Original article

Ability of bronchodilators to prevent bovine experimental respiratory distress

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Summary — This cross-over trial involving 6 Blue Belgian double-muscled calves aimed to investigate whether ipratropium bromide inhaled alone or in combination with clenbuterol hydrochloride could prevent the dramatic clinical and pulmonary disturbances that are observed during an experimentally induced bronchoconstriction. Inhaled bronchodilators significantly influenced the clinical and functional responses of bovines subjected to a 5-hydroxytryptamine perfusion. However, despite a mean (standard error) wash-out period of 11.2 (3.1) and 7.5 (0.9) d after the 1st and the 2nd one-day challenges, respectively, first-order carry-over effects (*ie* those remaining from the previous treatment), effects of the period during which the treatment was allocated and interaction effects did not allow a definitive interpretation of overall treatment differences.

aerosol / bronchodilator / clenbuterol / inhalation / ipratropium

Résumé — Aptitude des bronchodilatateurs à prévenir un syndrome de détresse respiratoire expérimentale chez le bovin. *Six veaux hyperviandeux de race Blanc Bleu Belge ont été introduits dans cette étude dont le but fut de déterminer si le bromure d'ipratropium, inhalé seul ou en association avec de l'hydrochlorure de clenbutérol, permet de prévenir les perturbations cliniques et fonctionnelles observées lors d'une bronchoconstriction expérimentale. Les bronchodilatateurs influencèrent, de manière significative, la réponse clinique et fonctionnelle des bovins soumis à une perfusion lente de 5-hydroxytryptamine. Néanmoins, malgré un intervalle d'attente moyen (erreur standard) de 11,2 [3,1] et 7,5 [0,9] j respectivement après le premier et le deuxième traitement, des interactions, des effets transférés du traitement précédent et des effets de la période durant laquelle le traitement fut administré n'ont pas permis une interprétation définitive des différences thérapeutiques globales observées.*

aérosol / bronchodilatateur / clenbutérol / inhalation / ipratropium

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INTRODUCTION

Due to the morphological and functional peculiarities of their lungs, cattle are prone to develop rapid respiratory failure (Lekeux *et al*, 1984). Deaths, reduced weight gains, additional labour and veterinary costs all contribute to the financial loss that is due to respiratory disease in calves. In the UK, such losses exceed £50 million annually (Gourlay *et al*, 1989).

In Belgium, acute respiratory distress syndrome is a common and dramatic syndrome in which the bovine respiratory syncytial virus (Wellemans and Leunen, 1975) plays an important role. Several studies concerning the pathogenesis and the physiological changes associated with the bovine respiratory syncytial virus have been undertaken (Lekeux *et al*, 1985; Kimman *et al*, 1989a,b; Leblanc *et al*,1991) and have shown that the syndrome can be associated with airway obstruction (Lekeux *et al*, 1985) and hyperreactivity (Leblanc *et al*, 1991). Consequently, an aerosol delivery equipment suitable for calves has been designed. Several studies have been dedicated to the performances of this aerosol delivery equipment and to the physical properties of therapeutic droplets atomized by means of this equipment (Genicot *et al*, 1994a,b).

On the basis of this background, the present study aimed to investigate whether ipratropium bromide inhaled alone or in combination with clenbuterol hydrochloride was able to prevent the dramatic clinical and pulmonary disturbances that are observed during an experimentally induced bronchoconstriction (BC).

MATERIALS AND METHODS

Animals

Six Blue Belgian double-muscled calves with a mean (se, standard error) bodyweight of 112.0 (2.9) kg were involved in this cross-over trial which lasted 32 d. The animals were randomly allocated to a treatment (A, B or C) sequence (table I). A mean (se) wash-out period of 11.2 (3.1) and 7.5 (0.9) d was introduced after the 1st and the 2nd one-day challenge, respectively.

Examination under normal breathing (NB) conditions was performed 7.5 (1.6) d after the 3rd challenge.

Treatments

As described in table II, bronchodilators (treatments BC–A and BC–B) and saline (treatment BC–C) were atomized into the calves' tidal volume. After a 1 h resting period, a continuous intravenous administration (0.015 mg.kg^{-1}.min^{-1} active base) of a 5-hydroxytryptamine creatinine sulfate (Sigma Chemical Co, Saint Louis, MO, USA) solution began in the 3 groups. This administration was performed by means of a perfusion pump (960 Volumetric Infusion Pump, Imed Ltd, San Diego, CA, USA). The main effects of this inflammatory mediator are an intense tachypnea, a diffuse bronchoconstriction and a pulmonary arterial hypertension (Desmecht *et al*, 1992).

After the sequence of treatments, each animal was submitted to a further treatment (NB) which was undertaken in order to test the influence of the experimental conditions on the respiratory pattern. During NB, neither inhalant nor bronchoconstrictor was administered.

Pulmonary function tests

Throughout this study, pulmonary function tests using the monofrequency forced oscillation method (Reinhold *et al*, 1992; Close *et al*, 1994) were carried out. A compact portable device (LF1, Ganshorn, Medizin Electronic GmbH, Germany) was used to measure the oscillatory resistance (R_{os}) and the phase angle (Ψ) while the animals were in a quiet state and wearing a snugly fitting face mask which had been checked for its airtightness and dead space. The R_{os} and Ψ values made it possible to split the impedance of the respiratory system (Z_{rs}) into its real (Re) and imaginary (Im) components. The respiratory system compliance (C_{rs}) was calculated from the Im value (Close *et al*, 1994).

Table I. Design table for randomly allocated treat-
ment (A, B or C) sequences.

Treatment	Animals					
	1	*2*	*3*	*4*	*5*	*6*
1	A	A	B	B	C	C
2	B	C	A	C	A	B
3	C	B	C	A	B	A

Timetable for collection of clinical and pulmonary function data

During treatments BC–A, BC–B and BC–C, the respiratory rate was determined and a pulmonary function test was performed before (time 0) the inhalation of therapy A, B or C and at the 5th (time 5) and the 24th (time 24) min of the 5-hydroxytryptamine challenge which lasted 26.1 (0.5) min.

During the treatment (NB), which was conducted with calves that were breathing normally, the respiratory rates and pulmonary function parameters were collected twice at a 24 min interval, *ie* at 0 and 24 min, respectively.

Data analysis

For each animal, the relative changes observed in respiratory rates and pulmonary function parameters were expressed as a percentage of their baseline value. All these changes (Δ) were assessed using a small sample inferential procedure (Mendenhall and Reinmuth, 1978), *ie* a 2-tailed Student's *t* testing the null hypothesis $\Delta = 0\%$ against the alternative that Δ is either greater than or less than 0%.

In order to compare the protective properties of substances atomized in the tidal volume of calves, a crude overall within-animal comparison (Pocock, 1984) of substances was done on the respiratory rate and the pulmonary function parameters. A 1-tailed *t* test for paired differences was used to assess whether the mean differences as large as those observed had a reasonable chance of occurring even if the substances were equally effective (Pocock, 1984). This relatively straightforward analysis makes the 3 following main assumptions: no period effect, no treatment-period interaction, and no carry-over effect. Consequently, these 3 assumptions were checked as described in Jones and Kenward (1989). This check was especially important in the detection of period and treatment effects, interactions and first-order carry-over effects, *ie* those remaining from the previous treatment.

The significance of differences was accepted at $P \leq 0.05$. The results are presented as mean (se). When several *P*-values are required to

Table II. Substances atomized into the calves' tidal volume.

Treatment	Drug	Dose (mg) inserted in nebulizers	Active principle in the solution (%)
BC–A	Ipratropium bromide	0.6	0.025
BC–B	Ipratropium bromide and clenbuterol hydrochloride	0.6 0.075	0.025 0.003
BC–C	Saline solution	45	0.9
NB	None	None	–

The atomization was performed at a pressure of 600 kPA for a period of 5 min.

express the degree of significant changes observed in more than 2 groups or at the 2 different times (5 and 24), the ranges of P-values are expressed.

RESULTS

Clinical parameters

The mean respiratory rates collected during the 4 treatments are displayed in table III. The period during which the treatment was allocated, the actual and previous treatments all significantly influenced the responses. Treatment B led to a significantly better protection against tachypnea when the previous treatment had not included any bronchodilator.

The results from the crude analysis were as follows. Under the normal experimental conditions (NB), ie neither inhalant nor bronchoconstrictor administered, the respiratory rate did not significantly change ($P = 0.8194$) over time. However, under treatments BC–A, BC–B and BC–C, ie when 1 of the 3 inhalants and the bronchoconstrictor were administered, a significant ($0.0002 \leq P \leq 0.0342$) increase in the respiratory rate was observed at both times 5 and 24. This was especially true ($0.0002 \leq P \leq 0.0013$) during treatment BC–C. In treatment BC–C, the respiratory rates collected during the 5-hydroxytryptamine perfusion reached 282.1% (22.0%) of their baseline values. After pretreatments A and B, this percentage reached 207.5% (18.7%) during the perfusion.

At time 24, the relative longitudinal changes observed in respiratory rates of

Table III. Mean (se) respiratory rate *(RR)*, oscillatory resistance (R_{os}) and respiratory system compliance (C_{rs}) collected before the pretreatment (0), and at the 5th and the 24th min of the 5-hydroxytryptamine (BC) challenge.

Parameters	Treatments	Time (min)		
		0	5	24
RR (min⁻¹)	BC–A	27.8 (3.4)	54.7* (7.3)	54.8* (7.0)
	BC–B	27.2 (3.9)	49.0* (7.9)	51.8** (4.7)
	BC–C	23.8 (3.1)	64.0** (6.0)	63.0** (5.7)
	NB	30.3 (4.6)	–	29.8 (3.2)
R_{os} (kPa.L⁻¹.s)	BC–A	0.38 (0.02)	0.45 (0.02)	0.48 (0.04)
	BC–B	0.40 (0.02)	0.49* (0.03)	0.49 (0.04)
	BC–C	0.33 (0.03)	0.50** (0.02)	0.55** (0.02)
	NB	0.37 (0.03)	–	0.35 (0.02)
C_{rs} (L.kPa⁻¹)	BC–A	0.037 (0.001)	0.032 (0.002)	0.031* (0.002)
	BC–B	0.039 (0.003)	0.030* (0.002)	0.029** (0.001)
	BC–C	0.041 (0.003)	0.029** (0.001)	0.028** (0.001)
	NB	0.040 (0.003)	–	0.040 (0.002)

Under normal breathing (NB), each parameter was collected twice at a 24 min interval, ie at 0 and 24. *, ** Significantly different from baseline values (* $P \leq 0.05$; ** $P \leq 0.01$). The animals were either pretreated with ipratropium bromide, 0.025% (BC–A), ipratropium bromide 0.025% and clenbuterol hydrochloride 0.003% (BC–B), saline 0.9% (BC–C) or were investigated in the absence (NB) of 5-hydroxytryptamine and inhalant.

each animal under treatments B and C, were significantly greater ($P = 0.0053$ and 0.0004, respectively) than those observed under treatment NB. Furthermore, the relative respiratory rates observed at time 24 under pretreatment A were significantly greater ($P = 0.0374$) than those observed under treatment NB. When comparing the enhancement of the respiratory rates observed (at times 5 and 24) under treatments BC–A and BC–B, no significant ($P = 0.2887$ at time 5 and $P = 0.4078$ at time 24) difference was detected. Under treatment BC–B, the observed relative enhancement in the respiratory rate was significantly ($P = 0.0275$ at time 5 and $P = 0.0389$ at time 24) lower than that observed under treatment BC–C. Under treatments BC–A and BC–C, the observed relative enhancement was not significantly different.

Functional parameters

The R_{os} and C_{rs} values are presented in table III. For both parameters, interactions between actual and previous treatments, between a treatment and the period during which it was administered were detected.

R_{os}

In the crude analysis, the major increase ($0.0016 \leq P \leq 0.0043$) in the R_{os} values was detected in pretreatment C. During treatments BC–A and NB, R_{os} did not ($0.0938 \leq P \leq 0.2588$) significantly change over time. In treatment BC-B, a significant enhancement of the R_{os} values was detected ($P = 0.0257$) at time 5. Values collected at time 24 showed that the rises in the R_{os} values observed in treatments BC–A, BC–B and BC–C were significantly greater ($P = 0.0232$, 0.0156 and 0.0009, respectively) than those in treatment NB. In treatments BC-A and BC–B, the R_{os} enhancement percentages were not ($0.3126 \leq P \leq 0.323$) significantly

different. Compared to the relative R_{os} enhancement observed in treatment BC–C, those observed during treatments BC–A and BC–B were significantly ($0.0085 \leq P \leq 0.0262$) lower. In treatment BC–C, R_{os} indeed reached 161.4% (10.4%) of its baseline value whereas it reached 121.9% (4.5%) in treatments BC–A and BC–B.

C_{rs}

The following results are also from the crude analysis. When the calves were submitted to treatment NB, no significant change was detected in the C_{rs} values. However, in treatments BC–A, BC–B and BC–C, a significant ($0.0022 \leq P \leq 0.0438$) decrease in the C_{rs} values was observed at times 5 and 24. This was especially true ($0.0022 \leq P \leq 0.0048$) for treatment BC–C.

The relative longitudinal decline observed in the C_{rs} values during treatment NB was significantly lower than that observed in treatments BC–A ($P = 0.0043$), BC–B ($P = 0.0019$) and BC–C ($P = 0.0001$). In treatments BC–A and BC–B, the relative decreases in the C_{rs} values were not significantly different ($0.0924 \leq P \leq 0.1326$). The same observation was made ($0.0601 \leq P \leq 0.1046$) when comparing the relative changes in C_{rs} values observed in treatments BC–B and BC–C. However, the relative decline in C_{rs} values observed in treatment BC–A was significantly ($P = 0.0109$ at time 5 and $P = 0.0183$ at time 24) lower than that observed in treatment BC–C. In treatment BC–C, during the 5-hydroxy-tryptamine perfusion, C_{rs} dropped to 69.4% (2.7%) of its baseline values. In treatments BC–A and BC–B, that parameter fell to 81.2% (2.8%) of its baseline values.

DISCUSSION

The results obtained during treatment NB convincingly support the fact that the experi-

mental conditions did not influence the respiratory pattern.

The present study did not generate data suggesting that 0.9% saline does not induce a bronchoconstriction in healthy animals. However, it seems unlikely that 0.9% saline may induce such a phenomenon. Indeed, in a previous study (Genicot *et al*, 1994c), 0.9% saline, which was administered to calves suffering from a natural acute respiratory distress syndrome, did not significantly influence the pulmonary function parameters.

The observed lower hyperpnea in animals pre-treated with bronchodilator(s) might be the result of a lower hypoxemia due to a lower diffuse bronchoconstriction. The fact that the pulmonary 5HT-induced changes were reduced by bronchodilators is in accordance with other studies. Indeed, atropine partially inhibits the respiratory effects of 5-HT in calves (Linden *et al*, 1993), dogs (Islam *et al*, 1974; Krell and Chakrin, 1976) and rats (Church, 1975). Moreover, β_2-adrenoreceptor stimulants have been shown to relax airway smooth muscles irrespective of whether they have been contracted by different potential asthma mediators such as carbachol, histamine, 5-HT, PGF2α, or substance P (Persson and Carlsson, 1987).

Effects due to the period during which the treatment was allocated, first-order carry-over and interaction were detected in this cross-over trial. These effects made it somewhat difficult to interpret overall treatment differences within patients. There is no biological explanation as yet for the detected first-order carry-over effects, *ie* those remaining from the previous challenge.

Although indirect components could have modulated serotonin-induced bronchoconstriction through the formation of cyclooxygenase products (Bakhle and Smith, 1977; Mitchell and Adcock, 1988) and activation of β-adrenergic receptors (Innes, 1962; Pluchino, 1972; Freeman *et al,* 1981), residual effects from the inhaled solution(s)

and/or from the 5-hydroxytryptamine perfusion seem unlikely. The following explanations may be advanced. During this experiment, the challenges were separated by a washout period of at least 6 d, during which time the subjects did not receive any medication. Tachyphylaxis to β_2-agonists is a well-known phenomenon both *in vitro* and *in vivo* (Conolly *et al*, 1987; Svedmyr and Löfdahl, 1987; Vathenen *et al*, 1988) but there is no convincing evidence for the development of a clinically significant tachyphylaxis of antiasthmatic effects (Larsson *et al*, 1977; Svedmyr and Löfdahl, 1987; Svedmyr, 1990). Moreover, in the present study, the calves were not exposed to more than 1 inhalation of β_2-agonist. In addition, the dissociation half-lives (mean ± SD) of ^3H-ipratropium iodide are 6.6 ± 0.3 min, 2.1 ± 0.3 min and 15.5 ± 1.2 min for H_{m1}, H_{m2} and H_{m3}-receptors, respectively (Disse *et al*, 1992; Speck and Hammer, 1993).

A long-term tolerance (a tolerance lasting several days) to repeated 5-hydroxytryptamine challenges has not been investigated before. In addition, the literature concerning the short-term tolerance to 5-hydroxytryptamine seems controversial, indicating either the existence (Buckner *et al,* 1991) or the absence (Colebatch *et al*, 1966) of such a tolerance. Despite this controversy, it is interesting to note that the clinical and functional parameters returned to baseline within 20 min after the end of a 5 min challenge with intravenously administered 5-hydroxytryptamine (Desmecht *et al*, 1992). Moreover, administration of 5-hydroxytryptamine results in minimal pulmonary oedema. Even lethal doses of 5-hydroxytryptamine produced only patchy pulmonary congestion (Aitken and Sanford, 1972). Finally, although methacholine is a cholinergic agonist, its repeated administration induces a short-term tolerance when more than 1 treatment is made within a 24 h period. This tolerance is reversible within a period of several hours (Beckett *et al*, 1992).

In conclusion, inhaled bronchodilators significantly influenced clinical and functional responses of animals subjected to a 5-hydroxytryptamine challenge. However, first-order carry-over effects, effects of the period during which the treatment was allocated and interaction effects did not permit a clear interpretation of the overall treatment differences within animals.

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