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Reduction of gastric acid secretion and ulcer formation by 3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan (Lu 3-010); an inhibitor of noradrenaline uptake

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Lu 3–010 [3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan], administered intraperitoneally, blocks the uptake of [^{3}H]noradrenaline into the mouse and rat heart and has an activity 5 times greater than imipramine and comparable to that of desipramine. Lu 3–010 inhibits basal gastric acid secretion in the rat and is 4 and 2 times more potent than imipramine and desipramine, respectively. The drug is about 9 times more potent than desipramine in preventing the pentagastrin-induced stimulation of gastric acid secretion in the rat. Lu 3–010 reduces the incidence of stress-induced gastric lesions in the rat, exhibiting an ED50 $\pm$ s.e. of 6·3 $\pm$ 1·4 mg/kg, and at 10 mg/kg, ulcer development in the 17-hour Shay test is reduced by 50%.

Noradrenaline is mainly inactivated through its uptake into the storage sites of sympathetic nerve endings (Kopin, Hertting & Gordon, 1962; Chidsey, Kahler, Kelminson & Braunwald, 1963; Thoenen, Huerlimann & Haefely, 1964). Blockade of this uptake process, which is at the level of the cell membrane, could lead to a higher level of noradrenaline at the receptor sites and thus result in a potentiation of its effects on the effector organs. Various drugs which block this uptake process potentiate the pharmacological effects of noradrenaline (Haefely, Huerlimann & Thoenen, 1964). The drug 3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan (I; Lu 3–010) blocks the uptake process and, since it is devoid of cholinergic activity, it appears to be the most specific inhibitor of this uptake mechanism (Waldeck, 1968). Various related pharmacological activities of Lu 3–010 are reported here.

EXPERIMENTAL

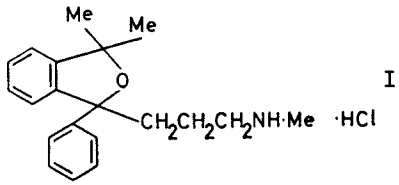
Radioactive noradrenaline levels

Male albino mice, 23–25 g, or rats, 60–80 g (Canadian Breeding Laboratories) were injected in the tail vein with 1·5 μCi ($\pm$)-7-[^{3}H]noradrenaline* HCl (Radiochemical Centre, Amersham) in 0·25 ml of a solution of 0·75% sodium chloride and 0·01N HCl. Drugs were injected intraperitoneally in 0·5 ml bidistilled water; the doses refer to the salt of the drug.

The tissue samples were homogenized in ice-cold 0·4N perchloric acid and centrifuged. A portion of the supernatant fluid was transferred to a vial containing a mixture of 1 ml methanol, 3 ml ethanol and 10 ml toluene phosphor [0·4% 2,5-diphenyloxazole and 0·005% 1,4-bis(5-phenyloxazol-2-yl)benzene] and the total radioactivity was measured by liquid scintillation counting (efficiency: 12%). The

* 2-Amino-1-(3,4-dihydroxyphenyl)-[1-^{3}H]ethanol.

radioactivity in the heart of the mouse (Burak & Draskoczy, 1964; Daly, Creveling & Witkop, 1966) and rat (Wurtman, Kopin & Axelrod, 1963) at times comparable to those of the present studies is almost entirely due to [^{3}H]noradrenaline.



I

Gastric acid secretion

The basal gastric acid secretory activity was determined by a modified method of Shay, Sun & Gruenstein (1954) in Charles River female albino rats (Canadian Breeding Laboratories; 170–190 g) caged individually and from which food had been withheld 48 h before pyloric ligation and drug administration. After the first 24 h of food deprivation the animals were given access to 8% sucrose in 0·2% sodium chloride for 8 h. Water was permitted freely except during the 8 h the animals were on sucrose and after drug treatment. The pylorus was ligated under ether anaesthesia and the sutured incision covered with collodion to prevent animals ingesting adhering blood. The stomachs were lavaged with 0·9% saline by No. 5 Argyle premature infant feeding tube until clear; immediately after, the drugs were given intraperitoneally. Three h after pyloric ligation, the animals were anaesthetized with ether, the stomachs tied at the oesophageal junction, removed, rinsed in water, opened along the greater curvature and the contents collected in centrifuge tubes. The amount of acid in the centrifuged gastric juice was determined by titration against 0·1N sodium hydroxide in a direct reading pH meter to pH 7·0. There were 4–9 animals in each group.

The effect on drug-stimulated secretory activity was determined according to Kim & Shore (1963) as modified by Levine (1965). Food was withheld from rats for 48 h but they received sucrose in the same way as did the animals used to determine basal gastric acid secretory activity. Under ether anaesthesia, the pylorus was ligated, the stomach lavaged with 0·9% saline until clear and the oesophagus ligated in the cervical region. Because of the oesophageal ligation, no collodion was necessary. Test drugs were given intraperitoneally immediately after the operations. Pentagastrin treatment (1 μg/kg, s.c.), according to Lippmann (1970), or saline, was started 20 min after the test drugs and continued at 20 min intervals for a total of 5 injections. There were 6–9 animals in each group. The animals were killed by a blow on the head 2 h after receiving the test drugs. The stomachs were removed in the same manner as described above and the gastric contents emptied into centrifuge tubes. The volumes of juice were noted and the stomachs were rinsed with bidistilled water to yield a final volume of 5 ml. The samples were centrifuged and titrated against 0·01N sodium hydroxide in a direct reading pH meter to pH 7·0 to obtain the total acid.

Development of gastric lesions

To assess the effects of the drugs on the development of multiple stress-induced gastric lesions, the method of Brodie & Valitski (1963) as modified by Senay & Levine (1967) was used. Food was withheld from male albino rats (150–180 g) for

24 h but water was freely available. Drugs were administered intraperitoneally to groups of 10 to 14 animals immediately before placing the rats into restrainers 45 min before exposure to a cold environment of 5°; the restrainer is made of plexiglass and restricts the movement of the rats to a minimum. In each experiment an equal number of untreated rats were run concurrently with treated rats. The cold exposure lasted 150 min and 1 h later the rats were killed with ether and their stomachs removed. The stomachs were opened along the larger curvature and then unfolded for inspection. Of the untreated rats, 80 to 100% developed gastric lesions consisting of one or several macroscopical erosions of the glandular mucosa often accompanied by haemorrhage. The results were scored on the basis of presence or absence of lesions regardless of their number and severity. The data were submitted to probit analysis and the ED50 (dose protecting 50% of the animals) was calculated.

Anti-ulcer activities were also assessed using the 17-h Shay test (Shay, Komarov & others, 1945). After the animals were fasted for 48 h the pylorus was ligated under ether anaesthesia and the abdominal cavity closed. Drugs were administered intraperitoneally immediately after the ligation. The rats were killed 17–19 h later and their stomachs tied at the cardia and removed. The stomachs were macroscopically examined and the severity of gastric lesion was graded according to the area showing glandular lesions. There were 5–10 animals in each group.

Student's *t*-test was used to evaluate all data except where otherwise noted.

RESULTS

The effects of the intraperitoneally-administered drugs on the uptake and release of [^{3}H]noradrenaline (^{3}H-NA) in the mouse and rat heart are shown in Table 1. In the mouse, the level of ^{3}H-NA, given after the drug, was decreased after Lu 3–010

Table 1. *Inhibition of uptake of [^{3}H]noradrenaline in the mouse and rat heart by Lu 3–010*

Drug	Dose mg/kg, i.p.	Radioactivity content: Drug given before ^{3}H-NA* counts/min g^{-1} ± s.e.	Inhibition %	Drug given after ^{3}H-NA† counts/min g^{-1} ± s.e.
Mouse				
Water		4631 ± 297		3443 ± 83
Lu 3–010	0·5	2284 ± 101 $P<0·001$	51	3451 ± 174
	0·25	2997 ± 202 $P<0·001$	35	
Imipramine ..	2·5	2861 ± 103 $P<0·001$	38	3643 ± 154
	1·0	3698 ± 162 $P<0·02$	20	
Desipramine ..	0·5	2687 ± 151 $P<0·001$	42	3747 ± 192
	0·25	3384 ± 112 $P<0·01$	27	
Rat				
Water		1630 ± 82		1501 ± 34
Lu 3–010	0·5	881 ± 29 $P<0·001$	46	1571 ± 49
	0·25	1263 ± 68 $P<0·01$	22	
Imipramine ..	2·5	733 ± 94 $P<0·001$	55	1677 ± 82
	1·0	1462 ± 111		
Desipramine ..	0·5	1068 ± 56 $P<0·001$	35	1523 ± 89
	0·25	1318 ± 103 $P<0·05$	19	

The animals were injected with drug 45 min before or after ^{3}H-NA and killed 75 min after the drug administration.

* There were 16 animals in the control and 8 in the treated groups.

† There were 10–12 animals in the control and 6–7 in the treated groups.

(51%), imipramine (38%) and desipramine (42%) at 0·5, 2·5 and 0·5 mg/kg, respectively. In the rat after the same doses of Lu 3–010, imipramine and desipramine there were declines of 46, 55 and 35%, respectively. Thus, in both animals Lu 3–010 was about 5 times more potent than imipramine and was similar in activity to desipramine.

In both the mouse and rat there were no reductions in heart ^{3}H-NA when the drugs were given after the ^{3}H-NA. Thus, the reductions in ^{3}H-NA caused by the drugs were due to blockade of uptake and not to increased release of ^{3}H-NA.

Table 2. *Inhibition of basal gastric acid secretion by Lu* 3–010

Drug	Dose mg/kg, i.p.	Gastric acid secretion m-equiv acid/3 h ± s.e. Exp I	Exp II	Exp III	% Inhibition Exp I	Exp II	Exp III
Water		0·47 ± 0·04	0·44 ± 0·03	0·43 ± 0·07			
Lu 3–010 ..	1·25		0·21 ± 0·03 ($P<0·001$)	0·19 ± 0·03 ($P<0·01$)		51	56
	0·63	0·27 ± 0·03 ($P<0·01$)	0·28 ± 0·05 ($P<0·02$)		43	36	
	0·32	0·40 ± 0·06					
Imipramine ..	5·0		0·20 ± 0·04 ($P<0·001$)			54	
	2·5	0·29 ± 0·05 ($P<0·02$)	0·30 ± 0·02 ($P<0·01$)		38	31	
	1·25	0·37 ± 0·02					
Desipramine ..	2·5			0·15 ± 0·01 ($P<0·001$)			64
	1·25	0·32 ± 0·04 ($P<0·05$)	0·29 ± 0·04 ($P<0·01$)		31	35	
	0·63	0·50 ± 0·06					

Table 2 shows the effects of the drugs on basal gastric acid secretion in the rat. At 1·25 and 0·63 mg/kg, Lu 3–010 inhibited gastric acid secretion 53 and 39%, respectively. Imipramine caused decreases at 5·0 (54%) and 2·5 (34%) mg/kg and the levels declined 64 and 33% after desipramine at 2·5 and 1·25 mg/kg, respectively. Lu 3–010 thus exhibited a higher inhibitory activity than desipramine (2 times) and was even more active (4 times) than imipramine.

In the pylorus-oesophagus-ligated rat, prior administration of Lu 3–010 or desipramine inhibited the stimulation of gastric acid secretion caused by pentagastrin. In animals receiving Lu 3–010, 1·25 mg/kg, the level of gastric acid secretion, was decreased to the level in the control animals; at 0·63 mg/kg, Lu 3–010 caused no significant change (Table 3). Desipramine, 10 mg/kg, prevented the pentagastrin-induced increase and 5·0 mg/kg partially prevented the increase. Lu 3–010 was thus 9 times the more potent.

The effect of Lu 3–010 and imipramine on the multiple stress ulcer is shown in Table 4. Both drugs protected against the development of gastric lesions in a dose-dependent manner. The ED50 ± s.e. was 6·3 ± 1·4 for Lu 3–010, while in the same test the dose of imipramine protecting 50% of the rats was 1·9 ± 1·8.

Results of the 17-h Shay test showed that both Lu 3–010 and imipramine inhibited the ulcer development about 50% at 10 mg/kg and at 20 mg/kg Lu 3–010 gave a 74% inhibition. At 5 mg/kg neither drug had an inhibiting effect.

Table 3. *Inhibition of pentagastrin-induced gastric acid secretion by Lu* 3–010

Drug	Dose mg/kg, i.p.	Gastric acid secretion μ-equiv acid/2 h + s.e. Exp I	Exp II	Exp III	% Inhibition Exp I	Exp II	Exp III
Water + saline ..		18 ± 4	10 ± 3	13 ± 2			
Water + pentagastrin* ..		78 ± 13	54 ± 10	56 ± 8			
Lu 3–010 + pentagastrin*	1·25	23 ± 7 ($P<0·01$)	13 ± 3 ($P<0·01$)		92	93	
	0·63		50 ± 5				
Desipramine + pentagastrin*	10·0	16 ± 4 $P<0·001$)	14 ± 2 ($P<0·01$)	10 ± 4 ($P<0·001$)	103	91	107
	5·0	41 ± 6 ($P<0·05$)	27 ± 1 ($P<0·05$)	26 ± 4 ($P<0·05$)	62	60	70

* Dose, 5 × 1 μg/kg, subcutaneously.

Table 4. *Inhibition by Lu* 3–010 *of the development of gastric lesions caused by stress*

Drug	Dose mg/kg, i.p.	Number of animals with gastric lesions	Inhibition %
Water		17/20	
Lu 3–010.. ..	2·5	7/8	0
	5·0	11/22	41
	10·0	5/14	58
Imipramine ..	2·5	8/21	55
	5·0	6/19	62
	10·0	8/17	45
	20·0	3/17	79

DISCUSSION

Lu 3–010 [3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan] inhibits the amine transport mechanism of the adrenergic cell membrane in the mouse heart when given intravenously (Waldeck, 1968) and in both the mouse and rat heart when administered intraperitoneally (present findings). In comparison with the known blockers of ^{3}H-NA uptake, imipramine (Axelrod, Hertting & Potter, 1962) and desipramine (Iversen, 1965), Lu 3–010 is about 5 times as potent as imipramine and is comparable in activity to desipramine in inhibiting the ^{3}H-NA uptake into the mouse and rat heart. That desipramine exhibits a higher activity than imipramine in the blockade of ^{3}H-NA uptake agrees with Iversen (1965).

As has been found in the present studies with Lu 3–010, imipramine also inhibits basal (Bonfils, Dubrasquet & Lambling, 1962; Bass & Patterson, 1967; Lippmann, 1969) and pentagrastrin-induced (Lippmann, 1970) gastric acid secretions and restraint-induced ulcer formation (Bonfils, Dubrasquet & others, 1960). Since imipramine reduces gastric acidity and relieves pain in ulcer patients (Varay, Bertheldt & others, 1960), Lu 3–010 may also be of value in these conditions.

It is relevant that catecholamines as well as 5-hydroxytryptamine inhibit gastric acid secretion (Forrest & Code, 1954; Harries, 1956; Haverback, Bogdanski & Hogben, 1958). Entry into the nerve ending, rather than enzymatic destruction, is the main mechanism for the termination of the biological action of noradrenaline

(Whitby, Axelrod & Weil-Malherbe, 1961). The ability of Lu 3–010 to block this uptake of noradrenaline, thereby preventing the inactivation, could thus be of significance in the observed antisecretory activity of this compound.

It is possible that other activities of such drugs are also relevant in relation to their antisecretory actions. Imipramine has been demonstrated to possess anticholinergic activity (Domenjoz & Theobald, 1959), while Lu 3–010 has the advantage of being devoid of anticholinergic activity (Petersen, Lassen & others, 1966).

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