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Species differences in the contractile response to two specific histamine H_1-receptor agonists: 2-(2-pyridyl)-ethylamine and 2-(2-aminoethyl)-thiazole

M. M. VOHRA, *Department of Pharmacology, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7*

Recently, it was reported (Vohra 1980) that guinea-pig and not rat vas deferens contains a histamine H_1-receptor. According to this report one would therefore predict that 2-(2-pyridyl)-ethylamine (PEA) and 2-(2-aminoethyl)-thiazole (ThEA), the two well-known specific histamine H_1-receptor agonists identified by Levi et al (1975) and Durant et al (1975), should be inactive in the rat vas deferens preparation but active in that of the guinea-pig. This study shows instead that both PEA and ThEA elicit marked dose-related contractile responses in the rat vas deferens. While ThEA was almost equally effective in both species, PEA was far more effective in the rat than in the guinea-pig. These observations led me to investigate the mechanism of action of the contractions caused by PEA and ThEA in the vas deferens of both species.

Stripped vasa deferentia from Wistar rats, 200–300 g, and guinea-pigs (mixed colour), 300–450 g, were prepared as described by Vohra (1980). They were set up individually under 0·5 g tension in double-jacketed organ baths, containing 25 ml of Krebs Ringer bicarbonate solution gassed with 95% O_2/5% CO_2 at 36 ± 1 °C; pH was 7·3–7·4. The contractions were measured isometrically using Grass FT.03 force displacement transducers on a Grass 7P polygraph. One hour was allowed for equilibration before the tissues were exposed to the test compounds. Dose-response curves were obtained by a stepwise increase in the concentration of the compound. Each concentration was added for 1 to 2 min and washed out thoroughly before the next concentration was added. There were 10 min intervals between successive concentrations. The tissues were incubated with an antagonist for at least 10 min before the test compound was added. Rats were pretreated with reserpine (10 mg kg^{-1}) (Serpasil) and guinea-pigs with reserpine (5 mg kg^{-1}), injected intraperitoneally 20 h before killing. The peak tension which developed after each concentration was used as the response in the construction of the dose-response curve. Only one compound was used on each tissue.

Drugs used were: (−)-noradrenaline HCl, tyramine HCl, scopolamine HCl, hexamethonium HCl, acetylcholine chloride (all from Sigma); mepyramine maleate (Poulenc); cocaine HCl (May and Baker); desipramine HCl (Geigy); phentolamine HCl, reserpine (Serpasil) (Ciba); cimetidine HCl, 2-(2-pyridyl)-ethylamine (SKF-71432-A_2), and 2-(2-amino ethyl)-thiazole (SKF-71481-A_2) (all from Smith, Kline and French).

Fig. 1 shows that both PEA and ThEA caused dose-related contractions in the vasa deferentia of the two species. PEA had far more efficacy in the rat than in the guinea-pig. ThEA was almost equally effective in both species. PEA had greater potency than ThEA in the rat but not in the guinea-pig. It also had less efficacy than ThEA in the guinea-pig.

In guinea-pig, the contractions caused by both compounds were not reduced or blocked by cimetidine (3·4–34·0 μM), a histamine H_2-receptor antagonist, but were completely abolished by mepyramine (0·05–0·10 μM), an H_1-receptor antagonist. The contractions were unaltered in the presence of 1 μM phentolamine

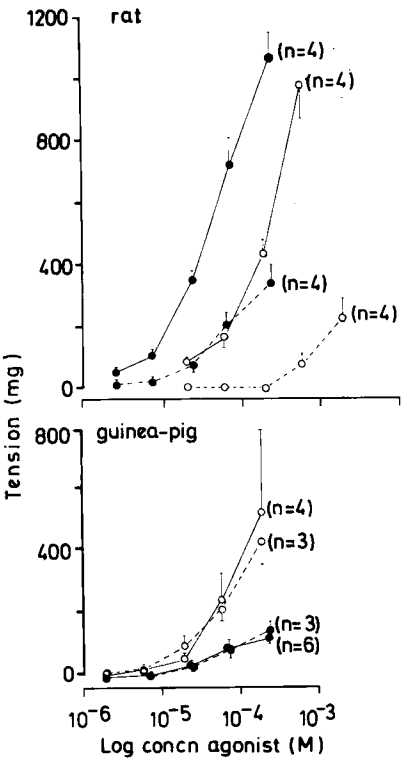

FIG. 1. Dose-response curves for 2-(2-pyridyl)-ethylamine (closed circles) and 2-(2-aminoethyl)-thiazole (open circles) on the rat and guinea-pig vas deferens. Solid lines represent responses before, dashed lines responses following, pretreatment with reserpine. Each value refers to mean ± s.e.m. (Vertical bars); n = number of tissues used.

and were only slightly reduced ($\simeq$ 10–15%; n = 4) at 5 μM concentration, though the contractions to added noradrenaline (24 μM) were completely blocked by 1 μM phentolamine. The contractions to PEA and ThEA were not abolished by desipramine (1–3 μM) and cocaine (3–10 μM), two well known blockers of the amine uptake process; the contractions caused by 2×10^{-4} M tyramine, however, were completely abolished by these concentrations of the inhibitors. Furthermore, neither hexamethonium (36 μM) nor scopolamine (2·8–14 μM) affected the contractions to either PEA or ThEA. In these concentrations, scopolamine completely blocked the contractions to acetylcholine (11 μM). As shown in Fig. 1, the contractions caused by both agents were unaffected following pretreatment with reserpine. Thus, findings in the guinea-pig show that contractions caused by PEA and ThEA are selectively blocked by the conventional antihistamine, mepyramine (Fig. 2), indicating that they are produced via activation of the H_1-receptors.

In the rat, however, contractions of the vas deferens caused by PEA and ThEA were neither antagonized by mepyramine (0·05–25·0 μM) nor by cimetidine (3·4–34·0 μM). They were also unaffected by hexamethonium (36 μM) or scopolamine (2·8–14·0 μM). The concentrations of scopolamine completely blocked the contractions to acetylcholine (52 μM). However, contractions to both agents were markedly reduced or completely antagonized by phentolamine (1–8 μM). In the presence of these concentrations of phentolamine, the contractions induced by adding exogenous noradrenaline were completely blocked. They were also markedly reduced or abolished by both desipramine (1–3 μM) and cocaine (3–10 μM). In these experiments, the contractions evoked by tyramine (29 μM) were blocked by desipramine (1–3 μM) but not by cocaine (3–10 μM). This lack of antagonism by cocaine of tyramine-evoked contractions in the rat vas deferens has been well documented previously (see Barnett et al 1968; Vohra 1969). These findings suggest that contractions of the rat vas deferens caused by the compounds are probably mediated via release of endogenous noradrenaline.

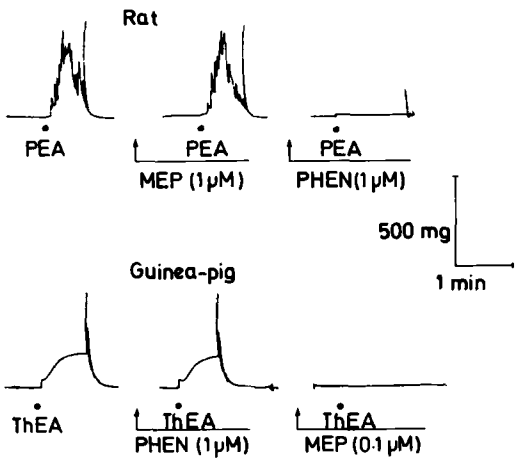

FIG. 2. Effect of phentolamine (PHEN) and mepyramine (MEP.) on contractions of rat and guinea-pig vas deferens caused by 2-(2-pyridyl)-ethylamine (PEA 75 μM) and 2-(2-aminoethyl)-thiazole (ThEA 60 μM).

To confirm these findings I investigated the effect of PEA and ThEA on the vasa deferentia of rats pretreated with reserpine (10 mg kg^{-1} i.p. for 20 h). As shown in Fig. 1, the contractions to both compounds were abolished or markedly reduced in such tissues, depending on the concentration used. Also, in these preparations, the contractions to low concentrations of tyramine (0·7–29·0 μM) were completely abolished although there were some residual contractions to high concentrations (58–115 μM). The residual contractions to both H_1-receptor agonists and to tyramine in the vas deferens of rats treated with reserpine were antagonized by 1 μM desipramine, indicating that the contractions in these tissues were due to insufficient depletion of noradrenaline by reserpine.

These results indicate that although PEA and ThEA cause dose-related contractions in the vasa deferentia of the rat and guinea-pig, the mechanism of their contractions is different in the two species. In the guinea-pig, contractions caused by these compounds are mediated through the stimulation of histamine H_1-receptor. Contractions caused in the rat vas deferens are secondary to release of endogenous noradrenaline. The mechanism by which PEA and ThEA release noradrenaline from the adrenergic nerve endings in the rat vas deferens is under investigation. Preliminary results indicate that the two compounds cause release of noradrenaline by entering the nerve endings and by probably displacing noradrenaline from its storage sites. This is consistent with the present finding that the contractile responses to both compounds were abolished by cocaine and desipramine. Thus, the results of this study support previous findings (Vohra 1980) that the rat vas deferens is virtually devoid of histamine H_1-receptors. Furthermore, the present findings also indicate that 2-(2-pyridyl)-ethylamine and 2-(2-aminoethyl)-thiazole, two well-known specific histamine H_1-receptor agonists, may affect not only histamine H_1-receptors. This emphasizes that caution is needed in interpreting data with these agonists.

Flynn et al (1979) also recently found a cimetidine-resistant component to positive chronotropic effect of PEA in guinea-pig heart. Since they found that this residual effect was blocked by propranolol but not by mepyramine, they said this indicated that PEA may interact with β_1-receptors. I suggest that their findings like the ones reported in the present paper, may have been due to release of noradrenaline.

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

G. B. FREGNAN*, M. PRADA, A. L. TORSELLO, M. VIDALI, *Divisione Ricerche Lusofarmaco, Via Carnia 26, 20132 Milano, Italy*

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.

* Correspondence.