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Effect of glipizide on dopamine synthesis, release and metabolism in PC12 cells

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Abstract

Sulfonylureas block ATP-dependent K^+ channels (K/ATP channels) in pancreatic β cells and brain γ-aminobutyric acid (GABA) containing neurons causing depolarization-evoked insulin or GABA release. In high concentrations, sulfonylureas also inhibit catecholamine release from bovine adrenal chromaffin cells and isolated guinea pig aorta. In this study, we examined the effect of glipizide, a sulfonylurea, on dopamine release from PC12 cells and found that neither basal nor K^+-stimulated dopamine release was affected. Although PC12 cells expressed mRNA for the K/ATP channel, functional K/ATP channels could not be demonstrated electrophysiologically, consistent with the lack of effect of glipizide on dopamine release. Glipizide did, however, increase cytoplasmic retention of the acidic dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), indicating blockade of their outward transport. The cellular accumulation of DOPAC was accompanied by reduced tyrosine hydroxylase activity and reduced formation of dopamine and its metabolites presumably by a negative feedback effect of the increased cytoplasmic concentrations of DOPAC.

Keywords: Dopamine; Tyrosine hydroxylase; Sulfonylurea; Glipizide; DOPAC (3,4-dihydroxyphenylacetic acid); HVA (homovalinic acid)

1. Introduction

Sulfonylureas are antidiabetic drugs that evoke insulin release by blocking ATP-sensitive inward rectifying K^+ channels (K/ATP channels) (for review see Isomoto et al., 1997), which leads to a depolarization-evoked increase in intracellular Ca^{2+} (Amoroso et al., 1990; Ben-Ari, 1990; Ashcroft, 1996; Ashcroft and Ashcroft, 1990). Apart from their well-known effects to stimulate insulin release from pancreatic β cells, sulfonylureas also stimulate neurotransmitter release from several neuronal cell types. In substantia nigra and globus pallidus slices, sulfonylureas increase γ-aminobutyric acid (GABA) release (Amoroso et al., 1990; Schimid-Antomarchi et al., 1990; Brotchie et al., 1993). These drugs also stimulate release of acetylcholine from striatal slices (Lee et al., 1997).

In contrast to the above stimulatory effects of sulfonylureas on insulin and GABA secretion, in high concentration these drugs inhibit catecholamine release from the adrenal gland and isolated guinea pig aorta (Hsu et al., 1975; Morita et al., 1988). Perhaps related to the inhibitory effect of sulfonylureas on catecholamine release, glibenclamide (a sulfonylurea) blocks the stimulation-induced elevation of intracellular Ca^{2+} and inhibits force development of the rabbit aorta (Yoshitake et al., 1991). Although, as expected, sulfonylureas enhance brain GABAergic transmission (Amoroso et al., 1990), glibenclamide does not affect dopaminergic neurotransmission in the striatum in vivo (Sommermeyer et al., 1994) despite the high density of sulfonylurea binding sites in this brain region (Mourre et al., 1990).

The discrepancies between the inhibitory effect of sulfonylureas on catecholamine release from the adrenal gland and guinea pig heart and stimulatory effects on insulin

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secretion from pancreatic β cells and neurotransmitter release from brain slices are probably due to additional actions of sulfonylureas at sites other than K/ATP channels. While sulfonylureas have highly specific actions on K/ATP channels in pancreatic β-cells and cardiac muscle, this selectivity may not be preserved in other tissues (Ashcroft, 1996; Ashcroft and Ashcroft, 1990). For example, at high concentrations, sulfonylureas also block voltage-gated and Ca^{2+}-activated K^+ channels (Galband et al., 1990; Reeve et al., 1992). Also, sulfonylurea receptors and K/ATP channels can exist as independent proteins (Khan et al., 1993) which may function differently than when combined to form the K/ATP channel.

To resolve the basis of the different effects of sulfonylureas on catecholamine compared to GABA and insulin release, we examined the effects of glipizide, a typical sulfonylurea, on synthesis, release and metabolism of dopamine in cultured undifferentiated PC12 cells. We assessed the presence of K/ATP channels in PC12 cells by reverse transcription polymerase chain reaction (RT-PCR), as well as by electrophysiological and fluorimetric methods. In addition, to further characterize the effects of glipizide on dopamine metabolism, we measured tyrosine hydroxylase activity and compared responses to glipizide to those obtained after monoamine oxidase inhibition.

2. Materials and methods

2.1. Cell culture and solutions

Stock PC12 cells were obtained from the American Type Culture Collection (Manassas, Virginia, USA) and maintained in Dulbecco's modified Eagle's medium (glucose, 4.5 g/l) (Gibco, MD, USA) supplemented with 10% heat-inactivated horse serum, 5% fetal calf serum (Gibco, MD, USA), 100 units/ml penicillin and 0.05 mg/ml streptomycin (Sigma, St. Louis, MO, USA) in 5% CO_2 atmosphere at 37°C. Medium was changed every 3 days and the cells were subcultured about once a week.

2.2. Biochemical studies

Ten to 100 ml of media or cell lysates were assayed for dopamine and its deaminated metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine, by high pressure liquid chromatography with electrochemical detection. Dopamine and its metabolites were separated on a 5 μm, 4.6 mm × 25 cm, ion pair C-18 ultrasphere column (Beckman, San Ramon, CA, USA), in line with a model 717 plus autosampler, a model 510 solvent delivery system at 0.8 ml/min (both from Waters Associates, Milford, MA, USA) and a model CB-100 amperometric electrochemical detector with the analytical cell set at a potential of 0.78 V. The mobile phase contained 1.4 g/l 1-heptanesulfonic acid sodium salt, 0.085 g/l EDTA, 1% triethylamine and 2.5% acetonitrile; and the pH was adjusted to 2.6 with 6.5 ml of 85% phosphoric acid. The results were recorded and analyzed using a Millennium 2010 Chromatography Manager software and hardware package (also from Waters Associates).

2.3. Reverse transcription polymerase chain reaction (RT-PCR)

RNA was extracted and isolated from PC12 and RINmf5 cells using an RNA ISOLATOR (Genosys Biotechnology, The Woodlands, TX, USA). RNA (2 mg) was reverse transcribed to a single-strand DNA (cDNA) using reverse primers and MoMLV-RT (Gibco, MD, USA). Portions (20 ng) of the cDNA pool were amplified using Kir6.2, SUR-1 or β-actin primers based on reported sequences; Kir6.2, f-5′-CGAGGTCCAGGTGACCATTGG-3′ (at 417) and r-5′-TGCGGTCCTCATCAAGCTGGC-3′ (at 1082) (accession no. U44897); SUR-1 f-5′-TCGCGCTGTGCCTTGTCACC-5′ (at 3483) and r-5′-ATGAGTACCACGCATGCTCCG-3′ (at 4021) (accession no. L40624). Rat β-actin f-5′-TTGTAACCAACTGGGACGATATGG-3′ (at 1552) and r-5′-GATCTTGATCTTCATGGTGCTAGG-3′ (at 2991) (Nudel et al., 1983). PCR was performed using a Perkin-Elmer Cetus thermal cycler in a volume of 100 ml containing cDNA, 10 mM dNTP, 100 mM KCl, 200 mM Tris–HCl (pH 8.75), 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1% Triton X-100, 1 mg/ml nuclease-free bovine serum albumin, 10 pmol of each primer and three units of TaqPlus long PCR system (Stratagene, La Jolla, CA, USA). The PCR conditions were: for Kir6.2, SUR-1 and β-actin primers initial denaturation at 94°C for 5 min followed by 35 (Kir6.2, SUR-1) or 27 (β-actin) cycles of denaturation at 94°C for 1 min, annealing at 65°C for 2 min and extension at 72°C for 3 min, with a final extension at 72°C for 10 min. The PCR products were separated by electrophoresis in 2% agarose gel, and visualized by ethidium bromide staining.

2.4. Distribution of metabolites between PC12 cells and culture medium

Cells [$(1 \text{ to } 2) \times 10^6$] were plated on poly-L-lysine-coated 35-mm tissue-culture dishes 48–72 h before the release experiments. Serum-containing media were removed and replaced with HEPES-buffered saline with the following composition (mM): 115 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$, 5.5 glucose, 1 NaH_2PO_4, 15 Na-HEPES (pH 7.2). After a 15-min incubation, the HEPES-buffered saline solution was replaced with fresh HEPES-buffered saline containing either no drug or the selected agent. The incubation at 37°C was continued for 15 min. One hundred and fifty ml of the medium was transferred into tubes containing 15 ml of 0.1 M perchloric acid containing 1% ethanol, and 0.02% disodium ethylenediamine tetraacetate (EDTA) for measurement of the re-

leased catecholamines and metabolites. Cellular contents of catecholamines and metabolites were determined after cell lysis with 0.1 M $HClO_4$ containing 0.5% Na_2EDTA and 0.1% $Na_2S_3O_5$. The amount of dopamine, DOPAC or HVA in the medium as a percent of the combined amounts present in both the medium and in the cells at the end of the incubation period provided a measure of the effects of the various treatments upon the efflux of dopamine, DOPAC or HVA from the cells into the medium.

2.5. Determination of tyrosine hydroxylase activity

Tyrosine hydroxylase activity was estimated according to Hayashi et al. (1988) by measuring the accumulation of 3,4-dihydroxyphenylalanine (DOPA) after inhibition of L-aromatic amino acid decarboxylase with 3-hydroxybenzylhydrazine (NSD-1015). The cells were incubated for 15 min in Krebs-saline buffer containing 1 mM NSD-1015. After the preincubation, the medium was replaced with a medium containing NSD-1015, with or without glipizide (100 mM). After a 15 min incubation, the cells were lysed by addition of 15 ml of 0.1 M perchloric acid containing 1% ethanol and 0.02% EDTA. After centrifugation at $10,000 \times g$ for 15 min, total DOPA accumulation in the medium and cells was determined in an aliquot of the supernatant fluid using high pressure liquid chromatography with electrochemical detection.

2.6. Patch clamp experiments

Cells were plated on 35-mm dishes and maintained in culture for 2–3 days at 37°C in an atmosphere of 95% air and 5% CO_2. Before experiments, the culture medium was replaced with a solution containing (mM): 140 NaCl, 4 KCl, 2.6 $CaCl_2$, 1 $MgCl_2$, 10 HEPES. Patch-clamp pipettes were made of soft capillary glass (Blue-Tip) using a BB-CH-PC puller (Mecanex, Geneva, Switzerland). For whole-cell recordings, pipets were filled with a high K^+ solution (mM): 140 KCl, 1 $MgCl_2$, 0.5 NaEGTA and 5 Na-HEPES and had tip resistances between 4–6 MW. For cell-attached measurements, pipets were filled with a solution of the following composition (mM): 140 KCl, 1 $MgCl_2$, 1 $CaCl_2$ and 5 Na-HEPES and had tip resistances from 8 to 10 MW. Experiments were carried out at room temperature (20–25°C). Whole-cell and single channel currents were recorded using an EPC-7 amplifier (List Electronics, Darmstadt-Eberstadt, Germany). For analysis, records were filtered at 50 Hz and digitized at 100 Hz, using the software packages from Axon Instrument (Foster City, CA, USA).

2.7. Measurement of relative membrane potential

Effects of glipizide on the membrane potential were estimated fluorimetrically using 0.5 mM concentration of the voltage-sensitive dye, diBA-C_4-(3) (Brauner et al., 1984). Cells (10^4) were seeded in each well of a 24-well poly-D-lysine-coated dish (CoStar). After 48 h incubation, the medium was removed and replaced with Krebs-saline medium containing diazoxide (100 mM), glipizide (100 mM), cromakalim (10 mM) or KCl (56 mM). After 15 min, diBA-C_4-(3) (0.5 mM, final concentration) was added to the medium. Intensities of the flourescence were measured at an excitation wavelength of 485 nm and an emission wavelength of 530 in a cytofluor II spectofluorometer (Perseptive Biosystems, MA, USA) 10 min after addition of the dye, when a steady fluorescence signal was obtained.

2.8. Measurement of intracellular Ca^{2+} levels

Changes in cytosolic Ca^{2+} ($[Ca^{2+}]i$) were monitored using the microfluorimetry technique with indo-1/AM (Molecular Probes, Oregon) as a $[Ca^{2+}]i$ indicator, as described in detail elsewhere (Rojas et al., 1994; Jaimovich and Rojas, 1994). Cells were loaded with indo-1/AM by incubating them for 1–2 h at room temperature (20–25°C) in a modified Krebs solution (mM) 145 NaCl, 5 KCl, 2.6 or 1.25 $CaCl_2$, 1 $MgCl_2$, 10 Na-HEPES, 5 $NaHCO_3$, 5.6 D-glucose, (pH 7.4) containing 2 mM indo-1/AM and 0.02% pluronic acid. The coverslips were then mounted in a perfusion chamber on the stage of an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan) and the cells were continuously superfused (0.5 ml/s) with modified Krebs solution. Drugs were added to the solutions used to superfuse the cells. To examine the effects of glipizide on KCl-induced increases in $[Ca^{2+}]i$, the cells were first perfused with Krebs solution containing 100 mM glipizide; the cells were then perfused with the solution of KCl (56 mM) also containing glipizide (100 mM). The loaded cells were excited at 355 nm and indo-1/AM fluorescence was measured at 410 and 485 nm. The output from each photomultiplier was sampled (at either 0.36 or 1.0 s/sample) and a computer program calculated the F_{410nm}/F_{485nm} ratio, obtained the corresponding $[Ca^{2+}]i$ from a calibration curve, and displayed the time course of F_{410nm}, F_{485nm}, and $[Ca^{2+}]i$. The system was calibrated as described previously (Rojas et al., 1994), using the same chamber as used for the experiments.

2.9. Data analysis and statistics

Retention in PC12 cells of DOPA, dopamine, DOPAC and HVA were expressed as the percentage of the total compound in the culture dish found in the cells after 15 min incubation [i.e., $(100 \times \text{cell content})/(\text{cell} + \text{media contents})$]. Dopamine release was defined as the amount of dopamine in the media as a percent of the total amount in cells and media. Statistical significance of the differences in the release and cellular retention of DOPA, dopamine and dopamine metabolites between control and treated groups were analyzed by Student's *t*-test, analysis of vari-

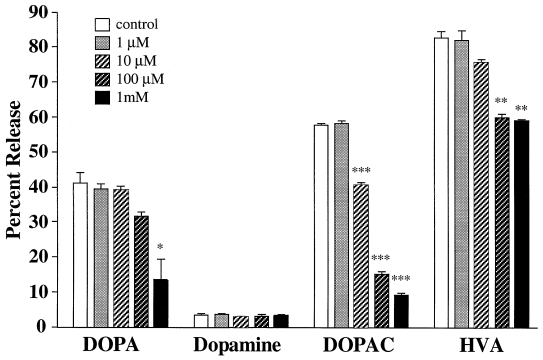


Fig. 1. Effect of glipizide on basal DOPA, dopamine, DOPAC and HVA release from PC12 cells. Cells were incubated for 15 min in HEPES buffered saline and after 15 min adaptation, the HEPES buffered saline was removed and replaced with fresh HEPES buffered saline containing glipizide (1–1000 mM) and the incubation at 37°C continued for 15 min. Amounts of DOPA, dopamine, DOPAC and HVA in the medium and in the cells were measured at the end of the incubation. Results are expressed as percent release [100 × amount in the medium/(amount in the medium + amount in the cells)] for each of the compounds measured. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$ compared to control.

ance and the Tukey–Kramer multiple comparison test where appropriate.

3. Results

3.1. Effect of glipizide on release of acidic dopamine metabolites

Incubation with glipizide affected neither basal (Fig. 1) nor K^+-induced dopamine release (Fig. 2), but dose dependently diminished release into the medium of DOPAC and, to a lesser extent, HVA. At a concentration of 10 mM or greater, glipizide dose-dependently reduced DOPAC release into the medium. At a concentration of 100 mM, glipizide also significantly reduced HVA release. Incubation with 1 mM glipizide significantly reduced DOPA release (Fig. 1). The reduction in DOPAC and HVA released into the medium was accompanied by increased levels of DOPAC in the cells and a dose-dependent decrease of DOPAC in the medium (Fig. 3). The total DOPAC present in the medium and cells was reduced significantly. Glipizide also enhanced the retention of HVA in PC12 cells (Fig. 3), but the total amount of HVA in the medium and cells was not significantly reduced.

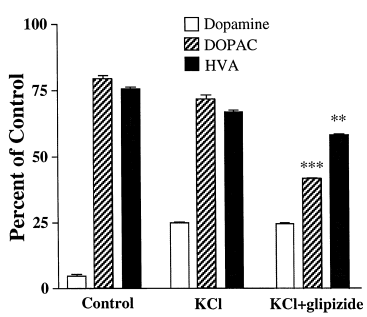


Fig. 2. Effect of glipizide on K^+-induced dopamine release from PC 12 cells. Cells were incubated for 15 min in HEPES buffered saline and after 15-min adaptation, the HEPES buffered saline was removed and replaced with fresh HEPES buffered saline containing KCl (56 mM) with or without glipizide (100 mM) and the incubation at 37°C continued for 15 min. Amounts of dopamine, DOPAC and HVA levels in the medium and in the cells were measured at the end of the incubation. Results are expressed as percent release [100 × amount in the medium/(amount in the medium + amount in the cells)] for each of the compounds measured. ** $p < 0.005$, *** $p < 0.001$ compared to KCl-treated group (KCl incubation induced significant increase in dopamine release, *** $p < 0.001$ compared to control group).

3.2. Glipizide did not affect intracellular Ca^{2+} levels

Baseline levels of free intracellular Ca^{2+} (166 ± 29 nM) were not significantly affected by incubation with glipizide (191 ± 37 nM). Furthermore, the K^+-induced

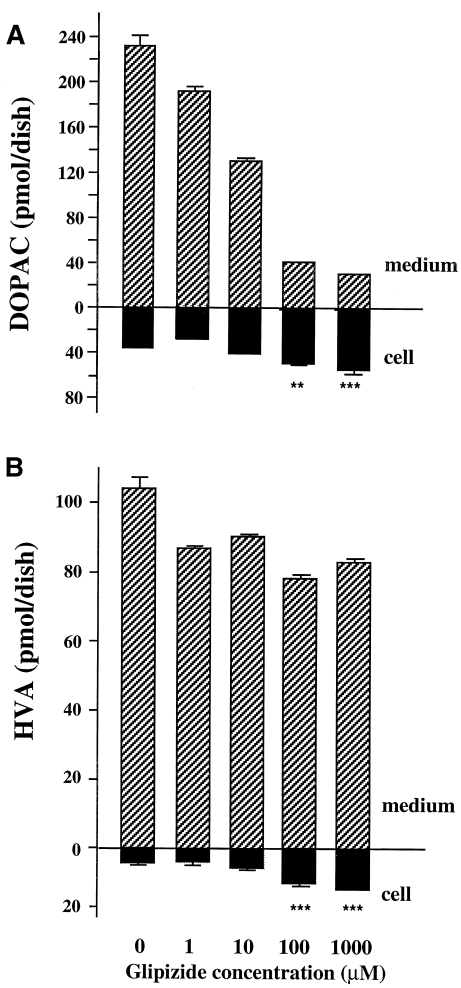


Fig. 3. Effect of glipizide on intra- and extracellular distributions of DOPAC (A) and HVA (B). PC12 cells were incubated for 15 min in HEPES buffered saline. After 15-min adaptation, the HEPES buffered saline was removed and replaced with fresh HEPES buffered saline containing glipizide (1–1000 mM) and the incubation at 37°C continued. After 15 min incubation, DOPAC and HVA contents in the media and cells were determined as described in Materials and Methods. Results are expressed as picomoles per dish. *** $p < 0.001$, ** $p < 0.01$.

increase in intracellular Ca^{2+} (253 ± 48 nM) was not affected by glipizide (267 ± 28 nM).

3.3. Accumulation of cytoplasmic dopamine metabolites is accompanied by reduced tyrosine hydroxylase activity

In addition to the redistribution of DOPAC and HVA between the cell cytoplasm and medium, incubation with glipizide reduced the total (cell + medium) formation of dopamine, its precursor, DOPA, and it's metabolites DOPAC, HVA and 3-methoxytyramine (Fig. 4A). Clorgyline, a monoamine oxidase A inhibitor, also reduced the formation of DOPAC and HVA, but its effects neither attenuated nor potentiated the effects of glipizide (Table 1). Whereas glipizide significantly reduced DOPAC and 3-methoxytyramine levels, clorgyline markedly increased total 3-methoxytyramine and diminished DOPAC levels (Table 1). After inhibition of L-aromatic amino acid decarboxylase by NSD-1015, DOPA accumulation was significantly reduced by glipizide (Fig. 4B).

3.4. Expression of K / ATP channels and its components in PC12 cells

Using RT-PCR, PC12 cells were found to express both Kir6.2 and SUR1 mRNA, but the level of expression of Kir6.2 and SUR1 was much lower than in RINmF5 cells (rat insulinoma cell line) (Fig. 5). To examine whether the expression of the mRNA was accompanied by the expression of a functional /typical (sulfonylurea sensitive) K/ATP channel, we measured the effect of glipizide on K^+-channel activity in PC12 cells.

It is well known that during prolonged application of the whole-cell patch clamp technique, there is a decrease in K^+ currents. To evaluate this decay we acquired two sets of records separated by 7-min intervals. In the presence of glipizide (100 mM) (Fig. 6B), the amplitude of the outward currents diminished similarly to that of controls (Fig. 6A). In addition, current–voltage relationships acquired from whole-cell current measurements revealed that glipizide had no significant affect on the current in any holding potentials that were applied (Fig. 6E).

We also examined the possible effects of glipizide on single channels. In the absence of glipizide, K^+-channel mean open time was 9.5 ms, the mean close time was

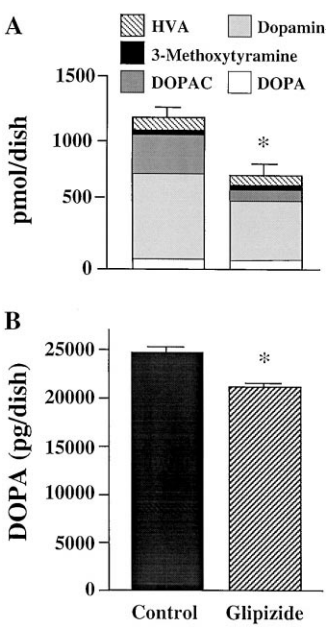


Fig. 4. Effects of glipizide (100 mM) on DOPA, dopamine, DOPAC, 3-methoxytyramine, and HVA levels in PC 12 cells, $^* p < 0.025$ (A) and on tyrosine hydroxylase activity (B). Tyrosine hydroxylase activity was estimated by measuring the accumulation of 3,4-dihydroxyphenylalanine (DOPA) after inhibition of L-aromatic amino acid decarboxylase with 3-hydroxybenzylhydrazine (NSD-1015). Cells were first incubated for 15 min in HEPES buffered saline with NSD-1015 (1 mM). After preincubation, the medium was replaced with a medium containing NSD-1015 and with or without glipizide (glip). After a second 15-min incubation, the cells were lysed and levels of DOPA level determined. Statistical significance of the difference in DOPA formation between control and glipizide, $^* p < 0.025$.

Table 1
Effects of glipizide and clorgyline on amounts of DOPAC, 3-methoxytyramine and total formation of dopamine and dopamine metabolites (Total) in PC12 cells. All results are expressed as a percent of levels in the untreated (control) group

Cells were incubated for 15 min in HEPES buffered saline. After 15 min adaptation, the HEPES buffered saline was removed and replaced with fresh HEPES buffered saline containing clorgyline (30 mM) with or without glipizide, or with glipizide (100 mM) without clorgyline, and the incubation at 37°C continued for 15 min. Contents of DOPA, dopamine, DOPAC, 3-methoxytyramine and HVA in the media and cells were determined at the end of the incubation. Results are expressed as percent ± SEM of untreated cells. Baseline levels in untreated cells were 74 ± 8 pmol/dish for DOPA, 706 ± 59 pmol/dish for dopamine, 267 ± 21 pmol/dish for DOPAC, 25 ± 2 pmol/dish for methoxytyramine, 109 ± 9 pmol/dish for HVA, and 1180 ± 96 pmol/dish for the total of DOPA, dopamine and all metabolites.

Treatment	DOPAC	3-Methoxtyramine	Total
Control	100 ± 8	100 ± 6	100 ± 8
Glipizide (100 mM)	34 ± 2	78 ± 4	61 ± 9
Clorgyline (30 mM)	7 ± 1	372 ± 23	52 ± 5
Clorgyline + glipizide	5 ± 1	392 ± 21	55 ± 8

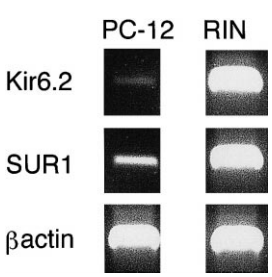
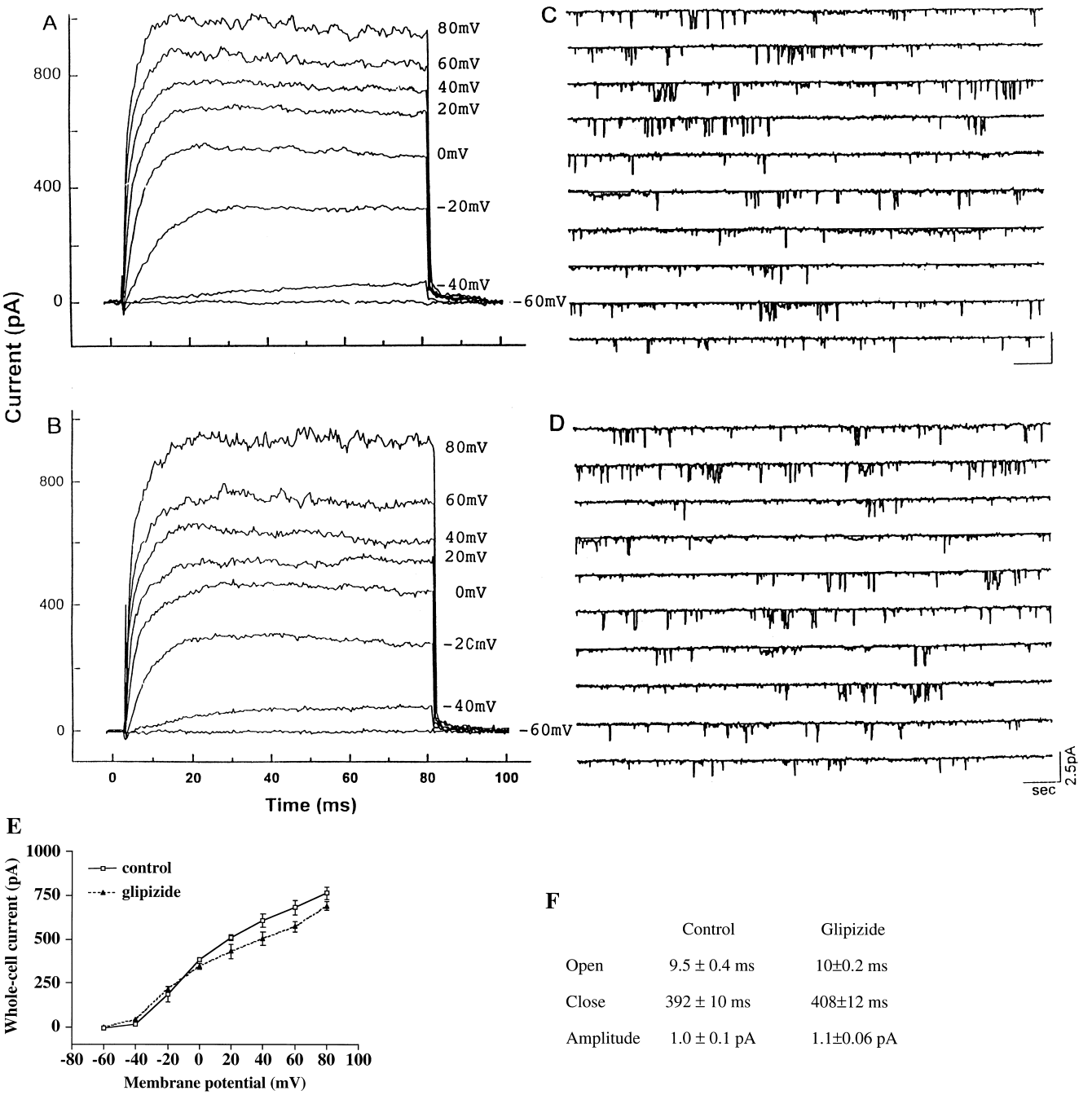


Fig. 5. Expression of K/ATP channel subunits Kir6.2, SUR1 and a control gene β-actin mRNA amplification products from PC12 cells and RIN cells (RIN = RINm5F rat insulinoma cell). Shown is an agarose gel of amplification products from polymerase chain reaction of 20 ng of cDNA pool. The PCR products were separated by electrophoresis in 2% agarose gel, and visualized by ethidium bromide staining.



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	Control	Glipizide
Open	9.5 ± 0.4 ms	10±0.2 ms
Close	392 ± 10 ms	408±12 ms
Amplitude	1.0 ± 0.1 pA	1.1±0.06 pA

Fig. 6. K^+-currents recorded from PC-12 cells are insensitive to glipizide. Left side panels (A,B) show two superimposed whole-cell membrane current records made by application of depolarizing pulses from the holding potential −60 mV to +80mV. Absolute membrane potential during the pulses is given next to the corresponding current record immediately prior to the application of glipizide (A) and 7 min after the application of 100 mM glipizide (B). (E) shows the average voltage–current relationships of whole cell recording obtained with (black triangle, $n = 4$) or without (open square, $n = 4$) glipizide. Right side panels show single K^+-channel activity before (C) and 7 min after (D) the addition of glipizide. Holding potential is 0 mV. Panel F shows the statistics for the single-channel recording from the results of four different cells incubated with or without (control) glipizide.

377.5 ms, and the corresponding open-channel current amplitude at resting potential was 1.0 pA. In the presence of glipizide (Fig. 6D), the mean open time, close time and current amplitude were 10.1 ms, 423.5 ms and 1.1 pA, respectively. Similar results from four different cells are shown in Fig. 6F (statistical analysis for open, close times and amplitude revealed no significant difference). Furthermore, using the membrane potential sensitive fluorescence dye, diBA-C_4-(3), (Brauner et al., 1984) we demonstrated that neither glipizide nor the K/ATP-channel openers, cromakalim and diazoxide, affected PC12 cell membrane potentials (2429 ± 26; 2532 ± 57; 2281 ± 144; 2464 ± 71; 4637 ± 98 fluorescence units control, glipizide, diazoxide, cromakalim 100 mM each and KCl 56 mM respectively).

4. Discussion

Incubation of PC12 cells with glipizide resulted in the cellular accumulation of DOPAC and, to a lesser extent, HVA. This was attended by significantly reduced production of dopamine and its metabolites, indicating reduced synthesis of dopamine. Measurement of DOPA accumulation after inhibition of L-aromatic amino acid decarboxylase confirmed that there was indeed a decrease in tyrosine hydroxylase activity during incubation with glipizide. The striking decrease in levels of DOPAC in the medium with a relatively small decrease in HVA suggests that the cellular retention of DOPAC was accompanied by its increased *O*-methylation to HVA.

At relatively low concentrations (in the nanomolar range) sulfonylureas have K/ATP-channel-dependent stimulatory effects on insulin and GABA release. At higher concentrations, these drugs inhibit catecholamine release and the stimulation-induced elevation of intracellular Ca^{2+} (Hsu et al., 1975; Morita et al., 1988; Yoshitake et al., 1991). The effects of glipizide on distribution of DOPAC and HVA and on tyrosine hydroxylase activity could be demonstrated only at relatively high concentrations. This suggests that these effects are probably due to additional actions of glipizide at a site other than the K/ATP channel.

PC12 cells possess tetraethylammonium-sensitive K^+ channels that influence Ca^{2+} entry and exocytotic release of dopamine (Taylor and Peers, 1998). However, we could not demonstrate the presence of functional ATP-sensitive K^+ channels, nor any effect of glipizide on other K^+ channels expressed in PC12 cells. Furthermore, blocking K/ATP channels in GABAergic neurons or the pancreatic β-cell increases intracellular Ca^{2+} levels inducing excoytotic release of GABA or insulin. However, since glipizide fails to elevate cytoplasmic Ca^{2+}, which would evoke catecholamine release, we believe that the effects of glipizide on PC12 cells are not modulated through the K/ATP channel. This conclusion is consistent with the reports by Latha et al. (1994) and Weille et al. (1989) demonstrating the lack of expression of functional K/ATP channel in PC12 cells.

Although glipizide did not appear to alter significantly basal dopamine release from PC12 cells (Fig. 1), glipizide markedly diminished DOPAC efflux and had a similar less striking effect on DOPA and HVA. The decreases in total production and efflux into the medium of DOPAC and HVA were not accompanied by any increase in 3-methoxytyramine (a pattern observed after clorgyline) indicating that the effect was not due to inhibition of monoamine oxidase.

The glipizide-induced decrease in secretion of DOPAC and HVA was accompanied by a general decrease in dopamine production, as assessed by total contents of DOPA, dopamine and dopamine metabolites in the cells and medium. Using NSD-1015 to block aromatic amino acid decarboxylase, we confirmed that the decrease in dopamine production was related to glipizide-induced inhibition of tyrosine hydroxylase. Another sulfonylurea, glibenclamide, also has been reported to decrease conversion of tyrosine to catecholamines in vitro (Morita et al., 1990).

Although total production of DOPAC and HVA was decreased, there was a paradoxical increase in the cytoplasmic concentrations of these metabolites. The increased retention of DOPAC and HVA in PC12 cells incubated with glipizide suggests inhibition of a sulfonylurea-sensitive transporter responsible for the extrusion of the acidic dopamine metabolites, DOPAC and HVA. Indeed, sulfonylurea drugs have been shown to inhibit the ATP-binding cassette type 1 transporter as well as P-glycoprotein and the cystic fibrosis transmembrane conductance regulator, all proteins that transport anions (Becq et al., 1997; Hamon et al., 1997; Golstein et al., 1999). Golstein et al. (1999) concluded that glibenclamide and a related compound appear to be general inhibitors of ATP-binding cassette transporters. Furthermore, in view of our finding that the export of DOPAC is inhibited by glipizide, the enhanced uptake of [^{14}C]tyrosine into bovine adrenal chromaffin cells during incubation with glibenclamide (Morita et al., 1990) could be explained by inhibition of tyrosine export from the cells.

Morita et al. (1990) attributed the glibenclamide-induced reduction in chromaffin cell tyrosine hydroxylation to inhibition of Ca^{2+} entry into the cells, as previously described by these investigators (Morita et al, 1988). This observation is consistent with the reported blockade of stimulation-induced increments in cytosolic calcium concentration and attenuation of vascular contraction of the rabbit aorta in the presence of glibenclamide (Yoshitake et al., 1991). In the present study, however, glipizide neither affected basal levels of intracellular Ca^{2+} nor blocked K^+ stimulation-induced increments in intracellular Ca^{2+} in PC12 cells. Thus, the inhibitory effect of glibenclamide on catecholamine synthesis is probably the result of feedback inhibition of tyrosine hydroxylase activity by the accumulated catechols in the intact cells. Laschinski et al. (1986) demonstrated that in PC12 cells, elevated levels of DOPAC or other catechols diminish tyrosine hydroxylase activity. Moreover, the glipizide-induced accumulation of DOPAC explains the findings by Morita et al. (1990) that the effects of glibenclamide on tyrosine hydroxylase could be demonstrated in vivo, but not when tyrosine hydroxylase activity was measured in the soluble fraction of bovine adrenal medullary tissues. These differences can be attributed to tyrosine hydroxylase feedback inhibition by DOPAC accumulation in the intact cells in vivo.

In summary, the discrepancy between the effects sulfonylureas in enhancing insulin release from pancreatic β cells and GABA release from brain slices and their lack of effect on dopamine release in PC12 cells appears to be due to the lack of functional K/ATP channels in PC12 cells

rather than blockade of calcium entry. The selective effect of glipizide on the cellular distribution of DOPAC and HVA suggests that PC12 cells express a sulfonylurea-sensitive transporter of the acidic dopamine metabolites, DOPAC and HVA. The reduction in catecholamine production elicited by glipizide appears to be due to feed back inhibition of tyrosine hydroxylase by the DOPAC accumulating in the cells.

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