The effects of single and repeated anorectic doses of 5-hydroxytryptamine uptake inhibitors on indole levels in rat brain

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1 The effects of acute and repeated equiactive anorectic doses (ED_{50}) of recently marketed 5-hydroxytryptamine (5-HT) uptake inhibitors on the content of brain indoles were compared in rats in relation to the brain regional concentrations of unchanged drug and its known active metabolite.

2 Single intraperitoneal (i.p.) doses of the anorectic ED_{50} of fluoxetine ($35 \mu\text{mol kg}^{-1}$), fluvoxamine ($60 \mu\text{mol kg}^{-1}$), paroxetine ($20 \mu\text{mol kg}^{-1}$) and sertraline ($49 \mu\text{mol kg}^{-1}$) slightly reduced brain 5-hydroxyindoleacetic acid (5-HIAA), with regional differences, this being compatible with 5-HT uptake blockade. Only fluvoxamine and sertraline significantly enhanced the content of 5-HT in the cortex.

3 The regional sensitivity to the acute effect of a given drug was not related to any preferential drug distribution, as these compounds distributed almost uniformly in the brain areas considered (cortex, striatum and hippocampus).

4 Repeating the same doses twice daily, i.p. for 14 days, however gave a different picture, fluvoxamine having little or no effect on the content of indoles and fluoxetine, paroxetine and sertraline lowering both 5-HT and 5-HIAA in all the brain regions compared to pair-fed control animals, 1 h after the last dose.

5 One week later only fluoxetine-treated animals still had reduced brain 5-HT, this probably being related to the accumulation of its main metabolite norfluoxetine in rat brain after chronic dosing.

6 Further studies on the relationship between the long-term neurochemical changes and anorectic activity are required but it appears from these results that anorectic drugs with similar acute effects on 5-HT uptake may differ in their long-term effects on 5-HT mechanisms.

Keywords: Fluoxetine; fluvoxamine; paroxetine; sertraline; anorectic drugs; brain; indoles; 5-hydroxytryptamine

Introduction

Recent studies indicate that an inhibitory 5-hydroxytryptaminergic system is involved in the control of mechanisms concerned with feeding. Accordingly many agents that increase the availability of 5-hydroxytryptamine (5-HT) show anorectic activity (Nathan & Rolland, 1987; Wong & Fuller, 1987; Garattini *et al.*, 1988, 1991; Samanin & Garattini 1990). They include several 5-HT uptake inhibitors which have proved effective in a number of experimental conditions of hyperphagia (see the above reviews) and some have also been reported to reduce body weight in man (Simpson *et al.*, 1981; Smedegaard *et al.*, 1981; Ferguson & Feighner 1987).

Consistent with an inhibition of reuptake, 5-HT uptake blockers given acutely at pharmacologically effective doses generally rapidly lower the concentrations of 5-hydroxyindoleacetic acid (5-HIAA) in whole brain or selected brain areas of rats, without appreciably affecting the levels of its precursor 5-HT (Ross *et al.*, 1981; Koe *et al.*, 1983; Schmidt *et al.*, 1988; Caccia *et al.*, 1992a).

Only fragmentary studies are available on the neurochemical effects of these drugs after repeated dosing in animals, which would allow a better assessment of the inhibition of amine uptake in relation to the therapeutic activity of the drugs. However, sertraline was reported to have no effect on 5-HIAA (and 5-HT) in selected rat brain areas after repeated administration, suggesting that its effect on 5-HT turnover is relatively short-lived (Leonard, 1988).

Fluvoxamine reduced 5-HT turnover in rat brain after short-term treatment ($25 \text{ mg kg}^{-1} \text{ day}^{-1} \times 7 \text{ days}$) but this effect was not apparent after three weeks of repeated dosing (5-HIAA was not measured) (Benfield & Ward, 1986).

Zimeldine, now withdrawn from the market, dose-dependently depleted both 5-HIAA and 5-HT after prolonged treatment (Ross *et al.*, 1981). Fluoxetine increased cortical 5-HT and lowered 5-HIAA when given for three days at a relatively high dose (30 mg kg^{-1}) (Sarkissian *et al.*, 1990) but reduced both indoles in cortex, hippocampus and striatum when given for longer times in the range of the anorectic ED_{50} ($7.5\text{--}15 \text{ mg kg}^{-1}$ for 7–21 days) (Caccia *et al.*, 1992b). Repeated full anorectic doses of (+)-fenfluramine, the prototype of the so called anorectic drugs which block 5-HT reuptake and enhance its release from nerve terminals, also reduce both 5-HT and 5-HIAA in animals, the effect being particularly marked and long-lasting at higher doses (Garattini *et al.*, 1988, 1991).

All these findings suggest that each 5-HT uptake inhibitor may affect 5-hydroxytryptaminergic mechanisms in rats differently, depending on its dose and treatment schedule, although a certain biochemical effect on 5-HT content and metabolism does not necessarily mean that it is essential to a functional effect such as the anorectic action. Hence our interest in comparing the acute and chronic effects on the central 5-hydroxytryptaminergic system of recently marketed 5-HT uptake inhibitors when given at anorectic doses to rats, in order to assess differences and similarities in their neurochemical behaviour.

The present study compared the effects of fluvoxamine, paroxetine and sertraline at equal anorectic doses (ED_{50}) on concentrations of 5-HT and 5-HIAA in discrete areas of the rat brain, in relation to the treatment schedule. The relationships between neurochemical effects on indole contents and the brain uptake and distribution of these drugs after acute and repeated administration of the ED_{50} were also examined. Fluoxetine was used as a reference drug for interaction with these aspects of 5-HT biochemistry.

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Methods

Animals and drug treatment

Male CD-COBS rats weighing 150–175 g (Charles River, Italy) were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85–23, 1985).

Fluoxetine hydrochloride (E. Lilly, Florence, Italy), fluvoxamine maleate (Unione Chimica Medicamenti, Solvay, Turin, Italy), paroxetine acetate (Ferrosan, Malmö, Sweden) and sertraline hydrochloride (Roering Farmaceutici Italiana, Pfizer, Rome, Italy) were dissolved in deionised water and injected intraperitoneally (i.p.). In separate experiments, animals were trained to eat their daily ration in 4 h.

On the day of the experiment the test drugs were injected i.p. and food was made available after 30 min. The amount of food eaten during the next 60 min was measured, as described before (Garattini *et al.*, 1991; Caccia *et al.*, 1992a; Anelli *et al.*, 1992).

The ED₅₀ ($\pm$ 95% confidence limits), calculated on five drug doses with four rats per group at each dose, according to the method of De Lean *et al.* (1978) adapted to a Macintosh computer (V. Guardabasso, personal communication) were 34.7 ($\pm$ 14.9) $\mu\text{mol kg}^{-1}$ (12.0 mg kg^{-1}) for fluoxetine; 59.9 ($\pm$ 37.8) $\mu\text{mol kg}^{-1}$ (22.2 mg kg^{-1}) for fluvoxamine, 20.4 ($\pm$ 5.7) $\mu\text{mol kg}^{-1}$ (7.9 mg kg^{-1}) for paroxetine and 48.7 ($\pm$ 25.4) $\mu\text{mol kg}^{-1}$ (16.7 mg kg^{-1}) for sertraline. These doses were then given to other groups of rats which were killed by decapitation 60 min thereafter for determination of the indole content and drug concentrations in selected brain areas (cortex, hippocampus and striatum).

Finally in other studies, the animals received i.p. injections of either vehicle or the test drug at approximately 12-h intervals for 14 days and were killed 60 min or 7 days after the last dose; the brains were immediately removed, as described above.

Since repeated treatment with 5-HT uptake inhibitors may reduce food intake (Wong & Fuller, 1987), the control animals were 'pair-fed', i.e. the amount of food treated animals ate was measured each day and given to controls the next day. According to this schedule, pair-fed animals started with the vehicle one day later than the test drug groups.

Chemical analysis

Concentrations of 5-HT, 5-HIAA, noradrenaline (NA), dopamine and its acidic metabolites homovanillic acid

(HVA) and dihydroxyphenylacetic acid (DOPAC) in brain areas were measured by high-performance liquid chromatography (h.p.l.c.) with electrochemical detection based on the method described by Achilli *et al.* (1985).

Plasma and brain concentrations of fluoxetine and sertraline and their nor-derivatives nor-fluoxetine and nor-sertraline were determined as described respectively by Caccia *et al.* (1990) and Tremaine *et al.* (1989). Fluvoxamine was extracted from plasma and brain homogenate (acetone-formic acid 85:15, v/v, as for fluoxetine), after adding fluoxetine as internal standard, and quantified by h.p.l.c. with ultraviolet detection (u.v.) as described by Foglia *et al.* (1989), with minor modifications (i.e. the chromatographic column was a $\mu\text{Bondapak C}_{18}$, 30 cm $\times$ 3.9 mm i.d., particle size 10 μm , Waters Assoc., maintained at room temperature). With these experimental conditions, approximate retention times were 10 min for fluvoxamine and 15 min for fluoxetine. The limit of detection, precision and reproducibility in plasma were similar to those reported by Foglia *et al.* (1989). The limit of detection in brain was 250 ng g^{-1} (about 0.8 nmol g^{-1}), using approximately 0.1 g of brain tissue. At this concentration the coefficient of variation (C.V.) was 13% ($n = 4$) and all higher concentrations gave C.V. less than 10%.

Paroxetine was extracted from plasma and brain homogenate as above and quantified by h.p.l.c. with u.v. detection at 240 nm. The analytical column was as for fluvoxamine but the mobile phase was 0.01 M $\text{KH}_2\text{PO}_4\text{-CH}_3\text{CN-CH}_3\text{OH}$ (60:35:5) buffered to pH 3 with H_3PO_4 , delivered isocratically at a flow-rate of 1 ml min^{-1} . Under these conditions, approximate retention times were 13 min for paroxetine and 22 min for fluoxetine (internal standard). The limit of detection was 25 ng ml^{-1} or 250 ng g^{-1} , using 1 ml of plasma or approximately 100 mg of tissue, and the C.V. were generally below 10% for identical samples containing 25–100 ng ml^{-1} or 250–2000 ng g^{-1} (i.e. the range of the calibration graphs for plasma and brain analysis, respectively).

Statistical analysis

Statistical analysis was done by one-way analysis of variance or two-way analysis of variance (when examining the effects of two factors, the brain areas and treatment schedule) and probabilities (P) less than 0.05 were considered statistically significant.

Results

Table 1 shows the effect of equiactive anorectic doses of fluoxetine, fluvoxamine, paroxetine and sertraline on the regional content of indoles 1 h after a single dose. Fluoxetine and paroxetine slightly lowered 5-HIAA in cortex (by

Table 1 Concentrations of indoles after equi-active anorectic doses (ED₅₀) of fluoxetine, fluvoxamine, paroxetine and sertraline to male rats

Drug ^a	ED ₅₀ ($\mu\text{mol kg}^{-1}$, i.p.)	Brain area	Content (% of control)	
			5-HT	5-HIAA
Fluoxetine	35	Cortex	95 $\pm$ 12	76 $\pm$ 7**
		Hippocampus	95 $\pm$ 8	83 $\pm$ 14*
		Striatum	106 $\pm$ 13	87 $\pm$ 16
Fluvoxamine	60	Cortex	120 $\pm$ 7**	68 $\pm$ 7**
		Hippocampus	107 $\pm$ 19	77 $\pm$ 11*
		Striatum	104 $\pm$ 13	74 $\pm$ 4*
Paroxetine	20	Cortex	102 $\pm$ 11	83 $\pm$ 14*
		Hippocampus	102 $\pm$ 19	94 $\pm$ 19
		Striatum	110 $\pm$ 11	85 $\pm$ 14
Sertraline	49	Cortex	116 $\pm$ 9*	80 $\pm$ 14*
		Hippocampus	111 $\pm$ 17	76 $\pm$ 10*
		Striatum	100 $\pm$ 13	80 $\pm$ 7*

^aResults are means $\pm$ s.d. of 5 animals.

** $P < 0.01$; * $P < 0.05$ compared to vehicle.

approximately 20% compared to the $0.26 \pm 0.04 \mu\text{g g}^{-1}$ 5-HIAA content of controls) with smaller or no effects (paroxetine) in hippocampus (controls $0.46 \pm 0.04 \mu\text{g g}^{-1}$) and striatum (controls $0.51 \pm 0.08 \mu\text{g g}^{-1}$); fluvoxamine and sertraline caused comparable reductions in all the brain regions examined. Brain 5-HT increased significantly only in the cortex of fluvoxamine- (0.53 ± 0.03 vs $0.43 \pm 0.03 \mu\text{g g}^{-1}$) and sertraline-treated rats (0.51 ± 0.04 vs $0.43 \pm 0.03 \mu\text{g g}^{-1}$). Cortical and hippocampal NA, striatal and cortical dopamine and striatal HVA and DOPAC were not affected 60 min after these doses of fluoxetine, fluvoxamine, paroxetine and sertraline (data not shown).

Twice-daily doses of fluoxetine, fluvoxamine, paroxetine and sertraline for 14 days slightly slowed the gain in body weight because food intake was reduced compared to controls, as expected. Final body weight averaged respectively 209 ± 12 g, 231 ± 5 , 240 ± 4 g and 244 ± 15 g in the groups treated with fluoxetine, fluvoxamine, paroxetine and sertraline, being 77–85% of that of the respective vehicle-treated groups (275–293 g). Food intake in the pair-fed control animals mimicked that of the drug-treated groups (with a delay of one day) over the period of drug administration and their final body weights (227 ± 10 , 235 ± 14 , 242 ± 14 and 257 ± 15 g for the pair-fed controls of fluoxetine, fluvoxamine, paroxetine and sertraline respectively) were comparable to the drug-treated group.

The content of indoles in the cortex, hippocampus and striatum, 1 h after the last dose of the 14-day regimen of fluoxetine, fluvoxamine and paroxetine are set out in Table 2.

Daily fluoxetine markedly reduced the brain content of 5-HT and 5-HIAA (30–60% reduction, depending on the indole and the brain region examined), compared to pair-fed control animals. This effect was shared by paroxetine (pair-fed control values 0.39 ± 0.02 , 0.21 ± 0.04 and $0.30 \pm 0.06 \mu\text{g g}^{-1}$ and 0.22 ± 0.02 , 0.26 ± 0.05 $0.51 \pm 0.13 \mu\text{g g}^{-1}$ for 5-HT and 5-HIAA in cortex, hippocampus and striatum respectively) and sertraline (pair-fed control values $0.40 \pm 0.02 \mu\text{g g}^{-1}$, 0.21 ± 0.04 and $0.28 \pm 0.06 \mu\text{g g}^{-1}$ and 0.22 ± 0.02 , 0.26 ± 0.05 , $0.51 \pm 0.13 \mu\text{g g}^{-1}$, respectively) but not by fluvoxamine which tended to reduce only 5-HIAA in the cortex (0.17 ± 0.01 vs $0.23 \pm 0.05 \mu\text{g g}^{-1}$). Concentrations of NA in cortex and hippocampus and dopamine and its metabolites DOPAC and HVA were not affected by the 14-day twice daily regimen of the four compounds, as after the single doses (data not shown).

The distribution of these drugs in cortex, hippocampus and striatum 1 h after the first and last injection of the ED_{50} is illustrated in Table 3. The 'anorectic' brain concentrations differed for the various compounds but fluvoxamine, paroxetine and sertraline distributed almost uniformly in the brain areas considered ($P > 0.05$, two-way analysis of variance), as previously reported for fluoxetine (Caccia *et al.*, 1990; 1992a). They all concentrated in brain tissues, achieving mean brain-to-plasma ratios ranging from 32–39 for fluvoxamine, 8–13 for paroxetine, and 70–80 for sertraline (80–90 for norsertraline) at the end of the 14-day regimen, when presumably pseudo-equilibrium between plasma and brain was achieved for all compounds.

Table 2 Content of indoles in cortex, hippocampus and striatum after repeated anorectic doses of fluoxetine, fluvoxamine, paroxetine and sertraline

Drug ($\mu\text{mol kg}^{-1}$; twice daily)	Brain area	Content (% of control) of:	
		5-HT	5-HIAA
Fluoxetine (35)	Cortex	$47 \pm 13^{**}$	$34 \pm 6^{**}$
	Hippocampus	$39 \pm 8^{**}$	$39 \pm 5^{**}$
	Striatum	$69 \pm 12^{**}$	$46 \pm 10^{**}$
Fluvoxamine (60)	Cortex	99 ± 11	$73 \pm 5^*$
	Hippocampus	94 ± 11	96 ± 13
	Striatum	110 ± 14	93 ± 7
Paroxetine (20)	Cortex	$55 \pm 7^{**}$	$61 \pm 11^{**}$
	Hippocampus	$49 \pm 10^{**}$	$40 \pm 5^{**}$
	Striatum	$53 \pm 11^{**}$	$48 \pm 10^{**}$
Sertraline (49)	Cortex	$80 \pm 6^{**}$	$60 \pm 5^{**}$
	Hippocampus	$86 \pm 6^{**}$	$62 \pm 8^{**}$
	Striatum	$84 \pm 11^{**}$	$70 \pm 8^{**}$

Drugs were injected intraperitoneally at approx 12 h intervals for 14 days. Assays were done 1 h after the last dose. Results are presented as percentage of the pair-fed control ($n = 5$). $^{**}P < 0.01$; $^*P < 0.05$ vs pair-fed control.

Table 3 Drug concentrations in brain areas after single and repeated anorectic doses of fluoxetine, fluvoxamine paroxetine and sertraline to male rats

Drug	(nmol $\text{kg}^{-1} \times \text{days}$)	Drug concentration (nmol g^{-1})		
		Cortex	Hippocampus	Striatum
Fluoxetine ^a	35×1	36 ± 13 (14 ± 5)	38 ± 13 (13 ± 7)	40 ± 14 (16 ± 4)
	35 , b.i.d., $\times 14$	$97 \pm 17^{**}$ (318 ± 35) **	$94 \pm 14^{**}$ (312 ± 56) **	$100 \pm 28^{**}$ (324 ± 64) **
Fluvoxamine	60×1	47 ± 18	45 ± 21	50 ± 23
	60 , b.i.d., $\times 14$	74 ± 31	72 ± 24	65 ± 34
Paroxetine	20×1	13 ± 2	13 ± 2	16 ± 5
	20 , b.i.d., $\times 14$	23 ± 9	20 ± 7	28 ± 15
Sertraline ^a	49×1	98 ± 26 (16 ± 5)	74 ± 21 (14 ± 2)	80 ± 18 (15 ± 5)
	49 , b.i.d., $\times 14$	77 ± 29 (49 ± 11) **	68 ± 9 (45 ± 13) **	71 ± 18 (44 ± 18) **

^aBrain concentrations of the active nor-metabolite nor-fluoxetine and nor-sertraline are shown in parentheses.

Each value is the mean $\pm$ s.d. of five rats. The determinations were made 60 min after the last dose.

$^{**}P < 0.01$; $^*P < 0.05$ compared to acute dosing.

After repeated sertraline the mean plasma (data not shown) and brain concentrations of unchanged drug tended to decrease ($P > 0.05$) and those of its metabolite nor-sertraline increased ($P < 0.05$), changing the metabolite-to-parent drug ratio in all brain regions compared to the acute dosing (0.6 vs 0.2). Although repeated treatment with fluvoxamine and paroxetine gave slightly higher mean plasma and brain concentrations than single doses in all tissues, the difference did not reach significance based on two-way analysis of variance ($P > 0.05$). In contrast, repeated doses of fluoxetine led to marked accumulation of the unchanged compound and its metabolite nor-fluoxetine in rat plasma and brain compared to acute dosing ($P < 0.05$) (Table 3).

Finally, additional groups of rats were given the anorectic ED_{50} of fluoxetine, paroxetine and sertraline or the vehicle twice daily for 14 days and were killed a week after the last doses for the measurement of the drug and indole concentrations in the brain. Fluvoxamine was not included in these studies because it has negligible effect on brain indole concentrations 1 h after the last dose (anorectic ED_{50}).

As shown in Table 4, the content of 5-HT in cortex and hippocampus (and other brain areas, data not shown) was still reduced by approximately 20–30% one week after fluoxetine (pair-fed controls 0.27 ± 0.02 and $0.35 \pm 0.04 \mu\text{g g}^{-1}$ for cortex and hippocampus) but not after paroxetine and sertraline. 5-HIAA was unchanged or even tended to be higher than the pair-fed control values (fluoxetine and paroxetine), although the differences did not always reach significance, probably because of the wide variability. By one week the drugs were undetectable in rat brain within the limits of sensitivity of the h.p.l.c. procedures. However, about 1 nmol g^{-1} of the active metabolite of fluoxetine, nor-fluoxetine, were still detected in the cortex and hippocampus of fluoxetine-treated rats.

Discussion

In agreement with previous pharmacological observations on 5-HT uptake blockers (Nathan & Rolland, 1987; Wong & Fuller, 1987; Samanin & Garattini, 1990; Garattini *et al.*, 1988; 1991) fluoxetine, fluvoxamine, paroxetine and sertraline showed dose-dependent anorectic activity in overnight food-deprived rats. On a molar dose basis, and taking into account the i.p. ED_{50} , paroxetine ($20 \mu\text{mol kg}^{-1}$) was slightly more active than fluoxetine ($35 \mu\text{mol kg}^{-1}$) and sertraline ($49 \mu\text{mol kg}^{-1}$) which in turn were slightly more active than fluvoxamine ($60 \mu\text{mol kg}^{-1}$) in this acute test in rats. These ED_{50} for anorexia are 2–5 times those inhibiting [^3H]-5-HT uptake *ex vivo*, with the exception of fluoxetine which shows comparable efficacy in inhibiting 5-HT uptake and causing anorexia (ED_{50} about $30 \mu\text{mol kg}^{-1}$) (Leonard, 1988; Johnson, 1989).

Paroxetine was also the most active compound in terms of the brain concentrations necessary to achieve an anorectic effect ($10\text{--}20 \mu\text{M}$) in rats, with sertraline giving the highest

($70\text{--}100 \mu\text{M}$) and fluvoxamine and fluoxetine intermediate 'anorectic' brain concentrations ($40\text{--}50 \mu\text{M}$), at the middle of the food intake period. Attempts to correlate brain concentrations with the *in vitro* potency in inhibiting 5-HT uptake (see Dechant & Clissold, 1991; Murdoch & McTavish, 1992 for a comparison of the relative potency) are unfortunately limited by the fact that fluoxetine and sertraline are biotransformed by hepatic N-dealkylation to the nor-derivatives nor-fluoxetine and nor-sertraline (Schmidt *et al.*, 1988; Tremaine *et al.*, 1989) and these fully retain the parent drug's anorectic activity in animals despite their lower efficacy in inhibiting 5-HT reuptake (Garattini *et al.*, 1991; Caccia *et al.*, 1992a; Anelli *et al.*, 1992). However, metabolites of fluvoxamine and paroxetine do not appear to have significant uptake inhibiting properties *in vitro* in animals (Benfield & Ward, 1986; Dechant & Clissold, 1991), although their activity in relation to 5-HT mechanisms other than uptake inhibition, and to the anorectic action, is still unknown.

Interestingly, brain concentrations of all these drugs (and their known active metabolites) are several hundred times the IC_{50} for inhibiting 5-HT uptake *in vitro* (ranging from $0.1\text{--}0.5 \mu\text{M}$ for fluoxetine to less than $0.01 \mu\text{M}$ for paroxetine) (Benfield & Ward, 1986; Leonard, 1988; Garattini *et al.*, 1991; Caccia *et al.*, 1992a), making it unlikely that they reduce food intake in rats by specifically blocking 5-HT neuronal uptake. Evidence now exists that fluoxetine, sertraline and their nor-derivatives (Garattini *et al.*, 1991) and paroxetine (T. Mennini, personal communication) can all release 5-HT from synaptosomes *in vitro* besides inhibiting 5-HT uptake. Fluoxetine (Gobbi *et al.*, 1992), sertraline and their active metabolites (T. Mennini, personal communication) induce tritium release from synaptosomes preloaded with [^3H]-5-HT, through a mechanism different from that of (+)-fenfluramine. The concentrations active on 5-HT release are in the same range as those reached after anorectic doses, suggesting another potential mechanism of action.

The neurochemical results of the present study confirm and extend previous findings on the effects of 5-HT uptake inhibitors on the content of indoles in rat brain. Acute treatment with anorectic doses of all these compounds typically reduces the content of 5-HIAA, although to different degrees and with regional differences in sensitivity. Fluvoxamine and sertraline, however, also tended to increase brain 5-HT, with regional differences again, an effect not shared by the other 5-HT uptake inhibitors mentioned, at least at doses in the range of anorectic activity. These findings also indicate that the regional sensitivity to the acute effect of a given drug was not due to differences in distribution as 5-HT uptake inhibitors distributed almost uniformly in the brain regions examined after either single or repeated doses.

After repeated doses of these compounds, however, the interaction with the 5-hydroxytryptaminergic system was different from after single doses. Fluvoxamine caused only a slight fall in the cortical content of 5-HIAA without appreciably affecting 5-HT. This was not related to any pharma-

Table 4 The effects of repeated administration of fluoxetine, paroxetine and sertraline on the content of indoles in cortex and hippocampus, 1 week after the last dose

Treatment ($\mu\text{mol kg}^{-1}$, twice daily)	Brain area	Indole content (% of control)		Drug concentration (nmol g^{-1})
		5-HT	5-HIAA	
Fluoxetine (35)	Cortex	$75 \pm 5^{**}$	127 ± 35	ND (0.9 ± 0.2) ^a
	Hippocampus	$82 \pm 9^{*}$	$124 \pm 11^{*}$	ND (1.2 ± 0.5) ^a
Paroxetine (20)	Cortex	106 ± 10	131 ± 36	ND
	Hippocampus	99 ± 14	106 ± 14	ND
Sertraline (49)	Cortex	97 ± 9	106 ± 12	ND (ND) ^a
	Hippocampus	99 ± 8	105 ± 9	ND (ND) ^a

Drugs were injected at the dose indicated, twice daily for 14 days. Each value is the mean $\pm$ s.d. of five animals.

(ND) = Below the level of quantitation (^abrain concentrations of the nor-metabolite).

$^{**}P < 0.01$; $^{*}P < 0.05$ vs pair-fed control.

cokinetic tolerance because the drug concentrations were, if anything, slightly higher in animals given the repeated schedule.

By contrast, paroxetine and sertraline caused pronounced decreases in the brain content of both 5-HT and 5-HIAA, as already known for fluoxetine (Caccia *et al.*, 1992b). Unlike fluoxetine, paroxetine accumulated only slightly in the brain with this schedule of repeated administration so the variations found should be mainly related to the different treatment schedule, as in the case of fluvoxamine. Brain concentrations of the active metabolite of sertraline, norsertraline, differed after repeated and acute dosing. In this case, therefore, the different neurochemical effects of acute and repeated sertraline may depend partly on kinetic factors leading to different metabolite-to-parent drug ratios at the site of action.

Sertraline and paroxetine, however, had no significant long-term effects in rat brain, indicating that their depleting effects on 5-HT content and metabolism are short-lasting. Fluoxetine on the other hand reduced 5-HT for at least a week after the last dose, although the content of 5-HIAA recovered fully or even tended to overshoot.

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(Received March 5, 1993)

Revised April 21, 1993

Accepted April 22, 1993)