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Comparative pharmacological profile of two imidazoline derivatives endowed with strong hypotensive activity: LR 99853 and clonidine

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Recently, a new imidazoline derivative (LR 99853, Table 1) has been reported to possess strong and long-lasting hypotensive activity (the oral threshold dose being 0·1 mg kg^{-1} in rats) (Manghisi et al 1979; Fregnan & Ferni 1980). Unlike clonidine, this chemically related compound did not evoke hypertensive episodes or cause sedation in animals up to the oral dose of 1 mg kg^{-1} (Fregnan & Ferni 1980; Fregnan et al 1980).

We have further characterized the pharmacological profile of the new drug. For this purpose tests known to be effectively influenced by clonidine were selected. These were on food and water intake, gastric and salivary secretion, gastrointestinal motility, glucose metabolism, prolactin secretion, pupil contractility and urine elimination. Experiments were on Sprague-Dawley rats (150–400 g) generally fasted for 16 h. The arterial pressure was measured in conscious animals by means of a cannula inserted into a carotid artery, under ether anaesthesia the day before the drug-treatment, and connected to a pressure transducer. Food intake was evaluated in unfasted rats by measuring the amount of potato eaten in a 2 h interval (Cross et al 1977). Water intake was estimated according to Le Douarec et al (1971) during 6 h. The total HCl, pH and volume of the gastric juice were determined 4 h after pylorus ligation (Shay et al 1945). The presence or not of sialorrhea was observed after treatment with an inactive dose of carbachol (3·2 μg kg^{-1} i.v.) for at least 10 min. Gastrointestinal motility was assayed 30 min after a charcoal meal (1 ml/rat of 10% suspension) according to Janssen & Jageneau (1957). Blood sugar values with or without a glucose load (25 ml kg^{-1} of a 16% solution) were determined enzymatically (Trinder 1969) every 0·5–1 h for 5 h. Plasma prolactin concentrations were determined by a radioimmune assay procedure (Niswender et al 1969). Pupil size was scored 0 = normal, 1 = medium dilatation, 2 = maximum dilatation and urine elimination was estimated by measuring the total volume at the end of a 6 h period of collection (Fregnan et al 1969). The drugs, solu-dispersed in 10%

Table 1. Influence of 2-[*N*-(2,6-dichlorophenyl)-*N*-(2-tetrahydropyranil)amino]-2-imidazoline (LR 99853) and clonidine on some pharmacological parameters after acute treatment.

Parameter	Effective dose in mg kg^{-1} (95% confidence limits) LR 99853	Clonidine
10% fall in blood pressure (late phase)	≈0·2	≈0·2
20% rise in blood pressure (early phase)	≈4·0	≈0·2
50% inhibition of food intake	5·7 (3·1 –10·6)	0·31 (0·19–0·51)
50% inhibition of water intake	1·5 (0·91–2·3)	0·08 (0·04–0·15)
50% inhibition of gastric HCl	1·1 (0·57–2·0)	0·04 (0·02–0·11)
50% increase of gastric pH	1·3 (0·80–1·9)	0·09 (0·04–0·21)
50% inhibition of gastric juice	1·8 (0·80–4·0)	0·10 (0·03–0·55)
30% inhibition of gastrointestinal motility	0·74 (0·53–1·0)	0·03 (0·01–0·09)
50% occurrence of sialorrhea after carbachol†	≈30	0·34 (0·19–0·61)
threshold dose increasing plasma prolactin	10	5
50% occurrence of mydriasis	2·5 (1·2–5·3)	0·45 (0·20–1·0)

≈ = approximate value due to a poor dose-response correlation. † = at the tested dose (3·2 μg kg^{-1} i.v.) carbachol did not provoke sialorrhea in control rats not receiving the hypotensive drugs.

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Table 2. Influence of acute or subacute (for about 45 days) treatment with LR 99853 and clonidine both on blood sugar level (with or without a glucose load) and on urine excretion.

Parameter	Type of treatment	Effective dose in mg kg^{-1} (95% confidence limits LR 99853	Clonidine
50% rise in blood sugar without a glucose load	acute	8·3 (5·3 –13·0)	0·1 (0·05 –0·30)
50% rise in blood sugar without a glucose load	subacute	[10 = +25]†	≈0·3
50% rise in blood sugar during a glucose load	acute	[1 = +16]†	0·024 (0·019–0·031)
50% rise in blood sugar during a glucose load	subacute	2·3 (0·8 – 2·9)	≈0·03
100% rise in urine excretion	acute	0·77 (0·59– 1·0)	0·034 (0·030–0·039)*
100% rise in urine excretion	subacute	0·59 (0·38– 0·94)	0·030 (0·015–0·040)*

≈ = approximate value due to a poor dose-response correlation.
* = calculated within the dose-range of 0·01–0·1 mg kg^{-1}, since at higher doses (0·3–3 mg kg^{-1}) clonidine progressively lost its diuretic activity and even became antidiuretic.
† = maximal administered dose and related % variation in activity.

arabic gum (in 0·9% NaCl) at various concentrations, were administered acutely or repeatedly (once a day for about 1 month and a half) in a constant volume of 5 ml kg^{-1} 1 h before either the final readings, the beginning of an evaluation period, or the administration of other substances. The drugs were generally administered by mouth to at least 10 rats for each dose level (excepting Shay's method which used the i.p. route). The effective doses and 95% confidence limits in the Tables were determined by plotting the log or ln of the doses versus the probit or the ln of the data.

The data depicted in Table 1 confirm the equal effectiveness of LR 99853 and clonidine in lowering the arterial pressure and give clear evidence of their different pharmacological profiles. As already reported (Fregnan & Ferni 1980), it appears clear that LR 99853 was unable to evoke the early phase of hypertension, unless very high doses were used, whereas clonidine always elicited a biphasic effect. Moreover, LR 99853 influenced other pharmacological parameters, listed in Table 1, less effectively than clonidine. The doses of the new substance causing anorexia, antidipsogenic effects, gastrointestinal blockade, mydriasis, potentiation of carbachol-induced sialorrhea and hyperprolactinaemia, were up to 30 times greater than that causing a 10% fall in blood pressure, while the doses of clonidine were generally the same or even smaller. The effects of the two imidazoline derivatives on blood glucose concentration and on urine excretion after single or repeated treatments are shown in Table 2. It appears evident that clonidine caused hyperglycaemia in both experimental conditions and impaired the glucose tolerance test at doses even smaller than the hypotensive ED10. In addition, clonidine also had marked effects on renal function, which varied according to the dose, progressively increasing the urine excretion with 0·01–0·1 mg, appearing almost inactive with 0·3–1 mg and even causing water retention with 3 mg. LR 99853 did not show this biphasic response on renal function up to the maximal tested dose of 10 mg kg^{-1}. On comparing the diuretic and hyperglycaemic effective doses reported on Table 2, it can be seen that LR 99853 was less potent than clonidine in both experimental conditions. Moreover, the doses of LR 99853 causing a significant hyperglycaemia after an acute or a subacute treatment were at least ten times greater than that reducing by 10% the blood pressure.

In conclusion, our present and previous experiments (Fregnan & Ferni 1980; Fregnan et al 1980) show that LR 99853 has a relative specificity for the hypotensive action and thus indicate that the compound differs from the chemical congener clonidine which has other pharmacological effects involving several peripheral or centrally regulated autonomic functions. Thus, clonidine causes early hypertension (Hoefke & Kobinger 1966), inhibition of food (Atkinson et al 1978) and water intake (Le Douarec et al 1971), blockade of gastric secretion (Hoefke & Kobinger 1966; Walz & Van Zwieten 1970) and of gastrointestinal motility (Bear & Steer 1976), potentiation of carbachol-induced sialorrhea (Green et al 1979), mydriasis (Gherezghiher & Koss 1979), hyperglycemia (Iwata 1969; Maling et al 1969), diuresis or urinary retention (Hoefke & Kobinger 1966; Le Douarec et al 1971; Roman et al 1979), sedation, depression of the conditioned avoidance responses (Laverty & Taylor 1969; Hawkins & Monti 1979) and hypothermia (Maskrey et al 1970). In addition, although the mechanism of action of the new imidazoline derivative is not yet well known, it is evident that LR99853 must be metabolized before being effective and that the metabolites formed are not mainly clonidine or clonidine metabolites (Fregnan & Ferni 1980).

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The effect of raw material purity on the acute toxicity and laxative effect of sennosides

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All commercial sennoside preparations contain chemically unidentified impurities besides sennosides A and B, and these may be responsible for many of the side effects of senna preparations (Schmid 1952; van Os 1976; Breimer & Baars 1976). We have compared the acute toxicity and laxative effect of pure sennoside glycosides with those of commercial senna extracts (containing 20–80% sennosides) not only by mouth but intravenously because although the drugs are never administered by that route it is possible that some systemic side effects of senna preparations are due to absorbed impurities (van Os 1976; Breimer & Baars 1976).

The following commercial sennosides or senna extracts used were: (1) sennoside A 99% (Salco Ltd), (2) sennoside B 99% (Salco Ltd), (3) sennosides A + B 99% (Salco Ltd) (I), (4) calcium sennosides A + B 82% (Medica Ltd) (II), (5) calcium sennosides A + B 60% (Andard-Mount Ltd) (III), and (6) calcium sennosides A + B 20% (Indian origin, supplied by Lehner AG) (IV). The percentage contents are those given by the manufacturers. As vehicles, 0·9% NaCl solution for calcium salts and 1·4% $NaHCO_3$ solution for free glycosides were used.

Acute toxicity was studied by intravenous and oral administration of the drugs. Female NMRI mice (20–25 g) were used. The mice for the oral toxicity test were fasted overnight but allowed free access to water, otherwise food and water were withdrawn on the morning before the experiments. Volumes of 10–50 ml kg^{-1} (according to the solubility) were used in the i.v. tests and 20 ml kg^{-1} in the oral tests. All solutions for i.v. injection were clear but, in oral tests, suspensions were also used. Ten mice were used for each dose level, dead animals were totalled 24 h after the drug had been given. The LD50 values were calculated according to the Nordic Pharmacopoeia and expressed as the amount of sennosides in the drug.

The laxative effect of the drugs was studied by the method of Lou (1949). Ten mice (20–30 g) were used for each test. Each animal was placed in a separate steel cage (6 × 18 cm) with a wire-grid floor and was supplied with a food preparation made by mixing 10 parts of powdered rat-cubes and 7 parts of water. Drugs were administered (10 ml kg^{-1}) into the stomach. Laxative activity was measured by counting the total number of wet faeces produced by the group of 10 mice over 24 h and expressed as the number of wet faeces kg^{-1} of mouse. The animals were used 4 or 5 times with intervening rest periods of one week. The drugs were administered in randomized order. The test were repeated so that results from five parallel experiments at each dose level were available. The regression lines for the dose response curves of each drug were calculated and the parallelity of the lines determined by comparing the regression coefficients ($P = 0.05$).

The results of the toxicity tests are seen in Table 1. No oral dose was lethal. Calcium sennosides A + B 20%

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