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Short Communication

Hypolipidemic properties of a diphenyl-methylen-ethylamine derivative with affinity for β_3-adrenoceptors in a model of hypercholesterolemia

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Abstract

β_3-Adrenergic agonists have been proposed as potential new drugs for the treatment of diabetes and/or obesity therapy, because of the hypoglycemic and lipolytic effects found with some of these compounds. Moreover, their application in other therapeutic areas such as hypercholesterolemia and atherosclerosis has been suggested. This experimental trial was conducted to assess the effects of Trecadrine, a new molecule with affinity for β_3-adrenoceptors, on a model of hypercholesterolemia in rats, and also to explore a possible beneficial role of these agents in lipid disturbances therapy. The results indicated a marked reduction in serum triglyceride levels (-40%; $P < 0.01$) and lipoprotein lipase activity in white fat (-49%, $P < 0.001$) of hypercholesterolemic rats treated with Trecadrine for 16 days as compared with hypercholesterolemic non-treated rats. Moreover, Trecadrine produced a significant increase in the oxygen consumption in brown adipose tissue ($+154\%$, $P < 0.01$). In relation to cholesterolemia, an improvement in total cholesterol (-20%) and total/HDL–cholesterol ratio (-25%) in serum was noted in the animals receiving the pharmacological treatment. In conclusion, the results of this trial support that Trecadrine administration may have a therapeutic potential in disorders associated with hypertriglyceridemia such as obesity and some types of hyperlipidaemias.

Keywords: β_3-Adrenergic agonist; Hypercholesterolemia; Hypotriglyceridemia

1. Introduction

In addition to β_1- and β_2-adrenoceptors, there is evidence for the occurrence of a third β-adrenoceptor (β_3-AR) in the brown and white tissue, esophagus, tracheal, gastric fundus, jejunum, ileum, colon, gall bladder, liver, skeletal muscle, and heart of some experimental animals [1]. In humans, the presence of β_3 in white and brown fat, gall bladder, small intestine, colon, stomach and prostate has been confirmed [2,3].

Since β_3-AR identification by pharmacological means and molecular biology techniques [4,5], a number of novel selective β_3-adrenergic agonists have been developed [6]. Most of these compounds stimulate lipolysis in white and brown adipocytes [7,8] and thermogenesis in brown fat [9], which has important implications regarding their possible use as antiobesity agents [10]. Moreover, the demonstration of β_3-AR in gastrointestinal tissue raises the question of the role of the β_3-adrenoceptor agonists in motility and secretory processes [11], as well as on intestinal absorptive and digestive functions [12]. At the moment, the major beneficial effects have been shown on obesity and diabetes [13], and new molecules are being synthesized for therapeutic-oriented applications in humans.

In that context, this experimental trial was conducted to assess the effects of a β_3-adrenergic agonist, Trecadrine, on a model of hypercholesterolemia in rats, in order to explore a possible beneficial role of these compounds in lipid disturbances therapy.

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2. Experimental

2.1. Chemistry

Trecadrine, used as a pure compound, is a diphenylmethylen-ethylamine derivative with affinity for β_3-adrenoceptors, whose formula has been previously published [14]. This product was generously supplied by Wassermann–Chiesi (Barcelona/Milan).

2.2. Groups

Male Wistar rats (24) weighing about 200 g, obtained from the Center of Applied Pharmacology (CIFA), were divided into two groups and housed in cages, with water and food freely available. The first group (control group, $n = 8$) received a semipurified control diet and the second group (hyper group, $n = 16$) was fed on a diet enriched in saturated fat (25%) and cholesterol (1%) as previously described [15]. After 26 days, the hypercholesterolemic state was reached [16] and then one group of hypercholesterolemic animals (hyper + β_3 group, $n = 8$) were given Trecadrine orally (0.5 mg kg^{-1} per day), a β_3-adrenergic agonist, for 16 days. Throughout the overall experimental period (42 days) these two groups received the same diet.

2.3. Methods

At the end of the experimental trial, the rats fasted for 16 h were killed by decapitation (9.00 and 11.00 am) and the blood was collected. Serum was separated from whole blood and glucose, triglycerides, total cholesterol and HDL–cholesterol were determined by using enzymatic colorimetric kits (Boehringer Mannheim, France) as well as insulin by means of a radioimmunoassay Kit of Biomedica (Sorin Biomedica, France). Lipoprotein lipase (LPL) activity was analyzed in adipose tissue by a fluorometric assay according to Del Prado et al. [17]. Dibutyrilfluoresceine was used as substrate for the enzyme and the fluoresceine liberated by enzymatic hydrolysis was measured. Finally, oxygen consumption in brown adipose tissue (BAT) was measured with a Clarke oxygen electrode [18].

2.4. Statistical analyses

The results are expressed as mean ± SEM and they were evaluated statistically using the one-way analysis of variance (ANOVA) followed by a Fisher PLSD test, or by Kruskal–Wallis and U-Mann–Whitney tests as appropriate. In all cases, $P < 0.05$ was used as the criterion of statistical significance.

3. Results and discussion

A number of studies have shown that β_3-adrenergic agonists can be useful in diabetes therapy [19] or obesity [20] and they have also been suggested to have a putative role in the treatment of some lipid disturbances [1], due to the hypoglycemic and lipolytic effects found with some of these compounds. Thus, much interest has been directed toward the development of new agents that stimulate these metabolic responses.

Different experimental trials carried out with Trecadrine have shown that this molecule with affinity by β_3-AR has beneficial properties on diet-induced obesity [21] and alloxan-diabetes models in rats [16]. The major advantages found in these pathological disorders have been in relation to fat deposition and serum triglycerides. In this way, a statistically significant decrease ($P < 0.01$) has been found in the serum triglyceride levels of animals treated with Trecadrine as compared with hypercholesterolemic non-treated rats (Table 1). Some authors have associated this outcome with alterations in fat assimilation in the digestive tract and changes in triglyceride storage and mobilization in adipose tissue [2]. Moreover, it has been reported that β_3-adrenergic agonists produce an increase in the rate of lipolysis and a decrease in lipid synthesis in adipose tissue [22]. The reduction in LPL activity in adipose

Table 1
Measurements in the different groups: control; hyper (rats fed a hypercholesterolemic diet) and hyper + β_3 (hypercholesterolemic rats treated with Trecadrine for the last 16 days) [a]

Measurements	Groups in study		
	Control	Hyper	Hyper + β_3
Serum triglycerides (mg dl^{-1})	125 ± 26^a	117 ± 12^a	70 ± 5^b
Total cholesterol (mg dl^{-1})	68 ± 7^a	314 ± 37^b	252 ± 49^b
Total/HDL–cholesterol	1.8 ± 0.1^a	24.9 ± 4.8^b	18.8 ± 3.7^b
O_2 consumption in BAT (μl O_2 g^{-1} min^{-1})	1.08 ± 0.66^a	0.54 ± 0.20^b	1.37 ± 0.26^a
LPL in adipose tissue (nmol min^{-1} mg $protein^{-1}$)	4.41 ± 0.29^a	13.49 ± 0.68^b	6.89 ± 0.60^c

[a] Values are means ± SEM of eight male Wistar rats in each group. Data with different superscripts in the same row are significantly different ($P < 0.05$).

tissue of hypercholesterolemic animals treated with Trecadrine (Table 1) may be explained by these findings. In addition, the elevation in the oxygen consumption in BAT of rats receiving Trecadrine (Table 1) confirms a possible lipolytic effect and an enhancement in thermogenesis of this compound [23]. With regard to cholesterol levels, the administration of Trecadrine produced no statistically significant changes on serum total cholesterol and HDL/cholesterol ratio, although a marked improvement reduction (about 20%) in cholesterolemia after the pharmacological treatment was observed. The results of this trial support that Trecadrine administration may have therapeutic potential in disorders associated with hypertriglyceridemia, such as obesity and some types of hyperlipidaemias.

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