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β-Adrenoceptor Profile of Ractopamine HCl in Isolated Smooth and Cardiac Muscle Tissues of Rat and Guinea-pig

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Abstract—The investigational sympathomimetic amine, ractopamine hydrochloride, has been profiled for adrenergic activity in selected smooth and cardiac muscle preparations. There was no significant interaction of ractopamine with α-adrenergic receptors in the rat vas deferens at concentrations up to 10^{-5} M. However, ractopamine produced a concentration-dependent increase in the force and rate of contractions of atria isolated from normal and reserpinized guinea-pigs (EC50 = 1×10^{-7} M). These increases were submaximal compared with isoprenaline (70–85%), suggesting partial agonist activity at the β_1-receptor site. Ractopamine completely relaxed the KCl-contracted guinea-pig trachea and rat costo-uterine smooth muscle to their resting tensions (EC50 = 3×10^{-7} and $5{\cdot}5 \times 10^{-8}$ M, respectively), indicative of full β_2-agonist properties. Propranolol blocked the response of ractopamine in isolated tracheal and atrial tissues ($pA_2 = 7{\cdot}70$), demonstrating a β-adrenergic mechanism of activity. Ractopamine also exhibited antagonism of the response of the guinea-pig trachea to the β-agonist, isoprenaline. Relative to other β-agonists, ractopamine was 100-fold more potent than the phenethanolamines, salbutamol and ritodrine, at the β_1-adrenoceptor, and approximately 7- to 11-fold more potent than ritodrine, but only one-sixth to one-tenth as potent as salbutamol at the β_2-adrenoceptor. Thus, ractopamine possesses significant β_1- and β_2-agonist properties. The submaximal stimulation of the force and rate of atrial contractions is indicative of a partial β_1-agonist, while the maximal relaxation of tracheal and costo-uterine smooth muscle is characteristic of a full β_2-agonist.

Recently, a class of compounds collectively referred to as phenethanolamines has been found to increase muscle mass and reduce fat content in domestic livestock (Baker et al 1984; Dalrymple et al 1984; Anderson et al 1987). It is generally thought that the effects of phenethanolamines in stimulating lipolysis and increasing muscle mass is due to β-adrenoceptor activation (Lands et al 1967; Bowman 1980). Ractopamine hydrochloride is a member of the phenethanolamine class and is being developed as the racemate for use as a feed additive to promote growth and carcass leanness in swine. Ractopamine, (±)-4-hydroxy-a-(((3-(4-hydroxyphenyl)-1-methylpropyl)-amino)methyl)benzmethanol hydrochloride (Fig. 1), is a sympathomimetic amine with β-agonist activity. Other compounds in this class, such as ritodrine and salbutamol, have been shown to be β_2-selective agonists, and are used in the management of preterm labour and asthma, respectively (O'Donnell 1972; Cullum et al 1969; Barden et al 1980; Gerritse et al 1985a,b; Broadley et al 1986; Grassby & Broadley 1986).

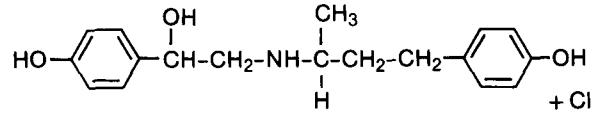

FIG. 1. Structure of ractopamine hydrochloride (mol. wt = 338).

Since β-adrenoceptor activity appears to be an important characteristic of phenethanolamines relative to their effects on growth promotion, the present studies were designed to provide an adrenergic profile of ractopamine hydrochloride in smooth and cardiac muscles. It was of additional interest to compare the β-adrenergic properties of ractopamine with the β_2 selective agonists, ritodrine and salbutamol.

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Materials and Methods

Tissue preparations

Male and virgin female Sprague-Dawley rats, 200–330 g (Harlan Industries, Inc., Indianapolis, IN, USA), and male Hartley guinea-pigs, 240–465 g (Charles River Laboratories, Wilmington, MA, USA), were used.

Rats were killed by cervical dislocation, and the tissues were removed from the animals. One end of the vas deferens or costo-uterine muscle was tied with thread to a stationary glass rod and the tissue placed in an organ bath containing Krebs bicarbonate solution. The other end was attached with thread to a force-displacement transducer. The tissues were placed under a resting tension of 250–500 mg. In some instances, the vas deferens was stimulated with square wave pulses at supramaximal voltage (40 V), 1·0 ms duration, and 0·1 Hz.

Guinea-pigs were killed by cervical dislocation. Some guinea-pigs were pretreated with reserpine (2·5 mg kg^{-1}, i.p.) 48 and 24 h before they were killed. Beating hearts were removed from each animal and placed in Krebs bicarbonate solution. Atria (right and left combined) were dissected and suspended in isolated organ baths by attaching one end of the tissue to a stationary glass rod and the other end to a force-displacement transducer. Atria preparations were placed under a passive force of 2 g. Trachea were placed in Krebs bicarbonate solution and cut into 4–5 mm rings (Blattner et al 1980). The tracheal rings were transferred to tissue supports and placed under a resting tension of approximately 2 g.

The isolated tissues were placed in Krebs bicarbonate

solution with the following composition (mM): 118·2 NaCl, 4·6 KCl, 1·6 $CaCl_2 \cdot 2H_2O$, 1·2 KH_2PO_4, 1·2 $MgSO_4 \cdot 7H_2O$, 24·8 $NaHCO_3$, 10·0 dextrose (pH 7·4), and allowed to equilibrate for approximately 1 or 2 h at their assigned resting tensions before drugs were tested. Tissue bath solutions were maintained at 37°C and aerated with 95% O_2–5% CO_2. Isometric measurements were made with either a Statham UC-3 transducer or Grass FT03 transducer and recorded on a Beckman Dynograph Model R-611 or R-411 as changes in grams of force. Atrial rate was determined with the use of a cardiotachometer.

Bioassay

Ractopamine was examined for its ability to contract each preparation from baseline tension, to antagonize the contraction or relaxation produced by standard agonists, and to relax the KCl-($3{\cdot}6 \times 10^{-2}$ M) contracted trachea and costo-uterine muscles. Vehicle controls were run to ensure that any noted action was due to the drug and not to vehicle or to time considerations. A minimum of three tissues per treatment (ractopamine or control) were utilized in all experiments. Phenoxybenzamine HCl (1×10^{-6} M) was kept in contact with the isolated costo-uterine tissues during the entire experiment to rule out the possibility of α-receptor activation. In experiments where propranolol was examined, two concentrations were used to antagonize the effects of the agonists.

Statistical analysis

Results were expressed as the mean ± s.e.m. Statistical differences between tissues treated with ractopamine or vehicle were determined by Student's *t*-test for unpaired groups (Haber & Runyon 1977). Statistical significance was evaluated at $P < 0{\cdot}05$. For comparative purposes, $pA_2(-K_b)$ (Arunlakshana & Schild 1959) values were calculated for each of the atrial experiments. The pA_2 is defined as that concentration of antagonist which would produce a two-fold (rightward) shift in the concentration-response curve to a given agonist (Tallarida & Jacob 1979).

Drugs and related compounds

All compounds were prepared in Milli-Q water. Dilutions of the stock solutions to achieve tissue bath concentrations were made in Krebs bicarbonate buffer. The sources of the compounds used were as follows: ractopamine HCl, Lilly Research Laboratories, Indianapolis, IN, USA; (±)-propranolol HCl, (−)-isoprenaline HCl, reserpine, salbutamol, and noradrenaline bitartrate, Sigma Chemical Co., St Louis, MO, USA; phenoxybenzamine HCl, Research Biochemicals Inc., Wayland, MA, USA; yutopar (ritodrine HCl), Astra. Ractopamine is a racemic mixture of four stereoisomers: 52% *RS*, *SR* and 48% *RR*, *SS*. The *RR* stereoisomer is the most pharmacologically active of the four stereoisomers and comprizes approximately 25% of the mixture.

Results

In the rat vas deferens, ractopamine (10^{-9}–10^{-5} M) did not antagonize the response of noradrenaline, did not contract or relax the tissue from baseline, and did not alter the response to field stimulation (data not shown).

Ractopamine relaxed the KCl- contracted costo-uterine muscle to pre-contracted levels at a concentation of 10^{-5} M (Fig. 2). The EC50 was $5{\cdot}5 \times 10^{-8}$ M (Table 1).

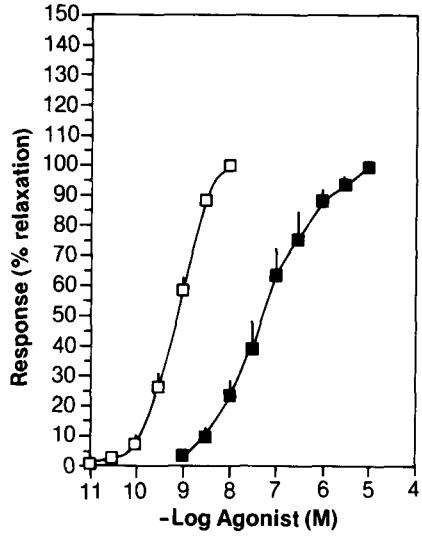

FIG. 2. The effects of isoprenaline (□, n = 18) or ractopamine HCl (10^{-9}–10^{-5} M) (■, n = 8) on the KCl ($3{\cdot}6 \times 10^{-2}$ M)-contracted Sprague-Dawley rat costo-uterine smooth muscle. Phenoxybenzamine HCl (1×10^{-6} M) was present in the tissue baths during the entire experiment. The average response to KCl was 0·7 ± 0·1 g (n = 8).

Table 1. EC50 values and potency estimates of isoprenaline, salbutamol, ritodrine, and ractopamine.

Tissue	Test	Isoprenaline	Salbutamol	Ritodrine	Ractopamine
Guinea-pig atria (β_1)	EC50 (M)	8×10^{-9} (11)	1×10^{-5} (3)	1×10^{-5} (4)	1×10^{-7} (8)
	Potency	1·00	0·0008	0·0008	0·08
	Fraction of isoprenaline max.	1·00	0·89	0·89	0·73
Guinea-pig trachea (β_2)	EC50 (M)	3×10^{-8} (14)	3×10^{-8} (3)	2×10^{-6} (3)	3×10^{-7} (8)
	Potency	1·00	1·00	0·015	0·10
	Fraction of isoprenaline max.	1·00	1·35	1·32	1·00
Rat costo-uterine (β_2)	EC50 (M)	6×10^{-10} (18)	1×10^{-8} (7)	6×10^{-7} (4)	$5{\cdot}5 \times 10^{-8}$ (8)
	Potency	1·00	0·06	0·001	0·011
	Fraction of isoprenaline max.	1·00	1·00	1·00	1·00

EC50 = concentration of an agonist that is required to produce 50% of the maximum response. Responses in the trachea and costo-uterine muscle were evaluated based upon contraction force, while responses in the atria were determined based upon the rate of contraction. ( ) = number of experiments. Potency = ratio of the EC50 value of isoprenaline to the EC50 values of isoprenaline, salbutamol, ritodrine, or ractopamine.

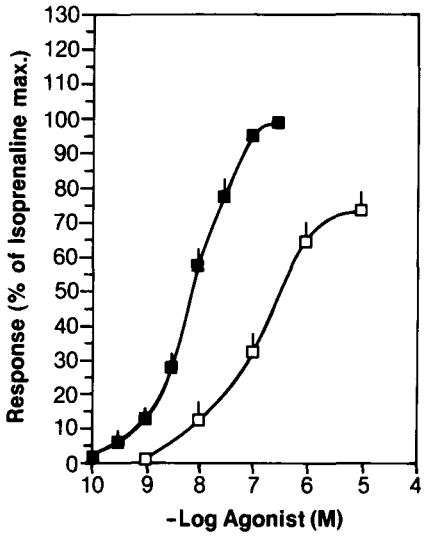

FIG. 3. The effect of isoprenaline (■, n = 11) or ractopamine HCl (□, n = 8) on the rate of contractions of the guinea-pig spontaneously beating atria. Each point represents the mean ± s.e. of the number of observations indicated in the legend. The average maximum response to isoprenaline was 150 ± 5 beats min^{-1} (n = 11).

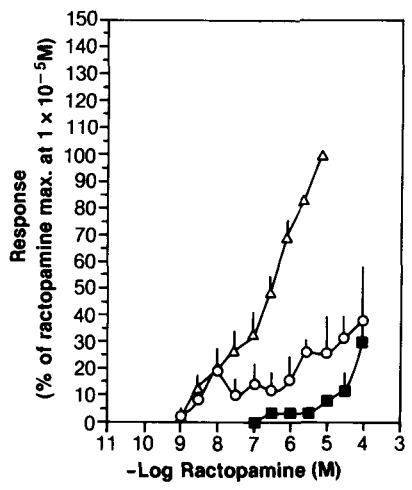

FIG. 4. The effect of propranolol (1×10^{-7} M ○, n = 3; and 1×10^{-6} M ■, n = 3) on the relaxation of the guinea-pig KCl-contracted trachea induced by ractopamine (△, n = 8). The initial average maximum response to KCl was 1·1 ± 0·1 g (n = 14). Each point represents the mean ± s.e. of the number of observations indicated in the legend. * $P < 0.05$ for the comparison of the ractopamine HCl group with the vehicle group by Student's t-test for unpaired data.

Ractopamine produced a significant concentration-dependent increase in both force and rate of contraction of the guinea-pig atrium as compared with the vehicle. Fig. 3 shows the rate of response as a percent of the isoprenaline maximum response. Propranolol antagonized the effect of ractopamine (rightward, parallel shift) on atrial rate ($pA_2 = 7.70$). The pA_2 value is slightly lower than that reported by Hartley & Pennefather (1984) for isoprenaline antagonism due to propranolol ($pA_2 = 8.86$). Atria from guinea-pigs pretreated with reserpine exhibited a similar concentration-dependent increase in both force and rate of contraction in response to ractopamine (data not shown). The EC50 values for the increase in force and rate of atrial preparations from animals pretreated with reserpine were similar to those of normal tissues with ranges of 6.5×10^{-8} to 1×10^{-7} and 1×10^{-7} to 2×10^{-7} M for normal and reserpinized atria, respectively.

The resting tension (1·78 g) in the (non-contracted, no KCl) guinea-pig trachea exposed cumulatively to 10^{-9} to 10^{-5} M ractopamine was unchanged except for a 0·14 g relaxation at 10^{-5} M ractopamine. Cumulative addition of 10^{-9} to 10^{-5} M ractopamine to the KCl contracted trachea, however, resulted in full relaxation of the contraction (0·94 g), an effect which was antagonized by propranolol (Fig. 4). Ractopamine also antagonized the response of the KCl-contracted trachea to isoprenaline (Fig. 5).

The β_2 selective adrenergic agonists, salbutamol and ritodrine, were also examined in the isolated atrial, tracheal, and costo-uterine preparations. Table 1 is a summary of the EC50 and potency values of salbutamol, ritodrine, and ractopamine compared with the standard, isoprenaline, in the isolated atria, trachea, and the costo-uterine smooth muscle.

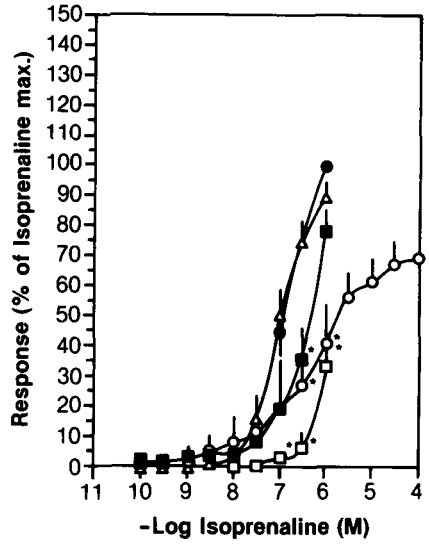

FIG. 5. The effect of ractopamine HCl (1×10^{-7} M ■, n = 3; 3×10^{-7} M ○, n = 4; and 1×10^{-6} M □, n = 3) or vehicle (△, n = 4) on the relaxation of the guinea-pig KCl-contracted trachea induced by isoprenaline (●, n = 14). The initial average maximum response to KCl was 1·1 ± 0·1 g (n = 14). Each point represents the mean ± s.e. of the number of observations indicated in the legend. * $P < 0.05$ for the comparison of the ractopamine HCl group with the vehicle group by Student's t-test for unpaired data.

Discussion

The pharmacological profiling of the investigational feed additive, ractopamine, in isolated smooth and cardiac muscle tissues revealed that this phenethanolamine does not appreciably interact with α_1- or α_2-adrenergic receptors in the rat vas deferens. On the other hand, ractopamine was a positive inotropic and chronotropic agent in the guinea-pig atria. Maximal β_1-agonist response was less than the maximum isoprenaline response in this tissue. Additionally, ractopamine also increased the rate and force of contractions in tissues from reserpinized animals indicating its mode of

action is direct β_1-adrenoreceptor activation rather than catecholamine release (Maling et al 1971). These effects were antagonized by the non-selective β-antagonist, propranolol, supporting the contention that the effect of ractopamine resulted from a direct interaction with β-adrenoceptors. Also, ractopamine was approximately 100-times more potent than the phenethanolamines, salbutamol and ritodrine, as a β_1-agonist. In the guinea-pig trachea and rat costo-uterine smooth muscles, ractopamine relaxed the tissues maximally (100%), effects which were blocked by propranolol. However, the block by propranolol was not parallel in nature, which might suggest a non-competitive component to the antagonism. Relative to the β_2-agonists, salbutamol and ritodrine, ractopamine is intermediate in potency as an agonist at the β_2-receptor (salbutamol > ractopamine > ritodrine). Previous reports on another β_2-agonist, terbutaline, indicate that ractopamine is approximately one-half as potent in the KCl-contracted costo-uterine smooth muscle as this sympathomimetic amine (Colbert et al 1991). The higher relative potency of ractopamine at the β_1-receptor site may be partially explained by Smith et al (personal communication) who characterized the β_1- and β_2-receptor affinities of ractopamine in C_6 rat glioma cells and found it to be a β_1 selective agonist. It should also be noted that Carswell & Nahorski (1983) reported a 15:85 proportion of β_1:β_2 receptor binding sites in guinea-pig trachea. Thus, a small percentage of the activity of the agonists observed in the trachea may be attributable to β_1 activity.

Ractopamine also acted as a β_2-antagonist by blocking the response of the trachea to isoprenaline. However, this antagonist activity appeared to be non-selective in nature since a non-parallel shift in the dose–response curve was observed.

By classical definition, full receptor agonists produce the maximal response for a given tissue while partial agonists produce a maximal response which is below the maximum for that tissue as defined by a full agonist, such as isoprenaline (Marano & Kauman 1976; Zaborowsky et al 1980; Cohen et al 1982). Therefore, based upon the data from these experiments, ractopamine would be characterized as a partial β_1-agonist and a full β_2-agonist.

Acknowledgement

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