Metabolic and Cardiovascular Effects of the Adenosine A<sub>1</sub> Receptor Agonist N<sup>6</sup>-(<i>p</i>-Sulfophenyl)Adenosine in Diabetic Zucker Rats: Influence of the Disease on the Selectivity of Action

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ABSTRACT
Studies were designed to investigate differences in pharmacokinetics and pharmacodynamics of the adenosine A<sub>1</sub> receptor agonist N<sup>6</sup>-(<i>p</i>-sulfophenyl)adenosine (SPA) between lean and obese Zucker rats. In conscious rats, time courses of the effect on heart rate and parameters of lipid metabolism (fatty acids, glycerol) were monitored in combination with the decline of drug concentrations after i.v. administration of 100 µg SPA in 15 min. Small differences in pharmacokinetics of SPA were observed between lean and obese rats. Values for clearance and volume of distribution were 1.2 ± 0.2 ml/min and 88 ± 10 ml in lean, and 1.6 ± 0.1 ml/min and 110 ± 7 ml in obese animals, respectively. Modelling of the concentration-heart rate relationship on the basis of the sigmoidal E<sub>max</sub> model revealed no difference in EC<sub>50</sub> (99 ± 12 and 118 ± 17 ng/ml) or E<sub>max</sub> (−191 ± 16 and −185 ± 22 bpm) between the lean and obese rats. The metabolic effects of SPA were totally different between lean and obese rats. Potent (EC<sub>50</sub> = 18 ± 3 ng/ml) inhibition of lipolysis was observed in the lean rats. In obese rats, SPA was less potent (EC<sub>50</sub> = 109 ± 36 ng/ml) resulting in short lasting antilipolytic effect. Furthermore, administration of SPA resulted in a significant decrease in insulin concentrations. These findings show that changes in glucose and lipid metabolism may be associated with an altered sensitivity to the antilipolytic actions of adenosine A<sub>1</sub> receptor agonists.

Adenosine A<sub>1</sub> receptor agonists may be used as modulators of glucose and lipid metabolism (Hoffman et al., 1986) in NIDDM. NIDDM is generally characterized by a decreased action of insulin, which is associated with an elevated circulating NEFA. These elevated NEFA concentrations diminish the insulin-induced glucose utilization (Ferrannini et al., 1983), glucose oxidation and suppression of hepatic glucose production in normal, obese and NIDDM subjects (Reaven and Chen, 1988). Inhibitors of fatty acid release from adipocytes (e.g., adenosine A<sub>1</sub> receptor agonists) suppress these elevated NEFA concentrations and offer the potential of reducing hyperglycemia via the so-called glucose-fatty acid cycle (Foley, 1992). Selective adenosine A<sub>1</sub> receptor agonists have been developed as antilipolytic agents and have been shown to lower plasma circulating NEFA concentrations in normal (Strong et al., 1993) and streptozotocin-induced diabetic rats (Reaven et al., 1988). A major drawback for the use of A<sub>1</sub> receptor agonists in type 2 diabetes is the occurrence of severe hemodynamic actions after peripheral administration (Jacobson et al., 1991). Recent in vitro studies, however, have shown that the antilipolytic effects occur at lower concentrations of A<sub>1</sub> receptor agonists than the hemodynamic depressor effects (Gurden et al., 1993). In normal Wistar rats, an integrated pharmacokinetic-pharmacodynamic modelling approach was used to investigate the in vivo selectivity of action of the adenosine A<sub>1</sub> receptor agonist SPA. In these experiments, the EC<sub>50</sub> for the antilipolytic effect was 6-fold lower than the EC<sub>50</sub> for the effect on heart rate (Van Schaick et al., 1997a).

It is, however, important to investigate whether changes in selectivity of action occur in disease due to adaptive alterations in pharmacokinetics or pharmacodynamics. NIDDM is frequently associated with obesity, which may influence the distribution and clearance of compounds (Shum and Jusko, 1984). In addition, high levels of free fatty acids may compete for plasma protein binding and as such affect active drug concentrations. Furthermore, alterations at the adenosine receptor level have been reported in animal models (Van-

ABBREVIATIONS: SPA, N<sup>6</sup>-(<i>p</i>-sulfophenyl)adenosine; NIDDM, non-insulin-dependent diabetes mellitus; NEFA, nonesterified fatty acids; CPA, N<sup>6</sup>-cyclopentyladenosine; P/B, plasma-to-blood ratio; f<sub>p</sub>, free fraction in plasma; HPLC, high performance liquid chromatography; AUC, area under the concentration-time curve; MAP, mean arterial pressure.
The purpose of the present study was to investigate the pharmacokinetic-pharmacodynamic relationship of the adenosine A1 receptor agonist SPA in an insulin-resistant animal model of NIDDM. SPA was chosen as a model compound since it is a selective A1 adenosine receptor agonist with a modest potency. As a result the plasma concentrations required for suppression of lipolysis are rather high. By selecting SPA as a model compound it is possible to determine the complete plasma concentration versus time profile in the pharmacokinetic-pharmacodynamic experiments. An additional advantage of SPA is that as a result of the low lipophilicity, it is expected to cross the blood-brain barrier with difficulty. This diminishes the contribution of central effects to the pharmacodynamics in vivo. The successful analysis of the haemodynamic and antilipolytic effects of SPA has been convincingly demonstrated in previous investigations (Van Schaick et al., 1997a; Van Schaick et al., in press). The genetically obese Zucker rat (fa/fa) is a useful animal model of insulin resistance in NIDDM (McCaleb and Sredy, 1992) and obesity (Bray, 1977). The obese Zucker rats are characterized by mild hyperglycaemia, abnormal oral glucose tolerance (Rohner-Jeanrenaud et al., 1986) and insulin-resistance of both liver and muscle tissue (Terrattaz et al., 1986). These metabolic abnormalities in the obese rats are inherited as a single gene defect (Zucker and Zucker, 1961) and are not present in the lean (Fa/?) Zucker rats. The effects on both hemodynamics and lipid metabolism were investigated after intravenous administration of SPA. For both effects, concentration-effect relationships were determined and quantified on the basis of integrated pharmacokinetic-pharmacodynamic models. The pharmacokinetic and pharmacodynamic parameters were used to detect differences between lean and obese animals and to investigate the selectivity of action of SPA between lipid metabolism and hemodynamics.

Methods

Chemicals. SPA (a gift through the NIMH synthesis programme) and CPA were obtained from Research Biochemicals (Natick, MA). Ethyl acetate (Baker Chemicals, Deventer, The Netherlands) was distilled prior to use. Acetonitrile (HPLC grade) was obtained from Westburg (Leusden, The Netherlands) and tetrahydronium-hydroxide from Aldrich (Axel, The Netherlands). Water was used from a Milli-Q system (Millipore SA, Molsheim, France). All other chemicals were of analytical grade (Baker, Deventer, The Netherlands).

Animal model and surgical procedures. Male obese (fa/fa) and lean (Fa/?) Zucker rats were purchased at 10 weeks of age from Charles River Farms, Woerden, The Netherlands and were used at 17 weeks of age. The animals were housed individually in plastic cages with a normal 12-h light-dark cycle, fed on laboratory chow (Standard Laboratory Rat, Mouse and Hamster Diets, SMR-A, Hope Farms, Woerden, The Netherlands) and allowed tap water ad libitum. Four days before experimentation indwelling cannulas were implanted into the right jugular vein for drug administration (polythene, 13.5 mm, 0.58 mm I.D.), and the right and left femoral artery (polythene, 18 mm, 0.58 mm I.D. + 4.5 mm, 0.28 mm I.D.) (24). The arterial cannulas were guided through the femoral artery into the abdominal aorta and were used for blood sampling and recording of arterial blood pressure, respectively. All cannulas were tunnelled subcutaneously to the back of the neck and exteriorized. After the operation the cannulas were filled with a 25% (g/v) solution of polyvinylpyrrolidone (Brocacef, Maarssen, The Netherlands) in 0.9% (w/v) sodium chloride containing 50 IU/ml heparin. One day before the experiment animals were fasted on water. During the experiment the animals were conscious, freely moving, and allowed tap water ad libitum.

Study design. Both lean and obese Zucker rats were randomly allocated to parallel groups of 6–7 animals that were either given an intravenous infusion of 100 μg SPA in 15 min or vehicle (saline) in 15 min. Each animal was given 100 μg SPA in a total volume of 765 μl (8.7 μg/ml min⁻¹). Doses were not adjusted to the weight of the animals, since it was not expected that the increase in adipose tissue mass in the obese rat would affect the distribution of the hydrophilic agonist SPA. A 2.0 mg/ml solution of SPA in water was prepared and stored at −20°C until use. On the day of the experiment this solution was diluted to the final dose. A motor-driven infusion pump (Braun Meleunngen, Germany) was used to infuse a constant volume to each rat.

All experiments were started between 9:00 and 10:00 in the morning to minimize the contribution of diurnal rhythm in the effect measurements. Arterial blood pressure was measured from the cannula in the left femoral artery using a miniature strain gauge P10EZ transducer, connected to a plastic diaphragm dome (TA1017, Disposable Critilfo Dome) (both Vigg-Spektreaded B.V., Bilthoven, The Netherlands). The pressure transducer was connected to a polygraph amplifier console (RMP9018, Nihon Kohden Corporation, Tokyo, Japan). Heart rate was captured from the blood pressure signal. The signals were passed through a CED 1401 interface (Cambridge Electronic Design, Cambridge, United Kingdom) into a 80486 computer using the data acquisition program Spike 2 (Spike 2 Software, Version 3.1, Cambridge, England) and stored on diskette. During the experiments the cannula connected to the pressure transducer was flushed continuously with a 0.9% sodium chloride solution at a flow rate of 500 μl/hr (Syringe infusion pump 22, Harvard apparatus, Plato B.V., Dieren, The Netherlands) to ensure a continuous recording of the blood pressure.

Small arterial blood samples were drawn frequently for determination of SPA blood concentrations and measurement of metabolic parameters (NEFA, glycerol, glucose and insulin). A total number of 15 arterial blood samples was drawn for the determination of the pharmacokinetic profile of SPA. Blood sample volumes (20, 50 or 100 μl) depended on the expected blood concentration. Blood samples were hemolized directly in 400 μl water and kept on ice until storage at −20°C. For determination of NEFAs, glycerol and insulin, 24 small blood samples were drawn according to a predefined time schedule. A total blood volume of 70 μl was sampled into plastic tubes prefillied with 75 μl saline containing 1% (w/v) EDTA. After centrifugation the plasma was pipetted into a clean tube and stored at −20°C until analysis. Blood glucose concentrations were determined directly using a small drop of blood. After sampling the arterial line was flushed with a few microliters of saline containing 20 IU/ml heparin to prevent clotting.

Plasma protein binding. The P/B and f u of SPA were determined after intravenous administration of SPA to a group of lean and obese Zucker rats which had previously been used in the control experiment (minimal 2 weeks’ recovery). The concentration dependency of the plasma protein binding was examined by its determination at two different SPA concentrations. Blood samples with a volume of 1 ml were drawn at 15 and 45 min after administration of 100 μg SPA in 15 min and transferred directly to heparinized tubes on ice. An aliquot of 50 μl of blood was hemolized in 400 μl Millipore water. The remaining blood was centrifuged at 4°C to separate the plasma. A sample of 50 or 100 μl was retained for analysis and the remaining plasma was subjected to ultrafiltration. Free compound was separated from plasma protein bound compound by filtration of the supernatant at 1090 × g at 37°C using the Amicon Microparti-
tion System in combination with an YMT ultrafiltration membrane (Amicon Divisions, Danvers, MA). Unbound SPA concentrations were determined in 100 μl of the ultrafiltrate. Corresponding SPA concentrations in blood, plasma, and ultrafiltrate were determined in each sample using HPLC.

**Determination of SPA concentrations in blood.** The blood concentrations of SPA were determined by ion-pair reversed phase HPLC using UV detection (wavelength = 302 nm) as has been reported recently (Van Schaick et al., 1997a). Briefly, the HPLC system consisted of a Waters 510 solvent delivery pump (Millipore-Waters, Milford, MA), a WISP 710B automatic sample injector (Millipore-Waters), a Spectroflow 757 U.V. detector (Applied Biosystems, Ramsey, NJ) and a Chromatopack C-R2A reporting integrator (Shimadzu, Kyoto, Japan). A stainless-steel Microsphere C-18 cartridge column (100 mm, 4.6 mm I.D., 3 mm particle size) (Chrompack Nederland NV, Bergen Op Zoom, The Netherlands) equipped with a hand packed (Pellicular C-18 material, particle size 20–40 mm, Chrompack Nederland BV) guard column (20 mm, 2 mm I.D.) (Upchurch Scientific, Oak Harbor, WA). The mobile phase consisted of a mixture of 20 mM acetate buffer (pH 4) and acetonitrile in a ratio of 82/18 (v/v) to which 20 mM tetrabutyl-ammoniumhydroxide was added as ion-pairing reagent. At a flow rate of 0.5 ml/min the retention times of SPA and the internal standard (CPA) were 7 and 9 min, respectively.

The biological samples were prepared for analysis according to the procedure described below. The hemolyzed blood samples were mixed with 50 μl of internal standard (18 μg/ml CPA) and deproteinized by successive addition of 2 ml acetonitrile, mixing on a vortex and centrifugation at 2000 × g. After having transferred the supernatant to a clean tube, 200 μl acetonitrile buffer (20 mM, pH 4) and 50 μl 1 M hydrochloric acid were added. This mixture was extracted with 5 ml ethyl acetate on a vortex for 1 min. After centrifugation (10 min, 2000 × g) the organic phase was discarded. Following the addition of 50 μl of 1 M sodium hydroxide the aqueous layer was evaporated to dryness in a vacuum vortex at 40°C. The residue was dissolved in 100 μl acetonitrile buffer containing 20 mM TBAB and 50 μl was injected into the chromatographic system. Recovery exceeded 52%.

SPA blood concentrations were calculated using the SPA/CPA peak-height ratio in the calibration curve. The detection limit was 3 ng/ml (signal to noise ratio of 3). In the concentration range of 7.5 ng/ml to 500 ng/ml the within-day coefficient of variation was <4%. The between-day variation was <15%, 8% and 6% for 10, 300 and 600 ng/ml, respectively.

**NEFA and glycerol assays.** Plasma NEFA concentrations were determined using the Wako NEFA C-kit (Wako Chemicals GmbH, Neuss, Germany). Total volumes of sample and reagent were reduced to 50 and 100 μl by using a 96-well microtiterplate. The assay was linear in a concentration range of 0.025 to 0.3 mM. The within-day coefficients of variation were <7% and 5% for 0.025 and 0.2 mM, respectively. Between-day variation was <20% and 4% for 0.05 and 0.2 mM.

Glycerol concentrations in plasma were determined using an enzymatic colorimetric method (Randox glycerol kit, Randox Labortories, LTD, Ardmore, United Kingdom). The glycerol concentrations were calculated using a calibration curve (0.005–0.1 mM) run in parallel. Within this concentration range, the within-day and between-day coefficients of variation were <3% (0.005 and 0.075 mM) and 14% (0.01 and 0.075 mM), respectively.

**Determination of insulin and glucose.** Insulin concentrations were determined using the rat insulin RIA kit from Linco (Linco Research Inc., St. Louis, MO). Blood glucose concentrations were directly measured using a diagnostic glucose analyzer (Accutrend Alpha glucose analyzer, Boehringer Mannheim B.V., Almere, The Netherlands).

**Data analysis.** The pharmacokinetics and pharmacodynamics of SPA were quantified in individual rats. The blood concentration-time profiles of SPA were described by a biexponential function in both lean and obese rats using the nonlinear least-squares regression program Siphar (Simed SA, Creteil, France):

\[
C(t) = \frac{\sum_{i=1}^{2} C_i}{\lambda_i T} \cdot \left(1 - e^{-\lambda_i t}\right) \quad t \leq T \quad (1A)
\]

\[
C(t) = \frac{\sum_{i=1}^{2} C_i}{\lambda_i T} \cdot \left(e^{-\lambda_i T} - e^{-\lambda_i t}\right) \quad t > T \quad (1B)
\]

where \(C_i\) is the concentration at time \(t\), \(T\) is the infusion duration, \(C_i\) and \(\lambda_i\) are respectively the coefficients and exponents of the equation. Total blood clearance (Cl), the elimination half-life (\(t_{1/2,\text{e}}\)), and the volume of distribution at steady state (\(V_{\text{ss}}\)) were calculated following standard procedures, using the coefficients and exponents of the fitted function. The functions were fitted to the data with weight 1/y². In each individual rat the fitted function of the concentration-time profile was used to calculate the concentrations at the measured effect-time points.

Heart rate has been shown to be an appropriate pharmacodynamic index for the activation of cardiac \(A_1\) receptors (Mathôt et al., 1994). The relationship between the SPA blood concentrations and heart rate was described on the basis of the sigmoidal \(E_{\text{max}}\) model:

\[
E = E_0 + \frac{E_{\text{max}} \cdot C^n}{EC_{50}^n + C^n} \quad (2)
\]

where \(E\) is the observed effect at SPA blood concentration \(C\), \(E_0\) is the no-drug response, \(E_{\text{max}}\) is the maximal effect, \(EC_{50}\) is the blood concentration at half maximal effect and \(n\) is a constant expressing the shape of the concentration-effect relationship. The no-drug response values were obtained by averaging the heart rate effect from 300 to 360 min postdose. This value for \(E_0\) was fixed in the equation.

The relationship between SPA blood concentration and the anti-lipolytic effect was quantified by a physiological indirect response model, which described the change in NEFA concentrations as being an indirect response to the inhibition of the factors controlling it (Van Schaick et al., 1997a). The rate of change of the NEFA concentrations is described by:

\[
\frac{dN}{dt} = k_r \cdot f(C) - k_{out} \cdot N \quad (3)
\]

where \(k_r\) represents the zero-order rate constant for the synthesis of NEFAs, \(k_{out}\) the first-order rate constant for the elimination of NEFAs and \(N\) the plasma NEFA concentration. The function \(f(C)\) represents the fractional inhibitory effect according to the sigmoidal \(E_{\text{max}}\) model:

\[
f(C) = 1 - \frac{E_{\text{max}} \cdot C^n}{EC_{50}^n + C^n} \quad (4)
\]

where \(C\) is the SPA concentration, \(E_{\text{max}}\) the maximal inhibition of lipolysis, \(EC_{50}\) is the SPA concentration at half-maximal inhibition and \(n\) is the Hill-factor expressing the steepness of the curve. The NEFA data were fitted according to equation 5, which is an empirical solution of the differential equation (eq. 3):

\[
N(t) = N_0 \cdot (1 - f(C)) \cdot e^{-k_{out} \cdot t} + N_0 \cdot f(C) \quad (5)
\]

where \(N_0\) is the base-line NEFA concentration. It has been demonstrated that with this empirical solution identical values of the pharmacodynamic parameters are obtained as when fitting the data to the differential equation directly. The use of the empirical solution offers a number of practical advantages such as the possibility to constrain the value of certain parameters (such as \(N_0\)) to a fixed value (Van Schaick et al., in press). The equations were fitted to the data.
using the nonlinear least-squares regression program Siphar (Simed SA, Creteil, France).

**Statistical analysis.** The parametric one-way analysis of variance or Student’s t test was used to compare the pharmacokinetic and pharmacodynamic parameter estimates that were obtained in the different treatments. The corresponding nonparametric tests were used in case of nonhomogeneity of the data. Parameters for heart rate and NEFA that were obtained within the same individual rats were compared using a paired t test. A significance level of 5% was selected. All data are reported as mean ± S.E., unless indicated otherwise.

### Results

**Biological parameters.** At the age of 17 weeks the obese Zucker rats differed significantly from their lean littermates (table 1). During aging the weight of the homozygote fa/fa Zucker rats increased rapidly resulting in severe obesity and an increase in subcutaneous fat. At 17 weeks the total body weight of the obese rats was ~50% higher than that of the lean rats. Furthermore, the obese rats developed hyperinsulinemia, hyperlipidemia and mild hyperglycaemia (table 1). The increase in fasting blood glucose concentrations was only moderate but significantly different from the lean rats.

**Pharmacokinetics.** The time courses of the SPA concentrations are depicted in figure 1. The pharmacokinetic parameters as obtained in the lean and obese Zucker rats are summarized in table 2. Administration of 100 μg SPA to obese rats resulted in slightly lower blood concentrations than in the lean rats. As a consequence the AUC was significantly lower in the obese rats (65 ± 7 μg/min/ml) in comparison to the lean rats (103 ± 14 μg/min/ml). The average total blood clearance and apparent volume of distribution at steady state were 1.2 ± 0.2 and 1.6 ± 0.1 ml/min and 88 ± 10 and 110 ± 7 ml for lean and obese rats, respectively. In both groups of rats the average terminal half-life of SPA was 55 ± 3 min.

The P/B and fu of SPA were determined in a separate group of lean and obese rats. The averaged values are summarized in table 2. The free fraction of SPA in plasma was significantly lower in the obese rats (65 ± 7 μg/min/ml) in comparison to the lean rats (103 ± 14 μg/min/ml). The average total blood clearance and apparent volume of distribution at steady state were 1.2 ± 0.2 and 1.6 ± 0.1 ml/min and 88 ± 10 and 110 ± 7 ml for lean and obese rats, respectively. In both groups of rats the average terminal half-life of SPA was 55 ± 3 min.

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**Hemodynamic effects.** To investigate alterations in hemodynamic responses, the bradycardic and hypotensive effects of SPA were assessed in both groups of rats. The time course of the effect on heart rate after administration of SPA or vehicle is depicted in figure 2. Administration of the vehicle produced no effect on either heart rate or blood pressure.

**TABLE 1**

| Characteristic differences between lean and obese Zucker rats at the age of 17 weeks |
|---------------------------------|---------------------------------|
| Lean Zucker rats                | Obese Zucker rats               |
| Weight (g)                      | 320 ± 6                         | 466 ± 10*                       |
| NEFA (μM)                       | 0.40 ± 0.01                     | 0.94 ± 0.06*                    |
| Glycerol (mM)                   | 0.10 ± 0.006                    | 0.39 ± 0.04*                    |
| Insulin (ng/ml)                 | 0.38 ± 0.03                     | 3.5 ± 0.2*                      |
| Glucose (mM)                    | 5.4 ± 0.15                      | 6.55 ± 0.13*                    |
| HR (bpm)                        | 395 ± 10                        | 393 ± 12                        |
| MAP (mm Hg)                     | 108 ± 3                         | 110 ± 4                         |

* Values significantly different between lean and obese rats (t test; P < .001). Mean ± S.E., n = 13–14.

**TABLE 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean Zucker rats</th>
<th>Obese Zucker rats</th>
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<tbody>
<tr>
<td>CI (ml/min)</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.1*</td>
</tr>
<tr>
<td>Vdss (ml)</td>
<td>88 ± 10</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>t1/2,n (min)</td>
<td>103 ± 14</td>
<td>65 ± 7*</td>
</tr>
<tr>
<td>AUC (μg min/ml)</td>
<td>2.0 ± 0.2</td>
<td>1.8 ± 0.06</td>
</tr>
<tr>
<td>P/B (%)</td>
<td>50% higher</td>
<td>110% higher</td>
</tr>
<tr>
<td>fu (%)</td>
<td>55% higher</td>
<td>46% higher</td>
</tr>
</tbody>
</table>

CL, clearance; Vdss, volume of distribution at steady state; t1/2,n, terminal half-life; AUC, area under the concentration-time curve; P/B, plasma-to-blood ratio; fu, free fraction in plasma.

* Values from lean and obese Zucker rats were significantly different (Student’s t test; P < .05). Mean ± S.E., n = 7.

**Fig. 1.** Plasma concentration vs. time profiles of SPA after intravenous infusions of 100 μg during 15 min to lean and obese Zucker rats; the infusion period is indicated by the solid bar; data are presented as mean ± S.D. and the fitted lines are the averages of 7 curves.

**Fig. 2.** Time course of heart rate after i.v. administration of 100 μg SPA during 15 min to conscious lean (●) and obese Zucker rats (■); effect of administration of the vehicle to lean (○) and obese rats (□); data are presented as mean ± S.E. of 6–7 animals; the horizontal bar represents the infusion period.

The time course of the SPA-mediated bradycardic effect was significantly different between both group of rats (t test; P < .05). Administration of SPA resulted in a similar maximal decrease in heart rate. However, the bradycardic effect lasted longer in the lean rats than in the obese rats. This longer duration of action was in agreement with the larger AUC in the lean rats.

No differences were observed in base-line heart rate and blood pressure between lean and obese Zucker rats. Base-line MAP values were 107 ± 3 and 106 ± 3 mm Hg (mean ± S.E., n = 7) in lean and obese rats, respectively (data not shown). In contrast to the severe reduction in heart rate (reduction of...
49% and 47% of base line), the reduction in MAP was limited (21% and 17% of base line). Furthermore, the blood pressure-time profiles of the lean and obese rats were superimposable (data not shown).

The individual biexponential pharmacokinetic fits were used to estimate SPA blood concentrations at the time points of the heart rate data. The bradycardic effect was related directly to blood agonist concentrations and the concentration-effect relationships were described according to the sigmoidal E_{max} model. The concentration-heart rate curves of a typical lean and obese rat are shown in figure 3. The estimated pharmacodynamic parameters are depicted in table 3. None of the parameters were significantly different (t test). The potency (EC_{50}) and intrinsic activity (E_{max}) of SPA was similar in both lean and obese and these values were comparable to those found previously in Wistar rats (Van Schaick et al., 1997a).

**Inhibition of lipolysis.** Plasma NEFA and glycerol concentrations were measured as indicators of inhibition of lipolysis. Adenosine A_{1} receptor-mediated inhibition of lipolysis resulted in a decrease in ambient NEFA or glycerol concentrations. The time profiles of the NEFA concentrations that were observed in both lean and obese animals are depicted in figure 4. In contrast to heart rate, the NEFA profiles were different between the lean controls and the obese diabetic animals. SPA elicited a prolonged reduction in NEFA concentrations in the lean Zucker rats. Typically, the NEFA concentrations decreased slowly and reached a maximal reduction of ~73% after 30–40 min. This maximal effect was maintained over a long period and returned to base-line values at low SPA concentrations. A slight increase in NEFA concentrations was observed in the vehicle-treated group of lean rats.

These antilipolytic effect profiles in the lean rats could be adequately described by the indirect response model. In this model the delay between concentrations and effect is accounted for by the slow elimination of NEFAs from blood and the inhibition of lipolysis is characterized by the sigmoidal E_{max} model. The pharmacodynamic parameters are summarized in table 4. In the lean rats the EC_{50} for the antilipolytic effect (18 ± 3 ng/ml) was ~5-fold lower than the EC_{50} for the reduction in heart rate (99 ± 12 ng/ml).

![Fig. 3. Concentration-heart rate relationships of SPA in an individual lean and obese Zucker rat that were given 100 μg in 15 min; the solid line represents the best fit of the data according to the sigmoidal E_{max} model; no-drug heart rate values were 374 bpm and 388 bpm for the lean and obese rat, respectively.](image)

![Fig. 4. Time profiles of the plasma NEFA concentrations in lean (top) and obese Zucker rats (bottom) after intravenous infusions of 100 μg SPA during 15 min or vehicle (765 ml saline) during 15 min; data are reported as mean ± S.E., n = 6–7).](image)

<table>
<thead>
<tr>
<th></th>
<th>E_{0} (bpm)</th>
<th>E_{max} (ng/ml)</th>
<th>EC_{50} (ng/ml)</th>
<th>EC_{50,u} (ng/ml)</th>
<th>Hill factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>386 ± 19</td>
<td>-191 ± 16</td>
<td>99 ± 12</td>
<td>80 ± 10</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Obese</td>
<td>393 ± 17</td>
<td>-185 ± 22</td>
<td>118 ± 17</td>
<td>114 ± 16</td>
<td>2.2 ± 0.5</td>
</tr>
</tbody>
</table>

No significant differences were observed between the parameters. Mean ± S.E., n = 7.

Base-line NEFA concentrations were 2-fold higher in the obese rats than in the lean rats (table 1). In both the SPA and vehicle treated obese Zucker rats a biphasic effect on plasma NEFA concentrations was observed. Before the start of the infusion plasma NEFA concentrations increased in both treatment groups. The increase lasted until 20 min after start of the SPA infusion, after which the NEFA levels decreased until a lower base-line level was reached. This decrease, however, progressed significantly faster in the SPA treated rats.

For all treatment groups the total area under the effect-time curve (AUE) was calculated as measure for the antilipolytic effect of SPA (fig. 5). This area was calculated from the effect values after subtraction of the placebo effect profiles. In figure 5 the AUEs are plotted as relative values to allow comparison of effects parameters with different units of measure (100 would indicate 100% effect over the entire time span of the experiment). In the lean rats lipolysis was more effectively reduced, resulting in a larger AUE (65% vs. 25% for the lean and obese rats, respectively). A clearer effect of SPA on lipolysis was observed after subtraction of the placebo-effect from the SPA-effect (fig. 6). The relative decrease in
The purpose of the present study was to investigate differences in pharmacokinetics and pharmacodynamics after

plasma NEFA concentrations was less pronounced and lasted shorter in the obese Zucker rats. The time-courses of the NEFA concentrations that were obtained after subtraction of the placebo effect were described on the basis of the indirect response model. The estimated pharmacodynamic parameters are summarized in table 4. In the obese Zucker rats the value for \( k_{\text{out}} \) was fixed to the value obtained in

modelling of the effect in the lean rats. In the obese rats the \( EC_{50} \) and \( E_{\text{max}} \) were significantly different from the lean rats. Figure 7 shows the averaged concentration-effect relationships for the effect on heart rate and NEFA in the lean and obese rats. The concentration-heart rate relationships were identical, whereas the concentration-antilipolytic effect relationship of SPA in the obese rats was shifted to higher concentrations. Furthermore, the maximum effect was significantly lower.

The effect of SPA on plasma glycerol levels was identical to the effect on NEFA concentrations (see fig. 5). In both the lean and obese rats the glycerol concentrations were lower than the NEFA concentrations. For this parameter of lipid metabolism the same biphasic response was observed in both placebo and SPA-treated obese rats (data not shown).

**Effect on insulin and glucose.** The effect of SPA or vehicle administration on ambient blood glucose levels and plasma insulin concentrations are summarized in table 5. Administration of the vehicle did not affect glucose and insulin levels, whereas administration of SPA resulted in a significant increase in blood glucose concentrations in both groups of rats. The glucose concentrations normalized towards the end of the experiment. At the same time, the plasma insulin concentrations decreased in the SPA-treated animals. Insulin concentrations decreased from 3.5 \( \pm \) 0.2 to 1.1 \( \pm \) 0.2 ng/ml in the obese Zucker rats. In the lean rats fasted insulin concentrations were low and near the detection limit of the assay. Due to these low concentrations the apparent decrease in insulin concentrations could not be adequately quantified. The decrease in insulin concentrations is likely to be a direct effect of SPA on excretion of insulin since the effect progressed faster than the effect on NEFA concentrations. In a pilot experiment, pharmacodynamic parameters for the effect on insulin concentrations were derived by relating the time-course of the insulin concentrations to the concomitant concentration-time profile of SPA. Preliminary parameter estimates for \( EC_{50}, E_{\text{max}} \), and \( k_{\text{out}} \) for the effect on insulin concentrations were 38 \( \pm \) 13 ng/ml, 69 \( \pm \) 6% and 0.35 \( \pm \) 0.06 min\(^{-1}\) (mean \( \pm \) S.E., \( n = 3 \)). The value for \( k_{\text{out}} \) is in agreement with an elimination half-life for insulin of \( \sim 2 \) min (Cañas et al., 1995).

**Discussion**

The purpose of the present study was to investigate differences in pharmacokinetics and pharmacodynamics after

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**Table 4**

Pharmacodynamic parameter estimates of N\(^{-6}\)-(p-sulfophenyl)adenosine (SPA) for the reduction in plasma NEFA concentrations after i.v. infusion of 100 \( \mu \)g in 15 min to fasted Zucker rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (n = 10)</th>
<th>Obese (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N_0 ) ( \text{mg} )</td>
<td>( 0.41 \pm 0.01 )</td>
<td>( 0.99 \pm 0.08 )</td>
</tr>
<tr>
<td>( E_{\text{max}} ) ( \text{mg} )</td>
<td>( 73 \pm 3 )</td>
<td>( 21 \pm 3 )</td>
</tr>
<tr>
<td>( EC_{50} ) ( \text{ng/ml} )</td>
<td>( 18 \pm 3 )</td>
<td>( 17 \pm 2 )</td>
</tr>
<tr>
<td>( EC_{50,u} ) ( \text{ng/ml} )</td>
<td>( 14 \pm 3 )</td>
<td>( 2.5 \pm 0.4 )</td>
</tr>
<tr>
<td>Hill factor</td>
<td>( 3.5 \pm 0.9 )</td>
<td>3.9 ( \pm ) 1.5</td>
</tr>
<tr>
<td>( k_{\text{out}} ) ( \text{min}^{-1} )</td>
<td>( 0.094 \pm 0.009 )</td>
<td>0.08 ( \pm ) 0.02</td>
</tr>
</tbody>
</table>

* Different from values in lean rats (P < .05).
* Different from maximal effect in lean animals (P < .001; t test).
* Different from estimates based on plasma concentrations (P < .01; paired t test).
* Values are fixed in the modelling procedure.
* Maximal reduction as percentage of base line.

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**Figure 5**

Average areas under the effect curves (AUE) for the effect of SPA on heart rate, NEFA and glycerol; AUE were calculated in the period 0–360 min for each effect measure and presented as percentage of effect in placebo group.

**Figure 6**

The effect of SPA on plasma NEFA concentrations after correction of the values for the effect observed in the placebo treated group; the data are presented as mean \( \pm \) S.E. (n = 6–7) for lean and obese Zucker rats, respectively. The solid line represents the fit of the pooled data to the indirect response model.
acute administration of the adenosine A₁ receptor agonist SPA to either lean or obese Zucker rats. The obese Zucker rats served as animal model for insulin-resistance in type 2 diabetes mellitus (Terrattaz et al., 1986). The animals were shown to develop severe hyperinsulinemia, increased NEFA and glycerol concentrations and mild hyperglycaemia (table 1). These metabolic changes are in line with previous reports (McCaleb and Sredy, 1992) and resemble the abnormalities observed in human NIDDM (Karasik and Hattori, 1994).

Obesity, independent of glucose intolerance, has been related to insulin-resistance (Bogardus et al., 1985) and is frequently observed in NIDDM patients. Apparently, the coexistence of obesity and NIDDM augments the underlying defect of insulin-resistance. Obesity causes physiological changes that may influence the disposition of drugs (Jaber et al., 1996). In the obese rat clearance and apparent volume of distribution at steady state were significantly larger than in the lean rats (P < .05). These differences resulted in a lower AUC in the obese rats and overall lower concentrations of SPA.

The observed differences in pharmacokinetics were small and not proportional to the differences in body weight. In the obese rats the increase in bodyweight is mainly caused by an excessive increase in adipose tissue. Since SPA is a highly hydrophilic adenosine receptor agonist (Jacobson et al., 1992) it is unlikely for this compound to accumulate in fat tissue. A correction of the pharmacokinetic parameters on the basis of lean body mass (LBM) could be more appropriate (Jusko and Chiang, 1982). Additionally, a slightly lower plasma protein binding of SPA was observed in the obese Zucker rats which is consistent with the possible competition of free fatty acids for binding to plasma proteins (Frayn et al., 1996).

The observed reduction in heart rate is in agreement with activation of adenosine A₁ receptors (Mathôt et al., 1994). In both groups of rats the base-line hemodynamic parameters were similar. Although mild hypertension has been observed in obese Zucker rats (Kurtz et al., 1989; Zemel et al., 1992), no differences in blood pressure and heart rate values were observed in the present experiment (table 1). The maximal reduction in heart rate upon administration of SPA was similar in both groups of rats. The duration of the bradycardia, however, was longer lasting in the lean rats which is consistent with the higher SPA concentrations observed in the lean rats.

In each individual rat the reduction in heart rate was related to individual SPA blood concentrations and adequately described on the basis of the sigmoidal E_{max} model. Recently, this approach has been successfully used to quantify the effects of analogues of CPA (Mathôt et al., 1995). The derived estimates of potency and intrinsic activity were shown to reflect activation of the adenosine A₁ receptor in vivo. In this respect it should be taken into consideration that SPA is only moderately selective for A₁ adenosine receptors (Jacobson et al., 1992). However at the dose selected in the present investigation (100 μg/kg) concentrations of the drug are such that no activation of A₂a receptors occurs (Van Schaick et al., 1997b). This is important since such receptor activation might have contributed to the observed haemodynamic effect.

No differences were observed between the pharmacodynamic parameters for heart rate of the lean and obese rats (table 3). Apparently, the concentration-heart rate relationship of the adenosine agonist was not influenced by disease induced patho-physiological changes (fig. 7). Thus, the difference in the time course of the bradycardic effect was not caused by a different response, but by alteration in pharmacokinetics. This illustrates, therefore, that these pharmacokinetic differences should preferably be accounted for in investigating in vivo drug action (Levy, 1985).

The estimates of potency (EC_{50}) and intrinsic activity (E_{max}) of SPA for heart rate are in line with estimates obtained in Wistar rats previously (Van Schaick et al., 1997a). Although pharmacokinetics and pharmacodynamics may differ between species (including man), integrated PK-PD parameters have been found to be more similar between species.

### TABLE 5

<table>
<thead>
<tr>
<th></th>
<th>Lean, vehicle</th>
<th>Lean, SPA</th>
<th>Obese, vehicle</th>
<th>Obese, SPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>ng/ml</td>
<td>0.38 ± 0.05</td>
<td>0.32 ± 0.06</td>
<td>5.7 ± 0.2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.40 ± 0.06</td>
<td>&lt;0.24</td>
<td>5.4 ± 0.3</td>
<td>10.4 ± 0.9</td>
</tr>
<tr>
<td>Post</td>
<td>3.7 ± 0.6</td>
<td>3.3 ± 0.3</td>
<td>6.7 ± 0.3</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>Lean, SPA</td>
<td>3.5 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>6.4 ± 0.2</td>
<td>16 ± 0.9</td>
</tr>
<tr>
<td>Obese, SPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*S* Significant different from preadministration values (paired *t* test; *P* < .01).

Mean ± S.E., *n* = 7.
The pharmacokinetic parameters $V_{ass}$ and clearance differed 2-fold between the Wistar and Zucker rats, whereas EC$_{50,u}$ values (EC$_{50}$ based on free drug concentrations) were almost identical ($88 \pm 21$ ng/ml, $80 \pm 10$ ng/ml and $111 \pm 14$ ng/ml in Wistar, and lean and obese Zucker rats, respectively).

The metabolic differences between the lean and obese rats were expected to lead to alterations in antilipolytic effects. Indeed, although the rats were treated under identical experimental conditions, the time courses of the NEFA and glycerol concentrations were totally different. In the lean Zucker rats the base-line NEFA concentrations were stable and effectively suppressed by SPA (fig. 4). These observed antilipolytic effects were consistent with activation of adenosine A$_1$ receptors in adipose tissue (Strong et al., 1993).

Typically, the onset of the reduction in NEFA concentrations was slow. In contrast to heart rate, this effect is not directly related to blood SPA concentrations, since the reduction in NEFA concentrations is not only determined by the inhibition of NEFA release (lipolysis) but also by the elimination of fatty acids from the circulation. This effect can be described by an indirect response model (Jusko and Ko, 1994) in which the delay between agonist concentrations and measured effect is accounted for by the elimination rate-constant ($k_{out}$) of the physiological substance (NEFAs). Recently, the applicability of this indirect response model was demonstrated by the observation that the parameter estimates were independent of the administered dose. Furthermore, the model was validated on the bases of a comparison of the value of the model parameter $k_{out}$ with the elimination rate constant of glycerol upon exogenous administration (Van Schaick et al., in press).

The indirect response model adequately predicted the time-course of the NEFA effect in individual lean rats. The relationship between SPA concentrations and inhibition of lipolysis was characterised by the sigmoidal $E_{max}$ equation. Similarly to heart rate, estimates for potency and intrinsic activity were obtained for the antilipolytic effect of SPA. The EC$_{50}$ value of SPA for the inhibition of lipolysis was $18 \pm 3$ ng/ml and thus 5-fold lower than the EC$_{50}$ value for heart rate. This difference in potency between the effects had been observed previously (Van Schaick et al., 1997a) and is consistent with differences in receptor-effector coupling between tissues. At high SPA concentrations a small amount of NEFAs remained present in the circulation ($E_{max} = 73 \pm 3\%$; reduction from base line) (Strong et al., 1993).

In the obese Zucker rat the time-profile of the NEFA and glycerol concentrations were complex. In contrast to the lean rats, the base-line NEFA and glycerol levels were unstable in obese rats receiving placebo. Initially an increase was observed, which was subsequently followed by a decrease. At present it is not completely understood, why there is such a pronounced base-line effect. In principle stress is minimal under the conditions of the experiment. However it cannot be completely excluded that the observed change in base-line NEFA levels is caused by handling of the rats and repeated blood sampling. Possibly the regulatory control mechanisms which control the NEFA blood levels in lean rats are less efficient in obese Zucker rats. Thus the observed fluctuations in NEFA levels may reflect the disregulated glucose and lipid metabolism in the diabetic animal (McCaleb and Sredy, 1992) and their inability to cope with even minor stress (Goetsch et al., 1993).

In order to compare the total effect of SPA between the lean and obese Zucker rats the areas under the effect curves (AUE) were calculated for the period of 0–360 min. To allow comparison of effect with different units, each effect was expressed as percentage of initial base-line (prior to administration). Subsequently, the areas were calculated from the effect values after correction for placebo effect over the period of 360 min. For heart rate the AUE between the lean and obese rats were almost equal. For the antilipolytic effect, however, a significantly lesser effect (lower AUE) was observed in the obese rats in comparison to the lean animals. Subtraction of the placebo effects from the active NEFA values in the SPA treated lean and obese rats, more clearly revealed the pharmacological effect of SPA (fig. 6). The indirect pharmacodynamic response model was used to quantify these corrected response profiles and to obtain concentration-antilipolytic effect relationships in both lean and obese rats.

In the lean Zucker rats, the pharmacodynamic parameter estimates were similar to the estimates obtained without the correction for the base-line effect (slightly different $E_{max}$ and hill factor) (table 4). Description of the effect in the obese rats was more difficult due to larger intra-individual variation. To avoid an incorrect estimation of the value for $k_{out}$, this parameter was fixed to the elimination rate-constant in the lean rats. In the obese rats both the potency and intrinsic activity for the effect on NEFA concentrations was reduced (fig. 7). The EC$_{50}$ for the antilipolytic effect (109 ng/ml) was similar to the EC$_{50}$ for heart rate (118 ng/ml). Thus, the favourable 5-fold selectivity between heart rate and NEFA was not observed in the obese rats. Furthermore, the intrinsic activity ($E_{max}$) of SPA appeared to be reduced in the obese animals.

Previous studies have demonstrated that there are no differences in ligand affinity and A$_1$ receptor density between adipocytes of lean and obese animals (Vannucci et al., 1990). An impaired coupling between receptor and G protein may account for the reduction in intrinsic activity and potency observed in the present study. Indeed, abnormalities in the coupling of adenosine receptors and G protein have been associated to the decreased insulin sensitivity in NIDDM (Green, 1987) and a reduction in the amount of $G_{11}$ has been observed in adipocytes of obese Zucker rats and human subjects (Kaartinen et al., 1994). Moreover, the blunted antilipolytic effect of SPA is in agreement with studies in isolated adipocytes from obese patients, in which high levels of lipolysis and low sensitivity to R-PIA were observed (Ohisalo et al., 1986).

To our knowledge there are only a few studies that compare the antilipolytic responses of adenosine agonists in normal and diabetic animals. The A$_1$ receptor agonist R-PIA has been reported to be a more sensitive inhibitor of hormone-stimulated lipolysis in adipocytes of obese Zucker rats in comparison to those of lean rats (Vannucci et al., 1989), whereas the opposite has been reported for R-PIA and CPA in adipocytes of streptozotocin (STZ)-induced diabetic rats (Cox et al., 1997). Interpretation of these results, however, is difficult since responses towards lipolytic hormones (e.g. norepinephrine, isoproterenol) have been reported to be attenuated in diabetic animals as well (Vannucci et al., 1989; Cox et al., 1997). Furthermore, the adenosine A$_1$ receptor has been re-
ported to be tonically active in the absence of agonists (LaNoe and Martin, 1994), and may in this way influence the response towards exogenously administered agonists. Although these in vitro results are difficult to compare to our in vivo results, they indicate that adaptive changes of the adenosine receptor function may occur and play a role in the disregulated lipolysis in obesity and diabetes. The decrease in insulin concentrations observed in the obese animals further complicated the identification of the exact role of the A₁ receptor in the altered antilipolytic response. In the obese rats, the plasma insulin concentrations were significantly decreased (table 5). This reduction seemed to be a direct and sensitive effect of SPA (EC₅₀ = 38 ± 18 ng/ml) and may reflect inhibition of insulin release from the pancreas (LeBlanc and Soucy, 1994). The higher EC₅₀ in the obese Zucker rat may therefore be caused by a combined interaction of the adenosine agonist and endogenous insulin. The reduction in insulin concentrations may stimulate lipolysis and as such counteract the response of SPA in the obese Zucker rat.

Although the obese Zucker rat is primarily a model for obesity, it has been reported to be a useful model for the first stages in human NIDDM (Karasaki and Hattori, 1994) which are characterized by insulin resistance and hyperinsulinaemia. Adenosine receptor agonists would be able to improve insulin resistance but may be relatively ineffective due to their inhibition of insulin release. Low efficacy agonists may be more useful due to their ability to display organ selective effects (Ijzerman et al., 1994). In later stages of NIDDM, the ability of the pancreas to overproduce insulin is lost resulting in hypoinsulinaemia. This situation is more adequately mimicked by the STZ-induced diabetic rat. In a recent study by Cox and coworkers (Cox et al., 1997), adenosine A₁ agonists were shown to be less potent in adipocytes from STZ-induced diabetic rats. Furthermore, oral administration of the CPA antagonist, was reported to be tonically active in the absence of agonists (LaNoe and Martin, 1994), and may in this way influence the response towards exogenously administered agonists. Although these in vitro results are difficult to compare to our in vivo results, they indicate that adaptive changes of the adenosine receptor function may occur and play a role in the disregulated lipolysis in obesity and diabetes. The decrease in insulin concentrations observed in the obese animals further complicated the identification of the exact role of the A₁ receptor in the altered antilipolytic response. In the obese rats, the plasma insulin concentrations were significantly decreased (table 5). This reduction seemed to be a direct and sensitive effect of SPA (EC₅₀ = 38 ± 18 ng/ml) and may reflect inhibition of insulin release from the pancreas (LeBlanc and Soucy, 1994). The higher EC₅₀ in the obese Zucker rat may therefore be caused by a combined interaction of the adenosine agonist and endogenous insulin. The reduction in insulin concentrations may stimulate lipolysis and as such counteract the response of SPA in the obese Zucker rat.

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peripheral insulin resistance as evidenced by euglymic clamps in genetically obese fa/fa rats. Endocrinology 188:674–678.


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