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Effects of the Oral Administration of a β_3-Adrenergic Agonist on Lipid Metabolism in Alloxan-Diabetic Rats

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Abstract

Previous studies have reported that β_3-adrenergic agonists regulate plasma glucose, triglycerides and free fatty acids in situations of hyperglycaemia and dyslipidaemia in rodents. In this study Trecadrine, a novel compound with affinity for β_3-adrenergic receptors, has been tested in an alloxan-induced model of hyperglycaemia in rats.

Alloxan-induced hyperglycaemic rats were orally treated with Trecadrine (1 mg/kg/day for 4 days), resulting in an improvement of hyperglycaemia (from 16·6 to 8·3 mmol L^{-1}, $P < 0{\cdot}001$). This effect was not associated with statistical differences in plasma insulin levels, which may be explained by changes in insulin resistance and carbohydrate oxidation in peripheral tissues. Furthermore, a reduction in internal white fat weight (−39%), which was not statistically significant, as well as in plasma triglycerides (from 1·89 to 0·33 mmol L^{-1}, $P < 0{\cdot}001$) and free fatty acids (from 0·70 to 0·39 mmol L^{-1}, $P < 0{\cdot}001$), was found after Trecadrine administration. Trecadrine apparently induced lipolytic activity in adipocytes, as suggested by the increase of oxygen consumption in white adipose tissue ($+282\%$, $P < 0{\cdot}001$), while free fatty acids decreased apparently through their utilisation in other tissues. Furthermore, the increase in brown adipose tissue oxygen consumption ($+50\%$, $P < 0{\cdot}01$) and in rectal temperature ($P < 0{\cdot}05$) suggests that both glucose and fatty acid oxidation may be enhanced in this tissue.

These results give support to the possible therapeutic use of β_3-adrenergic compounds in situations of hyperglycaemia, particularly when this is accompanied by hypertriglyceridaemia.

Different studies have shown that β_3-adrenergic agonists may play an important regulatory role in adipose tissue metabolism. For example, it is known that these agents stimulate adipose tissue thermogenesis through an increase in the mitochondrial uncoupling protein UCP1 expression in both brown (Arbeeny et al 1995) and white (Nagase et al 1996) adipose tissues, as well as through an increase in UCP3 (Gong et al 1997; Savontaus et al 1998), which involves a rise in energy dissipation as heat. Moreover, β_3-adrenoceptors markedly stimulate lipolysis in white adipose tissue (Liu & Stock 1995) with a subsequent release of fatty acids.

β_3-adrenergic agonists also reduce body weight and fat content, as well as increasing resting metabolic rate and energy expenditure when administered to diet-induced (Berraondo et al 1997) and genetically obese rodents (Grujic et al 1997). In lean animals, these agents improve glucose tolerance, at least in part, by reversing insulin resistance in fat tissues (De Souza et al 1997), although they do not induce hypoglycaemia. Furthermore, some researchers have found a direct stimulation of insulin release on pancreatic-islet β-cells in the perfused mouse pancreas via β_3-adrenoceptors (Yoshida 1992), while other studies have shown an increase in glucose uptake through the mediation of the β_3-adrenoceptors occurring in skeletal muscle independently of the action of insulin (Abe et al 1993; Liu et al 1996).

However, the antidiabetic action of these agents is not yet well understood. In particular, there are no conclusive data on the interactions between the β_3-adrenergic agonist-induced effects on glucose and lipid metabolism in diabetes. For example, in obese Zucker (*fa/fa*) rats, used as a model of insulin resistance, chronic treatment with β_3-adre-

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nergic agonists did not change plasma glucose, insulin and triglycerides levels, although insulin action on adipose tissue was promoted (Virtanen et al 1997). On the other hand, in *ob/ob* mice a chronic treatment with β_3-adrenergic agonists resulted in a marked decrease in plasma glucose, triglycerides and free fatty acids, but insulin levels remained unaltered (Arbeeny et al 1995).

In this context, few studies have used a model of insulinopenic diabetes in animals. Furthermore, most studies have focused on the hypoglycaemic effect of β_3-adrenergic agonists (Sennitt et al 1985) or their action on insulin release (De Souza et al 1997). For example, chronic administration of a β_3-adrenergic agonist in streptozotocin-induced diabetic rats caused a decrease in plasma glucose levels, but no effects were found on tissue glucose utilisation and plasma free fatty acids (Ferré et al 1992). Although hypertriglyceridaemia is one of the main alterations induced by insulinopenic diabetes, apparently no studies have paid attention to the effects of β_3-adrenergic agonist administration on lipid metabolism in a model of this disease in animals.

The purpose of this study was to examine the effects of a β_3-adrenergic agonist, Trecadrine, a diphenylmethylene-ethylamine derivative whose formula and affinity for β_3-adrenoceptors (Barrionuevo et al 1996), as well as its effect on sugar absorption (Díez-Sampedro et al 1997), have previously been reported, on several indicators of lipid metabolism in a rat alloxan-induced diabetes model.

Materials and Methods

Animals and experimental protocol

Female Wistar rats (180–200 g) supplied by the Centre of Applied Pharmacology (CIFA, Pamplona, Navarra, Spain) were used. The care of the rats was in accordance with the rules of the local and national animal committees. Rats were housed in individual metabolic cages in a temperature-controlled room ($22 \pm 2^{\circ}$C), with a 12-h light–dark cycle (lights on from 07:00 h). Standard laboratory chow and tap water were available ad libitum. Rats were randomly assigned into two groups: rats in the first group (n = 19) were injected with a single dose of alloxan (120 mg kg^{-1}, s.c.) in order to develop hyperglycaemia and, after 2 days, a subgroup of them (β_3-treated group, n = 10) were orally administered by gavage with Trecadrine (1 mg kg^{-1}) once a day for 4 days, while the remaining diabetic rats (diabetic group, n = 9) received a vehicle. Control rats (n = 8) did not receive either alloxan or Trecadrine, but a placebo (0·9% NaCl).

In-vivo measurements

Rectal temperature was measured with a YSI 432 probe (Yellow Springs Instruments Co. Inc., Yellow Springs, Ohio, USA) coupled to a Panlab 0331 thermometer (Panlab s.l., Barcelona, Spain) every 20 min after β_3-agonist oral administration. After 4 days of treatment, body composition was measured with a non-invasive electromagnetic device (EM-SCAN model SA-2, Springfield, Illinois, USA), which offers adequate accuracy ($< 3\%$) and repeatability ($< 1\%$) as described elsewhere (Morbach & Brans 1992). After 4 days of treatment, animals were fasted overnight, killed by decapitation and then bled. The following tissues and organs were immediately dissected and weighed: interscapular brown adipose tissue, perirovaric, retroperitoneal, and mesenteric white adipose tissue (internal white adipose tissue), gastrocnemius and liver.

Other analyses

Plasma glucose was determined by an enzymatic colorimetric method (Spotchem, Menarini, Firenze, Italy). Plasma insulin concentration was measured with a radioimmunoassay kit with proved high specificity to rat insulin (Insik-5, Sorin Biomedica, Saluggia, Vercelli, Italy). Plasma triglycerides were determined by an enzymatic colorimetric assay (BM-Hitachi system 717, Boehringer Mannheim, Barcelona, Spain). Plasma free fatty acids were measured with an enzymatic colorimetric method (Wako NEFA C test kit, Wako Chemicals, Neuss, Germany).

Oxygen consumption in white and brown adipose tissues was immediately measured after dissection with a Clark-type oxygen electrode (YSI model 5300, Yellow Springs Instruments Co. Inc., Yellow Springs, Ohio, USA). A sample of tissue (0·1 g) was minced with scissors and suspended in 5 mL of Krebs–Ringer phosphate buffer (pH 7·4) containing 6 mM glucose, as described elsewhere (Fain 1975). Samples were incubated for 10 min in continuously rotating round-bottomed 10-mL flasks at 37°C.

Lipoprotein lipase (E.C.3.1.1.34) activity was determined in frozen gastrocnemius by a fluorimetric assay (Del Prado et al 1993), using dibutyrilfluorescein as substrate for the enzyme, and the fluorescein released was measured. The activity of this enzyme is expressed as nanomoles of fluorescein released per minute per milligram of protein.

Muscle protein content was measured by the method of Bradford (1976). Plasma leptin levels were determined by ELISA-sandwich (Martínez-Ansó et al 1998).

Statistical analysis

All data were reported as mean ± s.e.m. Comparisons between groups were initially made using analysis of variance (or Wilcoxon signed-rank test in the case of rectal temperature) and the Fisher test was used for post hoc comparisons. Values of $P < 0.05$ were considered significant.

Results

Although the body weights of the three groups of rats did not differ significantly initially, both treated and non-treated diabetic rats had lost weight when compared to control rats by the end of the experimental trial (Table 1). Decreases in brown and white adipose tissue weight, and in gastrocnemius muscle weight were also observed after the administration of alloxan. However, increases in interscapular brown adipose tissue and gastrocnemius weights were found after treatment with Trecadrine of hyperglycaemic rats as compared with untreated hyperglycaemic animals. Moreover, slight (although not statistically significant) decreases in body fat mass and internal white adipose tissue weight were observed.

On the other hand, the marked hyperglycaemia found in the diabetic group (Table 1) was recovered after Trecadrine administration for 4 days (from 16·6 to 8·3 mmol L^{-1}, $P < 0.001$). This effect on circulating glucose was not accompanied by changes in plasma insulin levels. Nevertheless, the insulin–glucose ratio is markedly lower in the non-treated diabetic rats when compared with both control and β_3-treated diabetic groups ($P < 0.001$).

Data concerning lipid metabolism (Table 2) reveal a significant decrease in plasma triglyceride levels after Trecadrine administration for 4 days (from 1·89 to 0·33 mmol L^{-1}, $P < 0.001$). Likewise, although plasma free fatty acids were similar in the control and diabetic groups, they were markedly decreased (from 0·70 to 0·39 mmol L^{-1}, $P < 0.001$) in β_3-adrenergic-agonist-treated rats. Both plasma triglycerides and free fatty acid levels correlated well with those of glucose ($r^2 = 0.199$, $P = 0.020$, and $r^2 = 0.187$, $P = 0.024$, respectively), suggesting that the lipolytic and hypoglycaemic effects of Trecadrine are associated. Neither diabetes nor Trecadrine induced changes in plasma total cholesterol.

Table 1. Effects of Trecadrine administration for 4 days on animal and tissue weights, as well as on plasma glucose and insulin levels.

	Control rats (n = 8)	Diabetic rats (n = 9)	β_3-Treated diabetic rats (n = 10)
Final body weight (g)	199·4 ± 4·9	171·2 ± 3·0*	179·1 ± 3·6*
Body fat (%)	8·7 ± 0·8	5·9 ± 0·7*	4·9 ± 0·3*
Internal WAT weight (g)	3·33 ± 0·49	1·24 ± 0·24*	0·76 ± 0·14*
Interscapular BAT weight (g)	0·29 ± 0·03	0·14 ± 0·02*	0·20 ± 0·02*
Gastrocnemius weight (g)	1·19 ± 0·04	0·97 ± 0·03*	1·08 ± 0·04
Plasma glucose (mmol L^{-1})	5·9 ± 0·4	16·6 ± 1·7*	8·3 ± 1·8
Plasma insulin (pmol L^{-1})	91 ± 14	84 ± 115	74 ± 7
Insulin–glucose ratio	15·8 ± 2·5	4·5 ± 0·8*	11·8 ± 2·3

Data are mean ± s.e.m. *Groups are significantly different with at least $P < 0.05$ compared with controls. Analysis of variance was used comparing the three groups and the Fisher test for pairwise comparisons was subsequently applied. WAT, white adipose tissue; BAT, brown adipose tissue.

Table 2. Effects of Trecadrine administration for 4 days on lipid metabolism indicators.

	Control rats (n = 8)	Diabetic rats (n = 9)	β_3-Treated diabetic rats (n = 10)
Plasma triglycerides (mmol L^{-1})	0·67 ± 0·03	1·89 ± 0·65*	0·33 ± 0·02
Plasma FFAs (mmol L^{-1})	0·67 ± 0·06	0·70 ± 0·14	0·39 ± 0·03*
Plasma cholesterol (mmol L^{-1}	1·60 ± 0·16	1·42 ± 0.10	1·63 ± 0·13
Gastrocnemius LPL activity (nmol fluorescein/mg/min)	15·3 ± 1·8	16·4 ± 2·4	15·0 ± 1·3

Data are mean ± s.e.m. *Groups are significantly different with at least $P < 0.05$ compared with controls. Analysis of variance was used comparing the three groups and the Fisher test for pairwise comparisons was subsequently applied. FFAs, free fatty acids; LPL, lipoprotein lipase.

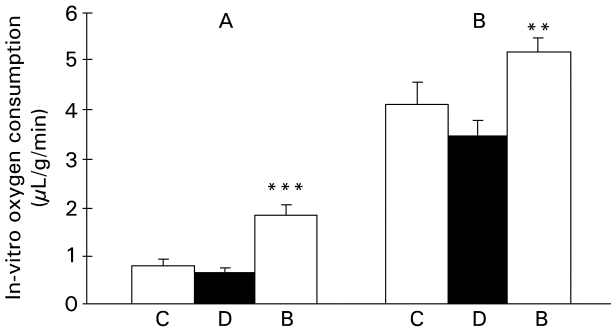

Figure 1. In-vitro oxygen consumption data in white (A) and brown (B) adipose tissue in control (C, n = 8), non-treated diabetic (D, n = 9) and Trecadrine-treated diabetic (B, n = 10) rats. Data are mean ± s.e.m. Analysis of variance was used to compare the three groups and the Fisher test for pairwise comparisons was subsequently applied. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$, significant differences as compared to the diabetic non-treated group.

Gastrocnemius lipoprotein lipase (LPL) activity remained unchanged after Trecadrine administration (Table 2).

No differences were found in plasma leptin levels when comparing Trecadrine- and placebo-treated hyperglycaemic rats (Table 1), although the values in both these groups were significantly lower than those for the control rats.

On the other hand, Trecadrine significantly increased rectal temperature ($+0{\cdot}3^{\circ}$C, $P < 0{\cdot}05$) 40 min after its oral administration to diabetic rats. However, no changes were observed in placebo-administered animals.

Finally, a significant increase in in-vitro oxygen consumption rates in both white and brown adipose tissues ($+282\%$, $P < 0{\cdot}001$, and $+50\%$, $P < 0{\cdot}01$, respectively; Figure 1) was observed in Trecadrine-treated rats as compared with non-treated diabetic and control rats.

Discussion

The diabetogenic effects of alloxan are attributed to a specific cytotoxic action mediated by hydroxyl radical generation on pancreatic β-cells (Takasu et al 1991). This damages a large number of β-cells, resulting in a decrease in endogenous insulin release. Alloxan-administered rats therefore become hyperglycaemic in a short period of time, followed by a hepatic glucose overproduction.

In this context, β_3-adrenergic agonists have been reported to elicit a potent hypoglycaemic effect in both genetically (Arbeeny et al 1995; Hashimoto et al 1997) and chemically induced (Yoshida 1992; Barrinuevo et al 1996) diabetic rats. Our data show a significant hypoglycaemic action of Trecadrine, which is not apparently linked to an improved pancreatic insulin release. A number of studies have shown an increase in endogenous insulin release after the acute administration of different β_3-adrenergic agonists (Yoshida 1992; Abe et al 1993; Grujic et al 1997). However, a decrease (Arbeeny et al 1995; Hashimoto et al 1997) or no variations (Liu & Stock 1995; Virtanen et al 1997) were observed after chronic treatments. Insulin release remained unaltered after Trecadrine administration for 4 days, although the insulin-glucose ratio was markedly lower in both control and treated diabetic rats as compared to non-treated diabetic rats. All of this suggests that an enhancement in insulin-dependent glucose uptake in peripheral tissues occurs. This has been attributed in diabetes type II models to an improvement in glucose tolerance by a partial reversion of insulin resistance in brown adipose tissue and skeletal muscle (Liu & Stock 1995), and also to the suppression of hepatic glucose output (D'Allaire et al 1996). In previous experiments (Berraondo et al 1997), the effects of the β_3-adrenergic agonist Trecadrine on normoglycaemic rats were only apparent in body fat content, not in plasma glucose levels.

Leptin is a hormone that appears to act as a signal of fat stores through the regulation of food intake and energy expenditure (Martínez & Frübeck 1997). Both white adipose tissue lipid content and β_3-adrenergic agonists have been reported to regulate leptin gene expression (Trayhurn et al 1996), although in the current trial, Trecadrine, a β_3-adrenergic agonist, did not inhibit circulating values of this hormone. However, leptin circulating values were statistically higher in the control rats than in the two diabetic groups, which is probably related to the higher body fat mass of the control rats.

On the other hand, a dramatic decrease in plasma triglycerides in those diabetic rats treated with Trecadrine to below the control values was observed, which could be associated with the well-documented lipolytic effect of Trecadrine on white adipose tissue (Martínez et al 1996). Some authors have found strong stimulation of white adipose tissue hormone-sensitive lipase after β_3-adrenergic agonist administration (Yamamoto et al 1997). This is supported by our own in-vitro oxygen consumption data in white adipose tissue, which shows a three-fold increase in Trecadrine-treated rats as compared to non-treated diabetic rats. Furthermore, our data show a plasma free fatty acid reduction in Trecadrine-treated rats, which could be explained by a significant enhancement in fatty acid utilisation by different tissues. This result is in agreement with other chronic treatments of animals with

hyperglycaemia and hypertriglyceridaemia with β_3-adrenergic agonists (Hashimoto et al 1997).

Brown adipose tissue oxygen consumption shows an enhanced thermogenic activity in this tissue. A number of studies have reported a marked β_3-adrenergic-induced increase in UCP1 (Arbeeny et al 1995; Umekawa et al 1997) and UCP3 (Gong et al 1997; Savontaus et al 1998) gene expressions in brown adipose tissue. The stimulation of fatty acid utilisation in brown adipose tissue seems to be associated with heat production, since this tissue preferentially uses this fuel (De Souza et al 1997). Jezek et al (1996) have posited that fatty acids are essential in the UCP1 mechanism of action, while some authors (Charon et al 1995) have found that β_3-adrenergic agonists stimulate lipoprotein lipase expression in brown adipose tissue, pointing to an enhanced triglyceride clearance from plasma in order to utilise the released fatty acids. Furthermore, some studies have found an increase in the number of brown adipocytes, even in tissues such as internal fat where they did not appear before the treatment with the β_3-adrenergic agonist (Himms-Hagen et al 1994; Ghorbani et al 1997). Finally, the fatty acids released from white adipocytes seem to be an important stimulus for thermogenesis in brown adipose tissue (Danforth & Himms-Hagen 1997).

Fatty acid muscle uptake tends to correlate with plasma free fatty acid levels. Thus, when skeletal muscle needs to oxidise more fatty acids, extracellular lipoprotein lipase increases its activity. However, the measurement of the enzyme activity in gastrocnemius revealed no differences when comparing Trecadrine-treated and non-treated rats, indicating that β_3-adrenergic agonists apparently do not stimulate fatty acid uptake in skeletal muscle.

Our data suggest a marked improvement in glucose utilisation in peripheral tissues, commonly explained by a β_3-agonist-induced partial reversion in insulin resistance and an improvement in glucose tolerance (Liu & Stock 1995), or even via an insulin-independent mechanism, as in-vitro studies have proved in isolated soleus muscle (Abe et al 1993) and extensor digitorum longus (Liu et al 1996). Moreover, some studies have found an improved glucose utilisation index in skeletal muscle and brown adipose tissue (Liu & Stock 1995), and even in white adipose tissue (De Souza et al 1997), in rats receiving chronic treatment with a β_3-adrenergic agonist.

Some of these results may be explained by brown adipose tissue hyperplasia and by oxygen consumption stimulation in brown and white adipose tissue, which can be promoted by UCP2 and UCP3 activation. β_3-adrenergic agonist administration has been reported to stimulate both UCP2 and UCP3 gene expression in white adipose tissue (Gong et al 1997; Emilsson et al 1998). Since the functions of uncoupling proteins are related to heat production by a leak of the proton electrochemical gradient across the inner mitochondrial membrane, an enhancement in brown and white adipose tissue thermogenesis is likely to be induced by β_3-adrenergic agonists.

In conclusion, this study has shown that Trecadrine has a strong hypoglycaemic effect, which could be attributed at least in part to an improvement in insulin responsiveness in peripheral tissues. Moreover, Trecadrine appears to normalise hypertriglyceridaemia by stimulating lipolysis in adipocytes, and also by enhancing fatty acid uptake and utilisation in both white and brown adipose tissue. These results suggest that this compound could be of great interest in the therapeutic alleviation of hyperglycaemia in both non-insulin- and insulin-dependent diabetes mellitus, especially when they are associated with obesity.

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