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Glucose increases activity and Ca^{2+} in Adult *Drosophila* Insulin Producing Cells

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Abstract

We sought to understand mechanisms underlying glucose sensing in *Drosophila melanogaster*. We found that adult insulin producing cells (IPCs) respond to glucose and glibenclamide with a burst-like pattern of activity. Under control conditions IPCs have a resting membrane potential of -62 ± 4 mV. In response to glucose or glibenclamide IPCs generate action potentials at a threshold of -36 ± 1.4 mV with an amplitude of 46 ± 4 mV and width of 9.3 ± 1.8 ms. Real-time Ca^{2+} imaging confirms that IPCs respond to glucose and glibenclamide with increased intracellular Ca^{2+} . These results provide the first detailed characterization of electrical properties of adult *Drosophila* IPCs and suggest that these cells sense glucose by a mechanism similar to mammalian pancreatic β cells.

Keywords

Drosophila melanogaster; K_{ATP} channels; glibenclamide; pancreatic β cells; Ca^{2+} imaging

INTRODUCTION

Functional similarities between flies and mammals in maintaining glucose homeostasis offer a unique opportunity for developing innovative molecular approaches for modeling type-2 diabetes in an invertebrate, genetically tractable system. Analogous to the opposing actions of insulin-secreting pancreatic β cells and glucagon-secreting pancreatic islet α cells in maintaining glucose homeostasis in mammals, insulin-like peptide-producing cells (IPCs) and adipokinetic hormone producing corpora cardiaca (CC) cells are neurosecretory cells functioning in glucose sensing in the fly [1,2]. Genetic ablation of IPCs induces hyperglycemia [1,3] whereas targeted ablation of CC cells renders larvae hypoglycemic [2]. While these genetic studies have validated the use of *Drosophila* as a potential model system for type-2 diabetes, the underlying mechanism responsible for glucose sensitivity remains to be determined. By measuring membrane potential of intact adult IPCs, we recently reported that similar to mammalian pancreatic β cells, membrane depolarization is recorded in response to glucose and a specific K_{ATP} channel blocker, glibenclamide, thus suggesting that K_{ATP} channels contribute to the mechanism whereby IPCs sense changes in circulating sugar [4]. Further, as demonstrated in mammalian pancreatic β cells [5], high

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glucose initiates a robust Ca^{2+} influx in adult IPCs [4]. In addition, *in situ* and real-time expression analysis show detectable levels the sulfonylurea receptor (Sur) subunit of the K_{ATP} channel in those cells [4].

To further establish that IPCs serve as endocrine control for insulin secretion in the adult fly, we demonstrate in this study electrophysiological properties recorded in adult IPCs are characteristic of β cells. A functional role of K_{ATP} channels in IPCs is confirmed with the use of glibenclamide. As K_{ATP} channels are recognized as a key component in regulating Ca^{2+} influx into β cells leading to glucose stimulated insulin secretion [6], we used real-time Ca^{2+} imaging techniques and recorded increased intracellular Ca^{2+} concentration in response to both glucose and glibenclamide in acutely dissociated adult IPCs. Additionally, we have developed patch-clamp techniques to monitor electrophysiological properties of adult *Drosophila* IPCs. The fact that glibenclamide elicits a robust firing rate response and increases intracellular Ca^{2+} suggests that adult IPCs sense and respond to glucose by a mechanism that likely involves inhibition of K_{ATP} channels.

METHODS

Whole brain preparation and electrophysiology

Whole brains were prepared from *dilp2-Gal4/UAS-GFP* flies and stored on poly-L-lysine coated cover slips in hemolymph-like (HL) solution as described previously [4]. Individual cover slips were transferred into recording chamber mounted on a fluorescence microscope (Zeiss Axioskop FS) and continuously perfused with a buffer solution (in mM): 3 KCl, 101 NaCl, 1 CaCl_2 , 4 MgCl_2 , 1.25 NaH_2PO_4 , 20.7 NaHCO_3 , 35 sucrose, 5 glucose, equilibrated with 5% CO_2 balance O_2 , pH 7.3 [4]. Individual IPCs were differentiated from non-IPCs by GFP fluorescence. Recordings of membrane potential were made by using an Axopatch 200B patch-clamp amplifier, digitized with a Digidata 1322A analog-to-digital converter and recorded using pCLAMP 10 software (Molecular Devices). Recordings were obtained at room temperature using electrodes with a DC resistance of 3–6 $\text{M}\Omega$ when filled with internal solution containing (mM): 120 KCH_3SO_3 , 4 NaCl, 1 MgCl_2 , 0.5 CaCl_2 , 10 HEPES, 10 EGTA, 0.3 GTP-Tris, pH 7.2; electrode tips were coated with Sylgard. Firing rate was measured in the loose-patch configuration as previously described (7).

Acute dissociation preparation and real-time Ca^{2+} imaging

Adult IPCs were acutely dissociated from *dilp2-Gal4/UAS-GFP* brains in modified HL solution and plated on poly-L-lysine coated cover slips as previously described [4]. Cells were allowed to settle for 1 hour at room temperature before loading with the cell permeable Ca^{2+} -dye rhod-3 AM according to manufacturer's instructions (Molecular Probes). Ca^{2+} imaging, acquisition of fluorescence images and subsequent data analysis has been described in detail previously [4]. Glibenclamide was bath applied at a concentration of 20 μM .

Data Analysis

Action potential properties were measured from the average waveform of 3–5 action potentials collected under control conditions shortly after gaining whole-cell access. As shown in Fig. 1D, several action potential properties were measured. Action potential threshold (T) was defined as the voltage at which the first time derivative of the action potential (AP') exceeds $\sim 1.0 \text{ V/s}$. Action potential amplitude (AP-H) was measured from T to peak of the depolarization while action potential width (W) was measured at 50% of AP-H. The amplitude of the afterhyperpolarization (AHP) was measured from resting membrane potential (RMP) to the most negative post-spike potential. We also used peak inflection points of the AP' to estimate maximum rates of depolarization (MD) and repolarization

(MR). Firing rate histograms were generated by integrating action potential discharge in 10-s bins and plotted using Spike 5.0 software. Instantaneous firing rate (1/time between spikes) was used to characterize time-dependent changes in neuronal activity and power spectrum analysis of instantaneous firing rate was used to detect oscillations in activity based on peaks in signal power distributed with frequency. To determine if activity oscillations were due to random changes in activity, we randomized instantaneous firing rate (15 times for each original signal), performed power spectrum analysis on each signal, and compared peak power values obtained experimentally to randomized surrogate data. Statistical significance was determined at $p < 0.05$ by ANOVA and Newman-Keuls multiple comparison test or t -test. Data are presented as mean $\pm$ SEM.

RESULTS

Electrophysiological properties of IPCs

The electrical properties of adult IPCs were measured shortly after gaining whole-cell access (summarized in Table 1). Under control conditions RMP was -62 ± 4 mV which is depolarized relative to the equilibrium potential for K^+ under these conditions (-94 mV), suggesting that channels in addition to K_{ATP} contribute to RMP. These cells have an average capacitance (C) of 3.5 ± 0.7 pF and input resistance (R_{in}) of 1126 ± 67 M Ω , indicating they are extremely small and of low conductance. For this reason, it is likely that only small currents will be required to change membrane potential. These passive electrical properties are similar to values reported for mammalian pancreatic β cells [8–10]. Under control conditions, IPCs were not spontaneously active and did not appear to receive synaptic input as evidenced by a lack of synaptic potentials. When depolarized by DC current injection or exposure to glucose, IPCs produced action potentials at a threshold of -36 ± 1.4 mV with an amplitude of 46 ± 4 mV and width of 9.3 ± 1.8 ms (Fig. 1D). The MD was 17.9 ± 4 V/s and the MR was -7.5 ± 1 V/s. In addition, IPCs also exhibit a prominent AHP of 8.1 ± 0.2 mV (Fig. 1D) that returned to RMP within ~ 100 ms.

Glucose and glibenclamide have similar effects on excitability of IPCs

It is well established in mammalian pancreatic β cells that glucose-mediated ATP production inhibits K_{ATP} channels to initiate membrane depolarization and formation of Ca^{2+} -dependent action potentials that leads to increased intracellular Ca^{2+} levels and insulin release [8,11]. We hypothesize that this mechanism of glucose homeostasis is highly conserved between mammals and fruit flies. This possibility is supported by our previous findings that glibenclamide mimicked effects of high glucose on membrane potential of IPCs *in situ* [10]. To further support the possibility that K_{ATP} channels contribute to glucose sensing in these cells, we characterized the effects of glucose and glibenclamide on the firing rate of IPCs. For these experiments we used a variant of the whole-cell patch technique known as loose-patch recording to minimize ‘washout’ of soluble intracellular molecules, including potentially vital components of the glucose signaling mechanism [12]. Exposure to high glucose increased firing rate by 2.9 ± 1.2 Hz (Figs. 1B1 & 1C). The effects of glucose on excitability of IPCs were mimicked by glibenclamide which increased firing rate to 1.6 ± 0.5 Hz (Fig. 1B–1C). Interestingly, the repetitive firing response of adult *Drosophila* IPCs to either glucose (Fig. 1B2) or glibenclamide (data not shown) exhibited an oscillatory pattern of activity that cycled every 24.7 ± 6.6 (N=11) seconds between bursts of activity (4.1 ± 1.0 Hz) followed by periods of decreased activity (1.2 ± 0.7 Hz). Power spectrum analysis of instantaneous firing rate during glucose exposure revealed an average (N=3) peak power of 153 ± 5 AU, which was greater than averaged (N=45) peak power of randomized (i.e., shuffled) instantaneous firing rate 54 ± 1 AU (inset, Fig. 1B2). This oscillatory firing rate response is reminiscent of the characteristic glucose response of mammalian pancreatic β cells [8,13,14].

Glucose and glibenclamide increase Ca^{2+} in IPCs

In β cells, an important link between changes in membrane potential and insulin release is Ca^{2+} influx resulting of the opening of voltage-gated Ca^{2+} channels. To extend our previous results where acutely dissociated IPCs respond to high glucose with an increase in intracellular Ca^{2+} concentration [4], here we demonstrate that glibenclamide elicits a similar increase in intracellular Ca^{2+} (Fig 2). In these experiments, acutely dissociated adult IPCs were loaded with a Ca^{2+} -sensitive fluorescent dye (rhod-3) and Ca^{2+} concentration was measured during ~5 min exposure to glucose or glibenclamide. Consistent with our electrophysiological results, both glucose and glibenclamide significantly increased Ca^{2+} in adult IPCs (Figs. 2A–B), whereas non-IPCs did not show a Ca^{2+} response to either glucose or glibenclamide. We did not observe oscillations in intracellular Ca^{2+} concentration with kinetics that parallel burst-like changes in membrane potential, likely because our Ca^{2+} fluorescent sampling rate (0.03 Hz) was too slow to resolve rapid membrane oscillations.

DISCUSSION

It has been proposed that *Drosophila* IPCs are functionally analogous to pancreatic β cells in maintaining glucose homeostasis, yet little is known regarding the electrical properties of IPCs or how these cells sense changes in circulating glucose. Here, we used electrophysiological and Ca^{2+} imaging techniques to characterize glucose sensitivity of adult *Drosophila* IPCs. We show that IPCs responded to glucose and glibenclamide with a robust increase in firing rate, indicating that IPCs express functional K_{ATP} channels that likely contribute to the glucose sensing mechanism. In addition, exposure to glucose or glibenclamide increased Ca^{2+} in acutely dissociated IPCs. These results strongly suggest that glucose-mediated activation of IPCs involves inhibition of K_{ATP} channels, and is coupled to insulin release by a corresponding rise in intracellular Ca^{2+} .

Our results show that electrical properties of IPCs are similar to those of mammalian β -cells. For example, β cells typically have a RMP near -70 [8] and generate action potentials at threshold potentials near -50 mV with an amplitude of ~ 30 mV, width >25 ms and MD of ~ 3 V/s [8,9,15]. In addition, when activated by glucose or glibenclamide, IPCs showed an oscillating pattern of activity similar to the characteristic response of mammalian pancreatic β cells [13,14]. In β cells, this pattern of activity has been shown to result from fluctuation in K_{ATP} channel activity [13] and intermittent activation of voltage-dependent Ca^{2+} channels and results in a Ca^{2+} influx that is required for insulin release [14]. We show in our studies that glucose- or glibenclamide-mediated activation of IPCs also resulted in increased intracellular Ca^{2+} , suggesting that IPCs and β cells share a common glucose sensing mechanism.

Somewhat surprisingly, we found that glucose-mediated activation of IPCs did not appear to correspond with changes in whole-cell conductance. The most likely explanation for this is that only a small proportion of K_{ATP} channels are inhibited by this level of glucose. Given the very high R_{in} measured in IPCs, it is conceivable that only a small current is sufficient to depolarize membrane potential and increase excitability of IPCs. For example, a previous study found that glucose-mediated activation of β cells occurs with less than 10% change in K_{ATP} channel activity [16]. Likewise, a change in K_{ATP} conductance of only 0.15 nS applied with dynamic clamp was shown to have a large effect on excitability of murine β cells [9]. However, it is also possible that multiple channels contribute to glucose sensing and result in offsetting effects on conductance. Nevertheless, our finding that inhibition of K_{ATP} channels with glibenclamide mimics the effects of glucose strongly suggests that K_{ATP} channels are key mediators of glucose sensing by adult IPCs.

In conclusion, our electrophysiological and Ca^{2+} imaging studies in adult IPCs substantiate a role of these neurosecretory cells in glucose sensing with a mechanism that appears to involve inhibition of K_{ATP} channels as found in mammalian pancreatic β cells. This functional conservation validates adult *Drosophila* IPCs as a highly relevant and well-suited experimental system to rapidly test interventions modulating insulin secretion in modeling type 2 diabetes.

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Figure 1. Glucose and glibenclamide stimulate activity of IPCs

A, A diagram shows adult *Drosophila* brain anatomy and location of 14 IPC cells [4]. The soma of each IPC is located in the superficial layer of the *pars intercerebralis* and sends a unipolar projection down midline to presumably release insulin at yet undetermined locations (M. Tatar, Brown University, personal communication). **B1**. Firing rate trace shows that glucose (20 mM) caused a large and reversible increase in firing rate. The effects of glucose were mimicked by glibenclamide (10 μ M). **B2**. Traces of membrane potential and instantaneous firing rate (1/time between spikes) from the same cell as in (B1) shows oscillating bursts of activity (bars above trace) during glucose exposure. **B2 inset**, power spectrum analysis of instantaneous firing rate during glucose exposure (original data) shows a peak power of 158 AU at ~0.1 Hz, whereas the trace of shuffled instantaneous firing rate does not exceed ~54 AU, suggesting that burst-like activity of this cell is not due to chance. **C**. summary data showing firing rate under control conditions (N=6) and in glucose (N=5) or glibenclamide (N=6). **D**, mean AP expanded in time to illustrate waveform parameters of interest (results of this analysis are summarized in table 1).

Figure 2. Acutely dissociated IPCs respond to glucose and glibenclamide with an increase in intracellular Ca^{2+}

Fluorescence trace from an IPC loaded with the Ca^{2+} -sensitive dye rhod-3 shows that exposure to glucose (80 mM) or glibenclamide (20 μM) reversibly increases intracellular Ca^{2+} concentration. A normalized increase in fluorescence is calculated as absolute increase in fluorescence divided by fluorescence under control conditions [4]. **B.** A bar graph summarizing the change in fluorescence of IPCs in response to glucose (N=8) or glibenclamide (N=5). Note that control non-IPC cells (N=3) did not respond to glucose ($p < 0.05$, *).

Table 1

Electrical properties of adult IPCs.

Resting properties		Action potential properties	
RMP	-62 ± 4 mV (N=10)	Threshold	-36 ± 1.4 mV (N=3)
Rin	1126 ± 67 M Ω (N=13)	Height	46 ± 4 mV (N=3)
C	3.5 ± 0.7 pF (N=5)	Width	9.3 ± 1.8 ms (N=3)
Spontaneous activity	None (N=10)	AHP	8.1 ± 0.2 mV (N=3)