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Neurochemical Characterization of a New Potent and Selective Serotonin Uptake Inhibitor: Lu 10-171

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Abstract. The neurochemical characteristics of a new bicyclic phthalane derivative – Lu 10-171 [1-(3-(dimethylamino)propyl)-1-(*p*-fluorophenyl)-5-phthalancarbonitrile; citalopram] – have been investigated. Lu 10-171 and its metabolites were compared with tricyclic thymoleptics in several tests for serotonin (5-HT), noradrenaline (NA), and dopamine (DA) uptake inhibition in vitro and in vivo. Lu 10-171 is a very potent and completely selective inhibitor of the 5-HT reuptake mechanism, being 2–10 times as active as chlorimipramine. The metabolites of Lu 10-171 show weak 5-HT uptake inhibiting properties. Lu 10-171 and its metabolites are devoid of NA uptake inhibiting properties and in this respect they clearly differ from the tricyclic antidepressants, which possess effects both on 5-HT and NA uptake. The inhibition of 5-HT uptake in vitro is competitive and not connected with an increased efflux of 5-HT. Lu 10-171 and its metabolites only inhibit DA uptake in extremely high concentrations and in this respect they are even weaker than chlorimipramine and other tricyclic thymoleptics. Like the tricyclic thymoleptics, Lu 10-171 is without effect on MAO and does not change the endogenous levels of brain monoamines. Due to the selective action on 5-HT uptake, Lu 10-171 seems to be a valuable tool in studying the role of central 5-HT neurone systems in experimental neuropharmacology as well as in the ethiology of depressive illness.

Key words: 5-HT uptake inhibition – NA uptake inhibition – Thymoleptic effect

The underlying ethiological factor of depressive illness may be a decreased function of the neurotransmitter systems for noradrenaline (NA) or serotonin (5-HT) (Schildkraut, 1965, 1973; Lapin and Oxenkrug, 1969; van Praag and Korf, 1971; Åsberg et al., 1973). The tricyclic thymoleptics possess NA as well as 5-HT uptake inhibiting properties, although in varying proportion, and in this way increase the functional extraneuronal concentrations of the transmitters. Due to the clinical efficacy of thymoleptics with preferential effect on either 5-HT uptake (chlorimipramine, imipramine, and amitriptyline) or on NA uptake (protriptyline, desipramine, and nortriptyline) (Carlsson et al., 1969a and b; Carlsson, 1970; Lidbrink et al., 1971), the hypothesis was advocated that facilitation of NA transmission was related to increse in drive, while facilitation of 5-HT transmission was related to mood elevation (Kielholz and Pöldinger, 1968a and b; Carlsson et al., 1969a and b).

Previously, a series of phthalanes and thiophthalanes (Petersen et al., 1966, 1970) have been developed. These compounds with specific NA uptake inhibiting properties but practically devoid of effect on 5-HT uptake (Carlsson et al., 1969c; Lingjaerde, 1970) proved to be rather activating (Zavidovskaya, 1969; Strömgren and Friderichsen, 1971). Substitutions in the ring systems of the phthalanes led to substances with strong, specific 5-HT uptake inhibiting, but devoid of NA uptake inhibiting properties (Bigler et al., to be published). A compound of this type may help to clarify the role of 5-HT neurone systems in the ethiology of depressive illness and may also be a useful tool in neuro-biochemical and -pharmacological research.

Therefore one compound of this type, 1-(3-(dimethylamino)propyl)-1-(*p*-fluorophenyl)-5-phthalancarbonitrile (Lu 10-171, citalopram, see Fig. 1), has been subjected to a more extensive biochemical and pharmacological investigation. The present paper describes the biochemical characteristics of Lu 10-171 and its metabolites on the monoaminergic neuronal systems.

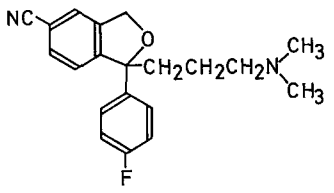

Fig. 1. Structural formula of Lu 10-171 – Citalopram

MATERIALS AND METHODS

Animals. Male albino mice, NMRI/BOM, SPF (18 – 25 g), male albino rats, Wistar/Af/Han/Mol (Han 67), SPF (180 – 220 g), New Zealand white rabbits, either sex (3 – 4 kg), pure-bred Beagle dogs, own breed, either sex (9.5 – 11 kg), were used.

Substances. The following compounds were used (the salt forms are given in parentheses): chlorimipramine (HCl), chlordesipramine (HCl), imipramine (HCl), desipramine (HCl), amitriptyline (HCl), nortriptyline (HCl), protriptyline (HCl), 4-methyl-α-ethyl-meta-tyramine-methylester (HCl) (H 75/12), 4,α-dimethyl-meta-tyramine-methylester (HCl) (H 77/77), pargyline (HCl), tranylcypromine ($^1/_2H_2SO_4$), benztropine (CH_3SO_3H), Lu 10-171 (HBr), Lu 11-109 (oxalate), Lu 11-161 (oxalate), and Lu 11-305 (HCl). Lu 11-109, Lu 11-161, and Lu 11-305 are the desmethyl-, didesmethyl-, and N-oxide metabolites of Lu 10-171, respectively (Fredricson Overø, personal communication).

The doses refer to the salts given. All substances were administered in physiological saline in a volume of 2.5 – 10 ml/kg body weight. 5-Hydroxy (side chain -2-^{14}C)tryptamine creatinine sulphate (54 – 58 mCi/mMole) was obtained from the Radiochemical Centre, Amersham. DL-Noradrenaline, L-bitartrate (7-^{3}H(N)) (4 – 10 Ci/mMole), and 3,4-dihydroxyphenylethyl-amine (ethyl-1-^{3}H(N)) (8.95 Ci/mMole) were obtained from New England Nuclear.

Statistics. Two-tailed Student's *t*-test, with the Smith-Satterthwaite modification (Miller and Freund, 1965) if necessary, was performed with results from Tables 3 and 5. IC_{50}-, ED_{25}-, and ED_{50}-values were evaluated by log-dose-response curves as the concentration or dose causing 50% or 25% inhibition of the response in control animals, respectively.

Inhibition of ^{14}C-5-HT Uptake in Rabbit Blood Platelets in vitro. Rabbit platelet rich plasma containing EDTA as an anticoagulant, was obtained by centrifugation at 290 g for 15 min and diluted by one volume of 0.05 M Na-P-buffer, pH 7.2. Four ml of this solution were incubated with test compound (100 µl) for 5 min at 37° C. Hereafter, 100 µl of ^{14}C-5-HT solution (final conc. 120 nM) were added. The incubation was continued for 15 min and was terminated by transferring the test tubes to an ice bath. The platelets were isolated by centrifugation (~ 4000 g, 5 min, 4° C). After draining, the platelets were gently washed with 4 ml of ice-cold saline, and the remaining radioactivity was determined by liquid scintillation counting.

Inhibition of the H 75/12-induced Depletion of 5-HT in Rat Brain in vivo. This effect was studied by a modification of the method developed by Carlsson et al. (1969a). By this method the depletion of 5-HT caused by H 75/12 is prevented by thymoleptic drugs by inhibiting the uptake of H 75/12 into 5-HT neurons. Drugs were given s.c. and after 20 min an intraperitoneal injection of H 75/12 (50 mg/kg) was given. Two hours after this injection the animals were killed by a blow to the head, immediately followed by decapitation. In the oral experiments the drugs were given in water 45 min before the injection of H 75/12. The brain was quickly removed, rinsed for blood, and immersed in crushed dry ice. Brains from two rats were pooled for the determination of 5-HT, which was performed fluorimetrically after purification by cation exchange chromatography as described by Hyttel and Fjalland (1972). The brains of rats receiving drug plus H 75/12 were always compared with rats receiving H 75/12 alone or vehicle alone.

Inhibition of ^{3}H-NA Uptake in Mouse Atria in vitro. For measuring the inhibition of uptake of ^{3}H-NA in mouse atria in vitro the method described by Sachs (1970) was used. Isolated atria were preincubated with drugs for 5 min at 37° C in oxygenated Krebs-Ringer phosphate-buffer, pH 7.4. Hereafter, ^{3}H-NA (final conc. 10^{-7} M) was added, and the incubation was continued for 15 min. Extra-cellular and loosely bound ^{3}H-NA was washed out in isotope-free buffer for 10 min. The atria were carefully blotted on filter paper, weighed and solubilized in 300 µl of Soluene® (Packard Inst. Co.), and the radioactivity determined by liquid scintillation counting.

Inhibition of ^{3}H-NA Uptake in Mouse Heart in vivo. Mice were fasted for 17 h before the experiment. Test substances were given subcutaneously 30 min before an intravenous injection of ^{3}H-NA (1 µg/kg). After 15 min the mice were killed by cervical dislocation and their hearts removed, cut open, and washed with cold saline. The radioactivity was extracted by heating the hearts to 95° C in 2 ml of 0.5 N NaOH for 30 min. After neutralization with 1.5 ml of 1.5 N acetic acid the samples were centrifuged for a few minutes and the radioactivity in the supernatant determined by liquid scintillation counting.

Inhibition of the H 77/77-induced Depletion of NA in Rat Brain in vivo. This effect was studied by a modification of the method developed by Carlsson et al. (1969b). By this method depletion of NA caused by H 77/77 is prevented by thymoleptic drugs through an inhibition of the uptake of H 77/77 into NA-neurones. Drugs were given s.c. in the nape. After 25 min, H 77/77 (5 mg/kg) was given s.c. in the back. One hour after the first drug dose, a second dose of drug was given s.c. in the nape. This dose was half the first dose injected. The rats were killed by a blow to the head, immediately followed by decapitation, 2 h and 25 min after the first drug dose. The brains were quickly removed, rinsed for blood, and immersed in crushed dry ice. The content of NA in the brains was determined fluorimetrically after purification by cation exchange chromatography as described by Hyttel (1974). The brains of rats receiving drug plus H 77/77 were always compared with rats receiving H 77/77 alone or vehicle alone. The ED-values calculated refer to the first dose of drug given.

Inhibition of ^{3}H-DA Uptake in Rat Striatal Synaptosomes. Rat brain striatum was gently homogenized in 40 volumes of ice-cold 0.32 M of sucrose containing 1 mM of nialamide. The P_2 fraction (synaptosomal fraction) was obtained by centrifugation (600 g, 10 min, 25000 g, 55 min) and suspended in 40 volumes of a modified Krebs-Ringer-phosphate buffer, pH 7.4 (122 mM NaCl, 4.8 mM KCl, 972 µM $CaCl_2$, 1.2 mM $MgSO_4$, 12.7 mM Na_2HPO_4, 3.0 mM NaH_2PO_4, 162 µM EDTA-Na_2, 1.14 mM ascorbic acid and 10.1 mM glucose, oxygenated with pure oxygen for 10 min before use). To 200 µl of the synaptosomal fraction on ice were added 3700 µl modified Krebs-Ringer-phosphate buffer-containing test compounds. After a preincubation at 37° C for 5 min, 100 µl of ^{3}H-DA (final conc. 12.5 nM) were added and the samples were incubated for 5 min at 37° C.

The incubation was terminated by filtering the samples under vacuum through Millipore filters (HAWPO 2500, 0.45 µ) with a wash of 5 ml buffer containing 10 µM of unlabeled DA. After solubilizing the filters in 1 ml of cellosolve the radioactivity was determined by liquid scintillation counting. The unspecific binding of ^{3}H-DA was determined by incubating control samples on ice instead of at 37° C.

Table 1. Effect of Lu 10-171 and its metabolites on 5-HT and NA uptake compared with known thymoleptics

Test compound	5-HT uptake			NA uptake			
	^{14}C-5-HT uptake in rabbit blood platelets in vitro IC_{50} nM	Reversal of H 75/12 induced 5-HT depletion from rat brain in vivo (mg/kg s.c.)		^{3}H-NA uptake in mouse atria in vitro IC_{50} nM	^{3}H-NA uptake in mouse heart in vivo (mg/kg s.c.) ED_{50}	Reversal of H 77/77 induced NA depletion from rat brain in vivo (mg/kg s.c.)	
		ED_{25}	ED_{50}			ED_{25}	ED_{50}
Lu 10-171	14	0.27	0.80	32000	> 160	> 160	> 160
Lu 11-109 (desmethyl)	31	52	> 160	19000	> 160	> 80	> 80
Lu 11-161 (didesmethyl)	250	> 80	> 80	5800	> 160	> 160	> 160
Lu 11-305 (N-oxide)	530	3.6	8.4	> 100000	> 160	> 160	> 160
Chlorimipramine	33	1.3	6.4	270	9.8	15	> 20
Chlordesipramine	270	34	133	3.7	1.1	4.0	50
Imipramine	320	7.5	32	75	3.3	2.1	6.8
Desipramine	1800	34	> 80	1.4	0.51	0.32	0.66
Amitriptyline	300	54	> 80	130	3.5	3.8	> 10
Nortriptyline	1500	14	60	29	1.3	1.9	9.7
Protriptyline	1300	> 80	> 80	0.83	0.34	0.53	1.2

Inhibition of ^{14}C-5-HT Uptake in Blood Platelets from Dogs Pretreated with Lu 10-171. Dogs were fasted overnight and cannulated in the cephalic vein from which blood was collected before and at different times after the oral administration of Lu 10-171 as tablets. Blood platelets were isolated and their uptake of ^{14}C-5-HT was determined as described above for rabbits. The number of blood platelets in each sample was determined by counting in a Coulter Counter apparatus after proper dilution. Uptake of ^{14}C-5-HT was calculated in cpm/10^6 blood platelets and expressed in per cent of the uptake in the daily control sample (mean of triple determinations 30, 15, and $^1/_2$ min before dosing the dog).

Efflux of ^{14}C-5-HT from Preloaded Rabbit Blood Platelets in vitro. Rabbit blood platelets (see above) were preloaded by incubation with ^{14}C-5-HT (1 µM) for 15 min at 37° C, isolated by centrifugation (12500 g, 15 min), and washed with cold 0.05 M Na-P-buffer pH 7.2 containing 75 mM NaCl and 5 mM KCl. The platelets were suspended in the same buffer (twice the original plasma volume) and to 4 ml of this suspension were added 100 µl of 5-HT (final conc. 10 µM) to inhibit reuptake of labeled ^{14}C-5-HT, and 100 µl of test drugs. The samples were incubated at 37° C or 0° C for 30 min. The incubation was stopped by placing the samples in ice water and the samples were centrifuged (7400 g, 3 min). The radioactivity in the supernatant and in the pellet was determined by liquid scintillation counting. The efflux was calculated in per cent of the efflux in control samples (after correction for efflux at 0° C) based on radioactivity either in the platelets or in the supernatant.

Efflux of ^{14}C-5-HT from Preloaded Rat Brain Synaptosomes in vitro. Synaptosomes (P_2-fraction) from whole rat brain (except cerebellum) was obtained as described for ^{3}H-DA uptake and were suspended in 100 volumes of modified Krebs-Ringer-phosphate buffer, pH 7.4. The synaptosomes were loaded with ^{14}C-5-HT by incubating the P_2-suspension at 37° C for 30 min in 0.4 µM of ^{14}C-5-HT. The synaptosomes were isolated (25000 g, 55 min), rinsed in buffer, and suspended in buffer. To 100 µl of this suspension were added 1800 µl buffer containing test compounds and 200 µl of unlabeled 5-HT (final conc. 10 µM). The samples were incubated at 37° C or 0° C for 30 min, and the incubation was terminated by filtering the samples under vacuum through Millipore filters (HAWPO 2500, 0.45 µ) with a wash of 3 ml ice-cold buffer containing 10 µM of unlabeled 5-HT. After solubilizing the filters in 1 ml of cellosolve the radioactivity was determined by liquid scintillation counting.

Inhibition of Monoamine Oxidase (MAO) in Rat Brain and Liver in vitro. The method of Krajl (1965), slightly modified, was used. Rat liver or brain mitochondrial preparations were used as sources of enzyme. The amount of 4-hydroxyquinoline formed by the action of MAO on kynuramine was measured spectrofluorimetrically.

Endogenous Levels of Monoamines in Rat Brain. Drugs were given s.c. 140 min before killing the rats. Endogenous amine levels were thus measured at the same time as uptake in the H 75/12 experiment. One group of rats was used for determination of 5-HT and another for determination of both NA and DA. 5-HT and NA were determined as described above and DA as described by Hyttel (1974), except that DA was oxidized as described by Atack (1973). The recovery of 5-HT, NA, and DA was approximately 60%, 90%, and 85%, respectively. No corrections have been made for these recoveries.

Liquid Scintillation Counting. All radioactive samples were determined by liquid scintillation counting after the addition of 10 ml of Instagel® (Packard).

RESULTS

Inhibition of 5-HT and NA uptake was studied in different experimental models, both in vitro and in vivo, and the effects of Lu 10-171 and its metabolites are compared with known thymoleptics in Table 1. Lu 10-171 was the most potent inhibitor of 5-HT uptake in vitro and in vivo, whereas Lu 10-171 and its metabolites were devoid of NA uptake inhibiting properties.

Concerning the effect on ^{14}C-5-HT uptake in rabbit blood platelets (Table 1, first column) it appears

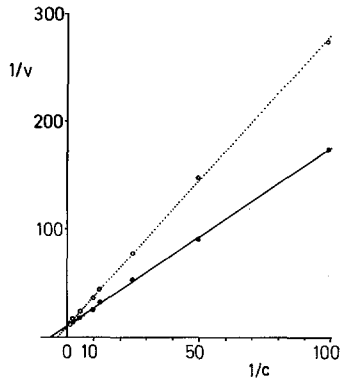

Fig. 2. Double reciprocal plots (Lineweaver-Burk) of the uptake rates (v) pmol 5-HT/min/10^6 blood platelets (*ordinate*) versus extracellular 5-HT concentration (c) µM in a medium with (○·····○) or without (●——●) Lu 10-171 (1 mM). Blood platelets were incubated for 5 min at 37° C. Regression lines were calculated according to the method of least squares. Each point represents the mean of triple determinations. V_{max}: 0.089 pmol 5-HT/min/10^6 blood platelets; K_m: 0.15 µM 5-HT; K_i: 1.9 nM Lu 10-171

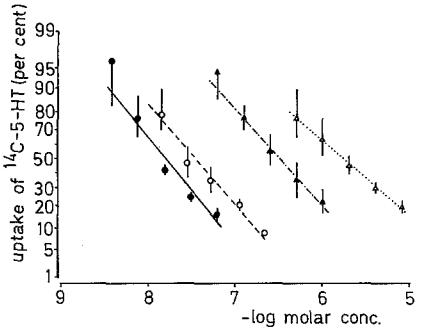

Fig. 3. Concentration–effect curves for inhibition of ^{14}C-5-HT uptake in rabbit blood platelets in vitro. Uptake of ^{14}C-5-HT is expressed in per cent of uptake in control samples and is depicted on a probability scale (*ordinate*). Each point represents mean ± S.D. of 5–12 determinations. Lu 10-171, ●——●, chlorimipramine, ○---○, imipramine, ▲·-·-▲, desipramine, △·····△

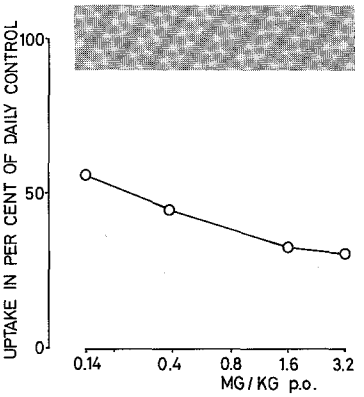

Fig. 4. Correlation between oral doses of Lu 10-171 and minimal uptake of ^{14}C-5-HT in isolated dog blood platelets within 2 h. Hatched area represent mean ± S.D. of the control group. Points on the curve are mean of triple determinations from the same plasma sample

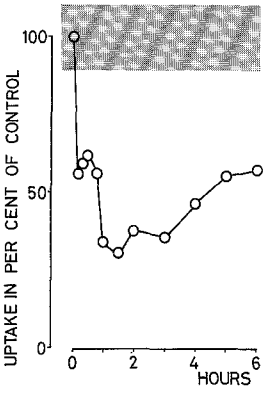

Fig. 5. Uptake of ^{14}C-5-HT in isolated blood platelets from a dog treated orally with 3.2 mg/kg of Lu 10-171. Hatched area represents mean ± S.D. of the control group. Points on the curve are mean of 3 determinations from the same plasma sample

that the dimethylamino derivatives were more potent uptake inhibitors than the corresponding monomethylamino derivatives. Lu 10-171 was twice as active and the desmethyl metabolite (Lu 11-109) as active as chlorimipramine, whereas the didesmethyl- and N-oxide-metabolites of Lu 10-171 (Lu 11-161 and Lu 11-305) were considerably weaker, although they were equipotent with imipramine and amitriptyline. The inhibition of ^{14}C-5-HT uptake by Lu 10-171 was competitive with an inhibitor constant of 1.9 nM as shown by the Lineweaver-Burk plot in Figure 2. In this respect, the tricyclic thymoleptics resemble Lu 10-171, since their dose-response curves for 5-HT uptake inhibition (Fig. 3) are parallel.

Also the in vivo test for central 5-HT uptake inhibition (H 75/12-test, Table 1, second column) the dimethylamino derivatives were more potent than the monomethylamino derivatives, except for amitriptyline/nortriptyline. Lu 10-171 was 5–10 times as active as chlorimipramine, while the demethylated metabolites of Lu 10-171 were considerably weaker and also less potent than imipramine and nortriptyline. The N-oxide metabolite (Lu 11-305) was rather potent in in vivo studies in contrast to the low potency in in vitro studies. After oral administration Lu 10-171 and chlorimipramine were much less active than after subcutaneous administration, but Lu 10-171 (ED_{25} 4.6, ED_{50} 11 mg/kg) was still 7 times as active as chlorimipramine (ED_{25} 31, ED_{50} 73 mg/kg). In all three experiments showing inhibition of NA uptake the monomethylamino derivatives of the tricyclic thymoleptics were more potent than the dimethyl-

amino derivatives in contrast to the effect on 5-HT uptake. Lu 10-171 and its metabolites were all without effect, whereas both chlorimipramine and especially its metabolite chlordesipramine had marked effect on NA uptake. Chlordesimipramine had the same potency as imipramine and amitriptyline, whereas chlorimipramine was 3–8 times less potent in the two in vivo tests (Table 1, fifth and sixth columns).

The inhibition of ^{14}C-5-HT uptake in blood platelets in vitro caused by Lu 10-171 was also seen in blood platelets derived from dogs dosed orally with Lu 10-171 tablets (0.14, 0.4, 1.6, or 3.2 mg/kg). Lu 10-171 caused a dose-dependent inhibition of ^{14}C-5-HT uptake (Fig. 4), with maximal effect within the first 2 h (Fig. 5) and with a tendency that the lower doses showed maximal effect earlier than the higher ones. The lowest dose tested (0.14 mg/kg p.o.) caused a 45% reduction of ^{14}C-5-HT uptake, indicating that even lower doses may be effective. An ED_{50}-value of 0.25 mg/kg p.o. was found from the dose-response curve.

Lu 10-171 and its metabolites were all extremely weak inhibitors of DA uptake (Table 2) and in this respect Lu 10-171 was even weaker than chlorimipramine and the other tricyclic thymoleptics. Compared to the potent DA uptake inhibitor, benztropine, Lu 10-171 was 370 times weaker.

Blood platelets lose approximately 15% of preloaded ^{14}C-5-HT after incubation at 37° C for 30 min. This efflux was increased by reserpine, H 75/12, ergotamine, and fenfluramine, all compounds known to deplete 5-HT (results not shown).

Lu 10-171 ($10^{-6} - 10^{-4}$ M) and chlorimipramine ($10^{-6} - 10^{-5}$ M) decreased or kept unchanged the efflux of ^{14}C-5-HT (Table 3), whereas only at higher concentrations (10^{-3} M) the platelets were almost emptied probably due to the lysis of the platelets.

Synaptosomes lose half of their ^{14}C-5-HT content after incubation at 25° C for 30 min. Lu 10-171 (10^{-3} and 10^{-6} M) had no effect or slightly decreased this efflux of ^{14}C-5-HT (Fig. 6).

Tricyclic thymoleptics showed weak or no effect on MAO (Table 4). They were at least 1000 to 10000 times weaker than pargyline and tranylcypromine. Like the tricyclics, Lu 10-171 was devoid of MAO-inhibiting properties at a concentration of 10^{-4} M.

The endogenous level of 5-HT, NA, and DA in rat brain was not influenced by Lu 10-171, Lu 11-109 (DA not determined), or chlorimipramine (Table 5) in doses where the compounds had pronounced effect on 5-HT uptake in rat brain.

Table 2. Effect of Lu 10-171, its metabolites and tricyclic thymoleptics on ^{3}H-DA uptake in rat striatal synaptosomes in vitro

Test compounds	IC_{50} μM
Lu 10-171	41
Lu 11-109 (desmethyl)	26
Lu 11-161 (didesmethyl)	12
Lu 11-305 (N-oxide)	> 100
Chlorimipramine	4.3
Chlordesipramine	2.2
Imipramine	18
Desipramine	9.1
Amitriptyline	5.4
Nortriptyline	3.6
Protriptyline	3.3
Benztropine	0.11

Table 3. Effect of Lu 10-171 and chlorimipramine on the efflux of ^{14}C-5-HT from preloaded rabbit blood platelets

Test compound	Conc. (μM)	Efflux of ^{14}C-5-HT derived from					
		blood platelets			supernatant		
No		100	100	100	100	100	100
Lu 10-171	1000	608**			672**		
	100	99	91	96	88	88*	77*
	10	66	74	79*	75*	71*	65*
	1			80*			69*
Chlorimipramine	1000	828**			750**		
	100	663**	604**		575**	481**	
	10	88	98		69	85*	
	1		80*			74*	

* Efflux less than control $P < 0.05$
** Efflux greater than control $P < 0.05$
Series of 3 experiments with triple determinations were performed. The efflux was calculated in per cent of the efflux in control samples based on radioactivity either in the platelets or in the supernatant

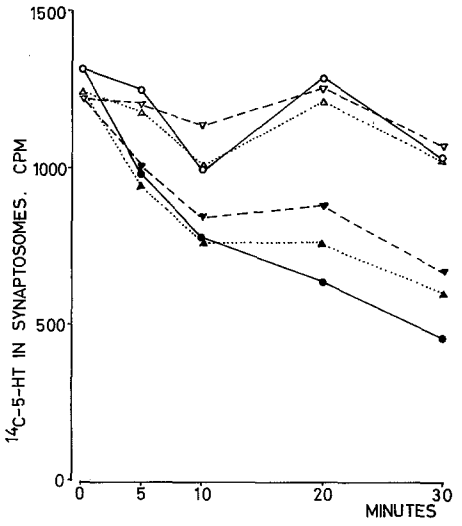

Fig. 6. Effect of Lu 10-171 on the efflux of ^{14}C-5-HT from preloaded rat brain synaptosomes in the presence of 10 µM of 5-HT. Contents of ^{14}C-5-HT in synaptosomes (mean of two determinations) were measured after incubation at 0° C (*upper*) and 25° C (*lower curves*) in the presence of Lu 10-171 (0 µM, ●——●, 1 µM, ▲·····▲, 1 mM, ▼– – – –▼)

Table 4. Inhibition in vitro of MAO derived from rat liver or brain. Comparison between Lu 10-171, tricyclic thymoleptics, and MAO-inhibitors

	IC_{50} µM	
	Liver	Brain
Imipramine	> 206	> 206
Desipramine	> 169	> 169
Amitriptyline	130	135
Nortriptyline	150	150
Chlorimipramine	31	41
Lu 10-171	> 100	> 100
Pargyline	0.037	0.055
Tranylcypromine	0.003	0.003

DISCUSSION

From the results presented it appears that Lu 10-171 is a rather potent inhibitor of 5-HT uptake in both the rabbit blood platelet test and in the H 75/12-test. Before concluding from these results that Lu 10-171 is a potent inhibitor of 5-HT uptake in serotoninergic neurones in the central nervous system some facts must be emphasized.

The blood platelets can be used as a model for serotoninergic neurones because blood platelets contain 5-HT, which they take up from the blood by an active transport mechanism at the level of the platelet membrane. (For reviews see: Pletscher et al., 1971; Lingjaerde, 1971; Cambell and Todrick, 1973). In the platelets 5-HT is stored in specific subcellular organelles, by which the amine is protected from metabolizing enzymes, i.e., MAO. The storage of 5-HT in platelets resembles in many respects that of other biogenic amines in various neurones (Pletscher et al., 1971). Like the uptake of 5-HT in cerebral slices (Carlsson, 1970), the uptake in blood platelets is inhibited by tricyclic antidepressants (Todrick and Tait, 1969; Ahtee and Saarnivaara, 1971; Tuomistro, 1974), and the order of potencies is identical. Thus, although the uptake and storage processes in platelets and in neurones may not be identical in every respect, the similarities mentioned above are so great that it is warranted to use the platelets as a tentative model of serotoninergic neurones.

Concerning the 5-HT depletion test, it was pointed out by Carlsson et al. (1969a), that H 75/12 causes displacement of monoamines owing to a high affinity for the amine uptake mechanism and to its resistance to MAO. The depletion of 5-HT caused by H 75/12 could be prevented by thymoleptic compounds by inhibiting the uptake of H 75/12 into 5-HT neurones. This was demonstrated biochemically by measuring the 5-HT content in brain and histochemically by examining brain slices using the fluorescence micro-

Table 5
Effect of chlorimipramine, Lu 10-171, and Lu 11-109 on the endogenous levels of 5-HT, NA, and DA in rat brain 140 min after treatment. Figures represent the mean ± S.D. (ng amine/g brain) of (*n*) determinations

All $P > 0.05$ between treated and control groups

	mg/kg s.c.	5-HT	NA	DA
Control	0	322 ± 52 (6)	373 ± 33 (8)	
Chlorimipramine	1.25	336 ± 24 (4)	386 ± 24 (4)	
Control	0		407 ± 39 (11)	558 ± 13 (6)
Chlorimipramine	5		374 ± 34 (9)	584 ± 47 (5)
Lu 10-171	5		397 ± 16 (9)	509 ± 88 (6)
Control	0	343 ± 52 (8)		
Lu 10-171	2.5	319 ± 48 (8)		
Control	0	299 ± 25 (6)	383 ± 33 (4)	
Lu 11-109	160	299 ± 22 (5)	338 ± 31 (4)	
	10	304 ± 24 (5)	339 ± 52 (4)	

scope technique (Carlsson et al., 1969a; Andén et al., 1969). By examining the effect of tricyclic thymoleptics Carlsson et al. (1969a) found that the secondary amines were weaker than the corresponding tertiary amines and that chlorimipramine was the most potent compound in this test.

This experimental evidence allows the conclusion that compounds active in the two tests – the blood platelet test and the H 75/12 test – are active inhibitors of 5-HT uptake in serotoninergic neurones.

That the inhibition of 5-HT uptake seen in the present study is real and not a consequence of an increased efflux of 5-HT initiated by the test compounds is seen from the efflux experiments (Table 3 and Fig. 6) in which Lu 10–171 did not increase but rather slightly decreased the efflux of ^{14}C-5-HT both from preloaded blood platelets and synaptosomes. The uptake of ^{14}C-5-HT into blood platelets was saturable with a K_m-value of 1.5×10^{-7} M. Lu 10-171 inhibited this uptake competitively (Fig. 2) with a K_i-value of 1.9×10^{-9} M. In this respect Lu 10-171 resembles the tricyclic thymoleptics (Tuomistro, 1974) as also shown by parallel dose-response curves (Fig. 3).

The activities of the compounds in the two tests for 5-HT uptake were not strictly parallel, probably due to different abilities to cross the blood-brain-barrier, to differential distribution in the central nervous system, and finally due to differential metabolism and excretion rates. The tertiary amines of the tricyclics were more potent than the corresponding secondary amines, which has previously been shown (Carlsson et al., 1969a; Carlsson, 1970; Lidbrink et al., 1971). This was also true for Lu 10-171 and its demethylated metabolite, Lu 11-109, whereas the primary amine (Lu 11-161) was without effect in vivo. Orally Lu 10-171 and chlorimipramine were less active than after subcutaneous injection, but the proportion between their effects is still in the order of 10. The in-vivo activity of the N-oxide, Lu 11-305, may be ascribed to transformation to the tertiary amine, Lu 10-171, since the N-oxide had almost no effect in vitro, where it is probable that no transformation takes place.

The ability of the mouse heart and with that the atria to take up and concentrate noradrenaline is due to the abundance of adrenergic neurones (Sachs, 1970; Jonsson and Sachs, 1971; Carlsson et al., 1969b; Sachs and Jonsson, 1972). Like H 75/12, the compound H 77/77 passes the blood-brain-barrier and is concentrated in monoaminergic neurones by the membrane pump (Carlsson et al., 1969b). The inhibition by thymoleptics of the NA depletion caused by H 77/77 is due to inhibition of the amine pump, which was demonstrated biochemically and histochemically by Carlsson et al. (1969b) and Andén et al. (1969), respectively. The effect of the compounds in the three tests (Table 1, columns 4 to 7) is therefore due to inhibition of the amine-pump in noradrenergic neurones either in the peripheral or in the central nervous system.

The results in the three tests are in good agreement. In the tricyclic series the monomethylamino derivatives are more potent than the corresponding dimethyl derivatives – the opposite to that found for inhibition of 5-HT uptake. This has previously been confirmed by many investigators (Carlsson et al., 1969b; Maxwell et al., 1970, 1971; Salama et al., 1971; Lidbrink et al., 1971; Frisk-Holmberg, 1972). Chlorimipramine, although weaker than the other dimethylamino-thymoleptics, possessed relatively good NA uptake inhibiting properties, and the metabolite of chlorimipramine, chlordesipramine (Mellström and Eksborg, 1976), which at steady state conditions is found in higher concentrations in plasma than the parent compound, possessed even greater activity than chlorimipramine itself. Lu 10-171 and its metabolites clearly deviated from the known thymoleptics by being without any effect on Na uptake. The small effects observed in the atria-test were found in unphysiological concentrations and cannot have any significance in vivo as also shown in the two other NA-tests.

The selectivity of Lu 10-171 and its metabolites on 5-HT uptake is further strengthened by their very poor effect on ^{3}H-DA uptake in synaptosomes (Table 2). Lu 10-171 were 10 times and the desmethylated metabolites 3–6 times weaker than chlorimipramine. Compared with the potent DA uptake inhibitor, benztropine, Lu 10-171 is 370 times weaker, and the tricyclic thymoleptics 30–200 times weaker in accordance with results published by Horn et al. (1971).

When dogs were treated with Lu 10-171 orally, inhibition of ^{14}C-5-HT uptake in isolated blood platelets was observed in vitro with peak effect after approximately 2 h, which is also the time where maximal plasma concentration of Lu 10-171 is found (Fredericson Overø, personal communication). From the data of Fredricson Overø, plasma levels of 7×10^{-7} to 3×10^{-8} M after doses of 3.2 to 0.14 mg/kg are attained, and with these concentrations inhibition of uptake in blood platelets from 100 to 70% would be expected from the rabbit blood platelets data and this is roughly what was found in the dogs. Since central effect (tryptophan potentiation in rats) is also found (Christensen et al., 1977) at those blood concentrations, the right dosing or blood niveau of Lu 10-171 may be monitored by measuring ^{14}C-5-HT uptake in blood platelets isolated from patients in treatment with Lu 10-171.

Like the tricyclic thymoleptics, Lu 10-171 showed so weak MAO-inhibiting properties in vitro (Table 4) that the effect must be considered to have no influence in vivo, as also expressed in unchanged monoamine levels in brain (Table 5) after Lu 10-171 or chlorimipramine.

Due to the abundance of 5-HT at postsynaptic receptors after inhibition of reuptake of 5-HT, a decrease in the firing and synthesis rate of 5-HT neurones would be expected (Aghajanian, 1972; Schubert et al., 1970). Indeed, when given to rats Lu 10-171 causes a reduction of 5-HT turnover (Hyttel, 1977). This was shown by a lowered level of the 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA), by a reduction in 5-HIAA accumulation after probenecid, and finally by a decreased fall in 5-HT and an increased fall in 5-HIAA after inhibiton of 5-HT synthesis by parachlorophenylalanine (PCPA) after treatment with Lu 10-171.

The biochemical effects have their correlate to the pharmacological effects of the compound (Christensen et al., 1977). Lu 10-171 is a very strong potentiator of 5-HT in the central nervous system as shown by its ability to potentiate the behavioral effects of 5-hydroxytryptophan (5-HTP) in mice and tryptophan in mice and rats. In agreement with its lack of inhibitory effect on NA uptake, Lu 10-171 was a very weak antagonist to reserpine- and tetrabenazine-induced ptosis and immobility.

The evidence presented here shows that Lu 10-171 is a very potent and completly selective inhibitor of the 5-HT reuptake mechanism, and does not share the inhibitory effect on NA reuptake known for tricyclic thymoleptics or the MAO inhibiting effect of MAO inhibitors used earlier in treatment of depression. Furthermore, Lu 10-171 is shown to have very weak anticholinergic and antihistaminergic properties compared to tricyclic antidepressants (Christensen et al., 1977).

Therefore, Lu 10-171 should be a useful tool in studying the role of central 5-HT neurone systems in experimental neuropharmacology as well as in the etiology of depressive illness.

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