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EFFECTS OF 4-(3-INDOLYL-ALKYL) PIPERIDINE DERIVATIVES ON BRAIN 5-HYDROXYTRYPTAMINE TURNOVER AND ON CARDIAC AND BRAIN NORADRENALINE OR 5-HYDROXYTRYPTAMINE DEPLETION INDUCED BY 6-HYDROXYDOPAMINE, H 75/12 AND 4-CHLOROAMPHETAMINE

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Abstract—4-(3-Indolyl-alkyl)piperidine derivatives (LM 5005, LM 5008 and LM 5015), like clomipramine, had no or little effects on brain 5-HT but reduced brain 5-HIAA levels and 5-HT turnover, after single or chronic treatment. Brain tryptophan levels remained unaffected by clomipramine, were slightly decreased by LM 5008 and increased by LM 5005 and LM 5015. The piperidine derivatives counteracted brain 5-HT depletion induced by H 75/12 and 4-chloroamphetamine more effectively than clomipramine, after i.p. or p.o. administration. On the contrary clomipramine was more effective in inhibiting cardiac NA depletion induced by 6-OH-dopamine than LM 5015; LM 5008 and LM 5005 were ineffective. The potential therapeutic effects of such compounds in mental diseases are discussed.

In a previous paper [1], 4-(3-indolyl-alkyl)piperidine derivatives were shown to be strong inhibitors of the uptake of 5-hydroxytryptamine (5-HT) by rat brain synaptosomes *in vitro* and *in vivo* and to possess a weaker inhibitory activity on the uptake of noradrenaline (NA) and dopamine (DA).

The mode of action of these compounds is, in spite of their higher selectivity, so similar to that shown by tricyclic antidepressive drugs that it suggests some identical effects on brain biogenic amine turnover [2, 3, 4] and on experimentally induced depletion of brain or cardiac monoamines [5, 6, 7].

This was to be ascertained, and the following report describes the activity of three 4-(3-indolyl-alkyl)piperidine derivatives (LM 5005, LM 5008 and LM 5015) and clomipramine on brain 5-HT turnover and on cardiac or brain monoamine depletion induced by 6-hydroxydopamine (6-OHDA), 4-methyl-α-ethylmetatyramine (H 75/12) and 4-chloroamphetamine (pCA).

MATERIAL AND METHODS

All reagents used were fluorometric grade. Levels of 5-HT, 5-hydroxyindole-acetic acid (5-HIAA), NA and tryptophan were determined respectively by the methods of Curzon *et al.* [8], Miller *et al.* [9], Denckla *et al.* [10] with slight modifications, it having been verified that there was no interference of the compounds tested in these fluorometric assays. Male rats of 200 g and male mice of 30 g, obtained from Charles River, were used for the experiments; they had free access to food and water. The drugs were administered as aqueous solutions of their hydrochloride salts.

Turnover of brain 5-HT. The rats were injected i.p. with the drugs (25 mg/kg). Fifteen min later, treated and control rats received probenecid (200 mg/kg i.p.). At intervals (0, 30 min, 60 min, 90 min, 120 min after probenecid) the animals were decapitated and the brains were removed and analyzed for 5-HT and 5-HIAA. Three determinations were made for each time and treatment. The values for brain 5-HIAA were transformed logarithmically for linear regression analysis. 5-HT turnover rates and times were calculated according to the method of Neff *et al.* [11, 12]. Brain 5-HT turnover was also studied after chronic LM 5008 and clomipramine treatment (10 days, 25 mg/kg i.p.).

Antagonism of H 75/12 induced depletion of brain 5-HT and NA. The rats (groups of 5) received H 75/12 (25 mg/kg i.p.) 2 and 4 hr before they were killed. The drugs were administered 30 min (i.p.) or 90 min (p.o.) before the first injection of H 75/12.

Antagonism of pCA induced depletion of brain 5-HT. The drugs were administered 15 min (i.p.) or 90 min (p.o.) before pCA (10 mg/kg i.p.). The rats (groups of 5) were killed 4 hr after pCA injection.

In another experiment pCA was injected 6 hr before sacrifice and the drugs (10 mg/kg i.p.) were administered 3 hr after pCA.

Antagonism of 6-OHDA induced depletion of cardiac NA. The mice (groups of 5) received 6-OHDA (7 mg/kg i.p.) 16 hr before they were killed. The drugs were injected 1 hr before 6-OHDA.

RESULTS

Effects on 5-HT, 5-HIAA and tryptophan levels in rat brain. After a single injection (25 mg/kg i.p.) the drugs tested had no or little effects on brain 5-HT but lowered brain 5-HIAA (Table 1). After chronic administration (10 days) clomipramine and LM 5008

Table 1. Effects of LM 5005, LM 5008, LM 5015 and clomipramine on brain 5-HT, 5-HIAA and tryptophan

Treatment			5-HT ng/g tissue (mean ± S.D.)	5-HIAA ng/g tissue (mean ± S.D.)	Tryptophan ng/g tissue (mean ± S.D.)
Control			400 ± 18*	416 ± 19	4000 ± 100
Clomipramine	Single	15 mn	342 ± 18*	362 ± 38	3800 ± 230
		45 mn	303 ± 17†	332 ± 18*	...
		75 mn	349 ± 23	316 ± 17†	...
	Chronic	15 mn	396 ± 23	254 ± 28†	—
LM 5008	Single	15 mn	350 ± 25	326 ± 5†	3570 ± 100*
		45 mn	310 ± 50	322 ± 9†	—
		75 mn	347 ± 7†	319 ± 24†	...
	Chronic	15 mn	447 ± 35	289 ± 21†	...
LM 5005	Single	15 mn	430 ± 10	349 ± 18*	4800 ± 220*
		45 mn	380 ± 42	294 ± 18†	—
		75 mn	380 ± 12	322 ± 9†	—
LM 5015	Single	15 mn	333 ± 13†	289 ± 10‡	4900 ± 230†
		45 mn	397 ± 9	263 ± 5‡	—
		75 mn	389 ± 18	270 ± 22†	—

Groups of 5 rats were injected intraperitoneally with the drugs (25 mg/kg) in single or repetitive (10 days) treatment. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

had no effect on 5-HT levels but decreased still more strongly 5-HIAA.

Brain tryptophan level remained unaffected by clomipramine; it was slightly decreased by LM 5008 and increased by LM 5005 and LM 5015.

Effects on brain 5-HT turnover. Injection of probenecid was followed by a linear increase ($r = 0.98$) in 5-HIAA level in rat brain. The turnover rate was in agreement with those found by different authors [4, 13]. The animals pretreated with the drugs accumulated 5-HIAA more slowly than controls (Table 2), the differences in slopes being statistically significant ($P < 0.001$). The decrease in 5-HT turnover caused by the different compounds was more pronounced with the 4-(3-indolyl-alkyl)piperidine derivatives than with clomipramine. After chronic administration of LM 5008 or clomipramine there was a greater decrease in the turnover rate than after a single dosage with these drugs.

Antagonism of H 75/12 induced depletion of brain 5-HT and NA. When the animals were treated with H 75/12 alone, brain 5-HT level decreased to about half the normal value whereas NA level decreased to a less extent. After injection of the compounds under study no antagonizing effect was observed on NA depletion, while 5-HT depletion was prevented by LM 5008 and to a lower degree by LM 5005, LM 5015 and clomipramine (Table 3). After oral administration the 4-(3-indolyl-alkyl)piperidine derivatives counteracted 5-HT depletion, whereas clomipramine remained ineffective until 50 mg/kg.

Antagonism of pCA induced depletion of brain 5-HT. pCA is a specific depletor of brain 5-HT. After i.p. injection all the drugs tested prevented its effect. The order of activity was: LM 5008 > LM 5015 > LM 5005 > clomipramine (Table 4). Orally, clomipramine, as in the case of H 75/12, was inactive up to 50 mg/kg, whereas the piperidine derivatives reversed pCA induced depletion.

In another experiment the drugs were injected 3 hr

Table 2. Influence of LM 5005, LM 5008, LM 5015 and clomipramine on turnover rates and turnover times of rat brain 5-HT, measured from 5-HIAA decline after probenecid administration

Treatment		Turnover rate of 5-HT (mcg/g/h)	Turnover time (mn)
Control		0.218 ± 0.027	113
Clomipramine	Single	0.105 ± 0.011	247
	Chronic	0.082 ± 0.016	290
LM 5008	Single	0.055 ± 0.014	488
	Chronic	0.043 ± 0.014	625
LM 5005	Single	0.062 ± 0.016	305
LM 5015	Single	0.050 ± 0.012	500

The drugs (25 mg/kg i.p.) in single or chronic (10 days) treatment were injected 15 min before probenecid (200 mg/kg i.p.) and the rats in groups of 5 were sacrificed 30, 60, 90 and 120 min later.

Table 3. Antagonism of H 75/12 induced depletion of brain 5-HT by LM 5005, LM 5008, LM 5015 and clomipramine

Treatment	Dose mg/kg i.p.	Brain 5-HT ng/g tissue (mean ± S.D.)	Inhibition %	ED_{50} mg/kg	Dose mg/kg p.o.	Brain 5-HT ng/g tissue (mean ± S.D.)	Inhibition %	ED_{50} mg/kg
Control		469 ± 12				621 ± 28		
H 75/12		263 ± 11				385 ± 13		
Clomipramine	25	429 ± 40	84	12	50	416 ± 12	15	> 50
	10	352 ± 26	43		25	379 ± 6	0	
	2.5	244 ± 7	0					
LM 5005	25	399 ± 40	76	5	50	602 ± 37	92	35
	10	389 ± 16	61		25	397 ± 19	7	
	2.5	342 ± 10	39					
LM 5008	25	539 ± 47	100	0.4	50	546 ± 25	69	40
	10	456 ± 9	94					
	2.5	436 ± 19	84		25	422 ± 25	14	
	0.25	368 ± 16	50					
	0.10	300 ± 7	18					
LM 5015	25	497 ± 34	100	5	50	534 ± 43	63	45
	15	460 ± 23	98					
	10	375 ± 20	55		25	379 ± 31	0	
	2.5	328 ± 9	32					

The rats in groups of 5 received H 75/12 (25 mg/kg i.p.) 2 and 4 hr before they were killed. Drugs were administered 30 min (i.p.) or 90 min (p.o.) before the first injection of H 75/12.

after pCA (Fig. 1). With pCA alone, the depletion was time dependent and greater at 6 hr than at 3 hr. The groups of rats which received LM 5005 and LM 5008 showed 5-HT levels similar to the control values, demonstrating a complete inhibition of the depleting effect of pCA by these compounds. This effect was only partially antagonized by LM 5015 and clomipramine.

Antagonism of 6-OHDA induced depletion of cardiac NA. 6-OHDA induced a large depletion of cardiac NA. Clomipramine prevented this depletion with an ED_{50} of 7.5 mg/kg (Table 5). LM 5015 was a weaker

Table 4. Antagonism of 4-chloroamphetamine (pCA) induced depletion of brain by LM 5005, LM 5008, LM 5015 and clomipramine

Treatment	Dose mg/kg i.p.	Brain 5-HT ng/g tissue (mean ± S.D.)	Inhibition %	ED_{50} mg/kg	Dose mg/kg p.o.	Brain 5-HT ng/g tissue (mean ± S.D.	Inhibition %	ED_{50} mg/kg
Control		563 ± 20				519 ± 16		
4-Chloroamphetamine		272 ± 18				267 ± 8.9		
Clomipramine	50	647 ± 39	100	37	50	291 ± 33	15	> 50
	25	372 ± 56	37					
	10	315 ± 28	19					
LM 5005	25	777 ± 56	100	14	50	380 ± 36	50	55
	15	434 ± 14	54		30	311 ± 19	19	
	5	360 ± 51	27		20	317 ± 10	15	
	3	372 ± 28	31					
LM 5008	5	473 ± 34	70	3	50	550 ± 36	100	21
	3	394 ± 39	44		30	489 ± 24	87	
	1	310 ± 79	17		20	369 ± 12	42	
LM 5015	15	524 ± 23	85	8	50	566 ± 29	100	27
	5	349 ± 28	22		30	405 ± 28	55	
	3	310 ± 28	8		20	317 ± 22	16	

Drugs were administered 15 min (i.p.) or 90 min (p.o.) before the i.p. injection of pCA (10 mg/kg). The rats in groups of 5 were killed 4 hr after pCA.

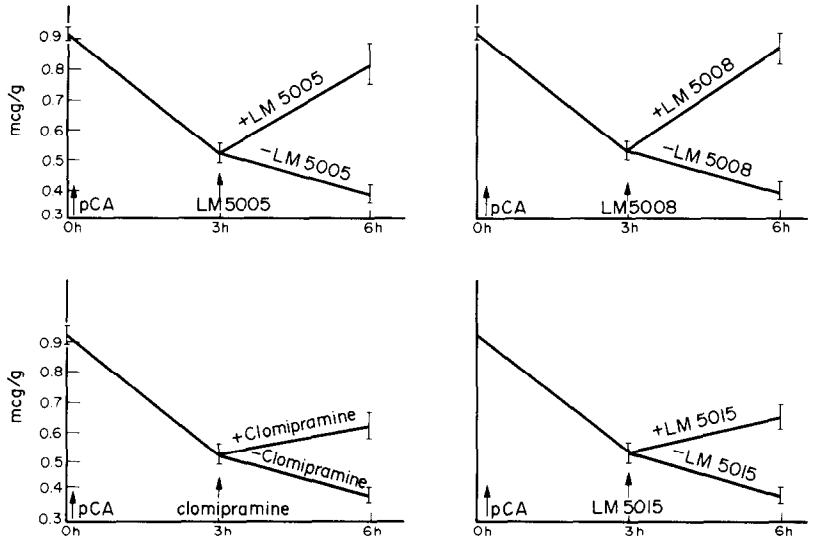

Fig. 1. Reversal effect of LM 5005, LM 5008, LM 5015 and clomipramine on the depletion of brain 5-HT induced by 4-chloroamphetamine (pCA). pCA (10 mg/kg i.p.) was injected 6 hr before the sacrifice and the drugs (10 mg/kg i.p.) 3 hr after pCA.

Table 5. Antagonism of 6-hydroxydopamine (6-OHDA) induced depletion of cardiac NA

Treatment	Dose mg/kg i.p.	Cardiac NA ng/g tissue (mean ± S.D.)	Inhibition %	ED_{50} mg/kg
Control		324 ± 11		
6-Hydroxydopamine		134 ± 7		
Clomipramine	5	221 ± 11	42	
	30	271 ± 15	72	7.5
	50	276 ± 23	75	
LM 5005	10	130 ± 12	0	0% at
	50	99 ± 14	0	50
LM 5008	10	117 ± 3	0	0% at
	50	128 ± 12	0	50
LM 5015	10	157 ± 8	12	
	30	191 ± 7	30	40
	50	269 ± 22	71	

The mice in groups of 5 received 6-OHDA (7 mg/kg i.p.) 16 hr before they were killed. Drugs were injected 1 hr before 6-OHDA.

inhibitor (ED_{50} = 40 mg/kg). In contrast LM 5005 and LM 5008 were ineffective at 50 mg/kg i.p.

DISCUSSION

4-(3-indolyl-alkyl)piperidine derivatives, like clomipramine, had no or little effects on brain 5-HT level but lowered brain 5-HIAA after single or chronic treatment. These results suggest that these derivatives decrease the brain 5-HT turnover but do not present any MAOI activity since otherwise a rise in the 5-HT level would have been observed after chronic treatment (10 days). The reduction of brain 5-HT turnover secondary to the inhibition of 5-HT uptake from the synaptic cleft may be due either to an increased stimulation of a presynaptic receptor or to a transsynaptic feedback mechanism.

LM 5005 and LM 5015 increased the brain tryptophan level. There are different possible mechanisms to explain their effect: displacement of plasmatic tryptophan from its specific binding proteins [14], increase in tryptophan uptake by the brain [15] or inhibition of hepatic tryptophan pyrrolase [16].

It is well known that H 75/12 and pCA are actively concentrated, like biogenic amines, inside the neurons by an uptake\mechanism located at the cell mem-

brane (membrane pump). Moreover, they have a high affinity for the intraneuronal synaptic vesicles. The injection of these compounds will lead to a displacement of the intraneuronal monoamines and thus entail a depletion. An inhibitor of the membrane pump should prevent such depletion. Thus, the antagonism of pCA or H 75/12 induced depletion of brain 5-HT is well representative of a 5-HT uptake inhibitory activity *in vivo*. This study confirms that 4-(3-indolyl-alkyl)piperidine derivatives possess a greater potency in inhibiting 5-HT uptake, after intraperitoneal or oral administration, than clomipramine which had a long time been considered as the most effective 5-HT uptake inhibitor.

The ED_{50} for antagonizing 6-OHDA induced depletion of cardiac NA are very comparable with those for inhibiting NA uptake by rat heart [1]. Clomipramine, which was less active than the piperidine derivatives in inhibiting 5-HT uptake, was also a potent inhibitor of NA uptake in rat heart *in vivo* and proved more effective in this respect than these compounds. These results demonstrate that 4-(3-indolyl-alkyl)piperidine derivatives inhibit *in vivo* the uptake of 5-HT with a much greater selectivity than clomipramine, hitherto recognized as the most specific reference product.

Because of this selectivity, these compounds might contribute to elucidate some brain 5-HT dependent mechanisms.

Considerable biochemical evidence relates tryptaminergic neurons to the regulation of different mental activities. For instance it has been suggested that there may be some dysfunction of indoleamine metabolism in depressive states [17, 18]. There is a decrease in the 5-HT and 5-HIAA levels in the brains of severely depressive patients having committed suicide [19, 20]. A diminished 5-HIAA response to probenecid has also been found in patients with endogenous depressions [21, 22]. Some workers have distinguished several components in the clinical actions of antidepressants, such as mood elevation, which is related to the inhibition of 5-HT uptake, and psychomotor activation, which is NA dependent [23, 24]. In the light of these findings it may be considered that 4-(3-indolyl-alkyl)piperidine derivatives constitute potential antidepressive drugs.

From another point of view, considering that destruction of tryptaminergic neurons of raphe nucleus [25] or inhibition of tryptophan hydroxylase by *p*-chlorophenylalanine [26] provokes insomnia, it may be postulated that 4-(3-indolyl-alkyl)piperidine derivatives could exert some effect on sleep and in particular suppress, like clomipramine [27], paradoxical sleep.

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