J. vet. Pharmacol. Therap. **30,** 496–499, doi: 10.1111/j.1365-2885.2007.00882.x. SHORT COMMUNICATION

Some pharmacodynamic effects of eformoterol in the horse

G. J. B. LADAGA*,†
F. P. LEZICA*
G. FERRARO‡ &
G. A. DE ERAUSQUIN*,§

*INCA Group, Buenos Aires, Argentina; †Laboratorio Fundación, Buenos Aires, Argentina; ‡Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina; §Department of Neurology, Washington University School of Medicine, St Louis, MO, USA

(Paper received 11 March 2007; accepted for publication 4 June 2007)

Gabriel A. de Erausquin, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8134, St Louis, MO 63110, USA. E-mail: erausquing@tci.wustl.edu

We recently showed that eformoterol is a rational choice for the treatment of exercise-induced pulmonary hemorrhage (Ladaga *et al.*, 2003). Administration of eformoterol results in bronchodilation, leading to reduced transmural alveolar pressure (Hoffman & Lefkowitz, 1982; Faulds *et al.*, 1991; Barnes, 1993; O'Donnell & Anderson, 1995). In addition, eformoterol inhibits bronchoconstriction induced by angiotensin II, adenosine, tachykinins and histamine (Advenier *et al.*, 1992; Verleden *et al.*, 1993; Ochsner, 1996; Nightingale *et al.*, 1999). Furthermore, it causes vasodilatation (Hoffman & Lefkowitz, 1982), increases mucociliary activity (Lindberg *et al.*, 1995), reduces endothelial permeability and endothelial fenestrations, and ensuing plasma exudation (Advenier *et al.*, 1992; Baluk & McDonald, 1994; Zink *et al.*, 1995), and inhibits release of inflammatory factors reducing eosinophil adhesion (Mita & Shida, 1983; Greiff *et al.*, 1998). In the present work, we studied the time course and dose response relationship of physiological and metabolic changes following the administration of eformoterol as a single dose to horses. This information is of importance for the potential use of this drug in clinical veterinary practice.

Measurements were carried out in horses located in a facility located in San Antonio de Areco, Buenos Aires, Argentina, during the spring, with field temperature: 15–20 °C. Thirty-three cross breed and thoroughbred horses, males and females, 2–12 years of age, weighing 450–500 kg were randomly assigned to one of six groups. Animals were stabled the night before field trial. From there on only water *ad libitum* was offered until the end of the sampling. All horses were clinically and with selected hematology variables within established reference ranges. Mares were not pregnant or lactating and were in anestrus. Eformoterol (Arterol®; Laboratorio Fundación, Buenos Aires, Argentina) was injected intramuscularly in a volume of 2 mL in the neck as a single dose of either 0.01, 0.02, 0.04, 0.08 or 0.12 mg. We collected data on heart rate (HR), respiratory rate (RR), depth of respiration, heart rhythm (auscultation), and data collected from a detailed clinical assessment. Depth of respiration was scored according to the following scale: 1, no respiratory sounds by auscultation in the middle diaphragmatic region and minimal or undetectable expansion by palpation; 2, light respiratory sounds by auscultation in the middle diaphragmatic region and partial expansion by palpation; 3, full respiratory sounds by auscultation in the middle diaphragmatic region and full expansion by palpation. Blood samples were collected from the jugular vein at 15, 30 and 45 min, and 1, 2, 3, 5, 6.5, 8 and 12 h following drug administration at 8 AM, and processed within 4 h of extraction. We measured potassium (2.5–4.0 mM/L), glucose (4.0–8.0 mM/L), aspartate methyl transferase (AST) (100–230 U/L), gamma glutamyl transferase (GGT) (0–5 U/L), creatine phosphokinase (CPK) (5–80 U/L), alkaline phosphatase (AP) (25–90 U/L), bilirubin (direct: 3.4–13 μmol/L and total: 15–44 μmol/L), BUN (11–24 mmol/L), creatinine (80–180 μmol/L), total protein (60–70 g/L) and albumin (30–35 g/L) by spectrophotometry (Metrolab 2300, Buenos Aires, Argentina). Glutamate dehydrogenase (GLDH) was measured by immunoassay. Statistical comparisons for main effects (dose and time) were carried out by two way ANOVA, followed by *post hoc* comparisons with Tukey test (SPSS 14.0; Lead Technologies, Inc., Charlotte, NC, USA).

Following administration of eformoterol, presence of sweat was evident at the injection site, usually affecting a small area of 3–5 cm in diameter and beginning 15 min after injection and lasting up to 5 h. Prolonged sweating was routinely observed with doses higher than 0.08 mg, being profuse at the base of the ear, neck, inguinal fossa, medial aspect of the thigh and in three cases extended to a great part of the body. Piloerection begins shortly after diaphoresis but lasts somewhat longer. Thirty minutes after injection, muscular tremor involving supra- and sub-scapular muscles is evident, and it continues for a variable time thereafter. Increased peristaltismus, judged by rate and loudness of borborigmus appeared about 1 h after injection and was unrelenting for 4–5 h. In general it lead to defecation. Administration of 0.12 mg caused significant nervousness and increased excitability (i.e. stall walking and neighs) beginning 30 min after injection. There was no conjunctival irritation, penile relaxation or paradoxical bronchospasm. Following administration of eformoterol, animals experienced pronounced decrease in respiratory rate with a marked increase in depth (judged by ventilation auscultated in the diaphragmatic lung

lobe). Onset of respiratory changes was detected 15 min after administration and persisted beyond 6 h (Fig. 1a, ANOVA main effect $P < 0.001$, $F = 23.4$, df = 9). Some degree of diaphragmatic ventilation was preserved in all animals beyond 8 h. Changes in respiratory rate were dose dependent (Fig. 1c, ANOVA main effect $P < 0.001$, $F = 8.39$, df = 4), but the dose relationship followed a U shape, with maximum effect at 0.04 mg. Administration of eformoterol resulted in a fast increase in heart rate which became significantly different from baseline by 30 min (Fig. 1b, ANOVA main effect $P < 0.001$, $F = 3.513$, df = 4). Three hours after administration of eformoterol, heart rate became slower than baseline, but this change was not statistically significant. Small doses of eformoterol (0.01 or 0.02 mg) did not affect average heart rates over the observation period. Increasing doses had an effect that appeared best described by a U shape, with 0.04 mg resulting in decreased heart rate, and higher doses causing an increase in this parameter (Fig. 1d, ANOVA main effect $P < 0.001$, $F = 9.752$, df = 4). No significant changes were detected in liver function tests or major plasma electrolyte concentrations. On the other hand, administration of 0.08 or 0.12 mg of eformoterol resulted in hyperglycemia beginning 1 h after injection and surpassing the 110 mg% limit (Table 1).

In a previous communication we demonstrated that eformoterol in doses of 0.04–0.08 mg can reduce the risk of exercise-induced pulmonary hemorrhage in horses undergoing competitive training (Ladaga *et al.*, 2003). Such range of dose was determined on a preliminary open trial that suggested optimal clinical doses of 0.040 mg for animals weighing 300 kg and 0.020 mg for animals with <300 kg body weight and colts (Laboratorio Fundación, unpublished data). In the same open trial, we found that doses higher than 0.080 mg appeared to have clinical side effects, but laboratory parameters were not assessed. We now report the physiological and metabolic effects of eformoterol on a range of doses selected to span those that have been shown previously to produce clinical efficacy. Sweatiness is likely due to β_2 adrenergic stimulation of the apocrine glands, specific to equines. It is a clearly visible parameter suggestive of susceptibility to the drug. The response of piloerector muscles is in general an $\alpha 1$ response, but seems to be β_2 in equine. One interesting observation is that while in equine increases in intestinal motility with defecation are observed, in research animals motility is decreased (Takeda & Takagi, 1980; Kawakami, 1984). A profound and persistent change in the respiratory parameters follows intramuscular administration of eformoterol, including a decrease in the respiratory rate and a clear auscultation of the diaphragmatic lung lobe associated with a visible dilatation of the costal arcade. Heart rate was increased briefly, and decreased in a sustained fashion beginning 3 h after administration. In animal models and in humans, eformoterol has chronotropic and positive inotropic effects (Ida, 1976, 1980; Lecaillon *et al.*, 1999) which would explain initial tachycardia. A possible explanation for the delayed bradychardia, can be provided by the sustained increase in ventilation following eformoterol administration. Such increase could lead to improved hematosis, increased pO2, and secondary reduction in heart rate. In the range of doses observed, the only metabolic parameter altered was serum glucose. Thus, single administration appears to have no

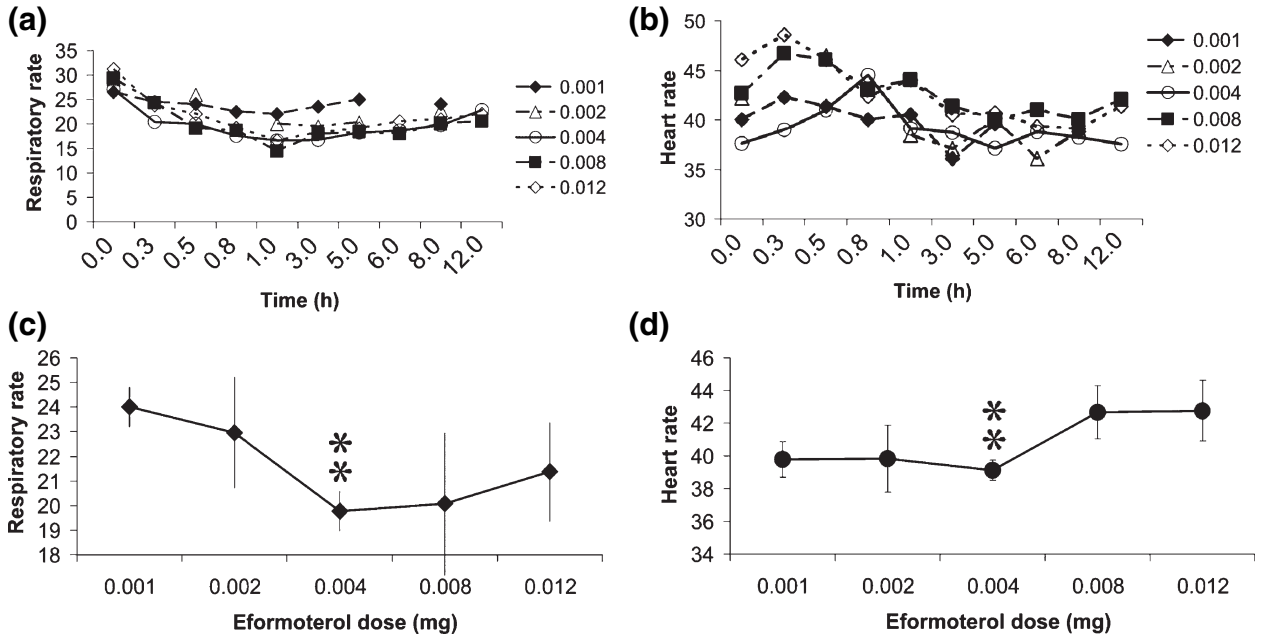

Fig. 1. Time and dose-dependency of physiological changes induced by eformoterol. Panels a and b show the time course of respiratory (a) and heart (b) rates with increasing doses of subcutaneous eformoterol (in mg). Each point represents mean (±standard deviation) of five or six animals. Panels c and d show dose-response curves for eformoterol-induced changes respiratory (c) and heart (d) rates at the time of the peak effect. Each point represents mean (±standard deviation) of five or six animals. $^{**}P < 0.01$ by ANOVA followed by Tukey *post hoc* comparison.

Table 1. Time course of changes in laboratory values after subcutaneous administration of eformoterol. Values represent mean (± standard deviation) of five or six animals. The left most column (*t*) indicates time (in h) after injection. For each laboratory measure the top row (d) indicates eformoterol dose (either 0.08 or 0.12) in milligram. Reference ranges for each of the laboratories are as follows: potassium (K, 2.5–4.0 mM/L), glucose (Glu, 4.0–8.0 mM/L), aspartate methyl transferase (AST, 100–230 U/L), gamma glutamyl transferase (GGT, 0–5 U/L), creatine phosphokinase (CPK, 5–80 U/L), alkaline phosphatase (Alk phos, 25–90 U/L), bilirubin (Bili, direct: 3.4–13 μmol/L and total: 15–44 μmol/L), BUN (11–24 mmol/L), creatinine (80–180 μmol/L), total protein (60–70 g/L) and albumin (30–35 g/L)

	K		Glu		Urea		Protein total		Globulins	
	0.08	0.12	0.08	0.12	0.08	0.12	0.08	0.12	0.08	0.12
0	3.95 ± 0.21	3.98 ± 0.25	90.50 ± 33.23	99.00 ± 8.49	28.00 ± 7.00	25.50 ± 2.50	6.15 ± 0.75	6.40 ± 0.10	2.75 ± 0.95	3.75 ± 0.45
0.25	3.70 ± 0.00	3.90 ± 0.14	98.50 ± 16.26	105.50 ± 7.78						
0.5	4.05 ± 0.21	3.78 ± 0.18	103.50 ± 20.51	108.50 ± 16.26	30.50 ± 5.50	25.00 ± 0.00	6.55 ± 0.65	6.55 ± 0.25	3.45 ± 0.55	3.70 ± 0.70
0.75	4.40 ± 0.21	3.73 ± 0.04	112.00 ± 36.77	108.00 ± 18.38						
1	4.15 ± 0.49	3.80 ± 0.14	110.50 ± 34.65	115.50 ± 3.54	31.50 ± 11.50	26.00 ± 0.00	7.10 ± 0.20	6.40 ± 0.10	3.90 ± 0.20	3.70 ± 0.50
2	3.95 ± 0.07	3.73 ± 0.53	140.50 ± 27.58	132.50 ± 9.19						
3	4.10 ± 0.28	3.95 ± 0.28	141.50 ± 0.71	148.50 ± 26.16	31.50 ± 6.50	27.00 ± 7.00	6.25 ± 0.45	6.60 ± 0.20	3.10 ± 0.60	3.50 ± 0.90
5	4.20 ± 0.64	4.15 ± 0.42	129.50 ± 24.75	144.00 ± 38.18						
6.5	4.48 ± 1.03	3.93 ± 1.17	112.50 ± 0.71	158.00 ± 9.90						
8	4.18 ± 0.11	4.18 ± 0.18	95.00 ± 4.24	137.00 ± 2.83	32.00 ± 12.00	30.00 ± 0.00	6.20 ± 0.30	6.65 ± 0.35	2.90 ± 0.40	3.70 ± 0.80
12	4.05 ± 0.21	3.75 ± 0.35	119.00 ± 0.00	126.00 ± 12.73	28.50 ± 9.50	27.50 ± 2.50	6.45 ± 0.55	6.50 ± 0.70	3.20 ± 0.60	3.75 ± 0.85
24					31.00 ± 9.00	30.00 ± 1.00	6.00 ± 0.70	6.70 ± 0.30	3.00 ± 0.80	3.90 ± 0.60

	Alk phos		GLDH		GGT		AST		CPK	
	0.08	0.12	0.08	0.12	0.08	0.12	0.08	0.12	0.08	0.12
0	164.00 ± 12.00	132.50 ± 23.50	3.30 ± 0.20	5.80 ± 3.70	11.90 ± 3.50	31.25 ± 15.25	218.00 ± 19.00	216.00 ± 58.00	134.50 ± 19.50	82.00 ± 5.00
0.5	161.50 ± 9.50	155.00 ± 22.00	3.35 ± 0.15	5.55 ± 3.05	9.50 ± 3.50	29.25 ± 10.25	233.00 ± 47.00	262.00 ± 30.00	103.50 ± 53.50	63.00 ± 3.00
1	166.00 ± 8.00	153.00 ± 33.00	4.05 ± 1.25	5.20 ± 2.00	12.50 ± 2.50	28.50 ± 13.50	250.50 ± 18.50	285.50 ± 67.50	106.50 ± 40.50	71.50 ± 1.50
3	180.50 ± 10.50	148.50 ± 27.50	6.00 ± 0.40	5.45 ± 1.55	13.75 ± 4.25	28.75 ± 10.75	259.50 ± 32.50	285.50 ± 58.50	102.00 ± 52.00	83.00 ± 8.00
8	166.00 ± 4.00	151.00 ± 34.00	5.25 ± 0.35	4.05 ± 1.25	13.10 ± 1.60	27.75 ± 9.25	241.50 ± 9.50	302.00 ± 79.00	80.50 ± 20.50	54.25 ± 1.75
12	150.50 ± 2.50	134.00 ± 19.00	4.55 ± 0.65	4.05 ± 0.55	26.15 ± 11.85	25.00 ± 9.00	237.00 ± 5.00	273.50 ± 74.50	87.50 ± 17.50	65.00 ± 15.00
24	148.00 ± 2.00	143.00 ± 30.00	6.35 ± 0.05	2.45 ± 0.05	30.15 ± 16.85	27.75 ± 13.25	159.00 ± 1.00	230.50 ± 49.50	86.50 ± 13.50	89.00 ± 19.00

	Bili dir		Bili ind		Bili total	
	0.08	0.12	0.08	0.12	0.08	0.12
0	0.25 ± 0.08	0.23 ± 0.01	0.93 ± 0.26	0.87 ± 0.08	1.18 ± 0.33	1.10 ± 0.08
0.5	0.20 ± 0.08	0.23 ± 0.05	0.97 ± 0.18	0.67 ± 0.01	1.16 ± 0.25	0.89 ± 0.05
1	0.28 ± 0.09	0.21 ± 0.03	0.99 ± 0.33	0.68 ± 0.09	1.26 ± 0.42	0.89 ± 0.12
3	0.17 ± 0.04	0.17 ± 0.00	0.95 ± 0.29	0.73 ± 0.02	1.09 ± 0.35	0.89 ± 0.02
8	0.26 ± 0.09	0.19 ± 0.02	0.93 ± 0.25	0.63 ± 0.01	1.18 ± 0.33	0.82 ± 0.03
12	0.24 ± 0.07	0.30 ± 0.05	0.91 ± 0.19	0.41 ± 0.01	1.14 ± 0.25	0.71 ± 0.03
24	0.23 ± 0.06	0.17 ± 0.02	0.95 ± 0.11	0.81 ± 0.19	1.18 ± 0.17	0.98 ± 0.21

deleterious effects on hepatic or muscle enzymes, and there is remarkable lack of effect on potassium ion concentrations. Notably, this is different from results observed in experimental animals and humans where a dose-dependent relationship in kalemia has been observed (Lecaillon *et al.*, 1999). A possible explanation for this discrepancy is the much larger muscle mass of the horse, which could provide for a greater potassium buffering capacity. Alternatively, the hyperglycemic effect is consistent with data in other species, accompanied by an increase in cAMP due to adenyl cyclase activation; an effect observed also in canine brain (Nishikori & Maeno, 1979; Mita & Shida, 1983).

In summary, we have found that eformoterol in a range of doses below and above those efficacious in the treatment of exercise-induced pulmonary hemorrhage, and after single administration, has few collateral effects and with no clinical significance. Furthermore, it results in profound changes in respiratory parameters consistent with improved gas exchange.

REFERENCES

Advenier, C., Qian, Y., Koune, J.D., Molimard, M., Candenas, M.L. & Naline, E. (1992) Formoterol and salbutamol inhibit bradykinin- and histamine-induced airway microvascular leakage in guinea-pig. *British Journal of Pharmacology*, **105,** 792–798.

Baluk, P. & McDonald, D.M. (1994) The beta 2-adrenergic receptor agonist formoterol reduces microvascular leakage by inhibiting endothelial gap formation. *American Journal of Physiology*, **266** (4 Pt 1), L461–468.

Barnes, P.J. (1993) Beta-adrenoceptors on smooth muscle, nerves and inflammatory cells. *Life Sciences*, **52,** 2101–2109.

Faulds, D., Hollingshead, L.M. & Goa, K.L. (1991) Formoterol. A review of its pharmacological properties and therapeutic potential in reversible obstructive airways disease. *Drugs*, **42,** 115–137.

Greiff, L., Wollmer, P., Andersson, M., Svensson, C. & Persson, C.G. (1998) Effects of formoterol on histamine induced plasma exudation in induced sputum from normal subjects. *Thorax*, **53,** 1010–1013.

Hoffman, B.B. & Lefkowitz, R.J. (1982) Agonist interactions with alpha-adrenergic receptors. *Journal of Cardiovascular Pharmacology*, **4** (Suppl. 1), S14–18.

Ida, H. (1976) Comparison of the action of BD 40 A and some other beta-adrenoceptor stimulants on the isolated trachea and atria of the guinea pig. *Arzneimittelforschung*, **26,** 839–842.

Ida, H. (1980) [General pharmacology of (alpha RS)-3-formamido-4-hydroxy-alpha-[[[(alpha RS)-p-methoxy-alpha-methylphenethyl]amino] methyl] benzyl alcohol fumarate dihydrate (BD 40A), a new bronchodilator agent]. *Nippon Yakurigaku Zasshi*, **76,** 633–654.

Kawakami, Y. (1984) Evaluation of therapeutic effects of formoterol, a b-adrenoceptor stimulating bronchodilator, on bronchial asthma. A multicenter double-blind single-dosis study. *Clinical Evaluation*, **12,** 697–727.

Ladaga, G.J.B., Pont Lezica, F., Ulloa, F., de Erausquin, G.A., Ruzzante, G., Negrelli, C. & del Carril, R. (2003) Evaluation of the efficacy of eformoterol on exercise-induced pulmonary hemorrhage in training thoruoughbred horses. *Online Journal of Veterinary Research*, **7,** 99–105.

Lecaillon, J.B., Kaiser, G., Palmisano, M., Morgan, J. & Della Cioppa, G. (1999) Pharmacokinetics and tolerability of formoterol in healthy volunteers after a single high dose of Foradil dry powder inhalation via Aerolizer. *European Journal of Clinical Pharmacology*, **55,** 131–138.

Lindberg, S., Khan, R. & Runer, T. (1995) The effects of formoterol, a long-acting beta 2-adrenoceptor agonist, on mucociliary activity. *European Journal of Pharmacology*, **285,** 275–280.

Mita, H. & Shida, T. (1983) Anti-allergic activity of formoterol, a new beta-adrenoceptor stimulant, and salbutamol in human leukocytes and human lung tissue. *Allergy*, **38,** 547–552.

Nightingale, J.A., Rogers, D.F. & Barnes, P.J. (1999) Differential effect of formoterol on adenosine monophosphate and histamine reactivity in asthma. *American Journal of Respiratory and Critical Care Medicine*, **159,** 1786–1790.

Nishikori, K. & Maeno, H. (1979) Characterization of beta-adrenergic receptor and adenylate cyclase in canine cerebellum. *Archives of Biochemistry and Biophysics*, **195,** 505–517.

O'Donnell, S.R. & Anderson, G.P. (1995) The effects of formoterol on plasma exudation produced by a localized acute inflammatory response to bradykinin in the tracheal mucosa of rats in vivo. *British Journal of Pharmacology*, **116,** 1571–1576.

Ochsner, M. (1996) Simultaneous measurement of Ca^{2+} transients and changes in the cell volume and microviscosity of the plasma membrane in smooth muscle cells. Evaluation of the effect of formoterol. *Biochemical Pharmacology*, **52,** 49–63.

Takeda, M. & Takagi, T. (1980) [Effects of a new bronchodilator, (alpha RS)-3-formamido-4-hydroxy-alpha-[[[(alpha RS)-p-methoxy-alpha-methylphenethyl] amino] methyl] benzyl alcohol fumarate dihydrate (BD 40A) on gastric acid secretion and gastrointestinal motility]. *Nippon Yakurigaku Zasshi*, **76,** 185–191.

Verleden, G.M., Belvisi, M.G., Rabe, K.F., Miura, M. & Barnes, P.J. (1993) Beta 2-adrenoceptor agonists inhibit NANC neural bronchoconstrictor responses in vitro. *Journal of Applied Physiology*, **74,** 1195–1199.

Zink, S., Rosen, P. & Lemoine, H. (1995) Micro- and macrovascular endothelial cells in beta-adrenergic regulation of transendothelial permeability. *American Journal of Physiology*, **269** (5 Pt 1), C1209–1218.