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Altered glucose homeostasis in α_{2A}-adrenoceptor knockout mice

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Abstract

To elucidate the functions of α_2-adrenoceptor subtypes in metabolic regulation, we determined plasma glucose and insulin levels and tissue uptake of the glucose analogue 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) in C57Bl/6J wild-type (WT) and α_{2A}-adrenoceptor knockout (α_{2A}-KO) mice at baseline and following α_2-adrenoceptor agonist ((+)-4-(*S*)-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole (dexmedetomidine)) and antagonist (4-[2-ethyl-2,3-dihydro-1*H*-inden-2-yl]-1*H*-imidazole (atipamezole)) administration. Basal glucose levels were 30% lower in α_{2A}-KO mice than in WT mice. In WT mice, dexmedetomidine lowered insulin and elevated glucose levels, and atipamezole reduced glucose levels. In α_{2A}-KO mice, neither drug affected the glucose or insulin levels. [^{18}F]FDG uptake was investigated in plasma, heart, liver, kidney, pancreas, lung, fat, and skeletal muscle. Cardiac [^{18}F]FDG uptake was a sensitive indicator of sympathetic function. Liver [^{18}F]FDG uptake conformed to the plasma glucose levels. In α_{2A}-KO mice, drug effects on [^{18}F]FDG tissue uptake were absent. Thus, the α_{2A}-adrenoceptor is the α_2-adrenoceptor subtype primarily involved in the regulation of blood glucose homeostasis in vivo.

Keywords: α_2-Adrenoceptor knockout; Atipamezole; Dexmedetomidine; ^{18}FDG; Fluorodeoxyglucose

1. Introduction

Presynaptic α_2-adrenoceptors are major regulators of the sympathetic nervous system, providing negative feedback control of noradrenaline release. In addition, postsynaptic α_2-adrenoceptors mediate physiological functions in numerous organs and tissues. In the brain, presynaptic α_2-adrenoceptors inhibit the release of noradrenaline and other neurotransmitters, but are also found postsynaptically. α_2-Adrenoceptors therefore directly or indirectly participate in all aspects of stress and arousal, including cognitive functions, cardiovascular responses, and metabolic effects. There are three α_2-adrenoceptor subtypes, α_{2A}, α_{2B}, and α_{2C}, encoded by separate genes. Studies on genetically engineered α_2-adrenoceptor knockout (α_2-KO) mice suggest that most of the previously recognised central α_2-adrenoceptor responses including sedation, hypothermia, analgesia, and hypotension are predominantly mediated through the α_{2A}-adrenoceptor. α_{2A}-, α_{2C}-, and most recently also α_{2B}-adrenoceptors have been implicated in presynaptic inhibition of transmitter release in sympathetically innervated tissues (Trendelenburg et al., 2003), and sympathetic output is enhanced in α_{2A}-KO mice, as evidenced by elevated plasma and urine catecholamine levels (Makaritsis et al., 1999). Peripherally mediated hypertension has been attributed mainly to the α_{2B}-adrenoceptor (Link et al., 1996), while the α_{2C}-adrenoceptor has been suggested to regulate adrenaline release from the adrenal gland (Brede et al., 2003).

Activation of postsynaptic α_2-adrenoceptors on pancreatic β-cells results in hyperglycemia through inhibition of glucose-stimulated insulin release (Metz et al., 1978; Angel

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et al., 1988). Pharmacological studies have indicated that the α_{2A}-adrenoceptor might be responsible for this effect (Angel et al., 1990). A role for postsynaptic pancreatic α_{2A}-adrenoceptors was later confirmed in mice with targeted β-cell overexpression of the human α_{2A}-adrenoceptor (Devedjian et al., 2000). These mice had normal basal insulin and glucose levels, but exhibited enhanced hypoinsulinemia and hyperglycemia in response to α_2-adrenoceptor activation. On the other hand, mRNA for multiple α_2-adrenoceptor subtypes has been detected in both rat and human pancreatic islets (Lacey et al., 1996; Chan et al., 1997). Furthermore, adrenaline was able to inhibit insulin secretion from pancreatic islets from α_{2A}- and α_{2C}-, but not from α_{2AC}-KO mice (Peterhoff et al., 2003), suggesting that insulin release may be inhibited by both α_{2A}- and α_{2C}-, but not α_{2B}-adrenoceptors.

2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is a positron emitting glucose analogue with a radioactive half-life of 110 min, commonly used for positron emission tomography (PET) imaging studies in humans (Wienhard, 2002). Various physiological processes alter cellular glucose metabolism, allowing detection of such changes with [^{18}F]FDG. Facilitative glucose transporters mediate cellular uptake of [^{18}F]FDG from the blood. Intracellularly, [^{18}F]FDG is phosphorylated by hexokinases to [^{18}F]FDG-6-phosphate. In most tissues, [^{18}F]FDG metabolism beyond [^{18}F]FDG-6-phosphate is very slow, and [^{18}F]FDG-6-phosphate is therefore trapped within cells. In the kidney cortex and in the liver, however, [^{18}F]FDG-6-phosphate is dephosphorylated by glucose-6-phosphatase (Gallagher et al., 1978; Stumvoll et al., 1997), resulting in re-formation of [^{18}F]FDG, which may re-enter the extracellular space. Hence, in these tissues, ^{18}F-radioactivity accumulation depends on both [^{18}F]FDG phosphorylation and [^{18}F]FDG-6-phosphate dephosphorylation rates. The high specific radioactivity of [^{18}F]FDG allows detection of minute amounts of label devoid of pharmacological effects. In addition, the high energy (511 keV) of the annihilation γ-rays produced during ^{18}F radioactive decay enables direct measurement of sample radioactivity, thus obviating the need for tissue homogenisation and precipitation procedures.

In the present study, wild-type (WT) control mice and mice lacking the α_{2A}-adrenoceptor were used to assess the roles of α_2-adrenoceptor subtypes in glucose regulation. To further elucidate α_2-adrenoceptor subtype functions, the mice were challenged with the α_2-adrenoceptor agonist (+)-4-(*S*)-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole (dexmedetomidine) or the α_2-adrenoceptor antagonist 4-[2-ethyl-2,3-dihydro-1*H*-inden-2-yl]-1*H*-imidazole (atipamezole). These compounds are α_2-adrenoceptor specific imidazole derivatives exhibiting high affinity for all three α_2-adrenoceptor subtypes (Virtanen et al., 1989; Haapalinna et al., 1997; Newman-Tancredi et al., 1998; Schwartz and Clark, 1998). We determined the glucose and insulin levels in plasma, and using receptor autoradiography, localised α_2-adrenoceptors in pancreatic sections. [^{18}F]FDG was used to monitor glucose uptake in tissues, as an indicator of metabolic activity.

2. Materials and methods

2.1. Animals

WT (control) C57Bl/6J mice, 2–3 months old, were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and allowed to acclimatise for at least 1 month at the local animal facility prior to experiments. Congenic α_{2A}-KO mice on a C57Bl/6J genetic background (Altman et al., 1999) were bred locally. Male mice were used in all experiments. The mice were maintained under standard conditions with lights on from 6:00 a.m. to 6:00 p.m. Animal care was in accordance with the guidelines of the International Council of Laboratory Animal Science (ICLAS). The study protocol was approved by the institutional animal care and use committee. The mice had free access to tap water and, unless stated otherwise, food, at all times. Eight groups of 4–6-month old mice, 10–17 in each group, were used in the [^{18}F]FDG experiments. In addition, 3 WT and 3 α_{2A}-KO mice, 2–3 months old, were used for α_2-adrenoceptor autoradiography, and 15 mice of each genotype, 3 months old, food deprived for 6 h, were used for determining fasting plasma glucose and insulin levels.

2.2. Drugs and chemicals

[^{18}F]FDG was synthesised at the Turku PET Centre Radiopharmaceutical Chemistry Laboratory with an automated device, using the method described by Hamacher et al. (1986), with slight modifications. The radiochemical purity exceeded 95% and the specific radioactivity exceeded 74 GBq/μmol at the end of synthesis.

Dexmedetomidine and atipamezole were gifts from Orion Pharma (Turku, Finland). All injected drugs were dissolved in physiological saline. Tritiated [8*aR*,12*aS*,13*aS*]-5,8,8*a*,9,10,11,12,12*a*,13,13*a*-decahydro-3-methoxy-12-(ethylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]naphthyridine ([ethyl-^{3}H]RS-79948-197), specific radioactivity 3.0 GBq/μmol, was purchased from Amersham Biosciences (Buckinghamshire, UK). 2-(*N*-[*m*-hydrophenyl]-*p*-[*N*-(3-hydroxyphenyl)-*p*-toluidinomethyl]-2-imidazolidine (phentolamine) was from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Experimental procedures

Accumulation of [^{18}F]FDG-derived radioactivity was assessed over 1 h from 30 to 90 min after the administration of 30 μg/kg dexmedetomidine or 1 mg/kg atipamezole. The drug administration scheme was based on a study by Lähdesmäki et al. (2003), investigating the effects of dexmedetomidine and atipamezole on locomotor activity

and body core temperature in WT and α_{2A}-KO mice, because these parameters can be assumed to directly affect tissue glucose utilisation. Dexmedetomidine at 30 μg/kg almost eliminated locomotor activity through sedation, measured from 30 to 90 min after drug administration, and reduced the body temperature by approximately 8 °C in WT, but not in α_{2A}-KO mice. Atipamezole at 1 mg/kg slightly increased locomotor activity in α_{2A}-KO mice, but not in WT mice, with no effect on body temperature in either genotype. A one hour [^{18}F]FDG accumulation time was appropriate also considering the 110 min radioactive half-life of ^{18}F, and has been previously validated in [^{18}F]FDG biodistribution studies in mice (Gallagher et al., 1977, 1978).

Between 9:00 a.m. and 10:00 a.m., 2–4 mice were placed in clean cages and transferred from the animal facility to a laboratory designated for radioactive experimental animal work situated in the same building. The mice were weighed, colour labelled on their tails, and allowed to rest for approximately one hour before the experiment. The mice were injected subcutaneously (injection volume 5 ml/kg body weight) with drugs or saline, or received no subcutaneous injection. After 30 min, all mice were given [^{18}F]FDG as a bolus into a tail vein (injection volume less than 100 μl). One hour after [^{18}F]FDG injection, the mice were anaesthetised with CO_2 gas, and blood was immediately collected by cardiac puncture into heparinised tubes. Plasma and blood cells were separated by centrifugation. Heart (left ventricle), liver, kidney, pancreas, lung, epididymal fat and hind limb thigh muscle samples were dissected. The plasma and tissue samples were weighed and their radioactivities measured in a 7.62×7.62 cm (3×3 in.) NaI(Tl) crystal well counter (Bicron 3MW3/3P, Bicron, Newbury, OH, USA). Background counts were subtracted and the radioactivity decay was corrected for. The plasma was frozen at −20 °C until used for glucose and insulin determinations. The ^{18}F-radioactivity, representing both [^{18}F]FDG and [^{18}F]FDG-6-phosphate that had accumulated in the tissue samples over the one-hour period following [^{18}F]FDG injection, was expressed as percentage of the injected dose per gram of tissue (%ID/g).

To assess the plasma glucose and insulin levels of WT and α_{2A}-KO mice in the fasted state, food was removed at 8:00 a.m., and blood was collected by cardiac puncture under CO_2 anaesthesia at 2:00 p.m. During the fast, the mice remained in their home cages.

2.4. Plasma glucose and insulin determinations

Plasma glucose levels were measured with an Analox GM9 glucose analyser (Analox Instruments, London, UK) using the glucose oxidase method. Plasma insulin levels were determined with an enzyme-linked immunosorbent assay (ELISA) (Mercodia Ultrasensitive Mouse Insulin ELISA from Mercodia, Uppsala, Sweden), using mouse insulin as reference.

2.5. [Ethyl-^{3}H]RS-79948-197 α_2-adrenoceptor autoradiography

Pancreatic cryo-sections, 20 μm thick, from three WT and three α_{2A}-KO mice were incubated with the α_2-adrenoceptor subtype-nonselective antagonist radioligand [ethyl-^{3}H]RS-79948-197 (Uhlén et al., 1998) in 50 mM potassium phosphate buffer, pH 7.4, for 1 h (Fagerholm et al., 2004). Non-specific binding was defined as binding in the presence of 10 μM of the α-adrenoceptor antagonist phentolamine. The sections were washed in fresh ice-cold buffer for 20 min and dipped in ice-cold de-ionised water to remove salts. The dried sections were apposed to Kodak BioMax MR autoradiographic film (Kodak, Rochester, NY, USA) for 15 weeks. The same pancreatic sections were subsequently stained with haematoxylin and eosin for histological identification of pancreatic islets.

2.6. Statistics

Statistical analyses were performed with SAS, version 8.2 (SAS Institute, Cary, NC, USA) and GraphPad Prism, version 2.01 (GraphPad Software, San Diego, CA, USA). Two-way analysis of variance (ANOVA) was used to assess genotype (WT, α_{2A}-KO) and treatment (saline, drug, or no pre-treatment prior to [^{18}F]FDG injection) effects, and their interaction. ANOVA was performed on log-transformed data in order to correct for deviations from normal distribution and for non-homogenous between-groups variances, thereby validating the use of ANOVA. Following ANOVA, the Tukey–Kramer test was used for pairwise comparisons of interest. Correlation analyses were performed with the Spearman non-parametric test. In fasted WT and α_{2A}-KO mice, plasma glucose levels were compared using independent two-tailed Student's *t*-test, and plasma insulin levels using independent Student's *t*-test with Welch's correction for unequal variances. The limit for statistical significance was set at $P<0.05$.

3. Results

3.1. Plasma glucose and insulin

Plasma glucose levels in the fed state (Fig. 1A) were 30% lower in α_{2A}-KO than in WT mice. In WT mice, plasma glucose levels were increased 58% by dexmedetomidine and decreased 23% by atipamezole. In α_{2A}-KO mice, dexmedetomidine and atipamezole did not significantly affect plasma glucose levels. Insulin levels in fed mice (Fig. 1B) were 70% lower in dexmedetomidine-treated WT mice than in saline-injected controls, with no statistically significant differences for the other experimental groups.

α_{2A}-KO mice have been shown to exhibit enhanced gastro-intestinal transit speed (Scheibner et al., 2002) and

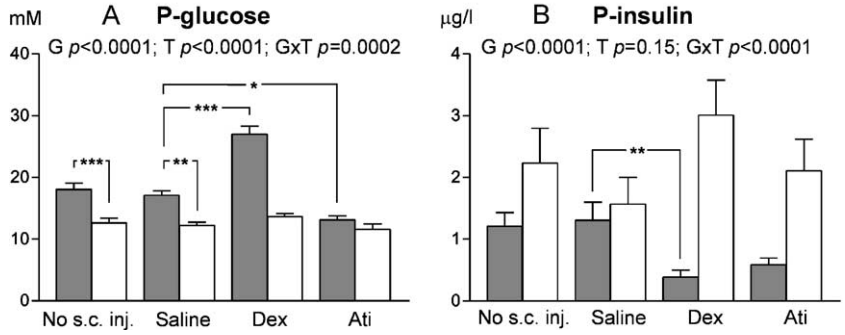

Fig. 1. Glucose (A) and insulin (B) plasma levels in fed WT (filled bars) and α_{2A}-KO (open bars) mice. The mice had received no subcutaneous injection (No s.c. inj.) or were subcutaneously injected with vehicle (Saline), the subtype-nonselective α_2-adrenoceptor agonist dexmedetomidine, 30 µg/kg (Dex), or the subtype-nonselective α_2-adrenoceptor antagonist atipamezole, 1 mg/kg (Ati). Bars represent arithmetic means±S.E.M. of 9–17 mice per group. ANOVA: G, genotype effect; T, treatment effect; G×T, genotype×treatment interaction. Post hoc: *P<0.05; **P<0.01; ***P<0.001.

attenuated nocturnal locomotor activity (Lähdesmäki et al., 2002). As both of these traits could affect the nutritional state of the freely fed animals, we additionally determined the plasma glucose and insulin levels in fasted mice. As in the fed state, fasting glucose levels (mean±S.E.M., n=15) were lower in α_{2A}-KO mice (11.4±0.6 mM) than in WT mice (15.8±0.7 mM), P<0.0001. Fasting plasma insulin levels (mean±S.E.M.) measured from the same animals tended to be higher in α_{2A}-KO (5.4±1.8 µg/l) than in WT mice (2.0±0.2 µg/l), P=0.08. Surprisingly, the plasma insulin levels in the fasted mice were higher than those of the fed [^{18}F]FDG administered mice, although the plasma glucose levels, as expected, were lower in the fasted state. Within experimental groups, there was no correlation between plasma glucose and insulin levels.

3.2. α_2-Adrenoceptor localisation in pancreas

In WT mice, [ethyl-^{3}H]RS-79948-197 receptor autoradiography revealed high-affinity α_2-adrenoceptor radioligand binding in pancreatic islets. In α_{2A}-KO mice, no specific binding was seen, indicating that the α_{2A}-adrenoceptor was the most numerous α_2-adrenoceptor subtype in the pancreatic islets. Binding of 1.0 nM [ethyl-^{3}H]RS-79948-197, and hematoxylin–eosin staining of pancreatic islets in WT and α_{2A}-KO mice are shown in Fig. 2.

3.3. [^{18}F]FDG tissue uptake

^{18}F-radioactivity was measured in tissues from WT and α_{2A}-KO mice that had received no subcutaneous injection, or subcutaneous injections of physiological saline, the α_2-adrenoceptor agonist dexmedetomidine, or the α_2-adrenoceptor antagonist atipamezole prior to an intravenous bolus of [^{18}F]FDG. Relevant animal characteristics for the experimental groups are given in Table 1.

Since α_{2A}-KO mice are potentially more sensitive to stress than WT mice (Schramm et al., 2001; Lähdesmäki et al., 2002), the effect of subcutaneous injection (saline injection versus no saline injection) was investigated. In saline-injected WT mice, cardiac [^{18}F]FDG uptake was 51% lower than in WT mice not subcutaneously injected (Fig. 3B). In α_{2A}-KO mice, subcutaneous saline injection did not alter ^{18}F-radioactivity accumulation in any of the tissues studied (Fig. 3A–H).

In α_{2A}-KO mice, enhanced sympathetic tone due to lack of presynaptic α_{2A}-adrenoceptors together with abolished postsynaptic α_{2A}-adrenoceptor functions were expected to result in altered basal [^{18}F]FDG biodistribution compared to WT mice. In mice not subcutaneously injected prior to [^{18}F]FDG administration, ^{18}F-radioactivity in the liver (Fig. 3C) was 41% lower, and in the lung (Fig. 3F) 29% lower, in α_{2A}-KO than in WT mice. When comparing saline-injected α_{2A}-KO and WT mice, ^{18}F-radioactivity was likewise lower in α_{2A}-KO liver (Fig. 3C) (51%), but in the lung (Fig. 3F), the genotype difference was not quite significant (P=0.052). Furthermore, cardiac [^{18}F]FDG uptake (Fig. 3B) was 115% higher and plasma [^{18}F]FDG (Fig. 3A) 41% lower in α_{2A}-KO than in WT mice; similar differences were not noted in animals not subcutaneously injected. Thus, basal glucose metabolism not only differed between the mouse genotypes, but also the physiological state of the animals influenced the responses.

The effects of dexmedetomidine and atipamezole on ^{18}F-radioactivity biodistribution in WT and α_{2A}-KO mice were compared with saline-injected controls. In WT mice, both α_2-adrenoceptor agonist and antagonist treatment resulted in prominent changes in [^{18}F]FDG biodistribution (Fig. 3A–H). In these mice, dexmedetomidine increased ^{18}F-radioactivity by 357% in plasma (Fig. 3A), by 167% in the liver (Fig. 3C), and by 298% in kidney (Fig. 3D), while ^{18}F-radioactivity was reduced by 79% in heart (Fig. 3B) and by 56% in hind limb muscle (Fig. 3H). Atipamezole treatment of WT mice decreased ^{18}F-radioactivity by 46% in plasma (Fig. 3A), by 45% in the liver (Fig. 3C), by 37% in pancreas (Fig. 3E), and by 32% in the lung (Fig. 3F), and increased ^{18}F-radioactivity by 261% in heart (Fig. 3B) and by 140% in epididymal fat (Fig. 3G). In α_{2A}-KO mice, the drug treatments had no statistically significant effects (Fig. 3A–H).

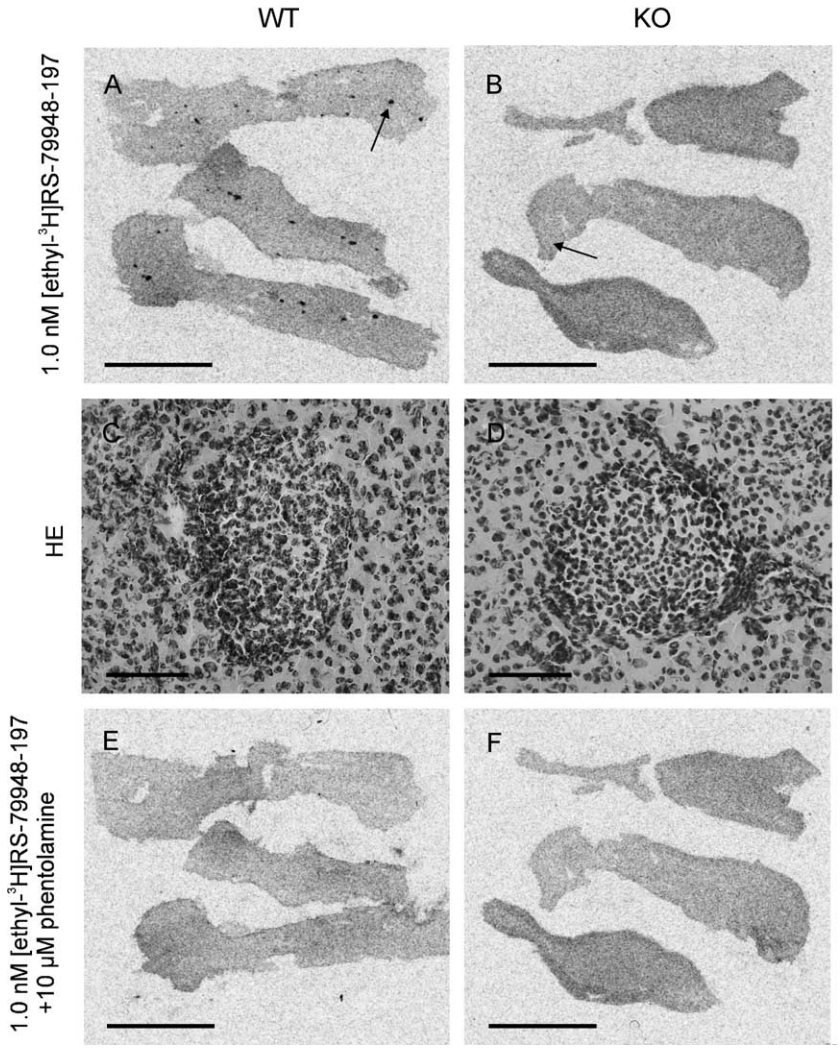

Fig. 2. Pancreatic sections from three WT and three α_{2A}-KO mice labelled with 1 nM of the subtype-nonselective α_2-adrenoceptor antagonist radioligand [ethyl-^{3}H]RS-79948-197. Binding was evident in pancreatic islets in WT (A), but not in α_{2A}-KO (B) mice. Arrows in A and B indicate the hematoxylin–eosin stained pancreatic islets shown in C and D, respectively. Non-specific binding was defined in adjacent sections by adding an excess of the α-adrenoceptor antagonist phentolamine to the radioligand buffer (E, F). Scale bars for A, B, E, and F are 5 mm, and for C and D 100 μm.

The heart is a major site of [^{18}F]FDG uptake, with virtually its entire ^{18}F-radioactivity present in the form of [^{18}F]FDG-6-phosphate already 1 min after intravenous [^{18}F]FDG injection (Gallagher et al., 1978). The rate of cardiac glucose utilisation could therefore possibly have influenced the [^{18}F]FDG amount in plasma, and thereby the amount of [^{18}F]FDG available to and phosphorylated by other tissues. Correlation analysis within the experimental groups revealed covariation of cardiac [^{18}F]FDG with ^{18}F-radioactivity in other tissues, including plasma, only for the liver (r=0.70, P=0.03), pancreas (r=0.66, P=0.04), and lung (r=0.71, P=0.03) in dexmedetomidine-treated WT mice. When additionally considering that the found correlations were positive, the results imply that

Table 1
Number of animals, age, weight, and the amount [^{18}F]FDG injected intravenously for each of the eight experimental groups

	WT	WT Sal	WT Dex	WT Ati	KO	KO Sal	KO Dex	KO Ati
N	17	10	10	10	10	10	10	10
Age (weeks)	18±1	19±1	22±1	21±1	20±1	21±1	20±1	20±1
Weight (g)	28±1	31±1	30±1	30±1	32±1	30±1	30±1	29±1
ID (MBq)	16±2	13±1	13±2	13±1	14±2	14±1	14±2	10±2

WT, wild-type; KO, α_{2A}-KO; Sal, physiological saline; Dex, 30 μg/kg dexmedetomidine; Ati, 1 mg/kg atipamezole; ID, injected dose. Results are means±S.E.M.

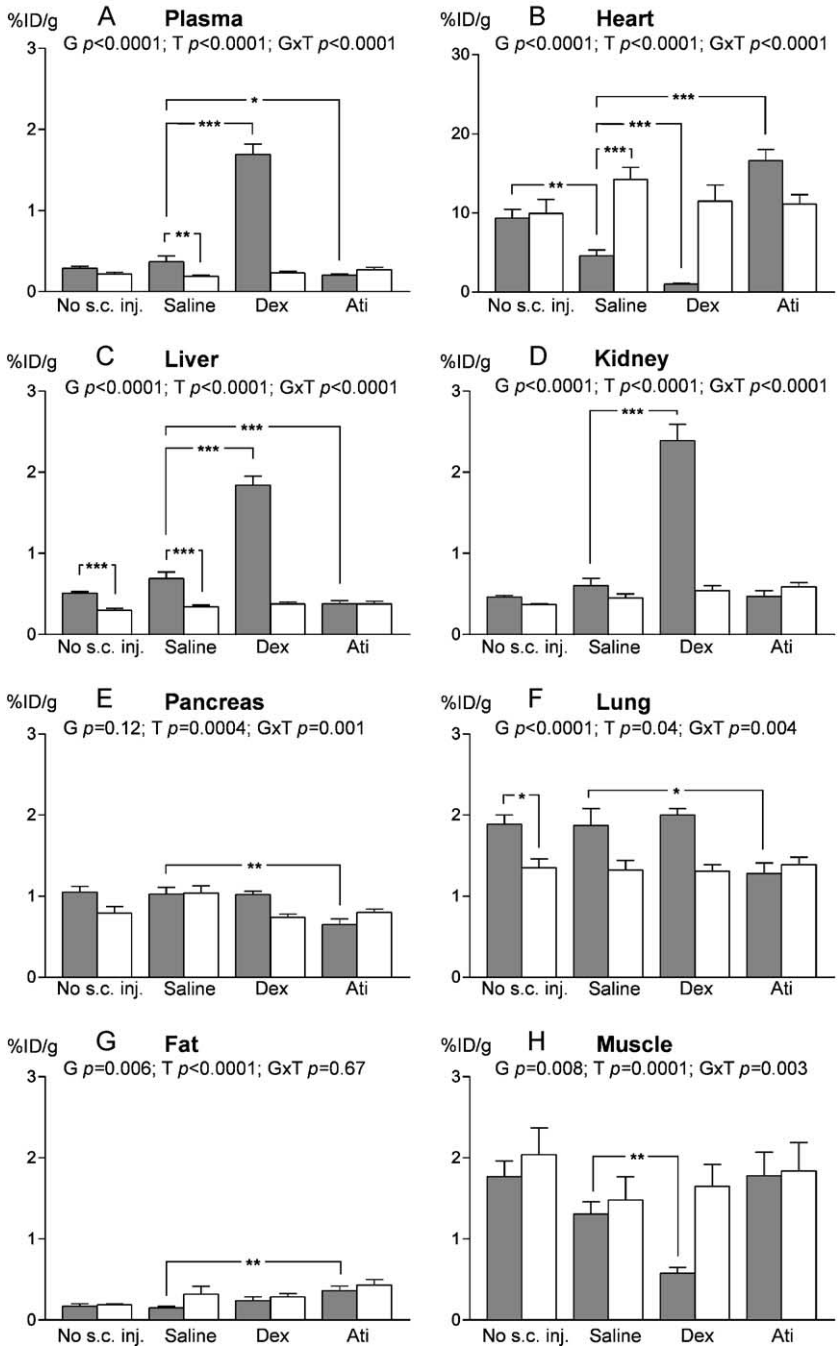

Fig. 3. Tissue ^{18}F-radioactivity accumulation expressed as % uptake of the injected dose of [^{18}F]FDG per gram of tissue (%ID/g) in fed WT (filled bars) and α_{2A}-KO (open bars) mice, 1 h after intravenous [^{18}F]FDG injection. The mice received either no pre-treatment (No s.c. inj.) or were 30 min prior to [^{18}F]FDG administration subcutaneously injected with vehicle (Saline), 30 μg/kg of the subtype-nonselective α_2-adrenoceptor agonist dexmedetomidine (Dex), or 1 mg/kg of the subtype-nonselective α_2-adrenoceptor antagonist atipamezole (Ati). Bars represent arithmetic means±S.E.M. of 9–17 mice per group. Note the different scale in panel B. ANOVA: G, genotype effect; T, treatment effect; G×T, genotype×treatment interaction. Post hoc: *P<0.05; **P<0.01; ***P<0.001.

cardiac [^{18}F]FDG uptake is not a major determinant of ^{18}F-radioactivity accumulation in other tissues. However, since the differences in cardiac [^{18}F]FDG uptake, and probably also in vascular resistance and blood flow, between some of the experimental groups were substantial compared to the within-groups differences, extrapolation from the within-groups results to between-groups results must be made with caution.

4. Discussion

4.1. Plasma glucose and insulin

One of the main findings in this study is that basal plasma glucose levels were lower in α_{2A}-KO than in WT mice. Furthermore, the variability of the plasma insulin levels was notably large in α_{2A}-KO mice, possibly reflecting

diminished noradrenergic control of insulin release, or variability in peripheral insulin sensitivity in this genotype. It is noteworthy that mice lacking dopamine β-hydroxylase, and thus lacking both noradrenaline and adrenaline, were hyperinsulinemic and had a similar reduction in plasma glucose levels as the α_{2A}-KO mice in the present study (Ste Marie and Palmiter, 2003). Together, these results suggest that activation of α_{2A}-adrenoceptors by noradrenaline plays an important role in glucoregulation in vivo, although the possibility of developmental compensatory effects on other glucoregulatory pathways remains.

Dexmedetomidine elevated and atipamezole reduced plasma glucose levels in WT mice, with no effect in α_{2A}-KO mice. The observed reduction in plasma insulin levels in WT mice by dexmedetomidine is in agreement with an inhibitory action of pancreatic α_{2A}-adrenoceptors on insulin release, and provides an explanation for the enhanced plasma glucose levels. In contrast, when considering the reduction of plasma glucose levels by atipamezole in WT mice, a concomitant lack of elevated plasma insulin levels was somewhat surprising. It is possible that the lower glucose levels in the atipamezole-treated WT mice determined the insulin levels at the investigated time point (one and a half hour after drug injection), and studies with glucose levels held constant are warranted to further clarify the effects of dexmedetomidine and atipamezole on insulin release in α_{2A}-KO and WT mice. There are numerous reports on the hyperinsulinemic and hypoglycemic effects of α_2-adrenoceptor antagonists, including those of atipamezole in mice (Durcan et al., 1991), although also negative results have been presented (see Abdel-Zaher et al., 2001, for references). Furthermore, imidazole-type compounds may augment insulin secretion through α_2-adrenoceptor independent mechanisms such as by direct inhibition of ATP-sensitive K^+ (K_{ATP}) channels on pancreatic β-cells (Proks and Ashcroft, 1997), but in the present study, the finding that atipamezole reduced plasma glucose levels in WT, but not in α_{2A}-KO mice, argues for either direct or indirect involvement of α_{2A}-adrenoceptors in this effect.

With α_2-adrenoceptor autoradiography, only α_{2A}-adrenoceptors were detected in pancreatic islets. While the expression levels of other α_2-adrenoceptor subtypes may have been below the detection limit, the autoradiographic evidence does show that α_{2A} is the most abundant α_2-adrenoceptor subtype in mouse pancreatic islets, and adds indirect evidence that inhibition of insulin release may be at least one of the mechanisms by which α_{2A}-adrenoceptor activation mediates hyperglycemia. In isolated cultured pancreatic islets from WT and α_2-adrenoceptor knockout mice, some inhibition of glucose-stimulated insulin release by adrenaline, mediated by α_{2C}-adrenoceptors, still occurred in α_{2A}-KO mice (Peterhoff et al., 2003). Different signalling mechanisms for α_{2A}- and α_{2C}-adrenoceptors were implied, because hyperpolarisation of islet cells and inhibition of cAMP accumulation was evident only in the presence of α_{2A}-adrenoceptors. We failed to detect significant effects of dexmedetomidine or atipamezole on plasma insulin and glucose levels in α_{2A}-KO mice, and a possible glucoregulatory action of α_{2C}-adrenoceptors therefore seems to be more subtle, or to occur under different circumstances, compared to α_{2A}-adrenoceptors. Our results do, however, suggest that stress may reduce insulin levels also in the absence of α_{2A}-adrenoceptors. In both investigated genotypes, plasma insulin levels were clearly lower in the freely fed [^{18}F]FDG administered handled mice than in the fasted mice that had been resting in their home cages. Neurotransmitters/neuromodulators such as neuropeptide Y and galanin co-released with noradrenaline from pancreatic sympathetic nerve endings have been shown to attenuate insulin release (Ahrén, 2000). Parasympathetic activity augments insulin release, and parasympathetic tone may also have contributed to the differences in plasma insulin levels between the experimental groups.

4.2. [^{18}F]FDG tissue uptake

The metabolic activities of selected insulin sensitive and insensitive tissues were investigated using [^{18}F]FDG. A bolus of [^{18}F]FDG was injected intravenously, and the relative accumulation of radioactivity in the tissues was assessed. Compared to the mM glucose levels present in the blood, the injected amounts of [^{18}F]FDG, not exceeding 35 nmol/kg as calculated from the specific radioactivity of [^{18}F]FDG at the time of injection, were very low. Slight variations between different animals in injection volume and specific radioactivity of the tracer are therefore not expected to affect the cell membrane transport or the intracellular phosphorylation of [^{18}F]FDG relative to glucose. In contrast, the differences in plasma glucose levels noted between the experimental groups may have resulted in differential competition for [^{18}F]FDG uptake and phosphorylation. Furthermore, plasma [^{18}F]FDG clearance occurs also by renal secretion, the kinetics of which may have differed between groups. Differences in plasma glucose or [^{18}F]FDG levels would, however, affect the uptake of [^{18}F]FDG into all tissues equally, and no such pattern was noticed when comparing the experimental groups. [^{18}F]FDG tissue uptake was therefore primarily determined by the metabolic activity of the tissues.

Altered net glucose utilisation in some of the experimental groups was evident from the differences in relative plasma [^{18}F]FDG contents. Plasma [^{18}F]FDG levels were lower in saline-injected α_{2A}-KO mice and in atipamezole-treated WT mice than in saline-injected WT controls. The differences in cardiac [^{18}F]FDG uptake between these groups likely accounts for the altered plasma [^{18}F]FDG levels. In WT mice treated with dexmedetomidine, much of the [^{18}F]FDG was retained in the blood, suggesting a substantial reduction in the overall glucose metabolic rate.

Glucose attenuates hepatic glycogenolysis and gluconeogenesis both insulin dependently and independently, resulting in a net decrease in hepatic glucose production and an

increase in glucose uptake (Sweet et al., 1996; Moore et al., 1998). Sympathetic stimulation directly increases hepatic glucose production by promoting glycogenolysis via hepatic α_1- and β-adrenoceptors, and indirectly by acting on non-hepatic tissues to provide the liver with gluconeogenic precursors (Chu et al., 1997, 2000). The hepatic ^{18}F-radioactivity accumulation observed in the present study conformed to the blood glucose levels in the respective experimental groups. The excessive glycemia in dexmedetomidine-treated WT mice therefore probably resulted more from reduced glucose disposal, rather than from increased hepatic glucose production. Also, renal ^{18}F-radioactivity was elevated in WT mice following dexmedetomidine. Under hyperglycemic conditions, excess glucose is removed from the plasma by kidney uptake, and like the liver, the kidneys are capable of both production and utilisation of substantial amounts of glucose (Stumvoll et al., 1997).

Elevated heart rate has been reported in α_{2A}-KO mice (Altman et al., 1999), but in the present study, cardiac [^{18}F]FDG accumulation was equal in WT and α_{2A}-KO mice that were not subcutaneously injected prior to [^{18}F]FDG. In contrast, cardiac [^{18}F]FDG uptake was significantly reduced in WT mice after saline injection. It thus appears that habituation to handling, i.e., the subcutaneous injection preceding the [^{18}F]FDG injection, occurred in WT, but not in α_{2A}-KO mice, resulting in attenuated [^{18}F]FDG injection stress and subsequent reduced cardiac energy requirements in saline-injected WT mice. Although [^{18}F]FDG accumulation over a 1-h period was assessed, it is conceivable that for the myocardium, with its rapid and extensive [^{18}F]FDG uptake, the glucose utilisation rate in the moments immediately following [^{18}F]FDG injection may be crucial.

In WT mice, dexmedetomidine decreased and atipamezole increased cardiac [^{18}F]FDG accumulation when compared to saline-injected WT controls. Centrally mediated reduction and enhancement of cardiac output in response to α_2-adrenoceptor agonists and antagonists, respectively, is well documented. α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors all appear capable of inhibiting noradrenaline release from cardiac atrial tissue in vitro (Trendelenburg et al., 2003), but neither dexmedetomidine nor atipamezole affected cardiac [^{18}F]FDG uptake in α_{2A}-KO mice, suggesting that α_{2A}-adrenoceptors in vivo are more important regulators of cardiac sympathetic activity than the other subtypes. Furthermore, the cardiac baroreceptor reflex is significantly attenuated in α_{2A}-KO mice (Niederhoffer et al., 2004), and changes in blood pressure are therefore expected to have a smaller impact on cardiac glucose utilisation in α_{2A}-KO than in WT mice.

In the lung, sympathetic stimulation causes vasoconstriction (Dawson, 1984), something which could explain the attenuated accumulation of ^{18}F-radioactivity in α_{2A}-KO mice and atipamezole-administered WT mice. The [^{18}F]FDG uptake results for pancreas and fat, on the other hand, were unconvincing. In the pancreas, noradrenaline, α-adrenoceptor activation, and electrical stimulation of pancreatic sympathetic nerves result in vasoconstriction and reduced pancreatic blood flow (Vaysse et al., 1977; Elisha et al., 1984; Hillaire-Buys et al., 1985; Ahrén et al., 1987). The regulation of adipose tissue metabolism by catecholamines is complex, with α_1-, α_2-, β_1-, β_2-, and β_3-adrenoceptors expressed in adipocytes at levels varying with, e.g., species, site of fat deposit, sex, age, and level of obesity. α_1-Adrenoceptors stimulate glycogenolysis (Lawrence and Larner, 1977, 1978; Garcia-Sainz, 1983) and β_3-adrenoceptors inhibit insulin-stimulated glucose uptake in rat adipocytes (Carpene et al., 1993). Lipolysis is inhibited by α_2-adrenoceptors and stimulated by β-adrenoceptors, but in rodents, in which adipocyte levels of α_2-adrenoceptors are low, α_2-adrenoceptors are less important for adipose tissue metabolism than in humans (Lafontan and Berlan, 1993).

4.3. Conclusions

The present results show that α_{2A}-adrenoceptors participate in the physiological control of blood glucose levels, and mediate the metabolic effects of acute α_2-adrenoceptor agonist and antagonist challenge in mice. Elevation of plasma glucose and reduction of plasma insulin levels by dexmedetomidine was dependent on α_{2A}-adrenoceptors. These results suggest an important role for α_{2A}-adrenoceptors in the integration of sympathetic activity and glucose metabolism.

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