Mechanisms of Low-Glucose Sensitivity in Carotid Body Glomus Cells

María García-Fernández, Patricia Ortega-Sáenz, Antonio Castellano, and José López-Barneo

OBJECTIVE—Glucose sensing is essential for the adaptive counterregulatory responses to hypoglycemia. We investigated the mechanisms underlying carotid body (CB) glomus cells activation by low glucose.

RESEARCH DESIGN/METHODS AND RESULTS—Removal of extracellular glucose elicited a cell secretory response, abolished by blockade of plasma membrane Ca^{2+} channels, and a reversible increase in cytosolic Ca^{2+} concentration. These data indicated that glucopenia induces transmembrane Ca^{2+} influx and transmitter secretion. In patch-clamped glomus cells, exposure to low glucose resulted in inhibition of macroscopic outward K^+ currents and in the generation of a depolarizing receptor potential (DRP). The DRP was abolished upon removal of extracellular Na^+. The membrane-permeable 1-oleoyl-2-acetyl-sn-glycerol induced inward currents of similar characteristics as the current triggered by glucose deficiency. The functional and pharmacological analyses suggest that low glucose activates background cationic Na^+-permeant channels, possibly of the transient receptor potential C subtype. Rotenone, a drug that occludes glomus cell sensitivity to hypoxia, did not abolish responsiveness to low glucose. The association of Glut2 and glucokinase, characteristic of some high glucose–sensing cells, did not seem to be needed for low glucose detection.

CONCLUSIONS—Altogether, these data support the view that the CB is a multimodal chemoreceptor with a physiological role in glucose homeostasis. ***Diabetes* 56:2893–2900, 2007**

The counterregulatory response to hypoglycemia is essential for life and particularly important for insulin-treated diabetic subjects; however, the underlying mechanisms remain largely unknown (1–3). The existence of low glucose–sensitive neurons in ventromedial and lateral hypothalamus (glucose-inhibited neurons) (3–5), as well as in other brain areas (6), is well documented; nevertheless, there is also considerable evidence suggesting that peripheral glucose control is necessary for the proper adaptation to hypoglycemia. Besides α-cells of the pancreas, systemic low-glucose receptors have been proposed to exist at the portal vein (7) and in the carotid body (CB) (8–11).

The CB, the main O_2-sensing peripheral arterial chemoreceptor in mammals, is composed of innervated clusters of glomus cells that, when activated by hypoxia, release transmitters, which either have predominantly an autocrine effect (dopamine) or stimulate afferent sensory fibers (ATP) (11–13). It is known that besides O_2 tension, CB glomus cells can sense changes of blood CO_2 and pH (12), and it has recently been shown that in vitro glomus cells are also low-glucose detectors (10,11,14). The glucose-sensing role of the CB was suggested by reports that anesthetized animals show a rapid increase in the output of hepatic glucose after infusion of the CB region with sodium cyanide (15,16) or alterations of carbohydrate metabolism in acute hypoxia (17). In addition, it was known that the counterregulatory response to insulin-induced mild hypoglycemia (8) or the neurosecretory response in exercise (9) in dogs was impaired after resection of the carotid body and surrounding tissues. It has also been hypothesized that CB dysfunction could be one of the factors underlying type 2 diabetes (18).

The molecular mechanisms used to sense glucopenia are poorly studied, although it is known that the most frequent response recorded in low glucose–sensing cells is a depolarization, which leads to the release of transmitters or hormones. This electrophysiological response could involve ATP-regulated potassium or chloride channels, nonselective cationic channels, or an ATP-dependent Na^+/K^+ pump (19–22). In CB glomus cells, low glucose has been shown to produce external Ca^{2+}-dependent catecholamine secretion in a dose-dependent manner and inhibition of macroscopic voltage-dependent K^+ currents (10). Nevertheless, the precise mechanisms whereby glomus cells are activated by low glucose are unknown. Herein, we describe the ionic mechanisms underlying glomus cell stimulation by low glucose and the relationship between oxygen and glucose sensing. We also report the main features of glucose metabolism in glomus cells.

From the Laboratorio de Investigaciones Biomédicas, Departamento de Fisiología Médica y Biofísica, and Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Sevilla, Spain.

Address correspondence and reprint requests to Dr. José López-Barneo, Laboratorio de Investigaciones Biomédicas, Edificio de Laboratorios, 2ª planta, Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot s/n, E-41013 Sevilla, Spain. E-mail: jose.l.barneo.sspa@juntadeandalucia.es.

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M.G.-F. is currently affiliated with the Howard Hughes Medical Institute and The Rockefeller University, Laboratory of Apoptosis and Cancer Biology, New York, New York.

CB, carotid body; TRP, transient receptor potential; TRPC, TRP C subtype.

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RESEARCH DESIGN AND METHODS

Enzymatic dispersion of glomus cells. Wistar rats aged between postnatal days 10 and 20 were deeply anesthetized by intraperitoneal injection of 350 mg/kg chloral hydrate (Panreac, Barcelona, Spain). Animal care strictly followed the institutional committee guidelines. Carotid body dissection and cell dispersion were done as indicated by Ortega-Sáenz et al. (23). Dispersed cells were plated onto poly-L-lysine–coated coverslips and kept at 37°C in a humidified incubator (5% CO_2, balance air) for up to 24–48 h before they were used for the experiments.

Preparation of carotid body thin slices. Carotid bodies were included in agarose as described by Pardal et al. (24), and slices were obtained with a vibratome. Slices (150-μm thick) were placed on 35-mm Petri dishes with the same culture medium used for dispersed cell culture and maintained at 37°C in a 5% CO_2 incubator for 24–48 h before use.

Amperometric measurement of secretion. To measure secretory activity in glomus cells, we monitored catecholamine release by amperometry. The slice was continuously perfused by gravity (flow 1–2 ml/min) with a control

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solution containing (in mmol/l): 117 NaCl, 4.5 KCl, 23 $NaHCO_3$, 1 $MgCl_2$, 2.5 $CaCl_2$, 5 glucose, and 5 sucrose, bubbled with a gas mixture of 5% CO_2, 20% O_2, and 75% N_2 (normoglycemic and normoxic situation). The low glucose experiments were done upon exposure of the cells to a glucose-free solution (117 NaCl, 4.5 KCl, 23 $NaHCO_3$, 1 $MgCl_2$, 2.5 $CaCl_2$, 0 glucose, and 10 sucrose). To test the effect of hypoxia, the normoglycemic solution was bubbled with 5% CO_2 and 95% N_2 to reach a PO_2 in the chamber of ~15 mmHg. The osmolality of the solutions was maintained constant at ~275 mosmol/kg, and all amperometric recordings were done at ~36°C. All reagents and pharmacological compounds (obtained from Sigma) were added to the external solution.

For amperometric recording, we used the procedures described previously for carotid body slices (10,24). Briefly, dopamine release was recorded with a 10-μm carbon-fiber electrode polarized to 750 mV and placed near a cell under visual control by using a piezoelectric manipulator. Amperometric currents were recorded with an EPC-8 patch-clamp amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany), filtered at 100 Hz, and digitized at 250 Hz before storage on a computer. The magnitude of the secretory response was estimated from the sum of the time integral of the secretory events (cumulative secretion) during a period of time (secretion rate in pC/min).

Patch-clamp recording. Voltage- and current-clamp recordings were performed on dispersed single glomus cells using the perforated-patch whole-cell configuration of the patch-clamp technique as adapted in our laboratory (24,25). The internal solution filling the pipette contained (in mmol/l): 30 KCl, 100 potassium glutamate, 1 EGTA, 10 HEPES, and 1 $MgCl_2$; the pH was adjusted to 7.2 with KOH, and the osmolality was ~280 mosmol/kg. To this solution, 240 μg/ml amphotericin B (prepared from a stock solution of 60 mg/ml in DMSO) was added. The control (5 mmol/l glucose) and low-glucose (0 mmol/l glucose) external solutions were those used in the amperometric studies. The effect of extracellular Na^+ removal was tested using *N*-methyl-D-glucamine as a substitute. Junction potentials were not corrected. Experiments were done at room temperature (~25°C). Ionic currents and membrane potential were recorded with an EPC-7 patch-clamp amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany) and stored on a computer for further analysis.

Microfluorimetric measurement of cytosolic Ca^{2+}. Dispersed glomus cells were incubated in a recording solution with 1–5 μmol/l Fura2-AM (Teflabs) at room temperature and light protected for 30 min. For the experiments, a coverslip with labeled cells was placed on a recording chamber (~0.2 ml) mounted on the stage of an inverted microscope (Axiovert 35; Zeiss) equipped with epifluorescence and photometry. A monochromator (Polychrome IV; TILL Photonics, Munich, Germany) was used for fluorescence excitation, and the light was deflected by a dichroic mirror BSP 430 nm into the microscope objective (Plan-Neofluar 40×, NA 0.75; Zeiss). Fluorescence was detected by a charge-coupled device (CCD) camera (C4880-80; Hamamatsu, Hamamatsu City, Japan). Alternating excitation wavelengths of 340 and 380 nm were used, and background fluorescence was subtracted before obtaining the F340/F380 ratio. Bathing solutions were the same as those used in the amperometry and patch-clamp studies. Experiments were done at room temperature.

Molecular biology. Poly(A+) RNA from rat carotid bodies was extracted using the Dynabeads mRNA Direct micro kit (Dynal, Oslo, Norway) as indicated by the manufacturer. Total RNA of the rest of the tissues analyzed (liver, brain, heart, or aorta) was obtained by the Trizol method (Trizol Reagent; Life Technologies, Gibco). RNA was reverse transcribed using SuperScript II RNase H reverse transcriptase (Invitrogen, Carlsbad, CA). After reverse transcription, cDNA was amplified by PCR using 1 μl first-strand cDNA. The primers used for the PCR and the size of the different fragments were as follows (degenerate oligonucleotides amplifying hexokinase types I–III are indicated by underlying the different bases for a same position): tyrosine hydroxylase (600 bp), sense: GGACATTGGACTTGCATCTCTGGG, antisense: TGAGAAGCAGTGTTGGGAGGATGG; hexokinase (306 bp), sense: *GCCAG*TGGTGAATGACACAGT, antisense: *GT*ACCAGCTC*AC*CCCA*AT*GTA; glucokinase (245 bp), sense: CATTGTGCGCCGTGCCTGTGA, antisense: TGC CGCTGCCCTCCTCTGATT; Glut1 (126 bp), sense: GCCTGAGACCAGTT GAAAGCA, antisense: TCAGCCTCCGAGGTCCTTCT; Glut2 (210 bp), sense: TGGCACATCCTACTTGGCCTAT, antisense: CTGCTCAGTCGACGCCTCTT; Glut3 (401 bp), sense: CCGCCTGATTATTGGCATCT, antisense: CAGTGACCT GCTTCTCCTGTGA; Glut4 (151 bp), sense: GGGCTGTGAGTGAGTGCTTTC, antisense: CAGCGAGGCAAGGCTAGATT; and Slo1 (78 bp), sense: CATGGCTTTCA ACGTGTTCTTC, antisense: GCCAGAACCACAGCTTATCATTG.

Cell sorting and enriched glomus cell preparation. Cells were quenched with staining medium (L15 medium containing 1 mg/ml BSA [Sigma A-3912], 10 mmol/l HEPES at pH 7.4, and 1% penicillin/streptomycin [BioWhittaker]). After centrifugation, the cells were resuspended in staining medium, filtered through a nylon screen (45 μm; Sefar America) and stained with antibody against HNK-1 (CD57 membrane glycoprotein; BD Pharmingen), a marker that identifies tyrosine hydroxylase–positive glomus cells (26). The analysis and sorting of HNK-1$^+$ cells was performed by a MoFlo cytometer (DAKO Cytomation). RT-PCR of the enriched glomus cell preparation was done as indicated in the previous section.

Statistical analysis. Samples were statistically compared with a Student's *t* test, and their differences were considered to be significant if $P < 0.05$. The data are expressed as means ± SE. Asterisks show statistically significant differences.

RESULTS

Responses of glomus cells to hypoglycemia. As we reported previously (10), removal of extracellular glucose elicited a powerful secretory response from glomus cells in CB slices qualitatively similar to the effect of direct cell depolarization with high extracellular K^+ (Fig. 1*A*). The hypoxia-induced secretion was abolished by blockade of plasma membrane Ca^{2+} channels with external Cd^{2+} or specific Ca^{2+} channel inhibitors such as nifedipine (Fig. 1*C*–*E*). Glomus cells respond to hypoglycemia in a dose-dependent manner (10,11) (see below); however, in this series of experiments, we used preferentially 0 mmol/l glucose to maximize the cellular responses (27,28). In Fura2-loaded cells, glucose deficiency evoked a reversible increase in cytosolic Ca^{2+} concentration (Fig. 1*B*). These data further supported the hypothesis that, as other stimuli (13), glucopenia induces glomus cell depolarization, transmembrane Ca^{2+} influx, and transmitter secretion. Glomus cell responses to low glucose were highly reproducible and when evoked sequentially in a given cell, they appeared with a progressively shorter latency (Fig. 1*F* and *G*).

Removal of extracellular glucose elicited in perforated patched glomus cells (resting potential, −42.5 ± 2.4 mV) a reversible depolarization averaging 9.6 ± 0.8 mV ($n = 25$) (Fig. 2*A* and *B*). The amplitude of the receptor potential varied with glucose concentration in the range between 0 and 5 mmol/l in a dose-dependent manner (Fig. 2*C* and *D*). Thus, low glucose induced in glomus cells a previously unnoticed graded depolarizing receptor potential, which is probably necessary to trigger Ca^{2+} channel opening and a full secretory response. We also observed a reversible inhibition of the macroscopic outward K^+ currents in perforated patched dispersed glomus cells upon exposure to low glucose (Fig. 3*A* and *B*, *upper recordings*), a response that confirmed our previous findings in dialyzed patch-clamped CB cells (10). Addition of either tetraethylammonium (a nonselective blocker of voltage-gated K^+ channels) or iberiotoxin (a selective blocker of voltage- and Ca^{2+}-activated maxi-K^+ channels) produced a marked reduction of K^+ current amplitude (Fig. 3*A* and *B*, *lower recordings*). In the presence of these K^+ channel blockers, application of 0 glucose did not produce any further decrease in the remaining K^+ current, and neither tetraethylammonium nor iberiotoxin prevented the secretory response of glomus cells to hypoglycemia. The effect of K^+ channel blockers and low glucose were additive (Fig. 3*C* and *D*). These results indicated that inhibition of voltage-dependent K^+ currents by glucose deficiency did not greatly contribute to stimulate glomus cell secretion. In fact, the amplitude of the 0 glucose–induced receptor potential ($\Delta V = 8.9 \pm 5.0$ mV; $n = 5$) was unchanged in cells where macroscopic K^+ currents were unaffected by glucose removal (Fig. 3*E*).

Activation of a background Na^+ conductance by low glucose. The low glucose–induced receptor potential was manifested in perforated-patched voltage-clamped glomus

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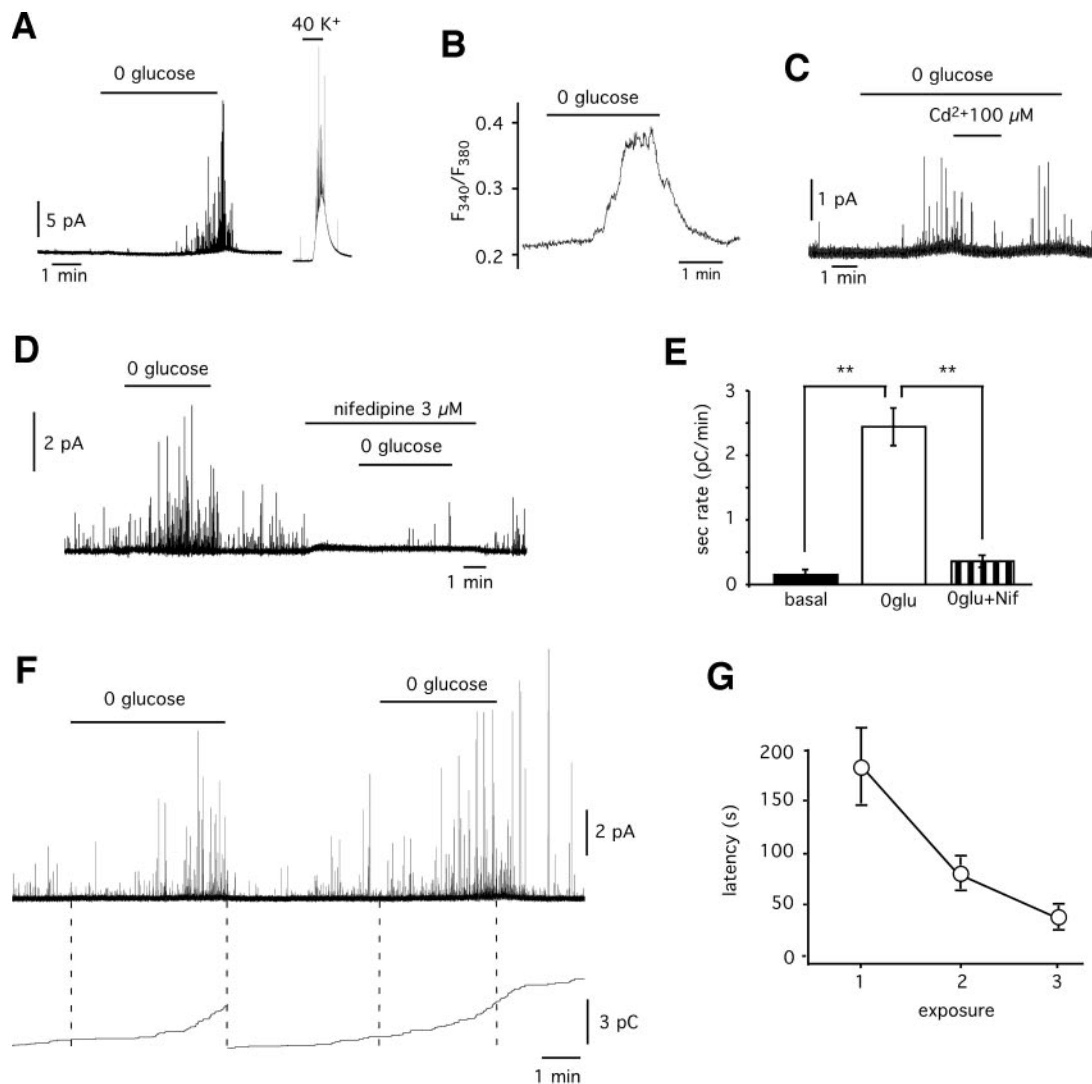

FIG. 1. Responses to low glucose of carotid body glomus cells before and after blockade of membrane voltage-dependent ion channels. *A*: *Left*, Secretory response to glucopenia of glomus cells in CB slices. *Right*, Secretory response to high extracellular (40 mmol/l) K^+. *B*: Reversible increase of cytosolic Ca^{2+} concentration in a Fura2-loaded glomus cell in response to low glucose. *C* and *D*: Abolishment of the secretory response to 0 mmol/l glucose by blockade of plasma membrane Ca^{2+} channels with Cd^{2+} (*C*) or nifedipine (*D*). *E*: Average secretion rate during exposure to the control solution (basal; 152 ± 76 fC/min), 0 glucose (0glu; 2,437 ± 291 fC/min), and 0 glucose plus nifedipine (0glu+Nf; 359 ± 90 fC/min) (basal/0glu, $P = 0.001$; 0glu/0glu+Nif, $P = 0.002$; $n = 3$). *F*: Repeated exposure to hypoxia in the same cell. The cumulative secretion signals (in picoCoulombs) are indicated at the bottom. The dotted lines indicate the onset and termination of the exposure to hypoxia. *G*: Latency of the secretory response upon exposure to hypoxia measured from the onset to the time at which there was a marked change in the slope of the cumulative secretion signal. The number of experiments for each data point are between 5 and 11.

cells as a reversible increase in holding current amplitude. This signal was accompanied by fast transient inward currents resembling the opening and closing of membrane channels (Fig. 4*A*). A plot representing the increments in peak current amplitude for each voltage is shown in Fig. 4*B*. The amplitude of both the envelope inward holding current and the transient currents decreased when the membrane was depolarized and increased with membrane hyperpolarization, thus suggesting that it was mediated by activation of an inward cationic (Na^+ and/or Ca^{2+}) conductance. In fact, a background Na^+ current that maintains the resting membrane potential to relatively depolarized values (around −40 to −50 mV) has been described in glomus (29) and the closely related chromaffin (25) cells. The precise nature of the low glucose–activated inward current was studied by ion substitution experiments (Fig. 4*C* and *D*). In voltage-clamped cells, replacement of extracellular Na^+ with the membrane impermeant cation *N*-methyl-D-glucamine produced a reversible decrease of the holding inward current and the amplitude of the single channel events as well as abolishment of the response to low glucose (Fig. 4*C*). Similarly, in current-clamped cells, removal of external Na^+ gave rise to a marked cell hyperpolarization and disappearance of the low glucose–induced receptor potential (Fig. 4*D*; see histograms on the *right panels* in Figs. 4*C* and *D*).

Several members of the C subclass of transient receptor potential (TRP) channels seem to be expressed in CB cells (30). TRP channels constitute a broad family of Na^+-permeant nonselective cation channels involved in numerous cellular functions including sensing temperature and osmolarity (31,32). The amplitude of the Na^+-dependent background current in glomus cells was reversibly decreased by the application of 100 μmol/l $LaCl_3$ (Fig. 5*A*), which, although nonspecifically, is known to block TRP C subtype (TRPC) channels (33). The most abundant TRPC

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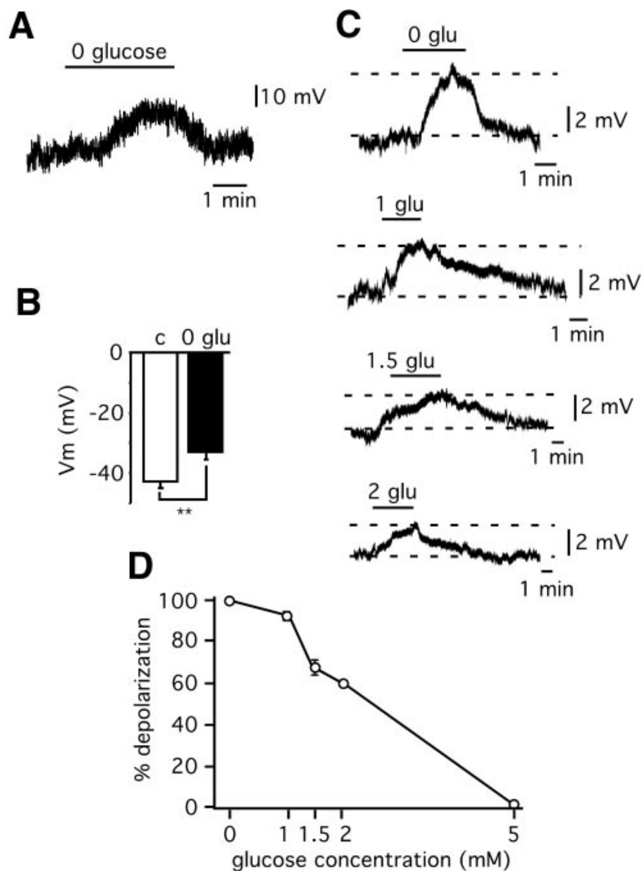

FIG. 2. Depolarizing receptor potential induced by low glucose (glu). *A*: Representative depolarizing response to glucopenia in a current-clamped glomus cell. Resting potential −42 mV. *B*: Membrane potential in control (c, 5 mmol/l glucose; −42.57 ± 2.27 mV, *n* = 25) and in 0 glucose (−32.97 ± 2.43 mV, *n* = 25) (*P* < 0.01). *C*: Graded depolarizations to various levels of extracellular glucose in a same cell. Resting potential was held at −40 mV by current injection. *D*: Summary of the amplitude of the depolarizing potential as a function of extracellular glucose measured in several cells (*n* = 2–5) exposed to at least three different glucose concentrations. Data are normalized to the maximal depolarization obtained with 0 glucose. In *C* and *D*, oxygen tension in the external solution was 15 mmHg.

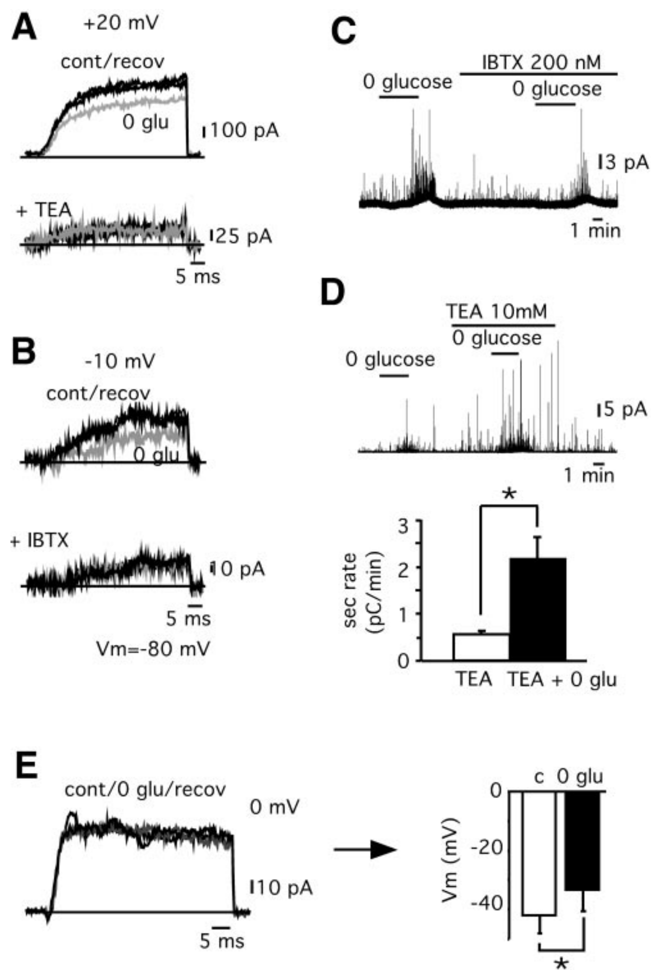

FIG. 3. Effect of low glucose and potassium channel blockers on low-glucose responsiveness of glomus cells. *A* and *B*: Decrease of macroscopic voltage-dependent K^+ current amplitude by glucopenia (*top*; *n* = 14) and inhibition of the currents by tetraethylammonium (TEA) and iberiotoxin (IBTX) (*bottom*: *n* = 3). *C* and *D*: Responsiveness to glucopenia of glomus cells in CB slices is unaltered by the K^+ channel blockers IBTX and TEA. *D*: *Bottom*: Secretion rate induced by TEA (5–10 mmol/l) and TEA plus 0 glucose (*n* = 5; *P* = 0.019). *E*: Depolarizing response to 0 glucose in cells in which voltage-dependent K^+ current was unaffected by glucopenia. Resting potential in control (c, 5 mmol/l glucose; −41.2 ± 6.44 mV, *n* = 5) changed to −32.72 ± 7.13 mV (*n* = 5) in 0 glucose (*P* = 0.02). cont, control; recov, recovery.

channels expressed in glomus cell membrane are TRPC3 and TRPC6 (30), which belong to a subfamily whose members are all activated by diacylglycerol, a product of phospholipase C activation (34). Application of the membrane-permeable diacylglycerol analog 1-oleoyl-2-acetyl-sn-glycerol induced inward currents of similar characteristics as the current triggered by glucose deficiency, which were also abolished by application of $LaCl_3$ (Fig. 5*B*). Although we must be aware that the pharmacology of TRP channels is quite nonspecific, altogether these results suggest that one or several subtypes of TRPC channels mediate the low glucose–induced background Na^+ current in glomus cells. In fair agreement with the results presented above, inhibition of phospholipase C with U73122 (1 μmol/l) prevented the responses (inward current activation and catecholamine release) of glomus cells to 0 glucose, thus suggesting that activation of this enzyme might participate in sensing low glucose. However, these data must be interpreted cautiously, since at higher concentrations (5–10 μmol/l), U73122 also blocks the macroscopic Ca^{2+} current in glomus cells (data not shown).

Combined glucose and oxygen sensing in glomus cells. Stimulation of CB glomus cells by low glucose is markedly potentiated in hypoxia, and both stimuli (glucose deficiency and hypoxia) act additively to induce transmitter release (10,11). However, the transduction mechanisms underlying glomus cell activation by low glucose appear to differ from those involved in O_2 sensing. Although most glomus cells in the slice preparation are activated by both hypoxia and low glucose (*n* > 50 cells) (Fig. 6*A*), there are cells that respond specifically to either hypoxia (*n* = 12) or glucose deficiency (*n* = 3) (Fig. 6*B*). In addition, it is known that the background low glucose–activated Na^+ current is unaffected by hypoxia (29). We have previously shown that rotenone (0.1–1 μmol/l) induces secretion in glomus cells and that it selectively occludes any further effect of hypoxia, thus suggesting that a rotenone binding molecule is involved in acute O_2 sensing by glomus cells (23). In all the cells tested, we confirmed the blockade of responsiveness to hypoxia by rotenone (Fig. 6*C*). However, this drug did not prevent cell

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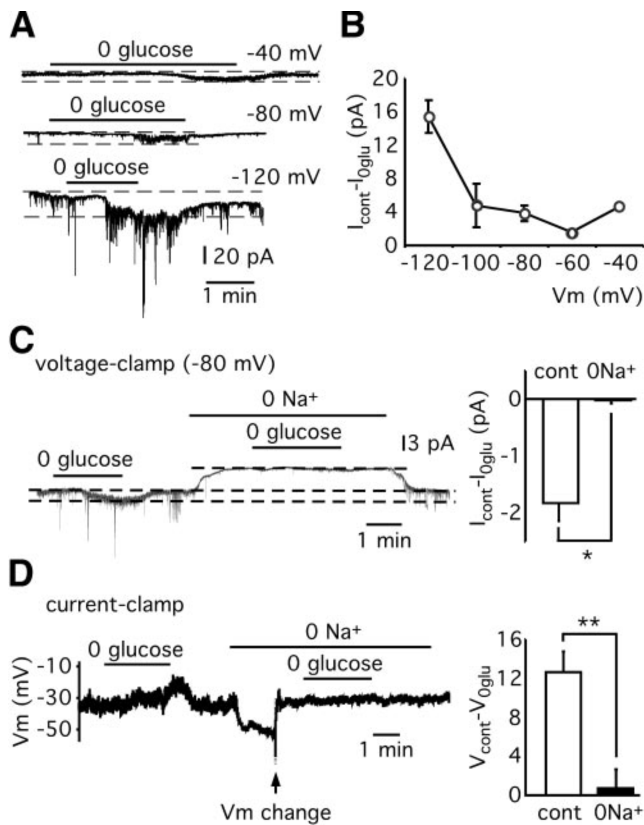

FIG. 4. Characterization of the low glucose–activated conductance in isolated glomus cells. *A*: Reversible increase of inward current upon exposure to low glucose at different voltages. *B*: Increment in the current induced by 0 glucose ($I_{control}$–I_{0glu}) at different membrane potentials (Vm). The number of experiments for each data point are between 2 and 11. *C* and *D*: Replacement of extracellular Na^+ with *N*-methyl-D-glucamine in voltage-clamped (*C*) or current-clamped (*D*) glomus cells exposed to 0 glucose. To compensate for the hyperpolarization induced by 0 Na^+, Vm was changed manually to the previous resting value (*arrow*) (plot in *C*: control −1.82 ± 0.32 pA, 0 Na^+ 0.02 ± 0.09 pA, $P = 0.02$, $n = 3$; plot in *D*: control 12.58 ± 1.93 mV, 0 Na^+ 0.66 ± 1.85 mV, $P < 0.01$, $n = 3$).

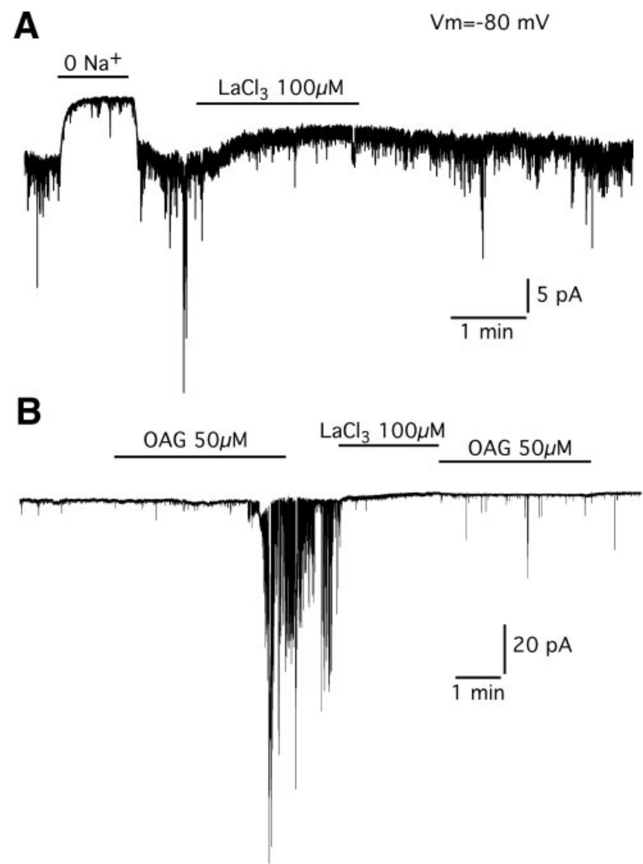

FIG. 5. Pharmacology of persistent Na^+ current in glomus cells. *A*: Decrease of the envelope inward current and the current transients in a perforated patched glomus cell upon removal of external Na^+ or the application of $LaCl_3$. *B*: Induction of transient inward current events by 1-oleoyl-2-acetyl-sn-glycerol and blockade of this activity by $LaCl_3$. Similar qualitative data have been observed in two other cells.

activation by low glucose (Fig. 6*D*). In fact, rotenone and low glucose had additive effects. Therefore, it seems that hypoxia and low glucose act on separate signal transduction mechanisms that share a common final pathway involving extracellular Ca^{2+} influx, the rise of cytosolic Ca^2, and neurotransmitter secretion.

Glucose transport and metabolism in the carotid body. In cells exposed repeatedly to low glucose, the time elapsed between the switch to the external solution and the initiation of the secretory response was markedly reduced from the first (175 ± 22 s) to the second (77 ± 14 s; $n = 11$ cells) trial (Fig. 1*G*). This lag between the application of 0 glucose and the initiation of secretion, also observed for the low glucose–induced inward currents, was not due to emptying of calcium stores from the endoplasmic reticulum, since the application of thapsigargine did not mimic or prevent the action of glucose removal (data not shown). This observation suggested that the variable that is being sensed is either intracellular glucose or a glucose-derived metabolite whose intracellular concentration changes in the absence of extracellular glucose. This idea was also supported by the inability of the nonmetabolizable glucose analogs 2-deoxyglucose (transported across the membrane and phosphorylated to glucose-6-phosphate; data not shown) or 3-*O*-methylglucose (transported but unaffected by hexokinases) to prevent the secretory response to glucose deficiency in glomus cells (Fig. 7*A*).

Among the glucose transporters of the Glut family, we found Glut1, Glut3, and Glut4 to be expressed in the CB. However Glut2, which appears to be specifically expressed in glucose-sensing cells (35,36), was undetectable (Fig. 7*B*). Glucokinase (hexokinase IV), a low-affinity hexokinase isoform that is characteristic of pancreatic β-cells, α-cells, and some glucose-sensitive neurons (27,35,37,38), was detected with some specificity in CB tissue (Fig. 7*C*). However, a detailed study in a purified preparation of catecholaminergic CB cells (see RESEARCH DESIGN AND METHODS) failed to demonstrate unequivocally the presence of glucokinase mRNA in glomus cells. In the same preparation, other genes, such as tyrosine hydroxylase or the maxi-K^+ channel α-subunit (Slo1), known to be expressed in glomus cells, were easily identified (Fig. 7*D*). Both in the whole carotid body and purified glomus cell preparations, the hexokinase form expressed is hexokinase I, as confirmed by sequencing the corresponding PCR products.

DISCUSSION

The main findings in this article are *1*) low glucose induces in neurosecretory glomus cells a dose-dependent and reversible depolarizing receptor potential due to activation

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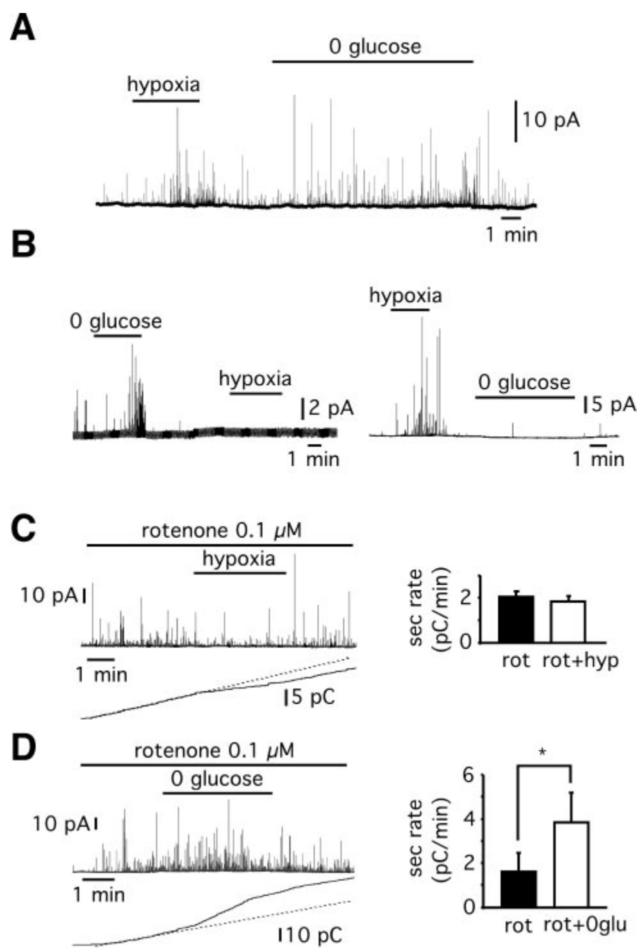

FIG. 6. Differential sensitivity of glomus cells to oxygen and low glucose. *A*: Secretory responses of a representative glomus cell to both hypoxia (PO_2, ~15 mmHg) and glucopenia. *B*: Examples of cells responding differentially to hypoxia or to low glucose. *C*: Blockade of responsiveness to hypoxia (hyp) by rotenone (rot) (n = 14; rot 2,037 ± 237, rot+hyp 1,825 ± 241 fC/min, P = 0.30). *D*: Responsiveness to low glucose in the presence of rotenone (n = 5; rot 1,622 ± 835, rot+0glu 3,851 ± 1,326 fC/min, $P < 0.05$). The traces below the amperometric recordings in *C* and *D* are the cumulative secretion.

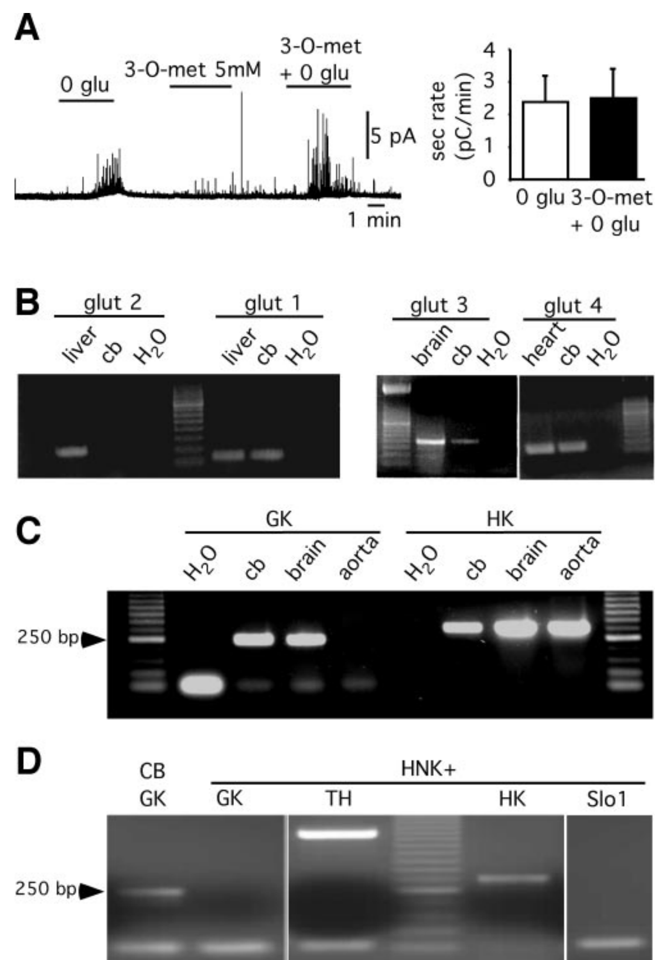

FIG. 7. Glucose transport and metabolism in the carotid body. *A*: Secretory response to glucopenia in the presence of 3-*O*-methyl-glucose in glomus cells (0glu 2,400 ± 800, 3-*O*-meth+0glu 2,500 ± 900 fC/min, n = 3, P = 0.5). *B* and *C*: RT-PCR showing expression of Glut1–4 (*B*) and glucokinase (GK)/hexokinase (HK) (*C*) in the whole carotid body and different tissues showed for comparison. *D*: RT-PCR analysis of the expression of glucokinase in a purified preparation of HNK$^+$ (tyrosine hydroxylase positive) glomus cells. Tyrosine hydroxylase (TH), hexokinase I (HK), and maxi-K$^+$ channel α-subunit (Slo1) genes are shown for comparison.

of an inward Na^+-permeable cationic conductance. We have shown that this Na^+ current is directly involved in regulating membrane potential responses to low glucose and likely the downstream Ca^{2+} response. This Na^+ conductance has characteristics compatible with currents mediated by TRPC channels. *2*) Sensitivity to low glucose and to hypoxia appear to depend on separate signal transduction mechanisms, although they converge on the final steps causing transmembrane Ca^{2+} influx and transmitter release. *3*) Glucose metabolism appears to be necessary for the regulation of glomus cell electrophysiological responses. The association of Glut2 and glucokinase characteristic of some high glucose–sensing cells does not seem to be needed for low-glucose detection. Altogether, these data support the view that the CB is a multimodal chemoreceptor with a physiological role in glucose homeostasis.

Using the perforated-patch clamp technique, we have shown that glucopenia produces inhibition of the voltage-dependent K^+ channels in glomus cells. However, the most critical electrophysiological effect of glucose removal on these cells is a depolarizing receptor potential generated by activation of background Na^+-permeable channels. This receptor potential has also been recently confirmed by another laboratory supporting the data presented in this article (11). Combination of an increase in Na^+ current and inhibition of voltage-dependent K^+ channels surely contributes to the maintained activation of Ca^{2+} channels, thus allowing the entry of Ca^{2+} that is necessary to trigger neurotransmitter release. Cell depolarization has been reported to occur during low-glucose sensing in numerous neurons, but the underlying mechanisms were not completely clarified (3). ATP-sensitive K^+ channels have been proposed to have a key role in sensing hypoglycemia by hypothalamic neurons (28); however, these channels do not seem to have a major contribution in CB glomus cells, since glibenclamide (an ATP-sensitive K^+ channel blocker) does not alter the neurosecretory response to glucopenia in CB slices (10). In addition, the electrophysiological experiments reported here rule out the involvement of background K^+ channels in the low-glucose activation of CB cells. Tandem pore K^+ channels have, however, been suggested to mediate inhibition of orexin neurons by glucose (5). Depolarization of striatal spiny neurons with glucose deprivation has been attrib-

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uted to either inactivation of the Na^+/K^+ pump or to potentiation of a nonselective cationic conductance (6,20). It is therefore possible that the Na^+-permeable channels generating the receptor potential in CB cells also participate in the depolarization of low glucose–sensitive neurons. These channels appear to belong to the TRPC3/6 subtypes, highly expressed in CB glomus cells (30) and having a pharmacology compatible with that observed for the background Na^+ current (33,34). Heterologously expressed recombinant TRPC6 channels exhibit single-channel events quite similar to those observed in glomus cells after activation by low glucose or 1-oleoyl-2-acetyl-sn-glycerol (39). Interestingly, TRPC3 channels have been shown to be directly involved in Ca^{2+} signaling, since Na^+ influx is coupled with reversal activation of the Na^+/Ca^{2+} exchanger, thus leading to the rise of cytosolic Ca^{2+} (40).

The fact that glucose analogs (deoxyglucose and methyl glucose) cannot substitute for glucose in glomus cells strongly suggests that glucose sensing does not depend on membrane transport and requires partial or complete glucose metabolization. Regarding the nature of the glucose-derived metabolite(s) sensed by glomus cells, there are several candidates. For instance, TRP and various subtypes of voltage-gated channels can be modulated by NAD/NADH, ADP-ribose, 2,3 diphosphoglycerate, or glyceraldehyde phosphate (41–44). Another possibility is that glucopenia leads to an increase in the AMP/ATP ratio in glomus cells and to activation of AMP kinase, which in turn activates the Na^+-permeable channels. AMP kinase has been proposed to be involved in low-glucose sensing by hypothalamic neurons (45,46), and mRNA for this enzyme can be detected in CB tissue (M.G.-F., P.O.-S., A.C., J.L.-B., unpublished data). However, inhibition of AMP kinase (C compound, 20 μmol/l) did not prevent a full secretory response to 0 glucose in glomus cells (data not shown). The clarification of which of these plausible mechanisms operates in glomus cells must await future experimental work. The existence of glomus cells responding only to hypoxia or hypoglycemia, and the different effect of these stimuli when applied concomitantly with rotenone, suggests that distinct pathways are used to sense oxygen and glucose in the CB.

We have shown that among the various glucose transporters studied, CB cells express Glut1, Glut3, and Glut4. Glut2, associated with some classes of glucose-sensitive neurons (35,36), was not expressed. These data are in accord with a recent study on glucose-sensing neurons in the ventromedial hypothalamus. Glut4 mRNA was found abundantly and Glut2 less frequently but without any apparent relationship to glucosensing capacity (47). Hexokinase I is expressed in CB tissue as well as in isolated glomus cells. In contrast, glucokinase mRNA was detected in the whole CB but not in isolated glomus cells. It is possible that the level of expression of this enzyme is below the resolution of our preparation of enriched glomus cells. Several commercially available antibodies against glucokinase were tested but resulted to be quite nonspecific. Another possibility is that glucokinase (although present in the CB tissue) is not required for low-glucose sensing by glomus cells. Glucokinase has been proposed to be necessary for glucose sensing by hypothalamic glucose-inhibited neurons (27); however, in other studies using single-cell PCR of identified glucose-sensing hypothalamic neurons, glucokinase was detected only in 43% of glucose-inhibited cells (47).

In summary, our data strongly support the view that glomus cells, the chemoreceptor elements in the CB, are physiologically relevant low-glucose sensors. The strategically located CB could be of special importance for brain homeostasis, since they could act as combined oxygen and glucose sensors to facilitate activation of the counterregulatory measures in response to small reductions of either variable.

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