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On-line analysis of gap junctions reveals more efficient electrical than dye coupling between islet cells

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Quesada, Ivan, Esther Fuentes, Etelvina Andreu, Paolo Meda, Angel Nadal, and Bernat Soria. On-line analysis of gap junctions reveals more efficient electrical than dye coupling between islet cells. *Am J Physiol Endocrinol Metab* 284: E980–E987, 2003. First published January 7, 2003; 10.1152/ajpendo.00473.2002.—Pancreatic β-cells constitute a well-communicating multicellular network that permits a coordinated and synchronized signal transmission within the islet of Langerhans that is necessary for proper insulin release. Gap junctions are the molecular keys that mediate functional cellular connections, which are responsible for electrical and metabolic coupling in the majority of cell types. Although the role of gap junctions in β-cell electrical coupling is well documented, metabolic communication is still a matter of discussion. Here, we have addressed this issue by use of a fluorescence recovery after photobleaching (FRAP) approach. This technique has been validated as a reliable and noninvasive approach to monitor functional gap junctions in real time. We show that control pancreatic islet cells did not exchange a gap junction-permeant molecule in either clustered cells or intact islets of Langerhans under conditions that allowed cell-to-cell exchange of current-carrying ions. Conversely, we have detected that the same probe was extensively transferred between islet cells of transgenic mice expressing connexin 32 (Cx32) that have enhanced junctional coupling properties. The results indicate that the electrical coupling of native islet cells is more extensive than dye communication. Dye-coupling domains in islet cells appear more restricted than previously inferred with other methods.

islets of Langerhans; gap junctions; intercellular communication

INSULIN SECRETION from the islets of Langerhans is a multicellular event that arises as an emergent property due to β-cell intercellular communication (16). The physiological heterogeneity found in individual β-cells, when the response to glucose and signaling patterns are considered (5, 13, 14, 35, 38), is switched to a coordinated and synchronized behavior when the β-cell population communicates (31, 39). Such signal coordination yields a more vigorous secretion and biosynthesis of insulin (5, 35, 38). Furthermore, when cellular connections are severed or defective, insulin secretion is markedly reduced (23, 50). Thus cell communication within the islets of Langerhans mainly mediates signal synchronization (39) and leads to a more effective insulin release.

Among the several mechanisms that control cell-to-cell communication within pancreatic islets (6), the one mediated by gap junctions is believed to be essential for the recruitment and synchronization of insulin-secreting cells (50). Gap junctions, which improve cell contacts, establish transmission pathways across the islet of Langerhans, allowing cell-to-cell interaction (50). This physical link is responsible for electrical and metabolic communication in several types of cells (19). However, whereas electrical coupling has been well documented in pancreatic islets (10) and is believed to account for signal synchronization of islet cells (39), the extent of metabolic coupling and its influence on islet synchronization and physiological response are still a matter of debate. Iontophoretic injection of dye and the evaluation of its intercellular transfer have been employed as a model for the passage of small molecules in several types of cells (3, 44). On the one hand, the iontophoretic injection of dyes (28) and metabolites (18) into pancreatic β-cells has indicated the existence of limited, spatially restricted territories of dye coupling, in agreement with the observations of intercellular exchanges of tritiated nucleotides and metabolites that have also suggested the presence of territories of metabolic cooperation (5, 22). On the other hand, studies on electrically coupled pairs of islet cells have failed to provide evidence for a significant dye coupling (24, 34), leading to controversial conclusions with regard to the extent of the intraislet communication network.

In this study, we have used the fluorescence recovery after photobleaching (FRAP) approach, which has been validated as a reliable technique to analyze functional gap junction-mediated communication, because of several advantages that it offers over alternative techniques (9, 51). First, FRAP is a noninvasive technique and thus is expected to avoid some of the cell injuries that may occur during cell injection or manipulation.

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Second, dye passage between cells through gap junctions takes place as a passive diffusional process, resembling the one that might be occurring in vivo. Third, the temporal resolution of the system stands for the on-line monitoring of gap junction function. We have used this technique to analyze dye coupling between native cells of control islets and cells from islets of transgenic mice that specifically overexpressed connexin 32 (Cx32) (7). These mice are hereafter referred to as rat insulin promoter (RIP)-Cx32. We show that, although electrical coupling was present in both control and transgenic cells, it was markedly enhanced in the latter (7). In contrast, dye transfer was not observed in native islet cells but was easily detected between cells of RIP-Cx32 mice. The results suggest that gap junctions of native β-cells ensure electrical coupling but restrict molecular exchanges.

METHODS

Islet cell isolation. All experiments were conducted according to regulations approved by our institution. Seven Swiss albino OF1 and five homozygotic transgenic (RIP-Cx32 strain) mice (7) were killed by cervical dislocation. The design of the RIP-Cx32 construct and the junctional and physiological characteristics of transgenic mice expressing Cx32 have been shown elsewhere (7). Pancreatic islets were isolated by collagenase digestion as previously described (31, 36) and were dispersed into single cells and clusters by enzymatic digestion in the presence of 0.05% trypsin and 0.02% EDTA for 2 min. Cells and clusters were plated onto coverslips and cultured for 24 h in RPMI 1640 supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 5.6 mmol/l glucose. Intact islets were used 2 h after isolation. No significant difference in cellular coupling was found between normal OF1 and C57B16 mice, which were used to control the experiments on transgenic mice (not shown) (7). We conclude that the majority of clustered cells that we used in FRAP experiments were pancreatic β-cells, because ~85% of cultured islet cells were identified as β-cells by means of immunofluorescence labeling of insulin content, as described elsewhere (36). In addition, β-cells can be easily distinguished by their large size and low nuclear-to-cytoplasmic ratio compared with other islet cell types (4, 36).

Astrocyte isolation. Astrocytes were isolated from cerebral cortices of 1- to 3-day-old rats and cultured for 2–4 wk, as previously described (30, 32). At least 98% of the cells expressed glial fibrillary acidic protein, a glial-specific marker, and had the characteristic flat, type 1-like astrocyte morphology. Astrocytes were plated onto coverslips 2–4 days before use. Coverslips were transferred from the culture medium (DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin) to Hanks' solution (140 mM NaCl, 5.4 KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Na-HEPES, pH 7.35) for photobleaching experiments.

FRAP. Cell clusters and intact islets were loaded with 5 μmol/l of the lypophyllic dye 5-(and-6)-carboxyfluorescein diacetate [5(6)-CFDA; Molecular Probes, Madrid, Spain] for 5 min at room temperature and then washed with the perfusion medium (see below). Once inside the cell, the dye is retained due to a conversion by cytoplasmic esterases into a nonpermeant and fluorescent carboxyfluorescein (CF) molecule. This Ca^{2+}-independent fluorescent dye of small size (molecular mass of the hydrolyzed derivative = 376 Da) has been shown to permeate gap junction channels of different kinds of cells (9, 30, 51) and of islet cells when iontophoretic techniques are used (18, 25). Cell clusters or islets were perfused with a modified Krebs-Ringer buffer containing (in mmol/l): 119 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 2.5 $CaCl_2$, which was constantly bubbled with a mixture of 95% O_2-5% CO_2, giving a final pH of 7.4. Glucose concentration was 16 mmol/l except when otherwise indicated. All experiments were performed at 37°C. Fluorescence was monitored under a Zeiss LSM 510 confocal microscope (Zeiss, Jena, Germany) by use of a ×40 oil immersion lens (numerical aperture 1.3) or a ×63 oil immersion lens (numerical aperture 1.25) and fluorescein filters. Photobleaching was performed by concentrating the confocal laser beam onto a selected cell and transiently increasing its intensity to the maximum (25 mW) for 30 or 60 s for each cell. After bleaching, recovery of fluorescence in this cell was monitored by recording an image every 4–6 s. Fluorescence was expressed in arbitrary units (from 0 to 255) as a function of time. The laser intensity used during image records did not produce photobleaching in any case. Results were plotted using commercial software (Sigmaplot; Jandel Scientific, Erkrath, Germany). Fluorescence recovery takes place as a result of dye transfer through gap junctions from surrounding nonphotobleached cells (30, 51). To control the specificity of the observed fluorescent changes, we first studied single cells. We observed no recovery of CF fluorescence after these cells were bleached, indicating that the recovery observed in pairs, clusters, and islets could not be accounted for by the incorporation of dye present in the medium. Fluorescence recovery was also reversibly blocked by 20 μmol/l 18α-glycyrrhetinic acid, a gap junction blocker, providing further evidence that connexin channels mediated the diffusion of CF from nonbleached into photobleached cells (17, 30).

After 5 min of exposure to CF, cells exhibited a bright fluorescence, indicating a rapid incorporation of the dye (30). To avoid toxic effects because of saturating concentrations, cells were not loaded for longer times. In the whole islets, loading for 5 min led to a peripheral distribution of CF; however, the dye was accumulated more deeply in the transgenic islets than in the native ones (compare images in Fig. 4*C* and Fig. 5*B*), most likely due to the enhanced coupling properties of the former (7). Longer loading times led to CF accumulation in deeper cell layers, but the center of the islet did not incorporate the dye even after 1-h loading (31, 36, 46). A uniform distribution of Cx32 throughout the islet has been demonstrated in transgenic RIP-Cx32 mice (7), ruling out the possibility that peripheral dye allocation could be attributed to a preferential expression of Cx32 in peripheral cells in the case of transgenic islets. Similar difficulties with dye penetration have been reported not only in pancreatic islets (31, 36, 46) but also in other thick preparations like brain slices (32). Nevertheless, this handicap is offset, since all the cell types including β-cells are represented at the periphery of the islet (31, 36), where the measurements were performed.

Calcium measurements. Calcium measurements after bleaching were performed by loading cells with 4 μmol/l fluo 3-AM (Molecular Probes, Madrid, Spain) for 20 min at 37°C and by monitoring fluorescence changes under the aforementioned confocal microscope (31, 36).

Cell viability test. Viability of cells was tested after photobleaching by loading cells with 1 μmol/l ethidium homodimer-1 (Molecular Probes, Madrid, Spain).

Electrophysiological recordings. Microdissected islets were attached in a 50-μl chamber and perfused at 37°C with 0.8 ml/min modified Krebs medium supplemented with 11 mM glucose. Individual cells were impaled using borosilicate glass microelectrodes of 80–120 MΩ. Cell membrane poten-

tials were recorded using an Axoclamp 2B amplifier acquired using Axoscope 8.2 (Axon Instruments, Foster City, CA) and analyzed using Origin 5.0 (Microcal Software, Northampton, MA). To evaluate electrical synchronization and coupling, two islet cells were simultaneously recorded within a single islet by use of electrodes whose tips were apart from each other 85 ± 15 μm (1, 2). Synchronization was evaluated by measuring the interval between corresponding spikes on the recordings of the two cells.

RESULTS

Electrical coupling was assessed using two microelectrodes that simultaneously recorded events within two cells of a single islet. We observed that the glucose-induced typical bursts of electrical activity, which characterize the insulin-producing β-cells, occurred concomitantly in the cells monitored in both control (Fig. 1*A*) and transgenic islets (Fig. 1*B*). However, whereas small temporal delays (≤1 s) between spikes occurred in cells from control islets (Fig. 1, *A* and *C*), β-cells of RIP-Cx32 transgenic mice were fully synchronous (Fig. 1, *B* and *D*). Although the duration of the active and silent phases was longer in transgenic than control cells, the degree of electrical activity, which is measured by the ratio of active phase to total period, was the same in both cells (7). Some differences in the electrical activity may arise because of the existence of two different sets of connexins mediating intercellular connections, mainly Cx36 in native islets (41) and Cx32 in transgenic cells (7), which may provide different junctional conductances (43, 45). Actually, variations of the gap-junctional conductance can modify synchrony, duration of bursts, and electrical pattern of the islet network (42). When the cable properties of the system were evaluated by measuring the propagation of pulses between cells separated by ~85 μm, we noted that currents injected into β-cells of RIP-Cx32 mice were able to propagate voltage deflections (Fig. 1*D*), whereas control cells were not at a detectable level (Fig. 1*C*). Note in Fig. 1*D* that islet cells from transgenic mice could transmit voltage deflections even during the silent phase. In this situation, coupling conductance may be smaller than during the active period (2). These results are representative of eight experiments from three different preparations for native islet cells and three experiments from three preparations for transgenic islet cells. Thus, although electrical coupling is widespread in the islet of Langerhans (2, 10, 31, 39), transgenic islets exhibited enhanced electrical synchronization and coupling properties.

Dye coupling was tested by monitoring the intercellular transfer of CF by use of a FRAP approach. To validate the technique, we first set up the experimental conditions using astrocytes (Fig. 2), since these cells are coupled by large junctional conductances (11) and show a rapid recovery of fluorescence after bleaching (30). In these experiments, we observed that the conditions that we used were adequate to rapidly assess junctional coupling of astrocytes. FRAP had an exponential time course (Fig. 2*B*) with a time constant of 59.21 ± 13.45 s (n = 8 cells from 8 clusters from 2 different preparations). An exponential fluorescence decay was often recorded from the adjacent nonbleached cells, which were coupled to the photobleached one (Fig. 2*B*) and which functioned as donors

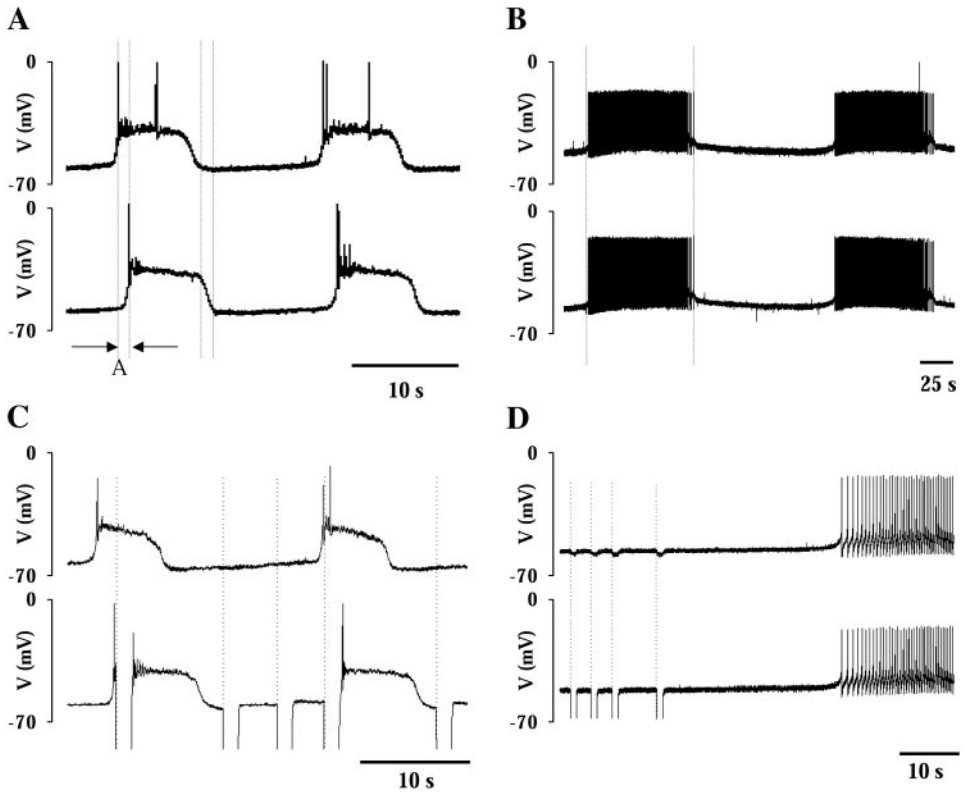

Fig. 1. Electrical coupling is detected in control islets and is improved in islets of rat insulin promoter (RIP)-connexin 32 (Cx32) transgenic mice. *Top*: simultaneous electrical activity of 2 pancreatic β-cells, at a distance of 85 ± 15 μm, in the presence of a stimulatory concentration of glucose (11 mM). Control islet is shown in *A* and transgenic islet in *B*. Compared with controls, bursts were longer and more synchronous in an islet of a RIP-Cx32 transgenic mice. Control cells revealed slight asynchronicity, as judged by small delays (A < 1 s) of both burst and spike patterns. *Bottom*: to evaluate current propagation between coupled cells, −0.5 nA electrical pulses were applied to 1 cell at the times indicated by the dotted vertical lines (*bottom trace*). Electrical activity was simultaneously recorded in this cell and a 2nd cell distant ~85 μm within the same islet (*top trace*). Injected currents did not provoke propagated voltage deflections in cells of a control islet (*C*), but did so in cells of an RIP-Cx32 transgenic mouse (*D*).

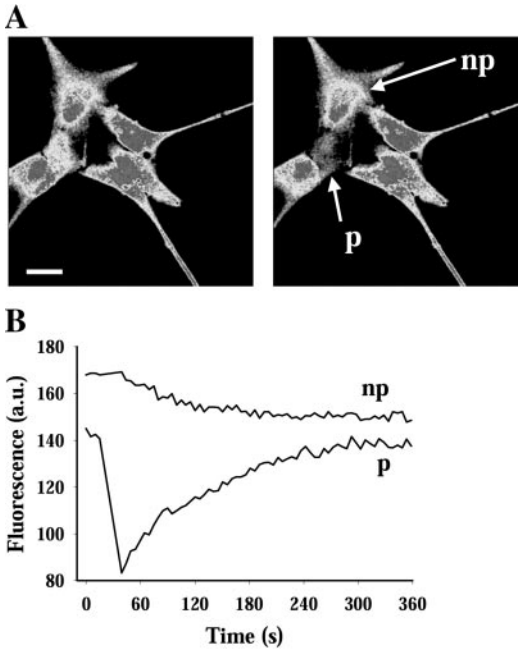

Fig. 2. Dye coupling detection in cultured astrocytes using the fluorescence recovery after photobleaching (FRAP) approach. *A*: cluster of 6 astrocytes is seen under the confocal microscope before (*left*) and after photobleaching (*right*) of 1 cell (p). The procedure resulted in a partial decrease in the fluorescence of carboxyfluorescein (CF) in the bleached cell but not in the adjacent cells. Calibration bar, 10 μm. *B*: plotting the fluorescence intensity of the bleached cell as a function of time typically revealed an exponential time course which corresponded to fluorescence recovery, due to transfer of CF from the nonbleached cells into the photobleached one. The nonbleached astrocyte, labeled np, exhibited a parallel fluorescence decay resulting from the dye passage via gap junction channels. a.u., Arbitrary units.

of the fluorescent probe. Thus astrocytes provided a reliable model and could be used as positive controls of FRAP experiments in native islet cells.

To investigate whether the laser may have damaged islet cells, we tested cell viability with ethidium homodimer-1 staining. We observed that, even under conditions more severe than those employed during the experiments, the impermeant DNA-binding probe did not stain the nuclei of photobleached cells (n = 7 from 3 different preparations), suggesting that these cells were still viable after laser bleaching (Fig. 3*A*). In contrast, nuclei stained red by ethidium homodimer-1 were occasionally observed in cells that were also unable to retain CF fluorescence after loading with 5(6)-CFDA (Fig. 3*A*). Additional CFDA incorporated into the medium resulted in reaccumulation of the dye in the photobleached cells. Measuring the glucose-induced Ca^{2+} changes, after cells were loaded with the Ca^{2+}-sensitive fluorescent probe fluo 3, provided another control for cell viability. We observed that cells that had been submitted to the FRAP protocol (n = 6 cells from 6 clusters from 2 different preparations) showed a typical response pattern to glucose (48) (Fig. 3*B*).

In islet cell clusters cultured for 24 h, no recovery of CF fluorescence was recorded after photobleaching (n = 16 cells from 10 clusters from 4 different preparations), whatever the number of clustered cells (Fig. 4, *A* and *B*). To evaluate whether lack of intercellular exchange of CF reflected alterations of gap junctions resulting from the cell isolation procedure, identical experiments were performed in freshly isolated islets of Langerhans (Fig. 4*C*). No case of fluorescence recovery was observed irrespective of whether islets were freshly isolated (n = 30 cells from 17 islets) or cultured for 24 h (n = 12 from 6 islets; not shown) or whether glucose was switched to different concentrations during fluorescence monitoring (n = 17 from 7 islets; not shown). Results were obtained from at least three different preparations.

Conversely, a consistent fluorescence recovery was recorded when the FRAP procedure was used to test cells in clusters and in intact islets of Langerhans obtained from transgenic mice that were forced to overexpress Cx32 (Fig. 5). Dye passage may take place

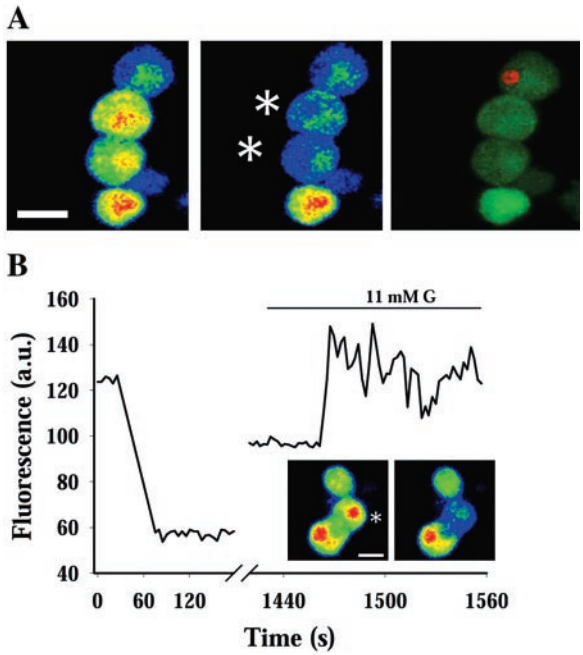

Fig. 3. FRAP did not impair the viability of islet cells. *A*, *left*: cluster of four islet cells was loaded with 5-(and 6)-carboxyfluorescein diacetate [5(6)-CFDA]. *Middle*: 2 of these cells (*) were photobleached during 2 min at maximal laser intensity under experimental conditions similar to those used for testing dye coupling. *Right*: cell viability was then tested by staining the cells with 1 μM ethidium homodimer-1 incubation. Red-stained nuclei were not observed in the 2 photobleached cells, indicating that these cells had remained viable after exposure to the laser beam. As a positive control for the staining, the *top* cell of the cluster shows a red nucleus, indicating that it was damaged. Accordingly, this cell also failed to incorporate and/or retain 5(6)-CFDA (*left*). *B*: glucose-induced Ca^{2+} responses recorded from bleached cells provided another control for cell viability. A group of 3 cells loaded with 5(6)-CFDA is shown before (*left*) and after photobleaching (*right*) of the central cell (*). The plot corresponds to the fluorescence intensity due to CF within the bleached cell. This fluorescence was high at the beginning of the experiment and sharply decreased immediately after photobleaching. A few minutes after this bleaching, cells were loaded with the Ca^{2+}-sensitive fluorescent probe fluo 3-AM for 20 min. Addition of 11 mM glucose (G) to the medium resulted in a typical Ca^{2+} response, as shown by the fluorescence pattern recorded from the bleached cell. Calibration bars, 10 μm.

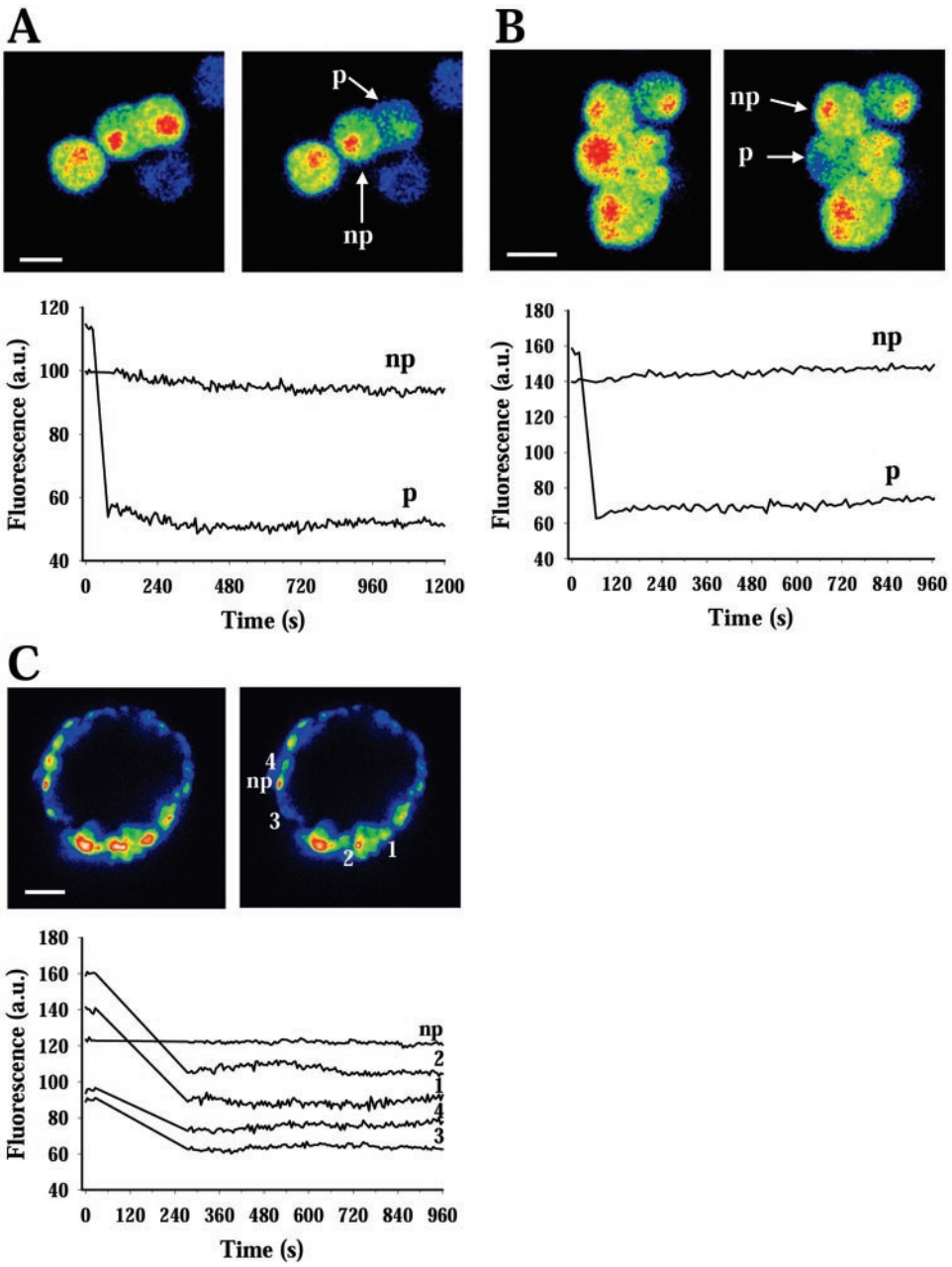

Fig. 4. FRAP did not detect dye coupling between control islet cells. *A*: cluster of 4 islet cells, of which 3 picked up 5(6)-CFDA, before (*left*) and after laser treatment (*right*). When fluorescence levels in both the photobleached cell (p) and a nonphotobleached neighbor (np) were plotted as a function of time, no change in fluorescence intensity was observed in either cell, indicating lack of dye coupling. *B*: when the experiment was repeated in a cluster of 6 cells, the same observations were made. *C*: isolated islet of Langerhans seen in a confocal section (10 μm) before (*left*) and after (*right*) photobleaching of some peripheral cells. Plots of fluorescence intensity as a function of time in several bleached cells (1, 2, 3, and 4) and in a nonbleached cell (np) provided no evidence of dye coupling. Changing the bleaching time led to the same results. Calibration bars, 10 μm for *A* and *B*, and 50 μm for *C*.

between β-cells, given that RIP-Cx32 is specifically expressed in this islet cell population (7, 15), and recent studies indicate an absence of coupling in non-β-cells (12, 31, 36). In these experiments, fluorescence recovery followed an exponential time course with a time constant of 225.9 ± 42.6 s in cell clusters (Fig. 5*A*; $n = 8$ cells from 8 clusters) and 182.3 ± 52.6 s in isolated islets of Langerhans (Fig. 5*B*; $n = 3$ cells from 3 islets). Results were obtained from three different preparations. The donor, nonphotobleached cells exhibited a parallel fluorescence decay, due to probe transfer to the coupled photobleached cell receiving this dye (Fig. 5, *A* and *B*). The intensity of this change, which was also observed in astrocytes, decreased with the increase in the number of clustered cells, presumably because the source of dye was then enlarged by the presence of more donor cells. The fluorescence values of recovery varied among different experiments. It might be attributable to divergences in the number of donor cells that were coupled in each case or to differences in tracer dilution due to diffusion as observed in other cell types (8). At any rate, we demonstrate that, under the same experimental conditions, native islet cells did not exchange CF, whereas transgenic cells with enhanced junctional properties allowed for the passage of the dye.

DISCUSSION

Gap junctions offer permeability to ions and metabolites, providing a mechanism for intercellular communication in multiple cellular systems (19). The system

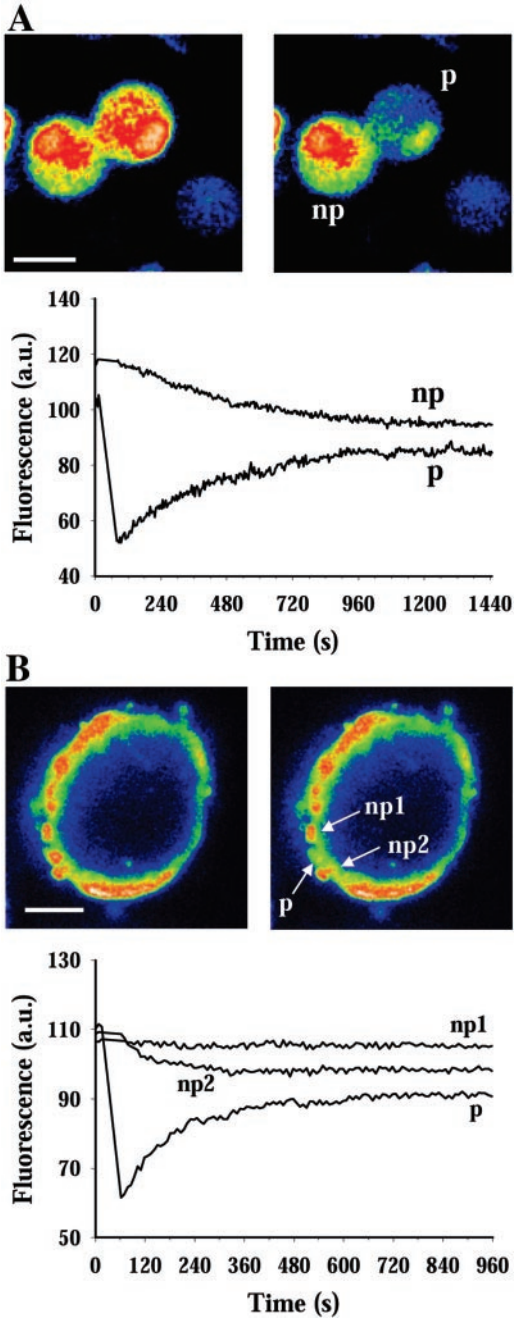

Fig. 5. FRAP detects dye coupling between islet cells of RIP-Cx32 transgenic mice. *A*: 2 cells loaded with 5(6)-CFDA are seen before (*left*) and after laser treatment (*right*) of 1 of them (p). Plotting fluorescence intensity as a function of time revealed a recovery that followed an exponential time course within the photobleached cell (p) and a concurrent decrease in fluorescence within the adjacent, nonbleached cell (np). Calibration bar, 10 μm. *B*: confocal section (10 μm) of an isolated transgenic mouse islet of Langerhans loaded with 5(6)-CFDA is shown in the image before (*left*) and after photobleaching (*right*). The bleached cell indicated in the image (p) exhibited a fluorescence recovery. The fluorescence decay observed in the nonphotobleached cell labeled np2 indicates that this cell may have been a donor coupled cell. Conversely, the lack of fluorescence changes in the nonbleached adjacent cell labeled np1 may indicate a lack of coupling with the bleached one. Calibration bar, 50 μm.

permits electrical and metabolic coupling of cells, affecting several cell functions including secretion (19, 52). Iontophoretic injection of dyes and monitoring of their transfer into neighboring cells have traditionally been utilized to trace molecular exchanges (3, 44). However, when applied to pancreatic β-cells, this technique has led to different conclusions. On the one hand, passage of dyes has been observed between β-cells of monolayer cultures (18, 25) and whole islets of Langerhans (7, 28), in agreement with the intercellular exchange of endogenous tritiated nucleotides and amino acids, which was monitored between the same cells without the use of cell microinjection (5, 22). On the other hand, the technique has failed to provide direct evidence for dye coupling in pairs of electrically coupled islet cells (24, 34). Furthermore, microinjection of dyes into islet cells has supported the idea of dye coupling between different types of endocrine islet cells (25, 28). Although the occurrence of such a coupling is consistent with the observation of typical gap junction plaques (33) between different types of islet cells, it does contrast with recent investigations indicating the absence of calcium signal synchronization and electrical coupling between β- and non-β-cells and between α- or δ-cells (12, 31, 36). Thus it is possible that the microinjection approach led to overestimated domains of dye coupling made of pancreatic β-cells, possibly because of secondary effects of cell injection and/or electrophoresis (20).

One way to bypass these problems is to use an alternative, noninvasive methodology such as the FRAP approach, which allows for the on-line monitoring of gap junction-mediated dye transfer between cells (9, 51). In contrast to microinjection (44), cells are not physically invaded during FRAP, with the exception of the exposure to a laser beam, which, however, can be performed under conditions that prevent major cell injury (9, 51). Instead, dyes available in a membrane-permeant form are allowed to diffuse into cells, where they are retained after enzymatic conversion into an impermeant, fluorescent derivative. Furthermore, and contrasting with microinjection procedures that were often used in combination with fixation of the injected cells for subsequent analysis (7, 28), FRAP combined with confocal sectioning allows for a direct measurement of dye passage in freshly isolated islets or living cultures, providing a real-time estimate of junctional permeability. Eventually, compared with microinjection conditions, which impose the prolonged injection of charged currents with various effects on cells (20, 27, 43), FRAP allows for a simple, diffusion-driven passage of dyes from one cell to another along concentration gradients, permitting experimental conditions that may be closer to those that islet cells face under physiological situations (9). In contrast, the electrical gradients imposed during iontophoresis (20) may affect the diffusion of charged fluorescent dyes. In this study, we have taken advantage of the potential benefits of the FRAP approach to revisit the existence of dye coupling between islet cells. Although earlier studies have sustained the idea of domains of metabolic coop-

eration in islet cells according to the intercellular transfer of several tracers, we show here that molecular exchange is much more constrained than previously reported. Our results suggest that electrical coupling, which is a widespread event in the islet of Langerhans (2, 10, 12, 31, 39), might have a major role in islet synchronization rather than metabolic communication.

We have first shown that FRAP did not affect the viability of islet cells, as judged by retention after photobleaching of both membrane impermeability to a DNA-binding dye and glucose-induced increase in intracellular calcium. We have then observed that the approach allowed for the detection of transfer of the small dye CF between astrocytes, as well as between the islet cells of transgenic RIP-Cx32 mice that show enhanced junctional properties (7). In the latter model, the observations made with the FRAP approach are fully consistent with those previously made using dye microinjection (7), indicating that the latter approach does not, per se, abolish molecular exchanges between islet cells. In contrast, similar experimental conditions did not provide evidence for a molecular exchange between control islet cells that were studied within either monolayer clusters or isolated islets of Langerhans. Electrical coupling in the absence of detectable dye passage has been observed in pairs of pancreatic β-cells (24, 34) and several other systems (29, 37, 40, 47, 49), possibly depending on the connexin isoform linking the cells under study (21). Islet cells express several connexin isoforms, among which Cx36 predominates between β-cells (41). Interestingly, the channels formed by this protein have a smaller unitary conductance than those formed by other mammalian connexins (43, 45). This property is well in agreement with the low junctional conductance recorded from β-cells in pairs (≈215 pS) (24, 34) and in whole islet of Langerhans (≈0.5–1 nS) (1, 2, 12). Conceivably, this small unitary conductance may also restrict the intercellular exchange of some dyes between cells coupled by Cx36 channels. Thus conflicting results about whether Cx36 channels allow dye coupling have been reported in monolayer cultures of HeLa cells transfected to express this protein (43, 47). Here, we provide novel evidence that molecules of the size, electrical charge, and hydrated volume similar to those of CF cannot permeate the channels, most likely made by Cx36 (41), that electrically couple mouse islet cells. The use of other gap junction tracers differing in sizes and electrical charges (9) is now required to assess whether this defect is absolute or provides for the cell-to-cell exchange of only selected types of molecules.

At any rate, the present results suggest that, although electrical coupling is widespread in the islets of Langerhans, dye coupling is more restricted, and possibly much more limited for selected molecules, than what has been previously proposed. The characteristically small conductance of Cx36 channels and the whole conductance provided by gap junctions at β- to β-cell interfaces ensure electrical communication but, at least when studied for short-term periods (15–25 min), restrict the exchange of molecules similar to CF.

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