Journal of Animal and Feed Sciences, 21, 2012, 493–502

Effects of active immunization against the recombinant second extracellular loop of sheep β_2-adrenoceptor on growth and blood biochemical parameters of female rats*

B. Yang, X.W. Bao, Q.Y. Guo, W.W. Dong, H.Y. Li, J.H. Gao and Y. Chen[1]

Xinjiang Agricultural University,
The Xinjiang Key Laboratory of Meat and Milk-Production Herbivore Nutrition
Urumqi 830052, P.R. China

(Received 29 December 2011; revised version 19 July 2012; accepted 14 September 2012)

ABSTRACT

The objective of this study was to determine effects of active immunization against the recombinant second extracellular loop of sheep β_2-adrenoceptor (rBAR) on growth performance, carcass parameters, internal organ weight, and some blood biochemical parameters of rats. Forty five six-week-old female Wistar rats were randomly allocated to three groups and received active immunization of rBAR (rBAR-immune group) or adjuvant by subcutaneous injection (contol goup), or oral administration of β-agonist clenbuterol hydrochloride (clenbuterol goup). Results showed that active immunization against rBAR displayed significant antibody titer responses. Growth performance, feed intake, carcass weight and leg muscle weight of rats were increased by oral administration of clenbuterol ($P<0.05$), but not by rBAR immunization. rBAR immunization increased weight of liver and lungs, and decreased blood urea nitrogen and triglycerides of rats ($P<0.05$). Abdomen fat weight and concentration of total cholesterol tended to decreased by rBAR immunization ($P=0.11$ and $P=0.087$). The results implied that antibody raised against the recombinant second extracellular loop of sheep β_2-AR can mimic part functions of β-agonist.

KEY WORDS: β_2-adrenoceptor immunization, growth, blood biochemical parameters, rat

---

* Supported by National Natural Science Foundation of P.R. China, Grant No. 30860195, and Foundation of Key Laboratory of Xinjiang Uygur Autonomous Region (2011)

[1] Corresponding author: e-mail: xjaucy@yahoo.com.cn

INTRODUCTION

Leaner carcass production in the husbandry has been emphasized because of the declining demand for animal fat and the increasing demand for lean meat. β-adrenoceptors (β-ARs) are involved in adipose tissue lipolysis and protein deposition in human and animal modulated by β-agonists (Langin, 2006). Carcass composition and feed efficiency of domestic animals can be improved by using β-agonists, such as clenbuterol, ractopamine and zilpaterol (Scramlin et al., 2010). However, detrimental effects of these compounds are also obviously. They are generally heat-stable and orally active, and have the potential to affect human's health if consumed accidentally (Mersmann, 1998). So β-agonists are forbidden to use in livestock production in many countries. Therefore, it is necessary to focus research efforts on developing new and safe ways to manipulate animal growth.

Results of accumulated researches showed that antibodies against receptors could recognize and activate the receptors in cell membranes, and imitate the effects of the corresponding hormones. For instance, monoclonal antibody 263 against extracellular domain of growth hormone (GH) showed GH agonist-like functions both *in vitro* and *in vivo* (Wan et al., 2003). Autoantibodies against α_1-, β_1- and β_2-AR showed some functions of their corresponding agonists (Zuo et al., 2006; Tutor et al., 2007; Yan et al., 2009). Antibodies, which directed against a peptide corresponding to the second loop of the human β_2-adrenergic receptor, were able to stimulate the L-type Ca^{2+} channels on isolated adult guinea-pig cardiomyocytes. This effect was similar to that obtained by zinterol, a specific β_2-agonist (Mijares et al., 1996). In another report, presence of antibodies against H24T, a peptide corresponded to the complete second outer loop of human β_2-AR, caused a leftward shift in a concentration-response curve for (−)-isoproterenol-induced relaxation of isolated bovine smooth muscle strips (Hill et al., 1998). This meant the antibodies recognized and activated the β_2-AR. These results implied that antibodies against the second extracellular loop of β_2-AR have the potential to mimic some functions of β-agonist *in vitro*. However, it is still unknown whether the antibodies can elicit agonist-like effects *in vivo*.

In previously study, a gene coding for the second extracellular loop of sheep β_2-AR was fused into the prokaryotic expression vector pET-32C (+) and expressed in *E. coli* BL21 (DE3) (Zhang et al., 2010). In the present report, we investigated effects of active immunization with the recombinant fusion protein (rBAR) on feed intake and growth of rats. Furthermore, changes of blood biochemical parameters related to protein, lipid and energy metabolism were also evaluated.

MATERIAL AND METHODS

Preparation of the recombinant fusion protein

The gene coding for the second extracellular loop peptide (H-W-Y-R-A-S-H-K-E-A-I-K-C-Y-A-K-E-T-C-C-D-F-F-T-N) of sheep β_2-AR was fused into 3' end of thioredoxin protein gene in the pET-32C (+) expression vector (Novagen®, Merck KGaA, Darmstadt, Germany) (Zhang et al., 2010). The recombinant fusion protein (rBAR) was expressed in *E. coli* BL21 (DE3), purified using His•Bind® purification Kit (Novagen®, Merck KGaA, Darmstadt, Germany), and dissolved in 0.1 mol/l phosphate buffered solution (PBS pH 7.4) to achieve the final concentration of 1.4 mg/ml.

Animals

All animal procedures were approved by the Academic Committee of Xinjiang Agricultural University. Forty five female Wistar rats (average body weight 175.27±10.13 g, 42±2 d of age) were obtained from the Laboratory Animal Center of Disease Control and Prevention of Xinjiang and randomly divided into three groups. One group of rats was immunized against rBAR (rBAR-immune group). Another group of rats was injected with adjuvant (control group) and the third group of rats was orally administrated clenbuterol hydrochloride (clenbuterol group).

Vaccine preparation and immunization treatments

Equivalent volume of rBAR solution and adjuvant were mixed, emulsified by sonication, and used as the vaccine. For the primary immunization, the control rats (n=15) received 100 μl emulsified sterile PBS (the sterile PBS:the complete Freund's adjuvant=1:1, (v/v)) for each injection. The rats in rBAR-immune group (n=15) received 70 μg of rBAR emulsified in the complete Freund's adjuvant for each injection. Rats in the two groups were boosted with 50 μl of emulsified sterile PBS or 35 μg of rBAR emulsified in incomplete Freund's adjuvant 2 wk later. The primary and booster injections were administered subcutaneously in the side of the neck and back.

Housing and feeding

All the rats were housed in individual pens and given free access to feed and water. The diet (Table 1) was fed in 2 equal allotments at 09.00 and 21.00.

Table 1. Ingredient and nutrient composition of the diet

Ingredient	Composition, DM basis, %	Nutrient	Composition, DM basis, %
Maize	36	Crude protein (CP)	20.19
Soyabean meal	15	Calcium (Ca)	0.96
Fish meal	4	Phosphorus (P)	0.82
Wheat bran with flour	24.5	Ether extract (EE)	2.88
Wheat bran	10	Metabolizable energy, ME, MJ/kg	18.13
Sunflower seeds meal (dehulled)	5		
Salt	1		
Limestone	0.6		
Meat and bone meal	3		
Calcium phosphate	0.9		

From d 0 to 7, rats were maintained for adaptation the diet and environment. On d 8, rats in the rBAR-immune and control group received primary immunization. On d 22, rats in the two groups received booster injection. Starting from d 22, rats in the clenbuterol group were fed with the diet containing clenbuterol hydrochloride (Sigma Chemical, St. Louis, MO, USA; 3 mg/kg DM diet). Feed intake and fasting body weight of each rat were recorded daily and weekly, respectively.

Blood sample collection

On d 43, after fasting body weight was recorded, rats were anaesthetized with ether, and then blood sample (2 ml) of each rat was collected by cardiac puncture. Vacutainers containing heparin sodium were used for blood collection and anti-coagulation. After being collected and gently mixed, all blood samples were placed immediately into refrigerator at 4ºC for 3 h, and then centrifuged at 3000 *g* for 10 min at 4ºC. Plasma was collected and stored at -20ºC until analysis.

Carcass parameters and internal organs weight

Immediately after blood collection, all rats were sacrificed and dissected. Carcass and internal organs including liver, heart and lungs of each rat were weighed, respectively. Then, abdominal fat was carefully isolated and weighed. Muscles of right leg including *gluteous superficialis*, *rectus femoris*, *gastrocnemius*, *biceps femoris*, *semitendinosus*, *vastus medialis*, *pectineus*, *adductor longus* and *gracilis* were dissected and weighed to obtain the leg muscle weight.

Antibody titers measurement

Plasma rBAR antibody titers were measured by ELISA. In brief, 96-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were coated with

100 µl of rBAR (2 µg/ml in PBS; pH 7.4) and incubated at 4°C overnight. Plates were washed extensively with PBS containing 0.05% (w/v) Tween 20 (PBST). Then, 100 µl of PBST containing 5% skimmed milk powder (w/v) was added and the plates were incubated at 37°C for 2 h. Plates were washed extensively with PBST, and 100 µl of plasma sample was added at a dilution of 1:2 000 and incubated at 37°C for 2 h. Then the plates were washed three times with PBST and 100 µl of horseradish peroxidase-conjugated goat monoclonal anti-rat IgG (Jackson ImmunoResearch Europe Ltd, Suffolk, UK) was added at a dilution of 1:8 000. After being incubated at 37°C for 1 h, the plates were washed three times with PBST. Then, 100 µl of tetramethylbenzidine substrate (Tiangen Biotech Co., Ltd, Beijing, China) was added to each well. The plates were incubated at 37°C for 5 min in the dark, and then 50 µl of 1 N sulphuric acid was added to stop reaction. The optical density at 450 nm (OD_{450}) was read with a microplate reader (Infinite M200, Tecan Group Ltd, Switzerland). Antibody titers against rBAR were expressed as OD_{450} value.

Blood biochemical parameters assays

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were determined spectrophotometrically by measuring the oxidation rate of reduced NADH (nicotinamide adenine dinucleotide) in thermostated cuvettes at 340 nm after incubation of samples with L-aspartate, L-alanine and Na-pyruvate, respectively. The activity of alkaline phosphatase (ALP) was determined using a colorimetric assay by evaluating the production of thymolphthalein at 590 nm after incubation of samples with thymolphthalein monophosphate. Enzyme activity was expressed in IU/l.

Plasma glucose was analysed by an automated glucose oxidase method. Blood urea nitrogen (BUN) was determined by a spectrophotometric assay based on the urease-Berthelot method. Total cholesterol (TC) assay was done by enzymatic-spectrophotometric method, and triglycerides (TG) were measured by enzymatic glycerol phosphate oxidase/peroxidase method. Determinations of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were carried out using homogenous enzymatic direct assay.

Statistical analyses

In the statistical comparisons between the groups, one-way analysis of variance (ANOVA) was used. The significance differences between the groups were determined by the Duncan Multiple Range Test. All statistical analyses were carried out using the PASW Statistics 18.0. P values <0.05 were accepted as statistically significant.

RESULTS

Antibody titers

No rBAR-specific antibodies were detected in rats in the control and clenbuterol group, whereas rats immune with rBAR displayed significant antibody titer responses ($P<0.05$; Figure 1).

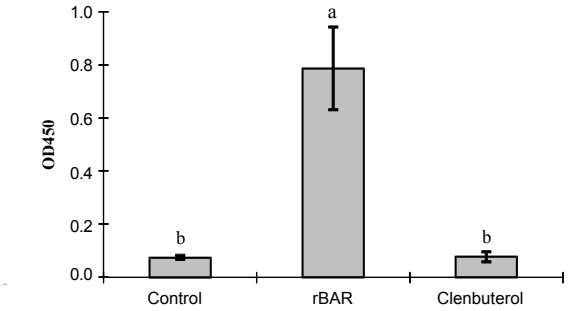

Figure 1. Antibody titer. The mean titers were expressed as average OD450 values of diluted serum (1:2 000) of fifteen rats in each group

Growth performance

Compared with the control group, oral administration of clenbuterol hydrochloride increased growth performance and feed intake of rats ($P<0.05$). Growth performance and feed intake of rats were not affected by active immunization with rBAR (Table 2).

Table 2. Effects of active immunization against the recombinant second extracellular loop of sheep β_2-AR on growth performance and feed intake of rats

Indices	Control	rBAR-immune	Clenbuterol
Initial weight, g	175.40 ± 10.51	175.25 ± 10.35	175.17 ± 10.44
Final weight, g	247.80 ± 20.10 ^{b}	253.82 ± 13.81^{ab}	266.25 ± 18.26^{a}
Average weekly gain, g	12.07 ± 2.35^{b}	13.10 ± 2.63^{ab}	15.18 ± 2.84^{a}
Average weekly feed intake, g	117.70 ± 9.47^{b}	118.00 ± 8.37^{b}	126.72 ± 10.72^{a}

data were expressed as mean ± standard deviation

$^{a, b}$ least square means within the same row without common superscript differ at $P<0.05$

Carcass parameters and internal organ weight

Liveweight and carcass weight of rats in the clenbuterol group were higher than that of the control and rBAR-immune group ($P<0.05$), and there was no different between the control and rBAR-immune group (Table 3). Compared with the control, weights of liver and lungs of rats in rBAR-immune group were increased

by 12.15% and 18.46% (P<0.05). Weight of abdomen fat of rBAR immunized rats decreased by 12.21% (P=0.11). Leg muscle weight of rats in the clenbuterol group was higher than that of the control and rBAR-immune group (P<0.05).

Table 3. Effects of active immunization against the recombinant second extracellular loop of sheep β_2-AR on growth internal organs, abdominal fat and leg muscle weight of rats

Indices	Control	rBAR-immune	Clenbuterol
Liveweight, g	254.96±16.74^{b}	258.27±11.61^{b}	278.19±13.59^{a}
Carcass, g	134.17±13.75^{b}	137.01±10.42^{b}	151.94±7.99^{a}
Liver, g	8.17±0.81^{b}	9.30±0.94^{a}	8.68±0.92^{ab}
Heart, g	0.83±0.07	0.86±0.10	0.90±0.09
Lungs, g	1.30±0.12^{b}	1.54±0.32^{a}	1.50±0.23^{a}
Abdomen fat, g	5.16±1.21	4.53±0.80	4.76±1.15
Leg muscle, g	15.12±1.52^{b}	14.70±1.53^{b}	16.68±1.26^{a}

data were expressed as mean ± standard deviation
^{a,b} least square means within the same row without common superscript differ at P<0.05

Blood biochemical parameters

Blood biochemical parameters including AST, ALT, LDH, HDL-C, LDL-C and GLU were unaffected by rBAR immunization or oral administration of clenbuterol hydrochloride (Table 4). Compared with the clenbuterol group, ALP activity was increased by immunization (P<0.05). TC tended to be decreased by immunization and clenbuterol (P=0.087). Concentrations of BUN and TG of rats in rBAR-immune group were decreased by 9.68% and 20.21% (P<0.05), respectively.

Table 4. Effects of active immunization against the recombinant second extracellular loop of sheep β_2-AR on blood biochemical parameters of rats

Biochemical parameters[1]	Control	rBAR-immune	Clenbuterol
U/l			
AST	191.93±53.39	195.07±74.34	185.93±131.68
ALT	41.93±8.61	47.71±13.72	46.79±22.43
ALP	115.07±27.28^{ab}	121.36±23.30^{a}	96.79±15.84^{b}
LDH	1942.93±528.53	2120.00±532.69	1732.14±711.44
mmol/l			
BUN	8.68±1.11^{a}	7.84±1.10^{b}	6.97±0.75^{b}
TC	1.59±0.26	1.45±0.20	1.43±0.17
HDL-C	0.69±0.12	0.69±0.09	0.74±0.13
LDL-C	0.13±0.02	0.14±0.03	0.13±0.02
TG	0.94±0.30^{a}	0.75±0.19^{b}	0.81±0.17^{ab}
GLU	7.11±2.00	7.40±1.86	7.12±0.79

data were expressed as mean ± standard deviation
^{a,b} least square means within the same row without common superscript differ at P<0.05
[1] AST - aspartate aminotransferase; ALT - alanine aminotransferase; ALP - alkaline phosphatase; LDH - lactate dehydrogenase; BUN - blood urea nitrogen; TC - total cholesterol; HDL-C- high density lipoprotein cholesterol; LDL-C- low density lipoprotein cholesterol; TG - triglycerides; GLU - glucose

DISCUSSION

Immunological approaches attract interest as an alternative method to direct administration of growth-promoting compounds to manipulate animal growth and body composition (Kim and Kim, 1997). Accumulated information shows that either active immunization using vaccines or passive immunization using monoclonal or polyclonal antibodies against some endogenous hormones or receptors provides a potential approach for improving growth performance. As a natural protein, antibody does not have drawbacks of chemical growth-promoting compounds. It can be easily destroyed by gentle cooking; and, even if ingested accidentally by humans, it can be prone to rapid degradation in the gut (Hill et al., 1998). Hill et al. (1998) raised antibody to the peptide which synthesized from the complete second outer loop of human β_2-AR. From their tests, the antibody worked *in vitro*. However, there is no further report about effects of this antibody on growth efficiency of animal *in vivo*. In the present study, growth performance and feed intake of rats were improved by clenbuterol but not by rBAR immunization. Cardoso and Stock (1996) reported that feed consumption was not affected by clenbuterol hydrochloride administration. Meanwhile, Mersmann (1998) considered that β-AR agonist effects on feed intake generally are small or none. The difference might be related to the animal species, age, gender, feed composition and feeding pattern.

In this research, the increase in weights of carcass and leg muscle caused by clenbuterol hydrochloride was consistent with the effects observed by Cardoso and Stock (1996) and Huang et al. (2000). In the rBAR-immune group, weights of carcass, heart and leg muscle were not affected by rBAR immunization, whereas liver and lungs weights were increased and abdomen fat mass tended to decrease. In the previous research, McNeel and Mersmann (1999) found that the abundance of β_2-AR on the cell surface of internal organs of rats was lung>liver>heart. Therefore, liver and lungs might be more sensitive to the rBAR antibodies. This might be one of the reasons that lead to weight change of liver and lungs in the present study.

Blood urea is generated in the liver by urea cycle by means of the detoxification of ammonia, a by-product of protein catabolism (Russell and Roussel, 2007). BUN has been suggested to indicate the relative rate of carcass protein accumulation (Chikhou et al., 1993). In the present work, BUN concentrations were decreased by clenbuterol hydrochloride and rBAR immunization and this agreed with the report of López-Carlos et al. (2012), who found that serum urea N concentration of feedlot ram lambs was reduced by β-adrenergic agonist administration regardless of feeding programme period. The decrease in serum urea N concentrations observed in the present trial could be explained by the mode of action of clenbuterol hydrochloride and the antibodies, which promoted muscle protein synthesis and utilization of N.

In previous studies, AST and ALT concentrations in steers were increased by cimaterol or clenbuterol (Chikhou et al., 1993). The increase in serum the activity of these enzymes is generally used as an indicator of tissue damage (Chikhou et al., 1993). In the present trail, activities of AST and ALT were within the range observed in healthy rats, and unaffected by clenbuterol or rBAR immunization. This result implied that active rBAR immunization did not have a negative impact on liver.

Another important role of clenbuterol hydrochloride is to affect body lipid metabolism. In this study, lipid metabolites TC, HDL-C and LDL-C were unaffected by clenbuterol administration and rBAR immunization, which was in agreement with previous reports in beef cattle and sheep (López-Carlos et al., 2012). However, TC and LDL-C concentrations were decreased and HDL-C concentration was increased in healthy men by oral albuterol, a β_2-selective adrenergic agonist. This might be subject and agonist related.

Serum triglycerides in this experiment decreased in rats in the rBAR-immune goup. Belahsen and Deshaies (1992) also found that serum triglycerides in Sprague-Dawley rats were decreased by clenbuterol injection. The decreased triglycerides might due to the fact that activation of β-AR in adipose tissue leads to activation and translocation of hormone-sensitive lipase with subsequent triglyceride hydrolysis (Kumar and Shanna, 2009).

The results from the present study implied that antibody raised against the recombinant second extracellular loop of sheep β_2-AR could mimic some functions of β-agonist. There were a number of possible mechanisms by which the antibodies might exert their effects at the β_2-AR. First, the antibodies might behave similarly to the conventional agonists, by causing a conformational change in the receptor protein upon binding. This proposed conformational change may then lead to receptor activation (Hill et al., 2008). Second, the binding sites on the receptor for the antibodies and conventional ligands would be different. Sterically, antibodies would be precluded from binding to the conventional ligand-binding sites deep within the receptor (Hill et al., 2008). Because the molecular mass of conventional agonists is ~1/1,000th that of IgG. This might be one of reasons that the actions of antibodies and clenbuterol were not exactly the same.

CONCLUSIONS

In summary, active immunization against the second extracellular loop of the sheep β_2-AR did not mimic the effects of β-agonists on the growth and carcass composition traits of rats. However, the antibodies elicited some agonistic functions as indicated lower concentration of blood urea nitrogen and triglycerides and higher weights of liver and lungs.

REFERENCES

Belahsen R., Deshaies Y., 1992. Modulation of lipoprotein lipase activity in the rat by the β_2-adrenergic agonist clenbuterol. Can. J. Physiol. Pharmacol. 70, 1555-1562

Cardoso L.A., Stock M.J., 1996. Effect of clenbuterol on growth and body composition during food restriction in rats. J. Anim. Sci. 74, 2245-2252

Chikhou F.H., Moloney A.P., Allen P., Quirke J.F., Austin F.H., Roche J.F., 1993. Long-term effects of cimaterol in Friesian steers: I. Growth, feed efficiency, and selected carcass traits. J. Anim. Sci. 71, 906-913

Hill R.A., Flint D.J., Pell J.M., 2008. Antibodies as molecular mimics of biomolecules roles in understanding physiological functions and mechanisms. Adv. Physiol. Educ. 32, 261-273

Hill R.A., Hoey A.J., Sillence M.N., 1998. Functional activity of antibodies at the bovine β_2-adrenoceptor. J. Anim. Sci. 76, 1651-1661

Huang H., Gazzola C., Pegg G.G., Sillence M.N., 2000. Differential effects of dexamethasone and clenbuterol on rat growth and on β_2-adrenoceptors in lung and skeletal muscle. J. Anim. Sci. 78, 604-608

Kim Y.H., Kim Y.S., 1997. Effects of active immunization against clenbuterol on the growth-promoting effect of clenbuterol in rats. J. Anim. Sci. 75, 446-453

Kumar R., Shanna S., 2009. Lipid profile changes in mouse gastrocnemius muscle after denervation and beta-adrenoceptor stimulation. Indian J. Exp. Biol. 47, 314-319

Langin D., 2006. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacol. Res. 53, 482-491

López-Carlos M.A., Ramírez R.G., Aguilera-Soto J.I., Rodríguez H., Aréchiga C.F., Méndez-Llorente F., Chavez J.J., Medina C.A., Silva J. M., 2012. Effect of the administration program of 2 β-adrenergic agonists on growth performance and carcass and meat characteristics of feedlot ram lambs. J. Anim. Sci. 90, 1521-1531

McNeel R.L., Mersmann H.J., 1999. Distribution and quantification of $beta_1$-, $beta_2$-, and $beta_3$-adrenergic receptor subtype transcripts in porcine tissues. J. Anim. Sci. 77, 611-621

Mersmann H.J., 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76, 160-172

Mijares A., Lebesgue D., Argibay J., Hoebeke J., 1996. Anti-peptide antibodies sensitive to the 'active' state of the β_2-adrenergic receptor. FEBS Lett. 399, 188-191

Russell K.E., Roussel A.J., 2007. Evaluation of the ruminant serum chemistry profile. Vet. Clin. N. Amer.- Food Anim. PR 23, 403-426

Scramlin S.M., Platter W.J., Gomex R.A., Choat W.T., McKeith F.K., Killefer J., 2010. Comparative effects of ractopamine hydrochloride and zilpaterol hydrochloride on growth performance, carcass traits, and longissimus tenderness of finishing steers. J. Anim. Sci. 88, 1823-1829

Tutor A.S., Penela P., Mayor Jr. F., 2007. Anti-β_1-adrenergic receptor autoantibodies are potent stimulators of the ERK1/2 pathway in cardiac cells. Cardiovasc. Res. 76, 51-60

Wan Y., Zheng Y.Z., Harris J.M., Brown R., Waters M.J., 2003. Epitope map for a growth hormone receptor agonist monoclonal antibody, MAb 263. Mol. Endocrinol. 17, 2240-2250

Yan L., Xu Y., Yao H., Xue W., Tian J., Ren H., Wu Y., Yang G., Ma X.L., Liu H., 2009. The effects of autoantibodies against the second extracellular loop of α_1-adrenoceptor on vasoconstriction. Basic Res. Cardiol. 104, 581-589

Zhang H.L., Chen X.P., Yang X., Chen Y., Han B., Wang Z.R., 2010. Molecular cloning of sheep β_2-adrenergic receptor (β_2-AR) gene and expression of the second extracellular loop in *Eschericha coli*. Chin. J. Agr. Biotechol. 18, 911-916

Zuo L., He Z.M., Liu L., Liu Z.B., Zhang L., Liu H.R., 2006. Preliminary study on the biological activity of autoantibody against cardiac β_2-adrenoceptor in rats. J. Shanxi Med. Univ. 37, 121-124