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The role of extraneuronal amine transport systems for the removal of extracellular catecholamines in the rabbit

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Abstract As selective inhibitors of the extraneuronal monoamine uptake system ($uptake_2$) suitable for in-vivo studies were not available, the question of whether $uptake_2$ plays a definite role in vivo is largely unresolved. We attempted to resolve the question by using 1,1′-diisopropyl-2,4′-cyanine iodide (disprocynium24), a novel agent that blocks $uptake_2$ in vitro with high potency. Anaesthetized rabbits were infused with ^{3}H-labelled noradrenaline, adrenaline and dopamine, and catecholamine plasma clearances as well as rates of spillover of endogenous catecholamines into plasma were measured before and during treatment with either disprocynium24 or vehicle. Four groups of animals were studied: group I, no further treatment; group II, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) inhibited; group III, neuronal uptake ($uptake_1$) inhibited; group IV, $uptake_1$ as well as MAO and COMT inhibited.

Disprocynium24 (270 nmol kg^{-1} i.v. followed by an i.v. infusion of 80 nmol kg^{-1} min^{-1}) did not alter heart rate and mean arterial blood pressure, but increased cardiac output by 22% and decreased the total peripheral vascular resistance by 16% with no difference between groups. When compared with vehicle controls, catecholamine clearances (normalized for the cardiac output of plasma) were decreased and spillover rates increased in response to disprocynium24. Although there were statistically significant between-group differences in baseline clearances (which decreased in the order: group I > group II > group III > group IV), the drug-induced clearance reductions relative to vehicle controls were similar in groups I to IV and amounted to 29–38% for noradrenaline, 22–31% for adrenaline and 16–22% for dopamine. Hence, there was still a significant % reduction in catecholamine clearances even after the combined inhibition of MAO and COMT, and there was no increase in the % reduction of clearances after inhibition of $uptake_1$. Noradrenaline spillover increased in response to disprocynium24 in all four groups by 1.6- to 1.9-fold, whereas a 1.5- to 2.0-fold increase in adrenaline and dopamine spillover was observed in groups II and IV only.

The results indicate that disprocynium24 interferes with the removal of circulating catecholamines not only by inhibiting $uptake_2$, but also by inhibiting related organic cation transporters. As disprocynium24 increased the spillover of endogenous catecholamines into plasma even after inhibition of MAO and COMT, organic cation transporters may also be involved in the removal of endogenous catecholamines before they enter the circulation.

Key words Plasma clearance of catecholamines · MAO-inhibition · COMT-inhibition · Disprocynium24 · $Uptake_2$ · Organic cation transporters

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Introduction

As the main enzymes involved in the metabolism of catecholamines, namely monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), are intracellular enzymes, cellular uptake processes are primarily responsible for the removal and, hence, for the termination of action of extracellular catecholamines. Figure 1 summarizes the transport systems known to effect cellular uptake of catecholamines. Uptake into noradrenergic neurones (neuronal uptake) is brought about by $uptake_1$ (Iversen 1967, 1975), whereas uptake into a variety of non-neuronal cells (extraneuronal uptake) is due to several transport processes. One of these is $uptake_2$ which mediates transport into myocardial cells, vascular as well as non-vascular smooth muscle

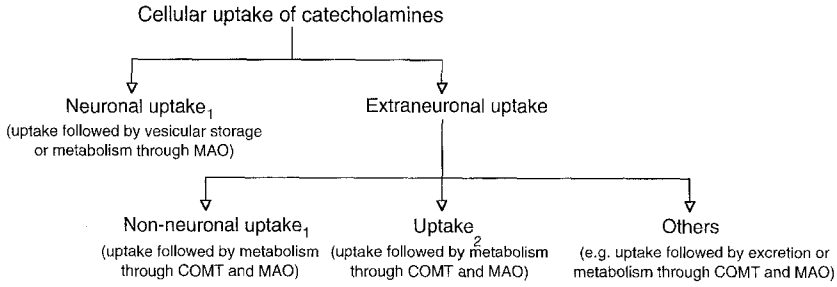

Fig. 1 Summary of the various membrane transport processes involved in the cellular uptake of extracellular catecholamines

cells (for reviews, see Iversen 1965, 1967; Trendelenburg 1976, 1980, 1988) and human glia cells (Staudt et al. 1993; Streich et al. 1996). Another extraneuronal uptake process is the non-neuronal type of uptake$_1$ which was shown to operate in the pulmonary circulation (Bryan-Lluka et al. 1992; Bryan-Lluka and O'Donnell 1992) and certain rabbit tissues such as dental pulp (Parker et al. 1987) and uterine endometrium (Kennedy and de la Lande 1987). Uptake$_1$ and uptake$_2$ are well defined plasma membrane-associated transporters for noradrenaline and other catecholamines. They have distinctly different substrate and inhibitor selectivities, strikingly different rate constants (V_{max}/K_m) for inward transport (uptake$_1$ >> uptake$_2$) and differ as to their function being dependent on (uptake$_1$) or independent of (uptake$_2$) the presence of sodium and chloride ions (for reviews, see Trendelenburg 1988; Graefe and Bönisch 1988). While the human and bovine types of uptake$_1$ have been cloned (Pacholczyk et al. 1991; Lingen et al. 1994), the protein structure of uptake$_2$ is unknown. Extraneuronal uptake of a kind not clearly defined yet ("others" in Fig. 1) takes place in cells that have the capacity to excrete catecholamines (e.g., in the kidney; Lappe et al. 1980; Rennick 1981). All these cellular uptake processes will function as irreversible sites of loss for catecholamines provided inward transport is followed by some mechanism that keeps the intracellular amine concentration low. The neuronal uptake$_1$, for instance, is followed by uptake into transmitter storage vesicles or by metabolism through MAO (Graefe and Bönisch 1988). Uptake$_2$, on the other hand, is associated with COMT and MAO activity (Trendelenburg 1980, 1988). This is true for all extraneuronal uptake processes (Fig. 1), although in certain cell types excretion may be a mechanism contributing to the removal of intracellular catecholamines.

Taking into consideration results obtained in vitro (Trendelenburg 1972; Iversen 1975) and in vivo (Kopin et al. 1984; Goldstein et al. 1986; Eisenhofer et al. 1989, 1991a), the importance of uptake$_1$ for the removal of noradrenaline from the extracellular fluid is well established. This includes evidence to show that in the rat (Eisenhofer et al. 1989) and rabbit (Eisenhofer et al. 1991b; Friedgen et al. 1994) about 40% of the total plasma clearance of noradrenaline is due to uptake$_1$ and that the non-neuronal type of uptake$_1$ in the lungs of the rabbit makes a definite contribution to the plasma clearance that is due to uptake$_1$ (Friedgen et al. 1994). On the other hand, our knowledge about the role of uptake$_2$ is limited. This is mainly due to the fact that the compounds traditionally known to inhibit uptake$_2$, namely O-methylated catecholamines, various corticosteroids and certain β-haloalkylamines (for review, see Trendelenburg 1988), have a relatively low potency and a variety of effects unrelated to inhibition of uptake$_2$. Recently, a novel class of uptake$_2$-blocking agents have been described. Studies in isolated tissues and cells in culture showed that certain cyanine derivatives, which did not interfere with uptake$_1$, inhibited uptake$_2$ with high potency (Russ et al. 1993a,b). One of these compounds, namely 1,1′-diisopropyl-2,4′-cyanine iodide (disprocynium24), is of particular interest. When tested in Caki-1 cells, which take up noradrenaline by uptake$_2$ (Schömig and Schönfeld 1990), this agent had an IC_{50} for uptake$_2$ inhibition of 14 nmol l^{-1} (Russ et al. 1993b).

In the present study, disprocynium24 was used as a tool to define the role played by uptake$_2$ in vivo. Anaesthetized rabbits were infused simultaneously with trace amounts of ^{3}H-labelled noradrenaline, adrenaline and dopamine, and the plasma clearance of these catecholamines as well as the spillover of their endogenous counterparts into plasma was measured both before and during exposure to disprocynium24. To determine whether the effects of disprocynium24 on clearance and spillover depend on the operation of uptake$_1$ and/or MAO and COMT, some of the animals were given desipramine (to block uptake$_1$) and/or inhibitors of both MAO and COMT. While inhibition of MAO type A and B was accomplished by pretreatment with both clorgyline and pargyline (Youdim et al. 1988), COMT inhibition was achieved by treatment with tolcapone (Friedgen et al. 1993). A preliminary account of the present results was communicated to the German Society for Clinical and Experimental Pharmacology and Toxicology (Friedgen et al. 1995).

Methods

Animal preparation. Sixty-six rabbits (Chinchilla bastards, 1.5–2.5 kg) of either sex were caged individually, fed on a laboratory diet, and allowed free access to water. The study protocol was approved by the local Animal Experimentation Committee.

Animals were anaesthetized with Saffan® (1:3 mixture of alfadolone and alfaxalone) and artificially ventilated with room air supplemented with O_2. The ventilation frequency ranged from 35 to 40 cycles per minute, and the inspiration pressure was held between 10 and 15 mbar. Blood gas analysis was used to adapt these parameters in each individual experiment. Anesthesia was induced by an i.v. injection of 9 mg kg^{-1} Saffan® and then maintained by i.v. infusion of 0.5 (during surgery) and 0.2 mg $kg^{-1}min^{-1}$ (after completion of surgery) Saffan®. In addition, animals were given fentanyl (15 µg kg^{-1} i.v.) at

the onset of surgery. Body temperature was maintained at 37.5–38.5°C. The right carotid artery, the right atrium and the left femoral vein were cannulated with polyethylene tubing filled with saline containing heparin (10 units ml^{-1}). After completion of surgery, gallamine (5 mg kg^{-1}) and heparin (2,500 units) were given i.v. Blood pressure and heart rate were recorded via the right carotid artery. To measure cardiac output by thermodilution, cold saline (300 µl; 1°C) was injected into the right atrium. The carotid artery catheter was equipped with a hemostatic valve through which a small thermistor (0.6 mm o.d.) was inserted into the ascending aorta and connected to a cardiac output computer (HMV 7905; Hoyer, Bremen, Germany). The cardiac output of plasma (CO_P) was calculated from the cardiac output and the haematocrit.

Experimental protocol. After a resting period of 20 min, animals were infused (left femoral vein) for 110 min with trace amounts of ^{3}H-labelled noradrenaline, adrenaline, and dopamine at rates of 53, 63, and 119 nCi kg^{-1} min^{-1} (corresponding to 5.0, 0.8 and 5.3 pmol $kg^{-1}min^{-1}$), respectively. Saline containing 20 mmol l^{-1} acetic acid was used as vehicle; it was infused at a rate of 1.2 ml h^{-1}. No changes in either blood pressure or heart rate were observed as a result of the ^{3}H-catecholamine infusion. To determine plasma levels of the ^{3}H-labelled and endogenous catecholamines, arterial blood (2 ml) was sampled from the right carotid artery during the infusion and collected in tubes placed on ice. Blood samples were centrifuged (2,600 g, 8 min, 4°C) immediately after collection. One milliliter of plasma was mixed with 0.5 ml of an ice-cold solution of 1% Na_2EDTA and 1.25% Na_2SO_3 and stored at –80°C until assayed. The blood cells obtained after centrifugation were resuspended in saline containing 5% (v/v) dextrane 40 and re-injected via the right atrial catheter.

To inhibit MAO type A and B, clorgyline (5 mg kg^{-1}) and pargyline (20 mg kg^{-1}) were administered i.v. Previous experiments had shown that pargyline given at cumulative doses of 20 to 80 mg kg^{-1} reduced the plasma level of endogenous 3,4-dihydroxyphenylglycol (DOPEG; the main presynaptic metabolite of noradrenaline) by 44 to 59% (Friedgen et al. 1996). Therefore, clorgyline was given in addition to pargyline in this study. The dose of clorgyline was selected on the basis of the following experiments. Three animals were given 20 mg kg^{-1} pargyline at 0 min and 2, 5 and 10 mg kg^{-1} clorgyline, respectively, at 60 min. Blood samples for measurements of plasma DOPEG were taken at –2, 58 and 120 min. While pargyline reduced plasma DOPEG to 47% of baseline, the additional treatment with 2, 5 and 10 mg kg^{-1} clorgyline resulted in a further decrease to 11, 5 and 6% of baseline, respectively. COMT inhibition was achieved by treatment with tolcapone (3 mg kg^{-1} i.v. to begin with, followed by doses of 1.5 mg kg^{-1} given every 30 min). This dose regimen was previously shown to produce full inhibition of COMT in the rabbit (Friedgen et al. 1993).

$Uptake_1$ was inhibited by desipramine; it was given at an i.v. dose of 2 mg kg^{-1} and then infused at a rate of 11 µg $kg^{-1}min^{-1}$. To inhibit $uptake_2$, disprocynium24 (Russ et al. 1993a, b) was used. Stock solutions of the drug (40 mmol l^{-1}) were prepared with dimethyl sulfoxide (DMSO) as solvent. Of the stock solution, 160 µl per kg body weight were diluted with saline to give a total volume of 6 ml. This working solution was administered i.v.: after a loading dose of 250 µl (270 nmol kg^{-1} disprocynium24), it was infused at a rate of 4.5 ml h^{-1} (80 nmol $kg^{-1}min^{-1}$ disprocynium24). Considering the pharmacokinetics of disprocynium24 in the rabbit (Russ et al. 1996), this dose regimen was expected to lead to a plateau drug concentration in plasma of about 450 nmol l^{-1}. In vehicle controls, working solution not containing disprocynium24 was given.

The cocktail of ^{3}H-catecholamines was infused from 0 to 110 min (t = 0 min was the point in time at which the ^{3}H-catecholamine infusion was started). Experiments consisted of a 45-min control period followed by a 65-min experimental period. When required, pargyline and clorgyline were given at –15 min. Treatments with tolcapone and desipramine also began at –15 min. Disprocynium24 or vehicle (DMSO in saline) was administered throughout the 65-min experimental period, with the loading dose being injected at 46 min (see above). Measurements of heart rate, mean arterial pressure (MAP), cardiac output (CO), and total peripheral resistance (calculated from the ratio of MAP/CO) were taken at 0, 15, 30, 40, 60, 75, 90 and 105 min. Arterial blood was sampled at 30, 40, 75, 90 and 105 min.

Four groups of animals were studied, each group comprising animals given vehicle and animals given disprocynium24. *Group I* consisted of 17 animals in which MAO, COMT and $uptake_1$ were intact (7 vehicle controls and 10 animals given disprocynium24). *Group II* involved 16 animals with MAO and COMT inhibited (7 vehicle controls and 9 animals given disprocynium24). *Group III* involved 16 animals with $uptake_1$ inhibited (6 vehicle controls and 10 animals given disprocynium24). *Group IV* consisted of 17 animals in which MAO, COMT and $uptake_1$ were inhibited (7 vehicle controls and 10 animals given disprocynium24).

Assay of catecholamines in plasma. Plasma samples were centrifuged at 4°C (6000 g, 10 min). The supernatant was mixed for 5 min with 20 mg Al_2O_3 and 300 µl TRIS buffer (2 mM, pH 8.7) by means of a rotatory mixer and filtered (GF 52, Schleicher & Schuell, Dassel, Germany) by centrifugation. The Al_2O_3 remaining on the filter was washed twice with 1 ml water. Catecholamines were then desorbed from Al_2O_3 with 2×100 µl of 0.1 M $HClO_4$. Of the pooled eluate fractions, 150 µl were injected into a HPLC system.

The HPLC system consisted of a type 364.00 pump (Knauer, Berlin, Germany), an automatic sample injector WISP 712 (Waters, Eschborn, Germany), a Coulochem II electrochemical detector connected to the detector cell model 5011 (ESA, Bedford, Mass., USA), and a 486 DLC 40 computer (Aquarius, Bad Homburg, Germany) equipped with Chromstar Winpeak ART hardware/software (Eppendorf Biotronic, Maintal, Germany). For fractionation of the mobile phase leaving the detector cell, a fraction collector model 1200 (ISCO, Lincoln, Nebrasca, USA) was used. The chromatographic separation was achieved by a 5 µm Hypersil ODS column (125×3.0 mm i.d.) maintained at 26°C. The mobile phase (containing 30 mmol l^{-1} citric acid, 15 mM Na_2HPO_4, 2 mmol l^{-1} EDTA, 2.1 mmol l^{-1} sodium octanesulfonate and 12% (v/v) methanol; pH 6.50) was pumped through the column at a flow of 0.7 ml min^{-1}. The oxidation potential of the detector cell was set to 280 mV vs. a solid state palladium reference electrode (corresponding to 680 mV vs. Ag/AgCl).

Quantification of the ^{3}H-labelled catecholamines was done by liquid scintillation counting, whereas electrochemical detection was used to quantify the total of endogenous plus ^{3}H-labelled catecholamines. Timed collections of the mobile phase leaving the detector cell allowed fractionation of the eluted radioactivity according to the retention times of the catecholamines. Plasma not containing ^{3}H-catecholamines was spiked with known amounts of the infusate containing ^{3}H-catecholamines and processed as all other plasma samples. In this way, infusion rates for the three ^{3}H-labelled catecholamines were determined in each individual experiment. The catecholamine recovery from plasma was determined in each experiment. Mean recoveries for noradrenaline, adrenaline and dopamine were 83, 82 and 78%, respectively; intraassay coefficients of variation for these recoveries were 6.8, 5.0 and 2.9% (n = 10), respectively, and interassay coefficients of variation 13.9, 12.1 and 11.9% (n = 66), respectively. Results were corrected for recoveries. The plasma concentrations of endogenous catecholamines given in Results were obtained after correction of total for ^{3}H-labelled catecholamines.

Assay of disprocynium24 in plasma. Samples of plasma obtained by filtration after Al_2O_3 extraction were deproteinized by the addition of 600 µl perchloric acid (1 mol l^{-1}) and subsequent centrifugation (6,000 g, 10 min, 4°C). Supernatants were filtered and stored at –20°C. On the day of analysis, samples were centrifuged again (12,000 g, 20 min, 4°C) and assayed for disprocynium24 by HPLC with fluorometric detection (Russ et al. 1993a). The detection limit in plasma was about 5 nmol l^{-1}.

Data analysis and statistics. The total-body plasma clearance (Cl) of the ^{3}H-catecholamines was obtained from Cl = infusion rate/plasma concentration. As the catecholamine clearances were linearly related to the cardiac output of plasma (CO_P), values of Cl were expressed as a percentage of CO_P. The spillover of endogenous catecholamines into plasma was calculated from the product "plasma concentration of endogenous catecholamine (pmol ml^{-1}) Cl (ml kg^{-1} min^{-1})".

Results are given as arithmetic means ±SEM or as geometric means with 95% confidence limits. Baseline values (Tables 1 and 2) represent the average results of two measurements taken at 30 and 40 min of the 45-min control period of ^{3}H-catecholamine infusion. For baseline values of clearance as well as catecholamine concentrations in and spillovers into plasma, geometric means are presented, because between-group comparisons by means of Bartlett's test showed homogeneity of variances only after log transformation. Geometric means are also shown when the results obtained during exposure to disprocynium24 or vehicle were expressed as a percentage of baseline values. This was done because ratios usually show a log normal distribution. The significance of differences between the effects of disprocynium24 and vehicle (within-group comparisons) was analysed by Student's *t*-test. Between-group differences were evaluated by analysis of variance followed by the Bonferroni test for multiple comparisons. *P* values of <0.05 were taken to indicate statistical significance.

Drugs used in the study. Saffan® ampoules (alfadolone: alfaxalone = 1: 3; 12 mg/ml; Schweizerisches Serum & Impfinstitut, Bern, Switzerland); Fentanyl-Janssen® ampoules (Janssen, Neuss, Germany); ^{3}H-7-(–)-noradrenaline (NET-377, 10.6 Ci/mmol), ^{3}H-(N-methyl)-(–)-adrenaline (NET-623, 78.8 Ci/mmol), and ^{3}H-7-dopamine (NET-131, 22.5 Ci/mmol) (NEN, Dreieich, Germany); (–)-noradrenaline bitartrate, (–)-adrenaline bitartrate, dopamine hydrochloride, (±)-3,4-dihydroxyphenylglycol (DOPEG), pargyline hydrochloride, clorgyline hydrochloride, gallamine triethiodide and dimethyl sulfoxide (Sigma, Deisenhofen, Germany); desipramine hydrochloride (Serva, Heidelberg, Germany); heparin (Thrombophob®; Nordmark, Hamburg, Germany); dextran 40 (Rheomacrodex 10%®; Schiwa, Glandorf, Germany); tolcapone (Hoffmann-La Roche, Basel, Switzerland); 1,1′-diisopropyl-2,4′-cyanine iodide (disprocynium24) (Nimbus, Hammelburg, Germany).

Results

Baseline observations

Tables 1 and 2 summarize the results obtained before disprocynium24 or vehicle was given. There was no difference between groups with respect to the haemodynamics

Table 1 Baseline haemodynamics observed at steady state during the control period of ^{3}H-catecholamine infusion

	Group I (n = 17)	Group II (n = 16)	Group III (n = 16)	Group IV (n = 17)
HR (beats/min)	293 ±6	297 ±8	312 ±7	292 ±7
MAP (mm Hg)	77 ±3	71 ±3	72 ±3	74 ±2
CO (ml/min)	354 ±20	351 ±22	363 ±19	349 ±28
CO_p (ml kg^{-1}min^{-1})	103 ±10	116 ±12	100 ±7	94 ±7
TPR (mm Hg min ml^{-1})	0.22±0.01	0.21±0.01	0.20±0.01	0.23±0.01

Values are arithmetic means ±SEM of heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), cardiac output of plasma (CO_p) and calculated total peripheral vascular resistance (TPR). Values represent mean results obtained at 30 and 40 min of the 45-min control period of ^{3}H-catecholamine infusion. Four groups of rabbits were studied: group I, no treatment; group II, MAO and COMT inhibited; group III, uptake$_1$ inhibited; group IV, uptake$_1$ as well as MAO and COMT inhibited. Variance analysis did not reveal group differences with regard to any of the haemodynamic variables given here

Table 2 Baseline values for catecholamine plasma concentrations and plasma kinetics observed at steady state of the control period of ^{3}H-catecholamine infusion

	Group I (n = 17)	Group II (n = 16)	Group III (n = 16)	Group IV (n = 17)
Plasma clearance (% of CO_p)				
^{3}H-noradrenaline	85.8	65.5**	46.7$^{\#\#}$	40.7$^{\#\#}$
	(76.0; 97.0)	(58.8; 72.9)	(43.6; 50.1)	(36.4; 45.6)
^{3}H-adrenaline	83.2	71.7**	71.2$^{\#\#}$	64.4
	(78.2; 88.5)	(66.4; 77.4)	(67.7; 74.9)	(59.1; 70.2)
^{3}H-dopamine	156.9	100.2**	94.4$^{\#\#}$	87.5$^{\#}$
	(137.3; 179.3)	(95.1; 105.7)	(89.4; 99.7)	(82.1; 93.2)
Plasma concentrations (pmol ml^{-1})				
Noradrenaline	2.16	3.23	3.68	4.15
	(1.47; 3.17)	(2.43; 4.29)	(2.66; 5.08)	(2.89; 5.96)
Adrenaline	1.41	1.23	0.87	1.32
	(0.94; 2.10)	(0.85; 1.79)	(0.52; 1.45)	(0.90; 1.92)
Dopamine	0.10	0.66**	0.15	0.83**
	(0.06; 0.16)	(0.49; 0.89)	(0.10; 0.23)	(0.70; 1.00)
Spillover into plasma (pmol kg^{-1}min^{-1})				
Noradrenaline	180	231	166	153
	(115; 283)	(161; 331)	(109; 253)	(103; 226)
Adrenaline	114	97	60	77
	(79; 165)	(63; 148)	(33; 109)	(51; 116)
Dopamine	15	72**	14	66**
	(9; 25)	(52; 100)	(9; 21)	(54; 80)

Values are geometric means (95% confidence limits) of the mean results obtained at 30 and 40 min of the 45-min control period of ^{3}H-catecholamine infusion. Group I, no treatment; group II, MAO and COMT inhibited; group III, uptake$_1$ inhibited; group IV, uptake$_1$ as well as MAO and COMT inhibited. CO_p, cardiac output of plasma. * P<0.05; ** P<0.01 for comparison between groups II and I as well as groups IV and III (effect of enzyme inhibition). $^{\#}$ P<0.05; $^{\#\#}$ P<0.01 for comparison between groups III and I as well as groups IV and II (effect of uptake$_1$ inhibition)

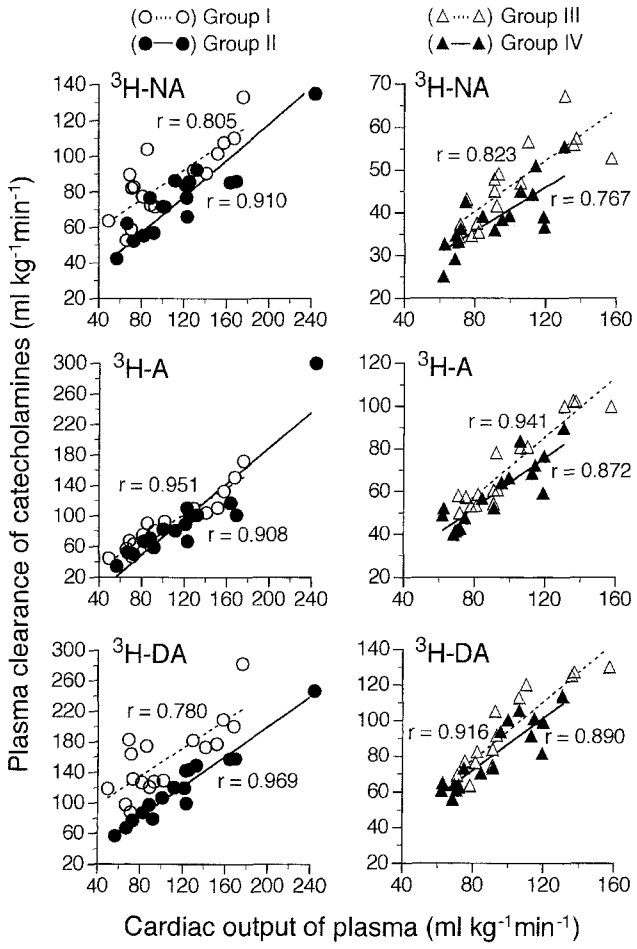

Fig. 2 Linear correlation between the plasma clearances of ^{3}H-catecholamines and the cardiac output of plasma. Anaesthetized rabbits were infused with ^{3}H-labelled noradrenaline (^{3}H-NA), adrenaline (^{3}H-A) and dopamine (^{3}H-DA) and the plasma catecholamine clearances as well as the cardiac output of plasma were measured at 30 and 40 min during the 45-min control period of the ^{3}H-catecholamine infusion (for details, see Methods). Four groups of animals were studied: group I, no treatment; group II, MAO and COMT inhibited; group III, uptake$_1$ inhibited; group IV, uptake$_1$ as well as MAO and COMT inhibited. Shown are results of individual experiments (with the data obtained at 30 and 40 min being averaged), the correlation coefficients (r) and the corresponding regression lines

(Table 1). The plasma clearance of the ^{3}H-catecholamines increased with increasing CO_p (Fig. 2). The linear correlation between the two parameters was highly significant ($P<0.001$) for all three catecholamines in each group of animals (Fig. 2). This is why ^{3}H-catecholamine clearances are expressed in Table 2 as a percentage of CO_p. The clearances normalized for CO_p showed less between-animal variation than clearance values expressed in units of ml kg^{-1} min^{-1}.

^{3}H-catecholamine clearances normalized for CO_p exhibited marked group differences (Table 2). The combined inhibition of MAO and COMT reduced the normalized clearance of ^{3}H-noradrenaline, ^{3}H-adrenaline and ^{3}H-dopamine by 24, 14 and 36%, respectively, in animals in which uptake$_1$ was intact (group II vs. group I), but only tended to decrease them in animals in which uptake$_1$ was inhibited (group IV vs. group III). Inhibition of uptake$_1$ caused the normalized clearance of ^{3}H-noradrenaline and ^{3}H-dopamine to drop by 46 and 40%, respectively, in animals with intact enzymes (group III vs. group I) and by 38 and 13%, respectively, in animals with MAO and COMT inhibited (group IV vs. group II). The decrease in the normalized ^{3}H-adrenaline clearance after uptake$_1$ inhibition was significant only in animals with intact enzymes (group II vs. group I) where it amounted to 13% (Table 2).

Plasma concentrations of endogenous noradrenaline and adrenaline did not exhibit group differences, although plasma noradrenaline tended to increase from group I to group IV (Table 2). Plasma dopamine, on the other hand, increased as a result of MAO and COMT inhibition: there was a 6.7-fold increase when uptake$_1$ was intact (group II vs. group I) and a 5.5-fold increase when uptake$_1$ was inhibited (group IV vs. group III). The increase in plasma dopamine by the combined inhibition of MAO and COMT was mainly due to an increase in dopamine spillover which increased 4.9-fold in animals with intact uptake$_1$ and 4.7-fold in animals with uptake$_1$ inhibited (Table 2). The spillovers of noradrenaline and adrenaline did not show any group differences (Table 2).

Plasma concentration of disprocynium24

Disprocynium24 was given as a loading dose of 270 nmol kg^{-1} and then infused at a rate of 80 nmol kg^{-1} min^{-1}. The four groups of animals did not differ as to the disprocynium24 concentrations in plasma. Therefore, the results of all experiments were pooled. The plasma concentrations observed 30, 45 and 60 min after the onset of infusion were 642±44, 663±44 and 641±47 nmol l^{-1} (means ±SEM; n = 39), respectively. This indicates that a steady state was reached. The plasma clearance of disprocynium24 calculated from these results was 146±9 ml kg^{-1} min^{-1}. In some of the animals (n = 23), the plasma concentration was also measured 20 min after the end of the disprocynium24 infusion. At that point in time it had fallen by 87±1% to 91±8 nmol l^{-1}.

Effects on haemodynamics

As revealed by analysis of variance, there was no group difference with regard to the effects of disprocynium24 and vehicle on haemodynamics. Therefore, to illustrate the effects of vehicle and disprocynium24, the data depicted in Fig. 3 represent pooled results from groups I to IV. When compared with vehicle controls, disprocynium24 did not alter heart rate and mean arterial blood pressure, but increased cardiac output by 22% and decreased total peripheral resistance by 16% (Fig. 3). The drug also produced a decrease in diastolic and an increase in systolic blood pressure so that the pulse pressure increased by 28% (not shown). These haemodynamic responses to the

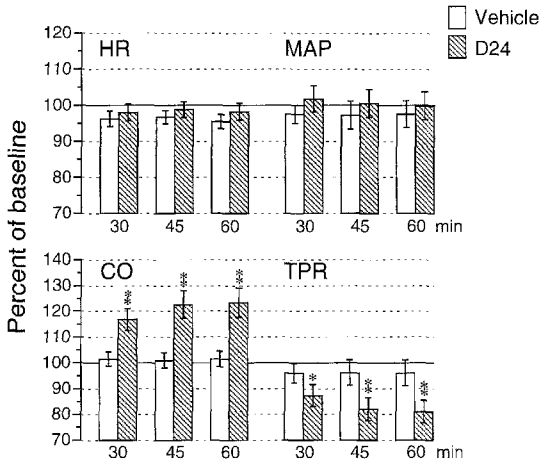

Fig. 3 Effects of disprocynium24 on haemodynamics. Heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO) and the calculated total peripheral vascular resistance (TPR) were measured at 30, 45 and 60 min after the onset of treatment with vehicle or disprocynium24 (D24). They are given in % baseline values which are the average of two measurements taken 15 and 5 min prior to the onset of treatment with vehicle or D24. Shown are geometric means (with 95% confidence limits as *vertical bars*) of 27 (vehicle controls) and 39 (D24-treated animals) observations; results obtained in all four groups of animals were pooled. Baseline values in vehicle controls and animals given D24 were (means ±SEM) HR: 299±6 and 297±4 beats/min, MAP: 74±2 and 73±2 mm Hg, CO: 364±16 and 345±15 ml/min, TPR: 0.213±0.009 and 0.220±0.008 mm Hg min ml^{-1}, respectively. * $P<0.01$, ** $P<0.001$ for differences between neighbouring columns

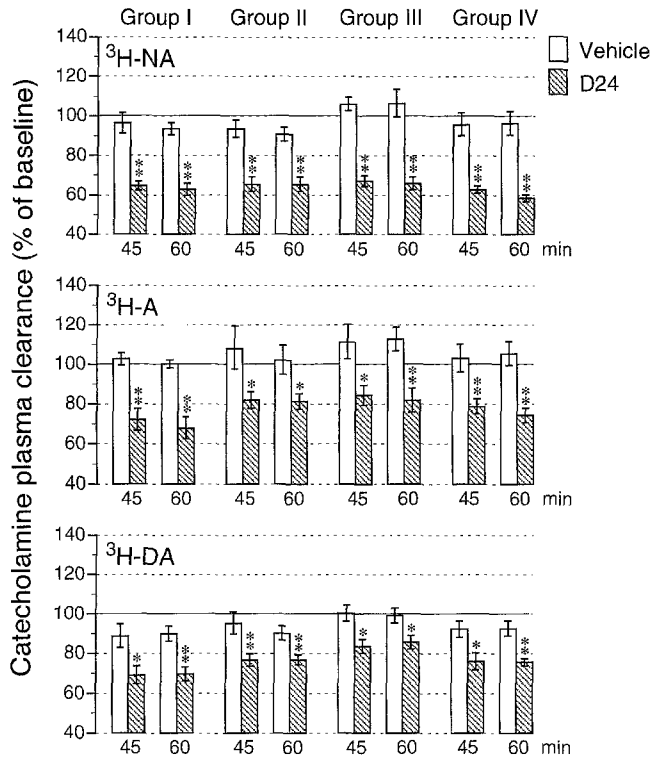

Fig. 4 Effects of disprocynium24 (D24) on the plasma clearance of ^{3}H-labelled noradrenaline (^{3}H-NA), adrenaline (^{3}H-A) and dopamine (^{3}H-DA). Measurements of catecholamine clearances were taken 45 and 60 min after the onset of treatment with vehicle or D24, and clearance values were expressed as a percentage of the cardiac output of plasma. Four groups of animals were studied: group I, no further treatment; group II, MAO and COMT inhibited; group III, $uptake_1$ inhibited; group IV, $uptake_1$ as well as MAO and COMT inhibited. Results are presented in percent of the baseline values summarized in Table 2. Shown are geometric means (±SEM as *vertical bars*) of 6–7 (vehicle controls) and 9–10 (animals given D24) observations. * $P<0.05$, ** $P<0.01$ for differences between neighbouring columns. Note that in all four groups vehicle treatment resulted in clearance values which did not differ from baseline values

drug reached a steady state after about 45 min of drug exposure (Fig. 3).

Effects on the plasma clearance of ^{3}H-catecholamines

The ^{3}H-catecholamine clearances observed during the experimental period of the ^{3}H-catecholamine infusion remained unaltered on vehicle infusion in groups I to IV: any changes above or below baseline observed after 45 and 60 min of vehicle infusion were not statistically significant. This is true irrespective of whether clearances were expressed in units of ml kg^{-1} min^{-1} or normalized for, and expressed as a percentage of, CO_p. The infusion of disprocynium24, on the other hand, was associated with a decrease in the plasma clearance of ^{3}H-catecholamines. The effect was more pronounced when clearances were normalized for CO_p than when absolute clearance values in units of ml kg^{-1} min^{-1} were considered. This is why normalized clearances relative to baseline values are used for analysis in Fig. 4. The use of normalized clearances is justified, because clearances were linearly dependent on cardiac output (Fig. 2), and because disprocynium24 increased cardiac output (Fig. 3). The cardiac output response to the drug had to be corrected for, because otherwise any increase in cardiac output would mask drug-induced decreases in ^{3}H-catecholamine clearance.

The fall in normalized ^{3}H-catecholamine clearances relative to baseline values were similar at 45 and 60 min during exposure to disprocynium24 (Fig. 4), whereas the decreases observed at 30 min (not shown) were usually smaller than those shown in Fig. 4. When the results obtained at 45 and 60 min were averaged, the normalized clearance of ^{3}H-noradrenaline fell relative to vehicle controls by 33% (group I), 29% (group II), 38% (group III) and 37% (group IV). The corresponding values for the clearance of ^{3}H-adrenaline were 31%, 22%, 26% and 26%, and for the clearance of ^{3}H-dopamine 22%, 17%, 16% and 18%, respectively (Fig. 4). Please note that similar percent clearance reductions in the four groups of animals imply that the absolute decreases in clearance differed between groups, because there were marked between-group differences in baseline clearances (Table 2).

As illustrated in Fig. 4, ^{3}H-catecholamine clearances did not show time-dependent changes during vehicle treatment. Therefore, the results of disprocynium24-treated animals were used to calculate the value of clearance brought about by disprocynium24-sensitive uptake. The calculation was carried out in each individual experiment with clearances being normalized for CO_p and involved subtraction

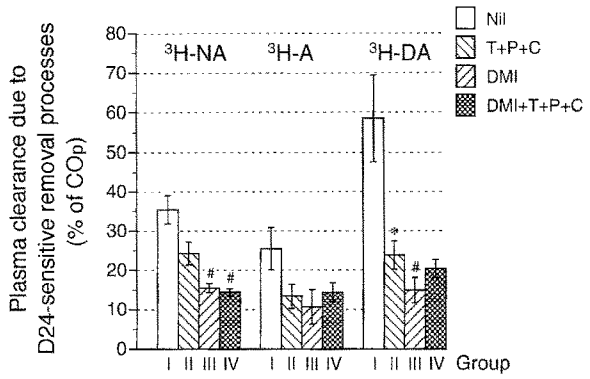

Fig. 5 Effects of MAO plus COMT and/or uptake$_1$ inhibition on the ^{3}H-catecholamine clearances brought about by disprocynium24-sensitive removal processes. Plasma clearances of ^{3}H-labelled noradrenaline (^{3}H-NA), adrenaline (^{3}H-A) and dopamine (^{3}H-DA) were measured both before and during treatment with disprocynium24 (D24) and normalized for the cardiac output of plasma (CO_p). The clearance due to D24-sensitive removal was calculated in each individual experiment by subtracting the value of clearance observed during D24 treatment from that observed before D24 treatment. The clearance before D24 treatment was the mean of two measurements taken 15 and 5 min prior to the onset of D24 treatment (baseline values), whereas the clearance during D24 treatment was the mean of two measurements taken 45 and 60 min after the onset of D24 treatment. Group I: no further treatment (Nil); group II: MAO and COMT inhibited by pargyline (P), clorgyline (C) and tolcapone (T); group III: uptake$_1$ inhibited by desipramine (DMI); group IV: MAO, COMT and uptake$_1$ inhibited. Shown are arithmetic means (±SEM as *vertical bars*) of 9–10 observations. * $P<0.05$ for comparison between groups II and I as well as groups IV and III (effect of enzyme inhibition). # $P<0.01$ for comparison between groups III and I as well as IV and II (effect of uptake$_1$ inhibition). Note that the D24-sensitive removal was statistically significant for each of the three ^{3}H-catecholamines in each of the four groups of animals

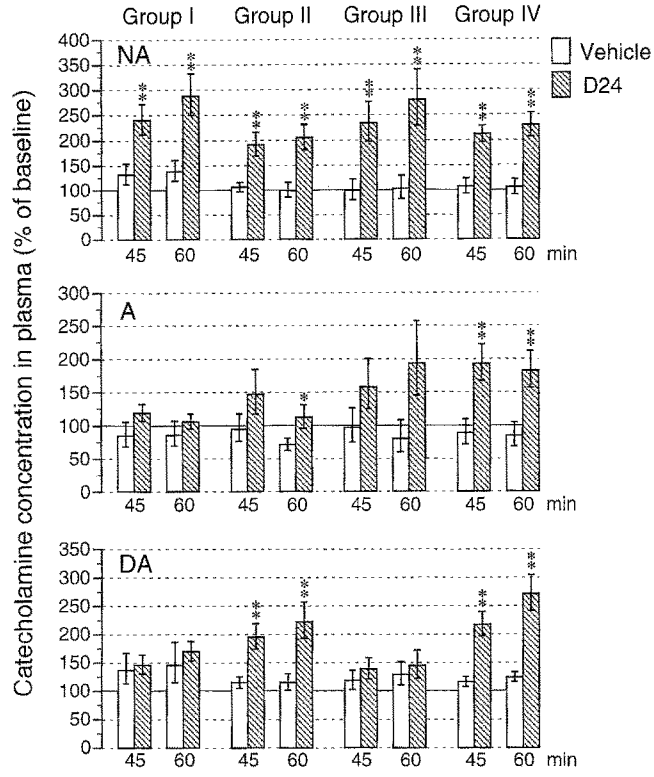

Fig. 6 Effects of disprocynium24 on the plasma concentration of endogenous noradrenaline (NA), adrenaline (A) and dopamine (DA). Measurements of catecholamine plasma concentrations were taken in four groups of anaesthetized rabbits 45 and 60 min after the onset of treatment with vehicle or disprocynium24 (D24). Group I, no further treatment; group II, MAO and COMT inhibited; group III, uptake$_1$ inhibited; group IV, uptake$_1$ as well as MAO and COMT inhibited. Results are presented as a percentage of the baseline values summarized in Table 2. Shown are geometric means (±SEM as *vertical bars*) of 6–7 (vehicle controls) and 9–10 (animals given D24) observations. * $P<0.05$, ** $P<0.01$ for differences between neighbouring columns

of the clearance observed during disprocynium24 treatment from that observed before disprocynium24 treatment. The results indicate that MAO and COMT inhibition decreased the disprocynium24-sensitive removal of ^{3}H-dopamine and tended to decrease that of ^{3}H-noradrenaline and ^{3}H-adrenaline (Fig. 5; group II vs. group I). Moreover, the ^{3}H-noradrenaline and ^{3}H-dopamine clearance due to disprocynium24-sensitive transport was smaller in animals treated with desipramine than in those not treated with desipramine (Fig. 5; group III vs. group I). The corresponding results concerning ^{3}H-adrenaline did not show any group difference (Fig. 5).

Effects on catecholamine concentration in and spillover into plasma

Treatment with vehicle did not alter plasma concentrations (Fig. 6) and spillover rates (Fig. 7) of endogenous catecholamines. Disprocynium24, on the other hand, enhanced the catecholamine plasma concentrations. When compared with vehicle controls, plasma noradrenaline increased in all groups of animals, whereas the plasma concentration of adrenaline and dopamine increased in group II and IV only (Fig. 6). The observed increases in plasma catecholamine concentrations were mainly due to increases in the rate of amine spillover into plasma. Considering averaged results after 45 and 60 min of drug exposure, noradrenaline spillover was increased relative to vehicle controls by 1.6-, 1.7-, 1.9- and 1.6-fold in group I, II, III and IV, respectively (Fig. 7). The corresponding results concerning adrenaline and dopamine spillover indicated a 1.5- and 1.9-fold increase, respectively, in group II and a 1.9- and 2.0-fold increase, respectively, in group IV (Fig. 7).

Discussion

The present study was carried out with the aim of quantifying the role of uptake$_2$ in the rabbit. If uptake$_2$ were to contribute to the removal of endogenous catecholamines before they enter the circulation (i.e., on the way from release sites to plasma), inhibition of uptake$_2$ should increase their spillover into plasma. If uptake$_2$ were to take part in the removal of catecholamines after they have entered the circulation, inhibition of uptake$_2$ should decrease the plasma clearance of infused ^{3}H-catecholamines. In

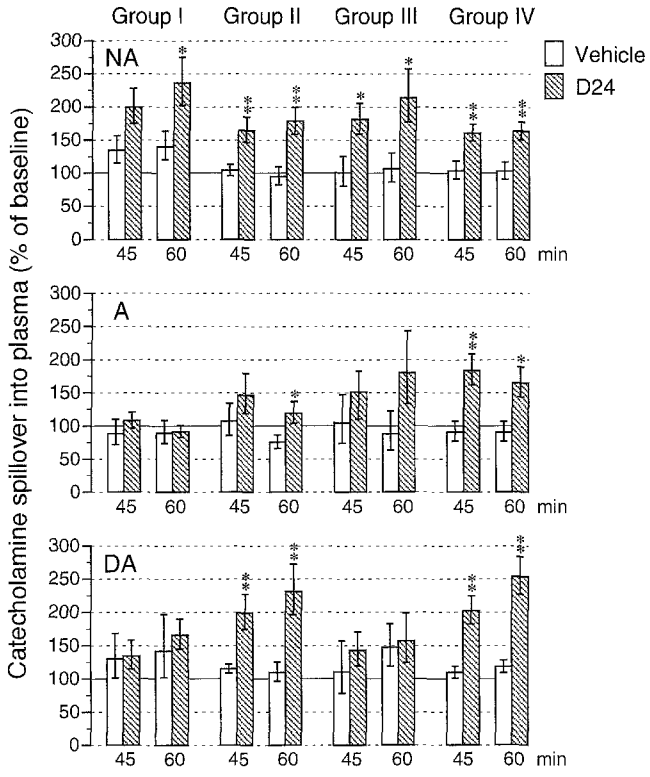

Fig. 7 Effects of disprocynium24 on the spillover of endogenous noradrenaline (NA), adrenaline (A) and dopamine (DA) into plasma. Rates of catecholamine spillover were measured in four groups of anaesthetized rabbits 45 and 60 min after the onset of treatment with vehicle or disprocynium24 (D24). Group I, no further treatment; group II, MAO and COMT inhibited; group III, $uptake_1$ inhibited; group IV, $uptake_1$ as well as MAO and COMT inhibited. Results are presented as a percentage of the baseline values summarized in Table 2. Shown are geometric means (±SEM as *vertical bars*) of 6–7 (vehicle controls) and 9–10 (animals given D24) observations. * $P<0.05$, ** $P<0.01$ for differences between neighbouring columns

other words, if $uptake_2$ were to play a distinct role in the catecholamine removal in vivo, disprocynium24 (at plasma concentrations exceeding its K_i for $uptake_2$ inhibition about 46-fold) should increase the spillover of catecholamines and decrease the clearance ^{3}H-catecholamines. The present results certainly go along with that. However, in many tissues $uptake_2$ ceases to function as an irreversible site of loss when MAO and COMT are inhibited (cf. Fig. 1), because uptake is then followed by amine accumulation within the cells so that net uptake quickly approaches zero (for reviews, see Trendelenburg 1976, 1980, 1988). Hence, the effects of a pure $uptake_2$ inhibitor on catecholamine spillover and clearance should be markedly reduced following the combined inhibition of MAO and COMT. In addition, the % decrease in ^{3}H-catecholamine clearance produced by a pure $uptake_2$ inhibitor should be more pronounced after inhibition $uptake_1$ than under normal conditions. Unexpectedly, disprocynium24 did not behave as predicted for a pure $uptake_2$ inhibitor. Therefore, our aim to quantify the role of $uptake_2$ in the rabbit was not achieved. Before discussing the effects of disprocynium24 on catecholamine plasma kinetics, group differences in baseline observations will be considered first.

Baseline observations

As the plasma clearances of the three ^{3}H-catecholamines are very high indeed, it is not surprising that they are flow-dependent and increase with increasing cardiac output. The present results indicate that this is true for the plasma clearance of ^{3}H-noradrenaline, ^{3}H-adrenaline and ^{3}H-dopamine, confirming earlier observations from this laboratory for ^{3}H-noradrenaline and ^{3}H-adrenaline (Halbrügge et al. 1991). That the cardiac output determines the value of ^{3}H-noradrenaline clearance is likewise known from various studies in humans (Esler et al. 1984). Therefore, ^{3}H-catecholamine clearances normalized for CO_p will be considered when the following discussion deals with the present results.

Inhibition of either MAO and COMT or $uptake_1$ caused the clearance of the three ^{3}H-catecholamines to decrease (Table 2). Comparison of group IV (MAO, COMT and $uptake_1$ inhibited) with group I (MAO, COMT and $uptake_1$ operative) shows that the combined activities of the three pathways were responsible for 53, 23 and 44% of the plasma clearance of ^{3}H-noradrenaline, ^{3}H-adrenaline and ^{3}H-dopamine, respectively. Interestingly enough, MAO plus COMT inhibition reduced the clearances in animals with intact $uptake_1$ but not in animals with $uptake_1$ inhibited, and, conversely, $uptake_1$ inhibition was more effective in reducing the clearances in animals with intact enzymes than in those with enzymes inhibited. Hence, the individual effects of inhibition of MAO and COMT on the one hand, and $uptake_1$ on the other hand, were not additive (Table 2). This indicates that the combined inhibition of MAO and COMT decreases not only the removal of circulating catecholamines mediated by $uptake_2$, but also the removal due to $uptake_1$.

Rates of noradrenaline and adrenaline spillover into plasma did not show between-group differences. As far as the noradrenaline spillover is concerned, $uptake_1$ inhibition by desipramine would be expected to produce an increase. However, it is now well established that $uptake_1$ inhibitors in general reduce sympathetic nerve firing rates through an action within the central nervous system (CNS) (Cohen et al. 1990; Szabo and Schultheiss 1990; Szabo et al. 1991). The central, sympathoinhibitory effect of $uptake_1$ blockers masks their peripheral effect on noradrenaline spillover. Like $uptake_1$ inhibition, the inhibition of MAO plus COMT also failed to alter the spillover of noradrenaline and adrenaline. But here again, our results may be confounded by effects of the enzyme inhibitors similar to those described above for $uptake_1$ inhibitors. For instance, MAO inhibitors may reduce the impulse traffic in sympathetic neurones either by an action within the CNS (Fuentes et al. 1979) or by blocking the ganglionic transmission (Pscheidt 1963).

Measurements of dopamine spillover gave straightforward results. Irrespective of whether $uptake_1$ was blocked or not, enzyme inhibition produced a 4.7- to 4.9-fold increase (Table 2). As most of the plasma dopamine is likely to be of non-neuronal origin (Cuche et al. 1990; Vieira-Coelho and Soares-da-Silva 1993; Eisenhofer et al. 1995;

Goldstein et al. 1995), our results show that MAO and COMT are very efficient pathways for the removal of this non-neuronal amine before it enters the circulation.

Effect of disprocynium24 on ^{3}H-catecholamine clearances

Disprocynium24 clearly reduced the plasma clearance of all three ^{3}H-catecholamines. The extent of the % reduction of clearance differed between ^{3}H-catecholamines, but was largely independent of whether MAO plus COMT and/or uptake$_1$ were inhibited. In the four groups of animals studied here, the decreases in ^{3}H-noradrenaline, ^{3}H-adrenaline and ^{3}H-dopamine clearance ranged from 29 to 38%, 22 to 31% and 16 to 22%, respectively. In the conscious rat, disprocynium24 (given at the same dose rate) was even more effective: it reduced the plasma clearance of ^{3}H-noradrenaline by about 60% (Eisenhofer et al. 1996). This disagreement in results may well be due to species differences in the functional expression of uptake$_2$ as noticed earlier (Gillespie 1976; Trendelenburg 1976, 1988). That extraneuronal uptake contributes to the removal of circulating catecholamines was shown in another study in rabbits (Friedgen et al. 1994): the plasma clearance of ^{3}H-isoprenaline, which is a good substrate for uptake$_2$ (but not for uptake$_1$) and is metabolized subsequent to cellular uptake by COMT only, fell by about 29% when COMT was inhibited.

As mentioned above, uptake$_2$ has the capacity to bring about a steady net removal of catecholamines only when inward transport is followed by amine metabolism through MAO and/or COMT. Therefore, if disprocynium24 were to block uptake$_2$ only, its inhibitory effect on ^{3}H-catecholamine clearances should be much less pronounced after inhibition of MAO and COMT (group II) than under normal conditions (group I). Contrary to expectation, the % inhibition of clearances produced by disprocynium24 was similar in groups I and II, and in group II there was still a disprocynium24-sensitive removal of ^{3}H-noradrenaline, ^{3}H-adrenaline and ^{3}H-dopamine (Fig. 5). Hence, disprocynium24 must have decreased catecholamine clearances not only by blocking uptake$_2$, but also by inhibition of related extraneuronal transport processes capable of mediating net removal of catecholamines even after inhibition of MAO and COMT. The difference between group I and II in the disprocynium24-sensitive clearance may reflect the clearance due to uptake$_2$; according to Fig. 5, it was highest for ^{3}H-dopamine. The disprocynium24-sensitive clearance remaining after MAO and COMT inhibition may reflect ^{3}H-catecholamine removal due to uptake followed by excretion (or removal due to ^{3}H-catecholamine excretion) (cf. Fig. 1) which may take place not only in the kidneys, but also in the liver, intestine and glandular tissues.

Cyanine analogues closely related to disprocynium24 are known inhibitors of the renal transport (and excretion) of organic cations including catecholamines (Rennick 1981; Schömig et al. 1993). Recently, a cDNA isolated from rat kidney was cloned which encodes a membrane protein (OCT1) that, when expressed in Xenopus oocytes, mediates cyanine-sensitive uptake of organic cations (Gründemann et al. 1994). OCT1 was found not only in the proximal tubules of the kidney, but also in hepatocytes and in the mucous membrane of the gut (Gründemann et al. 1994). Further experiments with OCT1 indicated that its pharmacological properties differed from those of uptake$_2$, but resembled those of a transporter for organic cations in rat hepatocytes (Schömig et al. 1995). They also showed that disprocynium24 inhibited OCT1-mediated transport with an IC_{50} of 110 nmol l^{-1} (Schömig et al. 1995). As the plasma concentration of disprocynium24 observed here exceeded the IC_{50} for OCT1 inhibition about 6-fold, it is very likely that disprocynium24 reduced catecholamine clearances not only by inhibition of uptake$_2$, but also by inhibition of organic cation transporters related to, but not identical with, uptake$_2$.

It was surprising to see that desipramine reduced the disprocynium24-sensitive clearances of noradrenaline and dopamine and that the additional inhibition of MAO and COMT then failed to reduce them any further (Fig. 5). A contributing factor may have been that desipramine, although highly selective for uptake$_1$ (Iversen 1967), caused at least a partial inhibition of uptake$_2$ and the related organic cation transporters. For uptake$_2$, a 29% inhibition in the presence of 1 μmol l^{-1} desipramine was reported (Salt 1972). For OCT1, desipramine had an IC_{50} of 2.8 μmol l^{-1} (Gründemann et al. 1994). Also, it should be emphasized that ^{3}H-catecholamine plasma clearances reflect the total-body removal of circulating catecholamines. Any change in regional blood flow may well alter plasma clearances by reducing the distribution of blood flow to those regions which are highly actively involved in the catecholamine removal due to uptake$_2$ and the related organic cation transporters. Because of the central sympathoinhibitory action of desipramine (see above), part of the effect of desipramine on the disprocynium24-sensitive clearance may have been due to such changes in regional blood flow distribution. Especially in the light of the fact that both uptake$_2$ (Gillespie 1976; Trendelenburg 1988) and organic cation transporters (Gründemann et al. 1994) are heterogeneously distributed in the body, consequences of changes in regional blood flow distribution are quite reasonable.

Effect of disprocynium24 on catecholamine spillover

The increase in noradrenaline spillover produced by disprocynium24 was similar in the four groups of animals, ranging from 1.6- to 1.9-fold (Fig. 7). When given to conscious rats in which uptake$_1$, MAO and COMT were intact, disprocynium24 (dosed as in the present study) was even more effective: it increased the noradrenaline spillover by a factor of 2.7 (Eisenhofer et al.1996). Our finding in the rabbit that the disprocynium24-induced increase in noradrenaline spillover was not altered even after the combined inhibition of enzymes and uptake$_1$ does not admit of an easy explanation. One possibility is that disprocynium24 increases the release of noradrenaline either by

a central or a peripheral (presynaptic) mode of action. However, as disprocynium24 failed to increase heart rate, this is unlikely to be the underlying mechanism. Another possibility is that organic cation transporters sensitive to disprocynium24 contribute to the removal of noradrenaline before it enters the circulation.

In contrast to noradrenaline spillover, the spillover of adrenaline and dopamine was increased in response to disprocynium24 only after MAO plus COMT inhibition (Fig. 7). As uptake$_2$ becomes non-functional when MAO and COMT are inhibited, the finding that inhibition of the two enzymes unmasked an effect of disprocynium24 is compatible with the view that uptake and/or excretion processes other than uptake$_2$ may help to remove adrenaline and dopamine at sites of their release or synthesis; inhibition of these pathways increases the proportion of the synthetized or released amine that reaches the circulation.

Effect of disprocynium24 on haemodynamics

The infusion of disprocynium24 at a rate of 80 nmol kg^{-1} min^{-1} produced an increase in cardiac output and a decrease in total peripheral resistance; heart rate and mean arterial blood pressure were not altered (Fig. 3). These haemodynamic effects were observed at steady-state drug concentrations in plasma of about 640 nmol l^{-1}. According to the accompanying report by Russ et al. (1996), marked disprocynium24-induced decreases in blood pressure appeared to be associated with plasma drug concentrations lower than 640 nmol l^{-1}. The reason for this apparent disagreement in results is due to the fact that Russ et al. (1996) gave bolus injections of disprocynium24 (4 μmol kg^{-1}) into the right atrium, with the first plasma sample being taken 1.5 min after the injection. Hence, the marked decrease in blood pressure observed by Russ et al. (1996) is probably a consequence of drug concentrations being present during and shortly after the bolus injection considerably higher than 640 nmol l^{-1}.

The cardiovascular effects of disprocynium24 are unlikely to be due to the drug's ability to block uptake$_2$ and related transporters for organic cations. Russ et al. (1996) showed that disprocynium24 antagonizes α_1-adrenoceptor-mediated contractions in the rabbit aorta and inhibits radioligand binding to α_1-adrenoceptors of the rat myocardium with a K_i of 240 nmol l^{-1}. Hence, the α_1-adrenoceptor-blocking properties of the drug together with drug level in plasma of about 640 nmol l^{-1} explains why the total peripheral resistance fell by 16%. The observed increase in cardiac output may be a consequence of the peripheral vasodilatation. However, as there was no increase in heart rate, the possibility must be entertained that disprocynium24 increases the myocardial contractility.

Conclusions

The present results indicate that the effects of disprocynium24 on catecholamine clearances from, and catecholamine spillovers into, plasma involve inhibition of both uptake$_2$ and related organic cation transporters. In animals with intact MAO and COMT, uptake$_1$ inhibition by desipramine reduced the clearances of noradrenaline, adrenaline and dopamine by 46, 13 and 40%, respectively, whereas disprocynium24 diminished them by 33, 31 and 22%, respectively. Taken together, the combined activities of uptake$_1$ and disprocynium24-sensitive transporters (uptake$_2$ plus related organic cation transporters) thus were responsible for 79% (noradrenaline), 44% (adrenaline) and 62% (dopamine) of the plasma clearances. A similar picture is obtained when the clearances of noradrenaline, adrenaline and dopamine observed in animals with intact uptake$_1$, MAO and COMT under baseline conditions (86, 83 and 157% of CO_p, respectively) are compared with those observed in the presence of disprocynium24 after inhibition of uptake$_1$, MAO and COMT (22, 48 and 64% of CO_p, respectively; calculated from measurements of Fig. 4, group IV): from these values it can be calculated that uptake$_1$, MAO, COMT and disprocynium24-sensitive transporters (including uptake$_2$) jointly account for 74% (noradrenaline), 42% (adrenaline) and 59% (dopamine) of the total plasma clearance of these catecholamines. Either assessment leaves a substantial fraction of the plasma catecholamine clearance unexplained – about 25% for noradrenaline, 60% for adrenaline, 40% for dopamine. The reason may be either an only partial inhibition of organic cation transport systems by disprocynium24 or the presence of unknown removal processes distinct from uptake$_1$, uptake$_2$, MAO, COMT and disprocynium24-sensitive organic cation transport.

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