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Effects of atipamezole — a selective α_2-adrenoceptor antagonist — on cardiac parasympathetic regulation in human subjects

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Summary

1 This double-blind, cross-over, placebo-controlled study on six healthy male volunteers was designed to evaluate the effects of α_2-adrenoceptor antagonism on cardiac parasympathetic regulation.

2 The subjects received atipamezole intravenously as a three-step infusion, which aimed at steady-state serum concentrations of 10, 30 and 90 ng ml^{-1} at 50-min intervals.

3 Drug effects were assessed with repeated recordings of blood pressure and electrocardiogram, in which the high-frequency (0.15–0.40 Hz) R-R interval variation is supposed to reflect cardiac parasympathetic efferent neuronal activity.

4 At the end of the three steps of the infusion, the mean (±SD) concentrations of atipamezole were 10.5 (3.9), 26.8 (5.6) and 81.3 (21.1) ng ml^{-1}.

5 Within this concentration range, atipamezole appeared to reduce slightly the high-frequency R-R interval fluctuations, indicating a minor vagolytic effect in the heart.

6 Atipamezole increased systolic and diastolic arterial pressure, on average by 20 and 14 mmHg (maxima at the second step of the infusion), which evidently reflects an overall sympathetic augmentation.

Keywords: atipamezole, heart rate variability, parasympathetic nervous system

Introduction

α_2-Adrenergic receptors have an essential role in cardiovascular autonomic regulation. Activation of central α_2-adrenoceptors in the brain stem decreases heart rate (HR), lowers blood pressure, sensitizes the cardiac baroreflex, and reduces blood pressure and HR oscillations (Elghozi, Laude & Janvier, 1991; Tulen *et al.*, 1993; Parlow *et al.*, 1999). On the contrary, peripheral postsynaptically located α_2-receptors control vasoconstriction (Civantos Calzada & Aleixandre de Artinano, 2001), thus being involved in the pressor response to catecholamines (Doda, 1997).

Atipamezole is a potent, specific and selective α_2-adrenoceptor antagonist suitable for the investigations of α_2-antagonistic activity in experimental models (Virtanen, Savola & Saano, 1989; Newman-Tancredi *et al.*, 1998). In addition, atipamezole may have some influence on the imidazoline-preferring receptors (Sjöholm, Savola & Scheinin, 1995). In veterinary medicine, atipamezole has been approved for the reversal of α_2-agonist-induced sedation or analgesia (Scheinin, MacDonald & Scheinin, 1988; Virtanen *et al.*, 1989). In humans, α_2-receptor blocking agents have been suggested to have therapeutic potential, e.g. in the treatment of adult-onset diabetes mellitus, depression, dementia and male sexual impotence (Chapleo, 1988; Berlan, Montastruc & Lafontan, 1992).

Large doses of atipamezole raise arterial blood pressure and noradrenaline plasma concentrations, and cause increased tension, alertness, coldness of hands and muscle tremor as manifestations of increased sympathetic nervous activity

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(Karhuvaara *et al.*, 1989, 1990). Where the typical effect of α_2-adrenoceptor antagonists in humans is an elevation of blood pressure, the HR response seems to be more variable (Goldberg, Hollister & Robertson, 1983; Elliott, Jones, Vincent, Lawrie & Reid, 1984). The effects of α_2-receptor antagonists on the HR or cardiac vagal tone may actually be biphasic, e.g. small doses of these drugs have been suggested to decrease, and large doses to increase vagal tone in the heart (Andrejak, Ward & Schmitt, 1983; Ramage & Tomlinson, 1985). In the few previous human studies on atipamezole, no marked effects on HR have been reported (Karhuvaara *et al.*, 1990; Huupponen, Karhuvaara, Anttila, Vuorilehto & Scheinin, 1995).

The measurement of high-frequency (HF, 0.15–0.40 Hz) fluctuations in HR has proved to be a sensitive method to detect gradual changes in cardiac parasympathetic modulation (see e.g. Pomeranz *et al.*, 1985; Hayano *et al.*, 1991; Scheinin *et al.*, 1999; Penttilä *et al.*, 2001a; Penttilä, Helminen, Luomala & Scheinin, 2001b). It should be noted that the changes in the HF variability of HR and the HR level do not always parallel each other (Medigue *et al.*, 2001), because the latter reflects the balance between vagal and sympathetic tone. We have recently demonstrated that the selective α_2-adrenoceptor agonist dexmedetomidine increases the parasympathetic-associated HF variability (Penttilä, Helminen, Anttila, Hinkka & Scheinin, 2004). In the present study, we wanted to explore whether the α_2-adrenoceptor antagonist, atipamezole, would, on the contrary, diminish the HF variability in a similar group of healthy subjects. We also evaluated the influence of atipamezole on cardiac repolarization (QT interval) and baroreflex sensitivity (phenylephrine test).

Methods

Study design and subjects

Atipamezole and placebo were given intravenously (i.v.) to six healthy non-smoking male volunteers (age 21–28 years; weight 71–78 kg; height 172–181 cm) using a double-blind, randomized, cross-over design. The washout period between consecutive infusions was at least 4 days. The general health of the volunteers was ascertained by clinical examinations including a 12-lead electrocardiogram (ECG). Medication and alcohol were forbidden for 48 h prior to the beginning of the study. The protocol was approved by the Ethics Committee of Turku University Hospital, Turku, Finland, and the study was performed in accordance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). The experiments were undertaken with the understanding and written consent of each subject.

Investigational drug

The active drug was atipamezole hydrochloride (Antisedan®, Orion Pharma, Espoo, Finland) diluted to 0.75 mg ml^{-1} final concentration and placebo was 0.9% sodium chloride solution. Atipamezole (and placebo) was administered as a continuous i.v. infusion using an infusion pump (Braun Perfusor ED 2, B. Braun AG, Melsungen, Germany) and the following infusion speeds: first step, loading dose 200 μg min^{-1} for 10 min, then maintenance at 25 μg min^{-1} for 40 min; second step, 400 μg min^{-1} for 10 min, then 50 μg min^{-1} for 40 min; third step, 1200 μg min^{-1} for 10 min, then 150 μg min^{-1} for 40 min. This dosing scheme was based on the earlier reported values of pharmacokinetic parameters of atipamezole (Karhuvaara *et al.*, 1990) and was supposed to produce steady-state serum concentrations of 10, 30 and 90 ng ml^{-1}, respectively. The total dose of atipamezole was approximately 27 mg within 2.5 h.

Study procedure

On the study days the subjects fasted from midnight, and the sessions started between 08:00 and 09:00 h. The subjects were connected to ECG and blood pressure devices, cannulated and baseline blood samples were drawn. During the study, the subjects were in supine position and they received a continuous 5% glucose infusion (100 ml h^{-1}). After a 30-min stabilization period, the session began with baseline cardiovascular measurements at rest, followed by the phenylephrine test (see below). The stepwise atipamezole/placebo infusion was then started and the measurements were repeated at each 50-min step, starting always 20 min after switching to the new concentration level. Blood samples were collected twice at each step (see Fig. 1). After the third step of the infusion, drug administration was discontinued and the measurements were repeated at 30 min, 1 and 2 h. The whole study session lasted approximately 5 h.

Cardiovascular measurements

Throughout the study, 5-min recordings of standard ECG signal, non-invasive blood pressure (Finapres 2300 device and Ohmeda 2300 BP Monitor; Ohmeda Inc., Louisville, CO, USA) and air flow (M909 flow-volume spirometer, Medikro Oy, Kuopio, Finland) were collected. During these recordings the subjects breathed at a fixed rate of 15 breaths min^{-1}. The spirometer data was needed for the calculations of tidal volume and minute ventilation (MV). The signals from the amplifier output were analogue-to-digital converted (M9401 serial interface unit, Medikro; temporal resolution 200 Hz), recorded and beat-by-beat time series of

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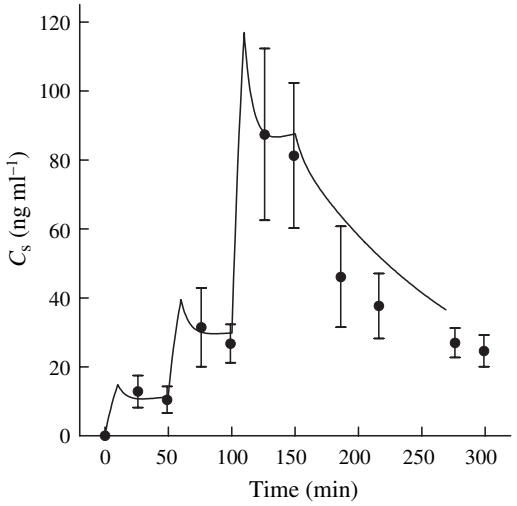

Figure 1 Simulated (continuous line; based on the values of pharmacokinetic parameters reported by Karhuvaara *et al*., 1990) and mean (SD) observed (circles) concentrations of atipamezole in serum during and after the infusion which targeted at three increasing steady-state serum concentrations (10, 30 and 90 ng ml^{-1}) with 50-min intervals.

R-R intervals (RRI), systolic and diastolic arterial pressures (SAP, DAP) and relative air flow were generated (CAFTS software package, Medikro).

The RRI time series were subjected to power spectral analysis in frequency domain using modified covariance autoregressive modelling with fixed model order of 14 (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Total power, i.e. the variance of RRI variability was generated after linear detrending of the signals. The low-frequency (LF, 0.07–0.15 Hz) and high-frequency (HF, 0.15–0.40 Hz) spectral powers were then computed by integration over the corresponding frequency bands. The coefficient of component variance for the HF spectral power (HF CCV) was also computed, using the formula (Hayano *et al*., 1991):

$$\text{HF CCV} = \frac{\sqrt{\text{HF power}}}{\text{Mean RRI}} \times 100. \quad (1)$$

In this study, power spectral variables are presented per MV, to take into consideration the possible changes in respiration (Brown, Beightol, Koh & Eckberg, 1993; Penttilä *et al*., 2001a). The ratio of the LF and HF powers was also computed, as this ratio has previously been suggested to describe cardiac sympathovagal balance (Pagani *et al*., 1986).

The QT intervals were analysed in 1-min representative periods of the ECG recordings collected at baseline and at the third step of the infusion (WinCPRS software version 1.153, Absolute Aliens Corp., Turku, Finland). Corrected QT intervals (QTc) were calculated by dividing QT by the square root of RRI given in seconds (Bazett's formula).

Baroreflex sensitivity (BRS) was estimated using the phenylephrine test, where a 150 μg i.v. injection of phenylephrine hydrochloride (Elkins-Sinn, Cherry Hill, NJ, USA) was given to raise blood pressure by 20–40 mmHg (see e.g. Parati, Di Rienzo & Mancia, 2000). In the analysis, a time window with a rise in SAP and lengthening in RRI was interactively selected and these RRIs were plotted beat-by-beat against the SAP values of the preceding cardiac cycle. BRS was then defined as the slope of the regression line fitting the SAP and RRI changes, and expressed in ms mmHg^{-1}. Only the analyses with Pearson's correlation coefficient (r) > 0.8 were accepted for statistics. The phenylephrine test was performed twice at each concentration and the mean of the two obtained BRS values was calculated.

Drug concentrations

The venous blood samples (7 ml) were centrifuged and the separated sera stored in plastic storage tubes at −20 °C until analysed. The analyses were performed at the Pharmacokinetics Laboratory of Orion Pharma (Turku, Finland) using a validated high-performance liquid chromatographic method (Huupponen *et al*., 1995). The lower limit of quantitation was 0.5 ng ml^{-1}.

Statistical analysis

The effects of atipamezole *vs*. placebo on the variables were examined from baseline up to the third step of the infusion (except for phenylephrine test where all time points were included in the analysis), using analysis of variance (ANOVA) for cross-over design with repeated measurement within periods. Whenever needed to normalize a skewed data distribution, log-transformations were performed. Statistical significance was established at the level $P < 0.05$. The following parameters were estimated in the model: carry-over or sequence effect, period, time and treatment effects as well as interactions of those effects. Possible treatment effect was primarily examined with time × treatment interaction. Interactions between time and treatment were examined by linear contrasts of cell means. The statistical analyses were carried out using MIXED PROCEDURE in SAS 6.11 statistical software (SAS Institute Inc., Cary, NC, USA).

Results

The main results of this study are presented in Table 1 and Figs 1 & 2. During the three-step infusion, the targeted atipamezole concentrations

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Table 1 Atipamezole concentrations (C_s) in serum and cardiovascular variables in six healthy subjects at baseline and at the end of each step of the infusion

	Baseline	Step 1	Step 2	Step 3
Targeted C_s (ng ml^{-1})				
ati	0	10	30	90
Measured C_s (ng ml^{-1})				
ati	0 (0)	10.5 (3.9)	26.8 (5.6)	81.3 (21.1)
Mean RRI (ms)†				
ati	1158 (163)	1244 (154)**	1255 (164)*	1206 (152)
pla	1209 (159)	1209 (128)	1242 (137)	1245 (124)
SAP (mmHg)				
ati	130 (12.4)	141 (15.4)	150 (15.1)**	141 (18.6)
pla	133 (18.1)	138 (15.2)	140 (15.6)	146 (14.0)
DAP (mmHg)				
ati	67.5 (7.2)	73.2 (9.1)	81.3 (12.1)*	76.2 (10.0)
pla	67.3 (9.7)	71.3 (8.4)	71.8 (7.2)	75.7 (7.1)
MV (l min^{-1})				
ati	10.5 (1.6)	10.8 (2.3)	11.4 (2.9)	12.8 (4.2)
pla	10.2 (1.2)	9.8 (1.1)	9.6 (1.1)	9.6 (0.7)
HF power (ms^2) MV^{-1}				
ati	378 (267)	313 (263)**	283 (231)	310 (345)*
pla	307 (238)	440 (328)	308 (198)	404 (314)
HF CCV/MV				
ati	0.493 (0.16)	0.399 (0.16)***	0.366 (0.12)**	0.359 (0.15)***
pla	0.422 (0.15)	0.512 (0.21)	0.435 (0.13)	0.482 (0.19)
LF power/HF power				
ati	0.29 (0.38)	0.23 (0.24)	0.28 (0.29)	0.26 (0.27)
pla	0.29 (0.31)	0.26 (0.22)	0.19 (0.09)	0.28 (0.19)
BRS (ms mmHg^{-1})‡				
ati	23.2 (6.0)	20.2 (16.1)	19.8 (11.2)	15.6 (6.5)
pla	20.8 (12.2)	22.7 (12.6)	17.2 (11.3)	14.5 (6.1)

Statistically significant differences between atipamezole (ati) and placebo (pla) treatments are indicated as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are presented as mean (SD).

†Also a significant period × time interaction ($P = 0.004$).

‡Due to technical problems, $n = 4$ in the phenylephrine test (see text for details).

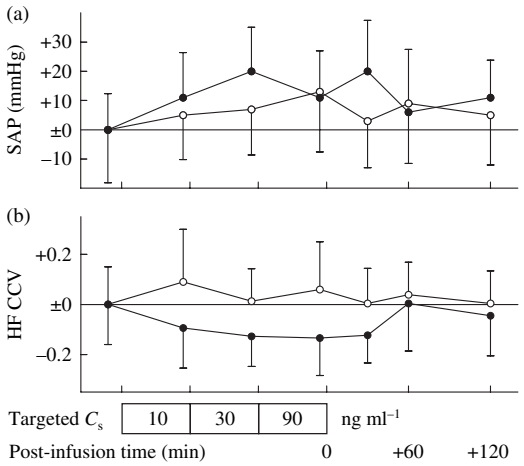

Figure 2 Systolic arterial pressure (a) and minute ventilation-corrected HF CCV (b) during and after the infusion of atipamezole (solid circles) and placebo (open circles). The variables are presented as changes from the average baseline of each session (atipamezole/placebo). Data are shown as mean (±SD); $n = 6$.

were well achieved. On average, atipamezole increased SAP by 20 mmHg and DAP by 14 mmHg (maxima at the 30 ng ml^{-1} level), and there was a minor decrease in HR, from 52 to 48 beats min^{-1}. The influence on HR was not concentration-dependent: at the highest 90 ng ml^{-1} level, the effect of atipamezole on the mean RRI did not differ from placebo. In MV and tidal volume (data not shown), there were no differences between the treatments.

Atipamezole decreased the HF spectral power ($P = 0.041$ in comparison with placebo) as well as HF CCV ($P < 0.001$), and in HF CCV the reduction was significant at all three steps of the infusion (Table 1 and Fig. 2b). At the highest concentration of atipamezole, the average MV-corrected HF CCV was decreased by 27% from the baseline level. Atipamezole had no statistically significant influences on the total power, the LF power or the LF power/HF power ratio (data not shown).

There were no statistically significant differences in QT or QTc interval between atipamezole and placebo. The maximal individual QTc interval, 396.4 ms, was measured at baseline, before drug administration.

The phenylephrine test was technically problematic as slopes with $r > 0.8$ were seen in only four of the six subjects. In these four persons, the BRS

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values appeared to decrease during the study days, but no significant drug effects were detected.

Discussion

As anticipated, and contrary to the α_2-agonist dexmedetomidine (Penttilä *et al.*, 2004), the α_2-antagonist atipamezole was found to reduce respiration-related HF HR variability, which mainly reflects cardiac parasympathetic regulation (Pomeranz *et al.*, 1985; Hayano *et al.*, 1991). Significant drug effects were observed both in the HF spectral power and its derivative HF CCV. Our finding seems to be in conformity with earlier results in anaesthetized dogs that a small dose of an α_2-adrenoceptor blocking agent, yohimbine, may increase sympathetic nervous activity and simultaneously decrease cardiac vagal activity (Andrejak *et al.*, 1983). The small fluctuations in the HF variability level during the placebo sessions can probably be attributed to the intra-individual variability of these sensitive measures combined with the small number of study subjects.

Because atipamezole is not supposed to bind to muscarinic acetylcholine receptors (Virtanen *et al.*, 1989), the inhibition of HF HR oscillations is obviously mediated by other mechanisms. α_2-Adrenoceptor antagonists are known to increase sympathetic nervous activity and raise noradrenaline concentrations at the neuroeffector junctions and in plasma (McCall, Schuette, Humphrey, Lahti & Barsuhn, 1983; Elliott *et al.*, 1984; Ramage & Tomlinson, 1985; Karhuvaara *et al.*, 1989, 1990). Experimental studies on animals have shown that noradrenaline acting via different α_2-adrenoceptor subtypes can inhibit acetylcholine release from central and peripheral cholinergic nerve terminals (Loiacono & Story, 1986; Tellez, Colpaert & Marien, 1999). Furthermore, if the atipamezole-induced release of noradrenaline from sympathetic nerve endings also takes place in the dorsal motor nucleus of the vagus nerve (Unnerstall, Kopajtic & Kuhar, 1984), it may augment the central noradrenergic inhibition of this parasympathetic nucleus, and lead to impaired vagal regulation of HR. In fact, this possible central effect has been proposed to be the mechanism behind the anticholinergic-like effects of reboxetine, a selective noradrenaline reuptake inhibitor with low affinity for muscarinic receptors (Szabadi, Bradshaw, Boston & Langley, 1998; Penttilä, Syvälahti, Hinkka, Kuusela & Scheinin, 2001c).

The low resting HR of 52 beats min^{-1} reflects the fact that the volunteers were healthy, physically well trained and evidently had a high resting vagal tone. Because endurance training improves the vagal modulation of HR and, hence, increases the HF variability (Shin, Minamitani, Onishi, Yamazaki & Lee, 1997), it can be assumed that the baseline values of and the changes in the HF spectral power observed in our study are of greater magnitude than would be in a group of untrained persons – or patients with impaired cardiac autonomic regulation. In consequence, it is very difficult to draw conclusions concerning the use of atipamezole in patients. After all, the vagolytic action of atipamezole in serum concentrations up to 90 ng ml^{-1} is evidently rather slight when compared with total parasympathetic blockade. We have previously shown that (supra)maximal doses of the muscarinic antagonist glycopyrrolate reduce the HF spectral power by more than 99% (Penttilä *et al.*, 2001a).

The maximal increase of blood pressure was observed at the end of the second step of the infusion (30 ng ml^{-1} level), when the subjects had received 9 mg dose of the drug. The hypertensive effect was somewhat more pronounced than in previous reports, in which the administration of 25 mg atipamezole as a 20-min infusion increased SAP by 12 mmHg, while 100 mg was needed to increase SAP by 20 mmHg (Karhuvaara *et al.*, 1989, 1990). This discrepancy may be due to the phenylephrine tests: although the transient α_1-receptor-mediated blood pressure rise during the test is considered to be short-lasting, it is possible that repeated phenylephrine tests could interfere with the blood pressure measurements at the subsequent step of the infusion. Such a carry-over effect could also explain the apparent increases of SAP and DAP during the placebo infusion. Nevertheless, this possible effect of phenylephrine should not have influenced the detected differences between atipamezole and placebo (see below), because the effect, if present, should have had an equal impact on the measurements in both sessions.

At 30 ng ml^{-1}, the hypertensive effect of atipamezole differed significantly from placebo, which evidently reflects the already described overall sympathetic activation. This interpretation is also supported by previous studies on yohimbine, where the α_2-antagonist increased noradrenaline levels together with blood pressure levels (Damase-Michel *et al.*, 1993; Senard *et al.*, 1993; Mosqueda-Garcia *et al.*, 1998). At the concentrations used in the current study (10–90 ng ml^{-1}), the α_1-agonist effects seen in rats after high doses of atipamezole are not likely to contribute to the hypertensive effect (McCall *et al.*, 1983; Virtanen *et al.*, 1989; Vayssettes-Courchay, Bouysset, Cordi, Laubie & Verbeuren, 1996). In clinical practice, the sympathetic-excitatory and hypertensive effects of atipamezole should be taken into account, e.g. when treating haemodynamically compromized patients.

Although the slight lengthening of RRI at low atipamezole concentrations appeared to differ statistically significantly from placebo, we consider this finding solely incidental and related to the lower baseline RRI in the atipamezole session (the reason for which remains unclear). In the earlier studies on healthy human subjects, various doses of

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atipamezole given intravenously (10–100 mg in 20 min), orally (20–100 mg) and buccally (5–40 mg) have not had any significant influences on HR (Karhuvaara *et al.*, 1990; Huupponen *et al.*, 1995).

Conclusions

Within the studied concentration range from 10 to 90 ng ml^{-1}, atipamezole appears to exert a slight but measurable vagolytic effect in the heart, manifested as a reduction of respiration-related HF HR variability. As anticipated, atipamezole increased arterial blood pressure, which evidently reflects an overall sympathetic augmentation. Atipamezole did not affect cardiac repolarization. The close agreement between simulated and measured serum concentration levels of atipamezole seems to confirm the earlier reported pharmacokinetic parameters of the drug (Karhuvaara *et al.*, 1990).

Acknowledgments

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