Effects of acute $\alpha_2$-blockade on insulin action and secretion in humans

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Effects of acute $\alpha_2$-blockade on insulin action and secretion in humans. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E57–E64, 1998.—We tested whether acute $\alpha_2$-blockade affects insulin secretion, glucose and fat metabolism, thermogenesis, and hemodynamics in humans. During a 5-h epinephrine infusion (50 ng·min$^{-1}$·kg$^{-1}$) in five volunteers, deriglidole, a selective $\alpha_2$-receptor inhibitor, led to a more sustained rise in plasma insulin and C-peptide levels ($+59 \pm 14 \text{ vs. } +28 \pm 6$, and $+273 \pm 18 \text{ vs. } +53 \pm 14 \text{ pmol/L}$, $P < 0.01 \text{ vs. placebo}$) despite a smaller rise in plasma glucose ($+0.90 \pm 0.4 \text{ vs. } +1.5 \pm 0.3$ mM, $P < 0.01$). Another 10 subjects were studied in the postabsorptive state and during a 4-h hyperglycemic (+4 mM clamp, coupled with the ingestion of 75 g of glucose at 2 h). In the postabsorptive state, hepatic glucose production, resting energy expenditure, and plasma insulin, free fatty acid (FFA), and potassium concentrations were not affected by acute $\alpha_2$-blockade. Hyperglycemia elicited a biphasic rise in plasma insulin (to a peak of $140 \pm 24$ pmol/L), C-peptide levels ($1,520 \pm 344$ pmol/L), and insulin secretion (to $410 \pm 22$ pmol/min); superimposed glucose ingestion elicited a further twofold rise in insulin and C-peptide levels, and insulin secretion. However, $\alpha_2$-blockade failed to change these secretory responses. Fasting blood $\beta$-hydroxybutyrate and glycerol and plasma FFA and potassium concentrations all declined with hyperglycemia; time course and extent of these changes were not affected by $\alpha_2$-blockade. Resting energy expenditure (+25 vs. +16%, $P < 0.01$) and external cardiac work (+28% vs. +19%, $P < 0.01$) showed larger increments after $\alpha_2$-blockade. We conclude that acute $\alpha_2$-blockade in humans 1) prevents epinephrine-induced inhibition of insulin secretion, 2) does not potentiate basal or intravenous-or oral glucose-induced insulin release, 3) enhances thermogenesis, and 4) increases cardiac work.

$\alpha_2$-adrenoceptors; thermogenesis

ANIMAL AND IN VITRO STUDIES have provided clear evidence that postsynaptic $\alpha_2$-adrenoceptors are present on pancreatic $\beta$-cells (30) and that their selective stimulation inhibits glucose-induced insulin release (17). In humans, the pioneer studies of Robertson and Porte (28) showed that in the postabsorptive state phentolamine, an $\alpha$-receptor antagonist, increased while propranolol, a nonselective $\beta$-blocker, inhibited insulin release. In addition, phentolamine potentiated and propranolol depressed insulin release in response to epinephrine-induced hyperglycemia. Normal subjects and particularly diabetic patients were found to have improved insulin secretion in response to intravenous glucose pulses when $\alpha$-adrenoceptors were blocked with phentolamine (27). Since then, the experimental work in this area in humans has been limited and controversial. Basal insulin secretion and the insulin response to arginine under hyperglycemic conditions were found to be enhanced by $\alpha$-blockade in diabetic but not in nondiabetic individuals (24). In contrast, selective $\alpha_2$-blockade with midazolide in healthy humans was found to lower both fasting plasma glucose and the glucose response to a mixed meal, although the effect on peripheral insulin concentrations was negligible (15). More recently, the role of $\alpha_2$-adrenergic tone on insulin secretion has been reported to be null (25) or limited to maximal secretory capacity (24). In the aggregate, it is still unclear whether a physiologically relevant $\alpha_2$-receptor tone is present in human $\beta$-cells in the postabsorptive state, nor is it clear whether this adrenergic tone is modulated by hyperglycemia.

The interactions of the sympathetic nervous system with glucose metabolism in humans are complex. Insulin sensitivity and glucose-induced thermogenesis are only marginally (~15%) reduced by acute $\beta$-receptor blockade, whereas acute $\alpha$-blockade with phentolamine is without effect on either (9). In postabsorptive humans, exogenous epinephrine (a potent $\alpha$- and $\beta$-agonist) infusion causes hyperglycemia through both increased hepatic glucose production and reduced glucose clearance. The former action is transient and biphasic (i.e., an initial acceleration of liver glycogenolysis is followed by enhanced gluconeogenesis) and is entirely explained by liver $\beta$-adrenoceptor stimulation (32). The reduced glucose clearance is secondary to the limitation of insulin secretion but also to a peripheral effect, which is limited to the insulin-dependent pathways (18) (noninsulin-mediated glucose uptake being unaffected; Ref. 4), and is probably mediated by $\beta_3$-receptors (19). Information on the effect of norepinephrine (whose action is mediated mainly by $\alpha$-receptors) in humans is scarce. In the fasting state, norepinephrine infusion, reproducing circulating levels similar to those attained during moderate stress, produces a transient stimulation of hepatic glucose output without altering fasting glucose clearance (31). In the insulinized state, norepinephrine appears to reduce glucose tolerance and insulin-stimulated glucose uptake leaving insulin secretion unaltered (21). In contrast to this finding, more recent (3) studies have shown that norepinephrine infusion increases both basal and insulin-mediated whole body glucose uptake. The interplay between insulin action and the adrenergic system is
further complicated by the observation that glucose ingestion as well as exogenous insulin infusion activates the sympathetic nervous system (particularly noradrenergic arm; Refs. 5, 29), establishing a physiological feedback system.

Although the relevance of all these interactions is clearer under stress conditions, when the sympathetic nervous system is strongly activated, it is not known whether α2-adrenoceptor activity interferes with thermogenesis or glucose and/or fat metabolism under more physiological conditions. The aim of the present study was to test in healthy subjects the effects of a selective removal of α2-receptor tone (using deriglidole, a selective α2-blocker) on insulin secretion, glucose utilization, energy expenditure, and hemodynamics in the postabsorptive as well as the insulinized state. Because the efficacy of deriglidole has been directly tested only in vitro and animal studies, we carried out a preliminary series of studies to verify that the drug, at the dosage we planned to use, reaches α2-adrenoceptors on pancreatic β-cell and prevents the inhibitory action of epinephrine on insulin secretion.

METHODS

Subjects

Informed written consent was obtained from 15 healthy male volunteers aged 20–32 yr. All subjects were within 10% of their ideal body weight and had a body mass index (BMI) ranging between 22 and 25 kg/m2. None of them had a positive family history of diabetes mellitus or essential hypertension. Before entering the study, each subject completed an extensive clinical workup (history, physical examination, blood chemistry, urinalysis, and electrocardiogram) to exclude concomitant diseases or diet and/or drug treatment. All subjects were requested to maintain their habitual lifestyle and diet for the duration of the study and to avoid strenuous physical exercise on the day before each test.

Experimental Protocol

Each subject was studied twice, 1 wk apart. After a double-blind design, subjects were randomly assigned either to the active treatment [deriglidole: (+)-1,2,4,5-tetrahydro-2-propylpyrrol[3,2,1-hi]-indole hydrochloride, manufactured by Synthélabo Recherche Laboratories, Paris, France] or placebo. Subjects were admitted as outpatients to the Metabolic Unit between 7:00 and 8:00 AM after an overnight fast (11–12 h). For the entire duration of the study, the subjects rested supine in a quiet, air-conditioned room. An antecubital vein (for infusion of test substances) and a wrist vein (for blood sampling) were cannulated, and the hand was then inserted into a heated box (70°C) to achieve arterialization of venous blood.

Study 1. Five subjects participated in the study. Thirty minutes after catheter placement, a 30-min baseline period started during which blood samples were obtained for substrate [glucose, glycerol, free fatty acids (FFA)] and hormone (plasma glucose, insulin, and C-peptide) determination. At 0 min, epinephrine (ISM, Milan, Italy) was infused at a constant rate (50 ng·min⁻¹·kg⁻¹) for the following 300 min. Twice during the study, at 0 and 180 min, subjects ingested a tablet of either deriglidole (15 mg) or placebo. Blood pressure and heart rate were measured hourly. Blood samples for glucose determination were obtained every 10 min for the 1st h and every 30 min thereafter. Plasma insulin, FFA, and potassium were determined at 30-min intervals.

Study 2. After catheter placement, subjects (n = 10) were asked to void, and thereafter a primed (30 μCi) constant (0.27 μCi/min) infusion of [3-H]glucose (NEN, Boston, MA) was started and continued for 3 h (from −120 min to 60 min). At −60 min, the subject’s head was placed in a ventilated Plexiglas hood, and gas exchange was measured by using a computerized, continuous, open-circuit system (Metabolic Measurement Cart Horizon, Sensor Medics, Anaheim, CA). Twice during the study, at 0 and 180 min, subjects ingested a tablet of either deriglidole (15 mg) or placebo. Three baseline blood samples (at −10, −5, and 0 min) were collected for the determination of plasma C-peptide, insulin, FFA, potassium, and glucose specific activity. The same assays were done on subsequent blood samples taken at 10-min intervals until 60 min. At this time, the [3-H]glucose infusion was stopped, the subjects voided, and a hyperglycemic clamp (raising plasma glucose 4.0 mM above fasting value) was then started following the method of DeFronzo et al. (10). Plasma glucose was measured every 5 min, and the frequency of blood sampling for plasma insulin and C-peptide was as depicted in the Fig. 3. At 180 min (after 2 h of hyperglycemia), urine was again collected; subjects then drank 150 ml of a 50% glucose-water solution and ingested a second tablet (15 mg of deriglidole or placebo). For the following 2 h (until 300 min), plasma glucose was maintained at +4.0 mM above baseline by modifying the exogenous glucose infusion rate according to the observed plasma glucose changes. Plasma FFA and potassium, blood β-hydroxybutyrate, and glycerol were measured every 30 min throughout. A third urinary collection was made at the end of the study. Gas exchange was also measured during the last 60 min of the hyperglycemic clamp (120–180 min) and the final 60 min after glucose ingestion (240–300 min). Arterial blood pressure, with a standard mercury sphygmomanometer, and heart rate were measured every 20 min.

Analytical Procedures

Plasma glucose was assayed by the glucose oxidase method (Glucose Analyzer, Beckman Instruments, Fullerton, CA). Plasma potassium was assayed in duplicate immediately after blood drawing by an ion-selective electrode method (Microlytes 6 Selective Ion Analyzer, Kone Instruments, Espoo, Finland). Plasma FFA were determined by an enzymatic method (Wako Chemical, Neuss, Germany). For determination of glycerol and β-hydroxybutyrate concentrations, blood was drawn into chilled tubes containing 1 mM perchloric acid (1:1 wt/wt) and immediately centrifuged; the deproteinized supernatants were stored at −70°C and assayed within 3 mo, in duplicate, by an automated spectrophotometric method (Eris Analyzer 6170, Eppendorf Geratebau, Hamburg, Germany). Plasma insulin and C-peptide were determined, in duplicate, by radioimmunoaassay (INSK 5 and C-Peptide, Sorin Biomedica, Vercelli, Italy). All samples from the same subject were assayed in the same run, with each placebo time point followed by its deriglidole pair. The technicians performing the assays were blind to the experimental design. Plasma glucose specific activity was measured in arterialized blood samples deproteinized with barium hydroxide and zinc sulfate. Nonprotein urinary nitrogen was measured by the Kjeldhal method. Plasma catecholamine concentrations were measured by high-performance liquid chromatography (HPLC; Catecholamine Analyzer, HLC-725CA, Thoso, Tokyo, Japan). Plasma deriglidole concentrations were assayed on timed blood samples (baseline and 1, 3,
and 5 h) by HPLC on a C8 column with ultraviolet detection after liquid-liquid extraction.

Data Analysis

Glucose utilization (M) values were calculated according to DeFronzo et al. (10). Protein oxidation was estimated from the nonprotein urinary nitrogen excretion rate over the three collection periods (~20 to 80, 80–180, and 180–240 min). Net carbohydrate and lipid oxidation rates were computed from oxygen consumption (V\textsubscript{O2}) and carbon dioxide production (V\textsubscript{CO2}) using standard calorimetric equations. Basal glucose rate of appearance (Ra) was calculated as the ratio of \([\text{3H}]\)glucose infusion rate to steady-state plasma [\text{3H}]glucose specific activity. After the ingestion of the first capsule, Ra was calculated with the use of the two-compartment model described in detail elsewhere (22). Insulin secretion rate was estimated from plasma C-peptide by deconvolution analysis and linear regularization as described in detail elsewhere (7). C-peptide kinetics was assumed to be biexponential, and the exponential parameters were taken to be equal to the mean values, as validated by Van Cauter et al. (33). All data are expressed as means ± SE. To test the statistical significance of time-related changes (time effect), differences between placebo and treatment studies (treatment effect), and differences related to the time × treatment interaction (interaction), a two-way analysis of variance (ANOVA) with doubly repeated measures was used on the entire data base or on specified time periods.

RESULTS

Study 1

Basal plasma epinephrine (average of ~60- and 0-min values) was similar in the placebo and deriglidole studies (43 ± 4 vs. 31 ± 6 pg/ml). Epinephrine infusion produced a plateau increase in plasma epinephrine levels that was similar in the two sets of experiments (1,205 ± 162 and 1,294 ± 188 pg/ml in placebo and deriglidole study, respectively). In contrast, \(\alpha\)-blockade was associated with a rise in plasma norepinephrine (from 144 ± 28 to 382 ± 110 pg/ml, \(P < 0.05\)), whereas baseline values were maintained during placebo administration (basal: 152 ± 19, test: 183 ± 33 pg/ml, \(P = NS\)). Before epinephrine infusion, both systolic (115 ± 4 vs. 119 ± 8 mmHg) and diastolic (74 ± 5 vs. 73 ± 5 mmHg) blood pressure and heart rate (66 ± 4 vs. 71 ± 6 beats/min) values were similar in the placebo and deriglidole study. Epinephrine infusion raised systolic blood pressure slightly but significantly in both studies (+9 ± 2 vs. +10 ± 2 mmHg, placebo vs. control, \(P < 0.05\) by ANOVA for time effect), whereas diastolic blood pressure did not change. Heart rate increased in response to epinephrine, the increase being higher during the deriglidole study (+2.5 ± 1.0 vs. +5.2 ± 1.4 beats/min, \(P < 0.05\) by ANOVA for treatment effect).

At baseline, plasma glucose, insulin, and C-peptide concentrations were similar in the two experiments (Fig. 1). In the placebo study, plasma glucose gradually rose and reached a plateau at 1 h that was maintained for the following 4 h. This prolonged hyperglycemia induced only a minor increase in plasma insulin levels, averaging +28 ± 6 pM over 300 min. Deriglidole administration was associated with a blunted and more transient rise in plasma glucose levels (\(P < 0.05\) by ANOVA for treatment and time × treatment effect), which returned to baseline at the end of the study (Fig. 1). This glycemic excursion was associated with plasma insulin levels that were twice as high as in the placebo study (\(P < 0.05\) by ANOVA). When the efficiency of insulin secretion was estimated as the ratio of the incremental (above baseline) insulin area to the incremental glucose area, deriglidole was associated with an eightfold increase in insulin release compared with placebo (116 ± 44 vs. 15 ± 2 pmol/mmol, \(P < 0.02\) by paired t-test). Over 300 min, the excess of insulin secreted in response to deriglidole (as calculated by deconvolution analysis of plasma C-peptide) averaged 13.5 ± 3.6 nmol (1.9 ± 0.5 U). Fasting plasma FFA levels were similar in the two studies (Fig. 2). With placebo, the infusion of epinephrine elicited a sharp

![Fig. 1. Study 1. Time course of plasma glucose, insulin, and C-peptide concentrations and insulin secretion rate before and during intravenous epinephrine infusion in placebo (dotted lines) and \(\alpha\)-blockade (solid lines) study.](http://ajpendo.physiology.org/Downloadedfromhttp://ajpendo.physiology.org/10.220334onOctober14,2017)
150% rise of plasma FFA, followed by a rapid decline to values still 50% above baseline for the remaining 200 min. Deriglidole administration did not prevent the early rise but was associated with a greater decline of plasma FFA, which returned to baseline levels at 120 min (P < 0.01 by ANOVA for interaction time × treatment). As shown in Fig. 2, plasma potassium declined in response to epinephrine infusion, and the gradient tended to be more pronounced after deriglidole administration (P = 0.08 by ANOVA).

Study 2

Deriglidole. Plasma deriglidole concentrations reached 7.1 ± 2.5 ng/ml 60 min after the first capsule ingestion and averaged 6.5 ± 1.4 ng/ml at 180 min, immediately before the second capsule was ingested. A further rise, to 11.2 ± 4.2 ng/ml, was observed at 300 min (2 h after ingestion of 2nd capsule). In both series of studies, no deriglidole was detectable in plasma at baseline and after placebo.

Glucose, insulin, and C-peptide. The time course of glucose, insulin, and C-peptide concentrations is shown in Fig. 3. Basal plasma glucose was superimposable on the occasion of the two studies and did not change during the 60 min after either deriglidole or placebo ingestion. During the hyperglycemic clamp, plasma glucose reached a stable level within 40 min of glucose infusion. The mean increment during the 2nd h of the clamp was 3.8 ± 0.1 mM in the placebo study and 3.7 ± 0.1 mM in the active drug study (P = NS by paired t-test). After glucose ingestion at 180 min, plasma glucose was maintained relatively stable on both occasions, the intraindividual mean coefficients of variation for the entire hyperglycemic period being 6.8 ± 0.8% and 6.9 ± 0.9% (control vs. α₂-blockade). No statistically significant difference in plasma glucose between treatment and placebo was observed in any period of the study (P = NS).

In the placebo study, basal plasma insulin (38 ± 5 pM) remained stable until 60 min. A peak value of 153 ± 19 pM was reached within 4 min of starting the glucose infusion; thereafter, insulin rose almost linearly until 180 min. Superimposed glucose ingestion elicited a further 2.5-fold rise in insulin levels, followed by a stable plateau. No difference between α₂-blockade and placebo was observed in the overall data set or during any time block (P > 0.05 for treatment and for time × treatment interaction by ANOVA). Plasma C-peptide concentrations showed a pattern of changes that was completely superimposable on that of plasma insulin levels throughout the study. Insulin secretion (Fig. 3) showed no significant increase during the 1st h,
rose biphasically in response to intravenous glucose, and doubled after glucose ingestion in the face of unchanged hyperglycemia. No consistent difference was observed between placebo and α₂-blockade experiments.

Metabolites. Baseline plasma FFA (0.443 ± 0.036 vs. 0.478 ± 0.069 mM, placebo vs. treatment), blood β-hydroxybutyrate (0.107 ± 0.020 vs. 0.162 ± 0.046 mM), and glycerol (0.042 ± 0.007 vs. 0.055 ± 0.008 mM) concentrations were similar on the two occasions and did not change during the 60 min after ingestion of the first capsule. Hyperglycemia produced a marked decrease in plasma FFA, blood β-hydroxybutyrate, and glycerol concentrations (to nadirs of 0.083 ± 0.013, 0.020 ± 0.003, and 0.019 ± 0.005 mM, respectively). These metabolites showed similar profiles during the treatment and placebo study.

Potassium. Baseline plasma potassium levels (3.93 ± 0.05 vs. 3.93 ± 0.05 mM) were superimposable in the two studies, were unchanged after the first capsule, and showed a similar 10% decline during the hyperglycemic clamp (to 3.67 ± 0.06 vs. 3.66 ± 0.05 mM, placebo vs. treatment, respectively); a further 4% decline (to 3.55 ± 0.06 vs. 3.50 ± 0.06 mM, respectively) was observed after glucose ingestion similarly in the derigilode and placebo studies.

Glucose disposal. Basal plasma [³H]glucose specific activities had a mean coefficient of variation of 3.4 ± 0.4% and averaged 3,894 (P < 0.05 vs. placebo) during the hyperglycemic clamp, the first capsule was associated with no significant changes, and further rose biphasically in response to intravenous glucose, and doubled after glucose ingestion in the face of unchanged hyperglycemia. No consistent difference was observed between placebo and α₂-blockade experiments.

Table 1. Study 2: indirect calorimetry data

<table>
<thead>
<tr>
<th>Treatment (T)</th>
<th>Basal</th>
<th>60</th>
<th>180</th>
<th>300</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>VₐO₂, ml/min</td>
<td>Placebo</td>
<td>271 ± 16</td>
<td>270 ± 15</td>
<td>276 ± 15</td>
<td>307 ± 17</td>
</tr>
<tr>
<td>VₐCO₂, ml/min</td>
<td>α₂-Block</td>
<td>274 ± 15</td>
<td>275 ± 15</td>
<td>287 ± 17</td>
<td>329 ± 21</td>
</tr>
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<td>RQ</td>
<td>Placebo</td>
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<td>226 ± 12</td>
<td>244 ± 14</td>
<td>301 ± 18</td>
</tr>
<tr>
<td>Nitrogen ex, mg/min</td>
<td>α₂-Block</td>
<td>233 ± 12</td>
<td>232 ± 13</td>
<td>259 ± 13</td>
<td>332 ± 21</td>
</tr>
<tr>
<td>Glucose ox, µmol·kg⁻¹·min⁻¹</td>
<td>Placebo</td>
<td>0.0 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>0.88 ± 0.01</td>
<td>0.99 ± 0.02</td>
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<tr>
<td>Lipid ox, µmol·kg⁻¹·min⁻¹</td>
<td>α₂-Block</td>
<td>0.8 ± 0.01</td>
<td>0.85 ± 0.02</td>
<td>0.91 ± 0.01</td>
<td>1.01 ± 0.04</td>
</tr>
<tr>
<td>Protein ox, µmol·kg⁻¹·min⁻¹</td>
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<td>9.9 ± 1.1</td>
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<td>10.1 ± 1.4</td>
<td>11.1 ± 1.3</td>
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<tr>
<td>Energy exp, J·kg⁻¹·min⁻¹</td>
<td>α₂-Block</td>
<td>10.0 ± 1.4</td>
<td>9.7 ± 1.2</td>
<td>11.1 ± 1.3</td>
<td>11.1 ± 1.3</td>
</tr>
</tbody>
</table>

Data are given as means ± SE. VₐO₂ and VₐCO₂ were similar in the two sets of experiments and were not affected by the first capsule (0–60 min). During intravenous glucose alone, both VₐO₂ and VₐCO₂ increased slightly, though not significantly. In contrast, glucose ingestion was followed by a 13% rise in VₐO₂ and a 34% increase in VₐCO₂ (P < 0.01 for both), so that the calculated respiratory quotient (RQ) increased to values very close to 1. α₂-Blockade resulted in higher increments in both VₐO₂ (+20%) and VₐCO₂ (+43%) (P < 0.01 for both treatment effects by ANOVA) and a similar increase in RQ. Carbohydrate oxidation was significantly stimulated and lipid oxidation suppressed during hyperglycemia, but there was no systematic difference between α₂-blockade and placebo. On the other hand, glucose ingestion was associated with a marked stimulation in thermogenesis (i.e., a 16% increase in energy expenditure, P < 0.01), which was significantly enhanced (+25%, P < 0.01 vs. placebo) by α₂-blockade.

Gas exchange and indirect calorimetry. Gas exchange and indirect calorimetry values are given in Table 1. Baseline VₐO₂ and VₐCO₂ were similar in the two sets of experiments and were not affected by the first capsule (0–60 min). During intravenous glucose alone, both VₐO₂ and VₐCO₂ increased slightly, though not significantly. In contrast, glucose ingestion was followed by a 13% rise in VₐO₂ and a 34% increase in VₐCO₂ (P < 0.01 for both), so that the calculated respiratory quotient (RQ) increased to values very close to 1. α₂-Blockade resulted in higher increments in both VₐO₂ (+20%) and VₐCO₂ (+43%) (P < 0.01 for both treatment effects by ANOVA) and a similar increase in RQ. Carbohydrate oxidation was significantly stimulated and lipid oxidation suppressed during hyperglycemia, but there was no systematic difference between α₂-blockade and placebo. On the other hand, glucose ingestion was associated with a marked stimulation in thermogenesis (i.e., a 16% increase in energy expenditure, P < 0.01), which was significantly enhanced (+25%, P < 0.01 vs. placebo) by α₂-blockade.

Hemodynamics. During the placebo study, systolic and diastolic blood pressure and heart rate remained unchanged until 60 min (Fig. 4). Subsequently, there was a gradual increase in both systolic blood pressure and heart rate (P < 0.01 for time effect by ANOVA), whereas diastolic blood pressure fell by 5% (P < 0.01). These changes account for the 18% increase from basal values in the double product (systolic blood pressure × heart rate).
DISCUSSION

Deriglidole is a peripheral adrenoceptor antagonist with a high affinity for $\alpha_2$-adrenoceptors. It inhibits $[3H]$clonidine and $[3H]$idazoxan but not $[3H]$prazosin binding to rat cortical and human platelet $\alpha_2$-adrenoceptors and is more selective than idazoxan for peripheral receptors (1). Because its $\alpha_2$-antagonistic activity on pancreatic adrenergic receptors in humans has not been previously assessed, we carried out preliminary experiments to document the ability of deriglidole to prevent the inhibitory effect of epinephrine specifically on insulin secretion. Our results of study 1 confirm that epinephrine infusion, at doses that raise plasma epinephrine to levels typically encountered during severe stress, causes stable hyperglycemia, which is known to result from transient stimulation of hepatic glucose production, reduction of peripheral glucose clearance, and restraint of insulin secretion. Although the first two actions are mediated by $\beta_2$-adrenoceptors (19), the latter is largely dependent on $\alpha_2$-receptor activation (26). The time course of plasma glucose, insulin, C-peptide, and insulin secretion rate during these experiments (Fig. 1) unequivocally indicates that insulin secretion was improved by deriglidole administration. Furthermore, the relative hyperinsulinemia observed during $\alpha_2$-blockade ($+30\, \text{pM}$), though small in absolute terms, was enough to counteract also the lipolytic activity of epinephrine (Fig. 2), confirming the exquisite sensitivity of lipolysis to minor changes in insulin levels in normal subjects. In addition, deriglidole prevented the epinephrine-induced inhibition of insulin secretion despite the associated (and expected; Refs. 6, 24) increase in plasma norepinephrine concentrations, which per se might have enhanced the $\alpha_2$-inhibitory tone on $\beta$-cells. Whether the effect of deriglidole on insulin secretion is solely determined by the removal of excess $\alpha_2$-Receptor tone or is also dependent on a direct activation of ATP-dependent potassium channels through a class-specific (imidazoline) receptor (16, 30), as suggested by preliminary evidence (14), is not known. The latter possibility, however, is not supported by the results of the current study 2 experiments, in which deriglidole alone (i.e., in absence of excess epinephrine) was without effect on insulin secretion.

The second series of experiments was designed to determine the effects of acute $\alpha_2$-blockade on basal insulin secretion (0–60 min), glucose-induced insulin secretion (60–180 min), and gastrointestinal potentiation of glucose-induced insulin secretion (180–300 min). Although stimulated insulin secretion is closely reflected in circulating insulin levels, inferring changes in basal insulin secretion from measurements of peripheral plasma insulin concentrations may be insufficiently sensitive. Minor changes in portal insulin concentrations that are sufficient to exert metabolic actions on the liver may not be associated with detectable changes in the systemic hormone concentrations. In addition, the half-maximal effective dose of insulin for some peripheral actions (e.g., inhibition of lypolysis and stimulation of potassium uptake; Ref. 34) is in the range 60–120 pM, i.e., not too far outside the error boundary of fasting insulin levels. In addition to measuring insulin secretion (by deconvolution analysis of C-peptide), we used hepatic glucose production, plasma FFA, blood glycerol, $\beta$-hydroxybutyrate, and plasma potassium measurements as multiple tracings for changes in insulin secretion. The observation that none of these parameters showed any significant differences between placebo and deriglidole for 60 min after drug administration led us to the conclusion that, in healthy individuals, neither basal insulin secretion nor lipolysis are affected by acute $\alpha_2$-blockade. Thus, in the postabsorptive state, $\beta$-cells do not appear to be under tonic adrenergic inhibition, in agreement with previous work in rats (13) and in contrast to recent experimental work in dogs (12) and the finding of Robertson and Porte (28). The latter study was done in six subjects with a wide weight range (from 91 to 133%) of ideal body weight, and phentolamine infusion was associated with a very small rise in plasma insulin (from 10 to 12 $\mu$U/ml). We cannot exclude that our study design may have missed such a small change or, alternatively, that $\alpha_2$-blockade might have been more effective on basal insulin secretion in subgroups of subjects (as suggested by greater insulin changes observed in those with higher fasting plasma insulin levels). In addition, the discrepancy might depend on the lack of specificity of phentolamine for $\alpha_2$-adrenoceptors. More recently, it has been reported that, in conscious dogs, the ingestion
of deriglidole results in a prompt (within 30 min) increase in plasma insulin and FFA concentrations (12). The higher dose used (1 mg/kg) or species differences might explain these discrepancies. Obviously, our conclusions are limited to the time frame and dosage used here. However, the plasma deriglidole concentrations already measured after 60 min and throughout the study are within the half-maximal inhibitory concentration (1 ng/ml) for the in vitro inhibition of selective agonist binding to β2-receptors. We cannot exclude that higher doses may have an effect on basal insulin secretion via a sulfonylurea-like mechanism on ATP-sensitive potassium channels, as recently demonstrated in mouse islets (14). According to the latter data, we calculated that, if the plasma concentration achieved in the present study were reproduced in vitro, a mouse islet preparation would respond with a 10–15% greater insulin secretion to a 15 mM glucose challenge.

With regard to glucose-stimulated insulin secretion, our protocol reproduced both the biphasic insulin release that follows acute intravenous glucose administration and the potentiation of glucose-induced insulin secretion that is elicited by glucose ingestion (8). Of interest is that, although the previous study (8) has used +7 mM hyperglycemia to document gastrointestinal potentiation of insulin secretion, the current results show that this phenomenon is already evident with more physiological plasma glucose elevations.

Acute hyperinsulinemia obtained by means of intravenous glucose infusion as well as glucose ingestion (5, 29) is known to be associated with an activation of the adrenergic nervous system. One consequence of such activation could be limitation of glucose-induced insulin secretion, i.e., a negative-feedback loop on the β-cell. However, in the current experiments despite boosting α2-blockade with another dose of deriglidole, we were unable to detect any enhancement (or derepression) of pancreatic β-cells during either intravenous or intravenous plus oral glucose administration.

Under conditions of stable hyperglycemia, the exogenous glucose infusion rate equals total glucose disposal (i.e., M) provided that hepatic glucose production is completely suppressed. Although we did not measure hepatic glucose output during the glucose clamp, it may be safely assumed that the combination of portal hyperinsulinemia and hyperglycemia caused full inhibition of endogenous glucose production in our normal volunteers. Glucose metabolism increased during intravenous glucose administration and was further stimulated as endogenous insulin release was potentiated by oral glucose (Table 1, Fig. 3). After glucose ingestion, the calculated M value underestimates the total rate of glucose utilization by an amount equal to oral glucose absorption. α2-Blockade was associated with a small (12%) increase in M through the whole hyperglycemic period, which persisted when only the 60- to 180-min time block (intravenous glucose alone) was analyzed. Thus α2-blockade appears to have a favorable, if small, influence on peripheral glucose uptake.

During the final hour of the control study, energy expenditure was 16% higher than at baseline. This thermogenic response, which is twice as high as that observed under conditions of euglycemic hyperinsulinemia (11), is the combined result of a stronger stimulation of glucose disposal and gastrointestinal glucose processing. α2-Blockade was associated with a significantly higher thermogenic response (Table 1). The mechanism of this effect can be surmised by considering that, under euglycemic conditions, the thermogenic response to insulin is decreased by propranolol (a nonselective β-blocker) but not by a nonselective α-blocker (phenotamine) (9). Probably, blockade of presynaptic α2-inhibitory adrenoceptors, by favoring catecholamine release (20), provided an additional drive for β-activity. The biochemical basis of the observed enhancement of energy expenditure by α2-blockade cannot be determined from our data but is likely to be due to acceleration of futile metabolic cycles as occurs during stress (23).

In the control experiments, a marked hyperdynamic response to hyperglycemia was observed (Fig. 4), with the double product increasing linearly up to 20% above baseline. This is compatible with a systemic adrenergic activation resulting in an increase in heart rate, a fall in diastolic blood pressure, and a rise in systolic blood pressure, a response qualitatively similar to that observed during the epinephrine infusion (study 1). However, we cannot rule out a direct vasodilatory effect of insulin, although this effect is usually observed at higher insulin concentrations (2). Acute α2-blockade caused a further increase in heart rate and systolic blood pressure and abolished the fall in diastolic blood pressure (Fig. 4). The mechanism presumably is consistent with the removal of the presynaptic α2-mediated inhibition of norepinephrine release (20). It is here worth recalling that clonidine, a potent α2-agonist, when parenterally administered, after a transient (1–2 min) rise in blood pressure due to vascular postjunctional α2-receptor stimulation, induces prolonged and stable hypotension. Because neither blood flow nor muscle sympathetic nerve activity were measured in our study, we cannot exclude the possibility that α2-receptor blockade caused sympathetic activation via a hemodynamic reflex; the higher diastolic blood pressure values associated with α2-blockade (Fig. 4), however, argue against this mechanism.

In conclusion, in normal subjects, acute selective α2-blockade prevents epinephrine-mediated inhibition of insulin secretion but does not potentiate either basal or glucose-induced (intravenous or oral) insulin release. It causes a mild increase in blood pressure and a marked enhancement of thermogenesis. These effects are probably related to a shift in the adrenergic balance toward β- and α1-activity.

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REFERENCES


