Review

Correlation Analysis between Visceral Manifestation Theories on *Xuanfa* and Effect of Adrenergic Receptors

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ARTICLE INFO	ABSTRACT

Chinese visceral manifestation theory states that lung dominates *qi*, regulates breathing, and governs *Xuanfa* (dispersing) and *Sujiang* (descending). Clarifying this theory with modern physiological and pathological knowledge has been considered as an important part of complementary and alternative medicine therapy. Previous studies found that most *Xuanfa* drugs contained pharmacodynamic ingredients related to adrenergic receptors (ARs) signal transduction. The association of *Xuanfa*, with the control of breath movement, nutrient transfer, spreading heat to regulate temperature, and helping the heart control blood circulation, coincides with the physiological function of organs dominated by ARs-regulated sympathetic postganglionic fibers. Therefore, we hypotesize that *Xuanfa* is closely related to ARs-regulated signal transduction. By modern biological knowledge, we tried to evaluate and expound the correlation between the molecular mechanisms of modern physiology or pathology and *Xuanfa* or *Sujiang* theory. Ultimately, the research and development of modern drugs should fully expect the guidance from Chinese visceral manifestation theory, and the application of this principle will guide the prevention and clinical treatment of a variety of refractory diseases caused by a change in environment, climate, or lifestyle.

Key words

adrenergic receptor; molecular mechanism; *Xuanfa* (dispersing); *Sujiang* (descending); sympathetic nerves

1. Introduction

Traditional Chinese medicine (TCM) and Western medicine (WM) have different ideologies. In Chinese visceral manifestation positions, the variety of modes in which lung *qi* operates is defined as the effects or results, and these modes also concisely summarize the function of lung *qi* by macroscopic analysis such as observation, analogy, and reasoning. However, in WM, the physiology and pathology of pulmonary diseases are expressed at the anatomical structure and biochemical function levels based on the respiratory system by microscopic analytical methods. How to combine both approaches in modern scientific interpretation has been the core content for TCM modernization (Bai, 2013).

Visceral manifestation theory indicates that lung dominates *qi*, regulates breathing, and governs *Xuanfa* (dispersing) and *Sujiang* (descending), checking the balance with human physiological function and mainly regulating the body's *qi* through breathing. *Xuanfa* and *Sujiang* are two antithetical aspects of lung *qi* operation. If in balance, the function of lung is normal, and the physiological function will be steady. The function of *Xuanfa* means to make *qi* and body fluid spread throughout the whole surface of the body to warm and nourish the skin and hair, which is mainly reflected in the following aspects: 1) controlling breath movement, 2) helping nutrient transfer, 3) spreading heat and regulating the sweat secretion to adjust the body temperature, and 4) contracting the systemic veins to help the heart control blood circulation.

Anatomical physiologists believe that autonomic nerves, including the sympathetic and parasympathetic nerves, control and coordinate the functions of internal organs, blood vessels, and glands. Under normal circumstances, the sympathetic and parasympathetic nerves constrain and balance each other and together regulate the body's function. When the sympathetic nerves are stimulated, a signal is sent by spinal nerve fibers to the sympathetic ganglia and then by postganglionic neurons to the effectors such as organs, cardiovascular system, and glands. Sympathetic nerves can cause such reactions as mydriasis, rapid heartbeat, visceral vasoconstriction, coronary dilation, increased blood pressure, bronchial dilatation, reduced gastrointestinal motility, bladder wall muscle relaxation, reduction in the saliva secretion, sweat secretion, and contraction of arrector pili muscles. Sympathetic nerves are regulated by the neurotransmitter noradrenaline (NA) and target adrenergic receptors (ARs).

2. ARs and their function

ARs can be divided into two categories, α and β, which altogether include nine subtypes: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3 (http://www. iuphar-db.org/ DATABASE/GPCR List Forward). α_1-ARs are mainly distributed in the vascular smooth muscle and cause vasoconstriction; α_2-ARs mostly exist in the presynaptic membrane of noradrenergic nerves and exert a negative feedback effect in the regulation of NA release. β_1-ARs are predominantly found in the myocardium and can cause an increase in heart rate and myocardial contractility. Most of the β_2-ARs exist in bronchial or vascular smooth muscle and cause the relaxation of bronchi or blood vessels; β_3-ARs are mainly distributed in fat cells and cause the decomposition of fat when excited (Zheng et al, 2005).

ARs belong to the G-protein-coupled receptor superfamily and have similar structures, which include seven transmembrane domains with extracellular N-terminus and intracellular C-terminus. They share common endogenous neurotransmitters, catecholamines, NA, and epinephrine; Thus, there must be interactions among various subtypes (Kobilka, 2011; Antonelli-Incalzi and Pedone, 2007). Although α_1-ARs could exert their effects via subunits G_q or G_h, and β-ARs via G_s, β-ARs account for 86% of the effects

of endogenous NA on the myocardium, but 14% of these effects could be controlled by α_1-ARs. Although α_2-ARs could mediate the inhibitory effect of adenylate cyclase (AC) via the G_i subunit on vascular smooth muscle, like α_1-ARs, α_2-ARs also could induce vasoconstriction by activating Ca^{2+} channel (Hein and Mechele, 2007). Additionally, β-ARs could regulate a series of important bioactivities, including cardiac pacing, myocardium contraction, vascular and bronchial smooth muscle relaxation, and metabolism of carbohydrates or lipids (Taira et al, 2008). Currently, the cAMP-PKA pathway could be regarded as the classic AR signaling pathway. β-ARs were believed to transmit signals by coupling with $G_{\alpha s}$, but recently β_2- and β_3-ARs were found also to couple with $G_{\alpha i}$ subunit. Additionally, $G_{\beta\gamma}$ subunits were involved in a variety of cell signaling processes (Katritch et al, 2013). Nevertheless, each subtype of AR had been given a specific tissue distribution and a unique effect related precisely to terminal effectors (Kolinski et al, 2012). Thus, it is very important to screen and develop highly selective agonist or antagonist drugs against different ARs.

2.1 Breath movement

Lungs control the movement of breath. The essence of breath movement is air exchange between the body and the surrounding environment: inhaling oxygen, exhaling carbon dioxide, and taking part in energy metabolism processes in the body. Inhalation is caused by the contraction of respiratory muscles, resulting in active inspiration. In contrast to passive exhalation, the lung retracts due to the relaxation of the respiratory muscle. In the human lung, the ratios of β_1- and β_2-ARs are 20% and 80%, respectively. Although β_2-ARs are also distributed extensively in the ciliated epithelium, mucous glands, and vascular endothelium, such as alveolar epithelial cells and mast cells, 90% of β_2-ARs are located in the alveolar walls. The β_2-AR agonists can relax the airway smooth muscle by activating β_2-ARs, therefore becoming the currently preferred options for treating asthma and chronic obstructive pulmonary disease (COPD) (Mathew et al, 2012). However, finding novel drug candidates with β_2-AR specific affinity from complex Chinese mteria medica (CMM) mixtures has been facing a great challenge (Zhao et al, 2010).

2.2 Heart and vessel

β-ARs also play a major role in the regulation of cardiac contractility. Both β_1- and β_2-ARs in cardiac muscle can activate AC by coupling with $G_{\alpha s}$, regulate protein kinase A (PKA), phosphorylate myosin and calmodulin, and ultimately enhance myocardial contractility. β-ARs (80%) expressed in cardiomyocytes are recognized as β_1-ARs, and they play a vital role in the regulation of myocardial contraction. But selective activation of β_2-ARs could avail the treatment of heart failure (Taskén and Aandahl, 2004). This emphasized the key role of β_1- and β_2-ARs in the regulation of cardiac output and blood pressure (Woo and Xiao, 2012). β_3-ARs were also found in the myocardium at a lower level and β_3

receptor blockers showed the protective effects on heart failure (Rasmussen and Burkey, 2009). Currently, β-blockers had been widely used for the treatment of hypertension, angina pectoris, myocardial infarction, acute and chronic heart failure, as well as other diseases (Baker and Harada, 2011).

2.3 Transport of nutrients

Transfer of nutrients refers to the digestion and absorption processes. With respect to carbohydrate and lipid metabolism, ARs could regulate liver glycogen decomposition and β-ARs had a more physiological function than α-ARs (Katz et al, 1993). Catecholamines could enhance the rates of aerobic glycolysis, release glucose in the muscles, and inhibit insulin-mediated glycogenesis (Barth et al, 2007). Acting through β$_3$-ARs, epinephrine, and NA could lead to lipolysis and heat production in fat cells and increase glucose intake in skeletal muscle. Therefore, a β$_3$-AR agonist was considered as an attractive target for type 2 diabetes and obesity treatment (Souza and Burkey, 2001; Sawa and Harada, 2006).

2.4 Spreading heat to regulate temperature

The regulation of body temperature is mainly performed by the skin and lung. Normally, the skin is responsible for 90% of heat loss, and diaphoresis is the most effective way to lose heat. α and β-ARs coexist in sweat gland cells on the skin. α-ARs antagonists could reduce sympathetic nervous tension, relax small skin arteries, increase blood flow, and lead to sweat. Although relaxing small skin arteries by exciting β-ARs could also contribute to sweat, α-ARs still played a decisive role (Eshel et al, 2004). Additionally, β$_3$-ARs are abundant on smooth muscle cells within the urinary tract, and selective β$_3$-AR agonists that could relax bladder smooth muscle had been used in the treatment of bladder dysfunction (Michel, 2011). Selective α$_1$-AR antagonists had also been used clinically to treat benign prostatic hyperplasia (BPH) (Fine and Ginsberg, 2008). Current research on lower urinary tract dysfunction focused on blocking afferent mechanisms, but enhancing afferent information had become the major means for voiding dysfunction with β$_3$-AR agonists (Kanai et al, 2011). Therefore, ARs are closely involved with thermoregulatory heat spreading and diuretic effects.

3. AR related drugs

3.1 AR related agonists and antagonists

In 1956, Stephenson (1956) proposed a modified receptor theory that removed the absolute distinction between agonist and antagonist drugs and introduced an intermediate class of partial agonists. According to this theory, most β-AR agonists were developed from the basic chemical structure of adrenaline. For example, early bronchodilators such as ephedrine and isoprenaline had non-selective and short-acting characteristics. Since the 1950s, new bronchodilators had constantly specific targeted β$_2$-ARs to reduce the toxic effects

on the heart. Until now, oral bronchodilators, such as salbutamol, terbutaline, and clenbuterol, have not been only resistant to the metabolic enzymes, catechol-*O*-methyltransferase (COMT), and monoamine oxidase (MAO), but some inhaled long-acting β$_2$-agonists (LABAs) have also been used for the treatment of COPD. LABAs have been in use since the 1990s enabling persistent bronchodilation for 12 h. Compared with twice-daily LABAs, new LABAs with ultra-long duration could provide the improvements in efficacy and compliance with fast onset of action in 24-h bronchodilation (Malerba et al, 2012). Simultaneously, more and more selective β$_1$-AR blockers, such as metoprolol, atenolol, and bisoprolol, were widely developed and used for heart treatment (Galandrin et al, 2008; Wisler et al, 2007; Poirier et al, 2012). α$_1$-AR antagonists, such as doxazosin and terazosin, were once used clinically for hypertension treatment, but now they could be increasingly used to treat BPH (Schilit and Benzeroual, 2009). In the late 1970s, the α$_1$-AR antagonist phenoxybenzamine was first used for BPH, but serious side effects limited its use (Caine et al, 1978). Thereafter, quinazoline compounds, such as tamsulosin, serving as selective α$_1$-AR antagonists, played a significant role in the treatment of BPH (Koshimizua et al, 2007). In short, the AR-related agonists and antagonists are currently considered as the most important drugs for the treatment of asthma, heart disease, and diuretic system disease.

3.2 AR agonists in TCM theory

In TCM theory, *Xuanfa* therapy is used to cure the syndrome of superficial cold and interior heat, and the symptoms of lung *qi* congestion or obstruction. TCM differs from the therapeutics based on single-chemical entities. Some herbal medicines and ancient CMM prescriptions often have novel therapeutic regimens against chronic lung diseases. A classic CMM prescription, Maxing Shigan Decoction (consisting of ephedra = *Ephedrae Herba*, almond = *Armeniacae Amarum Semen*, gypsum = *Fibrosum Gypsum*, licorice = *Glycyrrhizae Radix*, etc) from the *Treatise on Febrile Diseases* has been used to treat pulmonary diseases since 200 BC. And herbal medicine, ephedra is artfully used as the representative drug, and it plays the role of monarch drug in the prescription. Ephedrine alkaloids are the main effective ingredients in ephedra, including 1*R*,2*S*-ephedrine, 1*S*,2*S*-pseudoephedrine, 1*R*,2*S*-norephedrine, 1*R*,2*S*-*N*-methyl ephedrine, and 1*S*,2*S*-norpseudo ephedrine. Until now, ephedrine has been generally recognized as a nonselective β-AR agonist, the typical material basis for the efficacy of *Xuanfa*. However, in recent research, ephedrine was identified acting directly as an antagonist to α-AR (Ma et al, 2007), which may agree with the role of ephedra in inducing sweat and dispelling exogenous evils. In confirming the existence of bioactive compounds for *Xuanfa*, some AR-related natural products, such as synephrine (Shi et al, 2009), higenamine (Bai et al, 2008), amygdalin (Zheng et al, 2007), and other AR antagonist alkaloids (Ma et al, 2010; Li et al, 2011) have been repeatedly found in CMM prescriptions.

3.3 Bioactivity-integrated UPLC-Q/ TOF-MS identification systems for β₂-AR agonist screen

Based on AR-cAMP-PKA signaling pathways, a bioactivity-guided ultra-performance liquid chromatography/ quadrupole time-of-flight mass spectrometry (UPLC-Q/ TOF-MS) characterization rapid isolation and identification system was proposed by coupling it with a dual luciferase reporter assay system for a β₂-AR agonist screening (Hou et al, 2012a). By using this method, many trace bioactive natural products in complicated samples can be found in a short time. This method integrates three key processes such as liquid phase separation (Figure 1A), mass spectrum identification (Figure 1B), and biological evaluation (Figure 1C) with a

detection sensitivity at the nanogram level. Using the alkaloid extract of *Alstonia scholaris* (L.) R. Br. as an example, with a single injection, we isolated and identified 18 alkaloid ingredients and found eight β₂-AR agonists of indole alkaloids (Hou et al, 2012b). Similarly, 44 ingredients were isolated and identified in the CMM preparation, such as Qingfei Xiaoyan Pellet evolved from the above classic Maxing Shigan Decoction, and only ephedrine was confirmed as the major β₂-AR related bioactive ingredient (Cheng et al, 2012). This strategy simplifies the screening and evaluation procedure for AR drugs and clearly demonstrates that bioactivity-integrated fingerprinting is a powerful tool to improve the screening efficiency and potential β₂-AR agonist identification from complex herbal medicines.
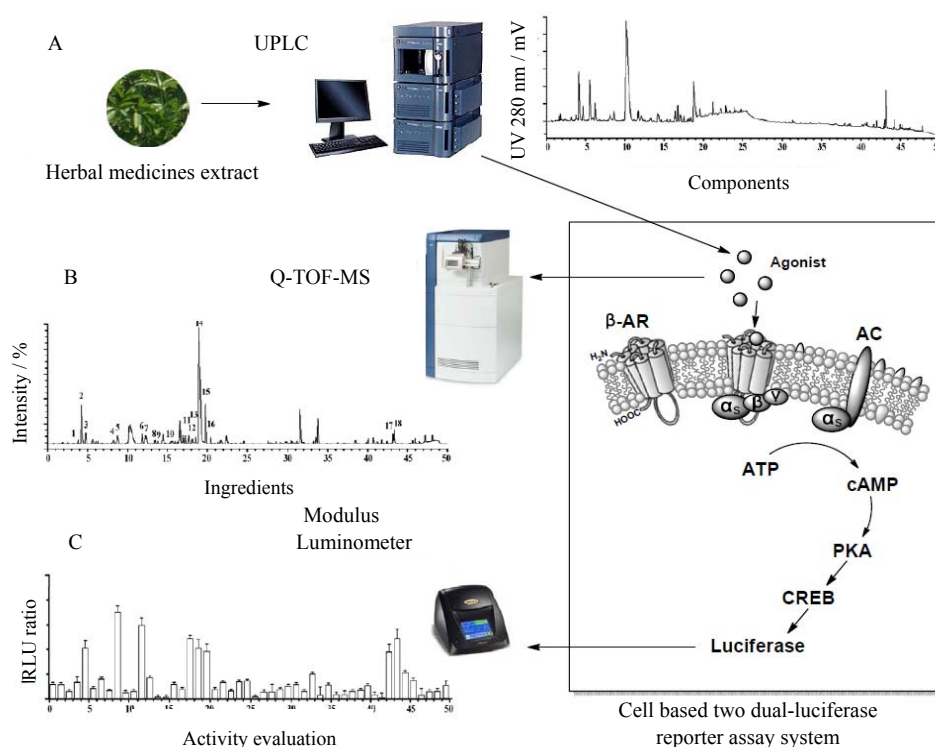
**Figure 1   Bioactivity-based UPLC-Q/TOF identification system for β-AR agonists from herbal medicines**

Concurrently, approximately 500 herbal drugs recorded in *Chinese Pharmacopoeia 2010* were screened for β₂-AR agonists. From this, more than 30 botanical-source herbal medicines with potential agonist activity were found (Figure 2A). Among them some medicinal materials were used for relieving exterior syndromes (*Jiebiao*), including ephedra, almond, asarum (*Asari Herba*), purple perilla (*Perillae Fructus*), and rattletop (*Cimicifugae Rhizoma*); some *qi-*regulating (*Liqi*) drugs including orange peel (*Citri Sinensis Pericarpium*), green tangerine peel (*Citri Reticulatae Viride Pericarpium*), and the fruit of immature citron (*Citri Grandis Fructus*); as well as some clearing heat and removing toxin (*Qingre Jiedu*) drugs, such as *Plumula Nelumbinis*, *Nelumbinis Folium*, *Menispermi Rhizoma*, and *Sinomenii Caulis*. In contrast, the structure except endogenic

catecholamine neurotransmitters (Figure 3A) generally included two types of compounds, phenethylamines (Figure 3B) and isoquinoline or indole alkaloids (Figure 3C).

Molecular docking (Autodock 4.0) could provide a possible mechanism to evaluate β₂-AR binding. Figure 4 shows that higenamine (isoquinoline type, Figure 4A) and ephedrine (phenethylamine type, Figure 4B) both can bind with human β₂-AR binding pocket, but with a much weaker intermolecular force than the classical agonist, salbutamol. Although isoquinoline and indole type alkaloids have weaker activity on β₂-AR, they still present anti-inflammatory effects (Ding et al, 2011; Yang et al, 2007; Shang et al, 2010). Whether the reason is a special structure of the phenolic hydroxyl group remains to be confirmed (Gomes et al, 2008).
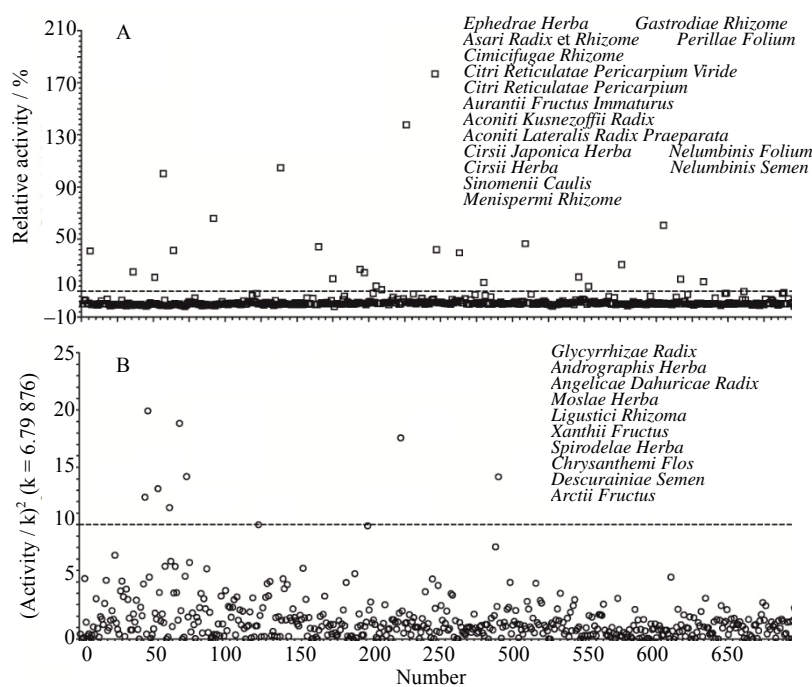
Figure 2   Screening of CMM for β-AR agonists and its synergistic compatibility drugs
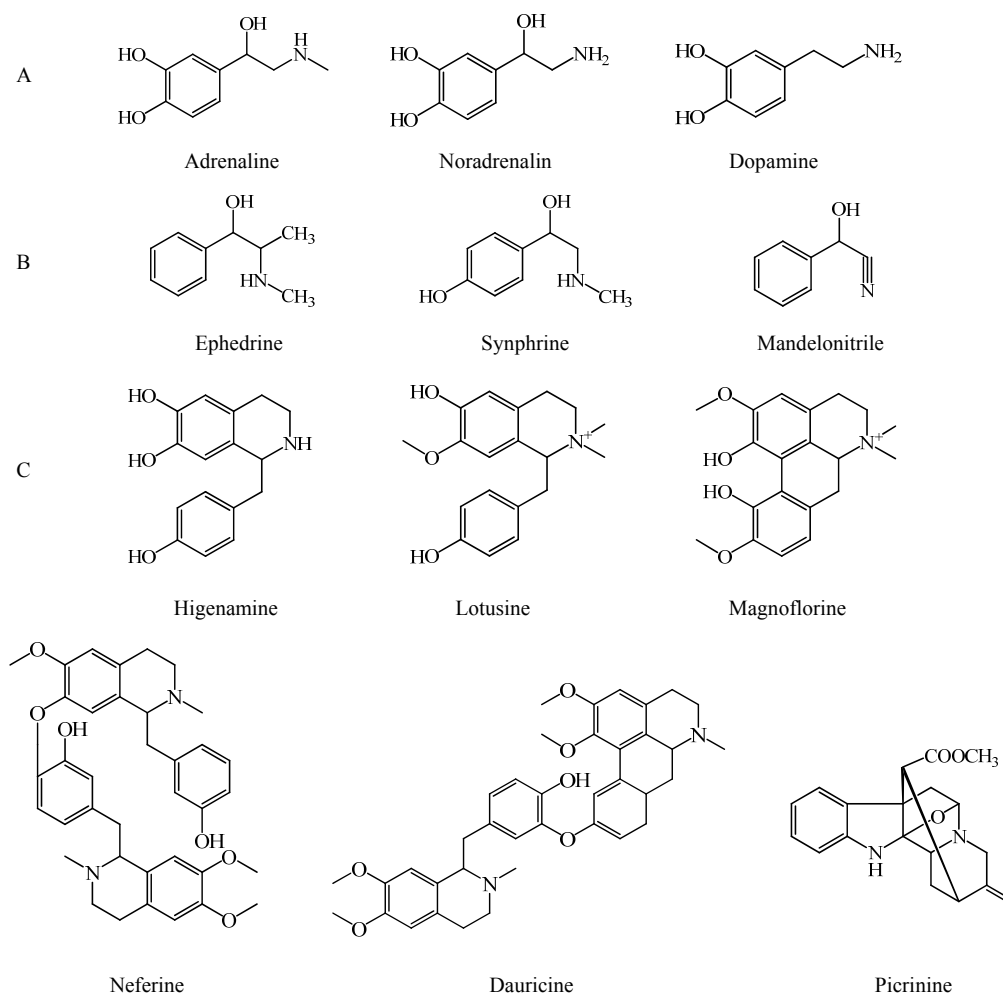
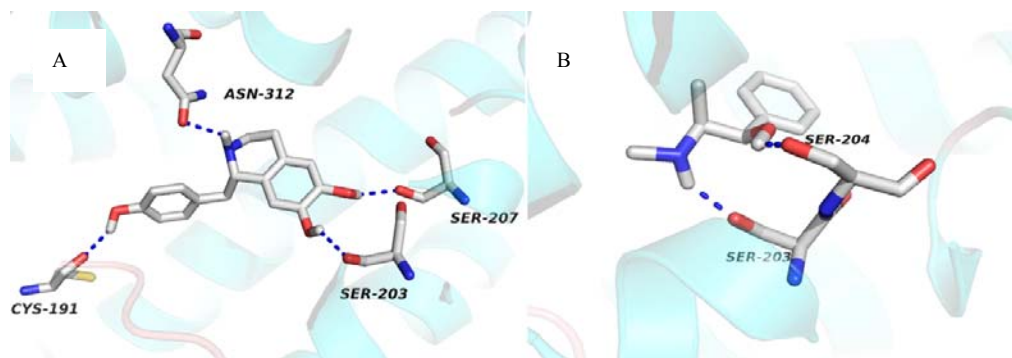**Figure 3   Molecular structures of endogenous (A) and natural (B and C) β-AR agonists**

Figure 4   Docking analysis of higenamine (A) and ephedrine (B) in human β₂-AR activity binding site

3.4   *Investigating mutually synergistic components in AR related pathway*

CMM formulae are prescribed that each herb is used to its greatest potential, thus improving the treatment results and reducing any adverse effects caused by the other combined herbal drugs. Recently, it was reported that the herbal drugs *Rehmanniae Radix* and *Anemarrhenae Rhizoma* could influence the expression of ARs or cAMP (Liu et al, 2004; Zhao et al, 2000). Based on the bioactivity-guided screening system described above, a mutually enhanced validation system was proposed by coupling it with a modified dual luciferase reporter assay for β₂-AR agonists. Consequently, other CMM that enhanced AR-cAMP-PKA pathway signal transduction such as *Angelicae Sinensis Radix*, *Xanthii Fructus*, *Elsholtziae Herba*, *Arctii Fructus*, *Lepidii seu Descurainiae Semen*, and other drugs were found (Figure 2B). This discovery will present the important information in illustrating the compatibility mechanism among *Xuanfa* formulae. If the synergistic mechanism can be clarified at the drug-target-pathway-network level, it will not only be important for explaining the mechanism of CMM prescription compatibility and helping drug discovery, but it can also guide the effectively combined use of clinical drugs related to ARs.

For example, glycyrrhetic acid (GA) has been reported for explaining the mechanism of CMM prescription compatibility and helping drug discovery, to exert synergistic anti-asthmatic effects via a β₂-AR mediated pathway (Shi et al, 2011). Using a chemical biologically labeled probe, azide-terminal GA, we found that GA localized predominantly on the cell membrane, decreased its cholesterol content, and changed the localization of G$_{αs}$ from lipid rafts to non-raft regions, which resulted in the binding of β₂-AR and G$_{αs}$, as well as the reduction of β₂-AR internalization (Shi et al, 2012). We speculate GA enhances β₂-AR activity by increasing the functional linkage through the subcomponents of the membrane β₂-AR-cAMP-PKA signaling pathway (Figure 5). As well known, many signal molecules are anchored in lipid rafts, and lipid rafts influence the mobility of membrane proteins and regulate the signal transmission of neurotransmitters and other receptors (Korade and Kenworthy, 2008; Pike, 2009). Therefore, we hypothesize that GA can affect many cell functions by regulating the contents of cholesterol in lipid rafts, which coincides precisely with the coordinating action of *Glycyrrhiza Radix* in CMM prescriptions.
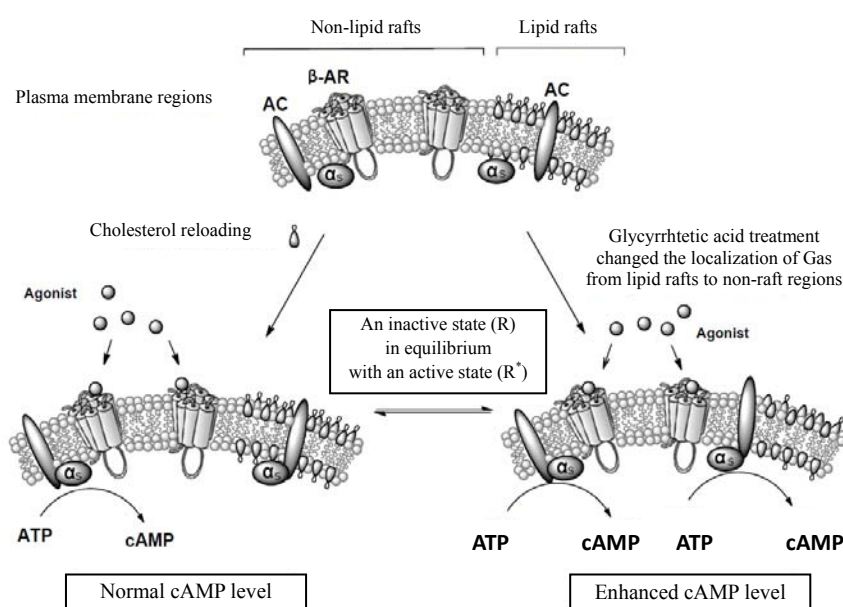

Figure 5   Changes of G$_{αs}$ localization by GA treatment from lipid rafts and promoted cAMP production of β-AR agonist

4. Hypotheses

Structural chemistry and structural biology have confirmed that ARs-related drugs share the catecholamine-like structures and regulate the physiological function of organs dominated by the ARs-regulated sympathetic postganglionic fibers (Brueckner et al, 2013; Kolinski et al, 2012). Conclusions derived from the clinical medicine coincide with connotation of TCM theory about *Xuanfa*. Consequently, we proposed that natural AR agonists or antagonists could participate in *Xuanfa* regulation process, and CMM prescription compatibility can enhance the regulation of the ARs related signal pathway more effectively (Figure 6).
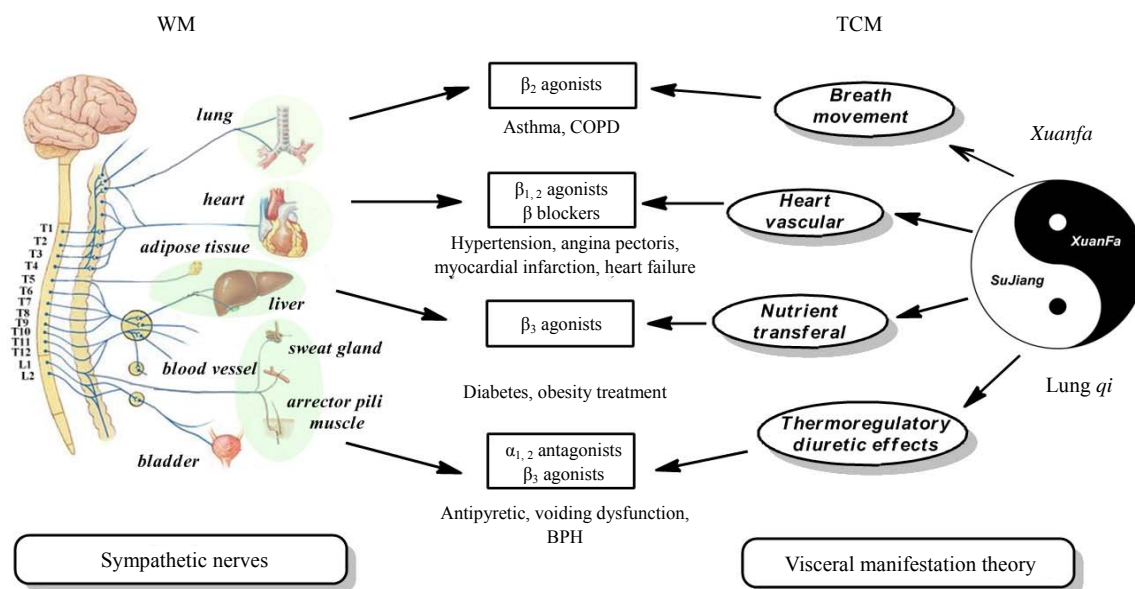

Figure 6   Modern biological hypothesis about lung dominates *Xuanfa*

5. Conclusion

The dispersion of lung *qi* is one of the fundamental forms of *Xuanfa* motion in visceral manifestation theory. Although theoretical basis, evidence, and history of TCM differ from WM and the understanding and therapies for disease are varied, different treatment modalities result in the same pathophysiological changes in the human body. And we believe that the processing and regulation target must be the same. Therefore, we assume *Xuanfa* is related to the physiological function of effector organs governed by sympathetic postganglionic fibers that are regulated by ARs and we hope to illustrate the correlation between lung *qi* motion and sympathetic nerve function by natural agonist or antagonist screening, receptor specificity analysis, and structure-activity relationship comparison. Ultimately, we try to clarify the scientific connotation of *Xuanfa* and deduce its theory at the molecular biology and network pharmacology level. With further research, we hope visceral manifestation theory of TCM will play a key role in modern drug research and development. Meanwhile, it is of profound importance on using this theory to guide the prevention and clinical treatment of a variety of refractory diseases including COPD, asthma, allergies, and immune dysfunction, which are caused by changes in the environment such as haze and air pollution, climate or life-style.

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