The relationship between antidopaminergic drugs and glucose has not been extensively studied, even though chronic neuroleptic treatment causes hyperinsulinemia in normal subjects or is associated with diabetes in psychiatric patients. We sought to evaluate dopamine D2 receptor (D2R) participation in pancreatic function. Glucose homeostasis was studied in D2R knockout mice (Drd2−/−) mice and in isolated islets from wild-type and Drd2−/− mice, using different pharmacological tools. Pancreas immunohistochemistry was performed. Drd2−/− male mice exhibited an impairment of insulin response to glucose and high fasting glucose levels and were glucose intolerant. Glucose intolerance resulted from a blunted insulin secretory response, rather than insulin resistance, as shown by glucose-stimulated insulin secretion tests (GSIS) in vivo and in vitro and by a conserved insulin tolerance test in vivo. On the other hand, short-term treatment with cabergoline, a dopamine agonist, resulted in glucose intolerance and decreased insulin response to glucose in wild-type but not in Drd2−/− mice; this effect was partially prevented by haloperidol, a D2R antagonist. In vitro results indicated that GSIS was impaired in islets from Drd2−/− mice and that only in wild-type islets did dopamine inhibit GSIS, an effect that was blocked by a D2R but not a D1R antagonist. Finally, immunohistochemistry showed a diminished pancreatic β-cell mass in Drd2−/− mice and decreased β-cell replication in 2-month-old Drd2−/− mice. Pancreatic D2Rs inhibit glucose-stimulated insulin release. Lack of dopaminergic inhibition throughout development may exert a gradual deteriorating effect on insulin homeostasis, so that eventually glucose intolerance develops. (Endocrinology 151: 1441–1450, 2010)
served (9). Insulin secretion is primarily controlled by metabolism-secretion coupling and is mainly regulated by glucose, but this process can be modulated by the central nervous system through parasympathetic and sympathetic nerves (10).

Dopamine exerts its actions by binding to specific membrane receptors, which belong to the family of seven transmembrane domain G protein-coupled receptors. Five distinct DR have been isolated, characterized, and subdivided into the D1- and D2-like subfamilies, on the basis of their biochemical and pharmacological properties (11). The D1-like subfamily comprises D1R and D5R, whereas the D2-like includes the D2R, D3R, and D4R. Some in vitro studies performed in isolated pancreatic islets suggested the participation of the D2R in insulin secretion (12–14), although the net effect of D2R stimulation observed in these studies and its functional importance remain controversial. In the present study, we sought to investigate the in vivo role of D2R in insulin secretion and glucose homeostasis. To this end, we studied β-cell function and glucose metabolism in mutant mice lacking D2R in comparison with their wild-type siblings. A battery of selective pharmacological tools was used to evaluate the in vivo and in vitro effects of D2R stimulation and blockade on insulin secretion and glucose metabolism in both wild-type and D2R-deficient mice (Drd2−/−). The results presented herein show that even though pancreatic D2R are inhibitory to glucose-stimulated insulin secretion, permanent loss of D2R causes glucose intolerance.

Materials and Methods

Animals

Male Drd2−/− mice, official strain designation B6;129S2-Drd2mtm1low by the Induced Mutant Resource at The Jackson Laboratory (Bar Harbor, ME), generated by targeted mutagenesis of the Drd2 gene in embryonic stem cells (15, 16), were used. The original F2 hybrid strain (129S2/Sv × C57BL/6J) containing the mutated Drd allele was backcrossed for at least 10 generations to wild-type C57BL/6J mice. Mutant and wild-type mice were the product of heterozygote crossings, and in all cases, control siblings were used. Mice of mixed genotypes were housed in groups of four or five in a temperature-controlled room with lights on at 0700 h and lights off at 1900 h and free access to laboratory chow and tap water. Animals were weighed and used at 2 and 7 months of age. We chose these two age groups to determine whether disruption of the D2R had a developmental effect on glucose homeostasis. Furthermore, prolactin and GH levels are similar between genotypes at 2 months of age (17), whereas at 7 months, prolactin levels are markedly increased, and pituitary GH release is decreased (17, 18).

Transgenic mice carrying an engineered bacterial artificial chromosome (BAC) in which coding sequences of the enhanced green fluorescent protein gene (EGFP) are under the transcriptional control of the mouse D2R gene (Drd2) were used to label D2R in pancreatic cells. These Drd2-EGFP mice were originally generated by the GENSAT (Gene Expression Nervous System Atlas) project at the Rockefeller University (New York, NY) and the National Institute of Neurological Disorders and Stroke, National Institutes of Health (Bethesda, MD) (19) and maintained in an outbred Swiss-Webster genetic background.

All experimental procedures were reviewed and approved by the institutional animal care and use committee of the Instituto de Biología y Medicina Experimental, Buenos Aires [in accordance with the Division of Animal Welfare, Office for Protection from Research Risks, National Institutes of Health, (A#5072-01)].

Reagents

Unless otherwise specified, all chemicals were purchased from Sigma (St. Louis, MO).

Intraperitoneal glucose tolerance test (IGTT)

IGTT was performed in conscious male Drd2−/−, wild-type, and Drd2−/− mice at 2 and 7 months of age. Briefly, after overnight fasting (12 h), an ip injection of glucose (2 mg/g body weight) was administered. Blood glucose levels (2 μl obtained from the tail of each mouse) were examined at 0, 15, 30, and 60 min after glucose injection with a hand-held glucose monitor (Ascensia Breeze, Bayer, Toronto, Ontario, Canada).

IGTT was also performed in cabergoline-pretreated mice of both genotypes. To this effect, cabergoline (2 mg/kg ip; Beta Laboratories, Buenos Aires, Argentina) was administered 1 h before IGTT (2 mg/g ip glucose). Glucose levels were measured before glucose administration (time 0) and 30 and 60 min thereafter. Insulin was measured at time 0 and 30 min. A group of animals was pretreated with haloperidol (3 mg/kg, ip) 30 min before cabergoline. The difference between pre- and postglucose levels was calculated as glucose level at 30 or 60 min minus glucose basal level (time 0). The doses of haloperidol and cabergoline were selected from our own previous experience and reports in the literature detailing their effect on DR subtypes (16, 20–22).

Glucose-stimulated insulin secretion (GSIS)

To examine glucose-stimulated insulin secretion, 12-h-fasted mice were used. Blood was collected from the tail vein before (0 min) and 5, 15, and 30 min after glucose administration (3 mg/g). Serum samples were immediately obtained by centrifugation at 3000 rpm for 10 min and stored at −20 C. Insulin secretion levels were assessed by a sensitive rat insulin ELISA kit (Crystal Chem, Chicago, IL).

GSIS was studied on isolated islets from 7-month-old male mice of both genotypes. Hand-picked islets were isolated after an intraductal collagenase V injection, as previously described (23). Pools of five islets were incubated in 250 μl RPMI 1640 medium, supplemented with 10% fetal bovine serum containing 2.8 mM glucose for 2 h, and then incubated with 0, 6.25, 12.5, or 25 mM glucose; 25 mM glucose plus dopamine hydrochloride (10−5 or 10−8 M) or 25 mM glucose plus dopamine hydrochloride (10−5 M) plus the D2R antagonist (+)-sulpiride (10−5 M Vipral; Laboratorios Roemmers, Buenos Aires, Argentina); or 25 mM glucose plus dopamine hydrochloride (10−5 M) plus the D1R antagonist SCH 23390 (1-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5 tetrahydro-1H-3-benzazepine (10−5 M) for 1 h. Insulin secretion levels were assessed by RIA. Insulin output was normalized to
the respective insulin content of the islets. To measure insulin content, tubes were centrifuged for 10 min at 800 × g, the supernatant was discarded, and acid-ethanol was added (250 μl/sample; 87.5% vol/vol ethanol plus 12.5% vol/vol 2 N HCl). Samples were kept overnight at 4 C, centrifuged for 10 min at 800 × g, and the pellet was discarded. Tris base (85 μl, 0.85 M) was added, and samples were stored at −20 C until measurement.

Intraperitoneal insulin tolerance test (ITT)

Mice were fasted for 2 h and then injected ip with human insulin (Humulin 1 U/kg body weight; Eli Lilly, Toronto, Canada). Blood glucose levels were measured at 0, 15, 30, 60, and 120 min.

RIA

Prolactin was measured by RIA using a kit provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Dr. A. F. Parlow, National Hormone and Pituitary Program, Torrance, CA). Results are expressed in terms of mouse prolactin standard RP3. Intra- and interassay coefficients of variation were 7.2 and 12.8%, respectively.

For IGF-I RIA, serum samples (15 μl) and IGF-I standards were subjected to the acid-ethanol cryoprecipitation method as previously described (24). IGF-I was determined using antibody (UB2-495) provided by Drs. L. Underwood and J. J. Van Wyk and distributed by the Hormone Distribution Program of the National Institute of Diabetes and Digestive and Kidney Diseases. Recombinant human IGF-I (Chiron Corp., Emeryville, CA) was used as radioligand and unlabeled ligand. The assay sensitivity was 6 pg/tube. Intra- and interassay coefficients of variation were 8.2 and 14.1%, respectively.

A specific insulin RIA was used as described previously (25) using human insulin for iodination and standard (Beta Laboratories), and anti-bovine insulin antibody (Sigma). The minimum detectable concentration was 2 ng, and the intra- and interassay coefficients of variation were 6.8 and 9.1%, respectively. Pancreatic tissue, 50 mg, was homogenized using a Polytron in 1 ml ice-cold acidic alcohol (0.18 N HCl/70% ethanol), incubated overnight at 4 C, and centrifuged at 12,000 rpm for 5 min. The supernatants were used for determination of insulin concentration by RIA. Results were normalized to the protein content of samples determined by Qubit Quant it protein assay kit (Invitrogen, Buenos Aires, Argentina) following manufacturer’s instructions.

Immunohistochemistry

Pancreata from 2- and 7-month-old animals fixed in formalin were embedded in paraffin, and immunohistochemistry was performed using a modified avidin-biotin peroxidase method as previously described (26). Antibodies for insulin (polyclonal guinea pig antinsulin antibody, 1:200 dilution; Abcam, Cambridge, MA), glucagon (rabbit antiglucagon, 1:200 dilution; Santa Cruz Biotechnologies, Santa Cruz, CA), and prolactin (rabbit antiglucagon, 1:200 dilution; Santa Cruz Biotechnologies, Santa Cruz, CA) were used. Proliferating cell nuclear antigen (PCNA) was visualized using an avidin-biotin kit coupled to alkaline phosphatase (Vector Laboratories, Burlingame, CA), and glucagon and EGFP immunoreactivities were visualized using an avidin-biotin kit coupled to peroxidase (Vector). As chromogens, diaminobenzidine was used for glucagon and EGFP, Vector Blue AP substrate kit III (SK 5300) for PCNA, and Vector Red AP substrate kit I (SK 5100) for insulin. Tissue sections were counterstained with hematoxylin.

Morphometric analysis

Morphometric analysis was performed using a Carl Zeiss transmitted light microscope at a magnification of ×250 and ×400. Image analysis of pancreatic sections for calculation of tissue areas was performed by Northern Eclipse, version 6.0 software (Empix, Imaging, Mississauga, Ontario, Canada). The number of islets (defined as insulin-positive aggregates of at least 20 μm diameter) was scored and used to calculate the islet numerical density (number of islets per square centimeter of tissue). Islets less than 5000 μm² were defined as small, those ranging from 5000 to 10,000 μm² as medium, and those exceeding 10,000 μm² as large. Mean islet size was calculated as the ratio of the total insulin cell area to the total islet number on the sections. The β-cell fraction was determined as the ratio of the insulin-positive cell area to the total tissue area on the entire section. The α-cell area was calculated by subtracting glucagon-positive cell area. The β-cell mass was obtained by multiplying the β-cell fraction by the pancreas weight. The number of cells that showed nuclear staining for PCNA was related to the total β- or α-cell area within the same sections. Data were calculated from three sections of each pancreas, representing the entire pancreas for each animal (head, body, and tail). Approximately 70–120 islets per section were analyzed. Four animals were studied per genotype and age, unless otherwise stated in the figure legends.

Statistical analysis

Plasma glucose, insulin, prolactin, and IGF-I titers and all morphometric data are expressed as means ± SEM. The differences between means were analyzed by the unpaired Student’s t test (in the case of only two groups) or by two-way ANOVA followed by Newman-Keuls test or Tukey’s honestly significant difference test for unequal n. Two-way ANOVA with repeated-measures design was used to analyze GSIS (effects of drug and genotype). P < 0.05 was considered significant.

Results

Body weight was decreased in 7-month-old Drd2−/− male mice compared with their normal wild-type littermates (23.4 ± 0.08 vs. 27.1 ± 0.5 g; P = 0.045). Serum prolactin levels were significantly increased in Drd2−/− vs. wild-type mice (154.1 ± 29.1 vs. 6.0 ± 0.2 ng/ml, P = 0.023), and even though there were no significant differences in circulating levels of GH, IGF-I concentration was lower in Drd2−/− mice (815 ± 61 vs. 1325 ± 89 ng/ml, P = 0.035).

Glucose and insulin responses to glucose overload in vivo in Drd2−/− mice and wild-type littermates

We investigated the impact of the lack of D2R on glucose homeostasis in vivo in male mice. Fasting glucose
levels in 7-month-old Drd2−/− mice were significantly higher than in wild-type mice (127 ± 6 vs. 105 ± 6 mg/dl, P = 0.040; n = 12 and 13, respectively), whereas no significant differences were found in fasting insulin levels (0.07 ± 0.02 vs. 0.12 ± 0.04 ng/ml, P = 0.36; n = 15 and 14). In 2-month-old Drd2−/− mice, glucose response to IGTT was similar to that observed in wild-type mice (Fig. 1A). However, 7-month-old Drd2−/− mice showed relative glucose intolerance; blood glucose levels were significantly higher than those observed in wild-type littermates 15, 30, and 60 min after the ip glucose load (Fig. 1B, P = 0.0012). On the other hand, basal glucose levels and IGTT in 7-month-old heterozygotes (Drd2+/−) were similar to those of wild-type mice (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org), and therefore, the rest of the experiments were performed using only Drd2−/− and wild-type mice.

To examine the insulin secretory response to glucose in vivo, 2- and 7-month-old mice of both genotypes were used after a 12-h fasting period. Serum insulin concentrations were measured before (time 0) and 5, 15, and 30 min after glucose injection (3 mg/g, ip). In wild-type 2-month-old mice plasma insulin concentrations rose 1.75-fold at 15 min, whereas no increase was observed in Drd2−/− mice (Fig. 1C; P = 0.028, Drd2−/− vs. wild-type at 15 min). In 7-month-old wild-type mice, there was a 2.7- and 4.6-fold increase in serum insulin concentration 15 and 30 min after glucose administration, whereas Drd2−/− mice displayed nonsignificant changes in insulin levels at all times [P = 0.049 and 0.020, wild-type vs. time-matched (15 and 30 min) Drd2−/− mice; Fig. 1D].

Adult female Drd2−/− mice also evidenced glucose intolerance and reduced insulin release evoked by glucose (Supplemental Fig. 2). Nevertheless, because time course of glucose elevation was slightly different and cycling pattern of ovarian hormones might modify responses in the wild-type group, we performed the rest of the studies in male mice.

**Glucose and insulin response to IGTT in vivo in both genotypes in the presence of dopamine agonists and antagonists**

We next sought to determine whether short-term administration of the D2-like agonist cabergoline could modify glucose homeostasis in vivo. Cabergoline (2 mg/kg) did not modify blood glucose levels significantly in fasted wild-type or Drd2−/− mice (not shown). Nevertheless, administration of cabergoline caused relative glucose intolerance in wild-type mice (Fig. 2A) but not in Drd2−/− mice (Fig. 2B). Blood glucose levels after an IGTT were increased by pretreatment with cabergoline in wild-type but not in Drd2−/− mice in comparison with saline-pretreated mice (P = 0.042 and 0.00040, cabergoline vs. time matched control at 30 and 60 min, respectively, in wild-type mice). Glucose levels decreased 60 min after glucose overload in Drd2−/− mice, either saline- or cabergoline-treated (P = 0.00038), and in saline-treated wild-type mice (P = 0.014) but remained elevated in wild-type cabergoline-treated mice (P = 0.83, Fig. 2, A and B).

Haloperidol, a dopamine antagonist, partially blocked glucose intolerance evoked by cabergoline at 60 min in wild-type animals; glu-
cose levels decreased at 60 compared with 30 min in wild-type and haloperidol-plus cabergoline-treated and not in cabergoline-treated mice ($P = 0.0014$, 0.0026, and 0.11 for time 60 compared with 30 min in wild-type, haloperidol-plus cabergoline-, and cabergoline-treated mice, respectively; Fig. 2C).

**In vitro insulin response to glucose**

We next investigated insulin secretion in isolated islets from 7-month-old male mice of both genotypes. Glucose (12.5 and 25 mM) increased insulin secretion in islets from wild-type animals ($P = 0.049$ and 0.0011, respectively, vs. control; Fig. 4A) and not in Drd2$^{−/−}$ mice ($P$ interaction for the effects of dose and genotype = 0.032). Insulin release in response to 25 mM glucose was significantly higher in islets obtained from wild-type compared with Drd2$^{−/−}$ mice ($P = 0.015$). Collectively, the *in vivo* and *in vitro* results show that Drd2$^{−/−}$ male mice have an impaired insulin response to glucose stimulation.

**In vitro insulin response to glucose in the presence of dopamine agonists and antagonists**

To determine the presence of D2R in pancreatic islets, we analyzed Drd2-EGFP transgenic mice, which express EGFP under the transcriptional regulation of the Drd2 locus. We detected EGFP immunoreactivity colocalizing with insulin in pancreatic islets of Drd2-EGFP transgenic mice (Fig. 4B) and not in islets from wild-type mice, as expected (Fig. 4C).

We next studied the physiological impact of the absence of functional pancreatic D2R in pancreatic islets *in vitro*. GSIS was compared in islets obtained from mice of both genotypes and in the presence or absence of dopamine ($10^{-5}$ and $10^{-8}$ M). A two-way ANOVA showed significant genotype × drug interaction ($P = 0.0033$). In islets from wild-type mice, glucose increased insulin secretion ($P = 0.0019$), and dopamine prevented this effect ($P = 0.0099$ for glucose vs. glucose plus $10^{-5}$ M dopamine, Fig. 4D). In contrast, neither glucose nor dopamine had any effect on GSIS in islets taken from Drd2$^{−/−}$ mice. Finally, we tested the effect of the D2R antagonist sulpiride or the D1R antagonist SCH 23390 on dopamine inhibition of GSIS in islets obtained from wild-type mice.
As shown in Fig. 4E, sulpiride but not SCH 23390 prevented dopamine inhibition, indicating that this effect is mediated by D2-like receptors (glucose and glucose plus dopamine plus sulpiride were significantly different from the control group; \( P = 0.0091 \) and 0.0025, respectively). Neither dopamine nor sulpiride modified basal glucose release (not shown).

**In vivo insulin action**

To assess the effects of D2R deficiency on insulin action *in vivo*, we measured the changes in plasma glucose concentrations after a single ip injection of insulin. As shown in Fig. 5, A and B, glucose disappearance curves were comparable in both genotypes in 2- and 7-month-old mice. In 7-month-old mice, glucose was measured up to 120 min to evaluate glucose rebound, and no significant differences were found. In females, a similar glucose response to ip insulin was found in both genotypes (Supplemental Fig. 2D).

**Islet and β-cell morphometry in Drd2\(^{-/-}\) and wild-type mice and pancreatic insulin content**

To further examine the impairment of insulin response to glucose observed both *in vivo* and *in vitro* in Drd2\(^{-/-}\)-mice, we performed a morphometric analysis of the pancreata of 7-month-old male mice of both genotypes. As shown in Table 1, the ratio of pancreas weight to body weight was not different between both genotypes, nor was islet density or average islet size. Islet density and distribution according to size were unaltered between genotypes (Fig. 6A). Percentage of β-cell area was not different between genotypes (Fig. 6B), but a significant decrease in absolute β-cell mass in 7-month-old Drd2\(^{-/-}\) males compared with wild-type males was observed (Fig. 6C), which might be related to the tendency to lower pancreas size in Drd2\(^{-/-}\) mice (Table 1). Furthermore, there was a decrease in pancreatic insulin concentration (nanograms per microgram protein) measured by RIA (Table 1). In contrast, we did not detect changes in α-cell mass or insulin to glucagon ratio between genotypes (Table 1).

**Pancreatic β-cell proliferation**

The preceding results suggested that the absence of D2R might be responsible for reduced β-cell mass and impaired insulin response to glucose, accounting for the subsequent impairment in glucose tolerance. To determine whether D2R might participate in the proliferation of β-cells within the islet, we examined costaining of PCNA with insulin or glucagon in pancreatic cell sections obtained from mice of both genotypes at 2 and 7 months of age. As shown in Fig. 7B, insulin cells undergoing replication, measured by PCNA-positive nuclei per given β-cell area, exhibited a 54% reduction in pancreas from 2-month-old Drd2\(^{-/-}\)-mice when compared with those of the wild-type pancreas, suggesting a decrease in β-cell proliferative capacity at this early stage. By 7 months, this decrease in β-cell proliferation was not evident (Fig. 7C). No differences in PCNA-positive cells per α-cell area were observed (not shown). Because at 2 months of age there was already a developmental effect related to the lack of D2R, we included immunohistochemistry data of a younger group (less than 1 month old) and could demonstrate that proliferation of β-cells (measured by the percentage of PCNA-stained nuclei in β-cells) was impaired already at 1 month (Fig. 7A).

**Discussion**

Even though the involvement and importance of dopamine as a neurotransmitter and neuromodulator in the regulation central nervous system function are well known, the effects of dopamine on insulin secretion and pancreatic β-cell function are poorly understood. The par-
TABLE 1. Effects of D2R deficiency on islet density, size, α-cell fraction and mass, insulin to glucagon ratio, and pancreatic insulin concentration in 7-month-old mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Drd2−/−</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas weight (mg)</td>
<td>0.197 ± 0.014</td>
<td>0.156 ± 0.016</td>
<td>0.075</td>
</tr>
<tr>
<td>Pancreas weight/BW</td>
<td>6.5 ± 0.30</td>
<td>7.4 ± 0.39</td>
<td>0.12</td>
</tr>
<tr>
<td>Islet density (n/cm²)</td>
<td>53.2 ± 18.6</td>
<td>45.8 ± 11.3</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean islet size (μm²)</td>
<td>17440 ± 1728</td>
<td>11975 ± 1728</td>
<td>0.38</td>
</tr>
<tr>
<td>α-Cell fraction (%)</td>
<td>0.091 ± 0.024</td>
<td>0.059 ± 0.005</td>
<td>0.26</td>
</tr>
<tr>
<td>Ratio insulin/glucagon area</td>
<td>5.4 ± 0.6</td>
<td>5.0 ± 0.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Pancreas insulin concentration (pg/μg protein)</td>
<td>8.82 ± 1.25</td>
<td>3.77 ± 0.77</td>
<td>0.017</td>
</tr>
</tbody>
</table>

For the first six parameters, n = 6 and 7; for insulin concentration measured by RIA, n = 7 and 5.

participation of the D2R on pancreatic function has been mainly studied using pharmacological agents with limited selectivity in vivo, or in vitro studies that preclude an integrative physiological analysis. To address the problem more directly, we combined the use of mice carrying targeted deletion of the D2R gene and different pharmacological agonists and antagonists, in vivo and in vitro. We report that the D2R play a crucial role in insulin secretion and glucose homeostasis; Drd2−/− male mice exhibit an impairment of insulin response to glucose overload and high fasting blood glucose levels, are glucose intolerant, and possess a reduced β-cell mass at 7 months of age. Decreased glucose tolerance was observed in Drd2−/− mice of both sexes, but we performed most experiments in male mice because cycling sexual hormones in females might influence glucose homeostasis (27, 28).

The reduction in β-cell mass, decreased insulin concentration, and a defect in glucose-sensing or stimulus-secretion coupling in the β-cells in D2R-deficient mice might explain the supra-normal rise in blood glucose levels that is evidenced after a glucose challenge. In turn, this reduction in pancreatic β-cell mass may be due to a decreased replication in earlier ages, as shown by the reduction in PCNA-positive β-cells in 1- and 2-month-old Drd2−/− mice. On the other hand, results of the glucose and insulin tolerance tests suggest that glucose intolerance is mainly caused by a blunted insulin secretory response rather than an increase in peripheral insulin resistance. This was confirmed by GSIS in vivo and in vitro. Nevertheless, the changes in glucose tolerance in Drd2−/− mice were modest; fasting blood sugar levels were 21% higher, and postinjection glucose levels were 22% higher than those in wild-type mice. Furthermore, Drd2−/− mice did not display an intermediate phenotype. In the absence of insulin resistance, it is likely that severe β-cell hypoplasia or dys-function is required to produce severe glucose intolerance or overt diabetes.

In wild-type mice, insulin secretion in response to glucose in vivo was blocked by cabergoline pretreatment. Therefore, to determine the role of pancreatic D2R in the insulin response to glucose, islets from wild-type and Drd2−/− mice were incubated in vitro and subjected to GSIS in the presence or absence of dopamine. Glucose stimulated insulin release only in islets obtained from wild-type mice, and dopamine inhibited this effect. Furthermore, a D2R antagonist but not a D1R antagonist blocked the inhibitory effect of dopamine on insulin secretion from islets of wild-type mice. These results demonstrate that pancreatic D2R inhibit insulin secretion in response to glucose.

A basis for the molecular mechanisms mediating dopamine action on glucose-stimulated insulin secretion in pancreatic β-cells has been revealed. DR were described in INS-1E β-cells as well as in dispersed rat, mouse, and human islets (13). In the present study, we confirmed the presence of D2R in mouse islets using Drd2-EGFP mice. It has been shown that dopamine exerts a differential effect on glucose-induced insulin secretion depending on the concentration used (14). Consequently, some reports documented inhibition (13, 29, 30), whereas others reported an increase of insulin secretion upon acute dopamine accumulation (30, 31). Because D2R agonists inhibit insulin secretion at lower concentrations, at higher concentrations, these agonists may act on other receptors or may not be absolutely specific for a given DR. Our present data demonstrate that even though pancreatic D2R are inhibitory to glucose-stimulated insulin secretion, permanent loss of D2R results in decreased insulin response to glucose in adult mice and that this defect is progressive because glucose intolerance is not observed in young animals. Our results also point to an important defect in glucose sensing or stimulus-secretion coupling in β-cells, because there was a complete lack of responsiveness of islets to glucose both in vivo and in vitro in 7-month-old mice. A possible explanation to reconcile results could be that the lack of D2R-mediated insulin inhibition throughout development in Drd2−/− mice exerts a gradual deteriorating effect on insulin response to glucose, and therefore, at 7 months of age, islets respond poorly to glucose stimulation, as occurs in type 2 diabetes. This hypothesis is consistent with the fact that in diabetes, the prolonged stimulation of β-cells depletes insulin granule stores, and eventually, β-cells become unable to secrete pulses of insulin and become insensitive to changes in glucose concentration (32).
epinephrine and norepinephrine from the adrenal medulla, which leads to the inhibition of insulin secretion in the pancreas (14). On the other hand, it has been reported that glucose-induced electrical activity of β-cells leads to the opening of voltage-gated calcium channels with subsequent Ca\(^{2+}\) influx and Ca\(^{2+}\)-dependent exocytosis of insulin (33) and that dopamine decreases cell membrane depolarization as well as cytosolic Ca\(^{2+}\) entry, and thus insulin secretion evoked by glucose stimulation is blunted (13).

On the other hand, variations in circulating hormones found in Drd2\(-/-\) mice might participate in altered pancreatic function. To begin with, prolactin levels are chronically elevated in Drd2\(-/-\) mice due to the lack of dopaminergic inhibition at the pituitary level (17). The physiological significance of prolactin in pancreatic function is mainly indicated by the increased insulin secretion and islet mass during pregnancy in both rodents and humans (34–36). Prolactin increases β-cell proliferation, insulin gene transcription, and glucose-dependent insulin secretion in isolated pancreatic islets and rat insulinoma cells (34–38). Moreover, in rats with chronic hyperprolactinemia, there is an increase in glucose and insulin concentrations after glucose overload. Men and women with chronic hyperprolactinemia have postprandial hyperinsulinemia and an exaggerated insulin secretory response to glucose (39–41). In addition, prolactin receptor deficiency is accompanied by islet and β-cell hypoplasia, reduced pancreatic insulin, and blunted insulin secretory response to glucose (42). Therefore, our present results are not consistent with a main effect of the hyperprolactinemic state on the pancreatic phenotype described, because hyperprolactinemia per se would evoke the opposite results in β-cell mass and insulin secretion to those we describe in the Drd2\(-/-\) mouse.

On the other hand, GH and serum IGF-I are decreased in Drd2\(-/-\) mice. Both hormones promote islet cell proliferation and increase insulin gene transcription and insulin secretion (43–46). Studies using animal models such as dwarf rats and GH receptor knockout mice show low glucose and insulin concentrations concordant with a state of insulin hypersensitivity (47). In humans, GH-deficient subjects have long been recognized as exhibiting increased insulin sensitivity, decreased insulin secretion, and hypoglycemia (48, 49), even though some reports observed insulin resistance in GH-deficient subjects (50, 51). Therefore, decreased β-cell mass and insulin secretion may be in part related to low GH action found in the Drd2\(-/-\) mice. Nevertheless, the fact that we did not find increased peripheral sensitivity to insulin or decreased basal glucose levels, which are generally present in animal models with low GH, and our in vitro results highlight the importance of pancreatic D2R in the phenotype described.

D2R deficiency on other tissues, particularly hypothalamus, may be influencing the phenotype. To this regard, it has been shown that bromocriptine improves glycemic control and glucose tolerance in obese type 2 diabetic patients (52) and in nondiabetic obese animals and humans and that bromocriptine can reverse many of the metabolic alterations associated with obesity by resetting central (hypothalamic) circadian organization of monoamine neuronal activity. Furthermore, glucose intolerance and insulin resistance result from decreased dopaminergic input to the area of the suprachiasmatic nucleus in animal models (53). Recently, bromocriptine was approved by the U.S. Food and Drug Administration for the treatment of type 2 diabetes. The drug appears to employ central mechanisms in ameliorating hyperglycemia. Nevertheless, our study strongly suggests that pancreatic D2R could also participate in the effect of dopamine agonists. We have recently studied mice lacking neural D2R (nDrd2\(-/-\)) generated by us (54) and found that glucose and insulin responses at 30 min after a glucose overload of 3 mg/g were similar to those of wild-type mice (unpublished results). These results further indicate that the insulin release impairment

**FIG. 7.** PCNA-positive β-cells. Number of PCNA-positive cells per insulin immunoreactive area (in square micrometers) in pancreatic sections from mice of both genotypes at 1 (A), 2 (B), and 7 (C) months of age. Three different pancreas sections from each animal (n = 3 animals) were immunostained for insulin and PCNA and subjected to morphometric analysis. *P < 0.05 vs. age-matched wild type.
found in the Drd2<sup>−/−</sup> mice was not mainly dependent on the lack of D2R in the central nervous system.

Our results are relevant in the analysis of some clinical findings such as the effect of chronic treatment with antipsychotic medications that can induce abnormalities in glucose metabolism that increase risk for cardiovascular disease and diabetes (55–57) or the altered glucose tolerance associated with prolonged treatment with atypical antipsychotics in humans (5) and in animal models (58). Furthermore, older diabetics who take antipsychotic medications have an increased risk of ending up in the hospital with elevated blood glucose levels, or hyperglycemia (59). These data together with our present results clearly demonstrate that D2R are modulators of insulin secretion.

We conclude that mice lacking D2R display an impaired glucose metabolism and that pancreatic islet D2R are involved in this effect. Our finding that the D2R plays an essential role in β-cell proliferation and insulin secretion adds a novel participant to the list of growth factors and hormones that control the fundamental and multifactorial process of glucose homeostasis. It is important to consider that a combination of defects, hormonal and genetic, might aggravate the metabolic dysfunction that accompanies an isolated signaling defect. Furthermore, this study constitutes a contribution to unraveling glucose intolerance found after prolonged treatments with neuroleptic drugs.

Acknowledgments

We thank the National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Pituitary Program and Dr. A. F. Parlow for prolactin and GH and prolactin RIA kits as well as the IGF-I antiserum. Dr. A. F. Parlow for prolactin and GH and prolactin RIA kits as well as the IGF-I antiserum.

We thank the National Institute of Diabetes and Digestive and Kidney Institutes of Health Research.

Disclosure Summary: The authors have nothing to disclose.

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