HAJER MOHAMED HMMAM

PHYSIOLOGY

MASTER' S DEGREE

2019

T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY

THE INVESTIGATION OF FLUOXETINE EFFECTS ON THE BRONCHIAL SMOOTH MUSCLES BY THE ISOLATED ORGAN BATH SYSTEM

MASTER'S THESIS

HAJER MOHAMED HMMAM

Istanbul-2019

T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY

THE INVESTIGATION OF FLUOXETINE EFFECTS ON THE BRONCHIAL SMOOTH MUSCLES BY THE ISOLATED ORGAN BATH SYSTEM

MASTER'S THESIS

HAJER MOHAMED HMMAM

SUPERVISOR
Assoc. Prof. Mehtap KACAR

Istanbul-2019

THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Programme : MSc in Physiology

Title of the Thesis : The Investigation of Fluoxetine Effects on the Bronchial Smooth Muscles by the Isolated Organ Bath System

Owner of the Thesis : Hajer Mohamed Hmmam

Examination Date : 29.07.2019

This study have approved as a Master Thesis in regard to content and quality by the Jury.

	Title, Name-Surname (Institution)	(Signature)
Chair of the Jury:	Prof. Dr. Bayram Yılmaz (Yeditepe University Faculty Medicine, Physiology Department)	
Supervisor:	Assoc. Prof. Mehtap Kacar (Yeditepe University Faculty Medicine, Physiology Department)	
Member/Examiner:	Assoc. Prof. Güldal İnal Gültekin (Okan University Faculty Medicine, Physiology Department)	

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 31.09.2019.... and numbered 2019/13-45

Prof. Dr. Bayram YILMAZ
Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

Hajer Mohamed Hmmam

ACKNOWLEDGMENTS

I would like to thank Head of Physiology Department Prof. Dr. Bayram Yılmaz for his continuous support and invaluable advice during my M.Sc. Programme.

I am deeply grateful to my supervisor Assoc. Prof. Mehtap Kacar, who gave me perfect opportunities during my scientific journey. I would like to thank for her support, encouragement and giving me valuable thoughts about science.

I am thankful to my lab partner Sinem Ethemoğlu for helping and supporting me whenever I needed.

During my thesis studies, thanks a lot to all those who supported me, and especially to my family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AC	Adenylate cyclase
ANP	A-Type Natriuretic Peptide
ARF	Acute respiratory failure
ASM	Airway Smooth Muscle
ASMC	Airway Smooth Muscle Cell
BNP	B-Type Natriuretic Peptide
CAM	Calmodulin
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
CNP	C-Type Natriuretic Peptide
DAG	Diacylglycerol
FDA	Food and Drug Administration
FSK	Forskolin
GC	Guanylate cyclase
IP_3	Inositol triphosphate
ISO	Isoproterenol
KDR	Delayed rectifier potassium channels
LS	Lithium salt
MAOI	Monoamine oxidase inhibitor
MDD	Major depression disorder
MLC	Myosin light chain
MPS	Micro-physiological system
PDE	Phosphodiesterase
PG	Prostaglandin
PIP	Phosphatidyl diphosphate inositol
PKA	Protein kinase A
PKG	Protein kinase G
PLC	Phospholipase C
PSS	Physiological salt solution
RyR	Ryanodine receptor
SNRI	Serotonin and norepinephrine reuptake inhibitor
SR	Sarcoplasmic reticulum
SRM	Serotonin receptor modulator
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressant
TRP	Transient receptor potential
TTCA	Tetracyclic antidepressant

ABSTRACT

Hmmam, Hajer Mohamed, 2019. The Investigation of Fluoxetine Effects on The Bronchial Smooth Muscles By The Isolated Organ Bath System. Yeditepe University Institute of Health Sciences, Department of Physiology, Master Thesis, İstanbul.

Airway smooth muscles (ASMs) are present in the respiratory system at the trachea and play a major role into protecting mechanical stability during breathing by contracting and relaxing as needed. Dysfunctionality in ASMs is directly related to respiratory system diseases, such as asthma and Chronic Obstructive Pulmonary Disease. The contractile mechanism of ASMs is complex. Therefore, research is necessary in this domain to identify issues and chemicals that can affect their contractions and impose health threats.

This study investigated the effects of fluoxetine on the smooth muscles of the ASM using an isolated organ bath system. Sprague Dawley rats used for the study were divided into 3 different groups following; spontaneous contractions with fluoxetine-added group from 10^{-9} to 10^{-1} concentrations (Fluoxetine; n = 6), the group induced by acetylcholine (Ach; n = 6) and acetylcholine induced fluoxetine group which fluoxetine concentrations are from 10^{-9} to 10^{-1} (Ach + Fluoxetine, n = 6). Sprague Dawley rats were sacrificed, and primary bronchial tissues were dissected and taken into Krebs solution. Primary bronchial tissues were suspended in the organ bath. After basal recordings, the strain was increased to 1.5 grams and the pharmacological agents were applied to respective groups. As a result of the experiment, in the acetylcholine-fluoxetine group has numerical decrease in comparison with acetylcholine with no statistical significance. On the other hand, there was a significant difference between the fluoxetine-only group and the acetylcholine-only group (*$p < 0.05$). In contrast to acetylcholine, which provides a strong contraction, fluoxetine has no such constrictive effect. The present study may contribute to the literature in this sense; therefore, future studies can be developed to evaluate the effects of oral usage of fluoxetine on the bronchial muscle in different experimental models.

Keywords: airway smooth muscles, fluoxetine, SSRI, organ bath system

ABSTRACT (Turkish)

Hmmam, Hajer Mohamed, 2019. Fluoksetinin Sıçan Bronş Düz Kaslarına Etkilerinin İzole Organ Banyosu Yöntemiyle Araştırılması. Yeditepe Universitesi Sağlık Bilimleri Enstitüsü, Fizyoloji Anabilim Dalı, Yüksek Lisans Tezi, İSTANBUL.

Solunum yolu düz kasları trakeanın iç bölgesinde yer alır ve solunum sırasında mekanik stabilitenin korunmasında önemli rol oynar. Solunum yolu düz kaslarında işlev bozukluğu, astım ve Kronik Obstrüktif Akciğer Hastalığı gibi solunum sistemi hastalıkları ile doğrudan ilgilidir. Solunum yolu düz kaslarının kasılma mekanizması birden fazla sebebe bağlı olduğu için, bu alanda kasılmalarını etkileyebilecek ve sağlık tehditleri oluşturabilecek sorunları ve kimyasalları tanımlamak için araştırma yapılması gerekmektedir. Bu araştırma, izole edilmiş organ banyosu sistemi kullanılarak fluoksetinin solunum yolu düz kasları üzerindeki etkileri araştırılmıştır. Çalışma için kullanılan Sprague Dawley sıçanlar 3 farklı gruba ayrıldı; spontan kasılmaları takiben 10^{-9} ve 10^{-1} arası konsantrasyonda fluoksetin eklenmiş grup (Fluoxetine; n=6), asetilkolin ile kasılma indüklenmesi yapılan grup (Ach; n=6) ve asetilkolin ile kasılma indüklemesi yapıldıktan sonra 10^{-9} ve 10^{-1} arası konsantrasyonda fluoksetin uygulanmış grup (Ach+Fluoxetine;n=6). Sprague Dawley sıçanlar sakrifiye edildikten sonra primer bronş dokuları disseke edilmiş ve Krebs solüsyonu içerisine alındı. Primer bronş dokuları organ banyosuna silindirik bir şekilde asıldı. Bazal kayıtlar alındıktan sonra gerim 1,5 grama getirilmiş ve her grup için kendi farmakolojik ajanları uygulandı. Deney sonuçları analiz edildiğinde; asetilkolin ile indüklenmiş fluoksetin grubunda, sadece asetilkolin ile indüklenen dokuya göre rakamsal olarak bir azalma görülse bile istatiksel bir anlamlılık bulunamamıştır. Diğer yandan sadece fluoksetin verilen grup ile sadece asetilkolin verilen grup arasında anlamlı fark bulunmuştur ($*p<0,05$). Güçlü bir kasılma sağlayan asetilkoline karşılık fluoksetinin kasıcı bir etkisi gözlenmemiştir. Araştırmamız literatüre bu anlamda katkı sağlayabilecek olup, bununla birlikte fluoksetinin oral olarak uygulanacağı farklı deney modellerinde bronş kasına olan etkilerinin araştırılacağı çalışmalar planlanabilir.

Anahtar kelimler: hava yolu düz kasları, fluoksetin, SSRI, organ banyosu

1. INTRODUCTION AND PURPOSE

1.1 Subject overview

The lower airways, from the trachea to the terminal bronchioles are surrounded by airway smooth muscle layer. This smooth muscle layer provides the contraction and relaxation of airways. This ability of airways is a key factor that provides ventilation and perfusion balance and gives mechanical stability (1). In interlobar bronchi, the organization of the smooth muscle is a sustained layer in the bronchial wall. The contraction of ASM in bronchial wall is under control via many extracellular messengers acting on specific membrane receptors. These messengers can be autonomous nervous system neurotransmitters, epithelial or inflammatory mediators. Therefore, basal tonus of airway is preserved by cholinergic innervation (2). In airways, major constrictor is parasympathetic nervous system. The airway smooth muscle cells (ASMCs) have specific membrane receptor that can stimulate by autonomous nervous system neurotransmitters, epithelial or inflammatory mediators, toxic substance, medicine (3). The parasympathetic stimulation of ASMCs leads to contraction of airways, and also is responsible for providing the basal tone of airways (4). The ASMCs express many β2-adrenoceptors that can be stimulate sympathetic nervous system. The stimulation of β2-adrenoceptors leads to relaxation of airways (5). The best-elaborate bronchodilators are β2-agonists, which stimulate β2-adrenergic receptors on ASM (6) Adrenergic agonists can alleviate the respiratory tribulation of the acute asthmatic attack and improve that of chronic asthma, and in addition to airway inflammation, contraction of airway smooth muscle plays an important function in asthma (7, 8). β2-adrenergic agonists do not seem to have any clear adverse effects on airway function. Most of the benefit in ASM lies in its abnormal behaviour in airway infirmity, in particular in asthma conditions. The pharmacology of ASM include understanding the factors that impact ASM function and affect these factors with drugs that may have therapeutic advantage (9).

Several substances such as inhaled toxic gases, anaesthetic gases may generate the contraction or altered reactivity of airways. The disturbance of airway reactivity plays important roles in pathogenesis of many pulmonary disease such as asthma (10, 11).

The airway smooth muscles are distinguished through their ultrastructure from the skeletal muscles as they are not ordered into sarcomeres, which makes their contractile mechanism more complex while enhancing their adaptability with regards to the force-length relationship (12). Airway smooth muscles are similar to other smooth muscles that are present in the vascular, uterine and gastrointestinal systems; however, there are differences in the neural regulation and mechanical responses that makes them unique and requires further investigation (13).

Furthermore, airway smooth muscles are continuously exhibiting contraction and relaxation during the breathing process, which decreases their stiffness and responsiveness to contractile stimuli. The vivo and vitro studies on ASM and their contractile apparatus hypnotize that mechanical forces modulate the cell structure and organization. The membrane adhesion plaques transfer the forces from the extracellular matrix to the cells' interiors. The filaments within the ASM transfer the force from one to another (14).

The drug tested in this research is fluoxetine, which is a medication used a treatment for depression. The drug is prescribed to treat depression but can also threaten cases that suffer from respiratory system disorders and diseases. Bronchial smooth muscles are located in the walls of the airways providing a certain level of flexibility during breathing (15). Fluoxetine is a chemical, classified as a selective serotonin reuptake inhibitor (SSRI) and binding with 5-HT receptors (16) that is used for other acute and long-term nervous system disorders, that was developed in the early 1970s as a successful substitute to other drugs that have more side effects (17). Nonetheless, studies have shown that Fluoxetine has direct influence on arterial and vascular smooth muscles (18). Due to similarities in origins, vascular and bronchial smooth muscles can be expected to react to the drug, especially as they have little differences in their contractile apparatus in terms of protein expression and gene transcriptional regulation (19).

Furthermore, ASM contraction is a key factor that provides ventilation and perfusion balance and gives mechanical stability. Inhaled toxic agents encounter a block from these non-cartilaginous airways, as a specific case (10, 11). Therefore, the aim of this research is to evaluate the effects of fluoxetine on the bronchial smooth muscles in order to understand its effects in these cases.

In 2017, researchers have shown that there could be a relationship between antipsychotic agents and risk of acute respiratory failure in patients with chronic obstructive pulmonary disease. But the exact mechanism of this relationship is unclear (20). The antidepressants that are used in treatment of depression may lead adverse effects in patients who suffer from respiratory system disorders. Therefore, we hypothesized that fluoxetine may affect ASMCs and change airway tone. The aim of this thesis is to investigate whether fluoxetine affects the tone of bronchial smooth muscle or not?

Fluoxetine commonly is used in treatment of depression and other neuropsychiatric disorders. It is usually well-tolerated. The common side effects of fluoxetine are associated with the gastrointestinal system and the nervous system (21). Fluoxetine treatment caused a significant increase in Na+ K+- ATPase activity in the synaptic plasma membranes of the cerebral cortex compared to control in rats (22). Fluoxetine has favourable safety/efficacy ratio and is considered a drug successfully used in treatment of depression and other neuropsychiatric disorders (23). Sherkawy et al. (24) studied fluoxetine in vivo effects on depression in asthma model and they demonstrated that fluoxetine could have protective effects. Although the several adverse or useful effects of fluoxetine have been explained in different models, previous studies have not focused on the effects of fluoxetine effects on the ASMCs. Finally, the effects of fluoxetine on the ASMCs is not completely demonstrated. Therefore, this thesis has original value.

Fluoxetine acts via blocking the reuptake of serotonin into presynaptic serotonin neurons by blocking the reuptake transporter protein situated in the presynaptic ends. Fluoxetine also has moderate activity at the 5HT2A and 5HT2C receptors. Fluoxetine has minimum of activity on noradrenergic re-uptake. Due to its re-uptake of serotonin, fluoxetine makes an activating effect (25).

In 2003, researchers have shown absorption of serotonin and metabolism via cultured guinea pig(A9) airway smooth muscle cells. The results of this study propose that cultured ASM cells are also capable of collecting and metabolizing serotonin, and elevate the possibility that ASM cells may share in to the inhibition of endogenous serotonin in the lungs (26).

1.2 Study purpose and significance

The main purpose of the research is to test the Fluoxetine on bronchial smooth muscles through a simulative environment inside an organ bath system, after extraction from adult male Sprague-Dawley rats. This research comes to add to the literature on the effects that are imposed on the respiratory system, specifically bronchial smooth muscles. As shown in the subject and purpose section of the proposal form, several studies have tested different chemicals, drugs or pollutants on rats' bronchial smooth muscles; however, none of the studies concerning the side effects of fluoxetine have tested its impacts on the bronchial smooth muscles (21, 22, 23).

Furthermore, providing the necessary data that describe the effects of the drug on the bronchial smooth muscles would be of a great value for medicine development and research, as having an understanding of the matter builds the base to enhance the drug, substitute it or provide a clear precaution for its side effects. Previous studies have shown that fluoxetine has anti-ejaculative effects on vas deferens on rats (16). In another study, researchers observed that fluoxetine has relaxation effect on rat aorta (18). Hence, it can be suggested that fluoxetine may have relaxation effects on the isolated bronchial smooth muscle.

1.3 Research question and method

The literature suggests that the side effects research of Fluoxetine is mainly focused on the general sides effects or the side effects on the nervous system. Few studies have researched the impacts of the drug on other body systems. As this study targets testing the effects of the drug on the respiratory system, opportunities are possible to develop the drug into a manner that eliminates side effects or further awareness can be raised in the medical field for complex cases related to respiratory system disorders and diseases and depression. Utilizing rats in testing drug effects is common in the literature. Altinisik, et al. (5) tested the impacts of $MgSO_4$ on respiratory system smooth muscles using an organ bath system of rats. Sakai, et al. (10) used the same method to test cigarette smoke extract on bronchial smooth muscles. Corboz, et al. (11) used the same targeted muscles from rats to test the impacts of furosemide.

In 2017, researchers said that there could be a relation between antipsychotic and acute respiratory failure (ARF) (20). Therefore, antipsychotics and antidepressants, such as fluoxetine, may have an effect on the bronchial smooth muscle cells.

The thesis hypothesizes a first-time by introducing fluoxetine, which is a medication used as depression treatment, on isolated bronchial smooth muscle. The material and equipment to be used in this experiment are available in the facilities of the university. The only anticipated resource-type that are needed are the Sprague Dawley rats, which can be provided with an approval from the specialized committees. The experiments will take place in the laboratories of the Faculty of Medicine, on department of Physiology, Yeditepe University.

In physiology laboratory, organ bath system is used for research since 2009. It is established on ex vivo experimental protocol. As tissue is dissected from rat, and in order to ensure its survival, it is puts in krebs solution and oxygen. Temperature and pH have been set up on optimum value. System binds to transducer and amplifier for recording. Pharmacological agents will be supplied. Adult male Sprague–Dawley rats, weighing 260–300g, were used in the experiments (Yeditepe University Medical School Experimental Research Centre, YUDETAM). All experimental procedures were approved by the local ethics committee. The experiment divides the animals into groups for comparison and tissue is maintained in a preserved environment in the Krebs solution, which designed as per the material and method part of this thesis. The drug is added in increments. When all data are collected, comparison of frequency and amplitudes of contractions will be calculated (27).

1.4 Thesis structure

The current chapter provided an overview of the research main elements; airway smooth muscles (ASM) and fluoxetine. A brief description of the contractile apparatus was provided, as well as the role of ASM in the respiratory system. Moreover, the first chapter introduced the purpose of the study and its significance on future research and pharmaceutical developments. Through understanding the literature gap, the question of the research is structured, and the expected outcomes are provided. The material and

method used in the experimental part of the research are reviewed with the controlled conditions that are provided to ensure the precision of the study results.

The second chapter performs a literature review for the main topics of the research, including the structure of airway smooth muscles and the studies that described their contractile apparatus. Furthermore, researchers that studied different chemical agents and drugs on airway smooth muscles and similar tissues are reviewed for discussion purposes. Additionally, the effects of fluoxetine on body systems and tissues are reviewed from the literature, as well as studies that focused on ASM and similar tissues. The third chapter describes the material and methods that are used in the experiments. Materials and synthesis methods are described for reliability, in addition to analytical methods that are used to interpret the data. Experiment synthesis observations are also recorded.

The fourth chapter provides the results of the experiments as recorded by the data acquisition unit and computer. Moreover, an interpretation and an analysis for the results are provided. The fifth chapter discusses the results of the experiments along with the findings of the literature review for comparison and conformity. A conclusion for the study is provided summarizing the findings of the literature review, methods and experimental results. Furthermore, recommendations based on the results are provided for related disciplines and future research.

2. LITERATURE REVIEW

2.1 Lung Physiology and Anatomy

The lung is divided into two unidentical parts; the right lung and the left lung. The overall shape of the lung is pyramidical asymmetric connected by the left and right bronchi to the trachea. The right lung is divided into three parts: superior lobe, middle lobe and inferior lobe, while the left lung is divided into two parts: superior lobe and inferior lobe. The lobes, which consists of multiple segments called bronchopulmonary, are separated from each other by the fissures (28). However, the isolation between the lobes is not complete, which allows ventilation between them (29). Moreover, a dent is present at the inner side of the left lung to accommodate the heart: cardiac notch (28). The overall physiology of the lungs is shown in Figure 2.1.

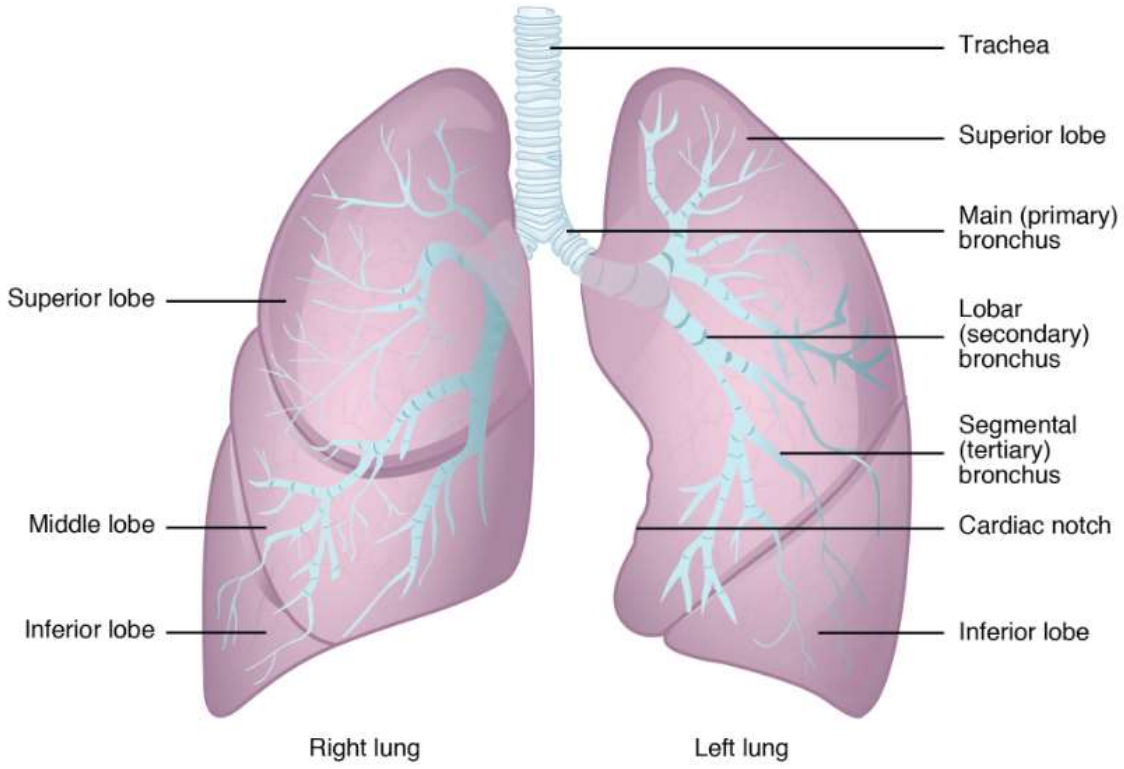


Figure 2.1: Lungs overall physiology (28)

Starting from the trachea down to the air exchanging terminals (alveoli), there are twenty-three generations of bronchioles, as shown in Figure 2.2. The first sixteen generations pass through an anatomic dead space (volume = 150 mL) and called the conducting zone, which does not have alveoli for gas exchange, while the successive bronchioles (also called acinus) are the transitional and respiratory zone that start to be occasionally covered with alveoli at the respiratory bronchioles and fully covered with them the alveolar ducts and alveolar sacs. The respiratory space of the lung forms the majority of the volume of the lung ranging between 2.5 to 3 litres. The third generation of the tracheobronchial airway coming from the main bronchus branches into the lung with 10 branches for the right lung and 8 branches for the left lung (30).

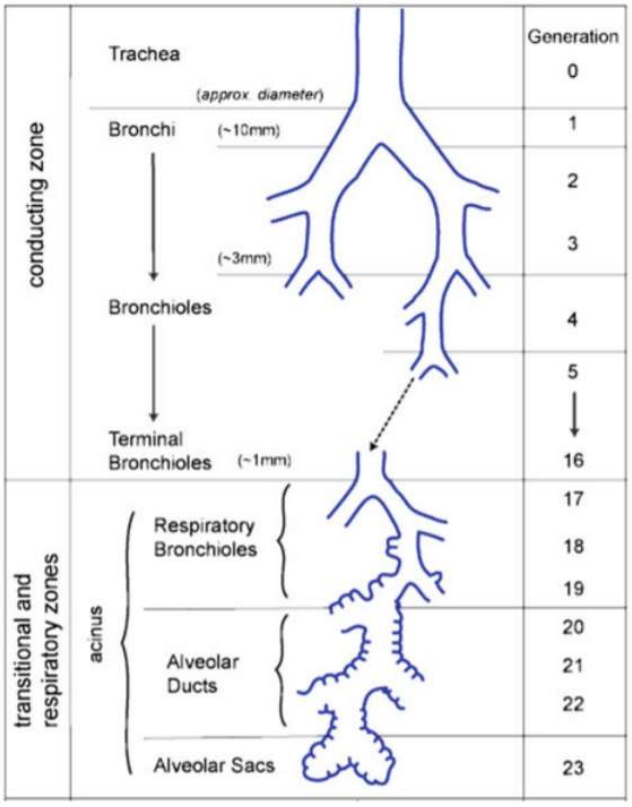


Figure 2.2: Generation of airway in human lungs (Schematic) (30)

The lungs are supplied with blood from the pulmonary circulation in order to perform the main function, which is the of exchange gases between the human body and the environment. The blood supply is carried by the pulmonary artery, which enters the

lungs with the primary bronchi and branches with the bronchioles until it reaches the alveoli (28), as shown in Figure 2.3. The exchange of gases occurs in the alveolus through pulmonary capillary networks with a wall type cells called Type I. Nevertheless, the supply of blood to the lung tissue is performed by the bronchial arteries (systematic blood vessels) entering from the hilum with pulmonary artery, bronchi and nerves. In the thoracic cavity, the lung is surrounded with a double lubricating layer that allows the expansion and recoiling of the lung easily (31). The parietal pleura and visceral pleura surround the lungs, and continues between them, at the fissures and around the heart, with the pleural fluid that provides the surface tension between the two layers (32). Figure 2.4 shows a section of the lungs at the pleural cavity.

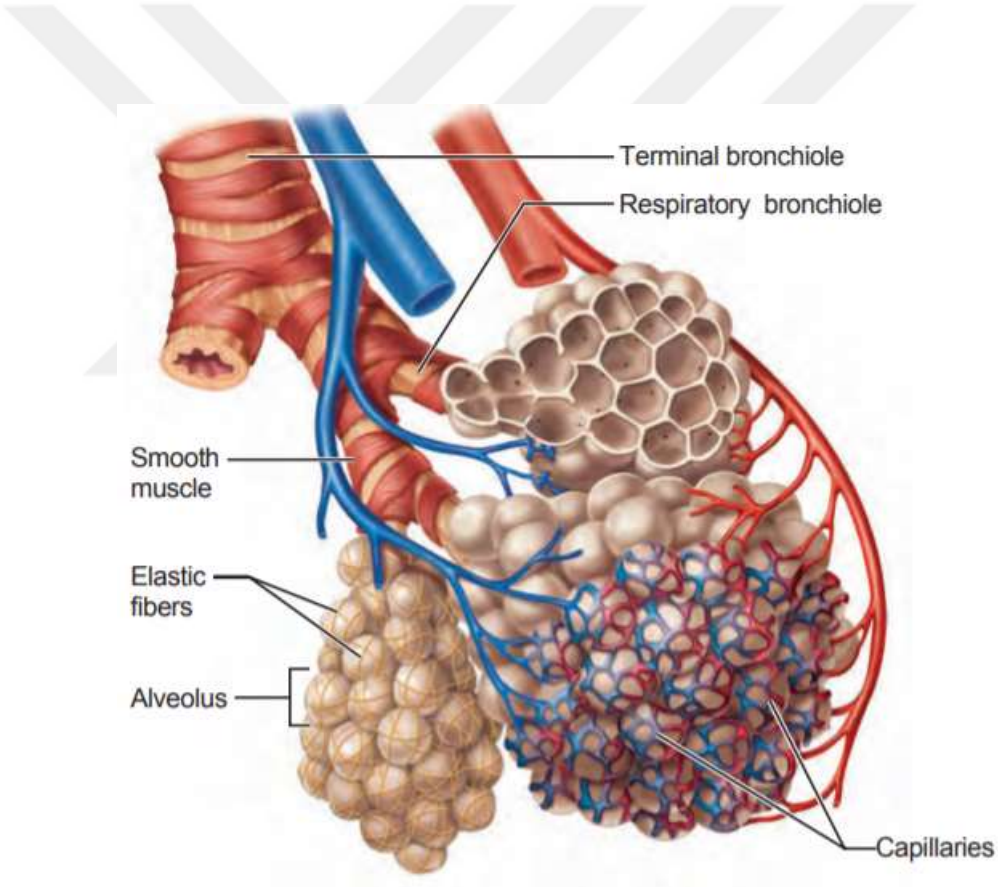


Figure 2.3: Pulmonary artery branching to the alveoli (32)

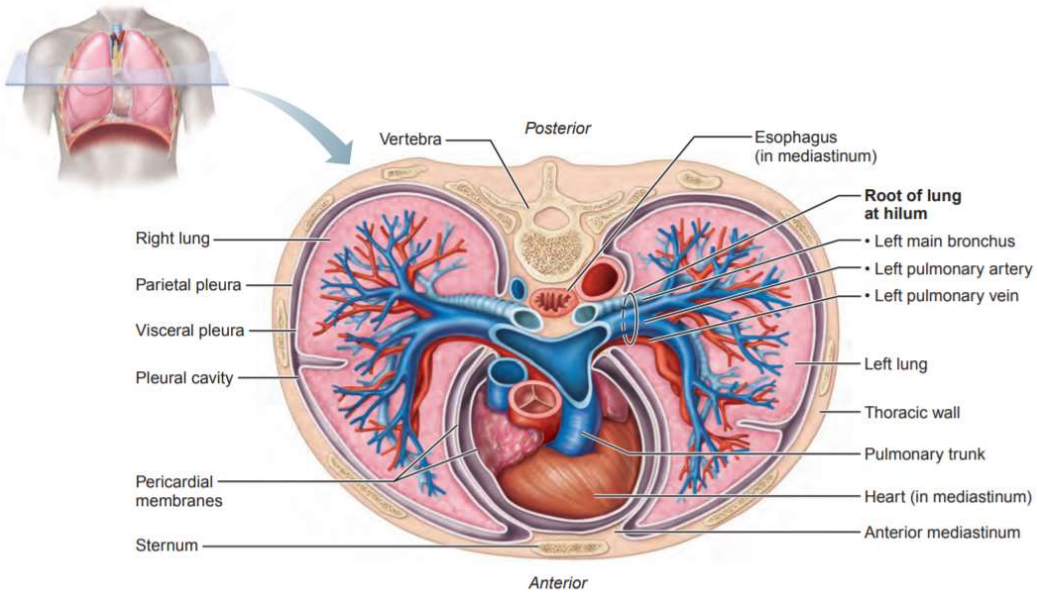


Figure 2.4: A section in thoracic cavity (32)

2.1.1 Primary Bronchi

The main bronchi, also termed as the primary bronchi, extends from the trachea and enter the lungs at the hilum. The two branches are each entering one lung and they are the main passageway for air into them. The main function of the primary bronchi is the distribution of the air that enters from the mouth through the trachea into each of the lungs towards the rest of the tracheobronchial tree (33). Although the primary bronchi are divided at the bottom of the trachea, their shapes are not identical. The right bronchus is shorter and enters the hilum at a more vertical position with a wider diameter, while the left bronchus enters in a more horizontal position at the hilum due to the obstacles imposed by the thoracic aorta and the arch of the aorta (34). The right bronchus branches into three secondary lobar bronchi at the right lung, while the left bronchus branches into two secondary lobar bronchi (35).

The anatomies of the trachea and primary bronchi are similar; however, the c-shaped cartilaginous rings of the trachea come in the form of cartilaginous sheets that are distributed irregularly and followed by bands of smooth muscles along the exterior wall of the primary bronchi (36), as shown in Figure 2.5. Both the cartilaginous sheets and the smooth muscles function at inhalation and exhalation to regulate the bronchi's lumen

diameter. The lumen contains the systematic blood vessels that supply oxygen to the bronchi and reserves the shape of the bronchi through the breathing (37). The luminal inner wall of the bronchi consists of a mucus membrane lining with cilia, hairy projections, to prevent foreign bodies from entering the lungs. The bronchi are connected other tissue of the lung through bands of adventitia (38).

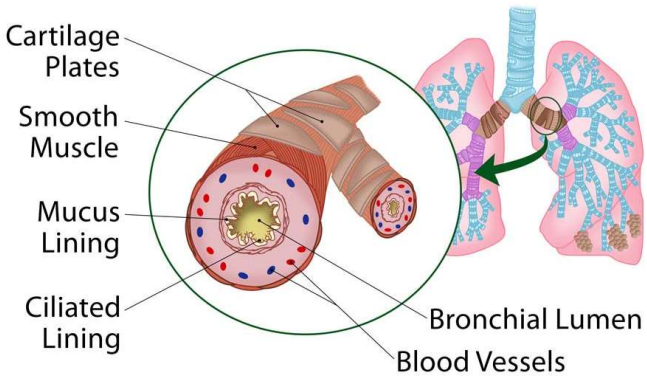


Figure 2.5: Anatomy of primary bronchi (39)

2.2 Smooth Muscles

Smooth muscles are spindle-shaped that are present in several internal organs in the body, where it is controlled by the autonomic nervous system, local metabolites and hormones. The contractility is simulated though receptors that available on the smooth muscle surface (40). Smooth muscles are available on the walls on the bronchi and responsible for its contractile apparatus. This section studies the structure, contractile apparatus and the receptors of the airway smooth muscles.

2.2.1 Development, Function and Structure of Airway Smooth Muscles

Despite the argument that airway smooth muscles (ASMs) are in terms of purpose similar to the vermiform appendix, which is misleadingly claimed of being “an evolutionary vestige” while it has proven immunological functions (41), research shows that the growth and development of ASM during the prenatal stage is essential to avoid several disorders during the postnatal stage, such as asthma and prematurity. There are

distinguished genetic characteristics that lead ASM to develop as the respiratory system becomes apparent in the first few weeks of embryo growth, when the intercellular signalling starts by laminin and Rho A structuring, as well as forming the unique mesenchymal cell shape. The characteristics of ASM, including its cell shape, stretch ability and distribution, is considered critical for lung growth (42). Nonetheless, an extensive review in the literature have compiled ten functions that are attributed to ASM, as follows (1):

1. Peristalsis to facilitate exhalation.
2. Peristalsis to facilitate mucus propulsion.
3. Peristaltic contraction in the prenatal lung to impose fluid pressure.
4. Facilitating lymphatic and venous flow.
5. Ventilation and perfusion matching.
6. Protection of the peripheral lung.
7. Protection of the structure of the airway.
8. Airways stabilization.
9. Improving cough effectiveness.
10. Optimization of anatomic dead space volume.

Therefore, the claim that assumes that airway smooth muscles (ASMs) are without a purpose or apparent beneficial functions are like those claims about the vermiform appendix; they are both not supported and should be discarded. The link between the changes in structure or function of airway smooth muscles and the hyperresponsiveness or asthmatic symptoms were first described systematically in the 1980s. The narrowing in the trachea that is observed in asthmatic attacks is a response to histamine leading to maximal ASM contraction (43). Several studies followed this observation in order to collect data to understand the link in a more elaborative manner. Scientists described the increase in smooth muscle mass as the most important structural change that cause the asthmatic narrowing of the trachea, in addition to the increase in the maximal shortening, velocity of shortening, and the reduction in relaxation and force caused by strain. In normal function, an equilibrium state is achieved through the detaching of myosin heads from the actin; however, in asthmatic cases, this equilibrium is disrupted leading to the frozen state in the contraction (44).

The obliquity of ASM is considered one of the factors that can potentially affect its functionality. In an experimental study and using morphomet-ric approach, Lei et al. measured the angel of orientation of ASM in four cats and one human specimens. The angel was measured for the ASM bundle orientation with reference to the transverse axis of the airway, as shown in Figure 2.6. The findings of the research show that the angel that varied between -20° and +20°, and had an absolute mean value of 13.1° in the human specimen is not expected to impose any physiological changes in the length of the airway during bronchoconstriction (45).

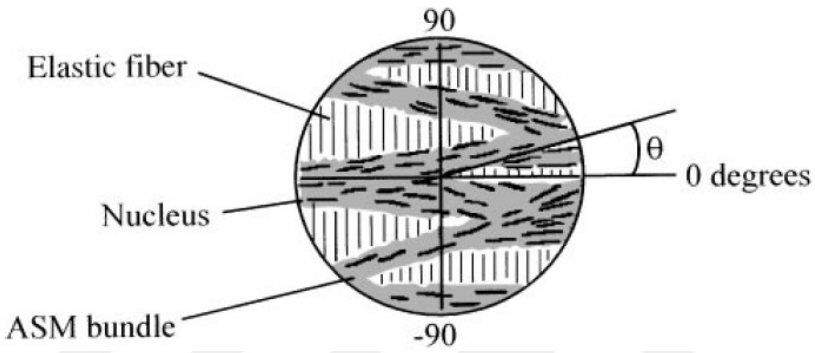


Figure 2.6: Measuring the angel of orientation of ASM bundles with relevance to the transverse axis (45)

2.2.2 Airway Smooth Muscle Contraction Mechanism

Through activating receptors on the cell membrane of the airway smooth muscle, the concentration of cytosolic calcium ions is increased or decreased, activating a certain pattern or response of the contractile apparatus of ASM. The influx of calcium ions is caused by an extracellular influx from the cell membrane through agonists opening L-type voltage operated channels for calcium ions or its release from sarcoplasmic reticulum. The depolarization of the cell membrane is controlled through K+ channels, maintaining an equilibrium potential at -60 mV, and low membrane voltage is induced through an outgoing K+ outgoing current. TRP proteins, TRPC3 specifically, have a major role in increasing the resting membrane potential to be higher than the equilibrium point. The current of K+ passes through its channels, such as KDR and calcium dependant K+ channels, which are activated by the increase of the calcium ion concentration. Several other channels control membrane depolarization as shown in Figure 2.7 (15). The

frequency of calcium ions Ca^{2+} oscillations within the cells determines the contractile status of the ASM (46).

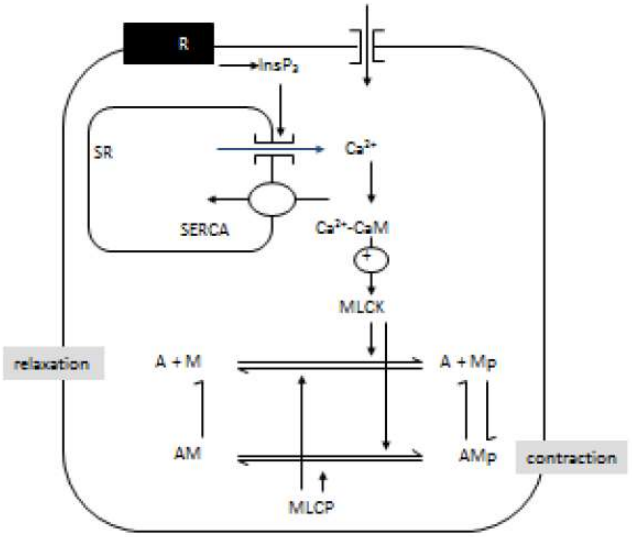


Figure 2.7: Contraction and relaxation of ASM induced through calcium ions and K+ channels (15)

The contractile apparatus of airway smooth muscles is mainly dependent on the cross bridge made mainly of myosin II protein, while the mechanism interacts with filamentous actin (47). The contraction occurs when actin and myosin cross bridges are activated, while myosin light chain is phosphorylated by myosin light chain kinase. The degree of phosphorylation determines the shortening extent and velocity (48). The recognized mechanisms that control ASM contraction and relaxation are shown in Figure 2.8. Ectoenzyme CD38 produces a second messenger (cyclic ADP ribose – cADPR), causing the release of Ca^{2+} from the channels of ryanodine receptor (RyR) in the sarcoplasmic reticulum (SR). G proteins play a major role in the contractile mechanism couples according to the type with their specific receptors, activating phospholipase C (PLC) and breaking up phosphatidyl diphosphate inositol 2 (PIP_2) to inositol triphosphate (IP_3) and diacylglycerol (DAG). Calmodulin (CAM) binds with intracellular Ca^{2+} to change the phosphorylation status of myosin light chain (MLC) in order to regulate the function of ASM (49).

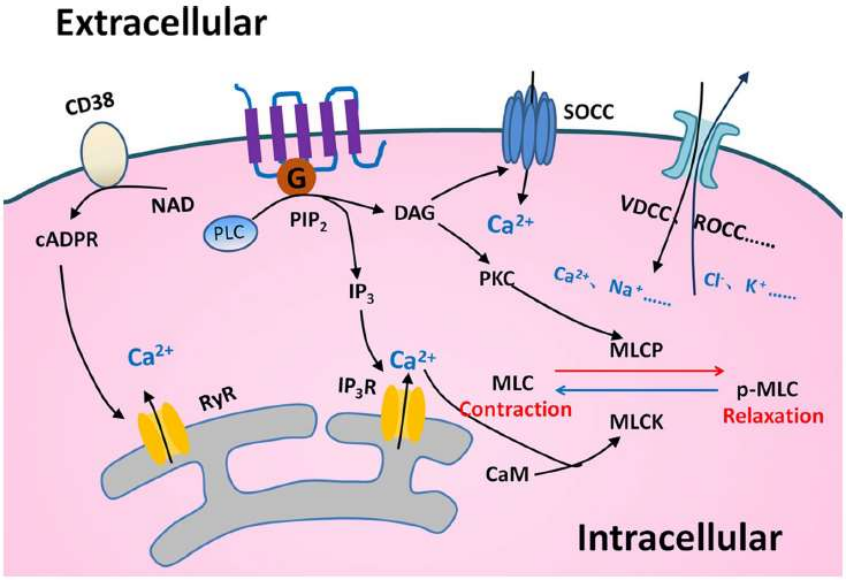


Figure 2.8: Contraction and relaxation mechanisms in ASM (49)

The relaxation mechanism in ASM is performed through cyclic nucleotide second messenger system through direct action on the membrane cell transporters or ion channels, or through the breakdown of cyclic nucleotides. The main action is simulated by cAMP and cGMP, as shown in Figure 2.9. Five mechanisms are described in the figure (2):

1. Through receptors linked to cAMP
2. Through receptors linked to cGMP
3. PDE inhabitation
4. K+ channels activation
5. Ca^{2+} channels inhabitation

Table 2.1 shows the different mechanisms that cause ASM relaxation in vitro and the agents that act in those mechanisms (2).

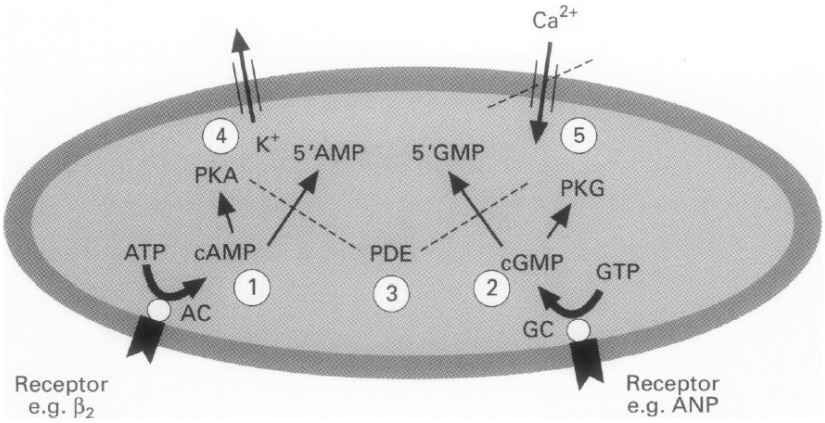


Figure 2.9: Pathways for ASM relaxation (2)

AC: adenylate cyclase; PKA: protein kinase A; GC: guanylate cyclase; PKG: protein kinase G

Table 2.1: ASM relaxation mechanisms and their agents (2)

ASM Relaxation Mechanism	Agents
Simulation of cAMP	B agonists; peptide histidine isoleucine; pituitary adenylate cyclase activating peptide; vasoactive intestinal peptide; PGE_2; PGI_2
Simulation of cGMP	Nitric oxide; sodium nitroprusside, CNP; BNP; ANP; nitrosothiols Nitrates
cAMP inhabitation/ GMP breakdown	Phosphodiesterase inhibitors
Ion channels	Calcium antagonists; sodium channel modulators; Potassium channel activators
Others	Lithium; Anaesthetic agents; Calmodulin antagonists; Protein kinase C inhibitors

The contraction and relaxation of airway smooth muscles is governed by extracellular messengers that activate specific receptors on the cell membrane (15). Barnes (4) shows three main functions of the airway smooth muscle; tone functions through contraction and relaxation, structure functions through proliferation, hypertrophy and transformation; secretion functions through mediators, cytokines, chemokines and

growth factors. The receptors of ASM have a major role in its reactivity as differences in receptors' type concentrations are found between proximal and distal airway cells. Bronchoconstrictors refers to the mediators and neurotransmitters that simulate ASM in the contraction, where the main contractile function of ASM is often facilitated through the increase and decrease in calcium ions in the intracellular region. Therefore, these agents play a major role in determining the tone of ASM through their direct and indirect effects. Table 2.2 shows the body produced bronchoconstrictors of ASM and their receptors. The action of bronchoconstrictors vary between a direct action and indirect action caused their release from inflammatory nerves and cells. Moreover, there are other agents that act as bronchodilators, which are responsible for the dilation of ASM and decreasing the resistance of the airway (3). These substances act through several receptors and a second messenger that is generally cAMP (4), as shown in Table 2.3.

Table 2.2: Mediators and neurotransmitters (bronchoconstrictors) and their receptors (4)

Mediator	Receptor	Neurotransmitter	Receptor
Histamine	H_1	Acetylcholine	M_3 (M_2)
Cys leukotrienes	Cys LT_1	Substance P	NK_2
Thromboxane	TP	Neurokinin A	NK_2
Prostaglandin D_2	TP (DP)	CGRP	CGRP1/2
Prostaglandin $F_{2\alpha}$	TP (FP)	Neuropeptide Y	Y_2
Isoprostanes	TP	Cholecystokinin	CCK_A
Platelet-activating factor	PAF	Bombesin/ GRP	BN
Serotonin	$5HT_2$		
Bradykinin	B_2		
Endothelin-1	ET_B		
Adenosine	A_{2b}		
Angiotensin II	AT-1		

Table 2.3: Bronchodilators of the respiratory system (4)

Bronchodilator	Receptor
Epinephrine	β_2-Adrenergic
Vasoactive intestinal peptide	PVR1
PACAP	PVR1
Prostaglandin E_2	EP
Prostacyclin	IP
Adrenomedullin	AM
Atrial natriuretic peptide	Guanylyl cyclase-A
Nitric oxide	Guanylyl cyclase (soluble)

It is considered critical to understand the conjunction between the contractile apparatus of the airway smooth muscle and the agents that simulate different receptors on their membrane, especially for asthmatic cases. Both bronchoconstrictors and bronchodilators signal pathways that control the tone of the ASM, which can be complex leading to other effects on the cell other than the contraction and the relaxation effects through Ca^{2+} oscillations (50). In a study performed to simulate the relation effect of three agents on the relaxation of ASM; isoproterenol ISO, forskolin FSK and 8 bromo cAMP, the three agents that are expected to elevate cAMP simulated a decrease in Ca^{2+} oscillations and subsequently relaxation of ASM, where the increase in concentration have induced further relaxation effect. By adding bronchoconstrictors; ionomycin, and Ca^{2+}- activated K^+ channels' blocker; iberiotoxin, the effect of elevated cAMP was not reversed (46).

The signalling and crosstalk of agents can be very complex within ASM, as some of them may not have a direct effect on the contractile apparatus but affect other receptors different agonists. In an experiment that examined signalling prostanoid-EP_1 receptor through prostaglandin E_2 agonist, there were no results from the signalling of the receptor on the contraction of ASM. Nonetheless, the activation of EP_1 receptor decreased the dilation effect of β_2-AR through its modification (51).

The understanding of the contractile apparatus of airway smooth muscles is observed through the interaction between the epithelial cells and ASM cells. The epithelium is a continuously renewed tissue of the respiratory system, which gives it the dynamic characteristic (52). G protein with C-terminal homology simulate Gs or cause

the inhabitation of the enzyme Gi. Therefore, vasoactive intestinal peptide (VIP) and beta-adrenoceptors simulate adenylate cyclase in ASM to increase cAMP causing relaxation, while acetylcholine simulate it to decrease cAMP causing constriction (53). Several factors have been identified as relaxation or contraction agents from the epithelium; PGs, Nitrite Oxide, ATP and endothelins, where PGs are the most important drivers. PGs have a major role in modulating the tone of ASM with different types; PGD, PGI, PGE, PGF and TX, which the ASM has specific receptors for each one of them, which are D prostanoid (DP), I prostanoid (IP), E prostanoid (EP), F prostanoid (FP) and T prostanoid (TP), respectively. The EP receptor has different four types depending on their signalling and action. As shown in Figure 2.10, the increase of cAMP concentration is derived by the DP, IP, EP_4, EP_2 and EP_3 receptors causing relaxation in the smooth muscle, while the increase in Ca^{2+} concentration is derived by EP_3, EP_1, FP and TP receptors causing contraction (54).

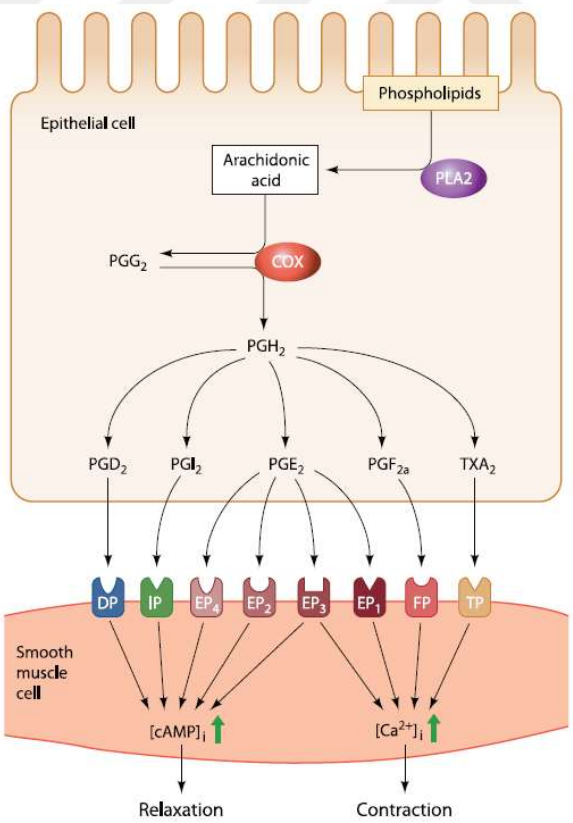


Figure 2.10: Regulation of ASM relaxation and contraction through different receptors by PGs from the epithelium (54)

5-hydroxytryptamine (5-HT) receptor is a serotonin inhabited type that causes contraction of ASM in several mammalian species; however, in humans the inhibition of the receptor causes contraction followed by relaxation (55). The contraction effect by simulating 5-HT receptors was proven in vitro by Dupont et al. through its 5-HT3 and 5-HT4 subtype receptors (56).

2.3 Antidepressants

Antidepressants are defined as a type of drugs that alter the chemical imbalance in the brain in order to treat the symptoms of anxiety and depression (57). These chemicals act on neurotransmitters in order to signal pathways (58). Neurotransmitters are chemicals that are endogenous to the body, which transmit signals through the synapse from the source neuron to the target neuron. Depression occurs through the lack of production of neurotransmitters, such as dopamine, melatonin and serotonin, which affects the emotions and behaviour of the patient. Therefore, antidepressants simulate or block the production of neurotransmitters in the brain that facilitates reuptakes in parts of the nervous system (57, 59).

2.3.1 Classification of Antidepressants

The mechanism of antidepressants is similar with few differences. However, all antidepressants target an effect on the brain through different neurotransmitters. Antidepressants are classified according to their mechanism of action into seven common categories (57):

- Serotonin and norepinephrine reuptake inhibitors (SNRIs)
- Selective serotonin reuptake inhibitors (SSRIs)
- Monoamine oxidase inhibitors (MAOIs)
- Serotonin receptor modulators (SRMs)
- Tetracyclic antidepressants (TTCAs)
- Tricyclic antidepressants (TCAs)
- Lithium salts (LSs)

Table 2.4 shows the most commonly used antidepressant, their classification, therapeutic index and toxicity in the case of overdosage.

Table 2.4: Classification, therapeutic index and toxicity of the most commonly used antidepressants (60)

Antidepressant	Mechanism/ Classification	Therapeutic Index	Overdosage Toxicity
Clomipramine	SNRI	Narrow	Moderate
Trimipramine	SNRI	Narrow	High
Amitriptyline	SNRI	Narrow	High
Nortriptyline	SNRI	Narrow	High
Maprotiline	SNRI	Narrow	Unidentified
Fluoxetine	SSRI	Wide	Low
Citalopram	SSRI	Wide	Moderate
Paroxetine	SSRI	Wide	Low
Sertraline	SSRI	Wide	Low
Fluvoxamine	SSRI	Wide	Low
Escitalopram	SSRI	Wide	Low
Moclobemide	MAOI	Wide	High

2.3.2 Fluoxetine

Fluoxetine is a compound developed by Eli Lilly in the mid-1980s in order to treat major depression disorder (MDD) and it is classified as a selective serotonin reuptake inhibitor (SSRI). After FDA approval in 1987, the drug was given a trade name of Prozac (17). The chemical is a racemic molecule with enantiomers R(-) and S(+) having equal potency to inhibit 5-HT receptors in vitro and vivo. The chemical structure of the compound is (p-trifluotomethylphenoxy)-N-methyl-3-phenylpropylamine, as shown in Figure 2.11. The metabolism of fluoxetine is performed by N-demethylation into the active metabolite of norfluoxetine, which is an SSRI compound with 20-fold stronger potency (61).

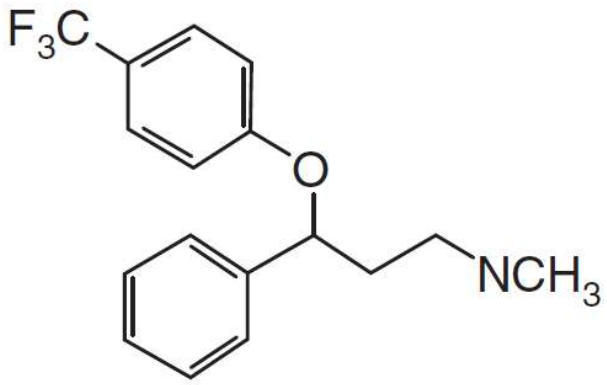


Figure 2.11: Fluoxetine chemical structure (61)

In studying the effect of fluoxetine on airway tissues in vitro, the samples were taken from human subjects undergoing surgery. None of the donors exhibited any symptoms of asthma. The main aim of the experiment was to evoke contraction in the tissue by adding drugs to simulate 5-HT type receptors. Organ bath prepared with krebs solution was used for the experiment, while samples were kept in krebs solution at 4 °C. Several 5-HT-evoking drugs were used. The results show that the contractile response increased with the increase of the drugs' concentrations, except for fluoxetine (56).

Another experiment tested fluoxetine on skeletal muscle tissue to inhibit serotonin receptors. Increasing the drug concentrations on the isolated skeletal muscle arterioles dilated them up to 160 μm, as shown in Figure 2.12. Activating the K^+ channels by adding 4-AP after adding fluoxetine did not have any effect on the response to the drug. When Ca^{2+} was added constrictions were observed on the tissue and continued to increase by increasing its concentration. The results of the research suggest that fluoxetine inhibited Ca^{2+} channels, which interfered with their signalling (62).

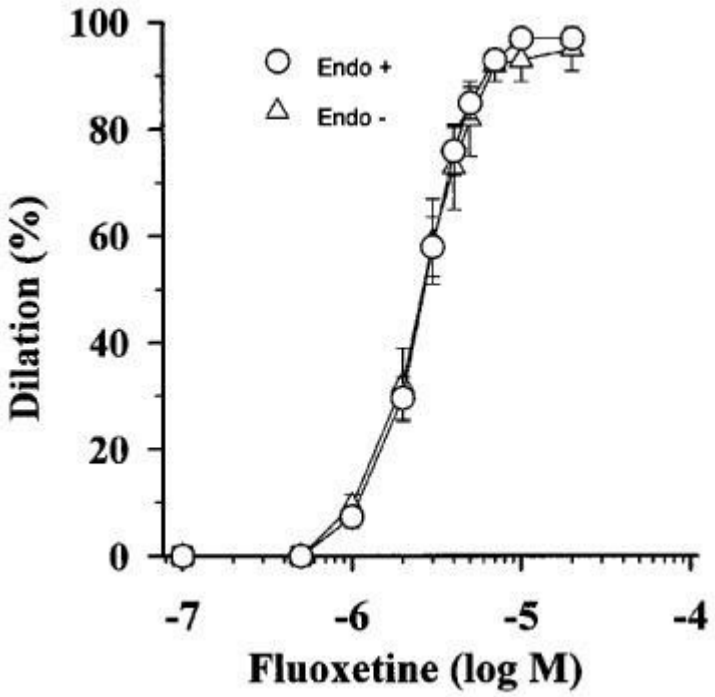


Figure 2.12: Increase in diameter in skeletal tissue with the increase of fluoxetine concentration (62)

Experimental research shows that fluoxetine targets mainly 5-HT_{2B} receptors through exhibiting SSRI response. Nonetheless, this effect was not found in mice whose genetics were modified to knock-out the receptor's gene in mice. Moreover, results show that fluoxetine targets all 5-HT receptors, including 5-HT_3 (63). Through a literature review, the use of fluoxetine to treat depression was found to induce respiratory system side effects in eight researches, which shows the potential of affecting 5-HT receptors in the system. The side effects were mainly cough and dyspnoea in periods ranging between a few weeks and 20 years (64). The risks for lung disease by SSRIs were found to be greater for elderly population (65).

2.4 Organ Bath System

The history of organ bath systems extends to the 1880s, when the first experiment was conducted on a dog's heart and lungs. In the past 140 years, several systems have emerged in order to enhance the accuracy and yields of the experiments. The organ bath system is an in vitro model classified as a micro-physiological system (MPS). The development of the system was motivated by increasing the speed, safety and efficiency

of the development and testing of pharmaceuticals. Furthermore, the aim of the system is to identify, characterise and neutralise the effect of chemicals and toxins on humans. The organ bath system is a construct that simulates the effect of a chemical or drug on an organ or tissue in a controlled environment that approximate the body conditions in order to maintain the integrity of the muscle tissue for several hours (66).

The organ bath system consists of a main chamber filled with a physiological solution that circulates from the bottom to the top (Figure 2.13). The tissue is placed on a hook near a supply of gas (95% Oxygen and 5% carbon dioxide). A second hook is fixed to the tissue with a line connected to the force transducer, which is connected to a machine for records of the changes in the tissue through its movement (67).

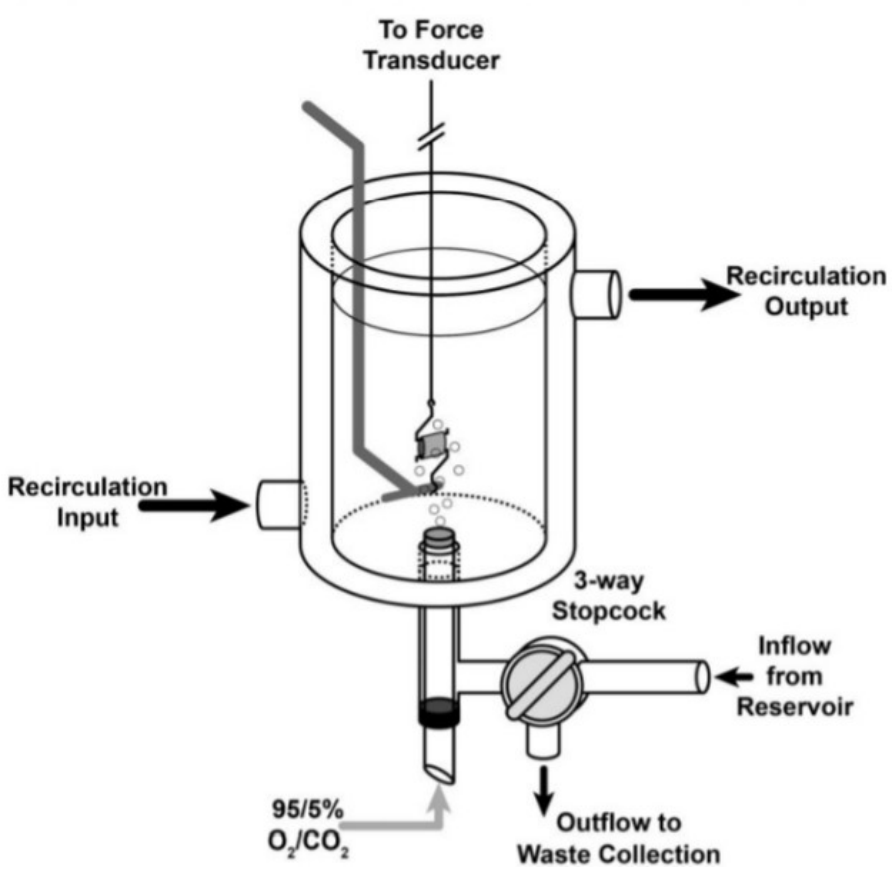


Figure 2.13: Organ bath system (67)

The preparation of the organ bath system starts with the preparation of the solution, which is generally 4 litres of water with a recipe of physiological salts and

calcium in order to prepare a physiological salt solution (PSS). The system is preheated to 37° through the water circulation until the system reaches equilibrium. The force transducer and the data acquisition system are switched on. The system is connected to medical gas of 95% Oxygen and 5% carbon dioxide, and system is pressurized after checking for leaks. The system is primed, and air bubbles are removed in order not to move the tissue and affect the readings. The aeration of the solution oxygenates the physiological salt solution buffer and gives Brownian motion for drug distribution. Once the system is ready, the prepared tissue is tied to the peg positioned on the stainless-steel rod with a silk suture and placed into the organ bath chamber. Passive tension is placed on the tissue until the tissue reaches plateau. The system is equilibrated for an hour after applying the passive tension and during this period the tissue is washed every 15 to 20 minutes. Thereafter, the PSS is replaced with another warmed one. The agonist, drug or chemical tested in the experiment is added with certain concentration, the contraction in the tissue is observed to its maximum and awaited until it reaches plateau. Different concentrations can be tested, and readings are recorded at the data acquisition system (67).

3. MATERIALS AND METHODS

3.1 Animals

Adult male Sprague Dawley rats (250-300g) were used in this study which were provided by Yeditepe University Experimental Research Centre. Experimental procedures were approved by Yeditepe University local ethic committee all animals were kept under optimized conditions as 12 hours light/dark cycle, temperature (22±1°C), free access to food and water (68, 69). All animals were randomly divided into 3 groups as follows; fluoxetine (Fluoxetine; n=5) (spontaneous contraction), fluoxetine with acetylcholine induced contraction (ACh+fluoxetine; n=5) and ACh induced group that refers to control (ACh; n=5).

3.2 Chemicals

Krebs-Henseleit solution was made of D+glucose (Riedel de Haen 16301; 1.98 g/L), NaCl (Sigma 746398; 8.88 g/L), KCl (Sigma P9541; 3.50 g/L), KH_2PO_4 (Sigma 60220; 0.136 g/L), $MgSO_4$ (Santa Cruz sc211764; 0.144 g/L), $NaHCO_3$ (S 5761; 1.26 g/L), $CaCl_2$ (Merck 102378.0500; 0.294 g/L). Acetylcholine (Sigma A2661; 10µM) was used to induce smooth muscle contraction (70). Fluoxetine (Prozac; 20 mg) was dissolved in water as 1 M. Final concentrations of fluoxetine was made up in Krebs solution. Serial dilution was made up from 10^{-1} M to 10^{-9} M. The serial dilution of Krebs solution is used for Ach induced group (27).

3.3 Preparation and Setup of System

Five litres of Krebs-Henseleit solution were prepared according to the recipe (Table 3.1). Reservoir Krebs-Henseleit solution was prepared in advance for usage after tissue washing and kept in a warmed reservoir. Organ bath system (Figure 3.1) was preheated through operating the heated water bath circulation until temperature reaches 37º C.

Table 3.1: Krebs-Henseleit solution recipe (pH 7.4)

Salt	mM
NaCl	118.0
KCl	4.7
$CaCl_2$	2.5
$MgCl_2$	1.2
$NaHCO_3$	25.0
KH_2PO_4	1.2
Glucose	10.0

Figure 3.1: Organ bath system used in experiment

Circulation direction was ensured to be flowing from the lowest barbed connection to the highest and connections between components were checked. Data acquisition system and force transducers were switched on at least 15 minutes prior commencing the experiment for temperature equilibration. Connection between data acquisition system and data acquisition software (Figure 3.2) was checked. Data acquisition software was launched.

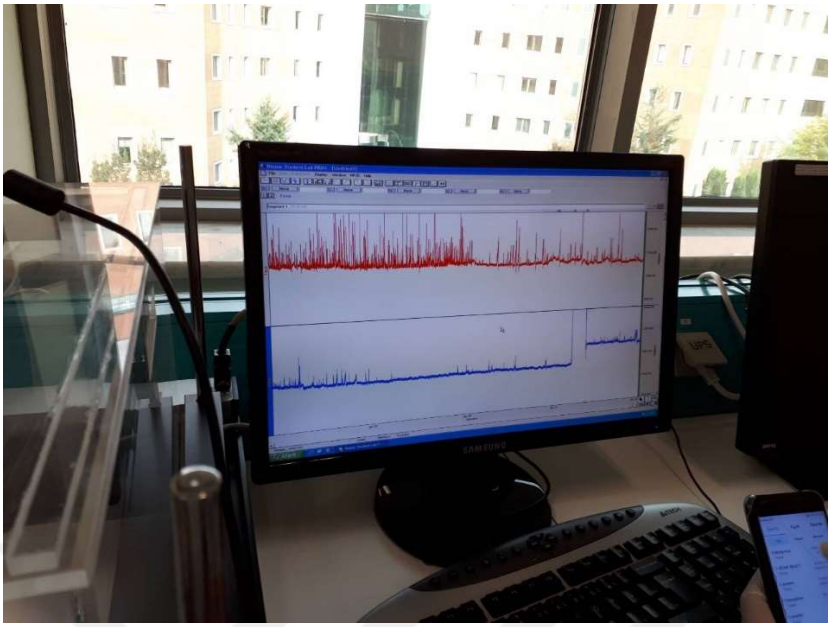


Figure 3.2: Data acquisition system operating the software creating a kymograph

Calibration of force transducers was checked prior tissue placement and prior commencement of data recording according to manufacturer instructions. A medical grade gas cylinder (95% O_2 and 5% CO_2) was connected to the system. Organ bath was checked for leaks and then system was pressurized. Organ bath system was filled with Krebs-Henseleit solution and circulation was kept running until solution reaches optimal temperature. System was primed and air bubbles were removed from the system and its tubing. Solution aeration was checked and adjusted to avoid tissue movements by the aeration or the bubbles, and to provide a Brownian motion movement (45).

3.4 Preparation of Airway Smooth Muscle

All animals were decapitated under anaesthesia. The primary bronchi were dissected. Small ring bronchial smooth muscle tissue strips (1 cm long) were prepared (Figure 3.3). Bronchial circular strips were placed in 20 ml jacketed organ cuvettes. Tissue was hanged from its top to the hook connected to force displacement transducer and the bottom was mixed hooked within the organ bath cuvette (Biopac Systems Inc. Organ Bath, Fully modular organ bath system). The organ cuvette contained Krebs

solution, adjusted pH 7.4 at 37 ºC and solution was aired with mixed 95% oxygen and 5% carbon dioxide during experiment protocol (the setup of the experiment after tissue placement is illustrated in Figure 3.4 for one of the specimens) (71). The organ cuvette was washed every twenty minutes with its solution. After equilibration period, bronchial strips were stabilized to 1.5 g stretch tension. Contraction activity was recorded with a fully modular organ bath system (Biopack Systems Inc. Turkey, Model MP35 with amplifier). Acetylcholine (10µM) was added in ACh and ACh+fluoxetine group circular strips. When acetylcholine saturation was reached, the lowest dose of fluoxetine was added. Every 15 minutes, new concentration was added to fluoxetine and ACh+fluoxetine groups. Finally, when all data are collected, mean contraction frequencies were compared (5, 72).

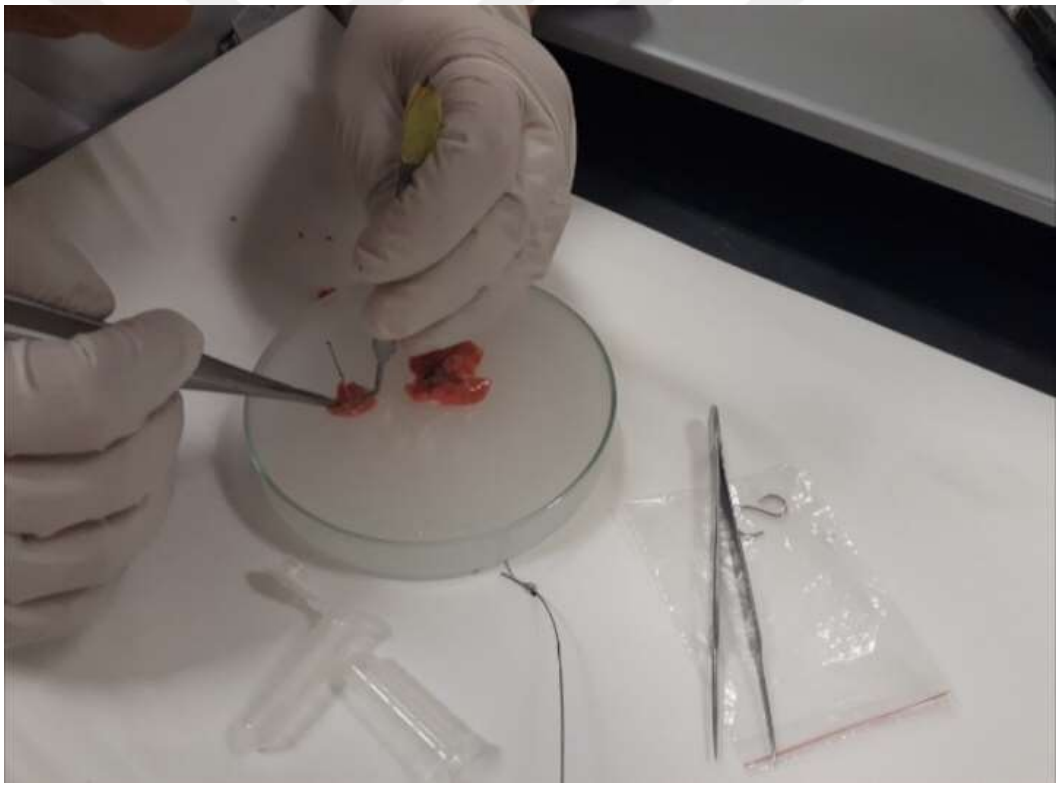


Figure 3.3: Hook placement on the tissue

Figure 3.4: Experiment setup for one specimen after tissue placement

3.5 Placement of Tissue in the System and Equilibrium

Prepared organ bath system was filled with warm Krebs-Henseleit solution and allowed to reach the required temperature. One end of the silk suture with the hooked tissue was tied to the peg stainless-steel rod and placed into the chamber of the system. After ensuring that the tissue was fully submersed into the Krebs-Henseleit solution, the rod was connected to a stand to stabilize it. The other silk suture end was tied to the force transducer with a slack in the suture. Tension was increased on the tissue to 2 grams and time was allowed for the tissue to reach plateau (passive tension). System was left for 60 minutes to reach equilibrium. Tissue was washed every 15 minutes, then system was drained from its Krebs-Henseleit solution and a new buffer was added from a warmed reservoir (5, 73).

3.6 Drug Preparation and the Experiment

Two grams of fluoxetine hydrochloride (fluoxetine HCl) were dissolved in 20 ml of distilled water. Then, nine different concentrations were prepared from 10^{-1} to 10^{-9} M. 30 minutes after adding acetylcholine to the first and third specimen, the lowest concentration of fluoxetine HCl was added to the three specimens. Contraction was recorded through the force transducer on the data acquisition software. Contraction was recorded within 5 minutes after addition of fluoxetine HCl to the 3 specimens. If contraction occurred, tissue was awaited to reach threshold and return to normal contractile behaviour. After checking the readiness of the tissue, the next concentration of fluoxetine HCl is added to the 3 specimens with an increase of 10^{-1} each time. Data recorded on data acquisition software are saved and data is analysed as presented in the next chapter.

3.7 Statistics

GraphPad Prism 8 was used for statistical analysis. Groups were compared by using either Two-Way ANOVA followed by TUKEY test within each concentration. All values were expressed as mean ± standard deviation (STD). $p<0.05$ were considered as statistically significant.

4. RESULTS

In this research, the effect of fluoxetine in the airway smooth muscle was investigated by using an organ bath system. This chapter provides the analysis of the results that were recorded on the data acquisition system.

4.1 Contraction Responses of Bronchial Smooth Muscle

Spontaneous contractile activity was recorded for nearly 3 hours with a fully modular organ bath system (Biopack Systems Inc. Turkey, Model MP35 with amplifier). Finally, when all data was collected, comparison of contractions were calculated according to mean values. Experiments were conducted for three different groups.

There was a significant difference between only fluoxetine treated group and only acetylcholine group, which was used as control. Initially, the contraction was evaluated according to the tension response. A significant difference was found between Ach group and fluoxetine group at concentrations of 10^{-9},10^{-8},10^{-6} and 10^{-5} (* $p < 0.05$) (Figure 4.1). Distribution graph is presented in Figure 4.3.

Secondly, all data were normalized according to baseline value. When normalized data were compared, there was a significant difference between Ach and fluoxetine group (* $p < 0.05$; ** $p < 0.01$) (Figure 4.2), and distribution graph (Figure 4.4) was made according to these data.

Finally, the comparison table, which is calculated by taking the difference according to baseline value, is provided in Table 4.1. However, there was no significant difference between acetylcholine + fluoxetine group and other groups. All electrophysiological recordings were converted to numerical data, their mean values were analysed statistical in GraphPad Prism 8. Tension values of all groups were analysed with Two-Way ANOVA test. According to results, p values that was under 0.05 was accepted as significant result.

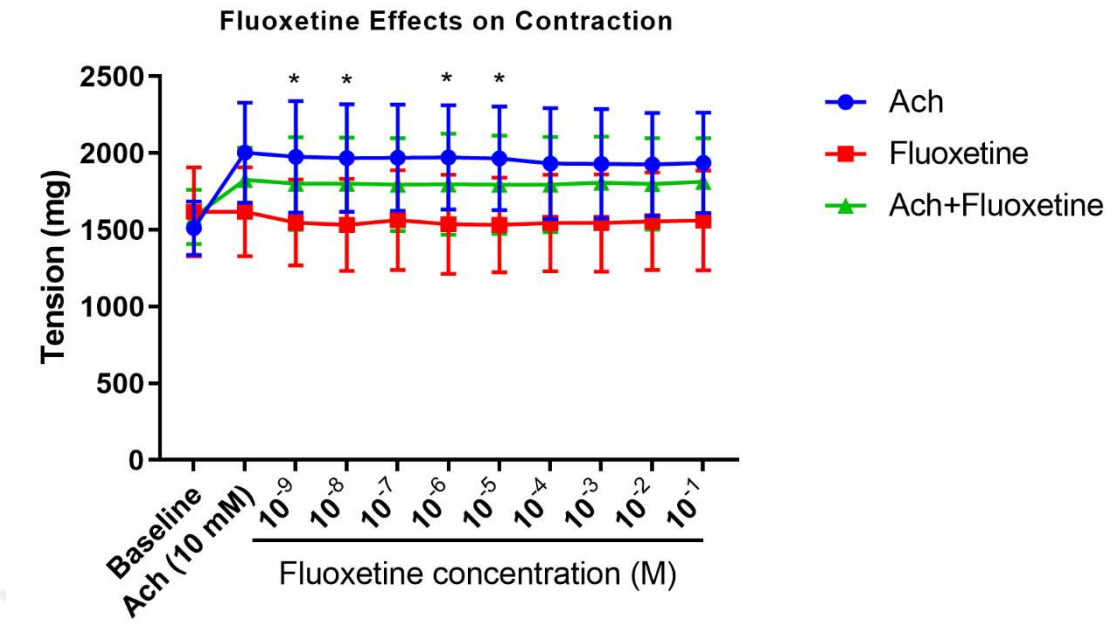


Figure 4.1: Fluoxetine Effects on Contraction Responses. Tension response of bronchial smooth muscle. The concentrations found to differ between the Ach and fluoxetine groups are: 10^{-9}, 10^{-8}, 10^{-6} and 10^{-5}. According to these results, while Ach caused contraction, fluoxetine does not generate contraction (* $p< 0.05$). Data are presented as mean ± SD (n=6 in each group).

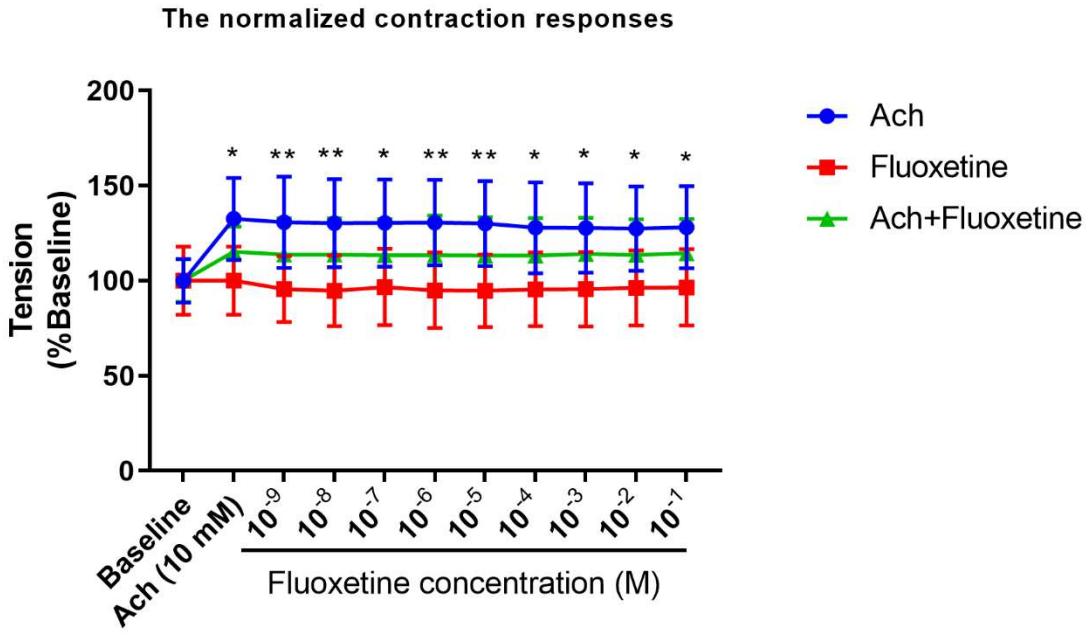


Figure 4.2: The normalized contraction responses. All data were normalized according to baseline value. According to these results, while Ach caused contraction, fluoxetine does not generate contraction. Significant changes were in the following concentrations; 10^{-9}, 10^{-8}, 10^{-6} and 10^{-5} (** $p<0.01$); Ach; 10^{-7}, 10^{-4} 10^{-3}, 10^{-2} and 10^{-1} (* $p< 0.05$). Data are presented as mean ± SD (n=6 in each group).

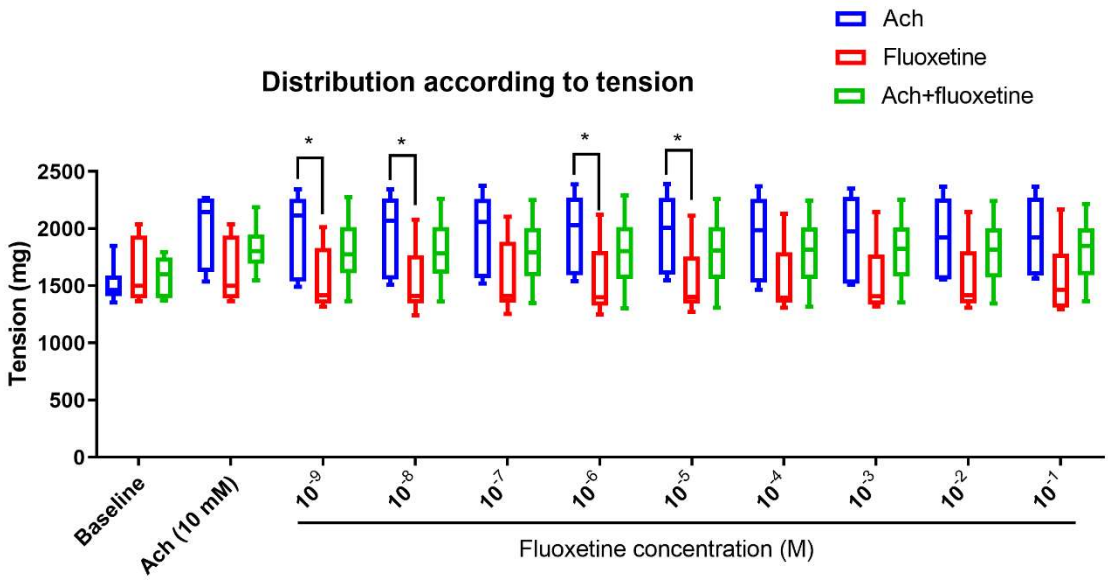


Figure 4.3: Distribution graph according to tension responses. Significant differences were found between Ach and fluoxetine groups (* $p<0.05$) at 10^{-5}, 10^{-6}, 10^{-8} and 10^{-9}. Data are presented as median ± (min-max) (n=6 in each group).

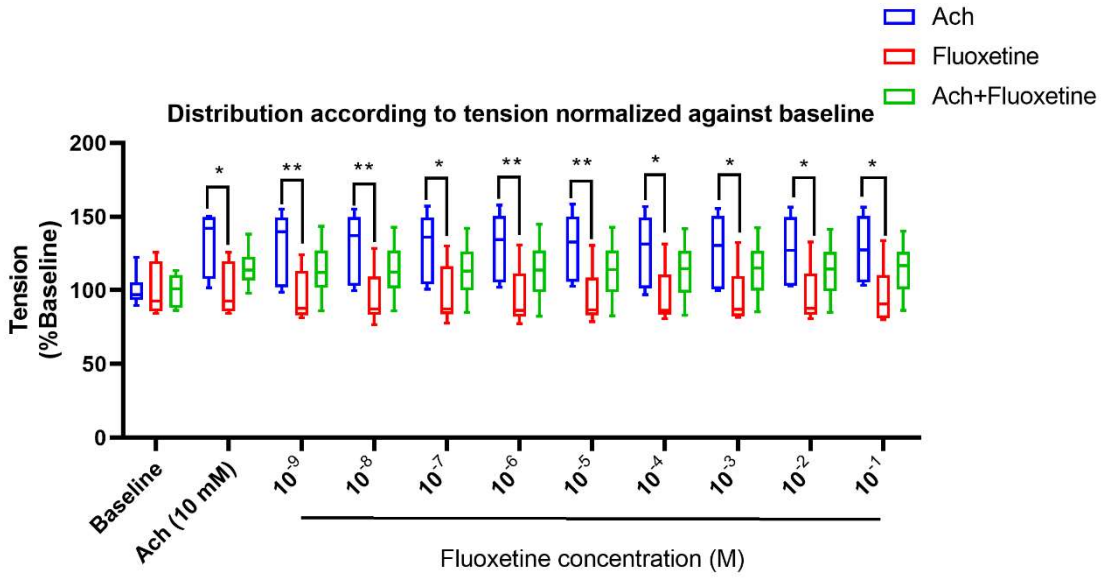
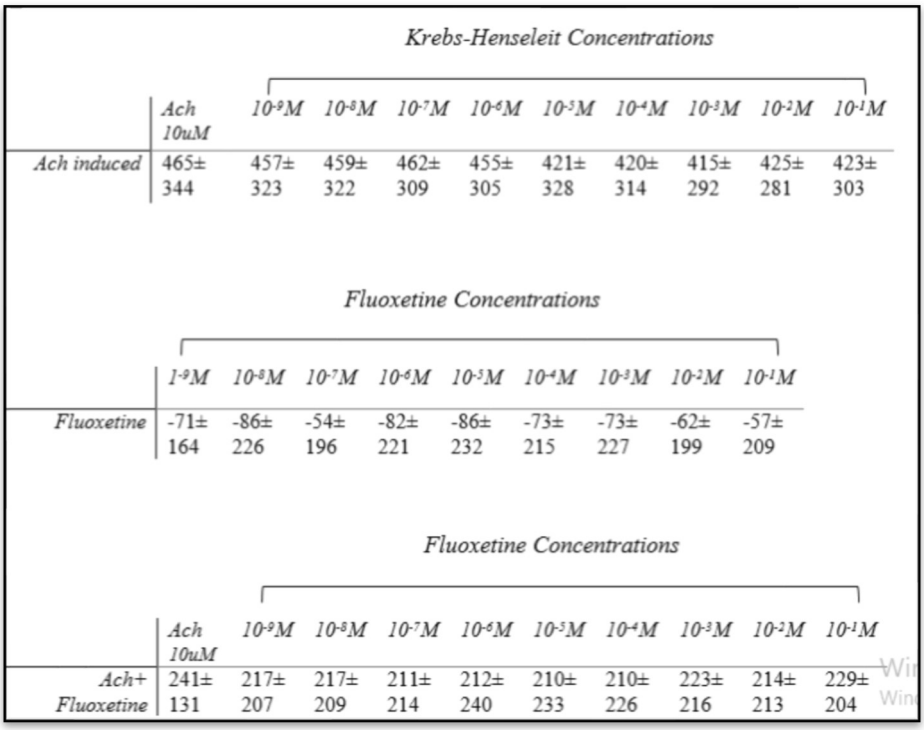


Figure 4.4: Distribution graph according to normalized tension responses. Significant differences were found between all Ach and fluoxetine groups (* $p<0.05$; ** $p<0.01$). Data are presented as median ± (min-max) (n=6 in each group).

Table 4.1: Differences in tension responses relative to baseline of each group



Krebs-Henseleit Concentrations

	Ach 10uM	*10^{-9}M*	*10^{-8}M*	*10^{-7}M*	*10^{-6}M*	*10^{-5}M*	*10^{-4}M*	*10^{-3}M*	*10^{-2}M*	*10^{-1}M*
Ach induced	465± 344	457± 323	459± 322	462± 309	455± 305	421± 328	420± 314	415± 292	425± 281	423± 303

Fluoxetine Concentrations

	1^{-9}M	*10^{-8}M*	*10^{-7}M*	*10^{-6}M*	*10^{-5}M*	*10^{-4}M*	*10^{-3}M*	*10^{-2}M*	*10^{-1}M*
Fluoxetine	-71± 164	-86± 226	-54± 196	-82± 221	-86± 232	-73± 215	-73± 227	-62± 199	-57± 209

Fluoxetine Concentrations

	Ach 10uM	*10^{-9}M*	*10^{-8}M*	*10^{-7}M*	*10^{-6}M*	*10^{-5}M*	*10^{-4}M*	*10^{-3}M*	*10^{-2}M*	*10^{-1}M*
Ach+ Fluoxetine	241± 131	217± 207	217± 209	211± 214	212± 240	210± 233	210± 226	223± 216	214± 213	229± 204

5. DISCUSSION AND CONCLUSION

Airway smooth muscles are located in the respiratory system in the trachea until the beginning of the terminal bronchioles as a layer around the airway. ASMs are responsible for the contraction and relaxation of the airway in order to provide mechanical stability during ventilation and perfusion. The contraction of ASMs is simulated through the activation of actin and myosin bridges as the myosin light chain is phosphorylated by myosin light chain kinase (5). The velocity and extent of the contraction force is determined by the degree of phosphorylation. Messengers initiate contraction and relaxation, which either causes the muscle to contract through the release of Ca^{2+} or relaxation through cAMP receptors, cGMP receptors, PDE inhabitation, activation of K^{+} channels or activation of Ca^{2+} channels. The relaxation of ASMCs occurs when β2 receptors are signalled. Therefore, β2-agonists that also act on the nervous system can also cause the relaxation of ASMCs (6).

Several experiments were performed in the organ bath systems in order to study the reaction of smooth muscles and tissues to different agents, chemicals or compounds. Diaz-Martin, et al. (2015) applied a methodology of organ bath in order to measure contractions in blood vessel rings (74). Undale, et al. (2012) used an organ bath to prove that alternative animals can be used along with an organ bath for medical and pharmaceutical experiments in order to measure the contractile of tissues (75). Abdur Rahman, et al. (2017) tested the effect of phylanodiflora on smooth muscles using an organ bath system in order to investigate its relaxant or contractile effects (76). The same authors used organ bath system recently to investigate the relaxation effects of herbal medicine (Ailanthus altissima), or heavy metal toxicity on smooth muscles (77). Koç et al. (2008) demonstrated that cadmium (Cd) toxicity reduced contractility of duodenal muscle by an organ bath system (68).

Due to the complexity of their contractile mechanism, it is important to understand the impacts of ASMCs' behaviour and reaction to different agents and chemicals. ASMs are part of the main mechanism that cause the Asthma and COPD reactions (7). There is no clear evidence on a negative relationship between β2-adrenergic agonists and the functionality of airway smooth muscles; however, due to the abnormal behaviour of

ASMs in asthmatic and COPD cases, studying the potential of β2-adrenergic agonists could provide a solution to these cases (8). Studying the contractile mechanism of airway smooth muscles is important for medical research, allows researchers to consider more data into their drug development with concern to respiratory system diseases and dysfunctions (9). Agents that signal relaxation in ASMs are considered as a potential threat to the coughing defence mechanism, while the ones that cause contraction can complicate asthma and COPD cases.

Göçmez, et al. (2010) studied the influence of long-term usage of fluoxetine on rats, where the results showed potential contribution of the antidepressant to male infertility (78). However, Souza, et al. (2014) performed a similar experiment to investigate the impact of fluoxetine's long-term treatment on rat vas deferens. The findings showed a positive effect of the drug on the denervation and reinnervation profiles; therefore, improvement in functional recovery was expected (79). The effect of fluoxetine on diabetic subjects was experimented by Habib, et al. (2015), where tested rats were having vascular abnormalities under chronic stress. The results show that fluoxetine reduced stress in diabetic and non-diabetic groups significantly, in addition to reduction of body weight, blood pressure and insulin response (80).

There are several studies in the literature that used organ bath systems in order to evaluate the contraction or relaxation of different muscles in vitro. Moreover, airway smooth muscles were evaluated for their contractility using different drugs and compounds. Nevertheless, there were no studies in the literature investigating the effects of fluoxetine on airway smooth muscles using an organ bath system. Wernicke (2004) studied the general side effects of fluoxetine (21). Zanatta, et al. (2001) described the mechanism of fluoxetine on cell membrane in vitro and in vivo (22). Rossi, et al. (2004) provided a literature review on the drug and its experiments (23). Although many studies investigated the effect of fluoxetine on other body tissues, none of these investigations described the contractile effects of the drug on ASMs using an organ bath system. In vivo research in the literature indicated that fluoxetine has protective effects in asthmatic cases (20). Due to the lack of studies on the subject, the current study adopted an experiment on the effects of fluoxetine on ASMs contractility; thus, contributing to the literature with original data.

Fluoxetine is an SSRI that is agonist of 5-HT type receptors (61). Studies have shown that 5-HT_{2B} receptors and 5-HT_3 receptors are mainly targeted by the drug. As nervous system cells and ASMCs have the 5-HT type receptors, the effect of fluoxetine is anticipated on both cell types (56).

For ASMs' research, many studies investigated the effects of different chemicals to induce contraction or relaxation. Bai and Sanderson (2006) experimented the relaxation mechanism of ASMs caused by the inhabitation of IP_3 receptor by forskolin (FSK) and isoproterenol (ISO) through laser scanning microscopy (81). Tatler, et al. (2011) studied two asthma models, in which the activation of β receptors caused relaxation in the ASMs and they attributed asthmatic reactions to the inhabitation or genetic loss of these receptors (82). These explanations for the relaxation effect in ASMs were confirmed by McGraw, et al. (1999), which eliminated the contraction response to methacholine by ASMs in mice through β2-agonist pre-treatment (83).

In this thesis, it was observed that 10μM ACh caused contraction in the bronchial smooth muscle. Ayar et. al. (2001) used ACh at the same dose for the myometrium contraction in the organ bath system (27). Altinisik et. al. (2016) used ACh at 10^{-6} M for the bronchial contraction in the organ bath system (5). Our results correspond with their results.

Vila, et al. (1999) investigated the effect of three antidepressants; sertraline, nortriptyline and amitriptyline on the contraction of mesenteric artery ring specimens taken from humans. An organ bath was used, and the artery rings were initially contracted with noradrenaline. The three antidepressants showed relaxation effects on the isolated tissue (84). Ribback, et al. (2012) showed that Fluoxetine has direct influence on arterial and vascular smooth muscles by testing the effects in an organ bath. The authors confirmed the relaxation effects of fluoxetine on vascular smooth muscles without any dependence on endothelium relaxation effects (18).

Pacher, et al. (1999) examined the effects of fluoxetine on skeletal muscle tissue to inhibit serotonin receptors. The increase of drug concentrations on the isolated skeletal muscle arterioles in an organ bath had a relaxation effect and dilatation reached to 160 μm. The authors attributed their observation to the activation of 5-HT receptors. In the experiment, K^+ channels were activated by adding 4-AP after adding fluoxetine, which

did not have any effect on the relaxation response of the drug. Contractions were observed, when Ca^{2+} was added and they continued to increase by increasing Ca^{2+} concentration. The results of the research suggest that fluoxetine inhibited Ca^{2+} channels, which interfered with their signalling (62).

Tuladhar, et al. (2002) concluded through their experiment that the signalling of the relaxation effect is simulated through 5-HT_4 receptors through testing the drug on rat ileum tissue in an organ bath (85). Mashhadi, et al. (2014) used similar tissue, rat ileum, in addition to tissues from the jejunum and duodenum from the small intestine in order to study the influence of 5-HT antagonists. Contraction was initially induced in the tissues through an electrical field; however, the 5-HT antagonists had limited effect on the contraction and relaxation of the tissues (86).

Experiments on fluoxetine prenatal effects were also performed in the literature. Fornaro, et al. (2007) tested fluoxetine on vascular smooth muscles in pregnant rats. After exposing pregnant rats to a constant concentration of the drug (10 mg/ kg) for eleven days, comparison of results of foetuses from experimental and control groups showed that pulmonary vascular smooth muscle proliferation was increase causing inducing pulmonary hypertension (87).

The results of the current study showed that the acetylcholine treatment induced tension, while fluoxetine treatment caused less tension in the acetylcholine induced contraction. Although the addition of fluoxetine caused a numerical decrease in ASM contraction, this relaxation effect was not statistically significant. It was observed that fluoxetine did not cause to the bronchial smooth muscle contraction in the organ bath system. However, Bootle et. al. (1998) demonstrated that intracisternal injections of fluoxetine caused facilitation bronchoconstriction response in the capsaicin-evoked bronchoconstriction model because of a reflex activation of bronchoconstrictor vagal preganglionic neurones (88). Capelozzi et. al. (2007) showed that fluoxetine treatment induced generation of bronchoconstriction through mononuclear infiltration and neutrophil recruitment into alveolar walls in the guinea pig model (89). On the other hand; Sherkawy et. al. (2018) demonstrated that anti-inflammatory, antioxidant effects of fluoxetine pre-treatment in ovalbumin induced asthma model in rat. They also showed

that fluoxetine caused decreased pathological changes in the lung tissues in the ovalbumin induced asthma model (24).

As seen through the literature, the effects of antidepressants, especially fluoxetine, on smooth muscles are evident by a relaxation effect. The influence is not only limited to airway smooth muscles, but also to mesenteric artery rings, vascular smooth muscles, skeletal smooth muscles and gastric tissue. The majority of research used organ path in order to conduct experiments, as well as inducing initial contraction through chemically or electrically. Most of the results confirm the relaxation effect of antidepressants on smooth muscles and body tissues with statistical significance, which is also performed in the current research through studying the effects of fluoxetine on airway smooth muscles.

The continuation of the current experiment in future research has several potentials. Further experiments can be performed on other SSRIs with low and over dosage effects, such as paroxetine, sertraline, fluvoxamine and citalopram, in order to identify the relationship between their ability to block 5-HT receptors and their effects on airway smooth muscles by using in vivo and in vitro models.

Conclusion: current study showed that fluoxetine does not affect bronchial smooth muscle contraction in the organ bath system. Although fluoxetine leads to a numerical decrease in ASM contraction during the ACh induced contraction, this relaxation effect was not statistically significant. More research can be performed on fluoxetine in order to explain its side effects and/or useful effects in the respiratory system for more safe usage in the patients.

6. REFERENCES

1. Mitzner W. Airway smooth muscle: The appendix of the lung. American Journal of Respiratory and Critical Care Medicine. 2004; 169(787-790).

2. Knox AJ, Tattersfield AE. Airway smooth muscle relaxation. Thorax. 1995; 50: p. 894-901.

3. Beehm KM. The Role of Bronchodilators in Preventing Exacerbations of Chronic Obstructive Pulmonary Disease. Tuberc Respir Dis (Seoul). 2016 October; 79(4): p. 241–247.

4. Barnes PJ. Pharmacology of airway smooth muscle. American Journal of Respiratory and Critical Care Medicine. 1998; 158: p. S123-S132.

5. Altinisik HB, Kirdemir P, Altinisik U, Gokalp O. Effects of magnesium sulfate on airway smooth muscle contraction in rats. Med Glas (Zenica). 2016; 13(2): p. 68-74.

6. Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic Visualization of Beta-Adrenoceptor Subtypes in Human Lung. American Review of Respiratory Disease. 1985 September; 132(3): p. 541-547.

7. Bramley AM, Thomson RJ, Roberts CR, Schellenberg RR. Hypothesis: excessive bronchoconstriction in asthma is due to decreased airway elastance. European Respiratory Journal. 1994 February; 7(2): p. 337-341.

8. de Jongste JC, Mons H, Bonta IL, Kerrebijn KF. Human asthmatic responses in vitro. In de Jongste JC. Human Airway Smooth Muscle. Rotterdam: CIP-DATA Koninklijke Bibliotheek, Den Haag; 1986. p. 167-177.

9. Kotlikoff MI, Barnes PJ. Pharmacology of airway smooth muscle. In Crystal RG, West EW, Barnes P. The Lung: Scientific Foundations. Philadelphia: Lippincott-Raven; 1997. p. 1345-1354.

10. Sakai H, Watanabe A, Fujita A, Misawa M, Narita M, Chiba Y. Augmented bronchial smooth muscle contractility induced by aqueous cigarette smoke extract in rats. Journal of Smooth Muscle Research. 2014; 50: p. 39-47.

11. Corboz MR, Ballard ST, Gao H, Benoit JN, Inglis SK, Taylor AE. Differential effects of furosemide on porcine bronchial arterial and airway smooth muscle. Journal of Applied Physiol. 2000; 89: p. 1360-1364.

12. Sieck GC, Gransee HM. Respiratory Muscles: Structure, function and regulation. Colloquium Series on Integrated Systems Physiology: From Molecule to Function. 2012 May; 4(3): p. 1-96.

13. Vodenicharov A. Structure and Function of Smooth Muscle with Special Reference to Mast Cells. In Sugi H. Current Basic and Pathological Approaches to the Function of Muscle Cells and Tissues – From Molecules to Humans.: InTech; 2012. p. 345-362.

14. Zhang W, Gunst SJ. Interactions of Airway Smooth Muscle Cells with Their Tissue Matrix. Proceedings of American Thoracic Society. 2008 January; 5(1): p. 32-39.

15. Ouedraogo N, Roux E. Physiology of Airway Smooth Muscle Contraction: An Overview. Journal of Pulmonary & Respiratory Medicine. 2014; 4(6).

16. Pedroso SP, de Souza BP, Jurkiewicz A, Juriewicz NH, da Silva Junior ED. Effects of in vitro, accute and chronic treatment with fluoxetine on the sympathetic neurotransmission of rat vas deferens. Autonomic Neuroscience: Basic and CLinical. 2017; 203: p. 17-24.

17. Wong DT, Perry KW, Bymaster FP. The discovery of Fluoxetine Hydrochloride (Prozac). Nature. 2005 September; 4.

18. Ribback S, Pavlovic D, Herbst D, Nedeljkov-Jancic R, Wendt M, Nedeljkov V, et al. Effects of Amitriptyline, Fluoxetine, Tranylcypromine and Venlafaxine on Rat Vascular smooth muscle in VITRO - The Role of the Endothelium. Journal of Physiology and Pharmacology. 2012; 63(2): p. 119-125.

19. Fernandes DJ, McConville JF, Stewart AG, Kalinichenko V, Solway J. Can we differentiate between airway and vascular smooth muscles? Clinical and Experimental Pharmacology and Physiology. 2004; 31: p. 805-810.

20. Wang MT, Tsai CL, Lin CW, Yeh CB, Wang YH, Lin HL. Association Between Antipsychotic Agents and Risk of Acute Respiratory Failure in Patients With Chronic Obstructive Pulmonary Disease. JAMA Psychiatry. 2017 January;: p. E1-E9.

21. Wernicke JF. Safety and side effect profile of fluoxetine. Expert Opinion on Drug Safety. 2004; 3(5): p. 495-504.

22. Zanatta LM, Nascimento FC, Barros SVT, Silva GRRS, Zugno AI, Netto CA, et al. In vivo and in vitro effect of imipramine and fluoxetine on Na+,K+-ATPase activity in synaptic plasma membranes from the cerebral cortex of rats. Brazilian Journal of Medical and Biological Research. 2001; 34: p. 1265-1269.

23. Rossi A, Barraco A, Donda P. Fluoxetine: a review on evidence based medicine. Annuals of General Hospital Psychiatry. 2004.

24. Sherkawy MM, Youssef A, M A, Salama AAA, Ismaiel IE. Fluoxetine protects against OVA induced bronchial asthma and depression in rats. European Journal of Pharmacology. 2018 October; 15(837): p. 25-32.

25. Sohel AJ, Molla M. Fluoxetine Treasure Island, FL: StatPearls Publishing LLC; 2018.

26. Youngson C, Nurse C, Yeger H, Cutz E. Oxygen sensing in airway chemoreceptors. Nature. 1993; 365: p. 153-155.

27. Ayar A, Kutlu S, Yilmaz B, Kelestimul H. Melatonin inhibits spontaneous and oxytocin-induced contractions of rat myometrium in vitro. Neuro Enocrinology Letters. 2001 June; 22(3): p. 199-207.

28. Young KA, Wise JA, DeSaix P, Kruse DH, Poe B, Johnson E, et al. The Respiratory System. In Young KA, Wise JA, DeSaix P, Kruse DH, Poe B, Johnson E, et al. Anatomy and Physiology. Houston: OpenStax College (Rice University); 2013. p. 973-1020.

29. Tomashefski JF, Farver CF. Anatomy and Histology of the Lung. In Tomashefski JF, editor. Dail and Hammar's Pulmonary Pathology. New York: Springer-Verlag; 2008. p. 20-48.

30. Tu J, Inthavong K, Ahmadi G. The Human Respiratory System. In Tu J, Inthavong K, Ahmadi G. Computational Fluid and Particle Dynamics in the Human Respiratory System. Amsterdam: Springer Netherlands; 2013.

31. Johnson Jr RL, Hsia CCW. Anatomy and physiology of the human respiratory system. WIT Transactions on State of the Art in Science and Engineering. 2006; 24.

32. Marieb EN, Hoehn K. The Respiratory System. In Marieb EN, Hoehn K. Human Anatomy & Physiology.: Pearson; 2013. p. 804-846.

33. Davis A, Moores C. The Respiratory System: Basic science and clinical conditions. 2nd ed.: Churchill Livingstone; 2010.

34. Shaik Z, Ramulu V, Hanimann KS. A study on anatomical dimensions of bronchial tree. International Journal of Research in Medical Sciences. 2016 July; 4(7): p. 2761-2765.

35. Amador C, Varacallo M. Anatomy, Thorax, Bronchial Treasure Island (FL): StatPearls Publishing; 2019.

36. Meyerholz DK, Suarez CJ, Dintzis SM, Frevert CW. Respiratory System (Bronchi). Comparative Anatomy and Histology (Second Edition). 2018;: p. 147-162.

37. López Diez Del Corral J, Gómez E, Sesma P. Anatomical study of the bronchial system and major blood vessels of the chicken lung (Gallus gallus) by means of a three-dimensional scale model. The Anatomical Record. 1992 October; 234(2).

38. Hyde DM, Hamid Q, Irvin CG. Anatomy, pathology, and physiology of the tracheobronchial tree: Emphasis on the distal airways. 9 American Academy of Allergy, Asthma & Immunology. 2009;: p. S72-S77.

39. The Respiratory System. The Respiratory System. [Online].; 2017 [cited 2019 June 2. Available from: https://www.therespiratorysystem.com/bronchi/.

40. Vodenicharov A. Structure and Function of Smooth Muscle with Special Reference to Mast Cells. In Sugi H, editor. Current Basic and Pathological Approaches to the Function of Muscle Cells and Tissues – From Molecules to Humans. Rijeka, Croatia: InTech; 2012. p. 345-362.

41. Kooij IA, Sahami S, Meijer SL, Buskens CJ, te Velde AA. The immunology of the vermiform appendix: a review of the literature. Clinical & Experimental Immunology. 2016 October; 186(1): p. 1-9.

42. Jesudason EC. Airway smooth muscle: an architect of the lung? Thorax. 2008 October; 64: p. 541-545.

43. Menzella F, Lusuardi M, Galeone C, Facciolongo N. Bronchial thermoplasty and the role of airway smooth muscle: are we on the right direction? Therapeutic and Clinical Risk Management. 2017; 13: p. 1213-1221.

44. Pascoe CD, Wang L, Syyong HTPPD. A brief history of airway smooth muscle's role in airway hyperresponsiveness. Journal of Allergy. 2012;: p. 1-8.

45. Lei M, Ghezzo H, Chen MF, Eidelamn DH. Airway smooth muscle orientation in intraparenchymal airways. the American Physiological Society. 1997;: p. 70-77.

46. Bai Y, Sanderson MJ. Airway smooth muscle relaxation results from a reduction in the frequency of Ca2+ oscillations induced by a cAMP-mediated inhibition of the IP3 receptor. Respiratory Research. 2006; 7(34).

47. Seow CY, Fredberg JJ. Signal transduction in smooth muscle historical perspective on airway smooth muscle: the saga of a frustrated cell. Journal of Applied Physiology. 2001; 91: p. 938-952.

48. Oliver BG, Balck JL. Airway smooth muscle and asthma. Allergology International. 2002; 55: p. 215-223.

49. Yan F, Gao H, Zhao H, Bhatia M, Zang Y. Roles of airway smooth muscle dysfunction in chronic obstructive pulmonary disease. Journal of Translational Medicine. 2018; 16(262).

50. Terzano C, Laurendi G, Capoccetta G, Rapisarda M, Pacilio R, Petroianni A. The smooth muscle and airway hyperresponsiveness. European Review for Medical and Pharmacological Sciences. 2007; 7: p. 9-26.

51. McGraw DW, Mihlbachler KA, Schwarb MR, Rahman FF, Small KM, Almoosa KF, et al. Airway smooth muscle prostaglandin-EP1 receptors directly modulate β2-adrenergic receptors within a unique heterodimeric complex. The Journal of Clinical Investigation. 2006 May; 116(5): p. 1400-1409.

52. Crystal RG, Randell SH, Engelhardt JF, Voynow J, Sunday ME. Airway Epithelial Cells: Current Concepts and Challenges. Proceedings of the American Thoracic Society. 2008 September; 5(7): p. 772-777.

53. Barnes PJ. Airway Receptors. Postgraduate Medical Journal. 1989; 65: p. 532-542.

54. Ruan YC, Zhou W, Chan HC. Regulation of smooth muscle contraction by the epithelium: Role of Prostaglandins. Physiology. 2011; 26: p. 156-170.

55. Campos-Bedolla P, Vargas MH, Segura P, Carbajal V, Calixto E, Figueroa A, et al. Airway smooth muscle relaxation induced by 5-HT(2A) receptors: role of Na(+)/K(+)-ATPase pump and Ca(2+)-activated K(+) channels. Life Science. 2008 September; 83(11-12): p. 438-446.

56. Dupont LJ, Pype J, Demedts MG, Leyn PD, Deneffe G, Verleden GM. The effects of 5-HT on cholinergic contraction in human airways in vitro. European Respiratory Journal. 1999; 14: p. 642-649.

57. Konduru J, Vanita P, Sabbavarapu L, Varall S. A Review on Antidepressant Drugs. Advances in Pharmacoepidemiology & Drug Safety. 2014; 3(1).

58. Taylor C, Fricker AD, Devi LA, Gomes I. Mechanisms of action of antidepressants: from neurotransmitter systems to signaling pathways. Cell Signal. 2005 May; 17(5): p. 549-557.

59. Khawam E, Laurencic G, Malone Jr DA. Side effects of antidepressants: An overview. Cleveland Clinic Journal of Medicine. 2006 April; 73(4): p. 351-361.

60. Khushboo SB. Antidepressants: mechanism of action, toxicity and possible amelioration. Journal of Applied Biotechnology & Bioengineering. 2017 September; 3(5): p. 437-448.

61. Perez-Caballero L, Torres-Sanchez S, Bravo L, Mico JA, Berrocoso E. Fluoxetine: a case history of its discovery and preclinical development. Expert Opinion on Drug Discovery. 2014 April.

62. Pacher P, Ungvari Z, kecskemeti V, Koller A. Serotonin reuptake inhibitor, fluoxetine, dilates isolated skeletal muscle arterioles. Possible role of altered Ca2+ sensitivity. British Journal of Pharmacology. 1999 June; 127(3): p. 740-746.

63. Peng L, Gu L, Li B, Hertz L. Fluoxetine and all other SSRIs are 5-HT2B Agonists - Importance for their Therapeutic Effects. Current Neuropharmacology. 2014 July; 12(4): p. 365-379.

64. Deidda A, Pisanu C, Micheletto L, Bocchetta A, Del Zompo M, Stochino ME. Interstitial lung disease induced by fluoxetine: Systematic review of literature and analysis of Vigiaccess, Eudravigilance and a national pharmacovigilance database. Pharmacological Research. 2017; 120: p. 294-301.

65. Rosenberg T, Lattimer R, Montgomery P, Wiens C, Levy L. The relationship of SSRI and SNRI usage with interstitial lung disease and bronchiectasis in an elderly population: a case–control study. Clinical Interventions in Aging. 2017 November; 12: p. 1977-1984.

66. Wikswo JP. The relevance and potential roles of microphysiological systems in biology and medicine. Experimental Biology and Medicine. 2014 September; 239(9).

67. Jespersen B, Tykocki NR, Watts SW, Cobbett PJ. Measurement of Smooth Muscle Function in the Isolated Tissue Bath: applications to Pharmacology Research. Journal of Visualized Experiments. 2015 January; 95.

68. Koç E, Koçak M, Akcil E. Cadmium reduces contractile responses of rat duodenum, in vitro. Biological Trace Element Research. 2008 February; 123: p. 154-160.

69. Reinheimer T, Mohlig T, Zimmermann S, Hohle KD, Wessler I. Muscarinic Control of Histamine Release from Airways: Inhibitory M1-receptors in Human Bronchi but Absence in Rat Trachea. American Journal of Respiratory and Critical Care Medicine. 2000; 162: p. 534-538.

70. Gosens R, Zaagsma J, Bromhaar MG, Nelemans A, Meurs H. Acetycholine: a novel regulator of airway smooth muscle remodelling? European Journal of Pharmacology. 2004 August; 500: p. 193-201.

71. Tavakoli R, Frossard N. 5-HT2-mediated bronchial responses after syngeneic lung trasplantation in the rate. British Journal of Pharmacology. 1995; 114: p. 1428-1432.

72. Sakai H, Shirai T, Yamamoto M, Chiba Y, Misawa M. Involvement of M3 Muscarinic Receptors in ACh-Induced Increase in Membrane-Associated RhoA of Rat Bronchial Smooth Muscle. Biological and Pharmaceutical Bulletin. 2005 April; 28(4): p. 625-628.

73. Van de Voorde J, Joos G. Regionally different influence of contractile agonists on isolated rat airway segments. Respiration Physiology. 1998 March; 112: p. 185-194.

74. Diaz-Martin D, Hernandez-Jimenez JG, Rodriguez-Valido M, Borges R. Measuring the Contractile Response of Isolated Tissue Using an Image Sensor. Sensors. 2015; 15: p. 9179-9188.

75. Undale VR, Jagtap PN, Yadav AV, Sangamnerkar SK, Upasani CD, Bhosale AV. An isolated chicken ileum: Alternative to laboratory animals for isolated tissue experimentation. IOSR Journal of Pharmacy. 2012 October; 2(5): p. 39-45.

76. Abdur Rahman HM, Ahmed K, Rasool MF, I I. Pharmacological evaluation of smooth muscle relaxant and cardiac-modulation potential of Phylanodiflora in ex-vivo and in-vivo experiments. Asian Pacific Journal of Tropical Medicine. 2017 October; 10(12): p. 1146-1153.

77. Abdur Rahman HM, Rasool MF, Imran I. harmacological Studies Pertaining to Smooth Muscle Relaxant, Platelet Aggregation Inhibitory and Hypotensive Effects of Ailanthus altissima. Evidence-Based Complementary and Alternative Medicine. 2019 March.

78. Göçmez SS, Utkan T, Ulak G, Gacar N, Erden F. Effects of long-term treatment with fluoxetine and venlafaxine on rat isolated vas deferens. Autonomic & Autacoid Pharmacology. 2010; 30(197-202).

79. Souza CF, Garcez-de-Carmo L, Conceicao IM, Freitas TA, Frussa-Filho R. Effects of long-term fluoxetine treatment on adrenergic plasticity in rat vas deferens. Brazilian Journal of Pharmaceutical Sciences. 2014 December; 50(4): p. 757-764.

80. Habib M, Shaker S, El-Gayar N, Aboul-Fotouh S. The Effects of Antidepressants “Fluoxetine and Imipramine” on Vascular Abnormalities and Toll Like Receptor-4 Expression in Diabetic and Non-Diabetic Rats Exposed to Chronic Stress. PLoS ONE. 2015; 10(3).

81. Bai Y, Sanderson MJ. Airway smooth muscle relaxation results from a reduction in the frequency of Ca2+ oscillations induced by a cAMP-mediated inhibition of the IP3 receptor. Respiratory Research. 2006 February; 7(1).

82. Tatler AL, John AE, Jolly L, Habgood A, Porte J, brightling C, et al. Integrin avb5-Mediated TGF-b Activation by Airway Smooth Muscle Cells in Asthma. The Journal of Immunology. 2011 October; 187: p. 6094-6107.

83. McGraw DW, Forbes SL, Kramer LA, Witte DP, Fortner CN, Paul RJ, et al. Transgenic Overexpression of β2-Adrenergic Receptors in Airway Smooth Muscle Alters Myocyte Function and Ablates Bronchial Hyperreactivity. The Journal of Biological Chemistry. 1999 November; 274(45): p. 32241-32247.

84. Vila JM, Medina P, Segarra G, Lluch P, Pallardo F, Flor B, et al. Relaxant effects of antidepressants on human isolated mesenteric arteries. British Journal of Clinical Pharmacology. 1999 April; 48: p. 223-229.

85. Tuladhar BR, Costall B, Naylor RJ. Modulation of 5-HT4 receptor function in the rat isolated ileum by ¯uoxetine: the involvement of endogenous 5-hydroxytryptamine. British Journal of Pharmacology. 2002; 136: p. 150-156.

86. Mashhadi FF, Naylor RJ, Javid FA. The Effects of Serotonin Receptor Antagonists on Contraction and Relaxation Responses Induced by Electrical Stimulation in the Rat Small Intestine. Gene Cell Tissue. 2014 April; 1(1).

87. Fornaro E, Li D, Pan J, Belik J. Prenatal Exposure to Fluoxetine Induces Fetal Pulmonary Hypertension in the Rat. American Journal of Respiratory and Critical Care Medicine. 2007; 176: p. 1035-1040.

88. Bootle DJ, Adcock JJ, Ramage AG. The role of central 5-HT receptors in the bronchoconstriction evoked by inhaled capsaicin in anaesthetised guinea-pigs. Neuropharmacology. 1998; 37(2): p. 243-250.

89. Capelozzi MA, Leick-Maldonado EA, Parra ER, Martins MA, Tibério IF, Capelozzi VL. Morphological and functional determinants of fluoxetine (Prozac)-induced pulmonary disease in an experimental model. Respiratory Physiology & Neurobiology. 2007 May; 156(2): p. 171-178.

APPENDIX 1. Curriculum Vitae

Personal Informations

Name	Hajer	**Surname**	Hmmam
Place of Birth	Libya	**Date of Birth**	14 February 1984
Nationality	Libyan	**TR ID Number**	99503546764
E-mail	Dr_hm8451@yahoo.com	**Phone number**	0 543 917 86 15

Education

Degree	**Department**	**The name of the Institution Graduated From**	**Graduation year**
Master	Department of Physiology	**Yeditepe University**	2019
University	Pathology Department	**Tripoli University**	2008
High school	Scientific Section	**Al-Orouba High School**	2002

Languages	**Grades**
Arabic	Mother Tongue
English	IELTS (London 2014) – Grade: 5.5

Work Experience

Position	**Institute**	**Duration (Year - Year)**
Pathology Technician	Ebn Ennafis Institute	2010 - Present

Computer Skills

Program	**Level**
Microsoft Office	Good
SPSS Statistics	Basic

APPENDIX 2. Ethical Committee Approval

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU

(YÜDHEK)

ETİK KURUL KARARI

Toplantı Tarihi	Karar No	İlgi	Proje Yürütücüsü
21.12.2018	712	14.12.2018	Doç. Dr. Mehtap KAÇAR

"Fluoksetinin Sıçan Bronş Düz Kaslarına Etkilerinin İzole Organ Banyosu Yöntemiyle Değerlendirilmesi" adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

Etik Onay Geçerlilik Süresi: 3 Yıl	**Hayvan Türü ve cinsiyeti:** Sıçan ♂	**Hayvan Sayısı:** 15

GÖREVİ	ADI SOYADI	
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Yardımcısı	Prof. Dr. Erdem YEŞİLADA	
Raportör	Vet. Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	**KATILMADI**
Üye	Prof. Dr. Rukset ATTAR	
Üye	Doç. Dr. Soner DOĞAN	
Üye	Doç. Dr. Ediz DENİZ	
Üye	Prof. Dr. Gamze TORUN KÖSE	**KATILMADI**
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Hakan GÖKSEL	
Üye	Ahmet ŞENKARDEŞLER	