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Quantification of the synergism between fenoterol and ipratropium bromide in the counteraction of experimental bronchospasm

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Abstract

The interaction between the β_2-agonist fenoterol and the antimuscarinic ipratropium bromide in the counteraction of experimental bronchospasm in the guinea-pig was studied qualitatively and quantitatively. Finney's analysis showed a significant overadditive interaction between the two constituents of the association, the 'overadditivity factor' being 2.15 if calculated on the peak activity or 2.68 if calculated on the area under the curve inhibition-time. In addition, the combination possesses both of the advantages of the two components, namely rapid onset of action (characteristic of the β-agonist) and long duration of action (characteristic of the antimuscarinic). A study of the principal cardiovascular and hemodynamic parameters in the cat ruled out any synergism between the two drugs at these levels.

Introduction

β_2-selective sympathomimetics like fenoterol [1, 2] are still the front-line drugs against bronchial asthma because of their effectiveness and rapidity of action. Recently, however, quaternary antimuscarinics, such as ipratropium bromide [3, 4] in the inhalatory form, have proved effective especially in chronic asthma. According to the classic models of molecular pharmacology [5, 6] β-agonists are classified as functional antagonists and antimuscarinics as competitive antagonists of cholinergic stimulation. In other words, an antimuscarinic interacts with the same receptor of the cholinomimetic and competes with it without possessing its specific activity, and so antagonizes the contracturant effect competitively, whereas a β_2-adrenoceptor stimulant interacts as an agonist with the β_2-receptor thereby expressing its own specific activity, muscle relaxation, thus antagonizing the contracturant effect of the cholinomimetic 'functionally'.

Earlier work both *in vitro* and *in vivo* [7] showed, even though only in qualitative terms, the existence of an overadditive interaction between fenoterol and ipratropium bromide in the counteraction of tracheobronchial smooth muscle spasm induced by cholinergic stimulation. These results provided experimental confirmation of the reasoning of ARIËNS et al. [5], according to which the combination of a competitive antagonist and a functional antagonist should yield an overadditive interaction.

The existence of an overadditive synergism against cholinergic stimulation makes an association of fenoterol with ipratropium bromide potentially very interesting in the treatment of bronchial asthma because of the important role of parasympathetic mechanisms in the disease [8–10].

This study aims to quantify the interaction between fenoterol hydrobromide and ipratropium bromide against acetylcholine bronchospasm in the guinea-pig, using an accurate inhalation procedure. Further, the interaction between the two drugs and their effect on the most important cardiovascular and hemodynamic parameters was studied in the cat. Both the antibronchospasmodic interaction study and the investigation of the cardiovascular and hemodynamic side effects were conducted on the fenoterol hydrobromide–ipratropium bromide combination in the fixed ponderal ratio of 2.5 : 1, a ratio similar to that existing between the doses at which the single drugs are generally used in clinical practice.

Materials and methods

Bronchospasm in the anesthetized guinea-pig

The method described by KONZETT and RÖSSLER [11] was used in 138 male Duncan–Hartley white guinea-pigs

weighing between 340 g and 360 g, anesthetized with urethan, 1.5 g/kg i.p., and kept at a constant temperature of 37°C. After tracheotomy, a tracheal cannula was connected to a Starling pump calibrated at 4–5 ml and 56 r.p.m. The administration of gallamine triethiodide, 5 mg/kg i.v., prevented spontaneous respiration in the course of the experiment. The ventilation of the animal was regulated to equal the pressure of 10 cm H_2O; the volume of over-flowed air was diverted to a piston recorder and recorded on a smoked cylinder. A series of bronchospasms at 5-min intervals were induced by i.v. acetylcholine chloride (Ach), at a dose (15–30 μg/kg) such as to induce an increase in tracheobronchial resistance equal to about 80% of the response to total occlusion of the tracheal cannula (maximum response).

Once the maximum bronchospasmodic effect of Ach was terminated, the total volume of air generated by the pump (4–5 ml) was channelled into the animal's respiratory tree, thereby ensuring a constant recovery of the baseline tracheobronchial resistance. The antibronchospasmodic agents, fenoterol hydrobromide and ipratropium bromide, singly or combined in the fixed weight ratio of 2.5:1, were administered directly into the tracheobronchial tree by 'forced insufflation', and inhalation procedure developed at out laboratory [12]: the substances in micronized powder (1–5 μm), mixed with micronized lactose powder, were insufflated into the respiratory tree suspended in 1 ml of pressurized air. This procedure ensures the uniform dispersal of the drugs in the respiratory tree and accurate dosing, and in no way affects the baseline conditions of the preparation.

Each animal received a single dosing of antibronchospasmodic in order to avoid after-effects of previous dosings. The antibronchospasmodic effect was evaluated for 41 min after administration. The interaction between fenoterol and ipratropium bromide was assessed quantitatively by the method of FINNEY [13], using the ID_{50} values calculated by the least squares method and applying the following formula which presupposes the hypothesis of additivity:

$$\frac{ID_{50}(F) \times ID_{50}(I)}{\alpha(F) \times ID_{50}(I) + \beta(I) \times ID_{50}(F)} = \text{predicted } ID_{50}$$

where $ID_{50}(F)$ and $ID_{50}(I)$ denote the ID_{50} of fenoterol hydrobromide and ipratropium bromide dosed singly, and $\alpha(F)$ and $\beta(I)$, the sum of which must be 1, represent the respective fractions of the compounds present in the association. The interaction between the two compounds was defined by the ratio between the predicted and observed ID_{50}. A ratio of more than 1 denoted overadditive interaction. The interaction was quantified both on the peak of inhibitory activity and on the area under the curve inhibition-time (AUC%). The AUC% expresses the inhibitory activity within a fixed time of 41 min. It was calculated by measuring the area under the curve inhibition-time. This datum was then converted into a percentage by rating as 100% the area under a hypothetical curve presenting 100% inhibition for the 41 min time-span considered.

Cardiovascular and hemodynamic analysis in the anesthetized cat

8 male cats in the weight range 2.2 to 2.7 kg were anesthetized with urethan, 400 mg/kg i.v. + chloralose 50 mg/kg i.v., kept at a constant temperature of 37°C and subjected to forced respiration by a Starling pump.

The following operations were performed: thoracotomy and application around the root of the aorta of an electromagnetic probe (ϕ = 6 mm) connected to a *Statham* square wave flowmeter; catherization of the left jugular vein for the intravenous administration of drugs; catheterization of the right femoral artery and left carotid artery for recording the left ventricular pressure and aortic pressure respectively. The following parameters were monitored and recorded on a Hewlett-Packard 8-channel polygraph: ECG; mean aortic pressure; left ventricular pressure; first derivative of the left ventricular pressure; heart rate; aortic blood flow (phase and mean); stroke volume.

The cardiac output and total peripheral resistance were calculated from the parameters recorded. The drugs, fenoterol hydrobromide and ipratropium bromide, singly or combined in the ponderal ratio 2.5:1, were administered at random by intravenous route at intervals of not less than 60 min. The peaks of the effects observed after administration were considered and expressed as percentage variations from the baseline values.

Results and discussion

Bronchospasm in the anesthetized guinea-pig

Figure 1 expresses the trends of the antibronchospasmodic activity of a series of logarithmically spaced doses of fenoterol hydrobromide and ipratropium bromide singly or combined in the fixed ratio of 2.5:1. The trend of the antibronchospasmodic activity of the combination presents features similar to those of the drugs given singly: swift onset of activity like fenoterol hydrobromide and long duration like ipratropium bromide.

The existence of an overadditive interaction emerges right from a first comparative analysis of the peaks and of the duration of antibronchospasmodic activity following administration of the two drugs given singly and from their combination at corresponding doses. Unequivocal proof of an overadditive synergism emerges from Finney's mathematical analysis of the ID_{50} values both for the peaks of inhibition and for the AUC% (Tables 1 and 2).

For both parameters the predicted ID_{50}/observed ID_{50} ratio was significantly greater than 1, which means that the combination of fenoterol hydrobromide and ipratropium bromide in the ponderal ratio of 2.5:1 induces significantly greater antibronchospasmodic effects than those that would be expected from a merely additive interaction. The 'overadditivity factor' is 2.15 (1.81–2.55) on the peak activity and 2.68 (2.37–3.02) on the AUC%.

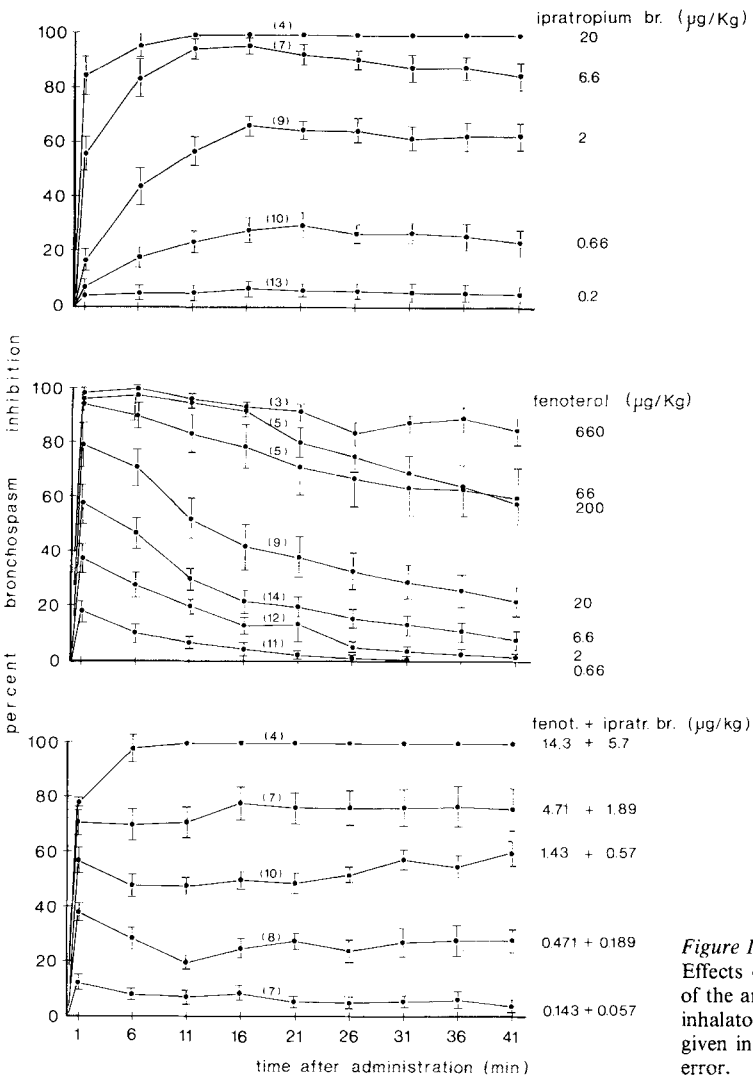

Figure 1
Effects on Ach bronchospasm in the guinea-pig, of the antibronchospasmodics indicated, given by inhalatory route. The number of experiments is given in brackets; vertical bars indicate standard error.

Cardiovascular and hemodynamic analysis in the anesthetized cat

Table 3 gives the mean percentage variations in the most representative cardiovascular and hemodynamic parameters induced by the i.v. administration of fenoterol hydrobromide, ipratropium bromide and their association in the ratio of 2.5:1. The mean baseline values observed for the various parameters (Table 3) were (means ± standard errors): LVP 216 ± 5 mmHg; *dP/dt* 7280 ± 304 mmHg/sec; MAP 120 ± 4 mmHg; HR 188 ± 14 beats/min; SV 1.60 ± 0.15 ml; CO 311 ± 43 ml/min; PR 37760 ± 7560 dynes·sec·cm^{-5}.

Fenoterol hydrobromide at both doses used induced an increase in all the parameters except MAP and PR, which fell sharply. These falls were obviously due to the well-known β_2-stimulant activity of the drug. The effects on the remaining parameters might be due to a 'residual' β_1-agonist activity. However, the positive chronotropic effect may be to some extent β_2-dependent, given the dense population of β_2-receptors present in the atrial cells of the cat [14], or, on the other hand, may be a reflex effect dependent from the sharp fall in blood pressure. Even the marked increase in *dP/dt* could be an indirect effect consequent to extracardiac changes, such as the sudden reduction in peripheral resistance.

Ipratropium bromide did not induce any

Table 1
Ach bronchospasm in the guinea-pig: inhibitory effects induced by inhaled fenoterol hydrobromide, ipratropium bromide and the combination in the ratio of 2.5:1. The effects were considered at the peak of inhibitory activity. The ID_{50} and confidence limits were calculated by the least squares method. The interaction was quantified by the method of FINNEY [13].

Drugs	Dose (μg/kg)	No. of experiments	Peak inhibition % ($\bar{X} \pm$ S.E.)	ID_{50} (95% confidence limits) (μg/kg)	Predicted ID_{50} / Observed ID_{50} (95% conf. limits)
Fenoterol · HBr	0.66	11	17.9 ± 3.4		
	2	12	38.6 ± 4.0		
	6.6	14	59.2 ± 5.3		
	20	9	86.1 ± 3.7	5.528 (7.385–4.137)	
	66	5	95.2 ± 2.2		
	200	5	99.0 ± 0.6		
	660	3	100 ± 0.0		
Ipratropium Br	0.20	13	9.2 ± 2.1		
	0.66	10	32.7 ± 3.3		
	2	9	68.5 ± 3.6	1.171 (1.329–1.031)	2.15 (1.81–2.55)
	6.6	7	96.3 ± 2.5		
	20	4	100 ± 0.0		
Fenoterol.HBr	0.20	7	14.5 ± 2.1		
+	0.66	8	39.1 ± 2.9		
Ipratropium Br	2	10	60.3 ± 3.9	1.246 (1.480–1.049)	
	6.6	7	84.6 ± 4.0		
	20	4	100 ± 0.0		

Table 2
Ach bronchospasm in the guinea-pig: inhibitory effects induced by inhaled fenoterol hydrobromide, ipratropium bromide and the combination in the ratio of 2.5:1. The effects were assessed in terms of area under the curve inhibition-time (AUC%) (see text). The ID_{50} and confidence limits were calculated by the least squares method. The interaction was quantified by the method of FINNEY [13].

Drugs	Dose (μg/kg)	No. of experiments	AUC% ($\bar{X} \pm$ S.E.)	ID_{50} (95% confidence limits) (μg/kg)	Predicted ID_{50} / Observed ID_{50} (95% conf. limits)
Fenoterol · HBr	0.66	11	4.5 ± 1.2		
	2	12	12.7 ± 1.6		
	6.6	14	24.4 ± 2.5		
	20	9	43.3 ± 1.8	23.46 (29.17–18.83)	
	66	5	70.8 ± 7.7		
	200	5	81.3 ± 3.4		
	660	3	91.7 ± 0.6		
Ipratropium Br	0.20	13	5.4 ± 1.8		
	0.66	10	24.2 ± 2.6		
	2	9	57.2 ± 3.3	1.574 (1.786–1.389)	2.68 (2.37–3.02)
	6.6	7	87.8 ± 3.5		
	20	4	98.4 ± 0.05		
Fenoterol · HBr	0.20	7	6.9 ± 1.4		
+	0.66	8	27.1 ± 3.2		
Ipratropium Br	2	10	52.4 ± 2.9	1.760 (1.986–1.560)	
	6.6	7	79.6 ± 3.6		
	20	4	98.1 ± 0.3		

appreciable variation in any of the parameters considered, even at a dose (30 μg/kg) appreciably higher than doses at which this drug was present in the combination under study.

The combination of fenoterol and ipratropium bromide induced variations that were not detectably different from those induced by fenoterol alone at corresponding doses. An example of the effects obtained with the different treatments is graphed in Figure 2.

Table 3
Cardiovascular and hemodynamic changes induced in the cat by intravenous administration of fenoterol hydrobromide, ipratropium bromide and the combination in the ratio of 2.5:1. The values were calculated at the peak variation from the baseline values. The parameters are as follows: left ventricular pressure (LVP); positive first derivative of left ventricular pressure (*dP/dt*); mean aortic pressure (MAP); heart rate (HR); stroke volume (SV); cardiac output (CO); total peripheral resistance (PR). The mean baseline values were ($\bar{X}$ ± S.E.): LVP 216 ± 5 mmHg; *dP/dt* 7280 ± 304 mmHg/sec; MAP 120 ± 4 mmHg; HR 188 ± 14 beats/min; SV 1.60 ± 0.15 ml; CO 311 ± 43 ml/min; PR 37760 ± 7560 dynes · sec · cm^{-5}.

Drugs	Dose (μg/kg i.v.)	No. of experiments	Percent changes from basal value ($\bar{X}$ ± S.E.)						
			LVP	*dP/dt*	MAP	HR	SV	CO	PR
Fenoterol · HBr	1.0	4	8.7 ± 2.3	24.9 ± 9.3	−28.7 ± 2.3	18.7 ± 5.3	14.9 ± 2.4	35.6 ± 3.5	−47.4 ± 2.9
Ipratropium Br	0.40	4	0	−0.1 ± 1.4	0	0	−0.9 ± 1.4	−0.2 ± 0.9	−1.5 ± 1.3
Fenoterol · HBr + Ipratropium Br	1.0 + 0.40	4	10.0 ± 3.0	26.2 ± 0.9	−32.8 ± 14.8	21.3 ± 8.7	16.8 ± 2.3	36.8 ± 6.5	−48.6 ± 2.9
Fenoterol · HBr	3.0	4	9.4 ± 0.9	41.7 ± 13.9	−43.4 ± 4.6	29.3 ± 9.7	19.4 ± 5.1	46.2 ± 5.3	−60.6 ± 4.5
Ipratropium Br	1.2	4	0	0	−0.4 ± 0.4	0	0.1 ± 3.4	−0.9 ± 2.8	0.8 ± 3.8
Fenoterol · HBr + Ipratropium Br	3.0 + 1.2	4	8.3 ± 1.5	29.7 ± 6.2	−42.2 ± 5.4	25.3 ± 6.8	18.8 ± 6.5	48.2 ± 7.9	−60.1 ± 5.5

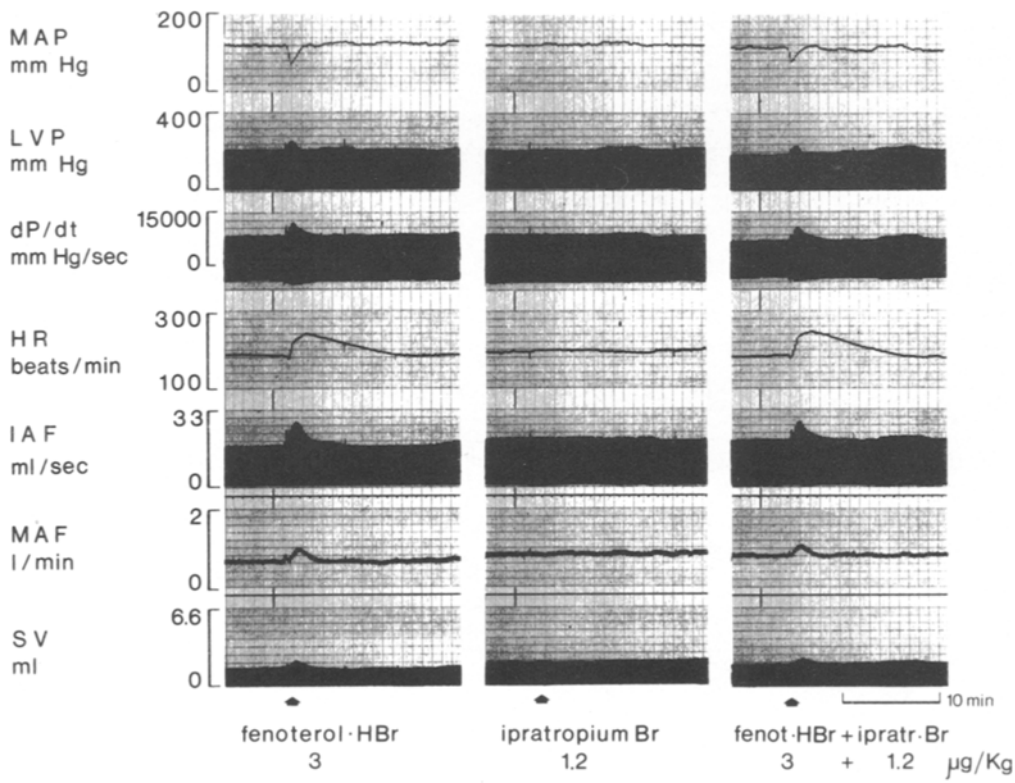

Figure 2
Example of the variations in some cardiovascular and hemodynamic parameters induced in the cat by intravenous fenoterol hydrobromide, ipratropium bromide and the combination of the two in the ratio of 2.5 : 1. The parameters are as follows: mean aortic pressure (MAP) left ventricular pressure (LVP); positive first derivative of left ventricular pressure (*dP/dt*); heart rate (HR); instant aortic flow (IAF); mean aortic flow (MAF); stroke volume (SV).

Conclusions

The results of this study show that the association of fenoterol hydrobromide and ipratropium bromide in the ratio of 2.5:1 is a rational combination for counteracting bronchial spasm induced by cholinergic stimulation. The rationality of the combination emerges from the following points:

(1) In the guinea-pig the combination induces an antibronchospasmodic effect some 2.5 times greater than would be obtained from a merely additive interaction. Further, as shown in Figure 1, the combination possesses both of the positive features of its constituents: the swift onset of action that characterizes fenoterol hydrobromide and the long duration of action that characterizes ipratropium bromide. The existence of these positive interactions *vis-à-vis* cholinergic stimulation make the combination especially promising in the treatment of bronchial asthma because of the important role of cholinergic mechanisms in this disease.

(2) The overadditive interaction against bronchial spasm is not accompanied by any similar interaction at the level of the cardiovascular and hemodynamic parameters. The test done in the cat shows that the effects induced by fenoterol hydrobromide on these systems are in no way affected by ipratropium bromide. In view of these facts, it may be presumed that the drug combination under study is potentially capable of exerting more intense and lasting antibronchospasmodic effects with the same incidence of side effects as fenoterol given at corresponding doses. In other words, it may be presumed that the combination studied produces equivalent or greater bronchial antispasmodic effects with lower doses of the β-agonist, the constituent responsible for the most important side effects.

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