Trachea Relaxing Effects and β_2-Selectivity of SPFF, a Newly Developed Bronchodilating Agent, in Guinea Pigs and Rabbits

Le-Ling GAN,*[,a] Min-Wei WANG,[a] Mao-Sheng CHENG,[b] and Li PAN[b]

[a] *Department of Pharmacology, Shenyang Pharmaceutical University; No. 103 Wenhua Rd. Shenyang 110016, China: and* [b] *Department of Pharmaceutical Engineering, Shenyang Pharmaceutical University; No. 103 Wenhua Rd. Shenyang 110016, China.* Received September 10, 2002; accepted November 12, 2002

In this paper we evaluated the bronchodilator effects of SPFF [2-(4-amino-3-chloro-5-trifluomethyl-phenyl)-2-*tert*-butylamino-ethanol chloride], a newly synthesized β_2 adrenergic agonist in guinea pigs and rabbits, in comparison with other β_2 adrenergic agonists, isoprenaline or salbutamol. We studied *in vitro* the bronchodilator effects of SPFF and isoprenaline on isolated guinea pig trachea strips with or without the precontraction of bronchocontractors (acetylcholine and histamie). The positive chronotropic effects of SPFF and isoprenaline on isolated guinea pig left atria were also tested *in vitro*. Potency values (pD_2, pA_2 or ED_{50}) were determined from the cumulative concentration–response curves. The results showed that SPFF and isoprenaline dose-dependently relaxed the isolated guinea pig trachea strips and the pD_2 values of both drugs were 7.66±0.68 and 8.79±0.19, respectively. Moreover, we confirmed that the bronchodilator effect of SPFF was due to the activation of β_2 adrenoceptor because this effect was easily antagonized by ICI-118551 (pA_2 8.90±0.01), a specific β_2 adrenoceptor antagonist. SPFF also dose-dependently relaxed the isolated guinea pig trachea strip precontraction with acetylcholine or histamine with ED_{50} values of 10.2±0.7 μM and 550±38.2 nM, respectively. Furthermore, the positive chronotropic effect of SPFF on isolated guinea pig left atria (pD_2 5.41±0.38) was much weaker than that of isoprenaline (pD_2 8.75±0.24), which implied that SPFF was more selective to airway β_2 adrenoceptor than isoprenaline; the β_1/β_2 selectivity assay also showed that SPFF was about 162 times more selective to β_2 adrenoceptor than isoprenaline. A radioligand binding experiment using guinea pig lung and cardiac ventricle as β_2 and β_1 adrenoceptor sources, respectively, also demonstrated that SPFF possesses high affinity (27.3 nM) and selectivity (4.6 fold) to β_2 adrenoceptors. The protective effects of SPFF and salbutamol on bronchospasm induced by bronchoconstrictor aerosol in guinea pigs *in vivo* were investigated, and the Konzett and Rössler experiment in rabbits *in vivo* was also carried out. SPFF significantly prolonged the latency time of histamine and acetylcholine induced asphyxiation collapse in guinea pigs: the ED_{50} value of SPFF i.g. was 0.32±0.05 mg·kg^{-1} in this experiment. Meanwhile, the ED_{50} values of salbutamol was 2.37±0.22, which meant that the bronchorelaxation effect of salbutamol was about 6 times less potent than that of SPFF. The Konzett and Rössler experiment performed in anesthetized rabbit showed that intraduodenal administration of SPFF exerted action of longer duration than salbutamol. From the results above we suggested that SPFF was a potent, long-acting bronchodilator with relatively higher β_2 adrenoceptor selectivity.

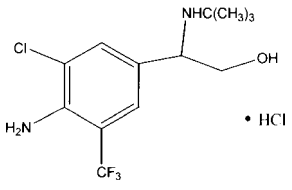
Key words β_2 adrenergic agonist; bronchodilator effect; receptor selectivity; 2-(4-amino-3-chloro-5-trifluomethyl-phenyl)-2-*tert*-butylamino-ethanol chloride (SPFF)

Asthma is a common respiratory disease characterized by reversible airway obstruction and airway hypersensitivity. β_2 adrenoceptor agonists have long been widely used as agents for the treatment of asthma, and the use of β_2 adrenoceptor agonists is based on the fact that bronchial muscles are mainly controlled by β_2 adrenoceptors, whose stimulation causes bronchodilation.[1)] High selectivity to β_2 compared to β_1 adrenoceptor is important for a β_2 adrenoceptor agonist, because low selectivity always induces side effects like tachycardia, caused by stimulation of the β_1 adrenoceptor; currently available drugs such as salbutamol and terbutaline are not as satisfactory in this sense.[2)]

We recently synthesized 2-(4-amino-3-chloro-5-trifluomethyl-phenyl)-2-*tert*-butylamino-ethanol chloride (SPFF) (Fig. 1) as a novel bronchodilator which possesses β_2 adrenoceptor stimulation activity. In this paper, we reported some preliminary results showing the potency, selectivity, and duration of action of SPFF in guinea pigs and rabbits for the first time; and the effects of SPFF are compared with those of other β_2 adrenoceptor agonists, isoprenaline and salbutamol. SPFF exhibited both a potent trachea relaxing activity and high β_2 selectivity.

MATERIAL AND METHODS

Drugs and Chemicals The composition of the Krebs–Hensleit solution was as follows (in g·l^{-1}): NaCl (6.92), KCl (0.35), $CaCl_2$ (0.28), $MgSO_4 \cdot 7H_2O$ (0.29), $NaHCO_3$ (2.1), KH_2PO_4 (0.16) and glucose (2.0). The Krebs solution used had the following composition (g·l^{-1}): NaCl (6.92), KCl (0.35), $MgSO_4 \cdot 7H_2O$ (0.15), $NaHCO_3$ (2.1), KH_2PO_4 (0.16), glucose (2.0) and $CaCl_2$ (0.24), isoprenaline hydrochloride was obtained from Hefeng Pharmaceutical Co., Ltd. (Shanghai, China). Salbutamol sulphate was from Yancheng Pharmaceutical Co. (Jiangsu, China). ICI-118551 [(±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)]-



SPFF

Fig. 1. Chemical Structure of SPFF

* To whom correspondence should be addressed. e-mail: gll206@sina.com

amino]-2-butanol] was obtained from Tocris Cookson Ltd. (Bristol, U.K.). Histamine phosphate and acetylcholine chloride were from Sigma (St. Louis MO, U.S.A.). [^{3}H]Dihydroalprenolol (1480 GBq/mmol) was from the National Institute of Atomic Energy (Beijng, China). SPFF was synthesized by Dr. M. S. Cheng and L. Pan (The Department of Pharmaceutical Engineering, Shengyang Pharmaceutical University, Shengyang, China).

Animals Guinea pigs (Hartley) and rabbits were provided by the Experimental Animal Center of Shenyang Pharmaceutical University. Animals for *in vitro* experiments were sacrificed by decapitation under light ether anesthesia.

Bronchorelaxation Effect on Resting Isolated Guinea Pig Trachea Strips Hartley guinea pigs of either sex weighing 355±35 g were sacrificed and the trachea strips were prepared; the preparation was then mounted under a resting tension of 2 g in an organ bath containing 10 ml of Krebs–Heinsleit solution (KH, pH 7.4) at 37 °C and superfused with a gas mixture (O_2 95%, CO_2 5%).[3] The trachea strips were left for 1.5 h and KH solution was changed at 15 min intervals before SPFF or isoprenaline was accumulatively added to the bath. The maximum relaxation produced by isoprenaline (10^{-6} M) was taken as 100% and dose–response curves of each drug were made.

Bronchorelaxation Effect on Precontracted Isolated Guinea Pig Trachea Strips The isolated guinea pig trachea strips were precontracted with acetylcholine (3 μM) or histamine (5 μM). The maximum contraction induced by histamine or acetylcholine was taken as 100% and the cumulative concentration–response curves for isoprenaline (10^{-9}—3×10^{-6} M) or SPFF (10^{-7}—10^{-4} M) were established. The concentration of acetylcholine and histamine used in this experiment produced 50—60% maximum response of each contractor (tested in preliminary experiments). The contact time was 20 min, enough to produce a steady level of contraction.[4] EC_{50}, the concentration needed to decrease the tone of trachea strips by 50%, was obtained from the dose–response curves to express the relaxant effects of SPFF and isoprenaline.

Competitive Effect of ICI-118551 on SPFF The isolated guinea pig trachea strips were precontracted with histamine (5 μM). Following the addition of a specific β_2 adrenoceptor antagonist, ICI-118551[5] (10^{-8}, 10^{-7} M), SPFF was added into the organ bath accumulatively, and the maximum relaxation produced by SPFF taken as 100%; the dose–response curves of SPFF in the presence of ICI-118551 were established.

Positive Chronotropic Effect of SPFF on Isolated Guinea Pig Left Atria Left atria were isolated from freshly excised hearts of male guinea pigs (250—350 g). The preparations were suspended in an organ bath containing 20 ml Krebs solution at 37 °C and superfused with 95% O_2 and 5% CO_2 under a resting tension of 0.2 g.[6] The spontaneous beating rate was measured with a heart rate counter triggered by atrial contraction. After an equilibration of 30 min, SPFF or isoprenaline was added to the organ bath accumulatively. Increases in beating rate were expressed as percentage of maximum increase caused by isoprenaline and the concentration–response curves were made.

[^{3}H]Dihydroalprenolol Binding in Guinea Pig Lung and Ventricular Membranes Male Hartley guinea pigs (300—350 g) were killed and the cardiac ventricle and lung were removed and homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.5) using a Polytron (setting 7—8, 30 s×2). The homogenate was centrifuged at 1500×g for 10 min, and the supernatant was recentrifuged at 45000×g for 30 min. The pellet was homogenized using a Potter type glass Teflon homogenizer with 7—8 passes. The homogenate was centrifuged again at 45000×g for 20 min. The final pellet was resuspended in the above buffer and stored in liquid nitrogen until use. The above procedure was conducted at 2—4 °C.[7]

The saturation experiments were performed in duplicate by incubating an aliquot of the membrane preparations (ventricle, 500 μg, lung 100 μg) with various concentrations of [^{3}H]Dihydroalprenolol (0.1—5 nM) for 20 min at 37 °C (final incubation volume of 250 μl). Incubation was terminated by the addition of 6 ml of ice-cold 50 mM Tris–HCl buffer. Membrane-bounded [^{3}H]Dihydroalprenolol was separated from free radioligand by filtration using a cell harvester (ZT-II, Weixin, China) over glass fiber filters (Whatman, GF/B). The filters were washed with an addition of 6 ml of ice-cold Tris–HCl buffer. The radioactivity of the membrane-bound [^{3}H]Dihydroalprenolol was determined with a liquid scintillation counter (LS-6500, Beckman, U.S.A). Specific binding was defined as the difference between the amount of [^{3}H]Dihydroalprenolol bound in the absence and presence of 400 nM propranolol.

In the competition experiment, an aliquot of the membrane suspension was incubated with [^{3}H]Dihydroalprenolol (2 nM) and various concentrations of competing drug, SPFF (ventricle: 1 nM—1 mM; lung: 0.1 nM—0.1 mM) or isoprenaline (ventricle: 1 nM—1 mM; lung: 0.1 nM—10 μM). The specific binding was obtained as described above.

Protein concentration was determined using the Coomassie brilliant blue assay reagent (Jiancheng, Nanjing, China).

The Protection Effects on the Bronchospasm Induced by Histamine–Acetylcholine Aerosol in Guinea Pigs Male Hartley guinea pigs weighing 150±30 g were put under a bell cover (4 l) and exposed to the aerosol (mixed with 2% acetylcholine and 0.1% histamine) produced by a nebulizer at a constant flow-rate of 2 ml·min^{-1} for 5 s.[8] SPFF (0.0625, 0.125, 0.25, 0.5, 1.0 mg·kg^{-1}) and salbutamol (1, 3, 9 mg·kg^{-1}) were dissolved in 1% CMC-Na, then administered i.g. 1 h before the aerosol exposure. The asphyxia response to the aerosol mixture was measured as latency time before collapse was developed. When asphyxia collapse did not develop within 6 min, the animals were considered "completely protected" and the latency time was taken as 6 min. Protection rates, the percentage of completely protected animals, were obtained and the ED_{50} values of each compound were estimated using the Bliss method.

Konzett and Rössler Experiment in Anesthetized Rabbits Male rabbits weighing 3.2±0.6 kg were anesthetized with pentobarbital (30 mg·kg^{-1}, i.v.). Body temperature was maintained at 36—37 °C with a heating pad and the Konzett and Rössler experiment[9] was performed. The trachea was cannulated and ventilated for 40 min^{-1} with a tidal air volume of 15 ml·kg^{-1} using a respirator. The overflow of air was measured by a flow transducer and recorded by a biosignal recording system (Ms2000, Guangdong College of Pharmacy, Guangzhou, China). Acetylcholine (30 μg·kg^{-1})

was given i.v. 30, 45, 60, 90, 120, 180, 240 min after intraduodenal administration of normal saline (N.S), SPFF (80, 8, 1 $\mu g \cdot kg^{-1}$) or salbutamol (80 $\mu g \cdot kg^{-1}$). The overflow induced by acetylcholine 1 min before N.S or bronchodilat or administration was taken as 100% and the inhibition rate of each bronchodilator was calculated at different time points.

Statistic Analysis The results are expressed as means± S.E. of n (number of experiments). Agonistic potency was expressed as pD_2 values, and antagonistic potency was expressed as pA_2 values, obtained from Schild plotting with a confidence limit of 95%.[10] Statistical significance of differences between groups was verified with unpaired Student's t test. The equilibrium dissolution constant (K_D) and maximum number of binding sites (B_{max}) for [^{3}H]Dihydroalprenolol binding to both membrane preparations were determined by Scatchard analysis. IC_{50} values of the radioligand competition curves were calculated by Hill plots.

RESULTS

Bronchorelaxation Effects on Resting Isolated Guinea Pig Trachea Strip SPFF decreased the tone of the trachea muscle at concentrations higher than 10^{-10} M and reached the maximum effect at 10^{-5} M (Fig. 2). The pD_2 values of SPFF and isoprenaline were 7.66±0.68 and 8.79±0.19, respectively (95% confidence limits), which meant that SPFF was about 10 times less potent than isoprenaline.

Effect on Precontracted Isolated Guinea Pig Trachea Strips In guinea pigs trachea strips precontracted with acetylcholine (3 μM), SPFF (10^{-7}—10^{-4} M) and isoprenaline (10^{-9}—3×10^{-6} M) induced a concentration-dependent relaxation (Fig. 3). Both drugs produced less than 100% relaxation of acetylcholine-induced contraction. The EC_{50} values of SPFF and isoprenaline were 10.2±0.7 and 3.8±0.6 μM (n=8), respectively. Meanwhile, SPFF (10^{-7}—10^{-4} M) and isoprenaline (10^{-9}—10^{-6} M) also produced a concentration-dependent relaxation on histamine (5 μM) precontracted trachea strips (Fig. 4). The maximum relaxation produced by both drugs was more than 100% of the histamine induced contraction, and the EC_{50} values were shown to be 550±38.2 and 67.8±40.0 nM, respectively.

Competitive Effect of ICI-118551 on SPFF ICI-118551 was shown as a competitive antagonist to SPFF by

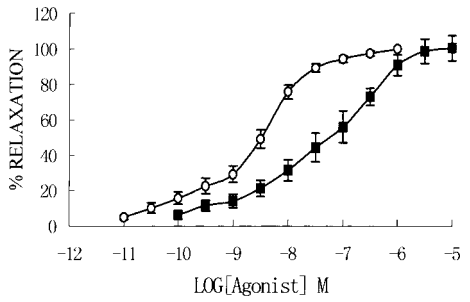


Fig. 2. Concentration Response Curve of SPFF (■) and Isoprenaline (○) in Relaxation of Normal Tone of Isolated Trachea of Guinea Pigs (n=8)

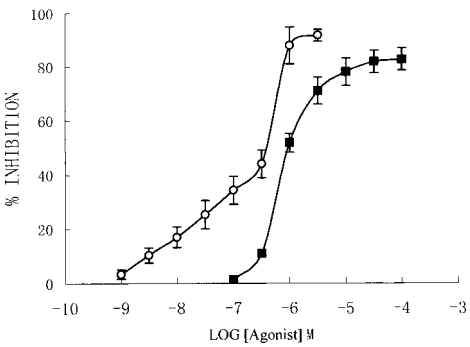


Fig. 3. Cumulative Concentration–Response Curves for SPFF (■, 10^{-7}—10^{-4} M) and Isoprenaline (○, 10^{-9}—3×10^{-6} M) on Isolated Guinea Pig Trachea Strips Precontracted by Acetylcholine (3 μM) (n=8)

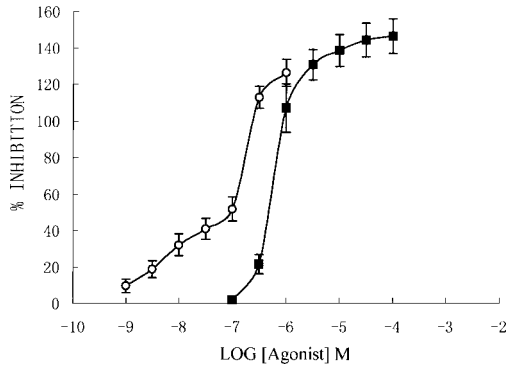


Fig. 4. Cumulative Concentration–Response Curves for SPFF (■, 10^{-7}—10^{-4} M) and Isoprenaline (○, 10^{-9}—3×10^{-6} M) on Isolated Guinea Pig Trachea Strips Precontracted by Histamine (5 μM) (n=8)

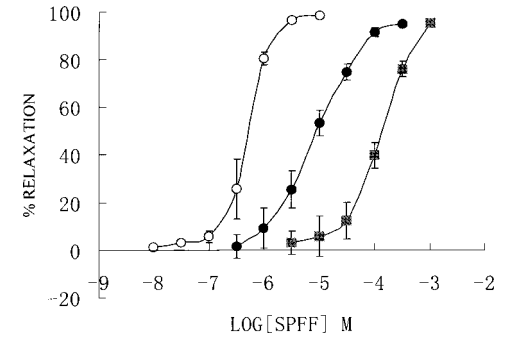


Fig. 5. Effect of ICI 118551 on Concentration–Response Curves for SPFF on Isolated Trachea of Guinea Pigs

○=SPFF (10^{-8}—10^{-5} M); ●=SPFF (3×10^{-6}—3×10^{-4} M) in the presence of ICI-118551 (10^{-8} M); ■=SPFF (3×10^{-5}—10^{-3} M) in the presence of ICI-118551 (10^{-7} M) (n=8).

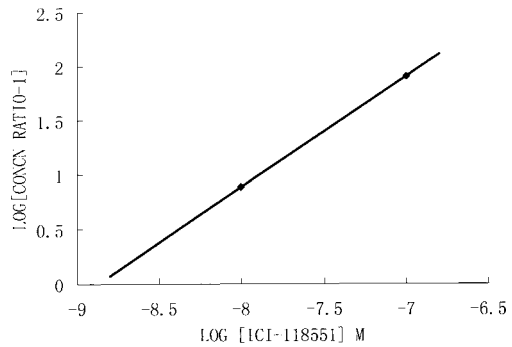


Fig. 6. Schild Plots for the Antagonistic Effect of ICI-118551 to SPFF on Isolated Trachea of Guinea Pigs (n=8)

The slope of this plot was 1.02±0.05.

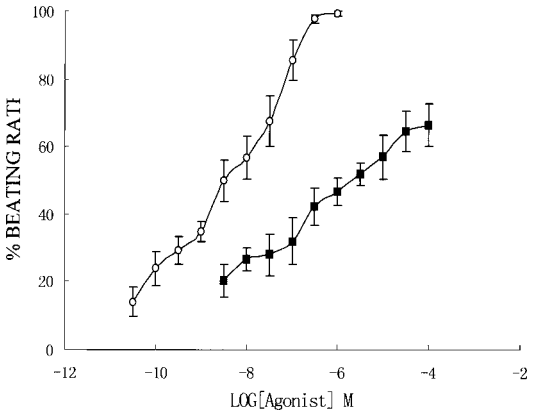


Fig. 7. The Positive Chronotropic Effects of SPFF (■, 3×10^{-8}—10^{-4} M) and Isoprenaline (○, 3×10^{-10}—10^{-6} M) on the Beating Rate of Isolated Guinea Pig Left Atria (n=8)

Table 1. The pD_2 Values for SPFF and Other Reference Compounds in *in Vitro* Studies

β-Agonist	Left atria		Trachea		β_2-Selectivity[b]
	pD_2	Intrinsic activity[a]	pD_2	Intrinsic activity[a]	
SPFF	5.41	0.65	7.66	0.98	178
Isoprenaline	8.75	1	8.79	1	1.1

a) The value of intrinsic activity was calculated as the ratio of the maximum response of each compound to the maximum response to isoprenaline; isoprenaline=1. *b*) Antilog [pD_2 (trachea)−pD_2 (atria)].

shifting right the dose–response curve of SPFF at low concentrations of 3×10^{-9} and 3×10^{-11} M in isolated guinea pig trachea strips (Figs. 5, 6). The pA_2 value of the antagonistic activity of ICI-118551 was 8.90±0.01.

Positive Chronotropic Effect on Isolated Guinea Pig Left Atria As shown in Fig. 7, SPFF (3×10^{-8}—10^{-4} M) induced concentration-related increase in the beating rate of contraction on guinea pig left atria, with a pD_2 of 5.41±0.38. The positive chronotropic effect of SPFF was about 2188 times less potent than that of isoprenaline, whose pD_2 value was 8.75±0.24.

β_1/β_2 Selectivity The β_1/β_2 adrenoceptor selectivity ratio (selectivity index) was obtained from the difference between the mean pD_2 values obtained from the isolated right atria and trachea strips in guinea pigs. The selectivity index values indicated that SPFF and isoprenaline were 178 and 1.1 times more effective on the trachea than on the right atria, respectively; therefore, SPFF was considered more highly selective to β_2 adrenoceptor than isoprenaline (Table 1).

Affinity and Selectivity for β Adrenoceptor Subtypes by Radioligand Binding Specific binding of [^{3}H]Dihydroalprenolol to the guinea pig ventricle was saturable, and Scatchard analysis indicated a single population of binding sites with a K_D of 8.5±3.7 nM, and B_{max} of 25.3±0.3 fmol/mg protein (4 experiments). In the lung membrane, specific binding was also saturable and the K_D and B_{max} were 8.3 ±0.2 nM and 351.2±6.2 fmol/mg protein (4 experiments), respectively. These data were similar to those previously reported.[11,12] The Scatchard plots were linear and the apparent Hill coefficients were unity in both membrane preparations,

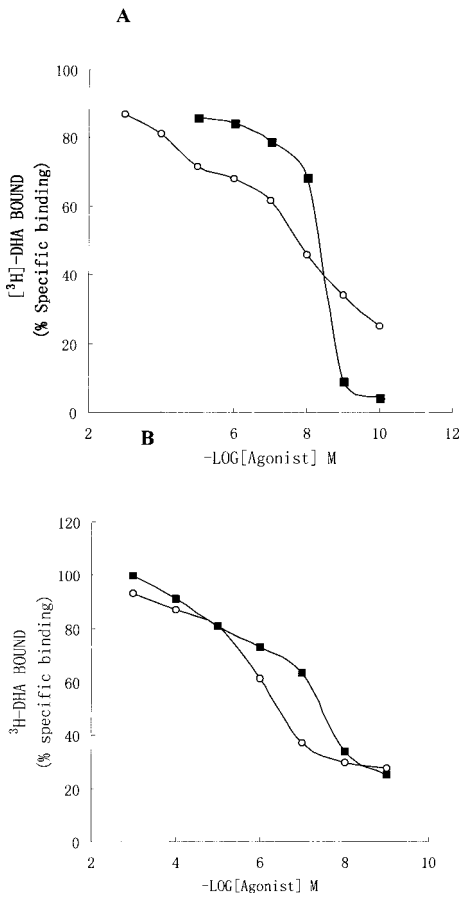


Fig. 8. Displacement Curves of SPFF (○) and Isoprenaline (■) on [^{3}H]Dihydroalprenolol ([^{3}H-DHA]) Binding to Guinea Pig Lung (A) and Ventricle (B) Membrane Preparation (n=4)

Table 2. Comparison of IC_{50} Values and Apparent Selectivity of SPFF and Isoprenaline Determined by Binding Inhibition Studies with [^{3}H]Dihydroalprenolol in Guinea Pig Ventricle and Lung Membrane Preparations (n=4)

Drug	Ventricle (β_1) IC_{50} (nM)[a]	Lung (β_2) IC_{50} (nM)[a]	β_2-Selectivity[b]
SPFF	126.1 (73.1—179.1)	27.3 (23.7—32.9)	4.6
Isoprenaline	43.8 (26.7—60.9)	120.2 (68.4—172.0)	0.36

a) Numbers in parenthesis indicate 95% confidence limits. *b*) IC_{50} (ventricle)/IC_{50} (lung).

indicating the absence of cooperative interactions.

Displacement curves for SPFF and isoprenaline of [^{3}H]Dihydroalprenolol binding to guinea pig lung and the ventricular membranes, which contain a relatively homogeneous population of β_2 and β_1 adrenoceptors, respectively, are depicted in Fig. 8. The IC_{50} values and overall β_2 selectivity ratios are shown in Table 2.

Protective Effect on the Bronchospasm Induced by Histamine–Acetylcholine Aerosol As shown in Table 3, both SPFF and salbutamol significantly prolonged the latency time of histamine and acetylcholine-induced collapse. The ED_{50} of each drug was 0.32±0.05 and 2.37±0.22 mg·kg^{-1}, respectively, meaning that SPFF was about 7 times more potent than salbutamol in inhibiting the bronchospasm induced by bronchocontractor aerosol in conscious guinea pigs (Table 3).

Table 3. Inhibition Effects of SPFF and Salbutamol on Bronchocontractor-Induced Bronchospasm in Guinea Pig

Group/(mg·kg^{-1})		Latent period/s	Protection rate (%)	ED_{50} (95% confidence limit)
Control	—	59.6±5.53	—	
SPFF	0.0625	118.3±32.6	12.5	
	0.125	160.5±40.5*	25	
	0.25	222.6±48.1*	50	0.32±0.05
	0.5	266.4±42.6**	62.5	
	1	322.5±23.3***	75	
Salbutamol	1	158.0±46.0	25	
	2	193.4±48.6*	37.5	1.82±0.19
	3	323.9±35.77***	87.5	

$*p<0.05$, $**p<0.01$, $***p<0.001$ *vs.* control group, $n=8$, Student's *t*-test.

Table 4. The Effect of Intraduodenal Administration of SPFF and Sabutamol in Rabbits, (Konzett and Rössler Experiment)

Group/μg·kg^{-1}	Inhibition rate (%) Time (min)						
	30	45	60	90	120	180	240
Control	7.1±9.3	13.9±6.21	8.4±8.2	11.4±7.3	2.6±6.4	3.3±6.1	10.4±6.75
SPFF group							
80	43.8±8.8*	47.0±3.9**	58.2±5.4***	45.3±7.1**	35.8±4.4**	39.8±6.2**,†	36.8±5.7*,†
8	39.3±10.4*	42.3±8.5*	59.8±6.0***	48.4±3.6**	33.9±6.5*	33.8±4.7**	25.8±5.8
1	15.5±6.8	37.5±6.57*	18.6±4.0	24.2±5.4	18.9±7.5	15.1±3.5	10.9±4.4
Sab 80	45.0±4.8**	50.2±7.2**	51.7±3.4**	27.4±5.4	31.8±6.21*	19.1±5.7	15.2±6.0

$*p<0.05$, $**p<0.01$, $***p<0.001$ *vs.* control group, †$p<0.05$ *vs.* Sab group $n=5$, Student's *t*-test.

Bronchodilation Effect on Anaesthetized Rabbits As shown in Table 4, intraduodenally administered SPFF and salbutamol significantly inhibited the increase of lung overflow induced intravenously by acetylcholine, but the effect of salbutamol diminished quickly 120 min after drug administration; the effect of SPFF, however, remained throughout the 240 min duration of all the experiments.

DISCUSSION

In isolated guinea pig trachea strips, the dose–response curves of SPFF were paralleled by the reference agonist, isoprenaline. The curves of SPFF were shifted right by the addition of ICI-118115, and the pA_2 value of ICI-118551 for SPFF was 8.9, which is nearly the same as that reported for the known selective β_2-adrenoceptor agonists, fenoterol and salbutamol.[13,14] The slope factor of the Schild plot of ICI-118551 for SPFF was almost 1.0 (1.02), indicating competitive antagonism of SPFF and ICI-118551 on the same receptor.[7] Furthermore, the intrinsic activity of SPFF in guinea pig trachea preparation was close to 1.0, indicating that SPFF behaved as a β_2-adrenoceptor full agonist like isoprenaline.

In precontracted trachea strips, the maximum relaxant effect of SPFF was roughly equivalent to isoprenaline in both models, consistent with what was observed in normal trachea strips. But interestingly, we found that despite the fact that the concentration of histamine used in the experiment was higher than acetylcholine, the contraction effect of acetylcholine was only partially inhibited. Meanwhile, the bronchocontraction effect of histamine was completely elicited by SPFF and isoprenaline. Comparable results were also reported in the studies of other β_2 agonists.[6,15]

In the studies of the β_2 adrenoceptor selectivity of SPFF, it was found that SPFF was 2188 times less potent than isoprenaline in increasing the beating rate of isolated guinea pig left atria. As the atria positive chronotropic effect was always considered a sign of the non-selective side effect of β_2 adrenoceptor agonist,[16] we assume that SPFF had higher selectivity to β_2 adrenoceptor than isoprenaline. The β_2 selectivity indexes showed that the selectivity of SPFF to β_2 adrenoceptor was 178, about 162 times greater than that of isoprenaline (1.1), and even greater than two widely used β_2 agonists, salbutamol and terbutaline, whose selectivity index values were reported to be 17 and 18, respectively, less than that of formoterol (204).[17]

To confirm the β_2 adrenoceptor selectivity of SPFF demonstrated by the functional experiments mentioned above, we also examined the affinity and selectivity of the compound for β-adrenoceptor subtypes by radioligand binding in comparison with isoprenaline. Results had showed that the specific binding in both membrane preparations was saturable, and the Scatchard analysis and hill plots indicated the validity of our experimental conditions. SPFF exhibited high affinity ($IC_{50}=27.3$ nM) in the lung membrane. Selectivity of SPFF for β_2 adrenoceptor assessed by the IC_{50} (ventricle)/IC_{50} (lung) was 4.6, which was 12.8 times higher than that of isoprenaline (0.36). But SPFF exhibited relatively lower selectivity in the binding experiment than in the functional experiment. Although the reason for this disagreement is not clear, it is possible that 20% of the adrenoceptors are of β_1 subtype in lung membranes[18,19]; the non-selective binding of [^{3}H]Dihydroalprenolol to the β_1 adrenoceptor resulted in an undesirable increase in the IC_{50} values of SPFF in lung membrane preparation.

The bronchodilator effect of SPFF was further validated *in vivo* by bronchocontractor-induced bronchospasm and Konzett and Rössler experiment, and SPFF was found to be more potent than salbutamol in both experiments. Since the

intrinsic activity values of SPFF and salbutamol obtained in isolated trachea strips were similar (0.98, 0.91, respectively), we presumed that the higher β_2 selectivity of SPFF could account for the stronger bronchodilator effect of SPFF *in vivo*.

SPFF also showed a longer duration of activity than that of salbutamol in rabbits. This seemed to be due to its low sensitivity to catechol-*O*-methyltransferase (COMT) because the chemical structure of SPFF lacks a ring hydroxyl group on the catechol nucleus[9] which will result in a longer effective plasma drug level. There may be some other possibilities to explain the longer duration of action of SPFF such as the appearance of some active metabolites, but all no other hypothesis has been validated so far.

In conclusion, SPFF was a potent and highly selective β_2-adrenergic receptor agonist with longer duration of action.

Acknowledgement The present work was supported by research grant 96-901-05-130 to Dr. Mao-Sheng Cheng from the National Science Council, China, P.R.

REFERENCES

1) Raffestin B., Cerrina J., Boullet C., Labat C., Benveniste J., *J. Pharmacol. Exp. Ther.*, **233**, 186—194 (1985).
2) Raghu P. M., Bahekar R. H., Rao A. R., *Pharmazie*, **55**, 475—482 (2000).
3) Coburn R., Tomita T., *Am. J. Physiol.*, **224**, 1072—1080 (1973).
4) Mariam Y., Olav T., *Drug Dev. Res.*, **51**, 169—176 (2000).
5) Bilski A. J., Halliday S. E., Fitzgerald J. D., Wale J. L., *J. Cardiovasc. Pharmacol.*, **5**, 430—437 (1983).
6) Kubo S., Matsubara I., Yamazaki M., Kasamatsu S., Koshinaka E., Kato H., Kase Y., *Arzneim.-Forsch.*, **30**, 1272—1278 (1980).
7) Kikkawa H., Naito K., Ikezawa K., *Jpn. J. Pharmacol.*, **57**, 175—185 (1991).
8) Xie Q. M., Zeng L. H., Zheng Y. X., Lu Y. B., Yang Q. H., *Acta Pharmacol. Sin.*, **20**, 651—654 (1998).
9) Murai T., Maejima T., Sanai K., Osada E., *Azneim.-Forsch.*, **34**, 1633—1640 (1984).
10) Arunlakshana O., Schild H. O., *Br. J. Pharmaco. Chemother.*, **14**, 48—58 (1959).
11) Jiang M. H., Liu L., Wang Q., Zhan W. X., *Acta Pharmacol. Sin.*, **8**, 318—320 (1987).
12) Chenieux-Guicheney P., Dausse J. P., Meyer P., Schmitt H., *Br. J. Pharmacol.*, **63**, 177—182 (1978).
13) O'Donnell S. R., Wanstall J. C., *Life Sci.*, **27**, 671—677 (1980).
14) Brandt H. D., Meyer C. L., *Arch. Int. Pharmcodyn.*, **289**, 46—59 (1987).
15) Craig M. S., *J. Pharmacol. Exp. Ther.*, **292**, 280—287 (2000).
16) Diana F., Lisa M. H., Karen L. G., *Drugs*, **42**, 115—137 (1991).
17) Decker N., Quennedey M. C., Rouot B., Schwartz J., Valley J., *J. Pharmacy Phamacol.*, **34**, 107—112 (1982).
18) Engel G., Hoyer D., Wagner H., *Naunyn Schmiedebergs Arch. Pharmacol.*, **317**, 277—285 (1981).
19) Stiles G. L., Taylor S., Lefkowitz R. J., *Mol. Pharmacol.*, **17**, 14—23 (1979).