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Inhibition of amine uptake in the mouse heart by some new "thymoleptic" drugs

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A number of new and established thymoleptic drugs were given to mice. Their inhibitory action on the uptake of [^{3}H]metaraminol and [^{3}H]noradrenaline in the heart was investigated. Among the compounds tested a monomethylamino-derivative was in general 2–3 times more potent than the corresponding dimethylamino-derivative. The phthalan derivative 3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan (Lu 3–010) was as efficient as protriptyline in all the tests performed. Changes in the substitution of the phthalan skeleton influenced the activity critically. As it is devoid of anticholinergic activity Lu 3–010 appears to be the most specific inhibitor of the amine transport mechanism of the adrenergic cell membrane found so far.

POTENTIATION of the effects of catecholamines and antagonism of the reserpine-induced syndrome have been recognized as characteristics of thymoleptic drugs (Haefely, Hürlimann & Thoenen, 1964; Sigg, 1959; Domenjoz & Theobald, 1959; Sulser, Watts & Brodie, 1962; Wilson & Tislow, 1962; Petersen, Lassen & others, 1966). It has also been claimed that this potentiation of the effect of the catecholamines is due to blockade of their inactivation (Schaeppi, 1960), specified further as inhibition of noradrenaline uptake into sympathetically innervated tissues (Axelrod, Hertting & Potter, 1962). This inhibition has been shown to be located in the amine transport mechanism at the level of the cell membrane, the "membrane pump" (Carlsson & Waldeck, 1965a; Malmfors, 1965). Previously we have tested the blocking action of some antidepressive and related agents using [^{3}H]-metaraminol (^{3}H–MA) as an indicator (Carlsson & Waldeck, 1965b). Since a new series of bicyclic "thymoleptics" (as judged by animal data) has become available (Petersen & others, 1966) it seemed to be of interest to include these compounds in our study.

Experimental

The present investigation was made on female mice. The general experimental and analytical procedure has been described elsewhere (Carlsson & Waldeck, 1963, 1965a). Other experimental details are given in the results section.

Results

The compounds tested were given intravenously (10 and sometimes 1 mg/kg) to mice 5 min before the intravenous injection of 0·02 mg/kg ^{3}H–MA, and the animals were killed 30 min thereafter. The hearts were removed and the level of ^{3}H–MA estimated. Animals which received only ^{3}H–MA served as controls.

The drugs are listed two by two, one dimethylamino- and one monomethylamino-derivative of each compound (Table 1). In all instances

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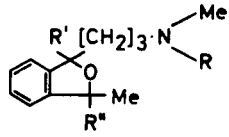
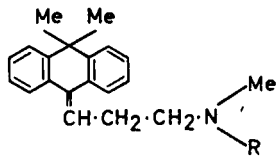
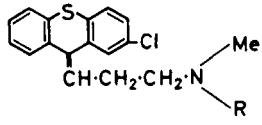
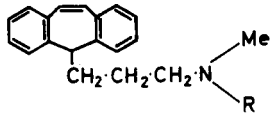
the monomethylamino-derivative was superior to its dimethylamino-analogue in preventing ^{3}H-MA uptake. The phthalan derivative 3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan (Lu 3–010) proved to be the most efficient of the compounds tested. The corresponding compound lacking the phenyl group in position 1 (Lu 3–071) had a

TABLE 1. INHIBITION OF [^{3}H]METARAMINOL UPTAKE IN THE MOUSE HEART BY SOME THYMOLEPTIC DRUGS. The drugs were given intravenously to mice (grouped six by six) 5 min before the intravenous administration of 20 μg/kg [^{3}H]-metaraminol. After another 30 min the animals were killed, the hearts removed and their content of [^{3}H]metaraminol determined. The mean ±s.d. of 10 control groups which received [^{3}H]metaraminol only was 46·4 ±7·3 ng/g. In general, the values, calculated as per cent of the control values are the mean of 2 experimental groups.

Drug	R	R′	R″	Formula	Inhibition as % of control at 10 mg/kg	Inhibition as % of control at 1 mg/kg
Lu 3–009	Me	Ph	Me		15	63
Lu 3–010	H	Ph	Me		7	18
Lu 3–028	Me	H	Me		81	—
Lu 3–071	H	H	Me		64	—
Lu 3–092	Me	CN	Me		13	61
Lu 4–012	H	CN	Me		10	39
Lu 3–035	Me	Ph	H		38	—
Lu 3–051	H	Ph	H		12	—
Melitracene	Me				46	85
Litracene	H				14	44
Chorprothixene ..	Me				27	—
Demethylchlor-prothixene ..	H				12	—
Protriptyline	H				9*	

* From Carlsson & Waldeck, 1965b.

much reduced activity, but the activity of the compound with a cyano-group replacing the phenyl group (Lu 4–012), however, was not appreciably less. A slightly lower potency than that of Lu 3–010 was observed in the compound in which one of the methyl groups in position 3 of Lu 3–010 was replaced by a hydrogen atom (Lu 3–051). The same structure-activity relations were observed for the corresponding dimethyl derivatives (Lu 3–009, Lu 3–028, Lu 3–092 and Lu 3–035).

In a subsequent experiment the dose-response relation of the blocking action of the most potent compounds in Table 1 was tested using [^{3}H]-noradrenaline (^{3}H–NA) as an indicator. For comparison, protriptyline was included in the test. The drugs were given in three different doses intravenously 5 min before the intravenous injection of 1 μg/kg of ^{3}H–NA. Controls were given ^{3}H–NA only. After another 30 min the animals were killed, the hearts removed and their content of ^{3}H–NA determined. In this test also Lu 3–010 appeared to be most potent and equal to protriptyline (Fig. 1). The three doses 0·5, 0·1 and 0·02 mg/kg blocked the

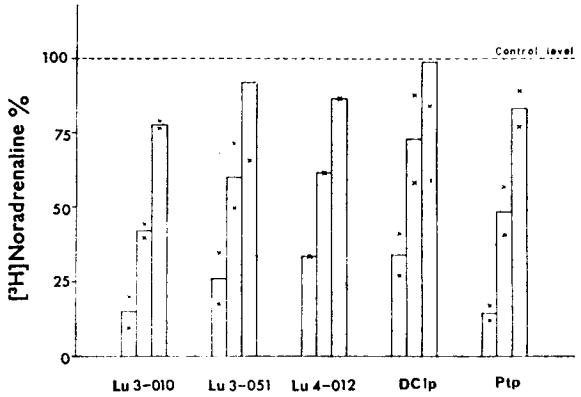


FIG. 1. Dose-response relationships for the inhibitory action of some thymoleptic drugs on the uptake of [^{3}H]noradrenaline in the mouse heart. The drugs were given intravenously to mice (grouped six by six) 5 min before the i.v. administration of 1 μg/kg [^{3}H]noradrenaline. After another 30 min the animals were killed and the content of [^{3}H]noradrenaline in the heart determined. Each substance was tested in 3 different doses, from the left: 0·5, 0·1 and 0·02 mg/kg. The mean $\pm$s.d. of 5 control groups which received [^{3}H]noradrenaline only was 2·61 $\pm$0·66 ng/g. The data are given in per cent of this control value. DClp = demethylchlorprothixene, Ptp = protriptyline.

^{3}H–NA uptake by 80, 40 and 15% respectively. The three drugs Lu 4–012, Lu 3–051, and Lu 3–092 appeared to be less potent but showed the same dose-response pattern.

The duration of the inhibitory action on the membrane pump was tested by giving the inhibitors intravenously (8–10 mg/kg) at different time intervals before the intravenous administration of ^{3}H–NA. In this series also, desipramine was included as a reference substance. Other experimental details were the same as in the previous experiment. The blockade of the ^{3}H–NA uptake induced by Lu 3–010 and protriptyline was prompt but appeared to be maximal for 1–2 hr only (Table 2). The blockade induced by desipramine disappeared even faster, because the ^{3}H–NA uptake was about 15 times higher when the drug was given 320 min rather than 5 min before the test amine. The compounds Lu 3–051 and demethylchlorprothixene appeared to be inefficient when given 320 min before the ^{3}H–NA although they caused a 90% blockade when given 20 min before.

Discussion

Metaraminol has proved to be a specific indicator of amine uptake at the level of the cell membrane of the adrenergic neuron (Carlsson & Waldeck, 1965a). It seems to be difficult, however, even with very potent inhibitors of the membrane pump, to reach ^{3}H–MA levels below 7% of the control value. This may in part be due to the metabolic stability of this compound, resulting in an extraneuronal retention of the amine. A high affinity for extraneuronal binding sites may also be a contributory factor. This is the reason why ^{3}H–NA was chosen for the study of dose-response relations and turn-over rates once the membrane-pump inhibiting qualities of a compound had been established by means of ^{3}H–MA.

TABLE 2. DURATION OF THE INHIBITORY ACTION OF SOME THYMOLEPTIC DRUGS ON THE [^{3}H]NORADRENALINE UPTAKE IN THE MOUSE HEART. The drugs were given intravenously to mice (grouped six by six) at various time intervals before the intravenous administration of 1 μg/kg [^{3}H]noradrenaline. After another 30 min the animals were killed and the content of [^{3}H]noradrenaline in the heart determined. The mean ±s.d. of 5 control groups which received [^{3}H]noradrenaline only was 2·30 ±0·94 ng/g. The data are single values given in per cent of the control value.

Drug	Dose mg/kg	Min drug given before			
		5	20	80	320
Lu 3–010	10	1	3	5	17
Lu 3–051	10	—	8	21	119
Demethylchlorprothixene ..	10	—	9	22	84
Protriptyline	8	2	2	3	6
Desipramine	10	2	4	10	37
		2	13	17	27

Of the compounds listed in Table 1, the monomethylamines in general showed 2–3 times higher activity in preventing ^{3}H–MA uptake than their corresponding dimethylamino-derivatives. This is in close agreement with previous data on other thymoleptics (Carlsson & Waldeck, 1965 a,b; Iversen, 1965). Further, changes in the substitution on the phthalan skeleton may influence the activity seriously (compare the activity of Lu 3–010 with that of Lu 3–071).

In the present study the activity of the most potent compound, Lu 3–010, did not appreciably differ from that of protriptyline in any of the tests. However, Petersen & others (1966) found Lu 3–010 about twice as active as protriptyline in the antagonism of reserpine and the potentiation of noradrenaline. As their test also included the adrenergic receptor mechanism, the discrepancy may reflect a higher sympatholytic activity of protriptyline than of Lu 3–010 (Petersen & others, 1966). In contrast to protriptyline, Lu 3–010 appears to be almost devoid of anticholinergic activity. It therefore appears to be the most specific inhibitor of the adrenergic membrane pump known so far.

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