Dissipation mechanisms for 5-hydroxytryptamine in the coronary circulation of the isolated perfused heart of the rat

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1 The contribution of uptake into vascular endothelial cells, of neuronal uptake and of extra-neuronal uptake in the dissipation of 5-hydroxytryptamine (5-HT) perfused through the coronary circulation of the rat heart was examined.

2 Hearts from reserpine-pretreated rats were isolated and perfused *in vitro* with 5-HT, in the absence or presence of inhibitors, and rates of appearance of the deaminated metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the venous effluent were measured using an h.p.l.c. assay.

3 The steady-state rates of 5-HIAA appearance in the venous effluent in hearts perfused with 1 μM 5-HT ($422 \pm 8.48 \text{ pmol g}^{-1} \text{ min}^{-1}$, $n = 12$) were reduced by pretreatment of the rats with 6-hydroxydopamine (22% inhibition), and by inclusion in the perfusion solution of 30 μM cocaine (28% inhibition), 100 μM 3-O-methylisoprenaline (64% inhibition), 100 μM corticosterone (58% inhibition), or 30 μM cocaine and 100 μM 3-O-methylisoprenaline (87% inhibition).

4 The extraneuronal deamination of 5-HT in the heart was saturable ($K_m = 101 \mu\text{M}$, $V_{max} = 31.2 \text{ nmol g}^{-1} \text{ min}^{-1}$). The neuronal deamination of 5-HT was saturated by about 50 fold lower concentrations of 5-HT than was extraneuronal deamination, but K_m and V_{max} values could not be determined.

5 In the coronary circulation of the rat heart, 5-HT was dissipated by the uptake processes for catecholamines, extraneuronal uptake (predominantly) and neuronal uptake, and subsequent metabolism by monoamine oxidase. There was no evidence that a cocaine-sensitive uptake of 5-HT into vascular endothelial cells made any significant contribution to 5-HT dissipation in the heart.

Introduction

The physiological role of 5-hydroxytryptamine (5-HT) is still not clear (Vanhoutte & Cohen, 1983), but the effects on local vascular function of 5-HT released from platelets have been implicated in various pathological conditions. These conditions include the coronary vasospasm of Prinzmetal's (variant) angina (Angus *et al.*, 1982; Bove & Dewey, 1983); cerebral vasospasm after subarachnoid haemorrhage (Allen *et al.*, 1974); digital vasospasm of Raynaud's phenomenon (Halpern *et al.*, 1960; Strandén *et al.*, 1982); and migraine (Raskin, 1981). Also, vasoconstrictor responses of blood vessels to 5-HT have been shown to be increased by atherosclerosis (Henry & Yokoyama, 1980; Heistad *et al.*, 1984). If 5-HT is involved in these conditions, then the presence of dissipation mechanisms for 5-HT in blood vessels

may be important in limiting its effect. It is well documented that 5-HT can be avidly taken up by platelets (Pletscher, 1968; Sneddon, 1969), and can be taken up and then metabolized by monoamine oxidase (MAO) in endothelial cells (Junod, 1972; Strum & Junod, 1972; Shepro *et al.*, 1975; Junod & Ody, 1977). There is also evidence for uptake and storage of 5-HT in noradrenergic neurones (Thoa *et al.*, 1969; Verbeuren *et al.*, 1983; McCulloch *et al.*, 1984; Paiva *et al.*, 1984) and for extraneuronal uptake and metabolism of 5-HT in vascular smooth muscle (Buchan *et al.*, 1974; Paiva *et al.*, 1984; Fukuda *et al.*, 1986a,b).

In the present study, the dissipation of 5-HT in the coronary circulation has been examined, in view of the suggestions that 5-HT may be involved in the coronary vasospasm of Prinzmetal's angina (*vide supra*) and in the cyclic variations in blood flow

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which occur in stenosed coronary arteries (Ashton *et al.*, 1986). The aim of the study was to examine the contributions of uptake into vascular endothelial cells, of neuronal uptake and of extraneuronal uptake in the dissipation of 5-HT perfused through the coronary circulation of the rat heart *in vitro*.

Some of the results of this work were presented to the August 1985 meeting of the Australian Physiological and Pharmacological Society (Williams *et al.*, 1985), the December 1985 meeting of the British Pharmacological Society (Bryan *et al.*, 1986), and the December 1985 meeting of the Australasian Society of Clinical and Experimental Pharmacologists (Bryan *et al.*, 1987).

Methods

Male albino Wistar rats (170–245 g) were injected intraperitoneally with 1 mg kg^{-1} reserpine 18–24 h before the experiments (shown in preliminary experiments to reduce endogenous noradrenaline and 5-HT in the heart to negligible levels), and intraperitoneally with 5000 U kg^{-1} heparin 20 min before the animals were killed by cervical dislocation and bleeding. Some rats were injected intravenously with 50 mg kg^{-1} 6-hydroxydopamine (6-OHDA) 24 h before the experiments to achieve chemical sympathectomy. This dose of 6-OHDA abolished the presence of noradrenergic neurones in the heart as determined by fluorescence histochemistry (Bryan & O'Donnell, unpublished observations). In the experiments with 6-OHDA-pretreated rats, other rats were injected intravenously with 1 ml kg^{-1} of the vehicle used for the 6-OHDA (0.53 mM ascorbic acid in normal saline, *vide infra*). The pretreatment of rats with the vehicle only did not affect the rates of 5-hydroxyindoleacetic acid (5-HIAA) appearance in the venous effluent, compared with untreated rats. Hence, the results from rats with and without the vehicle pretreatment were pooled. In some experiments, rats were injected intraperitoneally with 75 mg kg^{-1} pargyline, 18 h and 2 h before the experiment, to inhibit MAO.

The hearts from the rats were isolated and perfused using a Desaga PLG peristaltic pump by the Langendorff technique with Tyrode solution at a constant rate of about $10 \text{ ml g}^{-1} \text{ min}^{-1}$ at 36.5°C . The Tyrode solution contained (in mM): Na^+ 149.2, K^+ 2.7, Ca^{2+} 1.3, Mg^{2+} 1.05, Cl^- 144.3, H_2PO_4^- 0.4, HCO_3^- 11.9, glucose 5.0 and Na_2EDTA 0.04, and was aerated with 95% O_2 and 5% CO_2 . The perfusion pressure was monitored throughout the experiments and ranged from 25–30 mmHg. The hearts were initially perfused for 15 min with 5-HT-free Tyrode solution, and then with Tyrode solution containing 5-HT for 80.5 or 90.5 min. The perfusion

pressure was not affected by the 5-HT in the perfusion solution. 5-HT concentrations of $1 \mu\text{M}$ and greater were used throughout the study, so that, even when inhibitors were present, 5-HIAA in the venous effluent could be determined quantitatively using a high performance liquid chromatography (h.p.l.c.) assay (*vide infra*). In experiments with inhibitors (see Results), the inhibitor was included in the Tyrode solution from the 6th min of the initial perfusion and throughout the perfusion with 5-HT. Samples of venous effluent were collected at appropriate intervals during the perfusion with 5-HT (see Results). At the end of each experiment, the heart was removed from the cannula, blotted, weighed and placed in 0.31 M trichloroacetic acid containing 2.7 mM Na_2EDTA at $4\text{--}6^\circ\text{C}$. After at least 2 h, the hearts were homogenized and then centrifuged at $10,000 g$ for 10 min. Also, samples of the perfusion solution were collected immediately before and after each experiment (arterial solutions).

Samples (2 ml) of venous effluent and of the arterial solutions (after dilution with Tyrode solution for 5-HT concentrations greater than $30 \mu\text{M}$) were taken. Ice-cold 5 M acetic acid ($200 \mu\text{l}$) and 0.27 M Na_2EDTA ($20 \mu\text{l}$) were added immediately after collection of the samples. The samples were stored on ice and frozen until the h.p.l.c. assay of 5-HT and 5-HIAA was carried out. Samples of the supernatant from the heart homogenate were also frozen for subsequent h.p.l.c. assay.

H.p.l.c. assay of 5-HT and 5-HIAA

The h.p.l.c. apparatus consisted of a Waters Associates Model 510 pump and solvent delivery system, a Waters U6K universal injector, a Waters CN reversed-phase guard column and a Novapak C_{18} reversed-phase steel column ($5 \mu\text{m}$, 3.9 mm internal diameter $\times$ 15 cm). The electrochemical detection system comprised a Waters Model 410 electrochemical detector and a glassy carbon electrode, connected to a chart recorder. The optimal electrode potential was found to be $+0.65 \text{ V}$, relative to a Ag/AgCl reference electrode. The mobile phase contained Na_2HPO_4 70 mM, sodium heptane sulphonate 5 mM, Na_2EDTA 0.1 mM and acetonitrile 6.5%, and was adjusted to a pH of 3.6 with 8.7 M phosphoric acid. The flow rate was adjusted to 1 ml min^{-1} during assay of the samples and 0.1 ml min^{-1} when the h.p.l.c. was not in use for sample analysis. The mobile phase was recirculated through the system, was replaced at weekly intervals, and was degassed daily under vacuum in a sonicator.

Samples ($20\text{--}100 \mu\text{l}$) of venous effluent, arterial solution and supernatant of the heart homogenate were injected directly onto the h.p.l.c. without any sample clean-up. The arterial samples were injected

to assay the 5-HT concentration in the perfusion solution and to check that no peaks other than 5-HT appeared in the chromatograms. The retention times for 5-HIAA and 5-HT were 5.7 min and 9.7 min, respectively. All samples were analysed on the day of the experiment. Standards containing appropriate concentrations of 5-HIAA and 5-HT were prepared daily, in Tyrode solution containing inhibitors when required, and were treated as described above for experimental samples. The amounts of 5-HIAA and 5-HT in the samples were measured by comparison of the peak heights of the experimental and standard solutions.

The only inhibitor drug that was detected by the electrochemical detector was 3-O-methylisoprenaline (OMI), which had a retention time on the h.p.l.c. column of 14 min. Hence, in experiments with OMI, the h.p.l.c. assay time was increased, but the OMI did not interfere with the 5-HIAA or 5-HT peaks. Corticosterone presented some problems in that it appeared to bind to the h.p.l.c. column and increase the background noise of the detector, so the column was washed with dimethylsulphoxide (DMSO) to remove the corticosterone after each experiment. Cocaine did not interfere with the h.p.l.c. assay at all.

Drugs and solutions

The drugs used were: cocaine hydrochloride (Drug Houses of Australia, Sydney, N.S.W., Australia); corticosterone (Sigma Chemical Company, St. Louis, MO, U.S.A.); heparin sodium (as ampoules of 5000 u ml⁻¹; Weddel Pharmaceuticals Ltd, London, England); 6-hydroxydopamine (6-OHDA) hydrobromide (Sigma); 5-hydroxyindoleacetic acid (5-HIAA; Sigma); 5-hydroxytryptamine (5-HT) creatinine sulphate complex (Sigma); 3-O-methylisoprenaline (OMI) hydrochloride (Boehringer, Ingelheim, F.R.G.); pargyline hydrochloride (Sigma); reserpine (as Serpasil ampoules, 1 mg ml⁻¹; Ciba-Geigy Australia Ltd, Sydney, N.S.W. Australia).

Stock solutions of 5-HT, 5-HIAA and OMI (10 mM) were prepared in 10 mM HCl. Corticosterone (300 mM) was dissolved in DMSO and diluted with Tyrode solution, such that the final concentration of DMSO in the perfusion solution was 4.6 mM. Pargyline hydrochloride (92.5 mg ml⁻¹) was dissolved in normal saline (154 mM NaCl), and 6-OHDA hydrobromide (74 mg ml⁻¹) was dissolved in normal saline containing 0.53 mM ascorbic acid. Other drugs and all dilutions were prepared in Tyrode solution.

Calculation of results

Steady-state rates of 5-HIAA appearance were calculated as the mean of the rates of 5-HIAA appearance in venous effluent samples collected in the 66th, 71st,

76th and 80th min of perfusion with 1 μ M 5-HT, or in samples collected in the 80th, 84th, 87th and 90th min of perfusion with concentrations of 5-HT greater than 1 μ M.

Rate constants for efflux of 5-HIAA from the hearts were calculated as the steady-state rate of 5-HIAA appearance divided by the 5-HIAA content of the heart at the end of the experiment. The half-time for efflux of 5-HIAA was then calculated as 0.693 divided by the corresponding rate constant for efflux of 5-HIAA.

Rate constants for MAO activity k_{MAO} were calculated as the steady-state rate of 5-HIAA appearance divided by the intracellular concentration of 5-HT (s_i) in the heart at the end of the experiment (Grohmann, 1987). Values of s_i were calculated as the concentration of 5-HT in the perfusion solution multiplied by the tissue/medium ratio of 5-HT in the heart after correction for distribution of 5-HT in the extracellular space (0.365 ml g⁻¹, Bryan *et al.*, 1983).

Rate constants for efflux and for MAO are expressed as geometric means with 95% confidence limits. All other results are expressed as arithmetic means $\pm$ s.e. The significance of differences between values was assessed by Student's *t* test.

In the kinetic analysis of the deamination of 5-HT, the steady-state rates of neuronal or extraneuronal deamination (v) at various 5-HT concentrations (s) were calculated as differences between mean steady-state rates of deamination in the absence and presence of inhibitors and expressed with the s.e. of the differences. Values of s/v versus s for extraneuronal deamination were analysed by linear least squares regression analysis (Snedecor & Cochran, 1980), and K_m and V_{max} values were obtained by a method for least squares estimation of non-linear parameters (Marquardt, 1963).

Results

(A) Metabolism of 5-HT in hearts perfused with 1 μ M 5-HT

When rat hearts were perfused with 1 μ M 5-HT, venous effluent samples contained detectable amounts of 5-HIAA from the 10th min of perfusion, but not at the 2nd min of perfusion (Figure 1). The limit of sensitivity of the h.p.l.c. assay of 5-HIAA corresponded to a rate of appearance in the venous effluent of approximately 20 pmol g⁻¹ min⁻¹. 5-HIAA appeared to be the only metabolite of 5-HT that was formed in the heart, in that no other peaks were present in the chromatograph from the assay of either venous effluent samples or supernatant from the homogenate of the heart (Figure 2). The rates of 5-HIAA appearance in the venous effluent were

Figure 1 Rates of 5-hydroxyindoleacetic acid (5-HIAA) appearance in the venous effluent ($\text{pmol g}^{-1} \text{min}^{-1}$) plotted against perfusion time (min) for rat hearts perfused with $1 \mu\text{M}$ 5-hydroxytryptamine (5-HT) in the absence ($\bullet$, $n = 11$) or in the presence of inhibitors: $30 \mu\text{M}$ cocaine ($\blacktriangle$, $n = 7$), $100 \mu\text{M}$ corticosterone ($\triangle$, $n = 4$), $100 \mu\text{M}$ 3-O-methylisoprenaline (OMI, $\blacksquare$, $n = 4$) or $30 \mu\text{M}$ cocaine plus $100 \mu\text{M}$ OMI ($\blacktriangledown$, $n = 3$), or for hearts from rats that had been pretreated with 50 mg kg^{-1} 6-hydroxydopamine ($\square$, $n = 4$). Results are means of rates of 5-HIAA appearance from n hearts; vertical lines show s.e.

reduced by more than 95% (i.e. to levels that were not detectable) when the rats were pretreated with pargyline.

The rate of 5-HIAA appearance in the venous

Figure 2 Chromatograms of (a) a standard mixture of 1 pmol 5-hydroxyindoleacetic acid (5-HIAA) and 20 pmol 5-hydroxytryptamine (5-HT); (b) a $25 \mu\text{l}$ sample of venous effluent, collected over the 76th min of perfusion, from a heart perfused with $1 \mu\text{M}$ 5-HT; and (c) a $20 \mu\text{l}$ sample of supernatant from the homogenate of the heart in (b) at the end of the experiment.

effluent reached steady state after perfusion with $1 \mu\text{M}$ 5-HT for 65 min (Figure 1). Hence, steady-state rates of 5-HIAA appearance were determined from four samples taken between the 66th and 80th min of perfusion with 5-HT (Table 1).

Table 1 Effects of inhibitors and of 6-hydroxydopamine (6-OHDA) pretreatment on the steady-state rate of 5-hydroxyindoleacetic acid (5-HIAA) appearance in the venous effluent during perfusion of rat hearts with $1 \mu\text{M}$ 5-hydroxytryptamine (5-HT)

Inhibitor	Steady-state rate of 5-HIAA appearance in venous effluent ^a ($\text{pmol g}^{-1} \text{min}^{-1}$)	n	% inhibition ^b
No inhibitor	422 ± 8.48	12	—
6-OHDA pretreatment	$328 \pm 13.5^*$	4	22%
Cocaine $30 \mu\text{M}$	$305 \pm 11.2^*$	8	28%
OMI $100 \mu\text{M}$	$150 \pm 4.66^*$	4	64%
Corticosterone $100 \mu\text{M}$	$176 \pm 4.61^*$	4	58%
Cocaine $30 \mu\text{M}$ + OMI $100 \mu\text{M}$	$54 \pm 7.37^*$	3	87%

In all experiments, the rats were pretreated with reserpine. Inhibitors were present in the perfusion solution from the 6th min of the 15 min preliminary perfusion and throughout the 80.5 min perfusion with 5-HT (see Methods for details). Data are mean results from n hearts.

^a Steady-state rates of 5-HIAA appearance were calculated as the mean of rates determined at the 66th, 71st, 76th and 80th min of perfusion.

^b % inhibition was calculated as the difference between the steady-state rate of 5-HIAA appearance in the absence and presence of an inhibitor, expressed as a percentage of the rate with no inhibitor.

OMI = 3-O-methylisoprenaline.

* Significantly different from rate with no inhibitor, $P < 0.001$.

(B) *Effects of cocaine, 3-O-methylisoprenaline, corticosterone and pretreatment with 6-OHDA on metabolism of 5-HT*

In hearts perfused with $1 \mu\text{M}$ 5-HT, chemical sympathectomy, by pretreatment of rats with 6-OHDA, reduced the rates of 5-HIAA appearance in the venous effluent throughout the 80 min perfusion period (Figure 1). Cocaine ($30 \mu\text{M}$), which inhibits both neuronal uptake (Iversen, 1963) and uptake into vascular endothelial cells (Junod, 1972), also reduced the rate of 5-HIAA appearance in the venous effluent (Figure 1). The steady-state rate of 5-HIAA appearance (Table 1) in the presence of cocaine was not significantly different from that in hearts from 6-OHDA pretreated rats ($t = 1.23$, d.f. 10, $P > 0.05$).

The extraneuronal uptake inhibitors, OMI ($100 \mu\text{M}$) and corticosterone ($100 \mu\text{M}$), markedly reduced the rates of 5-HIAA appearance in the venous effluent throughout the 80 min perfusion period (Figure 1). Comparison of the steady-state rates of 5-HIAA appearance (Table 1) showed that the extraneuronal uptake inhibitors caused about 60% inhibition, and the inhibition by OMI was slightly, but significantly, greater than that by corticosterone ($t = 3.97$, d.f. 6, $0.01 > P > 0.001$). This difference between the effects of OMI and corticosterone was not due to an increase in efflux of 5-HIAA from the heart in the presence of corticosterone, since the rate constant for efflux of 5-HIAA from the heart was significantly lower in the presence of corticosterone (0.0476 min^{-1} , 95% confidence limits: 0.0399 and 0.0569 min^{-1} , $n = 4$) than in the presence of OMI (0.0775 min^{-1} , 95% confidence limits: 0.0696 and 0.0862 min^{-1} , $n = 4$).

Uptake into noradrenergic neurones, uptake into endothelial cells and extraneuronal uptake were inhibited by the inclusion of cocaine ($30 \mu\text{M}$) and OMI ($100 \mu\text{M}$) in the perfusion solution. This very markedly reduced the rate of 5-HIAA appearance (Figure 1), although there was still significant 5-HIAA appearance in the venous effluent at steady state (Table 1). The effect (Table 1) of the combined inhibitors (87% inhibition) was approximately equivalent to the additive effects of each inhibitor added separately to the perfusion solution (total of 92% inhibition).

(C) *Concentration-dependence of extraneuronal and neuronal deamination of 5-HT*

Hearts were perfused with 5-HT (1, 10, 30, 100 or $300 \mu\text{M}$) in the absence or presence of $100 \mu\text{M}$ OMI, and steady-state rates of 5-HIAA appearance in the venous effluent were determined (see Methods).

Steady-state rates of extraneuronal deamination of 5-HT were calculated as differences between the steady-state rates of 5-HIAA appearance in the absence and presence of OMI, and were corrected for incomplete inhibition of extraneuronal uptake by $100 \mu\text{M}$ OMI, as described by Bryan *et al.* (1984). The extraneuronal deamination of 5-HT in the hearts appeared to be saturable (Figure 3a), and obeyed Michaelis-Menten kinetics, in that a plot of s/v versus s was linear ($r = 0.993$, Figure 3b). The K_m value was $101 \mu\text{M}$ and V_{max} was $31.2 \text{ nmol g}^{-1} \text{ min}^{-1}$.

In another series of experiments, hearts were perfused with 5-HT ($1, 3, 10 \mu\text{M}$) in the absence or presence of $30 \mu\text{M}$ cocaine. Steady-state rates of neuronal deamination of 5-HT were calculated as the differences between the steady-state rates of 5-HIAA appearance in the absence and presence of cocaine. The neuronal deamination of 5-HT in the hearts appeared to be saturable (Figure 3c), but it was saturated by about 50 fold lower concentrations of 5-HT than was extraneuronal deamination (Figure 3a). It was not possible to carry out any further analysis of these data for neuronal deamination because of the large variability at the higher concentrations of 5-HT (Figure 3c). This variability was caused by the small contribution of neuronal deamination to the total deamination of 5-HT at these 5-HT concentrations. For example, when the concentration of 5-HT was $10 \mu\text{M}$, only 10% of the total deamination was inhibited by cocaine.

(D) *Activity of neuronal and extraneuronal monoamine oxidase*

The rate constant, k_{MAO} , characterizing the activity of MAO *in situ* in the heart, was calculated both for neuronal MAO (from data in section B with $1 \mu\text{M}$ 5-HT in the presence of $100 \mu\text{M}$ OMI) and for extraneuronal MAO (from data in section B with $1 \mu\text{M}$ 5-HT in the presence of $30 \mu\text{M}$ cocaine). The values of k_{MAO} were 0.188 min^{-1} (95% confidence limits: 0.108 and 0.325 min^{-1} , $n = 4$) for neuronal MAO and 0.222 min^{-1} (95% confidence limits: 0.117 and 0.420 min^{-1} , $n = 8$) for extraneuronal MAO. Hence, the activities of MAO for metabolism of 5-HT in neuronal and extraneuronal sites did not differ significantly. It should be noted that k_{MAO} for neuronal MAO may have been slightly underestimated, since a small degree of saturation of neuronal uptake of 5-HT would be evident at $1 \mu\text{M}$ 5-HT (see section C).

Discussion

Previous studies have shown that many cell types have an uptake process for 5-HT. After uptake, the 5-HT may be stored, e.g. in granules of platelets

Figure 3 Kinetic analysis of the extraneuronal (a and b) and neuronal (c) deamination of 5-hydroxytryptamine (5-HT) in the rat perfused heart. (a) and (c) Mean steady-state rates (v , in $\text{nmol g}^{-1} \text{min}^{-1}$) of (a) extraneuronal deamination or (c) neuronal deamination, calculated as described in Results, are plotted against 5-HT concentration (s , in μM) from data for 4 to 12 hearts; vertical lines show s.e. Note: The scales on the abscissal and ordinate axes are different for (a) and (c). (b) s/v is plotted against s from the data in (a) for extraneuronal deamination. The regression line shown was obtained by linear least squares regression analysis (Snedecor & Cochran, 1980).

(Stahl & Meltzer, 1978; Da Prada & Picotti, 1979) or in vesicles of either 5-hydroxytryptaminergic neurones (Aghajanian & Bloom, 1967) or noradrenergic neurones (Thoa *et al.*, 1969; Verbeuren *et al.*, 1983; McCulloch *et al.*, 1984; Paiva *et al.*, 1984). Alternatively, the 5-HT may be metabolized by MAO, e.g. in endothelial cells (Junod, 1972; Junod & Ody, 1977) or in smooth muscle cells (Paiva *et al.*, 1984). The present study has shown that, when 5-HT was perfused through the coronary circulation of the rat heart *in vitro*, 5-HT is dissipated by both extraneuronal uptake (inhibited by OMI or corticosterone) and by neuronal uptake into noradrenergic neurones (prevented by chemical sympathectomy with 6-OHDA). No evidence was obtained

for uptake into vascular endothelial cells in the heart. It should be noted that the rats were pretreated with reserpine, to prevent uptake into storage vesicles in the noradrenergic neurones, so that any 5-HT that entered these neurones would be exposed to intraneuronal MAO and detected as 5-HIAA after metabolism. When the hearts were perfused with $1 \mu\text{M}$ 5-HT, 13% of the metabolism of 5-HT was not inhibited by cocaine or OMI, and this probably represented a small amount of passive diffusion of 5-HT into MAO-containing cells in the heart. It has been shown previously that, at a concentration of $1 \mu\text{M}$, about 17% of the accumulation of isoprenaline in the rat heart occurred by passive diffusion (Bönisch, 1978).

Endothelial cells in the pulmonary circulation of perfused lung preparations have been shown to take up and metabolize 5-HT (Junod, 1972; Strum & Junod, 1972; Iwasawa *et al.*, 1973). In the systemic circulation, endothelial cells isolated from the aorta (Shepro *et al.*, 1975; Junod & Ody, 1977) and from capillaries of adipose tissue (Robinson-White *et al.*, 1981) have also been shown to take up and metabolize 5-HT. In the coronary circulation of the perfused heart of the rat, indirect evidence suggested that endothelial cells did not contribute to the dissipation of 5-HT. This evidence was that there was no significant difference between (i) the rates of metabolism of 5-HT in hearts from rats pretreated with 6-OHDA (to prevent neuronal uptake by destroying the noradrenergic neurones) and (ii) the rates in hearts perfused with solution containing cocaine, an inhibitor of both neuronal uptake (Iversen, 1963) and of the uptake of 5-HT into endothelial cells in perfused lungs (Junod, 1972). Thus, in contrast with the endothelial cells of perfused lungs, where there is avid cocaine-sensitive uptake and metabolism of 5-HT, there did not appear to be cocaine-sensitive uptake of 5-HT into vascular endothelial cells of the perfused heart of the rat. Cocaine was used in the experiments on rat hearts, instead of imipramine, an inhibitor that has been used to characterize uptake in both pulmonary endothelial cells (Junod, 1972) and endothelial cells from systemic blood vessels (Shepro *et al.*, 1975; Junod & Ody, 1977; Robinson-White *et al.*, 1981). This was because imipramine, in the concentrations required to inhibit neuronal uptake and uptake into endothelial cells ($5\mu\text{M}$), caused heart block in the rat hearts, as has been found previously by Callingham (1967).

The neuronal uptake and subsequent metabolism of 5-HT in the hearts of reserpine-pretreated rats is compatible with reports of 5-HT uptake into noradrenergic neurones in rat atria (McCulloch *et al.*, 1984), in blood vessels including dog saphenous vein (Verbeuren *et al.*, 1983; Paiva *et al.*, 1984) and dog cerebral arteries (Verbeuren *et al.*, 1983), and in guinea-pig vas deferens (Thoa *et al.*, 1969). It was not possible to determine values for the K_m and V_{max} of 5-HT for neuronal uptake in the heart (see Results, section C), but it was clear that neuronal uptake of 5-HT was saturated by 5-HT concentrations about 50 fold lower than those required to saturate extraneuronal uptake ($K_m = 101\mu\text{M}$). Hence, the K_m of 5-HT for neuronal uptake in the rat heart appears to be of the same order of magnitude as that for 5-HT uptake into endothelial cells of perfused lungs *in vitro* (rat, $5.9\mu\text{M}$; Junod, 1972) and *in vivo* (rabbit, $1.1\mu\text{M}$; Catravas & Gillis, 1983), and in cultured endothelial cells of the bovine pulmonary artery ($1\mu\text{M}$; Lee & Fanburg, 1986).

There are data on the extraneuronal uptake of

5-HT in various blood vessels, including those in the rat heart (Grohmann & Trendelenburg, 1984; present study), rat arteries (Fukuda *et al.*, 1986a,b), rabbit ear artery (Buchan *et al.*, 1974) and dog saphenous vein (Paiva *et al.*, 1984). In most of these studies, inhibition of 5-HT uptake by drugs that inhibit extraneuronal uptake of catecholamines has been used as evidence that the same uptake process transported both 5-HT and catecholamines. In an attempt to obtain direct evidence as to whether there was competition between 5-HT and isoprenaline for the same extraneuronal uptake sites, the effect of isoprenaline on 5-HT metabolism in the rat heart was examined. Unfortunately, isoprenaline interfered with the assay of 5-HIAA by h.p.l.c. However, the alternative experiment, showing that 5-HT inhibited extraneuronal uptake of isoprenaline (Grohmann & Trendelenburg, 1984), has demonstrated that these two amines enter extraneuronal sites in the rat heart by the same uptake process.

In the rat heart, the K_m and V_{max} values obtained for the extraneuronal deamination of 5-HT ($101\mu\text{M}$ and $31.2\text{nmol g}^{-1}\text{min}^{-1}$, respectively) were very close to the corresponding values for extraneuronal uptake of 5-HT ($181\mu\text{M}$ and $42.8\text{nmol g}^{-1}\text{min}^{-1}$, respectively; Grohmann & Trendelenburg, 1984). The similarity of these values suggested that the rate-determining step in the deamination of 5-HT in the heart was the uptake of 5-HT, and not MAO. The similarity also suggested that saturation of deamination of 5-HT reflected saturation of uptake of the amine, and not saturation of extraneuronal MAO. This relationship between kinetic constants has previously been shown for the extraneuronal uptake and deamination of noradrenaline in the rat heart (Fiebig & Trendelenburg, 1978) and also for the neuronal uptake and deamination of noradrenaline (Trendelenburg *et al.*, 1987).

The K_m of 5-HT for extraneuronal uptake in the rat heart is of the same order of magnitude as that of (–)-adrenaline and (–)-noradrenaline, but greater than that of (–)-isoprenaline (Grohmann & Trendelenburg, 1984). The only other reported K_m value of 5-HT for extraneuronal uptake is $391\mu\text{M}$ in the dog saphenous vein (Paiva *et al.*, 1984). The higher K_m of 5-HT for extraneuronal uptake in the dog saphenous vein compared with that in the rat heart may reflect a tissue or species difference, or may be due to the tendency for corresponding K_m values determined in incubated tissues to be higher than those determined in perfused organs (Grohmann & Trendelenburg, 1984).

In the rat heart perfused with $1\mu\text{M}$ 5-HT, extraneuronal deamination (64% of the total) exceeded neuronal deamination (22–28% of the total). Differences in uptake, and not differences in MAO activity, appeared to account for this, in that the activities of

neuronal and extraneuronal MAO in the rat heart were very similar (as shown by the calculated values of k_{MAO} ; see Results, section D). The relative amounts of neuronal and extraneuronal dissipation of 5-HT in different blood vessels, e.g. rat aorta, mesenteric artery and tail artery, has been shown to differ according to the density of noradrenergic innervation (Fukuda *et al.*, 1986b). In the dog saphenous vein, extraneuronal dissipation of 5-HT appeared to represent at least 30% of the total uptake and metabolism of 5-HT (Verbeuren *et al.*, 1983; Paiva *et al.*, 1984).

Since dissipation of 5-HT in the coronary circulation of the rat heart occurred via uptake processes for catecholamines, it is of interest to compare the results for 5-HT with those obtained previously for catecholamines. Steady-state rates of extraneuronal deamination (at non-saturating amine concentrations) were greater than steady-state rates of neuronal deamination for 5-HT (present study) and (–)-adrenaline (Grohmann, 1987), but the reverse was found for (–)-noradrenaline and dopamine (Grohmann, 1987). Hence, 5-HT and the catecholamine, (–)-adrenaline, appeared to be dissipated in the rat heart by the same processes, with the exception that extraneuronal adrenaline is metabolized by catechol-O-methyltransferase, as well as by MAO (Grohmann, 1987). Other comparisons that could be made between catecholamines (Grohmann, 1987) and 5-HT in the rat heart were the activities of neuronal and extraneuronal MAO. The rank order of k_{MAO} values for neuronal deamination was (–)-noradrenaline > dopamine > 5-HT > (–)-adrenaline, but that for extraneuronal deamination was dopamine > 5-HT > (–)-noradrenaline > (–)-

adrenaline. Hence, the relative activities of MAO for these amines differed in neuronal and extraneuronal sites, particularly for (–)-noradrenaline.

In summary, the study has shown that, in the coronary circulation of the rat heart, 5-HT was dissipated by the uptake processes for catecholamines, extraneuronal uptake and neuronal uptake. The contribution of extraneuronal uptake was greater than that of neuronal uptake, even at the low concentrations of 5-HT that have been measured free in human plasma under normal conditions (0.03–0.07 μ M; Genefke *et al.*, 1968; Frattini *et al.*, 1979). It is not possible to predict the local 5-HT concentrations that would occur if 5-HT were released in pathological conditions, e.g. Prinzmetal's angina, but the present results suggested that the dissipation of 5-HT in the coronary circulation would occur via extraneuronal uptake and neuronal uptake. There was no evidence that cocaine-sensitive uptake and metabolism of 5-HT in vascular endothelial cells made any significant contribution to 5-HT dissipation in the heart. Hence, to ensure that the pathological effects of any released 5-HT on vascular function in the coronary circulation would be terminated as quickly as possible, it would be important that drugs that inhibit the neuronal or extraneuronal uptake processes should not be present in the circulation.

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