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Interactions between an α_2-adrenergic antagonist and a β_3-adrenergic agonist on the expression of UCP2 and UCP3 in rats

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This experimental trial was devised to assess whether selective β_3-adrenergic receptor (AR) stimulation and simultaneous blockade of α_2-AR would affect thermoregulation. With this purpose, the individual and combined administration of a β_3-AR agonist, trecadrine, and an α_2-AR antagonist, yohimbine, were evaluated. Yohimbine produced a marked decrease ($p < 0.001$) in body temperature one hour after administration (5 mg kg^{-1}, i.p.) and blocked the thermogenic effect of trecadrine (1 mg kg^{-1}, i.p.) when simultaneously administered. Uncoupling protein-2 expression in skeletal muscle was downregulated ($p < 0.05$) by trecadrine, while yohimbine had no effect. White adipose tissue UCP2 and muscle UCP3 were not modified by either trecadrine or yohimbine administration. Liver UCP2 mRNA expression was significantly decreased by yohimbine ($p < 0.05$). However, this downregulation does not seem to explain the reduction in temperature produced by yohimbine given the fact that trecadrine produced a similar downregulation of hepatic UCP2 ($p < 0.05$). The present work indicates that α_2-AR antagonism blocks the thermogenic effects mediated by β_3-AR stimulation, contrary to our expectations, suggesting a possible interplay between both mechanisms. Moreover, these effects are not apparently explained by changes in UCP2 and UCP3.

Key words: Trecadrine, Yohimbine, β_3-adrenoceptor, α_2-adrenoceptor, Lipolysis, Thermogenesis.

The sympathetic nervous system plays an important role in energy expenditure through the regulation of thermogenesis, which constitutes a physiological defence against cold and excessive energy intake (23). Catecholamines exert a dual control on metabolism in rats and other species:

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stimulation by acting on β-adrenergic receptor (β-AR) and inhibition mediated through α_2-adrenoceptors (α_2-AR) (18). Thus, it would be reasonable to hypothesise that selective β_3-AR stimulation and simultaneous blockade of α_2-AR could result in an increase of the thermogenic response.

Uncoupling proteins (UCPs) are a family of mitochondrial proteins which affect oxidative phosphorylation by moving protons across the mitochondrial inner membrane toward the mitochondrial matrix (11). The first described member of this family, UCP1, is expressed uniquely in the brown adipose tissue (BAT), where it plays a main role in thermogenesis (15). Recently, two further members of the UCP family have been cloned; the ubiquitously expressed UCP2 and UCP3 which is expressed mainly in BAT and skeletal muscle (4, 7). β_3-AR agonists induce a strong induction of UCP1 expression in BAT, but have no clear effect on UCP2 and UCP3 expression (12, 13, 27, 28). In addition, it has been reported that administration of yohimbine increases oxygen consumption and heat production (10) and that yohimbine may regulate the expression of UCP1 in BAT (5). However, the thermogenic effect of α_2-AR blockade and its consequences on UCP2 and UCP3 expression remain unclear.

The aim of the present study was to study the effect of trecadrine and yohimbine on thermogenesis and the potential involvement of UCP2 and UCP3 from different tissues. In addition, we evaluated whether the α_2-AR blockade elicited by yohimbine induced a potentiation of the thermogenic effects of the β_3-AR agonist trecadrine in Wistar rats.

Materials and Methods

Animals and treatment.– Forty 5-month-old male Wistar rats weighing 420-430 g were obtained from CIFA (Centro de Investigación en Farmacobiología Aplicada, Pamplona, Spain), housed at 25 ± 1 °C with 12 h light cycle (8 am to 8 pm) and fed *ad libitum*. The animals were fasted for 24 h before drug administration and rectal temperature was measured as previously described (12). Rats, randomly assigned to four groups of ten animals each, received a single dose of: saline, the β_3-adrenoceptor agonist trecadrine (1 mg kg^{-1}, i.p.), yohimbine (5 mg kg^{-1}, i.p.) or a combination of both at the mentioned doses. After 1 h, rectal temperature was measured again and animals were killed by decapitation. Epididymal WAT, liver, and *gastrocnemius* muscle were excised, immediately frozen in liquid nitrogen and stored at -80 °C until analysis. All experimental procedures were performed according to institutional guidelines for Animal Care and Use at the University of Navarra.

Drugs.– Trecadrine, a diphenyl-methylene-ethylamine derived compound, whose formula and characterisation as a β_3-adrenoceptor agonist have been previously published (1) was a generous gift of Wassermann-Chiesi (Barcelona/Milan). Yohimbine-hydrocloride was purchased from Sigma (St. Louis, MO, USA). All other chemicals and organic solvents were of reagent grade.

RNA analysis by reverse transcription-polymerase chain reaction (RT-PCR).– Total RNA was isolated by ULTRASPEC-II (Biotecx Laboratories, Houston, TX, USA) from 100 mg of tissue according to the manufacturer's instructions.

Conditions of RT-PCR reactions have been previously reported (12). Primers used to amplify UCP2, UCP3 and β-actin cDNA were the same as described elsewhere (12). cDNA was amplified for 26 (UCP2), 27 (UCP3) and 21 cycles (β-actin) in skeletal muscle, 35 (UCP2) and 32 cycles (β-actin) in WAT and 35 (UCP2) and 27 cycles (β-actin) in liver. Levels of mRNA were expressed as the ratio of signal intensity for each UCP relative to that for β-actin. PCR band intensity was determined by densitometric analysis with the *Gel Doc 1000 UV fluorescent gel documentation system* and *Molecular Analyst 1.4.1* software for quantitation of images (Bio-Rad, Hercules, CA, USA).

For mRNA detection of the receptors studied, primers used to amplify β_3-adrenoceptor cDNA (GenBank S56481) were 5'-CTTCTACCTTCCC-CTCCTT-3' (sense, 638-656) and 5'-CTTCATAGCCATCAAACCTG -3' (antisense, 1172-1191) which were designed using the *Oligo® 4.05 Primer Analysis Software* (National Biosciences, Inc., Plymouth, MN, USA). Primers for α_{2A}-adrenoceptor were taken from the literature (6). RT-PCR reactions were performed with RNA obtained from control animals. The annealing temperature of amplifications was 56 °C for β_3-adrenoceptor and 64 °C for α_{2A}-adrenoceptor cDNA and 40 cycles were carried out in both cases. A negative control without reverse transcriptase was included for each tissue analysed to ensure that amplification did not proceed from residual genomic DNA.

Data and statistical analysis.– All results are expressed as mean ± SEM. Data were analysed using repeated-measure two-way ANOVA coupled to a two-tailed unpaired *t* test when an interaction was detected. The calculations were performed using the SPSS/Windows version 7.5.2S (SPSS, Chicago, IL, USA). A *p* value lower than 0.05 was considered statistically significant.

Results

The acute i.p. administration of trecadrine to fasted Wistar rats induced a significant rise ($p < 0.001$) in rectal temperature. On the contrary, yohimbine administration produced a marked decrease ($p < 0.001$) of body temperature, which could not be counteracted by trecadrine administration. Furthermore, a statistically significant interaction ($p < 0.05$) between both effects was found (Fig. 1).

Trecadrine produced a statistically significant ($p < 0.05$) downregulation of UCP2 mRNA expression in *gastrocnemius* muscle, which was more evident

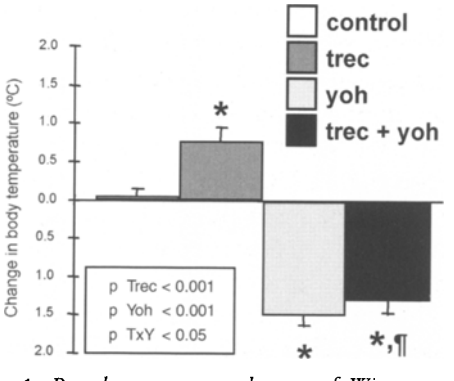

Fig. 1. *Rectal temperature changes of Wistar rats after acute (1 h) i.p. administration of trecadrine (1 mg kg^{-1}), yohimbine (5 mg kg^{-1}), both drugs combined at the same doses or vehicle.*
Values are mean ± SEM, n = 10 per group. Statistical analysis by two-way ANOVA is shown in the square. Two-tailed unpaired *t* test was performed after detecting interaction: * $p < 0.001$, *vs* control group. ¶ $p < 0.001$ *vs* trecadrine treated group.

when yohimbine was administered simultaneously, but there was no significant interaction. Muscle UCP2 was not changed by yohimbine (Fig. 2). Neither trecadrine nor yohimbine modified UCP3 mRNA expression in muscle one hour after i.p. administration (data not shown). UCP2 expression in WAT was not significantly affected by either trecadrine or yohimbine administration. UCP2 mRNA expression in liver was downregulated after acute trecadrine ($p < 0.05$) and yohimbine ($p < 0.05$) administration, although no additive effect was observed with simultaneous administration of both drugs.

β_3-AR mRNA was highly expressed in WAT, at a lesser extent in skeletal (*gastrocnemius*) muscle and was undetectable in liver, however, the RT-PCR assay performed was unable to accurately quantify levels of expression. The presence of α_{2A}-AR mRNA was demonstrated in white adipose tissue. High levels of α_{2A}-AR mRNA were found in *gastrocnemius* muscle, whereas it was undetectable in liver (Fig. 3). When no band was detected (β_3-AR and α_{2A}-AR in liver) a re-PCR was performed to support negative results (data not shown).

Discussion

Catecholamines appear to exert a dual control on metabolism in rats and other species by stimulating β-AR and inhibiting α_2-AR (18). Initially, it was believed that rat adipocytes did not have α_2-AR-mediated inhibition of lipolysis (20). This assumption, based mainly on the lack of effect of clonidine, was reassessed by using the selective α_2-AR agonist UK 14304 (25). Our RT-PCR results clearly show that rat white fat cells express α_{2A}-AR mRNA, supporting its involvement in the observed antilipolytic effect (19).

One hour after drug administration, trecadrine administration produced a marked thermogenic response as evidenced by the increase in rectal temperature, similar to results obtained with other

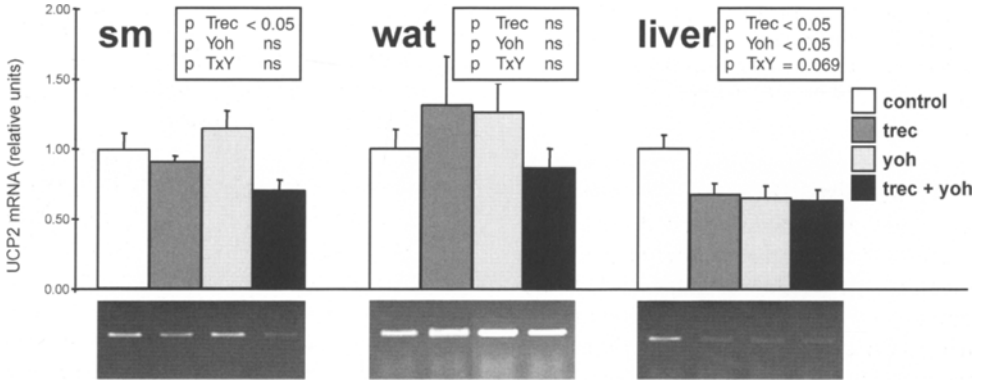

Fig. 2. *Effect of acute (1 h) i.p. administration of trecadrine (1 mg kg^{-1}), yohimbine (5 mg kg^{-1}), both drugs combined at the same doses or vehicle on UCP2 expression measured by RT-PCR.*
Data represent the mean ± SEM of the ratio between UCP2 to β-actin. The expression of UCP2 in control rats was assumed to be 1 (n = 6 in each group). Statistical analysis by two-way ANOVA is shown on top, ns = non-significant. Representative photographs of RT-PCR products are shown on bottom. sm: skeletal (*gastrocnemius*) muscle; wat: white adipose tissue.

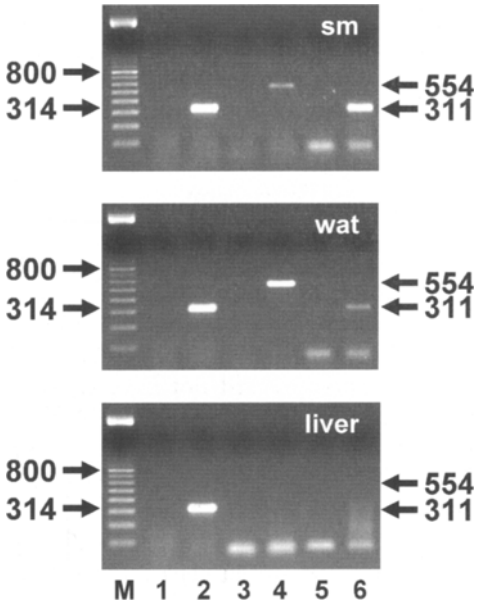

Fig. 3. *β_3-AR and α_{2A}-AR mRNA expression in gastrocnemius muscle (sm), white adipose tissue (wat), and liver of control Wistar rats. RT-PCR analyses were performed in the presence (lanes 2, 4, 6) or absence (lanes 1, 3, 5) of reverse transcriptase.* The sizes in base pairs of the PCR products for β-actin (lanes 1, 2), β_3-AR (lanes 3, 4), and α_{2A}-AR (lanes 5, 6) are shown. Sizes were determined from ethidium bromide stained gels by comparison with 100 DNA ladder (Pharmacia). Results are representative of three experiments performed at 40 cycles of amplification. The small-sized bands which appear in some of the lanes is due to primer-dimer amplification.

β_3-AR agonists (16, 21). By contrast, yohimbine produced a marked reduction in rectal temperature. Reported evidence concerning α_2-AR regulation of body temperature in rodents is inconsistent, with hypothermic responses described after both i.p. α_2-AR agonist (dexmedetomidine) administration (17) and s.c. α_2-AR antagonist (delequamine) administration (26). Yohimbine produces a hypothermic effect in rats (24, 29), but also attenuates/prevents the hypothermia produced by clonidine (3, 22). Our data show that yohimbine clearly reduces body temperature and that these effects are predominant over the thermogenic effect of trecadrine as supported by the detected interactions. The reduction in energy expenditure reported herein is in disagreement with the thermogenic effect previously described by GALITZKY *et al.* (10) in dogs, which may be explained by differences between species, depending on the different patterns of distribution of α_2-AR in brain.

In order to explore the possible mechanisms involved in the changes concerning thermogenesis, the expression of UCPs in several tissues was analysed. Skeletal muscle UCP2 was unaffected by yohimbine, but downregulated by trecadrine, in agreement with previous reports (2, 8). This downregulation could be due to a direct effect on the tissues, given the fact that β_3-AR are expressed in *gastrocnemius* muscle and may play a compensatory role, by avoiding an excessive energy loss as heat after the increase in whole body thermogenesis elicited by β_3-AR stimulation. Muscle UCP3 mRNA and WAT UCP2 were not modified by the administered treatments indicating that they do not appear to participate in the acute changes observed in thermogenesis.

In agreement with other authors (9) no detectable levels of β_3-AR mRNA were observed in liver. However, trecadrine decreased hepatic UCP2 mRNA expression, suggesting an indirect effect of trecadrine. Liver UCP2 mRNA levels were also reduced by yohimbine, however, the fall in body temperature produced by yohimbine cannot be attributed to the downregulation of liver UCP2 given the fact that a similar reduction was produced by trecadrine. The liver does not appear to express α_{2A}-AR mRNA (the subtype

studied) at levels detectable by RT-PCR, however, the expression of α_{2B}-AR mRNA in liver is well characterised (14). In this sense, the effect elicited by yohimbine may be due to a direct effect.

In summary, yohimbine produces a marked decrease in rectal temperature blocking the thermogenic effect of trecadrine, which is not fully explained by changes in UCPs expression in different tissues.

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Este estudio trata de comprobar si la estimulación selectiva de receptores adrenérgicos β_3 (β_3-AR) y el bloqueo simultáneo de α_2-AR tiene un efecto termogénico mediado por UCPs. Con este propósito, se evalúa la administración individual o conjunta de un agonista β_3-AR, trecadrine, y un antagonista adrenérgico α_2-AR, yohimbina. Una hora después de la administración de yohimbina (5 mg kg^{-1} i.p.), se observa un marcado descenso de la temperatura corporal ($p < 0.001$). Además, cuando ambos productos se administran conjuntamente, el efecto termogénico del trecadrine (1 mg kg^{-1} i.p.) se bloquea por la yohimbina. El trecadrine reduce la expresión de la proteína desacoplante-2 (UCP2) en el músculo esquelético ($p < 0.05$), mientras que la yohimbina carece de efecto. Ni el trecadrine, ni la yohimbina, producen modificaciones en la expresión de UCP2 en tejido adiposo blanco y UCP3 en el músculo esquelético. La yohimbina disminuye la expresión de UCP2 en el hígado ($p < 0.05$). Sin embargo, este cambio no parece explicar el efecto hipotérmico producido por la yohimbina, ya que el trecadrine produce una inhibición similar de la expresión hepática de la UCP2 ($p < 0.05$). Este estudio indica que el antagonismo de α_2-AR bloquea el efecto termogénico mediado por la estimulación de β_3-AR, contrariamente a lo que cabría esperar, lo que sugiere una posible relación funcional entre ambos receptores. Estos efectos no parecen explicarse por cambios en la expresión de UCP2 y UCP3.

Palabras clave: Trecadrine, Yohimbina, Receptores adrenérgicos α_2 y β_3, Lipolisis, Termogénesis.

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