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Pharmacological effects and pharmacokinetics of atipamezole, a novel α_2-adrenoceptor antagonist—a randomized, double-blind cross-over study in healthy male volunteers

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1 Single doses (10, 30 and 100 mg) of atipamezole (MPV-1248), a new potent and selective imidazole-type α_2-adrenoceptor antagonist, and saline placebo were administered as 20 min intravenous infusions to six healthy male volunteers in a randomized double-blind, cross-over phase I study. Later, 100 mg atipamezole was given orally to the same subjects in an open fashion.

2 The i.v. doses resulted in linearly dose-related concentrations of atipamezole in plasma. Pharmacokinetic calculations revealed an elimination half-life of 1.7–2.0 h, an apparent volume of distribution of 3.0–3.5 l kg^{-1} and a total plasma clearance of 1.1–1.5 l h^{-1} kg^{-1}. No atipamezole could be detected in plasma after oral dosing.

3 Subjective drug effects were seen mainly after the largest i.v. dose and included increased alertness and nervousness, coldness and sweating of hands and feet, tremor and shivering, motor restlessness, and increased salivation. Salivation was also quantitated using dental cotton rolls, with dose-related increases produced by the i.v. doses.

4 The 100 mg i.v. dose increased plasma noradrenaline concentrations on average by 484 ± 269 (s.d.) %, and also elevated both systolic and diastolic blood pressure (mean increases 17 ± 7/14 ± 2 mm Hg). The 30 mg dose had minor and the 10 mg dose no effects on these variables. Adrenaline and cyclic AMP levels in plasma were increased only after the largest dose. No drug effects were observed after oral dosing.

4 Plasma C-peptide and blood glucose levels were not markedly influenced by the drug, and cortisol secretion was not stimulated.

5 The observed effects are compatible with the presumed α_2-adrenoceptor antagonistic action of atipamezole and are in general concordance with the reported results of other α_2-adrenoceptor antagonists (yohimbine and idazoxan).

6 Although not orally active, atipamezole may prove to be a useful agent in studies of α_2-adrenoceptor function in man.

Keywords atipamezole α_2-adrenoceptor antagonist pharmacokinetics pharmacodynamics

Introduction

One of the primary actions of α_2-adrenoceptor antagonists is the blockade of inhibitory autoreceptors on noradrenergic nerve endings, leading to increased release of the neurotransmitter (Langer, 1981, 1987). Other pharmacological effects resulting from α_2-adrenoceptor blockade include presynaptic regulation of acetylcholine release and postsynaptic actions on smooth muscle contraction, energy metabolism, hormone secretion, and platelet aggregation (Chapleo, 1988; Goldberg & Robertson, 1983; Ruffolo *et al.*, 1988). The therapeutic potential of α_2-adrenoceptor antagonistic drugs is still relatively unexplored (Chapleo, 1988). They may, for example, constitute a new class of antidepressive drugs since idazoxan, a fairly specific and selective α_2-adrenoceptor antagonist (Doxey *et al.*, 1983), has shown promising antidepressive activity in early clinical trials (Crossley, 1984). Another, more obvious clinical use for α_2-adrenoceptor blocking drugs would be reversal of the effects induced by α_2-adrenoceptor agonists (Scheinin *et al.*, 1988).

Atipamezole [MPV-1248, 4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole, see Figure 1 for structure] is a novel potent, specific and selective α_2-adrenoceptor antagonist synthesized by Farmos Group Ltd, Finland (Karjalainen *et al.*, 1986). Compared with the α_2-adrenoceptor antagonists most often studied in humans, i.e. yohimbine and idazoxan, atipamezole is more specific and α_2-selective. It has an α_2/α_1-selectivity ratio of 8500 compared with 27 for idazoxan and 40 for yohimbine in receptor binding studies, and appears to be devoid of significant interactions with other neurotransmitter receptors (Virtanen *et al.*, 1988). In brains of intact rats, atipamezole increased the release and metabolism of noradrenaline in a dose-dependent manner, and it was also able to antagonize the neurochemical effects of two centrally acting α_2-adrenoceptor agonists, detomidine and medetomidine. It also caused rapid reversal of the sedative effects induced by these α_2-adrenoceptor agonists. These findings indicate that atipamezole rapidly penetrates into the central nervous system and is a potent antagonist at central α_2-adrenoceptors (Scheinin *et al.*, 1988).

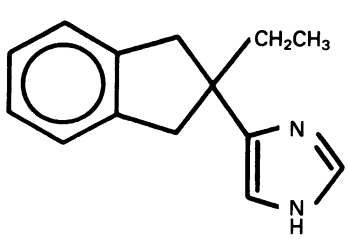

Figure 1 Chemical structure of atipamezole, 4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole (MPV-1248).

We have previously administered up to 100 mg i.v. doses of atipamezole to healthy male volunteers in a preliminary, open phase-I study aimed at assessing its tolerability and pharmacodynamic profile (Karhuvaara *et al.*, 1989). The present study was carried out in order to obtain a more detailed characterization of the pharmacological effects and pharmacokinetics of atipamezole in a dose range of 10 to 100 mg.

Methods

Subjects

Six healthy non-smoking males participated after providing written informed consent. Before inclusion in the study, the general health of the volunteers was ascertained by a detailed medical history, physical examination, ECG recording and clinical chemistry tests. Their mean (± s.d.) age was 23 ± 2 years (range 21–28 years), mean height 182 ± 2 cm (179–184 cm), and mean weight 73 ± 6 kg (67–83 kg). They had taken no medications in the month preceding this study. The study protocol was approved by the local Ethics Committee and the Finnish National Board of Health.

Design of the study

The study was double-blind and placebo-controlled, and followed a randomized cross-over design. Each subject received three different single doses (10, 30, and 100 mg) of atipamezole (supplied by Farmos Group Ltd, Turku, Finland) and saline placebo as i.v. infusions with intervals of at least 72 h. Subsequently, the study was continued in an open fashion, and the volunteers received 100 mg atipamezole solution orally.

Study outline

The subjects arrived at the laboratory at 07.30 h after fasting overnight. Two antecubital i.v. cannulae were inserted for blood sampling and drug administration. The subjects drank 200 ml water, and were then connected to an automated sphygmomanometer (Nippon Colin 203 Y, Tokyo, Japan) and an ECG monitor.

The drug (atipamezole hydrochloride diluted in 40 ml physiological saline), or saline placebo, was administered as an i.v. infusion over 20 min using an infusion pump (Perfusor ED 2, B. Braun, Melsungen, FRG).

Samples of venous blood were collected for determinations of blood glucose, plasma drug levels, and noradrenaline, adrenaline, cortisol, C-peptide, and cyclic adenosine 3',5'-monophosphate (cAMP) concentrations in plasma. Blood pressure and ECG were monitored until 6 h after drug administration. Subjective symptoms and side effects were evaluated repeatedly with a standardized questionnaire and a battery of visual analogue scales (100 mm long horizontal lines with the extremes drowsy—alert; calm—nervous; mentally slow—quick-witted; hostile—friendly; sad—happy; bored—interested; clumsy—skillful; lazy—energetic; withdrawn—social; satiated—hungry; contented—discontented; silent—talkative; strong—feeble; clear-headed—muzzy) (Bond & Lader, 1974; Mattila *et al.*, 1988), and in addition, the subjects were urged to report any possible subjective effects immediately on their occurrence. Non-stimulated saliva secretion was measured by placing three preweighed dental cotton rolls at the orifices of the parotid and sublingual salivary ducts for 2 min. Urine was collected in two fractions, 0–3 h and 3–6 h, and acidified with hydrochloric acid. The clinical chemistry screening tests were repeated after the last session.

The subjects had a light standard meal and were allowed to visit the lavatory at 180 min after drug administration. Otherwise they remained supine throughout the sessions.

Chemical determinations

Blood samples for determination of glucose concentrations were drawn into tubes containing sodium fluoride and analyzed with a glucose analyzer (GM 7 Analyzer, Analox Instruments Ltd, London, UK) utilizing the glucose oxidase method. Other blood samples were collected into pre-chilled EDTA tubes, placed on ice and centrifuged promptly at +4° C to separate the plasma. The plasma samples were stored at −70° C until analyzed.

Plasma catecholamine concentrations were determined using high performance liquid chromatography with coulometric detection (h.p.l.c.-EC) as reported previously (Scheinin *et al.*, 1987b). The method has intra-assay coefficients of variation (CV) of approximately 2% for noradrenaline and 2–10% for adrenaline in the relevant concentration ranges.

H.p.l.c.-EC was also used to quantitate catecholamine metabolites in urine. The deaminated metabolites 4-hydroxy-3-methoxymandelic acid (HMMA) and homovanillic acid (HVA) were analyzed after solid-phase extraction on Sephadex G-10 according to the procedure described by Bremmelgaard (1985). The non-deaminated, methylated metabolites metanephrine, normetanephrine, and 3-methoxytyramine were determined after acid hydrolysis according to Jouve *et al.* (1983). The urinary excretion of catecholamine metabolites was normalized for time and urine production by expressing the results in relation to the excretion of creatinine in the same sample.

The concentrations of cAMP in plasma were measured with the cAMP^{125} I assay system (Amersham, Buckinghamshire, UK). Plasma cortisol and C-peptide concentrations were determined with commercial radioimmunoassay kits (cortisol: Spectria®, Farmos Group Ltd. Turku, Finland; C-peptide: Novo Biolabs, Bagsvaerd, Denmark).

The atipamezole concentrations in plasma were determined using h.p.l.c. with u.v. detection at 215 nm (Kratos Spectroflow 783, Westwood, NJ, USA). Plasma samples (0.5 ml) were mixed with 0.5 ml water containing the internal standard, detomidine (Farmos Group Ltd, Turku, Finland; Scheinin *et al.*, 1988), and extracted with Extrelut®-columns (E. Merck, Darmstadt, FRG). The analytes were back-extracted from the 6 ml toluene eluate with 0.5 ml 50 mM phosphoric acid; 50 μl aliquots were injected into the h.p.l.c. system. Separation was achieved isocratically at room temperature using a mobile phase consisting of 22.5% (v/v) acetonitrile in 50 mM potassium phosphate buffer (pH 3.2, flow rate 1.5 ml min^{-1}) and a 150 × 3.9 mm Novapak® C18 column (Waters Ass., Milford, MA, USA). The detection limit was 5 μg l^{-1}, and the method was linear over the range tested, up to 2000 μg l^{-1}. The extraction efficiency was 95% at 40–2000 μg l^{-1}. Intra-assay CVs ranged from 9% at 20 μg l^{-1} to 1% at 2000 μg l^{-1}; the inter-assay CV was 5% at 100 μg l^{-1}.

Pharmacokinetic analysis

Linear one- or two-compartment open models with constant i.v. input and first-order output were used for pharmacokinetic calculations. Non-linear least squares fits of the data were performed with the computer programme PCNONLIN 3.0 (Metzler & Weiner, 1989). The pharmacokinetic parameters were defined and calculated as follows (Gibaldi & Perrier, 1982):

$t_{½}$ = $\ln 2/\lambda_z$
AUC = $C(0)/\lambda_z$ (one compartment), or
= $C_1/\lambda_1 + C_z/\lambda_z$ (two compartments)
CL = D/AUC
V_z = CL/λ_z,
where λ_1 and λ_z are the exponents of the computer-fitted functions of the post-infusion plasma concentration *vs* time curve and C_1 and C_z are the intercepts of the first and terminal (second) components of the predicted bolus decay curves. Further, $t_{½}$ = elimination half-life, AUC = area under the concentration-time curve, CL = total plasma clearance, and V_z = apparent volume of distribution during terminal (λ_z) phase.

Statistical analysis

The results are presented as means ± s.d. The statistical analysis of the results was performed using analysis of variance (ANOVA) or covariance (ANCOVA) for repeated measurements, with one (dose) or two (dose and time) within-factors, computed with BMDP2V programs (BMDP Statistical Software Inc., Los Angeles, CA, USA). When variances were unequal, log-transformation of the data was performed. When a significant drug effect or dose × time interaction was observed, the analysis was continued with separate ANCOVAs for each pair of dose levels. A P value of less than 0.05 was considered as statistically significant, and when pooled orthogonal components showed non-sphericity, Greenhouse-Geisser adjusted P values were used (Keselman & Keselman, 1984).

Results

Pharmacokinetics

The pharmacokinetic results after i.v. administration are summarized in Table 1. Plasma atipamezole concentrations and the areas under the concentration-time curves were linearly related to the administered dose (Figure 2). Total clearance, elimination half-life and the apparent volume of distribution were not dose-dependent, and showed relatively little variation between subjects (Table 1). Characterization of the initial distribution phase was not attempted, since the drug was given i.v. over 20 min. No atipamezole could be detected in plasma after oral administration of the drug.

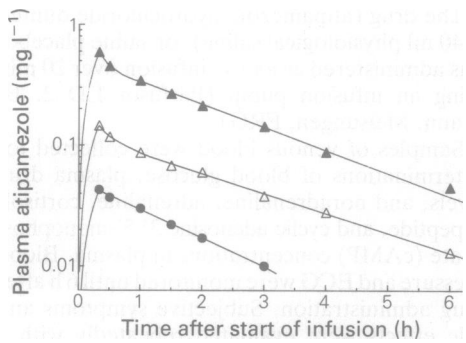

Figure 2 Mean atipamezole concentrations in plasma after 20 min intravenous infusions of 10 mg (●), 30 mg (△), and 100 mg (▲). Logarithmic scale; curve fitting by the programme PCNONLIN. Standard deviations have been omitted for clarity.

Safety and subjective drug effects

The 10 and 30 mg i.v. doses were well tolerated. The largest i.v. dose, 100 mg, caused some sympathomimetic-like subjective effects in all six volunteers (coldness or sweating of limbs, cold shivers: 3; tension or restlessness, irritability, tremor, increased salivation: 2; facial flush, light-headedness: 1), although none of them considered these as very unpleasant.

Table 1 Mean (± s.d.) pharmacokinetic parameters of atipamezole in six healthy subjects after intravenous doses of 10, 30 and 100 mg

	Atipamezole							
	10 mg		*30 mg*		*100 mg*		*ANOVA*	
Parameter	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	F	P
λ_z (h^{-1})	0.446	0.139	0.364	0.080	0.392	0.067	0.8	0.49
$t_{½}$ (h)	1.655	0.394	1.985	0.443	1.806	0.275	0.9	0.43
AUC (mg l^{-1} h)	0.095	0.019	0.333	0.047	1.191	0.071	901.2	< 0.001
CL (l h^{-1} kg^{-1})	1.465	0.296	1.237	0.218	1.137	0.137	3.2	0.09
V_z (l kg^{-1})	3.377	0.512	3.477	0.663	2.956	0.523	1.4	0.31

Note: λ_z = elimination rate constant of terminal phase; $t_{½}$ = elimination half-life, AUC = area under the concentration-time curve, CL = total plasma clearance, and V_z = apparent volume of distribution during terminal phase. ANOVA for AUC/dose: $F = 3.4$, $P = 0.08$.

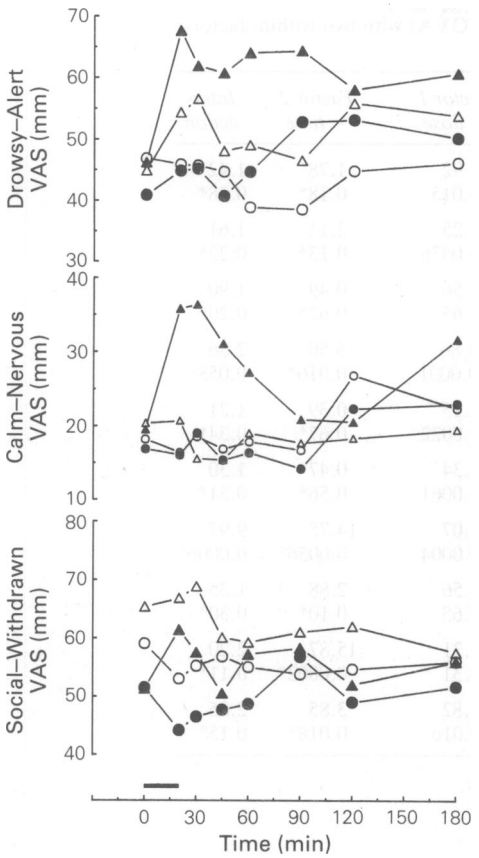

Figure 3 The effects of 20 min saline placebo (○) and 10 mg (●), 30 mg (△), or 100 mg (▲) atipamezole infusions on visual analogue scale (VAS) scores 'drowsy—alert' (top; $P = 0.03$ for effect of dose, ANOVA), 'calm—nervous' (middle; $P = 0.003$) and 'withdrawn—social' (bottom; $P = 0.04$). The means (in mm) of VAS readings of six subjects are presented. The horizontal bar represents the duration of the infusion.

Statistically significant rightward shifts occurred in VAS assessments of alertness (extremes: drowsy—alert), nervousness (extremes: calm—nervous), and on the scale 'withdrawn—social' (Figure 3). No statistically significant changes were seen in the other VAS measurements (data not shown). The clinical chemistry tests performed after the experiments did not reveal any clinically significant changes.

Saliva secretion

Basal (non-stimulated) salivation was statistically significantly increased after i.v. atipamezole (Table 2, Figure 4). Saliva secreted during 2 min

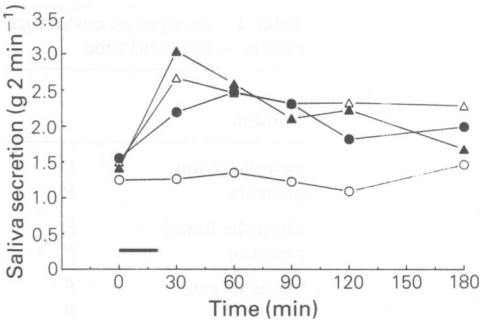

Figure 4 Saliva secretion after saline placebo and atipamezole infusions. Symbols as in Figure 3.

was increased on average by 151 ± 130% after the 100 mg dose (from 1.41 ± 1.15 to 3.04 ± 1.95 g) at 30 min after the start of the drug infusion compared with baseline values (ANCOVA: $F = 6.39$ $P = 0.0018$; dose × time interaction for 100 mg *vs* placebo). Saliva secretion was increased also after the two smaller doses, but pairwise ANCOVAs failed to reveal statistically significant differences from placebo.

Blood pressure and heart rate

Heart rate was increased in one subject after the largest i.v. dose (from 53 beats min^{-1} at start to 112 beats min^{-1} at 20 min). In the remaining subjects, heart rate was initially slightly reduced (from 58 ± 3 to 52 ± 6 beats min^{-1}) at 5 min, but returned to baseline values within 5–10 min. Thereafter no alterations were observed in heart rate. After the highest dose, the maximal average increase in diastolic blood pressure was 16 ± 7 mm Hg (from 65 ± 6 to 78 ± 9 mm Hg); systolic pressure increased by 20 ± 15 mm Hg (from 111 ± 10 to 130 ± 12 mm Hg) (Table 2, Figure 5). The highest values were seen between 15 and 30 min after the start of the drug infusion;

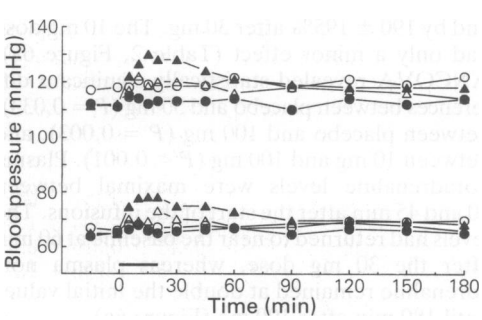

Figure 5 Systolic and diastolic blood pressures after saline and atipamezole infusions. The means of six subjects are presented. Symbols as in Figure 3.

Table 2 Analysis of covariance (ANCOVA) with two within- factors: effects of dose and time

Variable		*Factor 1 = dose*	*Factor 2 = time*	*Inter-action*
Systolic blood pressure	*F*	4.92	1.78	1.42
	P	0.015	0.18*	0.28*
Diastolic blood pressure	*F*	7.25	2.11	1.61
	P	0.0036	0.13*	0.22*
log heart rate	*F*	0.56	0.49	1.90
	P	0.65	0.62*	0.20*
log plasma noradrenaline	*F*	15.86	5.50	2.86
	P	0.0001	0.016*	0.058*
log Plasma adrenaline	*F*	8.15	0.39	1.21
	P	0.0022	0.67*	0.34*
Plasma cyclic AMP	*F*	6.34	0.47	1.30
	P	0.0061	0.56*	0.31*
Plasma cortisol	*F*	12.07	14.75	9.97
	P	0.0004	0.0056*	0.0006*
Blood glucose	*F*	0.56	2.88	1.35
	P	0.65	0.10*	0.30*
Plasma C-peptide	*F*	1.31	15.87	2.20
	P	0.31	0.0002*	0.11*
Saliva secretion	*F*	4.82	3.85	2.05
	P	0.016	0.018*	0.15*

* Greenhouse-Geisser adjusted *P* value

blood pressure then gradually declined and reached a plateau slightly above the values recorded at the start. Only a small, statistically insignificant rise in blood pressure was seen after 30 mg (Figure 5). The 10 mg dose had no influence on blood pressure.

Plasma catecholamines

Plasma noradrenaline concentrations were increased maximally by 484 ± 269% after 100 mg, and by 190 ± 195% after 30 mg. The 10 mg dose had only a minor effect (Table 2, Figure 6a). ANCOVA revealed statistically significant differences between placebo and 30 mg ($P = 0.033$), between placebo and 100 mg ($P = 0.003$), and between 10 mg and 100 mg ($P < 0.001$). Plasma noradrenaline levels were maximal between 20 and 45 min after the start of the infusions. The levels had returned to near the baseline at 60 min after the 30 mg dose, whereas plasma noradrenaline remained at double the initial values until 180 min after 100 mg (Figure 6a).

The concentration of adrenaline in plasma was also increased after atipamezole (Table 2, Figure 6b), but only the 100 mg i.v. dose dif-

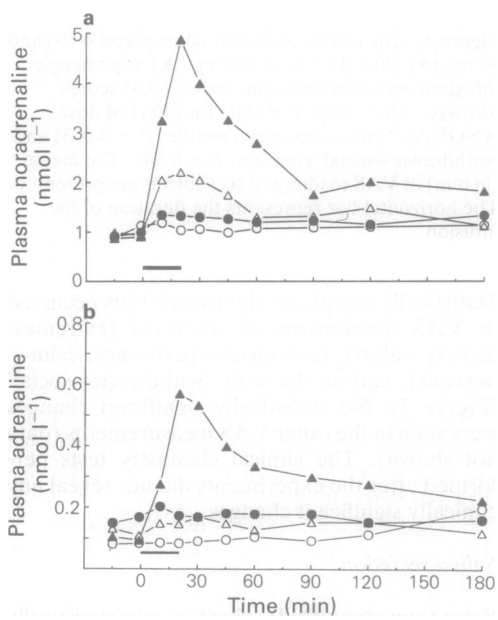

Figure 6 Mean concentrations of noradrenaline a) and adrenaline b) in venous plasma after saline and atipamezole infusions. Symbols as in Figure 3.

fered statistically significantly from placebo (mean increase from 0.09 ± 0.05 to 0.6 ± 0.7 nmol l^{-1} after 100 mg; no change after placebo; ANCOVA: $P = 0.031$). The adrenaline response varied markedly between subjects; the largest increase in plasma adrenaline concentration was from 0.09 to 1.98 nmol l^{-1}. This subject also had tachycardia (see above) and experienced the most prominent subjective effects.

Urinary excretion of catecholamine metabolites

The urinary excretion of catecholamine metabolites was not affected by atipamezole. The average excretion rates, expressed as mmol metabolite per mol creatinine, varied between 1.6–1.8 for HMMA, 1.5–2.0 for HVA, 0.05–0.06 for metanephrine, 0.08–0.09 for normetanephrine, and 0.06–0.07 for 3-methoxytyramine. Urine volumes and creatinine excretion were also similar after all doses (data not shown).

Cyclic AMP in plasma

The mean concentration of cAMP in plasma was increased by 50% (from 13.2 ± 4.5 to 21.7 ± 9.4 nmol l^{-1}) after 100 mg atipamezole (Table 2). This dose differed significantly from placebo and 10 mg, and ANCOVA revealed an almost significant difference ($P = 0.076$) between 100 and 30 mg. The increase in cAMP was temporally and quantitatively related to the increase in plasma adrenaline concentrations. The 10 and 30 mg doses did not significantly influence cAMP levels in plasma (data not shown).

Plasma cortisol and C-peptide and blood glucose

Plasma cortisol levels decreased markedly after all active i.v. drug doses (by 45–63%), but not after placebo (14% decrease), producing a clearly significant dose × time interaction in the overall ANCOVA (Table 2). Pairwise ANCOVAs revealed statistically significant differences between the placebo treatment and each active treatment.

Blood glucose levels were not affected by any of the doses. Plasma C-peptide concentrations decreased slowly during the placebo session (by about 30%), but were slightly and transiently, and not statistically significantly, increased after 100 mg (from 0.49 ± 0.13 at the start to 0.62 ± 0.11 nmol l^{-1} at 30 min; Table 2).

Oral administration

Neither any effects nor measurable plasma atipamezole concentrations were detected after oral dosing (data not shown).

Discussion

Preclinical studies have indicated that atipamezole is a potent, specific and selective antagonist of both centrally and peripherally located pre- and postsynaptic α_2-adrenoceptors (Scheinin *et al.*, 1988; Virtanen *et al.*, 1988). It has similar, high affinity to the two putative α_2-adrenoceptor subtypes, α_{2A} and α_{2B} (Bylund *et al.*, 1988), and has a more than two orders of magnitude higher α_2/α_1-selectivity ratio than either yohimbine or idazoxan (Virtanen *et al.*, 1988).

Most of the subjective effects (tremor, restlessness, cold hands, etc.) induced by the 100 mg i.v. dose of the drug were similar to those seen after the administration of sympathomimetic agents, and may have been secondary to the increased catecholamine release. In addition, the subjects reported increasing salivation soon after drug administration, and enhancement in alertness and nervousness were recorded with the visual analogue scales.

Dry mouth is one of the most common side effects of clonidine therapy (Hoefke, 1980). MK-912, a new α_2-adrenoceptor antagonist, was recently reported to increase basal saliva secretion in humans (Warren *et al.*, 1989). Increased salivation after atipamezole was demonstrated also in this study by objective measurements. A tendency to increase, although not statistically significant, was already seen after the 10 mg dose, which caused negligible changes in plasma catecholamine concentrations and few subjective effects. This suggests the existence of a physiological tonic regulation of salivation via α_2-adrenoceptors; both central and peripheral sites of action have been implicated (Green *et al.*, 1979).

Atipamezole increased plasma noradrenaline concentrations in a dose-related manner, resembling other α_2-adrenoceptor antagonists in this regard (Brown *et al.*, 1985a,c; Elliot *et al.*, 1984; Goldberg & Robertson, 1983). The administration of α_2-adrenoceptor agonists decreases the concentration of noradrenaline in plasma, mainly reflecting centrally mediated sympathetic inhibition (Reid, 1981; Scheinin *et al.*, 1987a), but a peripheral component mediated by inhibitory α_2-autoreceptors on sym-

pathetic nerve endings may contribute (Brown *et al.*, 1985b; Murphy *et al.*, 1984). The observed increase in plasma noradrenaline concentrations after atipamezole and other α_2-adrenoceptor antagonists is also likely to be derived mainly from a generalized increase in preganglionic sympathetic nervous activity following blockade of central α_2-adrenoceptors (Ramage & Tomlinson, 1985), although enhanced release of the transmitter from sympathetic nerve endings after blockade of peripheral presynaptic inhibitory autoreceptors can not be entirely ruled out (Brown *et al.*, 1985a). The observed increase in the concentration of adrenaline in plasma probably also resulted from increased central sympathetic activity, although a local α_2-adrenoceptor-mediated action on adrenal medullary chromaffin cells remains a possibility as well (Foucart *et al.*, 1987; Sakurai *et al.*, 1983).

Blood pressure was clearly elevated after the 100 mg i.v. dose of atipamezole. This was at least in part due to noradrenaline-induced, α_1-mediated vasoconstriction and increased peripheral resistance. Reflex bradycardia would be expected in connection with such a blood pressure increase as that observed in this study (Kallio *et al.*, 1989). However, heart rate was markedly affected only in one subject, and he had tachycardia. This was associated with prominent subjective effects and a high concentration of adrenaline in plasma. In the other five subjects, heart rate was very slightly reduced (by 5 beats min^{-1}) for a short period, whereas Elliot *et al.* (1984) reported a considerably more pronounced reduction (15 beats min^{-1}) after idazoxan, in spite of smaller blood pressure changes than those in the present study. Moreover, the bradycardic effect was seen at 5–10 min after the start of the infusion, whereas blood pressure peaked at 15–20 min, when heart rate was no longer different from the basal level. This indicates the existence of a cardio-excitatory factor in the haemodynamic effects of atipamezole; adrenaline could act as such a factor, with peripheral venous blood sampling possibly leading to underestimation of the increase in adrenaline release (Hjemdahl *et al.*, 1984). Increased release of noradrenaline from cardiac sympathetic nerves and increased levels of circulating noradrenaline may also have contributed by β-adrenergic positive inotropic effects on the myocardium.

The unaffected urinary catecholamine metabolite excretion in spite of marked changes in plasma catecholamine concentrations may be partly explained by the relatively short duration of action of the investigated drug, but also points to the relative insensitivity of urinary metabolite determinations in detecting transient changes in noradrenaline release.

α_2-Adrenoceptors mediate inhibition of adenylate cyclase activity in many target cells, leading to reduction of intracellular cAMP levels (Ruffolo *et al.*, 1988). Cyclic AMP functions as the intracellular second messenger for β-adrenoceptors, and the administration of β-adrenoceptor agonists leads to increased concentrations of cAMP in plasma (Fairfax *et al.*, 1984; Scheinin *et al.*, 1987b). The observed increase in plasma cAMP levels after the highest dose of atipamezole was probably mainly caused by β-adrenoceptor stimulation from increased sympathetic activity and increased levels of circulating adrenaline, and cannot be used as evidence for direct participation of α_2-adrenoceptors in the regulation of plasma cAMP levels.

α_2-Adrenoceptors are thought to be involved in the regulation of insulin secretion, their activation by clonidine and other agonists leading to suppression of insulin release from pancreatic β-cells (Limone *et al.*, 1983; Metz *et al.*, 1978; Nakaki *et al.*, 1980). The results obtained in humans with selective α_2-adrenoceptor antagonists are to some extent conflicting. Idazoxan has antagonized the guanfacine-induced reduction in plasma insulin (Brown *et al.*, 1985a,c) and facilitated the insulin response to adrenaline (Brown *et al.*, 1985a), but not to glucose (Östenson *et al.*, 1988; Struthers *et al.*, 1985). On the other hand, midaglizole (DG-5128), an α_2-adrenoceptor antagonist that does not readily penetrate into the central nervous system, has been reported to improve glucose-stimulated insulin secretion in non-insulin-dependent diabetics (Kashiwagi *et al.*, 1986). In the present study with fasting healthy subjects, the 100 mg i.v. dose of atipamezole induced only a small (and statistically insignificant) increase in plasma C-peptide concentrations (an indicator of insulin release), suggesting that α_2-adrenoceptors do not play a physiologically significant role in the regulation of basal insulin secretion.

The observed differences in plasma cortisol levels between the different doses of atipamezole and placebo are probably without clinical significance, and may rather have resulted from increased clearance of cortisol from plasma than its decreased release. The present observation is in line with the results of Price *et al.* (1986), who found increases in plasma cortisol levels after yohimbine only in depressed patients, and not in healthy volunteers.

All of the observed effects of atipamezole were generally consistent with earlier findings

obtained with other α_2-adrenoceptor antagonists (Brown *et al.*, 1985a,c; Elliot *et al.*, 1984; Goldberg & Robertson, 1983). Prominent effects were seen only after the 100 mg i.v. dose, whereas the 10 mg dose seemed to be almost totally devoid of any effects. However, we have recently observed that even a 5 mg dose of atipamezole is capable of reversing the effects induced by the potent and selective α_2-adrenoceptor agonist, dexmedetomidine (unpublished results). The doses used in this study were thus larger than actually needed to achieve substantial blockade of α_2-adrenoceptors.

Since atipamezole appears to have poor oral bioavailability, it is not suitable for clinical use in situations where oral dosing is desired, such as antidepressive therapy. The absence of pharmacodynamic effects after oral dosing provides evidence against the formation of significant amounts of active atipamezole metabolites after oral drug intake. However, because of its high specificity and selectivity and rapid elimination, atipamezole may prove to be a useful compound for studying α_2-adrenoceptor function in man. In veterinary medicine, it is under development as a specific reversal agent for the sedation and anaesthesia induced by α_2-adrenoceptor agonists such as xylazine, detomidine, and medetomidine (Scheinin *et al.*, 1988). A similar use may also be realized in human medicine, should the current interest in the anaesthetic applications of α_2-adrenoceptor agonists lead to their more widespread use in this setting (Hamilton, 1988; Longnecker, 1987).

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References

Bond, A. & Lader, M. (1974). The use of analogue scales in rating subjective feelings. *Br. J. med. Psychol.*, **47**, 211–218.

Bremmelgaard, A. (1985). Analysis of vanillylmandelic and homovanillic acids in urine using solid phase sample preparation and high pressure liquid chromatography with electrochemical detection. *Scand. J. clin. lab. Invest.*, **45**, 387–391.

Brown, M. J., Struthers, A. D., Burrin, J. M., di Silvio, L. & Brown, D. C. (1985a). The physiological and pharmacological role of presynaptic α- and β-adrenoceptors in man. *Br. J. clin. Pharmac.*, **20**, 649–658.

Brown, M. J., Struthers, A. D. & di Silvio, L. (1985b). Peripheral alpha$_2$-adrenoceptor stimulation contributes to the sympatholytic effect of guanfacine in man. *J. cardiovasc. Pharmac.*, **7** (Suppl 6), S159–161.

Brown, M. J., Struthers, A. D., di Silvio, L., Yeo, T., Ghatei, M. & Burrin, J. M. (1985c). Metabolic and hemodynamic effects of α_2-adrenoceptor stimulation and antagonism in man. *Clin. Sci.*, **68** (Suppl 10), 137s–139s.

Bylund, D. B., Ray-Prenger, C. & Murphy, T. J. (1988). Alpha-2A and alpha-2B adrenergic receptor subtypes: Antagonist binding in tissues and cell lines containing only one subtype. *J. Pharmac. exp. Ther.*, **245**, 600–607.

Chapleo, C. B. (1988). The discovery and therapeutic potential of selective α_2-adrenoceptor antagonists. In *Recent advances in receptor chemistry*, ed. Melchiorre, C. & Gianella, M., pp 85–106. Amsterdam: Elsevier Science Publishers.

Crossley, D. I. (1984). The effects of idazoxan, an alpha$_2$-adrenoceptor antagonist in depression—a preliminary investigation. *Abstract 1724P.* London: IUPHAR.

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. Pharmac.*, **78**, 489–505.

Elliot, H. L., Jones, C. R., Vincent, J., Lawrie, C. B. & Reid, J. L. (1984). The alpha adrenoceptor antagonist properties of idazoxan in normal subjects. *Clin. Pharmac. Ther.*, **36**, 190–196.

Fairfax, A. J., Rehahn, M., Jones, D. & O'Malley, B. (1984). Comparison between the effects of inhaled isoprenaline and fenoterol on plasma cyclic AMP and heart rate in normal subjects. *Br. J. clin. Pharmac.*, **17**, 165–170.

Foucart, S., Nadeau, R. & de Champlain, J. (1987). The release of catecholamines from the adrenal medulla and its modulation by α_2-adrenoceptors in the anaesthetized dog. *Can. J. Physiol. Pharmac.*, **65**, 550–557.

Gibaldi, M. & Perrier, D. (1982). *Pharmacokinetics*, 2nd edition. New York: Marcel Dekker.

Goldberg, M. R. & Robertson, D. (1983). Yohimbine: a pharmacological probe for study of the α_2-adrenoceptor. *Pharmac. Rev.*, **35**, 143–180.

Green, G. J., Wilson, H. & Yates, M. S. (1979). The effect of clonidine on centrally and peripherally evoked submaxillary salivation. *Eur. J. Pharmac.*, **53**, 297–300.

Hamilton, W. K. (1988). Fashion, Darwin, and anesthetics as poisons. *Anesthesiology*, **69**, 811–812.

Hjemdahl, P., Freyschuss, U., Juhlin-Dannfelt, A. & Linde, B. (1984). Differentiated sympathetic activation during mental stress evoked by the Stroop test. *Acta Physiol. Scand.*, Suppl 527, 25–29.

Hoefke, W. (1980). Clonidine. In *Pharmacology of antihypertensive drugs*, ed. Scriabine, A., pp 55–78. New York: Raven Press.

Jouve, J., Mariotte, N., Sureau, C. & Muh, J. P. (1983). High-performance liquid chromatography with electrochemical detection for the simultaneous determination of the methoxylated

amines, normetanephrine, metanephrine and 3-methoxytyramine, in urine. *J. Chromatogr.*, **274**, 53–62.

Kallio, A., Scheinin, M., Koulu, M., Ponkilainen, R., Ruskoaho, H., Viinamäki, O. & Scheinin, H. (1989). Effects of dexmedetomidine, a selective α_2-adrenoceptor agonist, on hemodynamic control mechanisms. *Clin. Pharmac. Ther.*, **46**, 33–42.

Karhuvaara, S., Kallio, A., Scheinin, M., Koulu, M., Scheinin, H. & Viikari, J. (1989). Tolerability and effects of atipamezole, a novel alpha$_2$-adrenoceptor antagonist, in healthy male volunteers. An open dose-finding study. *Curr. Ther. Res.*, **45**, 633–642.

Karjalainen, A., Virtanen, R. & Karjalainen, A. (1986). Synthesis of 4(5)-(2,3-dihydro-1H-inden-2-yl)imidazoles, a novel class of potent α_2-adrenoceptor antagonists. *IX Int. Symp. on Med. Chem.*, Berlin, p 268.

Kashiwagi, A., Harano, Y., Suzuki, M., Kojima, H., Harada, M., Nishio, Y. & Shigeta, Y. (1986). New α_2-adrenergic blocker (DG-5128) improves insulin secretion and *in vivo* glucose disposal in NIDDM patients. *Diabetes*, **35**, 1085–1089.

Keselman, H. J. & Keselman, J. C. (1984). The analysis of repeated measures design in medical research. *Stat. Med.*, **3**, 185–195.

Langer, S. Z. (1981). Presynaptic regulation of the release of catecholamines. *Pharmac. Rev.*, **32**, 337–362.

Langer, S. Z. (1987). Presynaptic regulation of monoaminergic neurons. In *Psychopharmacology—The third generation of progress*, ed. Meltzer, H. Y., 151–157. New York: Raven Press.

Limone, P., Cagliero, E., Massara, F. & Molinatti, G. M. (1983). Reduced glucose tolerance after guanfacine in normal and obese subjects. *Horm. metab. Res.*, **15**, 352.

Longnecker, D. E. (1987). Alpine anesthesia: can pretreatment with clonidine decrease the peaks and valleys? *Anesthesiology*, **67**, 1–2.

Mattila, M., Seppälä, T. & Mattila, M. J. (1988). Anxiogenic effects of yohimbine in healthy subjects: comparison with caffeine and antagonism by clonidine and diazepam. *Int. clin. Psychopharmac.*, **3**, 215–229.

Metz, S. A., Halter, J. B. & Robertson, R. P. (1978). Induction of defective insulin secretion and impaired glucose tolerance by clonidine. *Diabetes*, **27**, 554–562.

Metzler, C. M. & Weiner, D. L. (1989). *PCNONLIN (version 3.0), Software for the Statistical Analysis of Nonlinear Models on Micros*. Lexington, USA: Statistical Consultants, Inc.

Murphy, M. B., Brown, M. J. & Dollery, C. T. (1984). Evidence for a peripheral component in the sympatholytic actions of clonidine and guanfacine in man. *Eur. J. clin. Pharmac.*, **27**, 23–27.

Nakaki, T., Nakadate, T. & Kato, R. (1980). α_2-adrenoceptors modulating insulin release from isolated pancreatic islets. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **313**, 151–153.

Östenson, C.-G., Pigon, J., Doxey, J. C. & Efendic, S. (1988). α_2-adrenoceptor blockade does not enhance glucose-induced insulin release in normal subjects or patients with noninsulin-dependent diabetes. *J. clin. Endocrinol. Metab.*, **67**, 1054–1059.

Price, L. H., Charney, D. S., Rubin, A. L. & Heninger, G. R. (1986). α_2-adrenergic receptor function in depression. The cortisol response to yohimbine. *Arch. gen. Psychiatry*, **43**, 849–858.

Ramage, A. G. & Tomlinson, A. (1985). The effect of yohimbine, Wy 26392 and idazoxan on sympathetic nerve activity. *Eur. J. Pharmac.*, **109**, 153–160.

Reid, J. L. (1981). The clinical pharmacology of clonidine and related central antihypertensive agents. *Br. J. clin. Pharmac.*, **12**, 295–302.

Ruffolo, R. R., Nichols, A. J. & Hieble, P. (1988). Functions mediated by alpha-2 adrenergic receptors. In *The alpha-2 adrenergic receptors*, ed. Limbird, L. E., pp 187–280. Clifton: Humana Press.

Sakurai, S., Wada, A., Izumi, H., Kobayashi, H. & Yanagihara, N. (1983). Inhibition by α_2-adrenoceptor agonists of the secretion of catecholamines from isolated adrenal medullary cells. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **324**, 15–19.

Scheinin, H., MacDonald, E. & Scheinin, M. (1988). Behavioural and neurochemical effects of atipamezole, a novel α_2-adrenoceptor antagonist. *Eur. J. Pharmac.*, **151**, 35–42. Errata, **157**, 243–244.

Scheinin, M., Kallio, A., Koulu, M., Viikari, J. & Scheinin, H. (1987a). Sedative and cardiovascular effects of medetomidine, a novel selective α_2-adrenoceptor agonist, in healthy volunteers. *Br. J. clin. Pharmac.*, **24**, 443–451.

Scheinin, M., Koulu, M., Laurikainen, E. & Allonen H. (1987b). Hypokalaemia and other non-bronchial effects of inhaled fenoterol and salbutamol: A placebo-controlled dose-response study in healthy volunteers. *Br. J. clin. Pharmac.*, **24**, 645–653.

Struthers, A. D., Brown, D. C., Brown, M. J., Schumer, B. & Bloom, S. R. (1985). Selective α_2-receptor blockade facilitates the insulin response to adrenaline but not to glucose in man. *Clin. Endocrinol.*, **23**, 539– 546.

Virtanen, R., Savola, J.-M. & Saano, V. (1988). Highly selective and specific antagonism of central and peripheral α_2-adrenoceptors by atipamezole. *Arch. int. Pharmacodyn. Ther.*, **297**, 190–204.

Warren, J. B., Dollery, C. T., Fuller, R. W., Williams, V. C. & Gertz, B. J. (1989). Assessment of MK-912, an α_2-adrenoceptor antagonist, with use of intravenous clonidine. *Clin. Pharmac. Ther.*, **46**, 103–109.

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