Central and Peripheral Hypotensive Activity of Urapidil and its M1 and M2 Metabolites in the Cat

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Urapidil is a novel antihypertensive drug with a dual mode of action, namely the inhibition of α1-adrenoceptors, predominantly in the peripheral circulatory system, and stimulation of serotonin1A receptors in brain stem areas involved in central cardiovascular regulation (Kolassa et al. 1989). Urapidil thus leads to a decrease in peripheral vascular resistance without causing tachycardia or other relevant counter-regulatory reactions that might attenuate its long term efficacy. The therapeutic potential of urapidil in the treatment of hypertension was recently reviewed (Langtry et al. 1989).

After oral administration in man, urapidil is mainly metabolised to ρ-hydroxylated urapidil (M1) and, to a minor extent, to ω-demethylated urapidil (M2). The M1 metabolite reaches 20% of the serum concentration of the parent drug and the M2 metabolite reaches 10%, although, because the elimination half-life of M2 is 2.5 times longer, the area under the concentration-time curve (AUC) of this metabolite accounts for approximately 35% of the AUC of urapidil (Zech et al. 1986).

The question arises whether these metabolites contribute to the central and peripheral hypotensive effects of urapidil. We have previously shown that M1 has only a weak α1-antagonistic action in isolated organs in vitro, while M2 displays similar α1-adrenoceptor blocking activity to urapidil. In intact rats, M1 was without hypotensive activity, but M2 was as potent as urapidil (Zech et al. 1984).

The purpose of the present study was to investigate whether these metabolites contribute to the stimulation of central serotonin1A receptors. Thus, we studied their hypotensive effect in cats after injection into the vertebral artery (van Zwieten 1975). We also measured the affinity of urapidil and its metabolites for α1-adrenoceptors and serotonin1A receptors in vitro to determine whether there was a correlation between the central and peripheral hypotensive effects and the receptor affinity data.

1. Material and Methods

1.1 In Vivo Cardiovascular Experiments

Normotensive male mongrel cats (2.9 to 3.9kg bodyweight) were anaesthetised and prepared surgically for intravenous drug injections and arterial pressure recordings.

Dose-response relationships for each drug were obtained after injections into the femoral vein (peripheral administration) followed by injection of the same dose into the vertebral artery (central administration). With low doses, blood pressure was allowed to return to baseline before the next administration; with high doses, the injection interval was 60 minutes, irrespective of the degree of blood pressure recovery. Urapidil, M1 and M2 were administered in increasing doses of 1, 10, 100, 1000, 10 000 and, for M1 only, 100 000 nmol/kg. After the intravenous administration of the drugs, the sensitivity of α1-adrenoceptors was tested by measuring the blood pressure rise following an intravenous bolus injection of the α1-adrenoceptor agonist cirazoline (van Meel et al. 1981). Cirazoline 1 µg/kg was given 3 times (at 10-minute intervals) before, and once (10 minutes) after, each intravenous drug injection.

The dose of drug causing a reduction of 20mm Hg in mean arterial pressure (ED20mm Hg) was derived by interpolating the maximum mean
arterial pressure decreases induced by each drug dose. The maximal changes in heart rate in response to drug administration and the maximal increases in mean arterial pressure in response to cirazoline were also measured. The results are expressed as means of 6 experiments performed with either urapidil, M1 or M2.

1.2 In Vitro Receptor Binding Assays

All binding assays were carried out in duplicate using pig or rat cortical membranes, as described by Pazos et al. (1984). Saturation experiments with pig cortical membranes were performed as per Hall et al. (1986) in the presence of increasing concentrations (4 pmol/L to 2 nmol/L) of [3H]-8-OH-DPAT, a selective serotonin1A-receptor agonist. Competition experiments were performed in the presence of a fixed concentration of [3H]-8-OH-DPAT (0.1 to 0.3 nmol/L) and increasing concentrations of competing drugs. Nonspecific binding was determined in the presence of 10 μmol/L of serotonin.

Saturation experiments with rat cortical membranes were performed in the presence of increasing concentrations (8 pmol/L to 3 nmol/L) of [3H]prazosin, a selective adrenoceptor antagonist. Competition experiments were performed in the presence of a fixed concentration of [3H]prazosin (0.2 to 0.5 nmol/L) and increasing concentrations of competing compounds. Nonspecific binding was determined in the presence of 1 μmol/L of prazosin or 10 μmol/L phentolamine.

Bound and free radioactivity was separated by filtration and the radioactivity retained by the filter was quantified by liquid scintillation counting.

Radioligand binding data were analysed by the ligand programme, as modified by G.A. McPherson (1985) and distributed by Elsevier Biosoft (Amsterdam).

1.3 Statistics

The baseline values of heart rate and mean arterial pressure were compared using the Student’s t-test for unpaired observation with significance at p < 0.05. The radioligand binding data were fitted to a 1-site or 2-site binding model equation using the partial F-test with significance at p < 0.05.

2. Results
2.1 In Vivo Cardiovascular Experiments

The mean pretreatment arterial pressure in cats in the M2 treatment group was significantly lower (p < 0.05) than that in the M1 and urapidil treatment groups and there was a significant difference (p < 0.05) between the M1 and urapidil groups in the mean pretreatment heart rate; these differences are not considered important.

Injection of urapidil, M1 and M2 into the femoral vein lowered blood pressure in a dose-dependent manner, urapidil having the strongest and M1 the weakest hypotensive effect. After injection into the vertebral artery, urapidil and M2 lowered blood pressure dose-dependently, M2 being as effective as urapidil on a molar basis. Centrally administered M1 was much less effective (fig. 1).

The highest doses of urapidil, M1 and M2, when administered via the femoral vein, reduced blood pressure by 96, 98 and 67 mm Hg, respectively, and decreased mean heart rate by 60, 48 and 82 beats/min, respectively. Interestingly, heart rate decreased after central administration of urapidil and M2 (50 and 82 beats/min, respectively), but not of M1. The centrally administered doses of urapidil and M2 that reduced heart rate to this extent were 10 times lower than those administered by the peripheral route.

When the hypotensive effects of the drugs by the peripheral and central routes of administration were compared on the basis of their ED20 mm Hg, urapidil and M2 were 10 times more potent by the central than the peripheral route. In contrast, the ED20 mm Hg values for M1 did not differ.

Urapidil and its metabolites M1 and M2 antagonised the pressor action of the α-adrenoceptor agonist cirazoline. A dose-dependent decrease in α-receptor sensitivity could be demonstrated for all drugs, the blocking potency of urapidil and M2 being almost 10 times higher than that of M1.