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Anti-obesity effect of MPV-1743 A III, a novel imidazoline derivative, in genetic obesity

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Abstract

MPV-1743 A III ((±)-4-(5-fluoro-2,3-dihydro-1*H*-inden-2-yl)-1*H*-imidazole) is a novel imidazoline derivative. In this study, it was shown to bind with high affinity to α_2-adrenoceptor subtypes α_{2A} ($IC_{50} = 0.66 \pm 0.06$ nM), α_{2B} ($IC_{50} = 3.8 \pm 0.53$ nM), α_{2C} ($IC_{50} = 3.1 \pm 0.61$ nM) in the recombinant S115 cells and to α_{2D} ($IC_{50} = 0.94 \pm 0.10$ nM) in the rat submandibular gland. MPV-1743 A III also showed remarkably high affinity to α_1-adrenoceptors ($IC_{50} = 150 \pm 12$ nM) in the rat cerebral cortex and to imidazoline I_{2b}-binding sites ($IC_{50} = 150 \pm 5.0$ nM) in the rat liver. The functional α_2-adrenoceptor antagonistic effect of MPV-1743 A III was demonstrated by studying the ability of orally administered MPV-1743 A III to reverse and prevent the α_2-adrenoceptor agonist detomidine-induced mydriasis in rat. The anti-obesity effect of MPV-1743 A III was investigated in genetically obese (*fa/fa*) Zucker rats in two different phases of obesity. Chronic treatment with MPV-1743 A III (0.3–3 mg/kg per day p.o. for 3 weeks) dose dependently decreased weight gain in early-phase obesity. In fully established obesity, GDP binding to mitochondria and expression of uncoupling protein mRNA were increased in brown adipose tissue by MPV-1743 A III indicating an activation of non-shivering thermogenesis. The present study shows that MPV-1743 A III has a modest anti-obesity effect in the genetic rodent model of obesity. The relative importance of α_2- and α_1-adrenoceptors and imidazoline I_{2b}-binding sites in mediating the effects of MPV-1743 A III needs further evaluation.

Keywords: Brown fat; Zucker rat; α_1-Adrenoceptor agonist; α_2-Adrenoceptor antagonist; Imidazoline derivative; Obesity, drug therapy

1. Introduction

Brown adipose tissue is the major site for non-shivering thermogenesis (Ricquier and Mory, 1984) and contributes to energy balance by dissipating excessive dietary caloric intake in diet-induced thermogenesis (Rothwell et al., 1982). Heat production in brown adipose tissue is based on uncoupling of ATP synthesis from substrate oxidation by a mitochondrial membrane protein, the uncoupling protein (Himms-Hagen, 1992). Binding of the nucleotide guanosine diphosphate (GDP) to brown adipose tissue mitochondria can be used as an indicator of uncoupling protein amount and thermogenic activity of brown adipose tissue (Nicholls, 1976; Milner et al., 1988). The sympathetic nervous system is the primary pathway in the regulation of brown adipose tissue thermogenesis (Seydoux and Girardier, 1978). Since several models of experimental obesity are accompanied with lowered sympathetic tone and brown adipose tissue thermogenic activity (Bray, 1990), their activation has been brought up as a new approach in the treatment of obesity. β_3-Adrenoceptor agonists have already been shown to effectively activate brown adipose tissue thermogenesis and lower weight gain in experimental obesity (Arch et al., 1984; Cawthorne et al., 1992).

In addition to β_3-adrenoceptor agonists, also other possibilities to increase lipolysis and thermogenesis in adipose tissue, either directly or indirectly, exist. Theoretically, α_2-adrenoceptor antagonists could activate sympathetic nervous system and brown adipose tissue thermogenesis by two possible ways. First, blockade of the presynaptic

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α_2-adrenoceptors increases the release of noradrenaline from presynaptic nerve terminals. Second, antagonism of the inhibitory α_2-adrenoceptors by the unspecific α_2-adrenoceptor antagonist yohimbine has been shown to potentiate isoprenaline-induced adenylate cyclase formation in brown adipocytes (Domínguez et al., 1986). Indeed, there are some experimental data suggesting that α_2-adrenoceptor antagonists may have beneficial effect on energy balance in vivo. Yohimbine has been shown to increase lipolysis and thermogenesis in dogs (Galitzky et al., 1991). The lipolytic effect of yohimbine was also demonstrated in humans and shown to be mainly due to increased noradrenaline in adipose tissue (Berlan et al., 1991). Furthermore, α_2-adrenoceptor antagonists were shown to reverse the decreasing effect of α_2-adrenoceptor agonists on resting energy expenditure in rats, suggesting that α_2-adrenergic receptors are involved in the regulation of energy expenditure also in this species (Gazzola, 1993). However, the specific and potent α_2-adrenoceptor antagonist atipamezole failed to affect weight gain and brown adipose tissue thermogenesis in obese Zucker rats (Santti et al., 1994). In addition to α_2-adrenoceptors, there are α_1-adrenoceptors in brown fat, and their ability to potentiate β-adrenoceptor-mediated responses in brown adipose tissue has been demonstrated in the rat (Ma and Foster, 1984; Granneman, 1988; Raasmaja and Larsen, 1989).

In this study, a novel imidazoline derivative, MPV-1743 A III ((±)-4-(5-fluoro-2,3-dihydro-1*H*-inden-2-yl)-1*H*-imidazole, Fig. 1) was shown to bind with high affinity to all α_2-adrenoceptor subtypes and with a somewhat lower affinity to α_1-adrenoceptors and imidazoline I_{2b}-binding sites. In addition, the efficacy of orally administered MPV-1743 A III to antagonize central α_2-adrenoceptors was demonstrated. Finally, chronic treatment with MPV-1743 A III was shown to have an anti-obesity effect in genetically obese (*fa/fa*) Zucker rats at two different developmental phases of obesity.

2. Materials and methods

2.1. Receptor binding studies

Cultured S115 cell lines each expressing one of the human $\alpha_{2A,B,C}$-adrenoceptor subtypes (Marjamäki et al., 1993) were cultured in a hollow fibre bioreactor in the Centre for Biotechnology (Turku, Finland), harvested and washed by centrifugation as described earlier (Ala-Uotila et al., 1994). The cell pellet was suspended in 30 volumes of 50 mM Tris buffer with 5 mM EDTA (pH 7.5 at 4°C) and homogenized in a Teflon-glass homogeniser (Potter S, 10 strokes with 1000 rpm). Cell homogenate was then centrifuged twice at $47\,800 \times g$ (Sorvall RC-5C) for 30 min at 4°C with resuspension by two strokes in Tris/EDTA buffer. The final pellet was resuspended in 10 volumes of incubation buffer and distributed in aliquots to be stored at −80°C for later use.

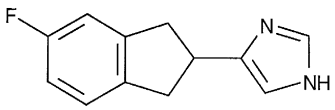

Fig. 1. Chemical structure of MPV-1743 A III.

For preparation of the rat submandibular gland and cerebral cortex, the tissues were removed from adult female Sprague-Dawley rats (160–200 g), washed and homogenized in 30 volumes of ice-cold buffer (50 mM Tris, 5 mM EDTA, pH 7.4 at 4°C for submandibular glands, and 50 mM Tris, 0.8 mM EDTA, pH 7.5 at 4°C for cerebral cortex) with an Ultra-Turrax. After an initial centrifugation at $500 \times g$ for 5 min, the supernatant was centrifuged and prepared to final storage aliquots as described above.

For preparation of the rat liver, the tissue was removed and cleaned from the connective tissue and washed in buffer of 0.25 M sucrose with 5 mM Hepes (pH 7.4 at 4°C), minced with scissors, frozen in liquid nitrogen and stored at −80°C. Frozen liver pieces were thawn and homogenized (Potter S, 500 rpm, eight strokes with the Teflon pestle) in 30 volumes of 0.25 M sucrose supplemented with 5 mM HEPES and 10 mM EDTA (pH 7.4 at 4°C) as well as with the protease inhibitors (0.1 mM phenylmethylsulphonyl fluoride, 2 μg/ml bacitracin, 2 μg/ml leupeptin, 2 μg/ml pepstatin A and 2 μg/ml soybean trypsin inhibitor). Stock solution of phenylmethylsulphonyl fluoride was made in isopropanol freshly prior to use. The liver suspension was centrifuged for 5 min at $600 \times g$ at 4°C, and the supernatant was poured through a double cheese-cloth, and the pellet was once homogenized and centrifuged and filtered as above. The supernatants were collected and centrifuged for 15 min at $47\,800 \times g$ at 4°C. After resuspension in 20 volumes of ice-cold 50 mM Tris buffer with 10 mM EDTA (pH 7.4 at 30°C), the homogenate was incubated for 30 min at 30°C to remove endogenous catecholamines, and then centrifuged as above. The pellet was resuspended in 50 mM Tris (pH 7.4 at 4°C), centrifuged, and final resuspension was done in the incubation buffer.

In a saturation binding experiment, ten concentrations of radioligands were with the cell and tissue suspension (20–50 μg per tube for the sources of α_{2A-C}-adrenoceptors and 100–200 μg of total protein for tissue sources) in a total volume of 0.25 ml in triplicate. Protein determinations were done using a colorimetric assay (Bradford, 1976). In a competition binding experiment, the radioligands were incubated at concentrations close to their K_d value with the cell and tissue suspension (protein concentration as above) with or without presence of a test compound in a total volume of 0.25 ml. After a 30 min incubation at 25°C, incubation was terminated by rapid filtration (Tomtec 96 harvester) through pre-wet glass fibre filter mats (Wallac, GF/B) and three washes of ice-cold

10 mM Tris buffer (pH 7.5 at 4°C). After drying, a solid scintillate (Meltilex, Wallac) was melted on the filter mats and the mats were measured for their radioactivity in a counter (Wallac BetaPlate) with 35% efficacy for tritium. Incubation buffers were as follows: 50 mM KH_2PO_4 buffer (pH 7.5 at 25°C) for [^{3}H]RX821002 experiments, 50 mM Tris (pH 7.4 at 30°C) for [^{3}H]idazoxan experiments, and 50 mM Tris (pH 7.7 at 25°C) for [^{3}H]prazosin experiments. Non-specific binding was determined using 0.1 mM oxymetazoline ([^{3}H]RX821002), 20 μM cirazoline ([^{3}H]idazoxan) or 10 μM phentolamine ([^{3}H]prazosin). Saturation experiments resulted in the following radioligand binding affinities and capacities: [^{3}H]prazosin K_d 99 ± 0.43 pM, B_{max} 180 ± 17 fmol/mg protein (slope = 0.89 ± 0.051, specific binding 85%, $n = 3$) at α_1-adrenoceptors in the rat cerebral cortex; [^{3}H]RX821002 K_d 0.55 ± 0.011 nM, B_{max} 460 ± 60 fmol/mg protein (slope = 1.1 ± 0.081, specific binding 95%, $n = 3$) at α_{2A}-adrenoceptors in the recombinant S115 cells, K_d 3.0 ± 0.22 nM, B_{max} 2000 ± 250 fmol/mg protein (slope = 0.99 ± 0.004, specific binding 94%, $n = 3$) at α_{2B}-adrenoceptors in the recombinant S115 cells, K_d 1.2 ± 0.034 nM, B_{max} 680 ± 81 fmol/mg protein (slope = 1.0 ± 0.013, specific binding 96%, $n = 3$) at α_{2C}-adrenoceptors in the recombinant S115 cells and K_d 0.36 ± 0.04 nM, B_{max} 140 ± 20 fmol/mg protein (slope = 0.99 ± 0.026, specific binding 97%, $n = 4$) at α_{2D}-adrenoceptors in rat submandibular gland; [^{3}H]idazoxan K_d 3.3 ± 0.45 nM, B_{max} 1200 ± 150 fmol/mg protein (slope = 1.1 ± 0.034, specific binding 90%, $n = 5$) at imidazoline I_{2b}-binding sites in the rat liver.

2.1.1. Chemicals

[^{3}H]RX821002, specific activity 48 Ci/mmol, and [^{3}H]idazoxan, specific activity 44 Ci/mmol, were from Amersham Life Science; [^{3}H]prazosin, specific activity 74.4 Ci/mmol, was from DuPont NEN. Cirazoline, phentolamine and oxymetazoline were from Sigma.

2.2. Efficacy and time-course of α_2-adrenoceptor blocking activity of oral MPV-1743 A III

The α_2-adrenoceptor blocking activity of oral MPV-1743 A III was demonstrated in rats. α_2-Adrenoceptor agonists are able to induce mydriasis in rats through central α_2-adrenoceptors located postsynaptically on the neurons of the Edinger-Westphal nucleus, the source of parasympathetic innervation to the iris (Berridge et al., 1983; Koss et al., 1984). Measuring the ability of an α_2-adrenoceptor antagonist to antagonize the α_2-adrenoceptor agonist-induced mydriasis can be used to determine the antagonistic potency. Potency and time-course of α_2-adrenoceptor blocking activity of orally given MPV-1743 A III was determined by measuring its ability to prevent or reverse the mydriasis induced by the α_2-adrenoceptor agonist detomidine (Virtanen and Nyman, 1985) in anaesthetized rats.

Adult Sprague-Dawley rats of both sexes (Alab, Sweden) weighing 200–300 g were used. In all experiments four rats were used in each group. The rats were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and 15 min later a polyethylene cannula was inserted into the lateral tail vein for intravenous drug administration (see below). Pupil diameter was measured by means of an operating microscope, which was provided with a 10 mm graduated line (0.1 mm divisions) in the ocular. The microscope had an internal light source and the light was maintained at a steady intensity throughout the experiments.

In the first experiment, the ability of MPV-1743 A III to prevent the detomidine-induced mydriasis was studied. The rats were pretreated with MPV-1743 A III (0.1 mg/kg, 0.3 mg/kg or 1 mg/kg, p.o.). 45 min later the animals were anaesthetized and cannulated, and after a further 15 min cumulative doses of detomidine were administered i.v. at 5 min intervals.

In the second experiment, the ability of MPV-1743 A III to reverse the detomidine-induced mydriasis was studied and compared with the effects of two reference α_2-adrenoceptor antagonists, atipamezole (Virtanen et al., 1989) and idazoxan (Doxey et al., 1983). The rats were injected 15 min after induction of anaesthesia with a maximally effective mydriatic dose of detomidine (100 μg/kg i.v.). 10 min later the cumulative dosing (i.v.) of the studied antagonists was started. The α_2-adrenoceptor antagonist potency was determined by calculating the cumulative dose which reversed the α_2-adrenoceptor agonist induced mydriasis by 50%.

In the third experiment, the time-course of α_2-adrenoceptor blocking activity was studied. Roughly equipotent doses of MPV-1743 A III or atipamezole were administered orally to groups of rats at various times (1, 2, 4, 7, 16 h) before challenge with detomidine. Detomidine was injected cumulatively at 5 min intervals 15 min after induction of anaesthesia as described above. Change in pupil diameter was then plotted graphically against the log-dose of detomidine for each group. The percentage antagonism of the mydriatic effects of a 30 μg/kg dose of detomidine was calculated for each pretreatment time point, which permitted the establishment of a time–effect curve.

2.2.1. Drugs

MPV-1743 A III, atipamezole, idazoxan, detomidine and sodium pentobarbitone (Mebunat) were from Orion Corporation Farmos, Finland.

2.3. The anti-obesity effect of MPV-1743 A III

2.3.1. Animals

Obese male Zucker rats were purchased from IFFA Credo (L'Arbresle, France). The rats in early phase obesity were 7 weeks old and weighed 230 ± 3 g at the beginning of the experiment, whereas the rats with fully established

obesity were approximately 13 weeks old and weighed 450 ± 5 g. The animals were individually housed and maintained under a constant light-dark cycle (lights on from 6.00 to 20.00 h). They had free access to normal laboratory rat chow (R36, Lactam, Sweden) and water ad libitum.

2.3.2. Experimental design

The rats in early phase obesity ($n = 38$) were divided into four groups. One group received tap water only (control group) and three intervention groups received different doses of MPV-1743 A III (0.3, 1 or 3 mg/kg per day) dissolved in drinking water for 21 days. The rats in fully established obesity received either MPV-1743 A III 1 mg/kg per day in the drinking water ($n = 8$) for 21 days or tap water only ($n = 8$). The intake of water was measured every day and the amount of MPV-1743 A III dissolved in the drinking fluid was adjusted every other day to ensure a correct drug intake.

During the treatments, 48 or 24 h food intake was measured throughout by placing a weighed amount of pelleted food in the cage and calculating the amount consumed. The cumulative food intake was calculated after the experiment. The body weights were measured every other day. The rats were decapitated on the morning of the 21st day. The early-phase obesity rats had free access to food and drinking water until the time of decapitation, whereas the rats with fully established obesity were fasted for 21 h before decapitation.

After decapitation blood was collected into prechilled EDTA tubes, whereafter plasma was separated and stored at -70°C until analyzed. Interscapular brown adipose tissue was quickly dissected from surrounding tissues. One lobe was used for the preparation of mitochondrial fraction and the other lobe was quickly frozen for further analysis of uncoupling protein mRNA expression. Epididymal and intraperitoneal fat pads were removed and weighed.

2.3.3. Analytical procedures

Binding of [^{3}H]GDP to brown adipose tissue mitochondria was measured as described earlier (Santti et al., 1994). In brief, the fresh brown fat pads were minced, diluted in 250 mM ice-cold sucrose buffer and homogenized. The homogenate was used for immediate preparation of mitochondria with differential centrifugation. The binding of [^{3}H]GDP was determined by incubating mitochondria in a basic medium containing 100 mM sucrose, 20 mM TES (*N*-tris-[hydroxymethyl]-methyl-2-aminoethane-sulphonic acid), 1 mM EDTA, 10 mM choline chloride, 2 μM rotenone, [^{14}C]sucrose and 10 μM [^{3}H]GDP.

The RNA for the determination of uncoupling protein mRNA content was extracted from brown fat by the acid guanidinium-phenol-chloroform method (Chomczynski and Sacchi, 1987). Levels of brown adipose tissue uncoupling protein mRNA were measured using slot-blot hybridization manifold (Schleicher & Schuell, Dassel, Germany) as described earlier (Rouru et al., 1993). Slot blots were quantitated with an OS/2 based image analysis system (MCID, Imaging Research, Ontario, Canada). The amount of uncoupling protein mRNA was expressed in relation to the amount of 28S mRNA.

Plasma insulin was measured with a rat insulin radioimmunoassay kit supplied by Novo Nordisk, Bagsvaerd, Denmark. Plasma glucose was analyzed with the glucose oxidase method with an Analox GM 7 measuring device (Analox Instruments, London, UK).

2.3.4. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by contrast analysis in the early-phase obesity experiment and by Student's *t*-test in the fully established obesity experiment. Weight gain from the beginning of the experiment was calculated for every second day and analyzed by ANOVA for repeated measurements to reveal the change in this parameter during the treatments. When pooled orthogonal components showed non-sphericity, Greenhouse-Geisser adjusted *P*-values were used. Logarithmic transformation of the data was performed when necessary. The calculations were performed using the BMDP software (BMDP Statistical Software, Los Angeles, CA, USA). A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Affinity of MPV-1743 A III to α_1-adrenoceptors, subtypes of α_2-adrenoceptors and imidazoline I_{2B}-binding sites

Similarly to atipamezole, MPV-1743 A III showed high affinity to α_2-adrenoceptors, with no remarkable selectivity to the subtypes (Table 1). In contrast to atipamezole, MPV-1743 A III had a remarkably high affinity to the α_1-adrenoceptors in the rat cerebral cortex as well as to the imidazoline I_{2b}-binding sites (Tesson et al., 1995) in the rat liver (Table 1).

3.2. Potency and time-course of α_2-adrenoceptor blocking activity of peroral MPV-1743 A III

Peroral pretreatment with MPV-1743 A III shifted the detomidine dose-response curve to the right in a parallel manner without reducing the maximal effect, suggesting competitive antagonism (Fig. 2). MPV-1743 A III and the reference α_2-adrenoceptor antagonists atipamezole and idazoxan reversed the detomidine-induced mydriasis (Fig. 3). The rank order of antagonist potencies to cause a 50% reversal was: MPV-1743 A III (ED_{50} 33 nmol/kg) ≥ atipamezole (ED_{50} 40 nmol/kg) > idazoxan (ED_{50} 91

Table 1
Affinities of atipamezole and MPV-1743 A III to α_1-adrenoceptors in the rat cerebral cortex (labelled by 0.15 nM [^{3}H]prazosin), α_2-adrenoceptor subtypes (labelled by 0.5 nM ($\alpha_{2A,D}$), 2.5 nM (α_{2B}) or 1 nM (α_{2C}) [^{3}H]RX821002) and imidazoline I_{2b}-binding sites (labelled by 0.4 nM [^{3}H]idazoxan)

Receptor/binding site	Atipamezole				MPV-1743 A III			
	Slope	IC_{50} (nM)	pK_i	n	Slope	IC_{50} (nM)	pK_i	n
α_1	1.08 ± 0.09	8 200 ± 630	5.32 ± 0.08	4	0.88 ± 0.01	150 ± 12	7.26 ± 0.03	4
α_{2A}	0.85 ± 0.08	1.4 ± 0.39	9.05 ± 0.08	3	0.95 ± 0.09	0.66 ± 0.06	9.35 ± 0.02	4
α_{2B}	1.03 ± 0.03	2.4 ± 0.34	8.93 ± 0.07	3	0.97 ± 0.03	3.8 ± 0.53	8.66 ± 0.06	3
α_{2C}	0.79 ± 0.05	2.6 ± 0.65	8.71 ± 0.06	3	0.95 ± 0.05	3.1 ± 0.61	8.72 ± 0.09	3
α_{2D}	0.92 ± 0.04	0.70 ± 0.05	9.49 ± 0.01	4	1.18 ± 0.02	0.94 ± 0.10	9.51 ± 0.03	4
I_{2b}	1.12 ± 0.02	18 000 ± 1 200	5.24 ± 0.03	5	1.00 ± 0.02	150 ± 5.0	7.11 ± 0.02	4

Slope values denote Hill number; IC_{50} values denote concentration of the compound that inhibited specific binding by 50%; pK_i values denote the negative logarithm of the inhibition constant (K_i value); n denotes number of experiments performed in triplicate. Numbers are arithmetic means (for pK_i values, geometric means) ± S.E.M.

nmol/kg). The time-course of central α_2-adrenoceptor blockade after peroral administration of MPV-1743 A III or atipamezole is given in Fig. 4. The duration of action of oral MPV-1743 A III is clearly longer ($t_{1/2}$ 5.7 h) than that of atipamezole ($t_{1/2}$ 2.1 h).

3.3. Anti-obesity effect of MPV-1743 A III

3.3.1. Early-phase obesity

Chronic treatment with MPV-1743 A III reduced weight gain (treatment effect: $P = 0.002$, time effect: $P < 0.001$, interaction of treatment and time: $P = 0.001$, ANOVA for repeated measurements). The two higher dose groups differed significantly from the control group (1 mg/kg per day: treatment effect: $P = 0.035$, time effect: $P < 0.001$, interaction: $P = 0.103$; 3 mg/kg per day: treatment effect: $P = 0.004$, time effect: $P < 0.001$, interaction: $P = 0.072$, ANOVA for repeated measurements, Fig. 5), whereas the smallest dose had no effect. When the weight gains up to the last day were compared, there was a significant treatment effect ($P = 0.003$, one-way ANOVA); the 3 mg/kg per day group differed significantly ($P = 0.009$, contrast analysis) and 1 mg/kg per day group somewhat less ($P = 0.056$) from the control. Cumulative food intake tended to be lower in these groups, the difference being almost significant ($P = 0.06$ one-way ANOVA, Table 2). None of the MPV-1743 A III doses had any effect on cumulative water intake (Table 2).

Binding of GDP to brown adipose tissue mitochondria was not affected by the treatment with MPV-1743 A III (Table 2). Similarly, there was no change in the expression of uncoupling protein mRNA in brown adipose tissue after the treatment when compared to the control (Table 2).

Chronic treatment with MPV-1743 A III had no effect on plasma concentration of insulin or glucose (Table 2). The weights of epididymal and intraperitoneal white fat pads were not changed when compared to the control (Table 2).

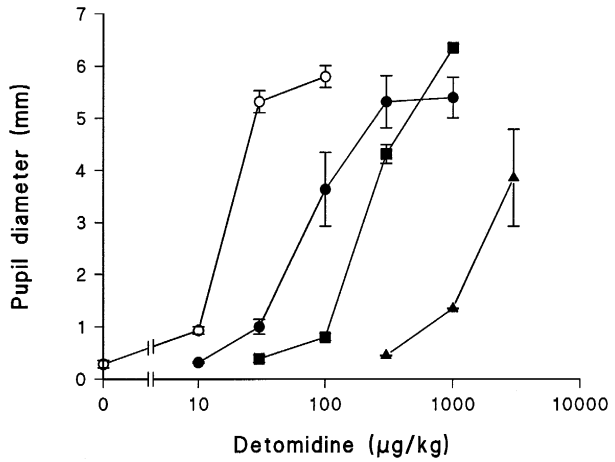

Fig. 2. Prevention of the mydriatic effect of detomidine (detomidine alone, ○) by MPV-1743 A III 0.1 mg/kg (●), 0.3 mg/kg (■), 1 mg/kg (▲) in anaesthetized rats. MPV-1743 A III was administered perorally 45 min before induction of anaesthesia and 60 min before challenge with cumulative i.v. doses of detomidine at 5-min intervals. Values are mean ± S.E.M., $n = 4$ in each group.

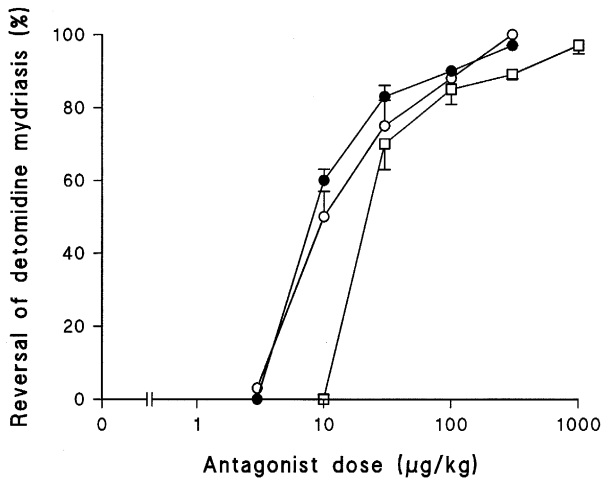

Fig. 3. Reversal of detomidine (100 μg/kg i.v. 10 min before) induced mydriasis in anaesthetized rats by MPV-1743 A III (●), atipamezole (○) and idazoxan (□). The antagonists were administered i.v. cumulatively at 5 min intervals. Values are mean ± S.E.M., $n = 4$ in each group.

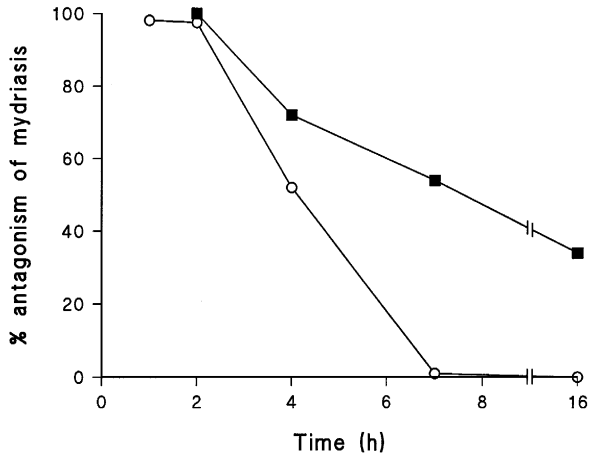

Fig. 4. Duration of central α_2-adrenoceptor blockade after administration of an oral dose of MPV-1743 A III 0.3 mg/kg (■) and atipamezole 1 mg/kg (○). $n = 4$ in each group.

3.3.2. Fully established obesity

Weight gain was lower in the MPV-1743 A III treated group than in the control, although the difference did not quite reach statistical significance ($P = 0.059$, ANOVA for repeated measurements; $P = 0.081$, Student's *t*-test in the last time point, Fig. 5). MPV-1743 A III had no effect on cumulative food or water intake in fully established obesity (Table 3).

Chronic treatment significantly increased GDP binding in this age group (67% higher than the control, Table 3). Similarly, the expression of uncoupling protein mRNA in brown adipose tissue was increased after the treatment (143% higher than the control, Table 3).

MPV-1743 A III did not change plasma insulin or glucose concentrations (Table 3). The weights of epididymal or intraperitoneal white fat pads were not affected when compared to the control (Table 3).

4. Discussion

In this study we found that a 3-week treatment with MPV-1743 A III, a novel imidazoline derivative, decreased weight gain in obese (*fa/fa*) Zucker rats in the early phase and in fully established obesity. In the latter, the binding of GDP to mitochondria and uncoupling protein mRNA expression were increased in brown adipose tissue indicating activation of non-shivering thermogenesis. In the former, food intake tended to be slightly lower in the MPV-1743 A III treated groups than in the control group.

The central α_2-adrenoceptor blocking properties of MPV-1743 A III were demonstrated in the present study by showing the ability of MPV-1743 A III to antagonize mydriasis induced by the α_2-adrenoceptor agonist detomidine. The two reference α_2-adrenoceptor antagonists, atipamezole and idazoxan, were slightly weaker with the rank order of potency being MPV-1743 A III ≥ atipamezole > idazoxan (Fig. 3). It was also shown that after oral administration MPV-1743 A III had a longer duration of action than atipamezole (Fig. 4). Furthermore, the receptor binding profile of MPV-1743 A III was investigated. MPV-1743 A III bound to α_2-adrenoceptors with high affinity showing no remarkable selectivity to the subtypes (Table 1). MPV-1743 A III had also a remarkably high affinity to the α_1-adrenoceptors and other studies have indicated that this reflects its activity as an agonist on α_1-adrenoceptors in various tissues in the rat (Savola, data not shown). In addition, MPV-1743 A III bound to the imidazoline I_{2b}-binding sites (Tesson et al., 1995). Although the lack of a suitable assay method to functionally characterize MPV-1743 A III at the imidazoline I_{2b}-binding sites prevents us from saying anything conclusive on the significance of its affinity at these sites, it is noteworthy that in another series of experiments (Tesson et al., 1995) a close correlation of binding affinity of a ligand to its property to inhibit

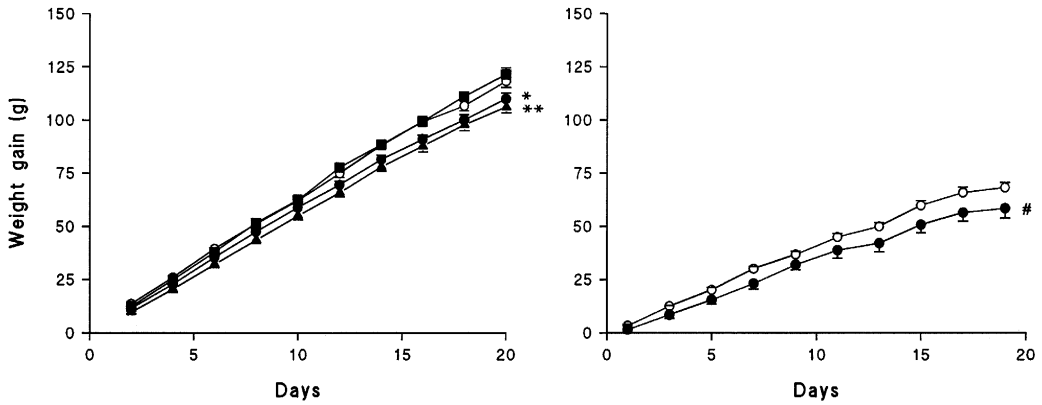

Fig. 5. Effect of MPV-1743 A III 0.3 mg/kg per day (■), 1 mg/kg per day (●), 3 mg/kg per day (▲) and control (○) on weight gain in early-phase obesity (left panel) and fully established obesity (right panel) in *fa/fa* Zucker rats. Drugs administered p.o. for 21 days, $n = 8–10$ in each group. Values are mean ± S.E.M. ** $P < 0.01$, * $P < 0.05$, $^{\#}$ $P = 0.059$ when compared to the control (ANOVA for repeated measures).

Table 2
Effects of MPV-1743 A III (p.o. for 3 weeks) or control treatments in early-phase obesity in *fa/fa* Zucker rats

	Control	MPV-1743 A III dose (mg/kg per day)		
		0.3	1	3
Cumulative food intake (g)	567 ± 10	570 ± 12	545 ± 9	537 ± 7
Cumulative water intake (ml)	591 ± 16	600 ± 16	575 ± 17	559 ± 14
GDP binding (pmol/g of tissue)	138 ± 15	175 ± 19	157 ± 15	148 ± 10
Uncoupling protein mRNA (relative density units)	5.4 ± 2.2	2.9 ± 1.0	1.9 ± 0.4	1.8 ± 0.6
Plasma insulin (ng/ml)	21.7 ± 2.6	23.1 ± 2.5	25.3 ± 3.7	24.7 ± 2.6
Plasma glucose (mmol/l)	7.8 ± 0.2	7.5 ± 0.2	8.0 ± 0.2	8.2 ± 0.2
Epididymal fat (g)	5.6 ± 0.3	5.5 ± 0.2	5.2 ± 0.2	5.6 ± 0.2
Intraperitoneal fat (g)	10.6 ± 0.5	10.2 ± 0.3	9.8 ± 0.3	10.1 ± 0.4

Values are mean ± S.E.M.; $n = 9–10$ in each group. No statistically significant differences observed between the control and any of the MPV-1743 A III groups.

monoamine oxidase was found. MPV-1743 A III also binds with micromolar affinity to muscarinic, serotonergic and histaminergic receptors, but whether they contribute to the pharmacological effects of MPV-1743 A III remains questionable (Savola, data not shown).

α_2-Adrenoceptor antagonists may increase sympathetic activation directly by blocking postsynaptic inhibitory α_2-adrenoceptors and indirectly through presynaptic receptors by increasing the release of noradrenaline. In addition, antagonism of central α_2-adrenoceptors may indirectly activate sympathetic outflow. Sympathetic activation leads to increased lipolysis and heat production in adipose tissue, and therefore, α_2-adrenoceptor antagonists have been studied as possible anti-obesity agents (Lafontan et al., 1992a; Galitzky et al., 1988). In contrast to the anti-obesity effect of MPV-1743 A III seen in this study, chronic treatment with the α_2-adrenoceptor antagonist atipamezole had no effect on weight gain or brown fat thermogenic activity in obese (*fa/fa*) Zucker rats in our previous study (Santti et al., 1994). As demonstrated in this study, the receptor binding profiles of the compounds are different. Both MPV-1743 A III and atipamezole show high affinity to α_2-adrenoceptors with no subtype selectivity, but in contrast to atipamezole, MPV-1743 A III has high affinity to α_1-adrenoceptors and to imidazoline I_{2b}-binding sites (Table 1).

The role of α_1-adrenoceptors in the regulation of brown adipose tissue thermogenesis is still controversial (Cannon et al., 1996). However, there is evidence that α_1-adrenoceptor agonism can potentiate β-adrenoceptor-mediated responses in brown adipose tissue (Ma and Foster, 1984; Granneman, 1988; Raasmaja and Larsen, 1989), and that cold acclimation and recruited states increase the number of α_1-adrenoceptors in brown adipose tissue, which suggest an increased importance of the α_1-adrenoceptor pathway in activated brown adipose tissue (Raasmaja and York, 1988). Imidazoline I_2-binding sites have been previously shown to exist in white adipose tissue in various species (MacKinnon et al., 1989; Langin et al., 1990a,b). They are located on monoamine oxidases, and ligand binding leads to inhibition of monoamine oxidase activity (Tesson et al., 1995). Although the role of imidazoline I_2-binding sites in white adipose tissue metabolism is still unknown (Carpéné et al., 1990a; Lafontan et al., 1992b), it is not possible to exclude that MPV-1743 A III could also indirectly affect sympathetically mediated regulation of non-shivering thermogenesis by modifying the function of monoamine oxidase and thereby metabolism of catecholamines in the adipose tissue. Eventually, MPV-1743 A III might be an interesting tool to elucidate the role of imidazoline I_2-binding sites in the regulation of adipose tissue metabolism.

Table 3
Effects of MPV-1743 A III (1 mg/kg per day p.o. for 3 weeks) or control treatments in fully established obesity in *fa/fa* Zucker rats

	Control	MPV-1743 A III
Cumulative food intake (g)	619 ± 13	608 ± 17
Cumulative water intake (g)	568 ± 17	517 ± 25
GDP binding (pmol/g tissue)	60 ± 9	101 ± 16 *
Uncoupling protein mRNA (relative density units)	0.6 ± 0.1	1.2 ± 0.4 *
Plasma insulin (ng/ml)	9.3 ± 1.5	8.7 ± 1.1
Plasma glucose (mmol/l)	7.4 ± 0.1	7.3 ± 0.3
Epididymal fat (g)	9.8 ± 0.4	9.3 ± 0.3
Intraperitoneal fat (g)	18.4 ± 0.9	18.7 ± 0.5

Values are mean ± S.E.M.; $n = 8$ in each group. * $P < 0.05$ when compared to control (Student's *t*-test).

Treatment with MPV-1743 A III activated brown adipose tissue thermogenesis only in fully established obesity. This is in line with earlier studies demonstrating abnormalities associated with obesity in both α_1- and α_2-adrenoceptor pathways in adipose tissue. The number of α_2-adrenoceptors in white adipose tissue increases with age and obesity in rats (Kobatake et al., 1991; Carpéné et al., 1990b) and the antilipolytic effect of the α_2-adrenoceptor agonist UK 14304 has been shown to increase with age and to be larger in obese than lean Zucker rats (Carpéné et al., 1990b). In contrast, the number of α_1-adrenoceptors in brown adipose tissue is decreased in obesity (Raasmaja and York, 1988).

Although the effects of MPV-1743 A III treatment on weight gain and brown adipose tissue thermogenesis after 3-week treatment were statistically significant, they were only modest. A similar type of treatment with a β_3-adrenoceptor agonist, BRL 35135, reduced weight gain by 19% and increased GDP binding to brown adipose tissue mitochondria 45-fold in obese Zucker rats (Santti et al., 1994). The far greater effect of BRL 35135 probably reflects the predominance of β_3-adrenoceptors over α_1- and α_2-adrenoceptors in rat adipose tissue. In several other species including man, α_2-adrenoceptors play a more important functional role in adipocytes (Lafontan et al., 1992a; Galitzky et al., 1988). The sympathetic tone is lowered in obese (*fa/fa*) Zucker rats (Godbole et al., 1978), and they also possess a reduced number of β_3-adrenoceptors compared to lean (*FA/FA*) Zucker rats (Muzzin et al., 1991). This could also partly explain the modest anti-obesity effect of MPV-1743 A III in obese Zucker rats.

In conclusion, the present study shows that MPV-1743 A III has an anti-obesity effect in a genetic rodent model of obesity. However, the relative importance of α_2- and α_1-adrenoceptors and imidazoline I_{2b}-binding sites in mediating the effects of MPV-1743 A III needs further evaluation.

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References

Ala-Uotila, S., Marjamäki, A., Matikainen, M.-T., Jalkanen, M., 1994. Use of a hollow fiber bioreactor for large-scale production of α_2-adrenoceptors in mammalian cells. J. Biotechnol. 37, 179–184.

Arch, J.R.S., Ainsworth, A.T., Cawthorne, M.A., Piercy, V., Sennitt, M.V., Thody, V.E., Wilson, C., Wilson, S., 1984. Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. Nature 309, 163–165.

Berlan, M., Galitzky, J., Rivière, D., Foureau, M., Tran, M.A., Flores, R., Louvet, J.P., Houin, G., Lafontan, M., 1991. Plasma catecholamine levels and lipid mobilization induced by yohimbine in obese and non-obese women. Int. J. Obes. 15, 305–315.

Berridge, T.L., Gadie, B., Roach, A.G., Tulloch, I.F., 1983. α_2-Adrenergic agonists induce mydriasis in the rat by an action within the central nervous system. Br. J. Pharmacol. 78, 507–515.

Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bray, G.A., 1990. The MONA LISA hypothesis: most obesities known are low in sympathetic activity. In: Oomura, Y., Tarui, S., Inoue, S., Shimazuogress, T. (Eds.), Progress in Obesity Research. John Libbey, London, pp. 61–66.

Cannon, B., Jacobsson, A., Rehnmark, S., Nedergaard, J., 1996. Signal transduction in brown adipose tissue recruitment: noradrenaline and beyond. Int. J. Obes. 20, S36–S42.

Carpéné, C., Galitzky, J., Larrouy, D., Langin, D., Lafontan, M., 1990a. Non-adrenergic sites for imidazolines are not directly involved in the α_2-adrenergic antilipolytic effect of UK 14304 in rat adipocytes. Biochem. Pharmacol. 40, 437–445.

Carpéné, C., Rebourcet, M.-C., Guichard, C., Lafontan, M., Lavau, M., 1990b. Increased α_2-adrenergic binding sites and antilipolytic effect in adipocytes from genetically obese rats. J. Lipid Res. 31, 811–819.

Cawthorne, M.A., Sennitt, M.V., Arch, J.R.S., Smith, S.A., 1992. BRL 35135, a potent and selective atypical β-adrenoceptor agonist. Am. J. Clin. Nutr. 55, 252S–257S.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Domínguez, M.J., Fernández, M., Elliott, K., Benito, M., 1986. Occurrence of α_2-adrenergic effects on adenylate cyclase activity and [^{3}H]-clonidine specific binding in brown adipose tissue from foetal rats. Biochem. Biophys. Res. Commun. 138, 1390–1394.

Doxey, J.C., Roach, A.G., Smith, C.F.C., 1983. Studies on RX 781094: a selective, potent and specific antagonist of α_2-adrenoceptors. Br. J. Pharmacol. 78, 489–505.

Galitzky, J., Taouis, M., Berlan, M., Rivière, D., Garrigues, M., Lafontan, M., 1988. α_2-Antagonist compound and lipid mobilization: evidence for lipid mobilizing effect of oral yohimbine in healthy male volunteers. Eur. J. Clin. Invest. 18, 587–594.

Galitzky, J., Vermorel, M., Lafontan, M., Montastruc, P., Berlan, M., 1991. Thermogenic and lipolytic effect of yohimbine in the dog. Br. J. Pharmacol. 104, 514–518.

Gazzola, C., 1993. α_2-Adrenoceptor-mediated effects on resting energy expenditure. Int. J. Obes. 17, 637–641.

Godbole, V., York, D.A., Bloxham, D.P., 1978. Developmental changes in the fatty (*fa/fa*) rat: evidence for defective thermogenesis preceding the hyperlipogenesis and hyperinsulinaemia. Diabetologia 15, 41–44.

Granneman, J., 1988. Norepinephrine infusions increase adenylate cyclase responsiveness in brown adipose tissue. J. Pharmacol. Exp. Ther. 245, 1075–1080.

Himms-Hagen, J., 1992. Brown adipose tissue metabolism. In: Björntorp, P., Brodoff, B.N. (Eds.), Obesity, 1st ed. J.B. Lippincott, Philadelphia, PA, pp. 15–34.

Kobatake, T., Watanabe, Y., Matsuzawa, Y., Tokunaga, K., Shigenori, F., Kawamoto, T., Keno, Y., Tarui, S., Yoshida, H., 1991. Age-related changes in adrenergic α_1, α_2, and β receptors of rat white fat cell membranes: an analysis using [^{3}H]bunazosin as a novel ligand for the α_1 adrenoceptor. J. Lipid Res. 32, 191–196.

Koss, M.C., Gherezghiher, T., Nomura, A., 1984. CNS adrenergic inhibition of parasympathetic oculomotor tone. J. Auton. Nerv. Syst. 10, 55–68.

Lafontan, M., Berlan, M., Galitzky, J., Montastruc, J., 1992a. Alpha-2 adrenoceptors in lipolysis: α_2-antagonists and lipid-mobilizing strategies. Am. J. Clin. Nutr. 55, 219S–227S.

Lafontan, M., Langin, D., Portillo, M., Paris, H., 1992b. Imidazoline binding sites in fat cells. Localization and pharmacologic differentiation from α_2-adrenergic receptors. Am. J. Hypertens. 5, 72S–79S.

Langin, D., Paris, H., Dauzats, M., Lafontan, M., 1990a. Discrimination between α_2-adrenoceptors and [^{3}H]idazoxan-labelled non-adrenergic sites in rabbit white fat cells. Eur. J. Pharmacol. 188, 261–272.

Langin, D., Paris, H., Lafontan, M., 1990b. Binding of [^{3}H]idazoxan and of its methoxy derivative [^{3}H]RX821002 in human fat cells: [^{3}H]idazoxan but not [^{3}H]RX821002 labels additional non-α_2-adrenergic binding sites. Mol. Pharmacol. 37, 876–885.

Ma, S.W., Foster, D.O., 1984. Potentiation of in vivo thermogenesis in rat brown adipose tissue by stimulation of alpha 1-adrenoceptors is associated with increased release of cyclic AMP. Can. J. Physiol. Pharmacol. 62, 943–948.

MacKinnon, A.C., Brown, C.M., Spedding, M., Kilpatrick, A.T., 1989. [^{3}H]-Idazoxan binds with high affinity to two sites on hamster adipocytes: an α_2-adrenoceptor and a non-adrenoceptor site. Br. J. Pharmacol. 98, 1143–1150.

Marjamäki, A., Luomala, K., Ala-Uotila, S., Scheinin, M., 1993. Use of recombinant human α_2-adrenoceptors to characterize subtype selectivity of antagonist binding. Eur. J. Pharmacol. 246, 219–226.

Milner, R.E., Wilson, S., Arch, J.R.S., Trayhurn, P., 1988. Acute effects of a β-adrenoceptor agonist (BRL 26830A) on rat brown-adipose-tissue mitochondria. Biochem. J. 249, 759–763.

Muzzin, P., Revelli, J.-P., Kuhne, F., Gocayne, J.D., McCombie, W.R., Venter, J.G., Giacobino, J.-P., Fraser, C.M., 1991. An adipose tissue-specific β-adrenergic receptor. J. Biol. Chem. 266, 24053–24058.

Nicholls, D.G., 1976. Hamster brown-adipose-tissue mitochondria. Purine nucleotide control of the inner membrane, the nature of the nucleotide binding site. Eur. J. Biochem. 62, 223–228.

Raasmaja, A., Larsen, P.R., 1989. α_1- and β-adrenergic agents cause synergistic stimulation of the iodothyronine deiodinase in rat brown adipocytes. Endocrinology 125, 2502–2509.

Raasmaja, A., York, D.A., 1988. α_1- and β-adrenergic receptors in brown adipose tissue of lean (*Fa/?*) and obese (*fa/fa*) Zucker rats. Effects of cold-acclimation, sucrose feeding and adrenalectomy. Biochem. J. 249, 831–838.

Ricquier, D., Mory, G., 1984. Factors affecting brown adipose tissue activity in animals and man. Endocrinol. Metab. Clin. North Am. 13, 501–521.

Rothwell, N.J., Stock, M.J., Stribling, D., 1982. Diet-induced thermogenesis. Pharmacol. Ther. 17, 251–268.

Rouru, J., Isaksson, K., Santti, E., Huupponen, R., Koulu, M., 1993. Metformin and brown adipose tissue thermogenetic activity in genetically obese Zucker rats. Eur. J. Pharmacol. 246, 67–71.

Santti, E., Huupponen, R., Rouru, J., Hänninen, V., Pesonen, U., Jhanwar-Uniyal, M., Koulu, M., 1994. Potentiation of the anti-obesity effect of the selective β_3-adrenoceptor agonist BRL 35135 in obese Zucker rats by exercise. Br. J. Pharmacol. 113, 1231–1236.

Seydoux, J., Girardier, L., 1978. Control of brown fat thermogenesis by the sympathetic nervous system. Experientia Suppl. 32, 153–167.

Tesson, F., Limon-Boulez, I., Urban, P., Puype, M., Vandekerckhove, J., Coupry, I., Pompon, D., Parini, A., 1995. Localization of I_2-imidazoline binding sites on monoamine oxidases. J. Biol. Chem. 270, 9856–9861.

Virtanen, R., Nyman, L., 1985. Evaluation of the α_1- and α_2-adrenoceptor effects of detomidine, a novel veterinary sedative analgesic. Eur. J. Pharmacol. 108, 163–169.

Virtanen, R., Savola, J.-M., Saano, V., 1989. Highly selective and specific antagonism of central and peripheral α_2-adrenoceptors by atipamezole. Arch. Int. Pharmacodyn. Ther. 297, 190–204.