium. The remaining steps took place under sterile conditions. To aid dissociation of the tissue, ALs were exposed to a hyperosmotic culture medium (BL-15 supplemented with an additional 20.0 g/l sucrose, 580 mOsm) for 10 min and transferred into a Ca2+- and Mg2+-free honey bee Ringer (in mM: 135 NaCl, 5 KCl, and 114.5 Tris-HCl; pH 7.2, 460 mOsm) for the same period. The tissue was rinsed briefly in culture medium (BL-15), excess fluid was removed, and the ALs (6/250 µl) were dissociated by trituration. The dissociated cells were transferred in 100-µl aliquots to the center of an uncoated plastic culture dish (Falcon, 3001) and allowed to adhere to the substrate for 3–5 min. The dishes were filled with 2 ml of culture medium (BL15) and placed in a humidified incubator at 28°C. Cells were maintained in culture for 4–5 days before use.

**Whole cell recording**

Patch-clamp recordings in whole cell configuration (Hamill et al. 1981) were used to examine DA modulation of ionic currents in honey bee AL neurons in vitro. The voltage-gated and Ca2+-dependent currents examined in this study have been described in detail elsewhere (for descriptions in Apis mellifera see Grünewald 2003; Kloppenburg et al. 1999b; Pelz et al. 1999; Schäfer et al. 1994; for review, see Wicher et al. 2001). These currents include a transient A-type K+ current (I_A), a sustained, delayed rectifier-like current (I_KV), Ca2+-activated K+ currents (I_CaK), a Ca2+ current (I_Ca), and a rapidly activating transient TTX-sensitive current carried by Na+ (I_TO). Cells were viewed under an IMT-2 microscope (Olympus) using phase-contrast optics. All experiments were conducted at room temperature. Recording electrodes (2–3 MΩ) were prepared from borosilicate glass (100-µl micropipettes, 1.71 mm OD, 1.32 mm ID; VWR Scientific, West Chester, CA) using a Flaming-Brown micropipette puller (P-87, Sutter Instruments) and backfilled with a solution containing (in mM) 100 K-aspartate, 40 KF, 20 KCl, 2.5 MgCl2, 1 EGTA, 160 sucrose, and 10 HEPES (pH 7.2). Throughout the recording period, cells were continuously superfused with artificial insect saline (AIS) containing (in mM) 130 NaCl, 6 KCl, 4 MgCl2, 5 CaCl2, 160 sucrose, 25 glucose, and 10 HEPES/NaOH (pH 7.2, 500 mOsm). Junction potentials were nullified before seal formation. Recordings were made using an Axopatch 1D amplifier in conjunction with a CV4 1/100 headstage and Digidata 1200 interface (Axon Instruments, Union City, CA). Data were acquired using pClamp6 software (Axon Instruments, Union City, CA) run on a 486 PC computer. Cells were clamped at a holding potential of –70 mV, and depolarizing voltage steps were used to activate voltage-gated channels in the cells. Membrane currents were filtered at 2 kHz using a low-pass four-pole Bessel filter and sampled at intervals of 100 µs. In most recordings, a P/4 protocol (see Armstrong and Bezanilla 1974) was used for digital subtraction of linear leak currents and capacitance artifacts. No compensation was made for series resistance, and therefore voltage errors may be present where currents measured were large. However, series resistance errors should not affect the central conclusions of this study.

**Isolation of currents**

Whole cell current profiles were examined initially in the absence of any blocking agents. Components of the whole cell current profile were divided into three categories: A-type K+ currents (A), delayed rectifier currents (Bi), and Ca2+ currents (Bii). Each category was divided further into two subcategories: A-type K+ currents were divided into slowly activating (Ai) and rapidly activating (Aii) subcategories, and delayed rectifier currents were divided into type 1 (Ai) and type 2 (Bi) subcategories. Current profile is dominated by large slowly activating outward currents that show significant inactivation during voltage pulse. Aii: I/V relationship measured 105 ms after voltage pulse onset (* in Aii). Bi: whole cell currents typical of type 2 cells. Voltage was stepped from a holding potential of –70 to +90 mV in 10-mV increments. Current profile is dominated by large slowly activating outward currents that show significant inactivation during voltage pulse. Bii: I/V relationship measured 105 ms after voltage pulse onset (* in Bii).

**FIG. 1.** Ai: whole cell currents typical of type 1 antennal lobe (AL) neurons. Voltage was stepped from a holding potential of –70 to +70 mV in 10-mV increments. Current profile is dominated by large outward currents that include a rapidly activating transient component and a sustained component that shows little or no inactivation during the voltage pulse. Aii: I/V relationship measured 105 ms after voltage pulse onset (* in Aii). Bi: whole cell currents typical of type 2 cells. Voltage was stepped from a holding potential of –70 to +90 mV in 10-mV increments. Current profile is dominated by large slowly activating outward currents that show significant inactivation during voltage pulse. Bii: I/V relationship measured 105 ms after voltage pulse onset (* in Bii).
isolated using routine pharmacological techniques described elsewhere (for Aps neurons, see Grünewald 2003; Kloppenburg et al. 1999b; Pelz et al. 1999; Schäfer et al. 1994; Wüstenberg et al. 2004). Na⁺ currents were blocked with TTX (10⁻⁷ M), whereas Ca²⁺ currents (and Ca²⁺-activated K⁺ currents) were blocked with 5 × 10⁻⁵ M CdCl₂. Rapidly activating, transient (A-type) current (IA) was blocked with 4-aminopyridine (4-AP; 5 × 10⁻⁵ M), quinidine (5 × 10⁻⁵ M) was used to block the delayed-rectifier-like current, IK, and K⁺ currents collectively were blocked by substituting K⁺ in the electrode solution with Cs⁺. To reveal the contribution that Ca²⁺-dependent K⁺ currents (IKCa) make to outward current profiles in Aps AL neurons, cells were exposed to Ca²⁺-free saline in which CaCl₂ had been replaced with MgCl₂. Effects of the IKCa blocker charybdotoxin (CTX; 2 × 10⁻⁸ M; reviewed by Garcia et al. 1995) were also tested in the cells. All chemicals were purchased from Sigma with the exception of TTX and CTX, both of which were purchased from Alomone Laboratories.

**DA application**

DA (dopamine hydrochloride, Sigma) was prepared in AIS immediately before use and pressure-ejected across the cell soma using a pipette concentration of 5 × 10⁻⁵ M (pipette concentration). At these concentrations, effects of DA on DA-sensitive cells were immediately obvious, and the chances of observing at least some recovery during DA washout were maximized. DA was applied in 20-ms pulses delivered immediately before each depolarizing voltage step. Continuous superfusion of the recording chamber with fresh AIS prevented the localized accumulation of DA around cells between individual voltage steps. The delivery pipette was removed from the recording chamber, and the cells were superfused with DA-free AIS to facilitate recovery from any DA effect. As a result of Ca²⁺ current rundown in the cells, total outward current amplitudes decreased progressively over time. For this reason, effects of DA were examined over a 10-min recording period and compared with time-dependent changes in current amplitudes occurring in control (untreated) cells.

**Statistical analysis**

Repeated-measures ANOVA was performed to determine the overall effect of DA application. This accounted for any serial correlation

![Image](https://example.com/image.png)

**FIG. 2.** Effect of dopamine (DA) on whole cell currents recorded from a type 1 AL neuron. Voltage was stepped from a holding potential of −70 to +50 mV in 10-mV increments. **Ai:** currents recorded before DA application. **Aii:** currents recorded from the same cell in the presence of DA (5 × 10⁻⁵ M). DA was applied in 20-ms pulses immediately before each depolarizing voltage pulse. **Aiii:** currents recorded after rinsing the neuron for 2 min in DA-free artificial insect saline (AIS). **B:** I/V relationship measured at −70 mV in **Ai,** −70 mV in **Aii,** and −70 mV in **Aiii,** 10 ms after the onset of each depolarizing voltage pulse. **C:** I/V relationship of profiles at −70 mV in **Ai,** −70 mV in **Aii,** and −70 mV in **Aiii,** measured 105 ms after the onset of each depolarizing voltage pulse. Effects of DA were partially but not fully reversible. **D:** effects of DA on mean outward current amplitudes. Voltage steps from a holding potential of −70 to +50 mV were used to compare the mean amplitude (±SE) of outward currents recorded in control (untreated) cells (n = 18) and in cells exposed to 5 × 10⁻⁵ M DA (DA-treated, n = 13) over time. Measurements were taken 105 ms after onset of each depolarizing voltage pulse. Current amplitudes for each cell were normalized to the initial value recorded at time 0 (0 min) and expressed as a fraction of that value. In cells receiving DA treatment, DA was applied 2 min after the 1st reading (arrow). Immediately after DA treatment, cells were washed in DA-free saline. Current amplitudes in DA-treated cells are compared with those recorded in control (untreated) cells. Significance of changes in current amplitude within each group was tested using repeated-measures ANOVA. Differences between groups at equivalent time-points were tested post hoc using independent samples Student’s t-test. Bonferroni’s correction was made for multiple t-tests (n = 4) and significance was accepted at P = 0.013, NS, not significant.
between data recorded from the same cell over time. In groups of cells recorded in the absence of blockers, and in cell groups recorded in the presence of TTX and 4-AP, small numbers of missing values at the +8-min time-point were estimated using multiple regression techniques, where the values of control or DA-treated groups recorded over the initial 6-min recording period were used to predict missing 8-min data points. Where significant overall, or group, effects were detected by ANOVA, Student’s t-test for independent samples were performed post hoc to determine at which specific time-points the current amplitudes of DA-treated and untreated cell groups were significantly different. Student’s t-test were also used to assess statistical differences in time-to-peak data. Significance was accepted at P < 0.05 except when multiple comparisons were made, where the level of significance was reduced according to Bonferroni’s correction. Mean values are expressed as mean ± SE. All analyses were performed using SPSS 11.0 (SPSS, Chicago, IL).

RESULTS

Current profiles in Apis AL neurons

Based on the composition of outward currents, AL neurons examined in this study (n = 51) could be grouped into two main categories (Fig. 1). In 71% of cells (n = 36), the outward current profile exhibited a rapidly activating transient component followed by a sustained component that showed little or no inactivation during the voltage step (Fig. 1Ai). With voltage steps above approximately −30 mV, the amplitude of the sustained outward currents in these cells increased linearly with each voltage step (Fig. 1Aii). Cells exhibiting this form of current profile are referred to here as type 1 cells. The remaining 29% of cells (type 2 cells, n = 15) exhibited outward currents in which the rapidly activating transient current seen in type 1 cells appeared to be absent, and the sustained component showed significant inactivation during the voltage step (Fig. 1Bi). In contrast to the outward current recorded in type 1 cells, the I/V curve for outward current in type 2 cells had a nonlinear relationship at potentials above +40 mV (Fig. 1Bii). The current profiles in type 2 cells are similar to those reported for AL projection (output) neurons (Grünewald 2003). We assume therefore that type 1 current profiles originate from cells belonging to the second major category of AL neurons, namely local AL interneurons, but this has yet to be confirmed. Here, attention is focused predominantly on cells exhibiting type 1 current profiles, although small numbers of type 2 cells were found to be responsive to DA (see following section).

Effects of DA on outward current profiles

Effects of DA on ionic currents were examined initially in the absence of any channel blockers. Changes in the amplitude of I_A recorded in type 1 AL neurons. I_A was isolated using TTX (10^-7 M) to block I_{Na}, quinidine (5 × 10^-3 M) to block I_{Kv}, and Cd^2+ (5 × 10^-5 M) to block Ca^2+-activated K^+ currents in the cells. A: current traces showing effects of DA on I_A. Holding potential was −70 mV. After prepulses to −100 mV (1 s), voltage was stepped from −100 to +90 mV in 10-mV increments. Ai: I_A before DA application. Aii: I_A recorded in the presence of 5 × 10^-5 M DA. Aiii: I_A recorded after rinsing neuron in DA-free saline for 2 min. B: I/V relationship of peak I_A amplitude in Ai-Aiii. C: effect of DA (5–50 × 10^-5 M) on mean peak I_A amplitude (±SE, n = 11) during a voltage step from −100 to +90 mV. Data from cells were normalized to their initial (0 min) value and are expressed as a fraction of that value. Significance of changes in current amplitude within each group was tested using repeated measures ANOVA. There was no significant change in I_A amplitude over time or as a result of DA application (P > 0.05).
of outward currents in cells exposed to DA (DA-treated; n = 13) were compared with those observed in cells receiving no DA treatment (untreated, n = 18). In the majority of DA-treated cells (54%), DA had no effect on the amplitude of currents contributing to the whole cell current profile. In 46% of the DA-treated cells, however, exposure to DA caused a pronounced reduction in the amplitude of outward currents in the cells (Fig. 2, Ai–Aiii). Among the DA-sensitive cells, there were four type 1 cells and two type 2 cells. Washing in DA-free saline partially reversed the effects of DA (see Fig. 2, A–C), but current rundown over time generally worked against the recovery of current amplitudes to levels recorded before DA application. Comparing the current profiles of control (untreated) cells and cells treated with DA revealed significant treatment-related differences between these two groups (Fig. 2D; ANOVA, F = 5.38, P = 0.028). Immediately after DA treatment, the mean amplitude of outward currents in DA-treated cells (measured 105 ms after the onset of a voltage step from −70 to +50 mV) was significantly lower than in untreated cells (Student’s t-test, t = 3.07, P = 0.009, significance at P < 0.013 after Bonferroni’s correction for 4 pairwise comparisons). After 2- to 6-min washing in DA-free saline, however, differences between the two groups of cells were no longer statistically significant.

Identification of outward currents modulated by DA

**A-TYPE K⁺ CURRENTS (Iₐ).** Outward currents modulated by DA were examined more closely in type 1 cells. Type 1 cells express a prominent, rapidly activating, transient A-type current, Iₐ (Fig. 3A). To assess the effects of DA on the amplitude of this current, Iₐ was isolated and identified in 11 type 1 cells. A series of 10-mV voltage steps from −100 to +90 mV were used to compare peak Iₐ recorded before DA application with peak current amplitudes recorded in the presence of DA [5 × 10⁻⁵ (n = 3) or 5 × 10⁻⁴ M (n = 8), data pooled for analysis; Fig. 3, B and C]. Iₐ amplitude remained stable over time and was not affected by treatment with DA (Fig. 3C; ANOVA, F = 0.82, P = 0.451).

**K⁺ CURRENTS OTHER THAN Iₐ.** Effects of DA on type 1 cells were examined further using cells in which the transient A-type current, Iₐ, was blocked by 4-AP (5 × 10⁻³ M; Fig. 4). Of the nine cells examined under these conditions, five showed no response to DA (5 × 10⁻⁵ M). In the four remaining cells, DA caused an immediate reduction in the amplitude of outward currents in the cells (Fig. 4, Ai and Aii). The responses of DA-sensitive cells were found to be significantly different from those of cells that were unresponsive to this amine (ANOVA, F = 13.41, P < 0.008). Immediately after DA application, outward current amplitudes in DA-sensitive cells were significantly lower than in cells in which no DA response was observed (Student’s t-test, t = 5.70, P = 0.006, significance at P < 0.017 after Bonferroni’s correction for 3 pairwise comparisons; Fig. 4B). However, 2–6 min after DA application, and with continuous rinsing in DA-free saline, current amplitudes in these two groups of cells were no longer significantly different (Fig. 4B).

The subtraction of currents recorded in DA-responsive cells immediately after DA treatment (Fig. 4Aii) from currents recorded in the same cells before DA application (Fig. 4Aii)
revealed that DA modulated two currents: 1) a rapidly activating transient outward current and 2) a slowly activating current that increased in amplitude throughout the 120-ms voltage step (Fig. 4Aiiii). To assist with the identification of these currents, their activation properties (times to peak) were compared with those of the A-type current, \( I_A \) (see Fig. 3A), and the delayed rectifier-like current, \( I_{KV} \) (see Fig. 5Aii). Using depolarizing voltage steps from −70 to +20 mV, the transient component of the DA-sensitive current reached peak levels in 2.28 ± 0.45 ms (n = 4), significantly faster than the time-to-peak \( I_K \) (3.82 ± 0.14 ms, n = 34) measured under the same conditions (Student’s t-test, \( t = −3.30, P = 0.046 \)). The relatively slowly activating sustained current, \( I_{KV} \), reached peak levels (also using voltage steps from −70 to +20 mV) in 87.30 ± 10.30 ms (n = 7), distinguishing \( I_{KV} \) also from the currents modulated by DA. Because the kinetics of \( I_A \) and \( I_{KV} \) suggest that they are not the targets of DA modulation, we examined the possibility that Ca\(^{2+}\)-activated K\(^+\) currents in type 1 AL neurons might be modulated by this amine.

\( \text{Ca}^{2+}\)-dependent currents in type 1 AL neurons

\( \text{Ca}^{2+}\)-dependent currents in type 1 neurons were identified using Ca\(^{2+}\)-free saline (Fig. 5). The amplitude of outward currents in the cells was dramatically reduced under these conditions (Fig. 5, Ai and Aii). Subtracting currents recorded in Ca\(^{2+}\)-free saline (Fig. 5Aii) from those recorded in the same cells under normal AIS (Fig. 5Ai) revealed that exposure to Ca\(^{2+}\)-free saline abolished a rapidly activating transient component as well as a sustained outward current in type 1 cells (Fig. 5Aiiii). Analysis of the Ca\(^{2+}\)-sensitive currents (Fig. 5Aiii) suggested that the two components share a similar activation threshold (around −20 mV; Fig. 5C). To explore the possibility that these Ca\(^{2+}\)-dependent K\(^+\) currents may be targets of DA modulation, we compared the modulatory actions of DA (5 × 10\(^{-5}\) M) with those of CTX (2 × 10\(^{-8}\) M; Fig. 6), a potent \( I_{KCa} \) channel blocker (Garcia et al. 1995). When bath-applied for 2–4 min immediately after a baseline recording, the effects of CTX (Fig. 6, Ai and Aii) were strikingly similar to the effects of DA (Fig. 6, Bi and Bii). Like DA (Fig. 6Biii), CTX blocked a rapidly activating transient outward current in type 1 AL neurons (\( I_{KCa \text{ transient}} \) with a time to peak current (using a depolarizing voltage step from −70 to +20 mV) of 2.76 ± 0.16 ms (n = 14), as well as a slowly activating, sustained current (\( I_{KCa \text{ sustained}} \)) that increased in amplitude throughout the 120-ms voltage step (Fig. 6Aiii). Exposing cells to CTX at a concentration of 2 × 10\(^{-8}\) M had a greater impact on the amplitude of these currents than 5 × 10\(^{-5}\) M DA, suggesting that CTX is a more potent inhibitor of calcium-dependent K\(^+\) channels in these cells than DA. Nonetheless, the kinetics of the currents blocked by CTX closely resembled those of the DA-sensitive currents described above. \( I/V \) curves generated from the CTX- and DA-sensitive currents (Fig. 6, Aiii and Biii, respectively) show that CTX-sensitive currents, like those modulated by DA, exhibit an activation threshold around −20 mV (Fig. 6, Aiv and Biv, respectively). The \( I/V \) curves reveal also that transient currents blocked by DA and CTX exhibit a more strongly nonlinear \( I/V \) relationship than sustained currents (Fig. 6, Aiv and Biv), suggesting that \( I_{KCa \text{ transient}} \) and

**FIG. 5.** Identification of Ca\(^{2+}\)-dependent currents in type 1 AL neurons using Ca\(^{2+}\)-free saline. TTX (10\(^{-7}\) M) was used to block \( I_{Na} \) and 4-AP (5 × 10\(^{-3}\) M) was used to block \( I_{K}\). Voltage steps were applied in 10-mV increments from a holding potential of −70 to +70 mV. Ai: outward currents recorded in normal saline (AIS). Aii: outward currents recorded in the same cell after 4-min exposure to modified AIS in which Ca\(^{2+}\) had been substituted with Mg\(^{2+}\). Aiii: Ca\(^{2+}\)-sensitive currents obtained by subtracting traces shown in Aii from traces shown in Ai. B: \( I/V \) relationships of currents at in Ai and in Aii, 115 ms after onset of each voltage step. There is a marked reduction in amplitude of outward currents in Ca\(^{2+}\)-free saline. C: \( I/V \) relationship of Ca\(^{2+}\)-sensitive outward current measured at peak amplitude (• in Aiiii) and 115 ms after onset of each voltage step (○ in Aiiii).

\( J \text{ Neurophysiol} \) • VOL 95 • FEBRUARY 2006 • www.jn.org
FIG. 6. Comparison of the effects of charybdotoxin and DA on non–A-type outward currents in type 1 AL neurons. TTX (10^{-7} M) was used to block \( I_{Na} \), and 4-AP (5 \times 10^{-7} M) was used to block \( I_{A} \). Voltage steps were applied in 10-mV increments from a holding potential of -70 to +60 mV. 

**A:** effects of charybdotoxin (CTX; 2 \times 10^{-8} M). 

- **Ai:** outward currents recorded before CTX exposure. 
- **Aii:** outward currents recorded in the same cell after 4 min of exposure to CTX. 
- **Aiii:** CTX-sensitive currents revealed by subtracting traces shown in **Aii** from traces shown in **Ai**. CTX blocks a rapidly activating transient current as well as a slowly activating sustained current. 
- **Aiv:** \( I/V \) relationships of CTX-sensitive currents. Peak transient current was measured at \( -70 \) in **Aiii**. Sustained component was measured 105 ms after onset of voltage step (● in **Aiii**).

**B:** effects of DA (5 \times 10^{-5} M). 

- **Bi:** outward currents before DA application. 
- **Bii:** outward currents recorded in the same cell in the presence of DA. 
- **Biii:** DA-sensitive currents revealed by subtracting traces shown in **Bii** from traces shown in **Bi**. \( I/V \) relationships of DA-sensitive currents. Peak transient current was measured at \( -70 \) in **Biii**. Sustained component was measured 105 ms after onset of the voltage step (● in **Biii**). Effects of DA are strikingly similar to those of CTX. 

**C:** effects of DA on outward currents recorded in cells exposed to CTX (2 \times 10^{-8} M). 

- **Ci:** voltage steps from a holding potential of -70 to +50 mV were used to compare mean amplitude (±SE) of outward currents recorded in control (untreated) cells (\( n = 8 \)) and in cells exposed to 5 \times 10^{-5} M DA (DA-treated, \( n = 10 \)) over time. Measurements were taken 105 ms after onset of each depolarizing voltage pulse. Current amplitudes for each cell were normalized to initial value recorded at time 0 (0 min) and expressed as a fraction of that value. In cells receiving DA treatment, DA was applied 2 min after the 1st recording (arrow). Immediately after DA treatment, cells were washed in DA-free saline. Current amplitudes in DA-treated cells are compared with those recorded in control (untreated) cells. Significance of changes in current amplitude within each group was tested using repeated-measures ANOVA. There was no significant change in outward current amplitude as a result of DA application (\( P > 0.05 \)).
These results suggest that Ca\textsuperscript{2+} and DA (ANOVA, F\textsubscript{1,517}=0.05) showed very little run-down in current amplitude over time in CTX-treated cells and no significant difference between control cells treated with CTX alone and cells treated with CTX plus DA (ANOVA, F\textsubscript{0.797}=0.68, P\textsubscript{0.05}=0.668). Taken together, these results suggest that Ca\textsuperscript{2+}-dependent K\textsuperscript{+} currents in type 1 AL neurons are the targets of DA modulation. One alternative possibility, however, is that these currents may be blocked indirectly through DA modulation of Ca\textsuperscript{2+} currents in the cells.

**DA MODULATION OF I\textsuperscript{CA}** To explore the possibility that DA affects Ca\textsuperscript{2+}-dependent K\textsuperscript{+} currents indirectly through modulation of I\textsubscript{CA}, we examined the effects of DA on isolated Ca\textsuperscript{2+} currents in type 1 cells (Fig. 7). Eight cells were exposed to DA (5–15 × 10\textsuperscript{-5} M, pooled for statistical analysis) and 10 (untreated) cells served as controls. The Ca\textsuperscript{2+} currents in these cells reached maximum amplitude within 30 ms of the onset of a depolarizing voltage step and declined over the remainder of the voltage pulse (Fig. 7A). Whether or not cells were exposed to DA, I\textsubscript{CA} activated between −50 and −40 mV, peaked around −10 mV, and reversed at approximately +45 mV (Fig. 7B). In all cells, there was a decrease in I\textsubscript{CA} amplitude over time (Fig. 7C). However, there was no significant difference in normalized I\textsubscript{CA} amplitudes between DA-treated cells and controls (ANOVA, F\textsubscript{0.797}=0.68, P\textsubscript{0.05}=0.797).

**DISCUSSION**

This study is the first to reveal the modulatory actions of DA on ionic currents in honey bee AL neurons. Our results show that a significant percentage of AL neurons are sensitive to DA and that DA reduces the amplitude of Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents in these cells. We show, in addition, that in type 1 cells, I\textsubscript{CA}, I\textsubscript{KV}, and most probably I\textsubscript{KV}, remain intact in the presence of this amine.

While the identity of cells responsive to DA has yet to be clearly established, our results suggest that type 1 cells are likely to represent a subpopulation of local AL interneurons (LNs). In the ALs of the bee, there are significantly more LNs than projection (output) neurons (PNs). Estimates of ~4,000 LNs per lobe (Withthöft 1967) and 800 PNs (Bicker et al. 1993) have been reported. That a majority of the cells in vitro exhibited type 1 current profiles suggests therefore that type 1 cells are more likely to be LNs than PNs. Examination of the electrophysiological properties of the cells supports this view. Outward current profiles exhibited by type 1 cells are markedly different from those reported for PNs (Grünewald 2003), which instead resemble current profiles observed in this study in cells of type 2.
targets of DA modulation. The kinetic properties of the two DA-sensitive currents closely resemble those of \( I_{KCa} \) transient and \( I_{KCa} \) sustained, and effects of DA on type 1 cells are mimicked by the \( I_{KCa} \) blocker, CTX. That no detectable DA modulation was observed in cells in which \( I_{KCa} \) had been blocked with CTX before DA application provides strong support for the conclusion that DA modulates \( Ca^{2+} \)-dependent \( K^+ \) currents in these neurons.

\( Ca^{2+} \)-dependent \( K^+ \) currents have been described in many insect species (Grolleau and Lapied 1995; Mercer and Hildebrand 2002b; Schäfer et al. 1994; Thomas 1984; Torkkeli and French 1995; Wegener et al. 1992; Zufall et al. 1991). Transient and sustained components of \( I_{KCa} \) have been reported, for example, in cockroach dorsal unpaired median neurons and, as in type 1 cells, both currents are blocked by CTX (Grolleau and Lapied 1995). The gene encoding the sustained \( I_{KCa} \) channel in cockroach neurons has been identified (Derst et al. 2003) and in cells heterologously expressing this channel, sustained \( I_{KCa} \) has an activation rate similar to that of the DA-sensitive sustained current observed in honey bee AL neurons.

Reports describing the outcome of \( I_{KCa} \) modulation in other invertebrate systems provide clues as to likely effects of DA modulation of \( Ca^{2+} \)-activated \( K^+ \) currents on the excitability of *Apis* AL neurons. In molluscan central pattern generator neurons, serotonin-induced attenuation of spike afterhyperpolarization, attributed to a reduction in \( I_{KCa} \) amplitude, promotes repetitive spiking in these neurons (Katz and Frost 1997). Applying DA to isolated pyloric dilator neurons in the lobster stomatogastric ganglion, on the other hand, increases the amplitude of total \( I_{KCa} \) contributing to an increase in interspike interval and a reduction in action potential frequency in these neurons (Kloppenburg et al. 1999c). These results, together with reports in other systems (e.g., Baxter and Byrne 1989; Baxter et al. 1999; Buchholtz et al. 1992), suggest that DA-induced attenuation of \( I_{KCa} \) will increase the excitability of honey bee AL neurons.

In developing nervous systems, levels of electrical activity have a significant impact on neuronal growth and differentiation (e.g., Baines et al. 2001; Duch and Levine 2000, 2002; Gu and Spitzer 1980; Kater and Mills 1991; Kater et al. 1988; Schilling et al. 1991; Spitzer et al. 1995, 2002) and on activity-dependent tuning of neuronal connections (e.g., Katz and Shatz 1996; Ruthazer and Stryker 1996; Shatz 1994; Sherrard and Bower 1998). DA-induced changes in cell excitability therefore could have developmental relevance in ALs of the bee. The early appearance of DA-immunoreactive processes in honey bee ALs (Kirchhof et al. 1999), evidence that DA-receptor genes in the bee brain are strongly developmentally regulated (Kurshan et al. 2003), and the identification in this study of DA-sensitive currents in developing AL neurons all suggest a developmental role for DA, in addition to its functions in the adult brain. In ALs of the sphinx moth, *Manduca sexta*, modulatory actions of serotonin on developing AL neurons (Mercer and Hildebrand 2002a; Mercer et al. 1995, 1996a,b; Oland et al. 1995) strongly foreshadow the actions of this amine in the brain of the adult moth (Kloppenburg and Heinbockel 2000; Kloppenburg and Hildebrand 1995; Kloppenburg et al. 1999a).

In adult honey bees, olfactory information is encoded by spiking patterns in AL projection neurons (Abel et al. 2001; Müller et al. 2002; Stopfer et al. 1997) and patterns of activity across the glomerular array (Galizia et al. 1999; Sachse et al. 1999). Both are likely to be affected by DA-induced changes in the activity of local AL interneurons and/or projection neurons. DA has the potential therefore to influence the detection and discrimination of odors and perhaps also olfactory learning and the formation of odotopic memories. An early investigation of DA function in honey bee ALs showed that DA applied to the lobes reduces the percentage of animals that respond to a conditioned olfactory stimulus (Macmillan and Mercer 1987). One possible target of DA modulation is the subpopulation of LNs that contain the inhibitory neurotransmitter GABA. Approximately 750 of the 4,000 LNs in the honey bee ALs are GABA immunoreactive (Schäfer and Bicker 1986; Wittchof 1967), and there is compelling evidence that these neurons play a role in AL functions such as odor discrimination (Sachse and Galizia 2002; Stopfer et al. 1997). However, DA may not target GABAergic pathways alone. In the vertebrate olfactory bulb, for example, DA not only regulates GABAergic inhibitory processing (Brüning et al. 1999; Davison et al. 2004; Duchamp-Viret et al. 1997), but also controls sensory input to the olfactory bulb through a presynaptic action on olfactory nerve terminals (Berkowitz and Trombly 2000; Ennis et al. 2001; Hsia et al. 1999; see also Duchamp-Viret et al. 1997). DA is likely to act at multiple sites also in primary olfactory centers of the honey bee brain. At least two DA receptor genes are expressed by deutocerebral neurons that surround the ALs (Kurshan et al. 2003; see also Kokay et al. 1998), and in this study, not only type 1 cells, but also a small number of type 2 AL neurons, responded to this amine.

While the mechanisms through which DA operates in the ALs have yet to be fully resolved, the results of this study provide an important step toward this goal. The aim of future studies will be to determine the functional consequences of DA modulation of \( Ca^{2+} \)-activated \( K^+ \) currents, both in the developing ALs and in AL neurons of the adult worker bee.

ACKNOWLEDGMENTS

We thank B. Niven for statistical advice, K. Miller for assistance with the illustrations, and K. Garrett for maintaining the honey bee colonies.

GRANTS

This work was funded by University of Otago Grant UORG 200100620 and Human Frontier Science Program Grant RG0014.

REFERENCES


