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Effects of monoamine uptake inhibitors on extracellular and platelet 5-hydroxytryptamine in rat blood: different effects of clomipramine and fluoxetine

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1 The concentration of 5-hydroxytryptamine (5-HT) in rat platelet-free plasma increased significantly 30 min after a single i.p. injection (10 mg kg^{-1}) of each of six inhibitors of the high-affinity 5-HT uptake (fluvoxamine, fluoxetine, alaproclate, paroxetine, sertraline and clomipramine). The increases ranged from 226% to 776% of control values. In contrast, imipramine, desipramine and femoxetine had no significant effect. The increase elicited by paroxetine was dependent on the dose (1, 5 and 10 mg kg^{-1}) and returned to control values after 4 h. That observed after clomipramine was also transient and paralleled the plasma concentration of the drug (Spearman-rank correlation $r = 0.43$).

2 *In vivo*, the rat pulmonary vascular endothelium removed trace amounts (8.8 nmol in a bolus) of intravenously injected [^{14}C]-5-HT. Paroxetine pretreatment (10 mg kg^{-1}, 30 min before-hand) reduced this uptake by 73%.

3 Repeated fluoxetine treatments reduced rat whole blood 5-HT concentration (ca. – 60% after daily 2 × 5 mg kg^{-1}, i.p. during 14 days). However, plasma (extracellular) 5-HT was not increased.

4 Various repeated treatments with clomipramine (i.p. injections or osmotic minipumps, up to 30 mg kg^{-1} day^{-1}), failed to decrease rat whole blood 5-HT concentrations. Platelet-free plasma 5-HT was also unchanged, even after treatments yielding plasma clomipramine levels 2.7 times higher than those that increased it acutely.

5 These results indicate that the extracellular pool of 5-HT in rat blood (measured in the platelet-free plasma) is physiologically under the control of high-affinity 5-HT uptake systems. The sustained 5-HT uptake inhibition does not result in an increase of 5-HT in platelet-free plasma, suggesting that adaptative mechanisms are triggered. The distinct long-term effects of the two antidepressants clomipramine and fluoxetine on rat whole blood 5-HT suggest a differential *in vivo* action on the rat 5-HT uptake.

Keywords: 5-Hydroxytryptamine uptake; antidepressant drugs; uptake inhibitors; endothelial uptake; clomipramine; fluoxetine; imipramine; paroxetine; platelets; plasma

Introduction

Central nervous system (CNS) 5-hydroxytryptaminergic neurotransmission is of key importance for the clinical effects of antidepressant drugs (Shopsin *et al.*, 1975; 1976; Delgado *et al.*, 1990). However, the study of the actions of antidepressants in human CNS is complicated by the fact that only indirect measures of neurotransmitter function can be used, which are scarce and difficult to interpret (see Murphy *et al.*, 1990 for review). Platelets have been used extensively because of their similarities with 5-hydroxytryptamine (5-HT) nerve terminals, particularly for the presence of amine storage granules and a high-affinity transporter for 5-HT (Stahl, 1985). However, platelets lack noticeable synthesis and metabolism of 5-HT, so that they provide only a restrictive view of the 5-HT system. In recent years, several laboratories have studied the effects of certain pathologies and pharmacological treatments on the extracellular 5-HT in the blood of psychiatric patients (Sarrias *et al.*, 1987; Cook *et al.*, 1988; Artigas *et al.*, 1989; Rupprecht *et al.*, 1989; Celada *et al.*, 1990). This variable is sensitive to rapid and sustained changes of 5-HT synthesis and metabolism (Ortiz *et al.*, 1988; Celada *et al.*, 1990) and therefore may overcome some of the limitations of the measure of 5-HT in platelets. Extracellular 5-HT concentrations in venous human blood (measured in platelet-free plasma) account for less than 1% of total blood 5-HT, the rest being stored in platelets (Ortiz *et al.*, 1988). The low concentration (5–15 nM) of extracellular 5-HT is a consequence of potent mechanisms removing 5-HT from the bloodstream (high-affinity uptake into platelets and endothelial cells and monoamine oxidase (MAO) deamination) after its release from enterochromaffin cells (Verbeuren, 1989). In different areas of rat brain, extracellular and tissue 5-HT maintain a similar proportion due to 5-HT reuptake (Adell *et al.*, 1991). Extracellular 5-HT in rat brain increases several fold after treatment with different uptake inhibitors (Kalén *et al.*, 1988; Auerbach *et al.*, 1989; Carboni & Di Chiara, 1989; Adell & Artigas, 1991) confirming *in vivo* that uptake is a basic mechanism of the control of extracellular- and therefore, receptor-available-5-HT. Since the 5-HT uptake systems also control the equilibrium between extra- and intracellular 5-HT in blood, the effects of 5-HT uptake inhibitors on extracellular 5-HT can also be studied *in vivo* outside the CNS. The aim of the present work was to study the effects of several high-affinity 5-HT uptake inhibitors, the working hypothesis being that they should increase the extracellular concentration of 5-HT in rat blood (measured in platelet-free plasma). Also, platelet 5-HT concentration should decrease only after sustained uptake inhibition. The hypothesis was confirmed after acute treatment, repeated treatment yielding unexpected results that are discussed.

Methods

Animal treatment

Male Wistar rats of 250–300 g (Iffa-Credo, Lyon, France) were kept in a controlled environment (22°C) with a light/

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dark periodicity of 12 h, at least for one week before the experiments. They were housed four per cage with water and food *ad libitum*.

Acute administration of uptake inhibitors was carried out i.p. to groups of 7–12 animals randomly chosen. In each experiment, a control group was injected with saline and processed along with the treated groups. Usually the acute effects of 2 or 3 different treatments were examined during the same experiment.

For the repeated administration of drugs, Alzet mini-osmotic pumps (Model 2002, Palo Alto, U.S.A.) were implanted subcutaneously under light ether anaesthesia. Clomipramine was dissolved in 2% Tween and fluoxetine in 50% dimethyl sulphoxide (DMSO) due to the low volume of mini-pumps. Control rats were implanted with pumps filled with the corresponding vehicles. Interindividual variance of weights was corrected by the method of Greenshaw (1986). Since the animals increased their weights during the treatment (two weeks), the drug doses reported correspond to those expected on the 7th day. Animals with implanted minipumps were housed individually to avoid reciprocal gnawing of sutures observed after some treatments.

Blood sampling

Blood sampling was routinely carried out between 16 h 00 min and 19 h 00 min. Under pentobarbitone anaesthesia (70 mg kg^{-1}, i.p. Sagatal, RMB, U.K.), the blood was drawn from the carotid artery through a polythene cannula (Portex, U.K.) cut bevelled (0.58 mm i.d., 4–15 cm long) into three Eppendorf tubes. The cannula and the sampling tubes contained K_2-EDTA calculated to give a final concentration of 0.5–1% (w/v) EDTA in blood. The use of this method of blood sampling is due to the necessity of avoiding 5-HT release from platelets that would occur after more traumatic methods. The blood of the first tube (0.8 ml) was used for the determination of whole blood 5-HT content. The second and third tubes (1.5 ml of blood each) were centrifuged (12,000 *g*, 5 min, 22°C). The resulting supernatant (platelet-free plasma) was carefully removed and frozen at − 80°C until analysed. Extracellular 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined in platelet-free plasma of the second tube. With this method, mean values of 5-HT in the platelet-free plasma of rats are usually in the low nM range (Ortiz *et al.*, 1991a,b).

High performance liquid chromatography analysis

The 5-HT concentration in platelet-free plasma was determined by high performance liquid chromatography (h.p.l.c.) coupled to electrochemical detection after a butanol/heptane/HCl extraction procedure (Artigas *et al.*, 1985). Whole blood 5-HT analysis was carried out as described in Ortiz *et al.* (1988). In both cases, bufotenine (N,N-dimethylserotonin) was used as internal standard. Plasma 5-HIAA determination was carried out as described for human blood (Martínez *et al.*, 1983). Minor modifications of these methods were introduced in order to use smaller volumes of sample. Plasma total tryptophan (TRP) was also analysed by h.p.l.c. and fluorescence detection (280/340 nm). Plasma clomipramine (CIM) levels were monitored by the method of Langerström *et al.* (1983), that involves a diethyl ether/HCl extraction from plasma at pH 3.5 and h.p.l.c. – u.v. determination with desipramine as internal standard.

Effect of paroxetine on the endothelial [^{14}C]-5-HT uptake

The pulmonary removal of [^{14}C]-5-HT from blood was measured *in vivo* by a modification of the method described by Catravas & Gillis (1980). Rats (350–450 g) were pretreated with saline or paroxetine (10 mg kg^{-1}, i.p., 30 min before, $n = 4$ rats/group) and anaesthetized with pentobarbitone (70 mg kg^{-1}, i.p.). Then, a bolus of 500 nCi of [^{14}C]-5-HT (8.8 nmol) (Amersham) was injected into the jugular vein. Blood was being collected simultaneously at 1-s intervals through a cannula placed in the carotid artery into tubes containing K_2-EDTA (120–150 µl of blood/tube). Blood (50 µl) from each tube was processed as for whole blood 5-HT determination and then subjected to liquid scintillation counting instead of h.p.l.c. ^{14}C d.p.m. ml^{-1} of blood was calculated in each 1 s sample, by use of a [^{14}C]-5-HT standard addition curve that was processed in parallel. To another set of rats ($n = 3$), 1 mg indocyanine green (cardiogreen) was injected as reference for the calculation of [^{14}C]-5-HT uptake. This compound does not leave the cirulation in a single passage through the lungs. Its concentration was measured by optic densitometry at 675 nm in plasma, in parallel to a standard addition curve of colorant. The area under the curve (d.p.m. or optic density of colorant) was used to calculate [^{14}C]-5-HT uptake with the formula:

$$\%\ 5\text{-HT uptake} = \left[1 - \frac{(\text{d.p.m. ml}^{-1})/\text{d.p.m. injected}}{(\text{mg ml}^{-1})/\text{mg injected}}\right] \times 100$$

^{14}C uptake represents the percentage of the total amine injected that was removed by endothelial 5-HT uptake during a single passage through the lungs (Catravas & Gillis, 1980).

Drugs

The following drugs were kindly provided: clomipramine, imipramine and desipramine (Ciba-Geigy), paroxetine (Beecham), fluoxetine (Eli, Lilly & Co.), fluvoxamine (Duphar), sertraline (Pfizer), alaproclate (Astra) and femoxetine (Ferrosan). The doses used correspond to the free compound. H.p.l.c. standards were from Sigma and the rest of the products were of the highest purity commercially available.

Statistics

The statistical significance of the changes induced by uptake inhibitors was assessed by one-way ANOVA followed by Student-Neuman-Keuls comparisons between the drug and saline groups. The effects of treatments on plasma 5-HT were evaluated by use of non-parametric statistics (Kruskal-Wallis test followed by Mann-Whitney U-test) because of the skewed statistical distribution of the variable in control rats (Ortiz *et al.*, 1991a). Accordingly, plasma 5-HT is represented in the figures by its median. Statistical significance was set at $P < 0.05$.

Results

Acute effects

Increase of plasma 5-HT by selective high-affinity 5-HT uptake inhibitors A single dose of the following drugs (10 mg kg^{-1}, i.p., 30 min before blood sampling) increased rat plasma 5-HT ($P < 0.05$) vs saline-treated rats: fluvoxamine, fluoxetine, alaproclate, paroxetine, sertraline and clomipramine (Table 1). In contrast, the increases induced by imipramine, desipramine and femoxetine did not reach statistical significance. Whole blood 5-HT (>99% in platelets; Ortiz *et al.*, 1988), was not modified in a consistent manner although slight decreases were observed after fluvoxamine, fluoxetine and imipramine.

Plasma 5-HIAA increased significantly after the acute administration of paroxetine (+110%), fluoxetine (+90%) and sertraline (+49%) ($P < 0.05$). More moderate changes were elicited by alaproclate (+32%) and imipramine (−16%). Plasma tryptophan was unchanged by these treatments.

Table 1 Increase of plasma 5-hydroxytryptamine (5-HT) produced by acute 5-HT uptake inhibitors

Drug	*Controls* median interquartile range (n)	*Treated* median interquartile range (n)	*% of controls*
Fluvoxamine	7.6 3.9–13.2 (10)	59 19.4–104 (11)	776%*
Fluoxetine	7.6 3.9–13.2 (10)	39.6 22–63 (11)	520%*
Alaproclate	8.1 5.4–28.8 (10)	37.5 18.8–61.9 (10)	465%*
Paroxetine	14.1 7.7–24.8 (10)	50.6 38.6–77.2 (11)	358%*
Sertraline	8.1 5.4–28.8 (10)	27 15.6–86 (11)	335%*
Desipramine	8.1 5.4–28.8 (10)	20.7 7.1–139 (12)	257%
Clomipramine	6.8 3.7–11.8 (9)	15.5 9.5–43 (8)	226%*
Femoxetine	8.1 5.4–28.8 (10)	15.1 10.5–131 (11)	187%
Imipramine	7.6 3.9–13.2 (10)	12.4 7.6–116 (11)	185%

Plasma 5-HT is given in nmol l^{-1}. *$P<0.05$ versus respective saline-treated animals, Kruskal-Wallis and Mann-Whitney tests. The data shown correspond to four different experiments in which median control values were not different (Kruskal-Wallis test).

Drug-effect relationships Different doses of paroxetine were administered to randomized groups of animals (0, 1, 5 or 10 mg kg^{-1}, i.p., 30 min before), eliciting dose-dependent increases in plasma 5-HT and 5-HIAA as shown in Figure 1. Whole blood 5-HT was not modified by such treatments.

The kinetics of the increase of extracellular 5-HT were studied after the administration of clomipramine and paroxetine. Groups of animals treated with a single dose of either drug (10 mg kg^{-1}, i.p.) were killed at different times post-administration: 15 min (only for paroxetine), 30 min, 1 h, 2 h and 4 h. With both drugs, plasma 5-HT rose maximaly at 30 min, returning to control values after 4 h. The increase of plasma 5-HT elicited by clomipramine (CIM) clearly paralleled the plasma concentration of the drug (Figure 2). In addition, the individual values of 5-HT and CIM in plasma correlated significantly (Spearman rank correlation $r = 0.43$, $n = 41$, $P<0.05$).

Effects of paroxetine pretreatment on the endothelial [^{14}C]-5-HT uptake The pretreatment with paroxetine (10 mg kg^{-1}, i.p., 30 min before) elevated the amount of [^{14}C]-5-HT left in blood after a single passage of the lungs. This is shown by the difference in the area under the curve represented in Figure 3. Saline-pretreated rats removed 47 ± 22% of the [^{14}C]-5-HT bolus, while the paroxetine group removed 13 ± 27% (means ± s.d. of $n = 4$ rats per group, $P<0.05$ Mann-Whitney test). Thus, paroxetine elicited a 73% reduction in the endothelial 5-HT removal.

Effects of sustained uptake inhibition

Effects of repeated clomipramine In a first experiment, repeated intraperitoneal administrations of CIM (15 mg kg^{-1} daily for 2, 4, 7, 14 or 21 days, killing 24 h after the last injection) did not modify the concentrations of total tryptophan, 5-HT and 5-HIAA in plasma nor 5-HT in whole blood (data not shown). The lack of effect on whole blood 5-HT was surprising, since lower doses decrease by more than 90% platelet 5-HT in depressive patients (Sarrias *et al.*, 1987). Given the short half-life of CIM in rat plasma after repeated administration (Friedman & Cooper, 1983), it is

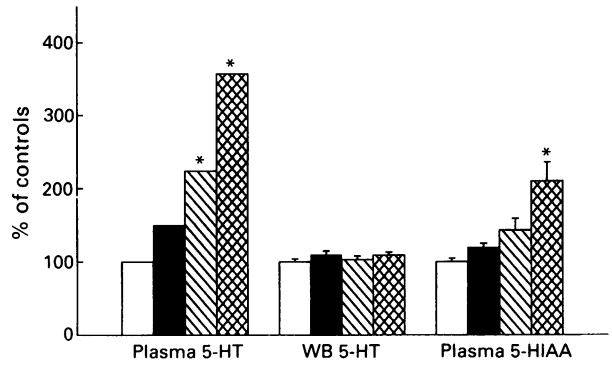

Figure 1 Dose-related increase of extracellular 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in plasma elicited by the selective high-affinity 5-HT uptake inhibitor paroxetine. Saline (open column) or paroxetine 1 mg kg^{-1} (solid column), 5 mg kg^{-1} (hatched column) or 10 mg kg^{-1} (cross-hatched column) were administered i.p. 30 min before blood sampling. Values are represented as medians (plasma 5-HT) or means ± s.e.mean (vertical lines) of 10 to 12 rats per group. Control values were: plasma 5-HT, 14.1 nM; whole blood (WB) 5-HT, 8.23 μM; plasma 5-HIAA, 81 nM. $P<0.05$ versus saline-treated animals, Kruskal-Wallis and Mann-Whitney tests (plasma 5-HT) or one-way ANOVA and Student-Newman-Keuls (plasma 5-HIAA).

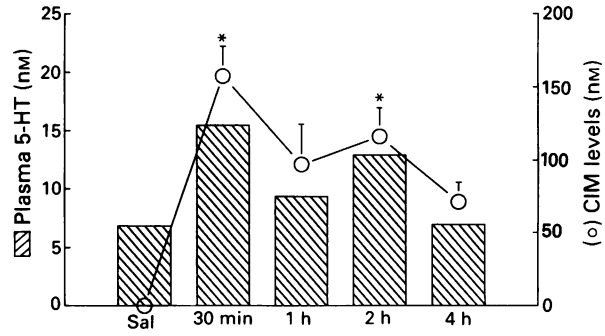

Figure 2 Time- and drug level-dependent increase of extracellular 5-hydroxytryptamine (5-HT) in plasma elicited by i.p. administration of 10 mg kg^{-1} of the high-affinity 5-HT uptake inhibitor clomipramine (CIM). Columns represent plasma 5-HT medians and (○) are means ± s.e.mean (vertical lines) of plasma CIM (7–10 rats per group). $P<0.05$ versus saline-treated animals, Kruskal-Wallis and Mann-Whitney tests. Plasma 5-HT and CIM levels correlated significantly (Spearman-rank correlation $r = 0.43$, $n = 41$, $P<0.05$).

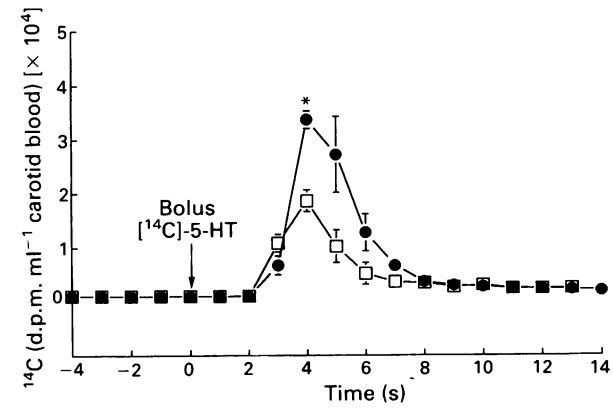

Figure 3 Effect of paroxetine pretreatment on the *in vivo* pulmonary vascular endothelial removal of [^{14}C]-5-hydroxytryptamine ([^{14}C]-5-HT), 30 min before the rats were pretreated with saline (□) or paroxetine 10 mg kg^{-1} (●). At time zero, 8.8 nmol of radiolabelled 5-HT was injected into the jugular vein while blood was being sampled each second through the carotid artery. The ordinate scale (d.p.m.) represents the radiolabelled 5-HT that escaped the endothelial removal of the amine. The data are means of four different experiments per group; s.e.mean shown by vertical lines. ($P<0.05$ two-tailed Student's t test.

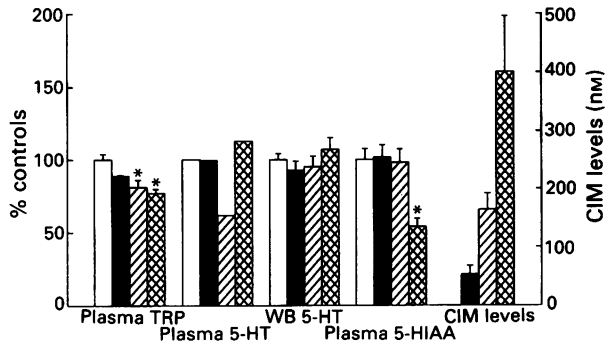

Figure 4 Dose-related effects of chronic clomipramine administration by osmotic mini-pumps. Treatments used were vehicle (open column), clomipramine 15 mg kg^{-1} day^{-1} for 7 days (solid column), 15 mg kg^{-1} day^{-1} for 14 days (hatched column) or 30 mg kg^{-1} day^{-1} for 14 days (cross-hatched column). No change was observed in plasma and whole blood 5-hydroxytryptamine (5-HT) even at the highest drug levels. Values are expressed as medians (plasma 5-HT) or means ± s.e.mean (vertical lines) of 7 to 19 rats per group. $P<0.05$ versus controls, one-way ANOVA and Student-Newman-Keuls comparisons. Control group pools data from two experiments with slightly different values: plasma tryptophan (TRP), 82 μM and 74 μM; plasma 5-HT, 13.5 nM and 12.2 nM; whole blood (WB) 5-HT, 7.2 μM and 8.6 μM ($P<0.05$ two-tailed Student's t test) and plasma 5-hydroxyindoleacetic acid (5-HIAA), 164 nM and 104 nM ($P<0.05$ two-tailed Student's t test). The statistical significances did not vary when each control group was considered alone.

likely that steady-state levels were not achieved. Therefore, we used a more sustained administration, by means of subcutaneously implanted mini-osmotic pumps. The treatments used were: 15 mg kg^{-1} daily for 7 or 14 days, and 30 mg kg^{-1} daily for 14 days, with no wash-out period at the end of the administration. Although the drug plasma levels obtained were within the therapeutic concentrations found in depressed patients (mean values: 54, 164 and 401 nM CIM in plasma, respectively), plasma and whole blood 5-HT were not modified. Plasma total tryptophan decreased moderately both in the 15 mg kg^{-1} day^{-1}, 14 days (−19%) and in the 30 mg kg^{-1} day^{-1}, groups (−23%, $P<0.05$). Plasma 5-HIAA concentration decreased only in the 30 mg kg^{-1} day^{-1} group (−46%, $P<0.05$) (Figure 4). Regression analysis showed a positive correlation between plasma TRP and 5-HIAA (Pearson's correlation $r=0.52$, $P<0.05$, $n=34$), and a dependence of both variables on CIM levels ($r=-0.47$ and -0.42 respectively, $P<0.05$). Multiple regression analysis showed that the correlation between 5-HIAA and CIM was due to that between tryptophan and CIM. Body weight gain during the treatments was lower in the clomipramine groups than in controls (data not shown).

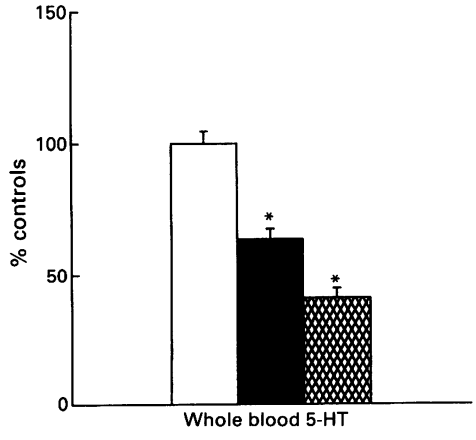

Figure 5 Dose-related effects of chronic fluoxetine administration. Treatments used were vehicle (open column), fluoxetine 5 mg kg^{-1} day^{-1} for 14 days using mini-osmotic pumps, no washout (solid column) or 2 × 5 mg kg^{-1} day^{-1} i.p. for 14 days, 48 h wash-out (cross-hatched column). *$P<0.05$ versus controls, one-way ANOVA and Student-Newman-Keuls. Respective mean control values were 8.6 μM and 9.4 μM (not significantly different, two-tailed Student's t test).

Effects of repeated fluoxetine Repeated administration of fluoxetine was carried out in two experiments, using either mini-osmotic pumps or i.p. injections. Subcutaneously implanted pumps (5 mg fluoxetine kg^{-1} day^{-1} for 14 days, no wash-out) decreased whole blood 5-HT (−36% vs. vehicle-treated rats, $P<0.05$ two-tailed Student's t test) (Figure 5). Plasma 5-HT was unaltered (median control values: 12.2 nM; fluoxetine: 11.6 nM). Neither plasma 5-HIAA nor plasma total tryptophan concentration were significantly changed. Intraperitoneal injections (5 mg kg^{-1}, twice daily for 14 days, followed by 48 h wash-out) decreased whole blood 5-HT (−59% vs. saline-treated controls, $P<0.05$ Student's t test).

Plasma 5-HIAA was not significantly changed by fluoxetine. Control values were significantly different between both experiments: Lower values were observed in the rats injected i.p. daily (80.4 ± 16.8 nM) than in those with an implanted pump (104.1 ± 13.7 nM; means ± s.d., $P<0.05$ two-tailed Student's t test). This difference may result from the stress of handling non-habituated animals (pump group) at the time of the experiment. This can moderately increase plasma 5-HIAA probably through activation of 5-hydroxytryptaminergic function (unpublished observations).

Discussion

In recent years it has been shown that an extracellular pool of 5-HT occurs in low nM concentrations in human blood, in addition to the well-known platelet pool (Artigas *et al.*, 1985; Anderson *et al.*, 1987; Ortiz *et al.*, 1988). When appropriate experimental conditions are used, the 5-HT found in platelet-free plasma is a good estimate of the extracellular pool. Independent changes of the extracellular and platelet pools in man have been reported in pathological conditions and after some pharmacological treatments known to affect the 5-HT system (Sarrias *et al.*, 1987; Cook *et al.*, 1989; Artigas *et al.*, 1989). In rats, little is known about this extracellular pool of 5-HT. Results from this laboratory show that its concentration is similar to that in man, in spite of the fact that rats contain about ten times more 5-HT than man in the platelet pool per unit of blood volume (Ortiz *et al.*, 1988; 1991a,b).

The present results indicate that in the rat, the plasma pool of 5-HT (but not the platelet one) is markedly increased after short-term uptake inhibition. This is consistent with the occurrence of high-affinity 5-HT uptake present in the platelets and vascular endothelial cells and perhaps in enterochromaffin cells and their physiological role in removing extracellular 5-HT (Verbeuren, 1989). The increase of plasma 5-HT was dependent on the dose of the uptake inhibitor (e.g. paroxetine). Also, it followed the evolution of drug plasma levels (clomipramine), which suggests that the turnover of extracellular 5-HT in blood is very rapid.

More marked plasma 5-HT increases were obtained with the new antidepressants which are selective 5-HT uptake inhibitors rather than with the tricyclic drugs. Also, the three drugs having the greatest effect (fluvoxamine, fluoxetine and alaproclate) displayed the same order of potencies as in the synaptosomal [^{3}H]-5-HT uptake inhibition (Wolf & Kuhn, 1991). This indicates that the increase of plasma 5-HT is representative of the actual effects of these drugs on the 5-HT transporter *in vivo*. In addition, unlike *in vitro* procedures (sometimes performed far from physiological conditions, such as the binding of [^{3}H]-imipramine to platelets at 0°C), the increase of plasma 5-HT takes into account other factors that may influence the interaction of the drug with its phar-

macological target (bioavailablity, metabolism and excretion, interaction with other mechanisms of control, etc).

Besides platelets, endothelial cells have a 5-HT transporter sensitive to imipramine (Catravas & Gillis, 1980; Bosin & Lahr, 1981; Robinson-White *et al.*, 1981; Lee & Fanburg, 1986). To examine the contribution of endothelial uptake to the increases of extracellular 5-HT in rats, we tested *in vivo* the effects of a single dose of paroxetine. Saline-treated animals removed almost 50% of the injected [^{14}C]-5-HT (8.8 nmol) in a single passage through the lungs. Paroxetine elicited an important reduction (− 73%) of the removal of this physiological amount of 5-HT (as [^{14}C]-5-HT) by the lungs, in parallel with a marked increase of plasma 5-HT (Table 1). These figures support the view that the endothelial uptake is a very important factor for the control of extracellular 5-HT in blood *in vivo*, confirming earlier data obtained with rat isolated lungs (Steinberg & Das, 1980). Also, the present results prove that the rat endothelial removal of 5-HT is sensitive to specific 5-HT uptake inhibitors, such as paroxetine.

Some of the specific 5-HT uptake inhibitors acutely increased plasma 5-HIAA concentration. This is formed by MAO-A in the liver and, after high-affinity 5-HT uptake, in the vascular endothelium (Verbeuren, 1989). The effects on the plasma 5-HIAA concentration probably derive from a major availability of extracellular 5-HT to the liver, since 5-HT can enter the hepatocytes from portal blood without specific uptake (Wiersma & Roth, 1980). However, the lack of a significant effect of fluvoxamine, which increased plasma 5-HT maximally, suggests that other factors may be differentially affected by the several drugs used (e.g. effects on the release of 5-HIAA by enterochromaffin cells, renal clearance, etc).

The next step was to examine the effects of repeated administration of 5-HT uptake inhibitors. Clomipramine and fluoxetine were chosen for their wide use in psychiatric practice for the treatment of depression, obsessive-compulsive and panic disorders. Two different repeated treatments with fluoxetine decreased whole blood 5-HT, indicating that the platelet high-affinity 5-HT uptake mechanism was effectively inhibited. Despite the 48 h wash-out used in the i.p. treatment, platelet 5-HT was markedly reduced. The slow metabolism of fluoxetine (Caccia *et al.*, 1990) probably accounts for this. However, plasma 5-HT was not increased as in the acute effects. This indicates the presence of adaptive mechanisms leading to a decrease of the circulating extracellular 5-HT. Repeated fluoxetine treatment does not increase extracellular 5-HT in the frontal cortex of rats (Sarkissian *et al.*, 1990), perhaps due to a reduction of brain 5-HT turnover (Fuller *et al.*, 1974). In the periphery, a decreased synthesis by or release from enterochromaffin cells could explain the reduction of 5-HT in the bloodstream after repeated fluoxetine treatment.

Long-term treatment with clomipramine failed to decrease rat whole blood 5-HT, despite achieving a stable plasma CIM concentration (up to 400 nM). In depressive patients, a two-week treatment with CIM (mean plasma levels: 205 nM) elicited a 90% reduction of platelet 5-HT (Sarrias *et al.*, 1987). Clomipramine, unlike other tricyclic antidepressants, has very potent and long-lasting effects on the [^{3}H]-imipramine binding and [^{3}H]-5-HT uptake in human platelets (Mellerup & Plenge, 1986; Poirier *et al.*, 1984; 1987). However, such long-lasting effects may not occur in rats. Acute CIM increased plasma 5-HT, but this change paralleled closely CIM levels in plasma. Therefore, interspecies differences in the interaction of CIM with the platelet 5-HT uptake transporter *in vivo* seem likely. Errebo *et al.* (1991) have studied in detail the drug-carrier interactions in synaptosomal preparations *in vitro*, revealing drug and species differences of the dissociation times of [^{3}H]-imipramine, [^{3}H]-paroxetine and [^{3}H]-citalopram from the 5-HT transporter. However, the relationship of these differences to the present results is uncertain. In addition, the CIM plasma levels obtained after long-term treatment were higher (up to 2.7 times) than those increasing plasma 5-HT acutely, but no change of this pool was observed. Thus, the triggering of adaptive mechanisms of peripheral 5-HT physiology is likely, as for fluoxetine.

In summary, the present work shows that plasma 5-HT is highly dependent on transient, but not sustained, inhibition of the high-affinity 5-HT uptake. Also, the marked differences in the effects elicited by fluoxetine and clomipramine suggest that their *in vivo* interaction with the rat platelet 5-HT transporter may be clearly distinct. Therefore, caution should be taken in extrapolating to man the effects of certain uptake inhibitors on the rat high-affinity 5-HT uptake.

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