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Effect of a new series of bicyclic compounds with potential thymoleptic properties on the reserpine-resistant uptake mechanism of central and peripheral monoamine neurones *in vivo* and *in vitro*

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1. Bicyclic compounds with potential thymoleptic properties (Lu-compounds) have recently become available, and their effects on the membrane pumps of the central and peripheral monoamine neurones have now been tested and compared with those of the tricyclic antidepressant drugs.

2. Biochemical and histochemical *in vivo* studies have been performed. The possible blocking action of Lu-compounds on the noradrenaline (NA) and 5-hydroxytryptamine (5-HT) displacement caused by 4,α-dimethyl-meta-tyramine (H 77/77) and 4-methyl-α-ethyl-meta-tyramine (H 75/12), respectively, has been studied, and a positive result has been taken as evidence for membrane pump blocking activity. No certain effects were obtained on the 5-HT displacement induced by H 75/12, whereas a partial blockade of the NA displacement by H 77/77 in central NA neurones was obtained after most of the Lu-compounds (Lu-3-010, 3-049, 3-092, 4-012) and especially after the thiophthalane derivative Lu 5-003. The ED50 of the latter drug was around 8 mg/kg, that is, somewhere between protriptyline (ED50 4 mg/kg) and desipramine (ED50 15 mg/kg) in potency.

3. Histochemical *in vivo* studies on the rat iris revealed that Lu 5-003 and especially the corresponding phthalane derivative Lu 3-010 were potent in blocking the uptake of α-methyl-NA in the adrenergic nerve terminals of the iris. The other Lu-compounds were less active. The releasing effects of the Lu-compounds on the extragranular accumulation of α-methyl-NA in the adrenergic terminals were weak compared with membrane blocking activity.

4. *In vitro* studies on the central and peripheral catecholamine (CA) neurones have also been performed. In the same way as, for example, protriptyline the Lu-compounds only blocked accumulation of α-methyl-NA in the NA terminals but not in the dopamine (DA) nerve terminals. Lu 5-003 and Lu 3-010 were the most potent of the Lu-drugs when added *in vitro*. The Lu-drugs were also injected *in vivo* after which the effect on the α-methyl-NA accumulation was studied *in vitro*. In isotope experiments with labelled α-methyl-NA it was found that desipramine, Lu-3-010, Lu 3-092 and Lu 4-012 were equally potent in blocking uptake in the central nervous system.

5. In general the results obtained with the various models are in agreement. The high activity of Lu 5-003 on the central NA neurones may be related to its high heptane/H_2O distribution coefficient.

6. It is concluded that the Lu-compounds have a selective action on the membrane pump mechanism of the central and peripheral NA neurones. In these respects they behave like desipramine and protriptyline.

An interesting series of bicyclic compounds with pharmacological properties similar to those of tricyclic thymoleptics has recently been described (Petersen, Lassen, Hansen, Huld, Hjortkjaer, Holmblad, Møller Nielsen, Nymark, Pedersen, Jørgensen & Hougs, 1966; Petersen & Møller Nielsen, 1967; Møller Nielsen, 1967). In previous publications we have investigated the ability of the latter group of drugs to block the reserpine-resistant amine uptake by central and peripheral monoamine neurones (see, for example, Malmfors, 1965; Carlsson & Waldeck, 1965; Carlsson, Fuxe, Hamberger & Lindqvist, 1966; Hamberger, 1967; Carlsson, Corrodi, Fuxe & Hökfelt, 1969a, b). In the present paper the effects of some of the new group of compounds on these uptake mechanisms are investigated.

Methods

The bicyclic compounds investigated are shown in Table 1. Male Sprague-Dawley rats (150–250 g body weight) and mice of the NMRI strain have been used. In the *in vivo* studies the following procedures have been utilized to test the effect of the bicyclic compounds on the reserpine-resistant uptake mechanism of the central DA and NA neurones biochemically and histochemically. The rats or the mice were treated twice with a 2 hr interval with 4,α-dimethyl-*m*-tyramine (H 77/77, 12.5 mg/kg, intraperitoneally), which can enter the brain and displace the intraneuronal DA and especially NA stores (Carlsson & Corrodi, unpublished data). H 77/77 apparently utilizes the reserpine-resistant uptake mechanism of the catecholamine neurones and can thus be used to examine drugs for a blocking

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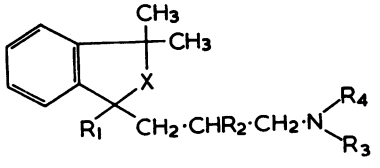
TABLE 1. *Structure of the bicyclic compounds investigated*



	X	R_1	R_2	R_3	R_3
Lu 5–003	S	C_6H_5	H	H	CH_3
Lu 4–074	S	C_6H_5	H	CH_3	CH_3
Lu 3–010	O	C_6H_5	H	H	CH_3
Lu 3–009	O	C_6H_5	H	CH_3	CH_3
Lu 3–049	CH_2	C_6H_5	H	H	CH_3
Lu 4–012	O	CN	H	H	CH_3
Lu 3–092	O	CN	H	CH_3	CH_3
Lu 3–048	O	C_6H_5	CH_3	CH_3	CH_3
Lu 4–004	O	C_6H_5	H	H	H
Lu 3–047	CH_2	C_6H_5	H	CH_3	CH_3

action on this mechanism centrally as well as peripherally. Thus it has been shown that potent thymoleptics—for example, protriptyline and desipramine—are able to block the displacement in the NA but not in the DA neurones (Carlsson *et al.*, 1969a). Each drug was injected intraperitoneally 30 min before each H 77/77 injection and at the time of the second injection only half of the dose was given. The animals were killed by decapitation 2 hr after the last H 77/77 injection. The brains of the rats were taken for histochemical analysis of DA, NA and 5-HT, using the procedure previously described (Dahlström & Fuxe, 1964 ; Hamberger, Malmfors & Sachs, 1965 ; Fuxe & Jonsson, 1967, see also Carlsson *et al.*, 1969a), while the brains of the mice were taken for biochemical analysis of DA (Carlsson & Waldeck, 1958 ; Carlsson & Lindqvist, 1962) and NA (Bertler, Carlsson & Rosengren, 1958).

In a few experiments the amine uptake by central 5-HT neurones was investigated using the same technique, using α-ethyl-4-methyl-metatyramine (H 75/12) as the displacing agent (see Carlsson *et al.*, 1969b). In the mouse experiments two doses of 100 mg/kg each were injected intraperitoneally, whereas in the rat experiments two doses of 25 mg/kg were used. The injection schedule was the same as in the H 77/77 experiments.

The effect on the reserpine-resistant uptake mechanism of the peripheral NA neurones was tested on the iris (see Malmfors, 1965). The drugs were injected 30 min before an intravenous dose of α-methyl-NA (Corbasil®, Hoechst) 0.1 mg/kg, given 15 min before killing. The duration of the blockade was tested by giving the drugs 30 min, 8 and 16 hr before the α-methyl-NA injection (0.1 mg/kg intravenously 15 min before killing) to rats also given reserpine (10 mg/kg, intraperitoneally, 12–14 hr before killing).

In a third type of experiment the releasing action of the agents on extragranular amines has been compared by giving these drugs in an intraperitoneal dose of 25 mg/kg (for (+)-amphetamine 5 mg/kg) 15 min after an α-methyl-NA injection (0.1 mg/kg intravenously) to reserpine-pretreated rats (10 mg/kg, intraperitoneally, 12–16 hr before killing). The rats were killed 30 min, 2, 4 and 8 hr after the α-methyl-NA injection. Immediately after decapitation, stretch-preparations were made of the iris which were reacted with formaldehyde gas as previously described by Malmfors (1965). The subjective determination of fluorescence intensity has recently been shown to be well correlated to quantitative measurements (Olson, Hamberger, Jonsson & Malmfors, 1968).

In the *in vitro* experiments both histochemical and isotope techniques were used and the studies performed on cerebral cortex slices, vas deferens slices and isolated iris. The drugs were either injected intraperitoneally 3 and 1 hr before killing with only half the dose the second time or added *in vitro* to the incubation medium to give concentrations of 0.03–300 μg/ml. 15 min before the addition of the amine. In the histochemical experiments all the rats were pretreated with reserpine (10 mg/kg, intraperitoneally, 12–14 hr before killing) and α-methyl-NA was added to the incubation medium to give a final concentration of the amine from 0.03 to 1 μg/ml. After incubation for 30 min the slices were prepared for fluorescence histochemistry. For further details on the *in vitro* technique in combination with histochemistry, see Hamberger (1967). In the isotope experiments both untreated and reserpine-pretreated rats were used. In the former case ^{3}H-NA (5 c/m-mole, New England Nuclear Co.) was used while in the latter case ^{3}H-α-methyl-NA (40 mc/m-mole, AB Hässle, Göteborg, Sweden) was used. After the incubation with

labelled amine the tissue was incubated in buffer without amine for 10 min at 37° C. The tissue was then homogenized in *n*-butanol, an aliquot of the butanol added to a toluol-ethanol scintillator and counted in a Packard liquid scintillation counter (see Jonsson, Hamberger, Malmfors & Sachs, 1969).

Results

Biochemical in vivo *studies on central CA and 5-HT neurones*

The biochemical results are summarized in Tables 2 and 3. All the compounds investigated caused a partial blockade of the NA displacement induced by

TABLE 2. *Effect of bicyclic derivatives on tissue noradrenaline (NA) depletion by* 4,α*-dimethyl-meta-tyramine (H* 77/77)

Treatment	First dose (mg/kg)	Brain NA (μg/g)		Heart NA (μg/g)		% Inhibition	
		Drug alone	Drug+H 77/77	Drug alone	Drug+H 77/77	Brain	Heart
None		0·41 (24) ±0·010	0·18 (18) ±0·004	0·62 (23) ±0·025	0·13 (18) ±0·008		
Lu 5–003	12·5	0·32 (1)	0·29 (2) ±0·035	0·50 (2) ±0·035	0·49 (2) ±0·075	79	97
	6·25	0·42 (5) ±0·031	0·27 (5) ±0·018	0·64 (5) ±0·060	0·42 (5) ±0·072	38	57
	3·13	0·41 (3) ±0·024	0·22 (3) ±0·019	0·76 (3) ±0·029	0·37 (3) ±0·035	17	38
	1·56	0·36 (2) ±0·015	0·17 (2) ±0·020	0·44 (2) ±0·035	0·26 (2) ±0·045	0	42
Lu 3–010	50	0·31 (2) ±0·045	0·27 (3) ±0·032	0·57 (2) ±0·055	0·48 (3) ±0·072	69	80
	25	0·33 (1)	0·24 (1)	0·63 (1)	0·39 (1)	40	52
	12·5	0·38 (2) ±0·060	0·25 (2) ±0·030	0·51 (2) ±0·045	0·41 (2) ±0·020	35	74
	6·25	0·36 (1)	0·21 (1)	0·53 (1)	0·39 (1)	17	65
	3·13	0·44 (2) ±0·055	0·21 (2) ±0·015	0·48 (2) ±0·030	0·33 (2) ±0·045	12	57
	0·78	0·39 (2) ±0·035	0·17 (2) ±0·015	0·58 (2) ±0·025	0·24 (1)	0	24
Lu 3–049	50	0·33 (3) ±0·034	0·27 (3) ±0·015	0·54 (3) ±0·078	0·43 (3) ±0·019	60	73
	12·5	0·34 (3) ±0·015	0·26 (3) ±0·015	0·53 (3) ±0·010	0·35 (3) ±0·017	50	55
	6·25	0·41 (1)	0·20 (1)	0·53 (1)	0·43 (1)	9	75
	3·13	0·43 (1)	0·18 (1)	0·51 (1)	0·30 (1)	0	45
Lu 4–012	50	0·29 (1)	0·25 (3) ±0·032	0·43 (1)	0·40 (3) ±0·041	64	90
	25	0·42 (1)	0·28 (1)	0·57 (1)	0·38 (1)	42	57
	12·5	0·37 (2) ±0·020	0·23 (2) ±0·005	0·47 (2) ±0·010	0·29 (2) ±0·025	26	47
	6·25	0·41 (1)	0·21 (1)	0·75 (1)	0·23 (1)	14	16
	3·13	0·35 (2) ±0·060	0·20 (2) ±0·030	0·57 (2) ±0·050	0·24 (2) ±0·040	12	25
Lu 3–092	50	0·25 (1)	0·24 (3) ±0·038	0·46 (1)	0·47 (3) ±0·038	86	103
	25	—	0·23 (1)	0·64 (1)	0·41 (1)		55
	12·5	0·38 (2) ±0·010	0·24 (2) ±0·010	0·53 (2) ±0·095	0·34 (2) ±0·085	30	53
	6·25	0·32 (1)	0·20 (1)	0·55 (1)	0·29 (1)	14	38
	3·13	0·40 (2) ±0·025	0·21 (2) ±0·045	0·53 (2) ±0·000	0·23 (2) ±0·065	14	25

Figures in brackets indicate number of experimental groups, each comprising six mice.

H 77/77. On central NA neurones the thiophthalane compound Lu 5-003 proved to be most potent. With an ED50 of about 8 mg/kg this compound appears to rank between protriptyline (ED50 about 4 mg/kg) and desipramine (ED50 about 15 mg/kg. Carlsson *et al.*, 1969a). Replacement of the sulphur atom of the ring system by oxygen (Lu 3-010) or carbon (Lu 3-049) resulted in loss of potency. A blockade was generally more easily obtained in the heart than in the brain. When given alone in high doses all the drugs investigated here, like the tricyclic thymoleptics, caused some reduction in brain NA levels. None of the drugs proved capable of blocking DA displacement by H 77/77, in agreement with the earlier observations on tricyclic thymoleptics (data not shown).

Three of the compounds were also tested for their ability to prevent 5-HT displacement by H 75/12. As shown in Table 3, the agents investigated showed, at most, borderline activity, apart from Lu 5-003 in the highest dosage (50 mg/kg). This dosage was toxic, however, in combination with H 75/12, and attempts to repeat the experiment with this dosage failed because of high mortality.

Histochemical in vitro *studies on central CA and 5-HT neurones*

The histochemical results from the studies on the effects of the bicyclic thymoleptic drugs on the NA and 5-HT displacement caused by H 77/77 and H 75/12, respectively, are summarized in Tables 4 and 5. It is obvious from Table 4 that the thiophthalane Lu 5-003 was the most potent blocker of the NA displacement induced by H 77/77 (Figs. 1–3). Clearcut effects were obtained with a dose of

TABLE 3. *Effect of bicyclic derivatives on 5-HT depletion by α-ethyl-4 methyl-metatyramine (H 75/12) in mouse brain*

Treatment	First dose (mg/kg)	Brain 5-HT (μg/g) Drug+H 75/12	% Inhibition
None		0·19 (66) ±0·005	
Lu 3–010	25	0·23 (3) ±0·029	14
	12·5	0·20 (1)	4
Lu 4–074	25	0·23 (2) ±0·030	14
Lu 5–003	50	0·37 (1)	58
	25	0·22 (2) ±0·015	13
	12·5	0·18 (1)	0
	6·25	0·17 (1)	0

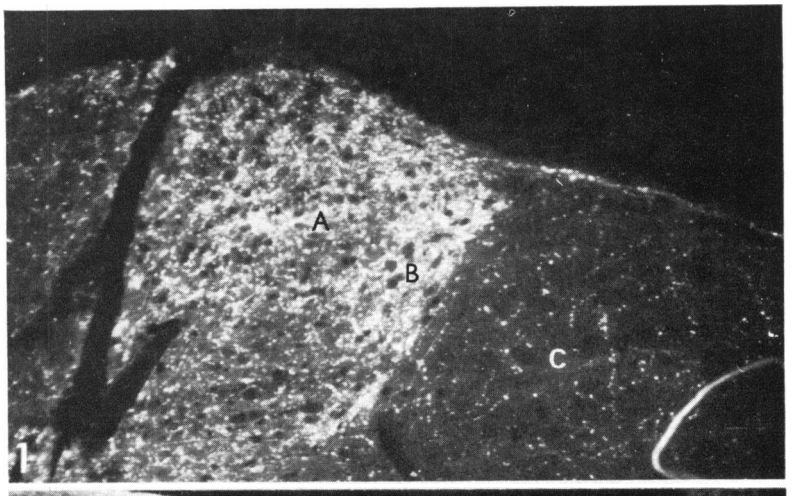
Figures in brackets indicate number of experimental groups, each comprising five mice.

LEGENDS FOR FIGURES ON FACING PAGE

FIG. 1. Dorsal motor nucleus of the vagus nerve (A), the solitary tract nucleus (B) and the hypoglossal nerve nucleus (C) of normal rat. A dense network of strongly green-fluorescent nerve terminals is observed in the two first-mentioned areas. (×120.)

FIG. 2. Same nuclei as in Fig. 1. A marked reduction in number and intensity of NA nerve terminals is observed compared with normal after injection of H 77/77 due to depletion of NA stores by H 77/77. For symbols see text to Fig. 1. (×120.)

FIG. 3. Same areas as in Fig. 1 after treatment with Lu 5-003 (6.25 and 3.13 mg/kg) plus H 77/77. There is only a slight reduction of the number and intensity of fluorescent NA nerve terminals due to a marked blockade of the H 77/77 induced NA depletion by Lu 5-003. For symbols see text to Fig. 1. (×120.)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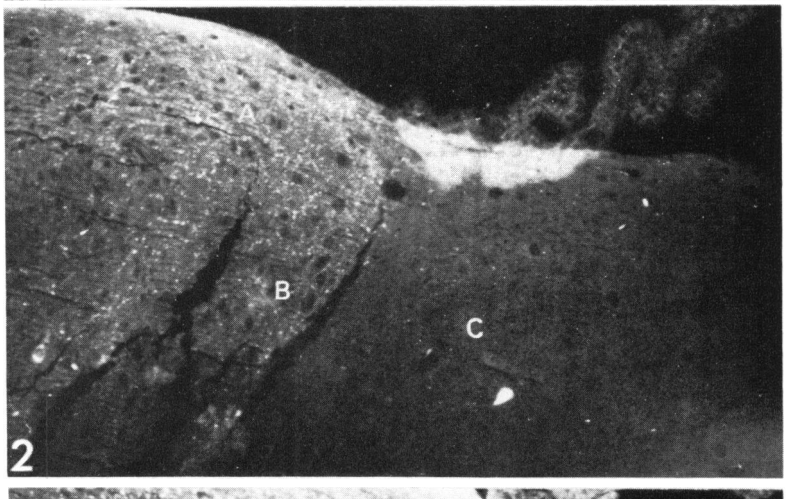
B
C
1


A
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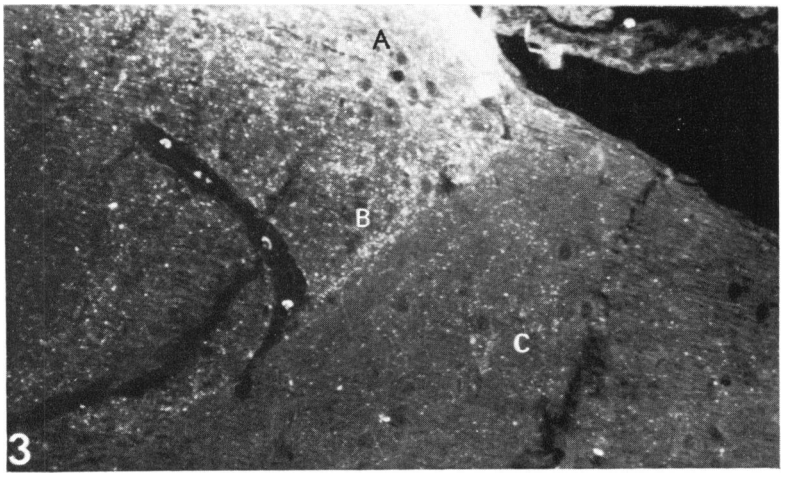
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6.25 mg/kg. The other compounds tested were about equally potent in blocking the NA displacement and were usually effective in doses between 12.5 and 50 mg/kg. Possibly with the highest doses used the Lu-compounds themselves caused a slight reduction in the number and intensity of NA nerve terminals. With the exception of Lu 5-003, the Lu-compounds were clearly less potent than desipramine in the H 77/77 model. None of the Lu-compounds proved capable of blocking the small depletion sometimes obtained in the DA nerve terminals after H 77/77 treatment.

TABLE 4. *Effect of bicyclic derivatives on the depletion of fluorescence caused by 4,α-dimethyl-meta-tyramine (H 77/77) in central NA nerve terminals*

Treatment	First dose (mg/kg)	Fluorescence intensity: Drug alone	Fluorescence intensity: Drug+H 77/77	Degree of blockade
None		3+ (7)	1+ (18) 2+ (4)	
Lu 5-003	25	2+ (3) 3+ (6)	2+ (3) 3+ (7)	Marked
	12·5	2+ (3) 3+ (6)	2+ (6) 3+ (4)	Marked
	6·25	3+ (5)	2+ (8) 3+ (1)	Moderate
	3·13	3+ (4)	1+ (3) 2+ (4)	Slight if any
Lu 3-010	50	2+ (4) 3+ (7)	2+ (7) 3+ (1)	Moderate
	25	2+ (2) 3+ (6)	1+ (2) 2+ (7)	Moderate
	12·5	3+ (4)	1+ (2) 2+ (5)	Moderate
	6·25	3+ (3)	1+ (5) 2+ (3)	None
Lu 3-049	50	2+ (2) 3+ (6)	1+ (1) 2+ (8)	Moderate
	12·5	3+ (4)	1+ (2) 2+ (6)	Moderate
	6·25	3+ (5)	1+ (6) 2+ (2)	None
Lu 4-012	25	3+ (5)	2+ (8) 3+ (1)	Moderate
	12·5	3+ (4)	1+ (4) 2+ (4)	Slight if any
	6·25	3+ (3)	1+ (5) 2+ (2)	None
Lu 3-092	25	2+ (3) 3+ (6)	1+ (2) 2+ (7)	Moderate
	12·5	3+ (5)	1+ (4) 2+ (4)	Slight if any
	6·25	3+ (4)	1+ (5) 2+ (2)	None

A semiquantitative estimation of the fluorescence intensity has been made.
3+, High intensity and number of terminals; 2+, moderate intensity and number of terminals; 1+, low intensity and number of terminals. Number of rats is within brackets.

TABLE 5. *Effect of bicyclic derivatives on the depletion of fluorescence caused by 4-methyl-α-ethyl-meta-tyramine (H 75/12, two intraperitoneal doses of 25 mg/kg) in the 5-HT nerve terminals of the nuc. suprachiasmaticus of the rat*

Treatment	First dose (mg/kg)	Fluorescence intensity: Drug alone	Fluorescence intensity: Drug+H 75/12	Degree of blockade
None		3+	1+ (18) 2+ (4)	
Lu 3-010	25	2+ (1) 3+ (4)	1+ (10) 2+ (2)	None
	12·5	3+ (3)	1+ (8) 2+ (2)	None
Lu 3-009	25	3+ (3)	1+ (9) 2+ (3)	None
	12·5	3+ (3)	1+ (11) 2+ (3)	None
Lu 5-003	25	3+ (3)	1+ (12) 2+ (3)	None
	12·5	3+ (3)	1+ (6) 2+ (1)	None
	6·25	3+ (3)	1+ (7) 2+ (1)	None
Lu 4-074	25	3+ (3)	1+ (10) 2+ (4)	None
	12·5	3+ (3)	1+ (6) 2+ (1)	None
Lu 4-012	25	3+ (3)	1+ (7) 2+ (1)	None
Lu 3-092	25	3+ (3)	1+ (6) 2+ (2)	None

A semiquantitative estimation of the fluorescence intensity has been made.
3+, High intensity; 2+, moderate intensity; 1+, weak or very weak intensity. Number of rats is within brackets.

As seen in Table 5, none of the tested bicyclic compounds could partially prevent the 5-HT depletion induced by H 75/12. The dimethylated compounds were not clearly more effective than the monomethylated Lu-compounds in this model.

Histochemical in vivo *studies on peripheral NA neurones*

The effect of bicyclic derivatives with thymoleptic properties and tricyclic thymoleptics on the reserpine-resistant accumulation of α-methyl-NA in peripheral NA nerve terminals was studied. The results are summarized in Tables 6 and 7.

Lu 5-003 and especially Lu 3-010 were found to be much more potent blocking agents than any of the other Lu-compounds used. Lu-3-010 was more potent than desipramine and comparable with protriptyline. The substances Lu 3-009 and Lu 4-012 were found to be somewhat more effective than imipramine, nortriptyline and especially amitriptyline.

TABLE 6. *Effect of bicyclic and tricyclic thymoleptics on the reserpine resistant accumulation of α-methyl-NA in the adrenergic nerves of rat iris as judged from the restitution of the nerve fluorescence*

	Dose (mg/kg)			
Drug	0·5	2	10	50
3–048		+++	++	+
4–004		+++	++	+
3–047			++	
3–092		++ (+)	+	−
3–049		++	(+)	
3–009		++	(+)	
4–012		++	(+)	−
5–003	++	+	−	
3–010	+	(+)	−	
Amitriptyline			++	+
Nortriptyline			+	(+)
Imipramine		++ (+)	+	−
Amphetamine		++	(+)	
Desipramine		+ (+)	−	
Protriptyline	+ (+)	(+)		
−	+++			

+++, Strong fluorescence; ++, moderate fluorescence; +, weak fluorescence.
The drugs were given 30 min before the administration of α-methyl-NA and the animals were treated with reserpine (10 mg/kg intraperitoneally) 16 hr before. The Lu-drugs are listed in increasing order of potency.

TABLE 7. *Duration of blockade of the reserpine-resistant accumulation of α-methyl-NA in the adrenergic nerves of the rat iris after treatment with Lu* 3–010, 3–092, 4–012 *and desipramine*

	Dose (mg/kg intraperitoneally)	Time before injection of α-methyl-NA 0·1 mg/kg (intravenously)			
Drug		0 hr	0·5 hr	8 hr	16 hr
3–010	2		(+)		
	5		−	(+)	++
4–012	10		(+)		
	25		−	+	+++
3–092	10		+		
	25		−	+ (+)	+++
Desipramine	4		(+)		
	10		−	−	+
Control (only α-methyl-NA)	−	+++			

For key to the symbols, see Table 6.

In the study on the duration of the blockade after administration of the drugs to be tested (Table 7) it was found that the blockade after Lu 3-010 and desipramine was still detectable 16 hr after injection. At this time-interval after injection, no blockade existed after Lu 4-012 and 3-092. The effect of the Lu-compounds on the release of extragranular NA in the NA nerve terminals of the iris was also studied. It was found that all the compounds studied had a rather small releasing action of about the same order as observed after desipramine. Thus the Lu 3-010, 4-012 and 3-092 did not behave as (+)-amphetamine, after which a rapid release is observed.

In vitro *studies on central and peripheral CA neurones*

Incubation of cerebral cortex and vas deferens slices from reserpine-pretreated rats with α-methyl-NA in concentrations from 0.001 to 10 μg/ml. results in the appearance of weakly to very strongly fluorescent NA nerve terminals, respectively (Hamberger, 1967).

It has previously been reported (Carlsson *et al.*, 1966; Hamberger, 1967) that desipramine, protriptyline and Lu 3-010 added *in vitro* inhibit the accumulation of α-methyl-NA in NA nerve terminals both in the peripheral and central nervous system while DA nerve terminals in the nucleus caudatus-putamen are unaffected. In the present study, the effects of Lu 3-049, 3-092, 4-012 and 5-003 were also investigated histochemically. All the substances inhibited the accumulation of α-methyl-NA into NA nerve terminals while the accumulation into DA nerve terminals was

TABLE 8. *Effect of desipramine, Lu* 3–010, *Lu* 3–092 *and Lu* 4–012 *on the retention of* ^{3}*H-α-methyl-NA in cerebral cortex and vas deferens slices of reserpine-pretreated rats*

Drug	Cerebral cortex	Vas deferens
No drug	100 (3)	100 (3)
Desipramine	35·5 (3)	2·7 (3)
	Range 27·1–46·0	Range 2·1– 3·6
Lu 3–010	43·5 (2)	2·5 (2)
	Range 37·4–49·6	Range 2·2– 2·8
Lu 3–092	43·5 (2)	27·4 (2)
	Range 36·0–51·4	Range 18·4–36·4
Lu 4–012	44·0 (3)	23·2 (3)
	Range 42·5–44·9	Range 13·2–37·5

The rats were given 50 and 25 mg/kg of the drugs 3 and 1 h before the experiments. The values are expressed as c.p.m./mg tissue and given as mean % of the control retention. Number of experiments in brackets.

TABLE 9. *Effect of Lu* 5–003 *on the retention of* ^{3}*H-noradrenaline in cerebral cortex slices and isolated iris*

Dose of Lu 5–003	Cerebral cortex	Iris
No drug	100± 7·6 (4)	100±6·5 (8)
3·1 + 1·5	121±20·0 (2)	53±3·0 (4)
6·25+ 3·1	88± 7·0 (4)	42±3·8 (8)
12·5 + 6·25	68± 8·4 (4)	25±2·1 (8)
25 +12·5	36± 2·3 (4)	12±0·5 (7)
10^{-5} M	38± 1·2 (2)	6±0·8 (4)

The rats were pretreated with Lu 5–003, 3 and 1 hr before the experiment or Lu 5–003 added *in vitro*. Slices of the cerebral cortex and iris incubated with ^{3}H-NA (2×10^{-6}M and 10^{-7}M ^{3}H-NA respectively). The values are expressed as c.p.m./mg cortex or/iris and given as mean %±S.E.M. of the control retention. Number of values within brackets.

unaffected. Although no detailed gradation can be done in these histochemical experiments the results show that desipramine, Lu 3-010 and Lu 5-003 were the most potent drugs and that Lu 3-092 was the least potent.

In other experiments the drugs were injected into the animals *in vivo* and the effect on the accumulation of NA or α-methyl-NA studied *in vitro*. In the histochemical experiments no marked differences between the drugs were seen. With tritiated α-methyl-NA the effect of desipramine, Lu 3-010, Lu 3-092 and Lu 4-012 on the retention of ^{3}H-α-methyl-NA was studied (see Table 8). Desipramine and Lu 3-010 were markedly more potent in vas deferens than in the brain. All the four drugs inhibited the uptake also in the brain. Unfortunately, Lu 5-003 was not available at the time of this study. However, later the ability of Lu 5-003 to inhibit accumulation of ^{3}H-noradrenaline in tissues has been investigated (Table 9). There was a marked inhibition of the retention of NA in both iris and brain, though lower doses were required for the former tissue.

Discussion

In an earlier study (Carlsson *et al.*, 1966) we compared Lu 3-010 with desipramine and protriptyline, using NA formation from L-DOPA as an indicator of membrane-pump blockade. We concluded that the activity of Lu 3-010 on peripheral adrenergic neurones was comparable with that of desipramine and protriptyline. On central NA neurones, however, we were unable to detect any marked action of Lu-3-010, in contrast to desipramine and protriptyline. The investigation of Waldeck (1968) as well as the present study underline the potency of Lu 3-010 on peripheral adrenergic neurones and the conclusions of Petersen *et al.* (1966) drawn from pharmacological data. As to the central NA neurones, the present displacement technique was apparently more sensitive in detecting blockade of the membrane pump than the method referred to above, because it was possible to demonstrate an effect of Lu 3-010 also on central NA neurones. However, the activity was weaker than that of protriptyline and desipramine.

Moreover in the *in vitro* experiments it was possible to demonstrate central activity of injected Lu 3-010 in the present experiments, in contrast to the earlier study. The reason for this difference is probably that in the present experiments larger and repeated dosage was employed and an isotope technique applied. Furthermore, a lower concentration of NA was used than before, which leads to a higher sensitivity (see Hamberger, 1967).

This relatively striking dissociation between central and peripheral activity exhibited by Lu 3-010 lead to speculations concerning a possible relationship between lipid solubility and the ratio of central to peripheral activity. On this basis the thio analogue of Lu 3-010—Lu 5-003—was synthesized. The distribution coefficient heptane/H_2O at pH 7.4 is 0.30 for Lu 3-010 and thus considerably lower than those of desipramine (0.82) and protriptyline (0.89). However, the corresponding value for Lu 5-003 is 3.8 (Petersen, personal communication). It is thus interesting to note that the ability of Lu 5-003 to block NA displacement centrally is distinctly superior to that of Lu 3-010 and even desipramine, thus approaching the potency of protriptyline. On the peripheral adrenergic neurones, however, the differences in activity tended to be the reverse. Thus in the iris Lu 3-010 is more potent than Lu 5-003 in blocking the amine uptake at the cell membrane of the NA

nerve terminals. Furthermore, this blockade is long-lasting as after desipramine. It therefore seems possible that the unexpectedly low central activity of Lu 3-010 is related to its low lipid solubility.

In spite of a potent ability of Lu 5-003 to block the membrane pump of the central NA neurones the corresponding pump in the central DA neurones appeared unaffected by Lu 5-003, for the displacement of the DA stores by H 77/77 did not appear to be blocked by Lu 5-003. Thus in this respect Lu 5-003 and also the other Lu-compounds appear to act like desipramine and protriptyline (Carlsson *et al.*, 1969a).

As seen from the release experiments on the NA nerve terminals of the iris, the Lu-compounds are also similar to tricyclic antidepressants such as desipramine and protriptyline in other respects. Thus they do not cause a rapid release of " extragranular " CA as is observed after amphetamine treatment (Carlsson *et al.*, 1966).

In the *in vitro* studies also Lu 5-003 proved to be a potent compound with respect to a blocking action on the membrane pump of the central NA neurones. Interestingly the effects of Lu 3-092 were less marked than those of Lu 3-010 and Lu 4-012 when added *in vitro* into the incubation bath, whereas after *in vivo* injections Lu 3-092 was as effective as Lu 3-010 and Lu 4-012. This may be due to a relatively high distribution coefficient heptane/H_2O at pH 7.4 for Lu 3-092 and/or to a demethylation occurring *in vivo* with formation of Lu 4-012.

Like desipramine and protriptyline, Lu 5-003 also proved relatively inactive in blocking 5-HT displacement by H 75/12. In view of our earlier considerations (Carlsson *et al.*, 1969b) Lu 5-003 would thus be expected to have an antidepressant spectrum like desipramine and protriptyline, an increase in drive being more prominent than elevation of mood, in contrast to imipramine for example. In any event clinical trials of Lu 5-003 in depressed patients seem warranted, especially in view of the fact that the Lu-compounds appear to be almost devoid of central depressant properties and have little or no anticholinergic activity (Petersen *et al.*, 1966 and personal communication).

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