PHARMACOLOGY OF THE HUMAN AND PORCINE ISOLATED MYOCARDIUM

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VAN MENS EN VARKEN

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For my parents
For Zhixiong and Tanxiang

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Contents

Chapter 1 General introduction

The heart, an important organ of the mammalian, operates as a pump. It is composed of two pumps: the right heart pumps the blood through the lungs while the left heart pumps the blood through peripheral organs. In turn, each of these separate pumps are composed of two pulsatile chambers, an atrium and a ventricle. Each minute, the heart of a resting adult pumps about 5 L of blood, or approximately the person's total blood volume. This works out to at least 720 L/day, the volume of blood weighting about 100 times more than the body. This heavy and incessant work is based on the fact that the heart muscle has a coordinated contraction. The cardiac muscle fibres are highly organized with strong inter-connections. A trigger pulse from the sinoatrial node, being the pacemaker, initiates and coordinates contraction, making possible the function of the heart as a pump and the contractility as the major parameter of the function of the heart.

1.1 Myocardial contraction

Since the effects on cardiac contractility are associated with changes in cellular mechanisms, the following brief review can be helpful to understand the mechanisms of actions of the inotropic agents.

The heart muscle cells are excitable, with spontaneous and triggered action potentials generated by voltage-dependent ion channels in the surface membrane. In mammalian heart, the cardiac muscle contraction-relaxation cycle is mainly controlled by myoplasmic Ca^{2+} concentration. As a striated muscle, the interaction between actin and myosin filaments of cardiac muscle at rest is blocked by the presence of tropomyosin bound to the actin filament. Tropomyosin forms part of the troponin complex, from which one component, troponin C, has binding sites for three or four calcium ions. When a sufficient amount of Ca^{2+} becomes available for binding, tropomyosin shifts its position, allowing the binding of the myosin cross-bridge to the actin filament, thus initiating the contractile process. The overall process, which begins with action potentials in the muscle fibers and causes release of Ca^{2+} from sarcoplasmic reticulum (SR), and in the end initiates contraction is called excitation-contraction coupling. The effects of substances on cardiac contraction are associated with alteration of intracellular Ca^{2+} concentration and/or Ca^{2+} sensitivity of myofilaments, and are shown in figure 1.1.

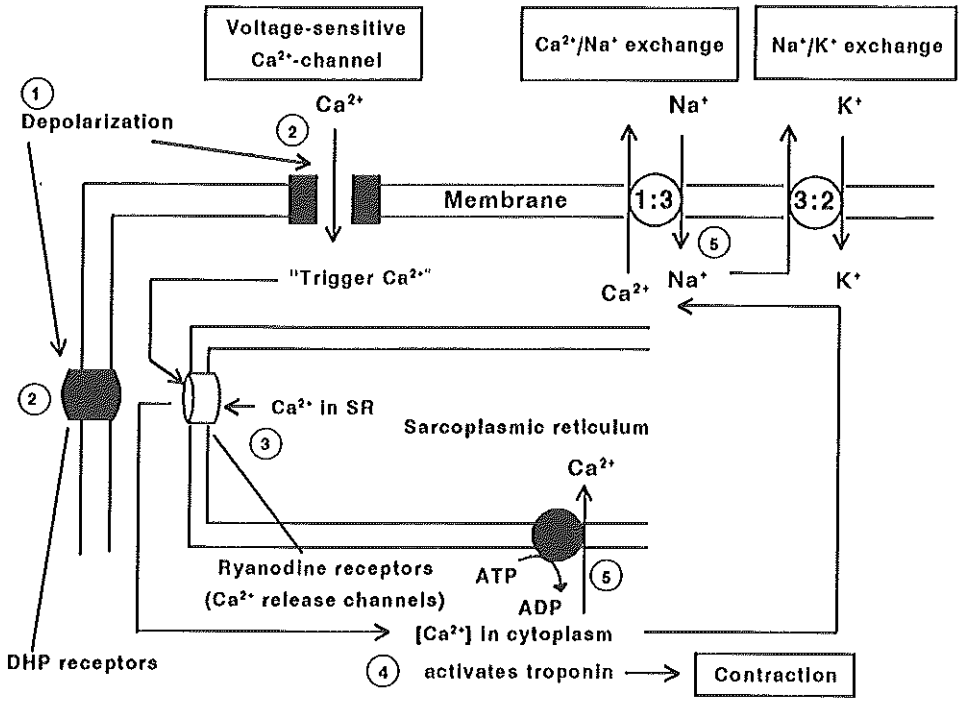


Figure 1.1. Pathways of excitation-contraction coupling in myocardial cells. Depolarization (1) opens (2) both membrane voltage-gated Ca^{2+} channels and SR Ca^{2+} channels controlled by sarcolemma located T tubule dihydropyridine (DHP) sensitive receptors. Entry of trigger Ca^{2+} potentiates release of (3) Ca^{2+} from the SR (Ca^{2+}-induced Ca^{2+} release). Crossbridges become active by the amount of Ca^{2+} released into the cytoplasm to troponin C (4). Relaxation occurs as Ca^{2+} is removed from the cytoplasm (5) by the Ca^{2+} pump of the SR and by Na^+/Ca^{2+} exchange across the cell membrane (Adapted from Moffett et al., 1993).

The increase in intracellular Ca^{2+} concentration results from Ca^{2+} entering though the voltage-sensitive calcium channels and Ca^{2+} released from SR. Both these actions could be initiated by depolarization. The latter also involves a Ca^{2+}-induced Ca^{2+} release mechanism: a small amount of Ca^{2+} through the voltage-gated L-type Ca^{2+} channels and/or Na^+/Ca^{2+} exchange across the surface membrane during the action potential would be insufficient by itself to activate the myofilaments, but would trigger a much larger release of Ca^{2+} from the intracellular stores in the SR. The released Ca^{2+} interacts with troponin C,

thereby initiating cardiac contraction. The amount of trigger Ca^{2+} exercises a strong influence on the final Ca^{2+} levels attained in the cytoplasm during systole, so changes in the amount of trigger Ca^{2+} are of major importance in the control of the myocardial contraction. Relaxation starts when myosin crossbridges are detached from actin. This requires removal of cytoplasmic Ca^{2+}, which is an active process energized by Ca^{2+} pumps located both in the SR and cell membrane. Via the Ca^{2+} pump in the SR, most of the total Ca^{2+} re-enters SR. Via the Na^{+}/Ca^{2+} pump in the cell membrane, the remaining Ca^{2+} are extruded in exchange for sodium ions, which are, in turn, extruded in exchange for potassium ions by the Na^{+}/K^{+} pump. Thus, activation or inhibition Na^{+}/K^{+} pump will speed up or slow down the Na^{+}/Ca^{2+} exchange and, secondarily, reduce or raise the intracellular Ca^{2+} concentration.

A change in Ca^{2+} sensitivity of myofilaments can also influence the Ca^{2+} binding and, hence, the contractility. One of the mechanismis to change Ca^{2+} sensitivity of the contractile structure could be troponin I phosphorylation. Evidence shows that certain forms of cardiac insufficiency may be due to the decrease of Ca^{2+} sensitivity of contractile proteins, rather than a lack of Ca^{2+} available for activation (Herzig, 1989). Therefore, in addition to changes in amount of cytosolic free Ca^{2+}, a change of muscle contractility could also be brought by alterations of Ca^{2+} sensitivity of myofilaments.

1.2 Receptor-mediated regulation of cardiac contractility

Many hormones and neurotransmitters, including catecholamines, as well as several neuropepitides affect/regulate the cardiac contractility by interacting with receptors located in cytoplasmic and membrane regions of cardiomyocytes.

It has become clear that many membrane receptors belong to a large "family" of receptors that couple to guanine nucleotide binding regulatory proteins (G-proteins). G-proteins are membrane-associated heterotrimers composed of α, ß, γ subunits. These subunits of the G-protein regulate effectors, such as adenylyl cyclase, phospholipase C and ion channels (reviewed by Kurachi, 1994). When the system is inactive, guanosine diphosphate (GDP) is bound to the α-subunit. Receptor-mediated activation of G-proteins occurs in a agonist dependent manner and involves the exchange of complexed GDP for guanosine triphosphate (GTP) (Strader et al., 1987). When occupied by agonists, the receptors are coupled to specific effector molecules linked via certain G-proteins. Different G-proteins interact with their relevant effectors and modulate intracellular levels of various second messenger systems (figure 1.2). It is well know that several second messenger systems modulated by G-proteins, such as adenylyl cyclase/ cyclic AMP, phospholipase C/

diacyglycerol/ inositol-1,4,5-trisphophate (PLC/DG/IP_3), Ca^{2+} channels/ Ca^{2+} and K^+ channels/ K^+ current, are involved in the regulation of cardiac contractility. Via activation of those systems, substances may enhance or depress the cardiac contractility.

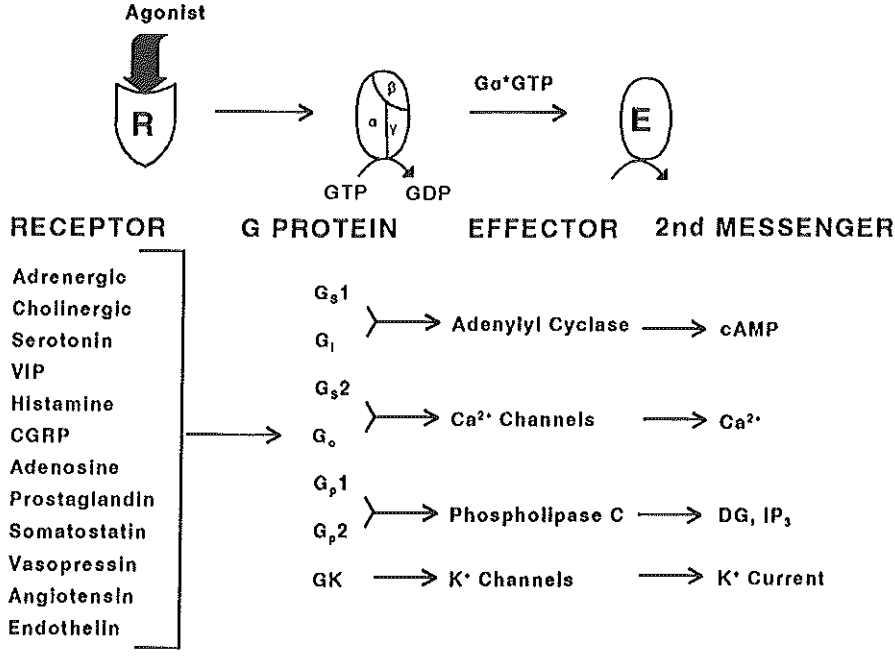


Figure 1.2. Diversity of receptors, guanine nucleotide binding regulatory proteins (G proteins), and effectors involved in membrane receptor systems. Once the agonist binds to the receptor (R), intermediary G proteins (shown with α, ß, γ, components) transduce the signal to effector (E) systems, which are coupled to second-messenger systems. G-protein function requires hydrolysis of guanosine 5'-triphosphate (GTP). The activated form of G proteins involves the α subunit and GTP (shown as G_α*GTP). DG = 1,2-diacylglycerol; IP_3 = inositol 1,4,5-triphosphate (adapted from Schwinn et al., 1991).

The stimulatory G-protein (G_s) activates the enzyme adenylyl cyclase resulting in an increase in intracellular adenosine 3'5'-cyclic monophosphate (cAMP). The main effect of cAMP is to activate the cAMP-dependent protein kinases (PKA) which control cell function in many different ways by causing phosphorylation of various enzymes, carriers

and other proteins (Krebs and Beavo, 1979), therewith promoting a slow Ca^{2+} inward current. This leads to an increased Ca^{2+} release from SR, either because it serves as a greater trigger for Ca^{2+}-dependent Ca^{2+} release or because it increases the filling of these stores with Ca^{2+} that can be released during subsequent beats (reviewed by Scholz, 1989), thus enhancing the inotropic response (England et al., 1984; Katz et al., 1990). In contrast to G_s protein, the inhibitory G-protein (G_i) inhibits adenylyl cyclase activity, thus leading to a reduction in the amount of cAMP generated by the enzyme and a depression in contractility.

Some receptors are coupled to a specific G-protein (G_p), which activates the enzyme phospholipase C, causing hydrolysis of phosphatidylinositol 4,5-bisphophate (PIP_2) in the plasma membrane. The products of this reaction are 1,2-diacylglycerol (DG), which is retained in the plasma membrane, and D-myo-inositol-1,4,5-trisphophate (IP_3). Both IP_3 and DG act as intracellular messengers with different functions that are eventually responsible for the increase of cytosolic free Ca^{2+}, thereby causing a positive inotropic effect. IP_3 causes the release of Ca^{2+} from intracellular non-mitochondrial stores (Berridge and Irvine, 1989). DG stimulates the activity of a Ca^{2+} sensitive enzyme, protein kinase C (PKC), another kinase involved in the phosphorylation of proteins. PKC is a Ca^{2+}- and phospholipid-dependent enzyme that is involved in the enhancement of Ca^{2+} current (DeReimer et al., 1985), the recruitment of new Ca^{2+} channels (Strong et al., 1987), the phosphorylation of contractile and cytoskeletal proteins (Naka et al., 1983; Fabiato, 1986) and the regulation of smooth muscle contraction (Menkes et al., 1986).

The direct or at least membrane-delimited regulation of ion channel function by G-proteins is a newly recognized mode of the remote-sensor-type regulation of ion channels (Hille, 1992). A number of the ion channels, such as K^+ channels, Ca^{2+} channels, Na^+ channels, and Cl^- channels, have been shown to be regulated by G-proteins in a variety of tissues (reviewed by Brown and Birnbaumer, 1990). In cardiac tissue, G_k activates the K^+ channel and causes outward current of K^+ (Kurachi, 1994), which may be responsible for a negative inotropic effect.

1.3 Positive inotropic effects and agents

A brief introduction of the receptor systems and their signal-transduction mechanisms of the known positive inotropic agents in the human heart is summarized in figure 1.3. There are two mechanisms which ultimately cause positive inotropism:

1. Increase in intracellular Ca^{2+} concentration;
2. Increase in the Ca^{2+} sensitivity of the contractile proteins.

1. Increase in intracellular Ca^{2+} concentration

The intracellular Ca^{2+} concentration can be increased via: i) increase in intracellular cAMP level; ii) stimulation of phosphoinositide hydrolysis (phosphoinositide turnover); iii) increase in intracellular Na^+ concentration.

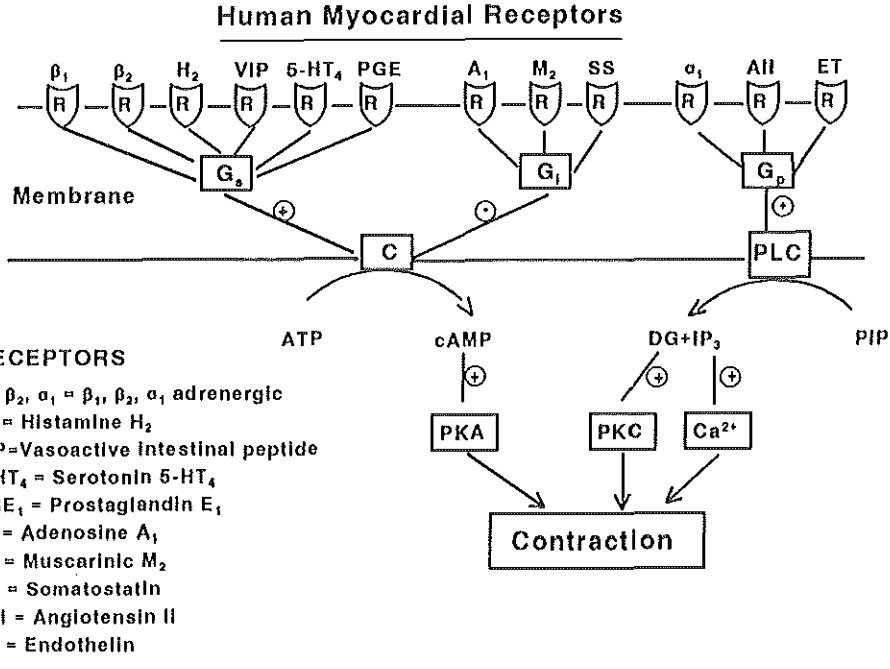


Figure 1.3. Receptor systems and their signal-transduction mechanisms in the human heart. G_s = stimulatory guanine nucleotide binding protein; G_i = inhibitory guanine nucleotide binding protein; C = catalytic unit of adenylyl cyclase; PLC = phospholipase C; PIP_2 = phosphatidylinositol 4,5-bis-phosphate; DG = 1,2-diacylglycerol; IP_3 = inositol-1,4,5-trisphosphate (Adapted from Brodde et al., 1992).

*Increase in intracellular cAMP level:*The adenylyl cyclase/cAMP system can be activated through interaction of substances with receptors, located on the plasma membrane and coupled to a G_s protein, resulting in increased intracellular cAMP levels. These substances include catecholamines, histamine, vasoactive intestinal peptide (VIP), 5-hydroxytryptamine (5-HT), calcitonin gene-related peptide (CGRP), and prostaglandin E_1 (PGE_1) (reviewed by Brodde et al., 1992).

The receptors for catecholamines, mediators of sympathetic nervous system, are coupled to two different second messenger systems. Acting through $ß_1$- and $ß_2$-adrenoceptor-adenylyl-cyclase pathway, catecholamines produce positive inotropic and chronotropic effects. This is considered the most powerful mechanism to increase heart rate and contractility (Brodde et al., 1992). Noradrenaline, which controls the cardiac contractility in different physiological conditions, evokes positive inotropic effect predominately via $ß_1$-adrenoceptor stimulation (Kaumann et al., 1989; Motomura et al., 1990). In addition, recent studies strongly suggested that in human heart, catecholamines also elicit positive inotropic effects through activation of the small proportion of α_1-adrenoceptors coupled to phospholipase C (PLC) pathway (see below).

Histamine exerts positive inotropic effects predominantly via H_2 receptors in both human atrium and ventricle (Bristow et al., 1982; Zerkowski et al., 1993). Studies indicated that the cardiac effects show species differences in the type of the receptors involved (Chiba et al., 1976; Hattori et al., 1983, 1986; Elizalde et al., 1986). The effects of histamine and the receptors involved in porcine heart are still unknown.

For 5-HT, there has been dramatic progress in receptor pharmacology in the recent past (Bradley et al., 1986; Hoyer et al., 1994), and it is well known that 5-HT causes profound, numerous and complex actions in the cardiovascular system (Saxena and Villalón, 1990; 1991); however, no data are available about the direct effects of 5-HT on ventricular tissue. 5-HT exerts positive chronotropic as well as inotropic effects in atrial tissue, which is mediated by 5-HT_4 receptors (Kaumann et al., 1990b, 1991b).

CGRP is a neuropeptide released by sensory nerve endings that are also present in the heart. CGRP may play an important role in the cardiovascular regulation. Previous studies showed that CGRP has positive inotropic and chronotropic effect in various mammalian hearts, including the human atrium (Mulderry et al., 1985; Marshall et al., 1986; Franco-Cereceda et al., 1987a,b; Ishikawa et al., 1988). However, CGRP may be species-specific and using the CGRP from one species in another may afflict its effects. VIP, a 28-amino acid-peptide neurotransmitter, was discovered as a potent vasodilator substance (Said and Mutt, 1970). It is present in nerves of the mammalian heart (Della et al., 1983; Lundberg et al., 1984) and elicits a positive inotropic effect in human atrial tissue (Franco-Cereceda et al., 1987b). The physiological effects of VIP are mediated by VIP-specific membrane receptors, that are coupled to adenylyl cyclase in human myocardial membrane (Taton et al., 1982).

Prostaglandins (PG_s) produce a complex effects on cardiac tissue, depending on the type of prostaglandin, the dose used, and the animal species in which the experiments are performed. In vivo experiments demonstrated that PGE_1 may increase contractile force and induce tachycardia in humans. However, the role of endogenous prostaglandins in

regulating or modulating cardiac function remains uncertain (Karmazyn and Dhalla, 1983; Maisch et al.; 1991, Brodde et al., 1992).

In addition to the receptor mediated mechanisms, an increase in intracellular cAMP level could also be brought about by forskolin, which directly stimulates the catalytic subunit of adenylyl cyclase. Other agents, such as pimobendan, milrinone, enoxinone and isobutylmethylxanthine (IBMX), increase cAMP by inhibiting cardiac phosphodiesterase III, an enzyme which converts cAMP into its inactive metabolite 5'-AMP.

*Stimulation of phosphoinositide hydrolysis:*Through the PLC/DG/IP_3 pathway, angiotensin II and endothelin increase the force of contraction in human atrium (Utrata et al., 1989; Moravec et al., 1990; Brodde et al., 1992; Zerkowski et al., 1993). The effects of angiotensin II are mediated by angiotensin AT_1 receptors (Urata et al., 1989; Zerkowski et al., 1993), which may be associated with an increase in phosphatidylinositol turnover (Allen et al., 1988). Effects and the subcellular mechanism of regulation of cardiac contractile function of angiotensin II in human heart and are still lacking. Endothelin is a 21-amino peptide hormone secreted by vascular and endocardial endothelial cells (Yanagisawa et al., 1988). Both ET_A and ET_B receptors exist in human myocardium (Bax et al., 1993), and it is unknown which of the two receptors mediate the positive inotropic effect.

The positive inotropic effect of the α_1-adrenoceptor agonists is accompanied by an increase in IP_3 and DG (Schmitz et al., 1987). It is suggested that α_1-adrenoceptors, coupled to a pertussis toxin-insensitive G-protein, activate PLC, leading to the release of intracellular Ca^{2+} and increased contractility (Schmitz et al., 1987; Bristow et al., 1988; Kohl et al., 1989; Maisch, 1991).

*Increase in intracellular Na^+ concentration:*Intracellular Na^+ concentration regulates cardiac contractility effectively by altering intracellular Ca^{2+} concentration via the Na^+/Ca^{2+} exchange. Two such mechanisms are known: inhibition of the Na^+/K^+-ATPase (cardiac glycosides), and prolongation of the open state of Na^+ channels (DPI-201, ceveratrum alkaloids). Both mechanisms increase intracellular Na^+, which is then exchanged for Ca^{2+}.

2. Increase in the Ca^{2+} sensitivity of the contractile proteins

An increase in Ca^{2+} sensitivity of the contractile proteins also enhances myocardial contractile force, since more Ca^{2+} could be bound to the contractile proteins. Some cardiotonic drugs, such as APP 201-533 (3-amino-6-methyl-5-phenyl-2(1H)-pyridinone) cause myofibrillar Ca^{2+} activation instead of enhancement of Ca^{2+} influx. These drugs may therefore provide a better therapeutic concept in the treatment of cardiac insufficiency associated with a reduced Ca^{2+} sensitivity. However, an enhanced contractility may occur

at the expense of impaired relaxation because of the easier Ca^{2+} binding. A selective positive inotropic "Ca^{2+} sensitizer" without other associated effects is still lacking.

1.4 Negative inotropic effects and agents

Biogenic substances that evoke negative inotropic effects have their receptors (also shown in figure 1.3) coupled to two kinds of G-proteins: one inhibits adenylyl cyclase, which has been designated G_i and the other activates K^+ channels, and has been named G_k.

In recent years, at least three receptor systems in the human heart were suggested to cause negative inotropic effects by a negative coupling to adenylyl cyclase: adenosine A_1 receptor, muscarinic M_2 cholinoceptor, and somatostatin receptor systems (Brodde et al., 1992).

Adenosine, a potent coronary vasodilator, is released from the human heart during the influence of catecholamines or ischemia/hypoxia and can produce negative inotropic effects in human atrial (both at baseline as well as after isoprenaline-stimulation) and ventricular (only after isoprenaline-stimulation) myocardium (Böhm et al., 1985, 1989, 1994). The effects of adenosine are mediated by adenosine A_1 receptors that are coupled to open potassium channels or to inhibit adenylyl cyclase (Böhm et al., 1989). Gupta et al. (1993) reported that adenosine agonists attenuated isoprenaline-stimulated effect in human ventricular myocytes via a cAMP-independent mechanism, mediated by adenosine A_1 receptors.

Similarly, acetylcholine, the main transmitter of the parasympathetic nervous system, induces negative inotropic responses in human myocardium (Brodde et al., 1992; Böhm et al., 1994). The effects of acetylcholine are mediated by muscarinic M_2 receptors which are coupled to both potassium channels and adenylyl cyclase in human heart (Brodde et al., 1992; Böhm et al., 1994). Animal studies show that acetylcholine may be associated with either inhibition of adenylyl cyclase (Korth and Kühlkamp, 1987; Eglen et al., 1988; Brodde et al., 1992) or stimulation of inositol-1,4,5-trisphosphate hydrolysis (Poggioli et al., 1986; Tsuji et al., 1987; Eglen et al., 1988), to elicit mixed inotropic effects mediated by the same M_2 receptors (Brodde et al., 1992).

Somatostatin, the growth hormone release-inhibiting factor, may function as a neurotransmitter in the heart and was reported to be used for the treatment of acromegaly patients (Tokgözoglu et al., 1993). It exerts negative inotropic effects in the atria of some species, including man, which are mediated via somatostatin receptors (Quirion et al., 1979; Campbell et al., 1982; Diez et al., 1985; Franco-Cerecede et al., 1987; Brodde et al., 1992). Somatostatin has been described to reduce the intracellular Ca^{2+} concentration (Hou

et al., 1987) in human atrial fibres and to inhibit the noradrenaline-induced stimulation of atrial contractility (Franco-Cereceda et al., 1987b), as well as to inhibit adenylyl cyclase activity in human ventricular myocardium (Hershberger et al., 1988). For these above three substances, further investigations are required to elucidate the effects and their mechanisms of actions in human isolated myocardium.

Vasopressin, a neurohypophyseal hormone, plays an important role in the regulation of cardiovascular function (Endoh et al., 1992). As a vasoconstrictor and antidiuretic agent, it is involved in orthostasis (Davies et al., 1976; Zerbe et al., 1983), cardiac syncope (Davies et al., 1976; Baylis and Heath, 1977), and haemorrhagic and other shock states (Hershey et al., 1964, 1965, 1968). The inotropic action of vasopressin shows a wide range of variation among mammalian species: positive inotropy in the rat (Walker et al., 1988) and cat (Schoemaker et al., 1990), but negative inotropy in the rabbit, dog (Montani et al., 1980; Endon et al., 1992) and ferret (Endon et al., 1992).

From the previous studies, it is interesting to notice that there are differences between responses in atrial and ventricular tissues. Several biogenic substances have been reported to cause negative inotropic effects in isolated atrial tissues, whereas few data are available about the direct negative inotropic effect of biogenic substances on basal contractility in ventricular tissues. Usually, ventricular contractility is regarded the most important parameter for cardiac function, but atrial tissue may contribute to a greater extent than considered.

1.5 Atrial function vs ventricular function

Existing evidence showed that the anatomical, biochemical, electrical and mechanical properties of atrial muscle are considerably different from those of ventricular muscle (Goldman et al., 1984). Atrial myocytes are smaller, branch less often, have fewer, if any, transverse tubules and contain specific granules that are not found in ventricular myocytes (McNutt and Fawett, 1969; Urthaler et al., 1975). The velocity of contraction is higher in the atrium than ventricle both in spontaneously beating heart (Korecky et al., 1974) as well as in isolated muscle preparations (Buccino et al., 1967; Korecky et al., 1974). Atrial muscle has been found to contain a unique myosin isozyme with a higher ATPase activity which is associated with the greater velocity of atrial contraction (Korecky et al., 1974; Long et al., 1977; Flink et al., 1978; Sartore et al., 1978). The results from Urthaler et al. (1975) indicate that the contractile performance of right atrial muscle is in many respects superior to that of right ventricular muscle. The atrial function is more affected by vagally mediated reflexes than ventricular function (Williams et al., 1965; Goldman et al., 1983).

In several mammalian species (human, dog, cat, and frog), the Ca^{2+}-induced release of Ca^{2+} is more developed in the atrium than in the ventricle (Fabiato et al., 1977, 1978, 1979, 1982). There are also significant differences between the pharmacological responses of atrial and ventricular muscles (Böhm et al., 1989; Kemmer et al. 1989; Schwinger et al., 1991; Jahnel et al., 1992; Holubarsch et al., 1993). These unique characteristics of atrial myocardium may have relevant physiological consequences, that should not be ignored.

The atrium functions principally as a blood reservoir and an entryway to the ventricle, but it also pumps weakly to help moving the blood into the ventricle. The ventricle in turn supplies the main force that propels the blood through either the pulmonary or the peripheral circulation. Because of the importance (as a power pump) in pumping blood to the peripheral circulation, abundant information about the mechanical characteristics of the left ventricle is available (Fry et al., 1964; Glick et al., 1965; Taylor et al., 1967). The left ventricle therefore has become a target for pharmacological therapy to increase cardiac function.

Atrial contraction usually causes an additional 20 to 30% of blood flow filling the ventricle. However, under normal resting conditions, the heart can continue to operate quite satisfactory without this extra 30% atrial contribution. It is capable of pumping 300 to 400% more blood, if the body needs that (Guyton, 1986). Accordingly, few investigations have devoted specific attention to the atrium.

However, atrial function may be underestimated. Evidence suggests that atrium may play a special and important physiological role in cardiac function. The importance of right atrium for heart function is obvious: the electrical impulse controlling contraction originates in sinoatrial node located in right atrium. In intact heart, the left atrium serves as a conduit for the passage of blood from the pulmonary vein to the left ventricle, as a reservoir for storing blood during left ventricular systole, and as a contractile chamber for augmentation of left ventricular filling (Matsuda et al., 1983). Specially in pathological conditions, left atrial contraction makes a significant contribution to left ventricular function. Atrial contraction made a larger contribution to left ventricular end-diastolic pressure (39 vs 20%), and stroke volume (35 vs 22%) in patients with myocardial infarction than in those without myocardial infarction, respectively (Rahimtoola et al., 1975). The ratio of active atrial emptying to left ventricular stroke volume in patients with myocardial infarction was significantly larger (42±12%) than in normal subjects (29±10%) (Matsuda et al., 1983). Thus, although under normal conditions, atrial contraction may not be crucial for cardiac function, under pathological conditions, such as myocardial infarction, the atrial contraction has become a substantial contribution to the impaired cardiac function.

Atrial tissue can be clearly distinguished from ventricular tissue, which may have consequences for pharmacological response. Although, it has been reported that atrial and ventricular tissues respond differently to pharmacological stimulation, the undergoing mechanisms, and the consequences of these differences are not elucidated. Moreover, in contractility studies, atrial tissue is commonly used as representative of "cardiac tissue". A study of the contractile regulation of atrial and ventricular myocardium in parallel is needed for better understanding the physiology of atrial and ventricular function.

1.6 Aim of the present thesis

The agonists and antagonists of biogenic substances, in particular, acetylcholine, catecholamines and histamine have been used in therapy for many years. Nowadays, more substances such as adenosine, angiotensin II, CGRP and somatostatin are under investigation as they may also play a role in the physiology and pathophysiology of the cardiovascular system, and thus providing novel therapeutical perspectives. With regard to the development of new inotropic agents, it is important to investigate the effects of potential drugs on human cardiac tissue.

In vivo, the changes in force of contraction in the heart can be markedly influenced by reflex mechanisms, and interactions with multiple factors, such as heart rate, preload, afterload, and neurohumoral factors. Therefore, isolated, electrically driven cardiac preparations provide a useful tool to examine the direct effects of substances on cardiac contractility under controlled conditions. To distinguish atrial and ventricular responses, these tissues should be studied separately, which requires in vitro methods.

This leads to the two major purposes of this thesis:

1. To investigate the effects and the possible mechanisms of several biogenic substances (5-HT, histamine, human α-CGRP, angiotensin II, adenosine, somatostatin and acetylcholine) on contractility in human isolated myocardium.
2. To explore the differences in the pharmacology of human isolated atrial and ventricular trabeculae. Special attention will be paid to responses of right atrial and left ventricular trabeculae from the same heart.

In addition, some studies will be performed on porcine cardiac tissue in parallel, to evaluate this as a model for human cardiac tissue. If the results from porcine heart are comparable with human's, it may serve as a relevant and convenient model for human myocardium. From the results, we anticipate to compare the responses in different tissues and different species. To our knowledge, this is the first study on contractile responses of the atrial and ventricular tissues from the same heart in parallel.

Chapter 2 5-Hydroxytryptamine increases contractile force in porcine right atrium but not in left ventricle

2.1 Summary

Positive chronotropic as well as inotropic effects of 5-hydroxytryptamine (5-HT) have been observed in pig atrial tissue, but no data are available about the direct effects of 5-HT on ventricular tissue. In the present study we investigated inotropic effects of 5-HT on atrial and ventricular trabeculae obtained from hearts of 3 months old pigs. The baseline isometric contractile force was significantly higher in ventricular (422±128 mg) than in atrial tissue (48±11 mg). A noradrenaline concentration-response curve (10^{-8} to 10^{-5} M) was used to check contractile responsiveness of the tissue and all responses were expressed as percentage of the response to 10^{-5} M noradrenaline. Noradrenaline caused a concentration-dependent increase in contractile force in both atrial and ventricular trabeculae. In contrast, though 5-HT (10^{-8} to 10^{-4} M) did increase force of contraction in atrial tissue (maximum: 72±20 % of the response to noradrenaline 10^{-5} M), the contractility of ventricular trabeculae was not significantly affected (maximum: 12±6 %). The present data show that, in contrast to atrial tissue, contractile force of ventricular tissue could not be significantly affected by 5-HT. To our knowledge, this is the first study to show that an agent which increased force of contraction in the atrium, did not have a corresponding effect on the ventricle. These findings may have important implications for a better understanding of the physiology and pharmacology of cardiac contractility.

2.2 Introduction

In 1985, Duncker et al. reported that 5-hydroxytryptamine (5-HT) elicited tachycardia in the anaesthetized pig, and that this response could not be attributed to stimulation of the then known 5-HT receptors. After extensive study, it was concluded that the 5-HT-induced tachycardia was mediated by a new 5-HT receptor type that was clearly different from 5-HT_1, 5-HT_2 or 5-HT_3 receptors (Bom et al., 1988). In parallel, a novel 5-HT receptor site, positively coupled to adenylyl cyclase and designated as 5-HT_4, was located in cultured neurones from mouse embryo colliculi (Dumuis et al., 1988, 1989). Indeed, the receptor involved in the tachycardic response to 5-HT in the anaesthetized pig could be characterized as a putative 5-HT_4 receptor, similar to the one found in the mouse embryo colliculi (Villalón et al., 1990, 1991).

The tachycardic response to 5-HT was suggested to result from a direct action on the cardiac pacemaker cells. *In vitro* studies in piglet isolated right atria confirmed a direct effect of 5-HT on sinus rhythm, mediated by 5-HT_4 receptors (Kaumann 1990a). In addition to the

chronotropic effect, a direct inotropic effect of 5-HT, also mediated by 5-HT_4 receptors, was observed in porcine isolated atrial tissue (Kaumann et al., 1991a).

The above mentioned studies clearly establish direct 5-HT_4 receptor-mediated chronotropic and inotropic effects on the atrial tissue. However, direct effects of 5-HT on the ventricle have not been described. Using the maximum rate of rise of left ventricular pressure (dP/dt_{max}) as an index for ventricular contractility, a recent *in vivo* study in pigs showed a significant inotropic response to 5-HT. However, when heart rate was kept constant by atrial pacing, the response was substantially attenuated, making it equivocal whether 5-HT had a direct effect on the left ventricle (Saxena et al., 1992). Therefore, the aim of the present study was to investigate whether a direct inotropic effect of 5-HT can be demonstrated in porcine isolated left ventricular tissue. For comparison, inotropic effects of 5-HT were studied in isolated right atrial tissue.

2.3 Material and methods

2.3.1 General

Right atrial and left ventricular tissues were obtained from 3 months old pigs, used as saline-controls in *in vivo* experiments (under pentobarbital aneasthesia, 20 mg/kg.h). The tissues were placed in ice-chilled oxygenated Kreb's buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 8.3), and trabeculae (1 mm thickness) were carefully dissected free. The trabeculae were mounted in organ baths (37°C, gassed with 95% O_2 and 5% CO_2), and attached to Harvard isometric transducers. Resting tension was set to approximately 750 mg for atrial and 1950 mg for ventricular tissue, to provide optimal loading conditions. Tissue was paced at 1.5 Hz, using field stimulation (3 ms, voltage 20% above threshold).

2.3.2 Experimental protocol

After a stabilization period of at least 60 min, baseline contractile force was recorded and a concentration-response curve for noradrenaline was obtained to check the viability of the tissue. Since the tissue was found to be irreversibly damaged after adding 10^{-4} M noradrenaline, the maximal concentration of noradrenaline was limited to 10^{-5} M, and all responses were expressed as percentage of the response to this concentration. Tissues with less than 25 mg response to 10^{-5} M noradrenaline were excluded from further analysis. After washing and restabilization a concentration-response curve for 5-HT (10^{-8} to 10^{-4} M) was obtained. Following another washing and stabilization period, the concentration-response

curve for noradrenaline was repeated. For two ventricular trabeculae the above protocol was performed during inhibition of neuronal uptake by cocaine (3×10^{-5} M) to check the influence of this process on the effects of 5-HT.

To evaluate the involvement of 5-HT_4 receptors in the atrial response to 5-HT, following the control noradrenaline concentration-response curve, 4 atrial preparations were incubated for 30 min with tropisetron (3×10^{-6} M), which blocks the 5-HT_4 receptors (Sanders and Kaumann, 1992). A concentration-response curve for 5-HT was then obtained, followed by another noradrenaline concentration-response curve.

2.3.3 Data presentation and analysis

Data are presented as mean ± S.E.M. Baseline values for atrial and ventricular tissue were compared using an unpaired Student's t-test. Effects of noradrenaline and 5-HT were analyzed using an ANOVA for repeated measurements. Differences were regarded statistically significant, if $p<0.05$.

2.3.4 Drugs

The following drugs were used in the present investigation: norepinephrine bitartrate and 5-hydroxytryptamine creatinine sulphate complex (Sigma, St. Louis, MO, U.S.A.), cocaine hydrochloride (O.P.G. Utrecht, The Netherlands) and tropisetron (ICS 205-390-ch, gift from Sandoz, Basle, Switzerland). Drugs were dissolved in distilled water (5-HT in 25 mM HCl) and fresh solutions were prepared each day.

2.4 Results

An example of the recording obtained from atrial and ventricular trabeculae is shown in figure 2.1. In both tissues noradrenaline increased the force of contraction in a concentration-dependent manner. 5-HT clearly affected the atrial contractile force, but, in contrast, was virtually inactive in the ventricular preparation.

Mean results from all experiments showed that baseline contractile force was significantly lower in atrial (48±11 mg, n=8) than in ventricular tissue (422±128 mg, n=13). As presented in figure 2.2, the first noradrenaline concentration-response curve again shows an increase in contractile force in both atrial and ventricular tissue. The noradrenaline-induced increase in contractile force was not maximal at 10^{-5} M, therefore EC_{50} values could not be calculated. The submaximal response to this noradrenaline concentration was significantly higher in ventricular than in atrial trabeculae; 293±71 mg compared to 94±20 mg.

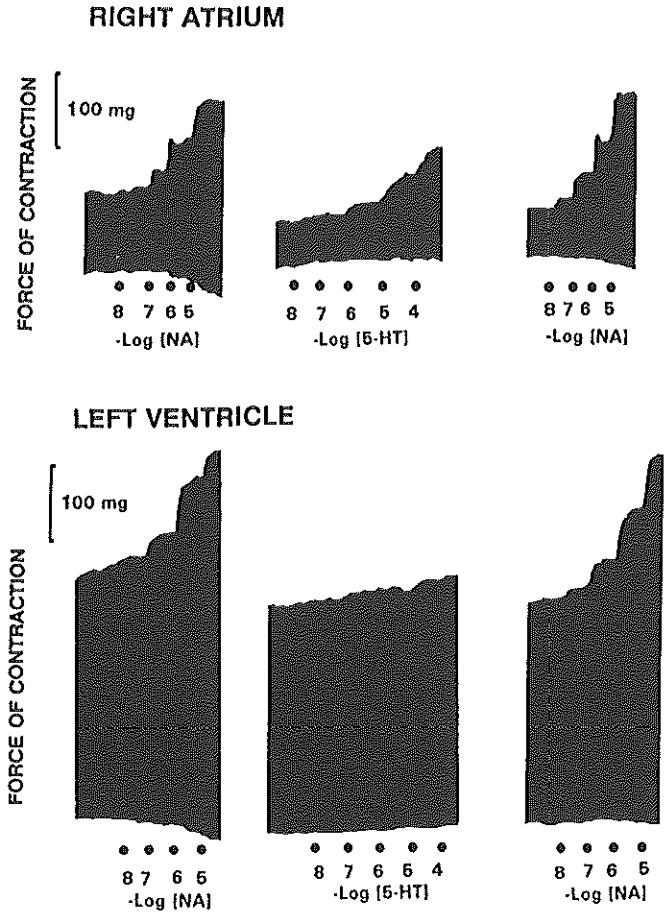


Figure 2.1. Recordings of the force of contraction in atrial and ventricular trabeculae, and the effects of increasing concentrations of 5-HT and noradrenaline, before and after 5-HT.

The concentration-response curves for 5-HT show a clear increase in atrial contractile force (maximum: 72±20 % of the response to 10^{-5} M noradrenaline) (figure 2.2A), whereas no significant effect could be demonstrated on ventricular contractile force (maximum: 12±6 % of the response to 10^{-5} M noradrenaline) (figure 2.2B). The atrial contractile force increased more than 100 % (from 48±11 mg at baseline to 104±13 mg at 10^{-4} M 5-HT), whereas the

ventricular tissue responded with only 7 % increase (from 407±112 mg at baseline to 439±118 mg at 10^{-4} M 5-HT). The absence of a significant ventricular response was also observed in the presence of cocaine (data not shown).

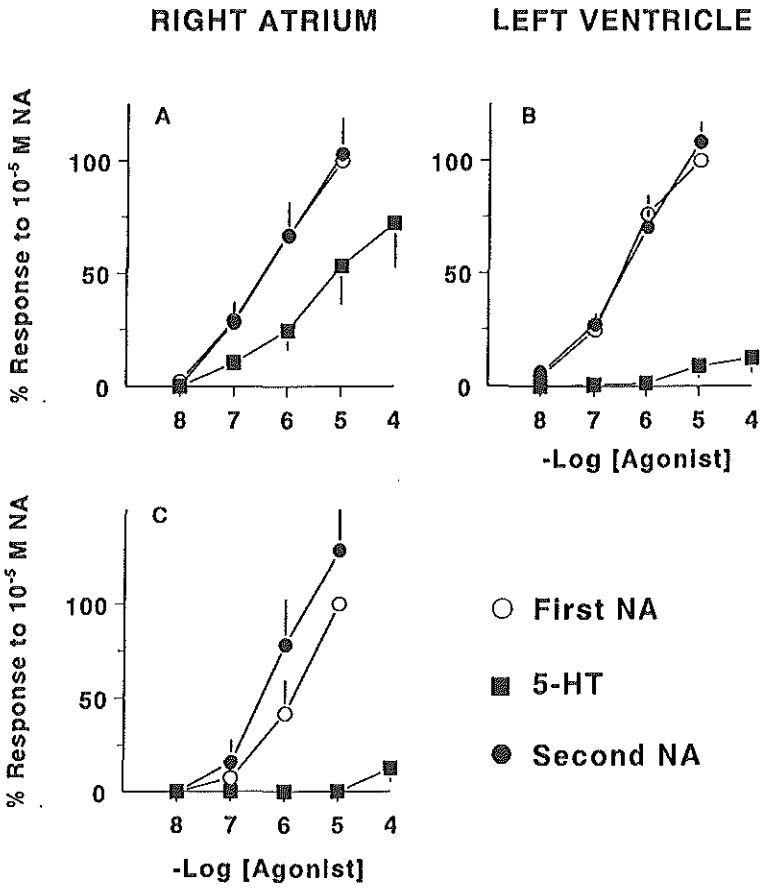


Figure 2.2. Concentration-effect curves for noradrenaline (NA) and 5-HT in pig atrial (n=8, panel A) and ventricular trabeculae (n=13, panel B), and in atrial trabeculae incubated with tropisetron (3×10^{-6} M) after the first noradrenaline curve (n=4, panel C). Responses to 5-HT are expressed as percentage of response to 10^{-5} M noradrenaline (First NA). The noradrenaline curve after 5-HT (Second NA) is presented to indicate that the lack of response to 5-HT in ventricular preparations was not due to deterioration of the tissue, and that tropisetron selectively blocked the 5-HT response.

During the course of the experiment no time related deterioration of the tissue occurred, since the response to noradrenaline remained unchanged (figure 2.2A+B); for atrial tissue, the response to 10^{-5} M noradrenaline after 5-HT was 103±15 % of the response to this concentration before 5-HT, while for ventricular tissue this was 108±8 %.

Tropisetron effectively blocked the inotropic effects of 5-HT in the atrial trabeculae (figure 2.2C); in the presence of tropisetron, 5-HT did bot increase contractile force at concentrations from 10^{-8} 10^{-5}, while 10^{-4} M 5-HT caused a minor effect of 12±7 % (n=4) of the response to noradrenaline 10^{-5} M. The response to noradrenaline was not attenuated by tropisetron (figure 2.2C); response to 10^{-5} M noradrenaline in the presence of tropisetron was 128±24 % of the response to this concentration during the control noradrenaline curve.

2.5 Discussion

In the present study, we compared the inotropic effects of 5-HT in pig right atrial trabeculae to those in left ventricular trabeculae. The baseline contractile force was significantly higher in ventricular than in atrial trabeculae. Since the amount of tissue for atrial and ventricular preparations was comparable, this difference may represent the different in-vivo functions of the two structures from which the trabeculae were obtained. Before administering 5-HT, we checked the viability of the tissues with noradrenaline, which induces inotropic effects using the same second messenger system, adenylyl-cyclase/cAMP (Brodde, 1991), as suggested for the 5-HT_4 receptor-mediated inotropic effects in atrial tissue (Kaumann et al., 1990a). Noradrenaline increased force of contraction in atrial as well as ventricular trabeculae in a concentration-related way, indicating that the mechanism of increasing force of contraction through the adenylyl cyclase/ cAMP pathway was intact.

5-HT caused concentration-dependent increases in contractile force to 72% of the response to 10^{-5} M noradrenaline in pig right atrial trabeculae, and 5-HT-induced responses could be effectively blocked by 3×10^{-6} M tropisetron. This is in accordance with earlier findings, where the positive inotropic (left atria) and chronotropic (spontaneously beating right atria) effects of 5-HT have been shown to be mediated by 5-HT_4 receptors, coupled to the adenylyl cyclase/ cAMP second messenger system (Kaumann, 1990a; Kaumann et al., 1991a). However, in contrast to the atrial tissue, we now report that 5-HT failed to significantly increase force of contraction in ventricular tissue, substantiating the poor *in vivo* inotropic effects of 5-HT during atrial pacing in anaesthetized pigs (Saxena et al., 1992). This absence of ventricular response could not be explained by a time related deterioration of the ventricular tissue. The unchanged noradrenaline responses (after 5-HT) would imply that the mechanism of increasing contractile force via the adenylyl-cyclase/cAMP pathway was still intact.

To our knowledge, our data demonstrate for the first time that in the mammalian heart an agent can elicit a positive inotropic response in the atria without having a corresponding effect on the ventricles. Although a functional explanation for the atrium/ventricle difference is not yet known, this phenomenon may contribute to a better insight in the physiology of atrial and ventricular contractility. Furthermore, positive inotropic effects of 5-HT, mediated by 5-HT_4 receptors, have been reported in human right (Kaumann et al., 1991b) and left atrial (Sanders and Kaumann, 1992) tissue. In view of the similarities between human and pig atrial tissue with regard to the effects of 5-HT, it would be important to establish the response to 5-HT in human ventricular tissue, with special emphasis on the development of 5-HT_4 agonists as positive inotropic agents.

In conclusion, the results of this study demonstrate that force of contraction can be studied in isolated right atrial and left ventricular trabeculae of the pig. Whereas in atrial trabeculae force of contraction can be significantly increased by noradrenaline and 5-HT, the ventricular trabeculae respond to noradrenaline, but not significantly to 5-HT. A similar lack of effect of 5-HT on human ventricle could have important consequences from the point of view of drug development.

Chapter 3 5-Hydroxytryptamine stimulates human isolated atrium but not ventricle

3.1 Summary

Although 5-hydroxytryptamine (5-HT) elicits positive inotropic effects on the human isolated atrium via 5-HT_4 receptors, no data are available about the effects on the ventricular myocardium. We investigated the inotropic effects of 5-HT in human healthy ventricular trabeculae and compared it to the effects in right atrial trabeculae. Baseline contractile force as well as the response to noradrenaline, used to check inotropic responsiveness of the tissue, were significantly higher in ventricular (391±10 and 719±126 mg, respectively) than in atrial tissue (189±5 and 383±79 mg, respectively). However, 5-HT increased force of contraction in atrial trabeculae up to 309±82 mg at 10^{-4} M, but completely failed to affect contractile force of ventricular tissue. We conclude that, in contrast to atrial tissue, 5-HT is ineffective as positive inotropic agent in human ventricular trabeculae. This finding obviously rules out development of 5-HT_4 receptor agonists for the treatment of heart failure, but suggests the absence of ventricular side effects with the potential use of 5-HT_4 receptor (ant)agonists in gastrointestinal disorders.

3.2 Introduction

5-Hydroxytryptamine (5-HT) affects the mammalian heart by a variety of receptors. The receptors involved vary with species; for example, 5-HT-induced tachycardia is mediated by 5-HT_1 (cat), 5-HT_2 (rat, dog), 5-HT_3 (rabbit, dog) or 5-HT_4 (pig) receptors (Villalón et al., 1990; Saxena and Villalón, 1991). Recently, 5-HT_4 receptors, which are positively coupled to adenylyl cyclase (Dumuis et al., 1989), have received more attention for being involved in the positive inotropic response to 5-HT. The 5-HT-induced increase in contractile force in porcine left atrium (Kaumann et al., 1991a) and human right (Kaumann et al., 1991b) as well as left (Sanders and Kaumann, 1992) isolated atrial tissue is mediated by 5-HT_4 receptors. Although, we recently reported a lack of inotropic responses to 5-HT in porcine left ventricular tissue (Schoemaker et al., 1992), direct effects of 5-HT on human ventricular tissue are not known. Despite the potential disadvantage of ensuing tachycardia, a contractile effect of 5-HT on the ventricular tissue may open opportunities to develop new positive inotropic agents. Therefore, in the present study we investigated the effects of 5-HT on human healthy left ventricular tissue. For comparison, inotropic responses to 5-HT were also studied in the right atrial tissue.

3.3 Material and methods

Hearts, obtained via Eurotransplant (Leiden/Rotterdam) from donor subjects dying from non-cardiac causes, were kept in cold cardioplegic solution for 4-20 h. Right atrial and left ventricular trabeculae of approximately 1 mm thickness were carefully dissected and mounted in the organ baths containing Krebs' solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 8.3), gassed with 95% O_2 and 5% CO_2 at 37 °C. Resting tension was set to 750 mg for atrial tissue and 1950 mg for ventricular tissue, to provide optimal loading conditions. The tissue was paced at 1 Hz and tension development was recorded using a Harvard isometric force transducer. After stabilization for at least 60 min, a cumulative concentration-response curve for noradrenaline was obtained to check tissue viability. Since trabeculae were irreversibly damaged at 10^{-4} M noradrenaline, the maximal concentration of noradrenaline was restricted to 10^{-5} M, and all effects were expressed as percentage of this response. Subsequently, after washing and restabilization for at least 30 min, a cumulative concentration-response curve for 5-HT (10^{-8} to 10^{-4} M) and, after again washing and stabilization, another one for noradrenaline were obtained. For two ventricular preparations the above protocol was performed during inhibition of neuronal uptake by cocaine (3×10^{-5} M) to check the influence of this process on the effects of 5-HT. To evaluate the involvement of 5-HT_4 receptors in the atrial response to 5-HT, following the control noradrenaline concentration-response curve, 6 atrial preparations were incubated for 30 min with 3×10^{-6} M tropisetron, used as an antagonist of 5-HT_4 receptors (Sanders and Kaumann, 1992). A concentration-response curve for 5-HT was then obtained, followed by another noradrenaline concentration-response curve.

Data are presented as means±S.E. Baseline values of atrial and ventricular tissue were compared using an unpaired Student' *t*-test. Effects of noradrenaline and 5-HT were analyzed using an ANOVA for repeated measurements. Differences were regarded statistically significant, if $p < 0.05$. The study was approved by our ethics committee.

The drugs used in the present investigation were: norepinephrine bitartrate and 5-hydroxytryptamine creatinine sulphate complex (Sigma, St. Louis, MO, U.S.A.), cocaine hydrochloride (O.P.G. Utrecht, The Netherlands) and tropisetron (ICS 205-390-ch, gift from Sandoz, Basle, Switzerland). Drugs were dissolved in distilled water (5-HT in 25 mN HCl) and fresh solutions were prepared each day.

3.4 Results

An example of the recording obtained from atrial and ventricular trabeculae is shown in figure 3.1. In both tissues noradrenaline increased the force of contraction in a concentration-dependent manner. 5-HT clearly affected the atrial contractile force, but, in contrast, was virtually inactive in the ventricular preparation.

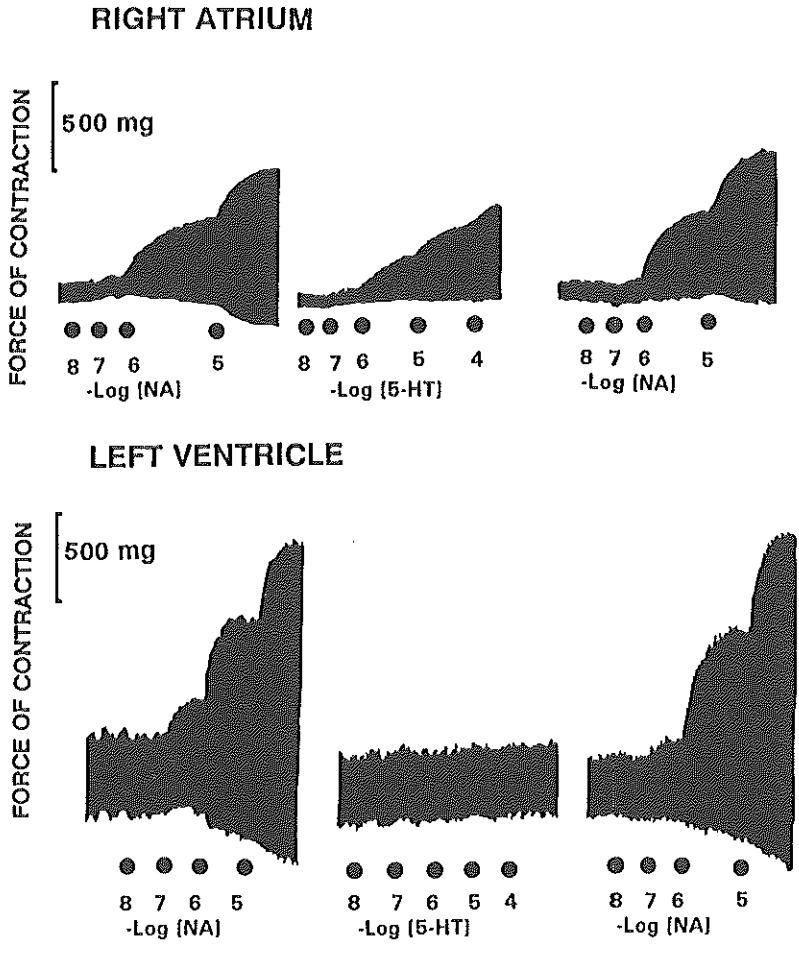


Figure 3.1. Recordings of the force of contraction in atrial and ventricular trabeculae, and the effects of increasing concentrations of 5-HT and noradrenaline, before and after 5-HT.

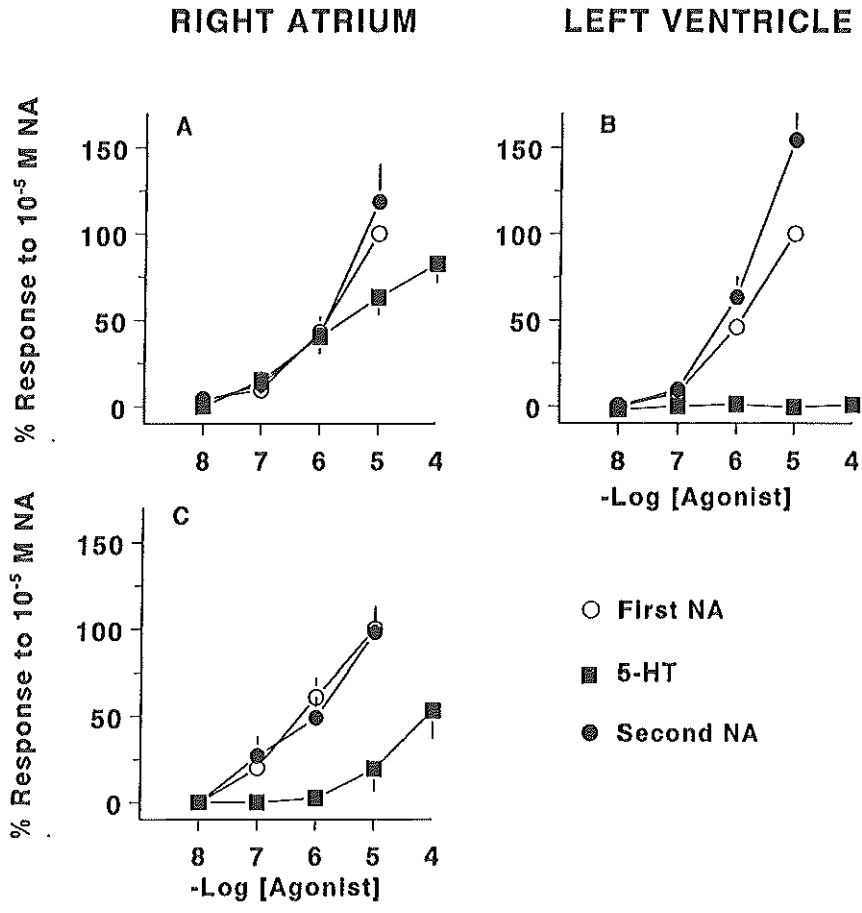


Figure 3.2. Concentration-effect curves for noradrenaline (NA) and 5-HT in atrial (n=12, panel A) and ventricular (n=10, panel B) trabeculae, and in atrial trabeculae incubated with tropisetron (3×10^{-6} M) after the first noradrenaline curve (n=6, panel C). Responses to 5-HT are expressed as percentage of response to 10^{-5} M noradrenaline (First NA). The noradrenaline curve after 5-HT (Second NA) is presented to indicate that the lack of response to 5-HT in ventricular preparations was not due to deterioration of the tissue, and that tropisetron selectively blocked the 5-HT response.

Mean results showed that baseline contractile force was significantly higher in ventricular (391±76 mg; n=10) than in atrial (189±55 mg; n=12) trabeculae. As presented in figure 3.2, noradrenaline caused concentration-dependent increases in atrial and ventricular contractile force, which were at the highest concentration, 10^{-5} M, 383±79 mg and 719±126 mg for atrial and ventricular tissue, respectively. Since the responses to 10^{-5} M noradrenaline were not maximal, EC_{50} values could not be calculated. 5-HT increased atrial force of contraction (figure 3.2, panel A) approximately 2.5 times (from 223±66 mg to 532±96 mg at 10^{-4} M). The atrial responses to 5-HT were effectively antagonized by $3x10^{-6}$ M tropisetron (a rightward shift in the 5-HT concentration response curve of approximately 1.6 log units), while the responses to noradrenaline were not affected (figure 3.2, panel C).

In contrast to the atrial tissue, no inotropic effect of 5-HT could be demonstrated in the ventricular tissue (figure 3.2, panel B). The absence of ventricular response was also observed in the presence of cocaine (data not shown). During the course of the experiment no time related deterioration of the tissue occurred, since the noradrenaline concentration-response curve obtained after 5-HT was not depressed compared to the one obtained before 5-HT (figure 3.2, panels A and B).

3.5 Discussion

In the present study, we compared the inotropic effects of 5-HT in human left ventricular trabeculae to those in right atrial trabeculae. The baseline contractile force was significantly higher in ventricular than in atrial trabeculae. Before administering 5-HT, we checked the viability of the tissues with noradrenaline, which induces inotropic effects using the same second messenger system, adenylyl-cyclase/cAMP (Brodde, 1991), as suggested for the 5-HT_4 receptor-mediated inotropic effects in atrial tissue (Kaumann et al., 1991b). Noradrenaline increased force of contraction in atrial as well as ventricular trabeculae in a concentration-related way, indicating that the mechanism of increasing force of contraction through the adenylyl cyclase/cAMP pathway was intact.

5-HT caused concentration-dependent increases in contractile force to 82% of the response to 10^{-5} M noradrenaline in human right atrial trabeculae, and the effect of 5-HT was effectively blocked by 3×10^{-6} M tropisetron, without the modification of the response noradrenaline. This is in accordance with earlier findings; the atrial positive inotropic effects of 5-HT were sensitive to tropisetron, indicating mediation by 5-HT_4 receptors, coupled to the adenylyl cyclase/cAMP second messenger system. Moreover, atrial 5-HT effects were insensitive to ß-blockade, indicating a lack of indirect effect via release of noradrenaline

(Kaumann et al., 1991b, Sanders and Kaumann, 1992). However, in contrast to the atrial tissue, we now report that 5-HT failed to increase force of contraction in ventricular tissue. The absence of a ventricular response supports the lack of 5-HT-induced release of noradrenaline from sympathetic neurons, and cannot be explained by a time related deterioration of the ventricular tissue. The unchanged noradrenaline responses after 5-HT imply that the mechanism of increasing contractile force via the adenylyl-cyclase/cAMP pathway was still intact. The results of the present study, showing tropisetron-sensitive inotropic responses to 5-HT in atrial but not in ventricular tissue, are similar to those we recently reported in hearts of 3 months old pigs (Schoemaker et al., 1992).

In conclusion, the most salient finding of the present study is that, in contrast to atria, 5-HT fails to affect the ventricular tissue. To our knowledge, this report demonstrates for the first time that in the human heart an agent can elicit a positive inotropic response in the atrium without having a corresponding effect on the ventricle. Besides possible contribution to a better insight in the physiology of atrial and ventricular contractility, the present findings have important pharmacological implications. They obviously rule out the development of 5-HT_4 receptor agonists for the treatment of heart failure and, in addition, predict lack of ventricular side-effects during anticipated use of 5-HT_4 receptor agonists and antagonists in gastrointestinal disorders (Clarke et al., 1989).

Chapter 4 Effects of histamine on porcine isolated myocardium: differentiation from effects on human tissue

4.1 Summary

Inotropic effects of histamine have been extensively studied in many species. However, data about the porcine myocardium, which is often used as a model for the human heart, are not available. We investigated inotropic effects of histamine on atrial and ventricular trabeculae obtained from porcine hearts. For comparison, we also evaluated the effects of histamine on human myocardium. Histamine caused concentration-dependent increases in contractile force in porcine and human atrial tissue (at 10^{-3} M: 267±70 mg and 317±81 mg, or 133±17% and 85±12% of the response to 10^{-5} M noradrenaline, respectively) as well as in porcine and human ventricular tissue (at 10^{-3} M: 592±148 mg and 773±203 mg, or 68±13% and 122±61% of response to 10^{-5} M noradrenaline, respectively). Cimetidine, but not mepyramine, antagonized the contractile effects of histamine in porcine and human atrial, and in human ventricular tissues. In contrast, the histamine-induced positive inotropic effect in porcine ventricular tissue was antagonized by mepyramine, but not by cimetidine. Propranolol failed to block the inotropic effect of histamine in all four tissues. These results indicate that, similar to human atrial trabeculae, the positive inotropic effect on porcine atrial trabeculae is mediated by H_2 receptors. In contrast to human ventricular trabeculae, however, the positive inotropic effect on porcine ventricular trabeculae appears to be mediated by H_1 receptors.

4.2 Introduction

Histamine elicits positive inotropic effects on the hearts of various animal species (Levi et al., 1982). The effects of histamine apparently are mediated in the dog by H_1 receptors (Chiba et al., 1976; Chiba, 1977), in the monkey by H_2 receptors (Hattori et al., 1883), in the guinea-pig (Black et al., 1972; Ledda et al., 1977; Verma et al., 1977) and rabbit (Elizalde, 1986; Hattori et al., 1988, 1991) by both H_1 and H_2 receptors, and in the cat and rat (Laher and McNeill, 1979, 1980) by endogenous catecholamine release. In the human heart, the effects of histamine on contractile function, both *in vivo* and *in vitro*, have been studied extensively. The increase in force of contraction in the atrium and ventricle are mainly mediated by H_2 receptors, although both receptor subtypes, H_1 and H_2, may exist in the heart (Ginsburg et al., 1980a,b, 1981; Guo et al., 1984). Thus, the cardiac effects of histamine show species differences in their mechanism of action and in the type of histamine receptors involved (Owen, 1977).

Another substance that shows clear species difference in its cardiac responses with respect to the receptor type involved is 5-hydroxytryptamine (5-HT) (Saxena and Villalòn, 1991). Recently, however, we demonstrated that the contractile effects of 5-HT were similar in human and porcine cardiac tissue. In both species, 5-HT increased contractile force in atrial tissue, mediated by 5-HT_4 receptors (Kaumann et al., 1991a,b; Schoemaker et al., 1992, 1993), but it failed to affect the force of ventricular contraction (Jahnel et al., 1992; Schoemaker et al., 1992, 1993). So far, the pig appears to be the only species in which the effects of 5-HT on cardiac tissues mimic those in humans. If the finding for 5-HT could be extended to other substances, such as histamine, the porcine isolated cardiac tissue may serve as a relevant and convenient model for the human myocardium.

We investigated the effects of histamine in porcine isolated atrial and ventricular myocardium, as well as the receptors involved in its effects. For comparison, the responses to histamine in the human myocardium were also evaluated.

4.3 Material and methods

4.3.1 Preparations

Right atrial and left ventricular trabeculae were obtained from hearts of 3 months old Yorkshire pigs used as saline-controls in other *in vivo* experiments (under pentobarbital anaesthesia, 20 mg/kg.h) and from 7 heart beating organ donors (5 male, 2 female; age 11-42 years), who died of non-cardiac disorders (1 cerebrovascular accident, 5 polytrauma, 1 encephalopathy) less than 24 h before the tissue was taken to the laboratory. The hearts were kindly provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation / Eurotransplant Foundation) after removal of the aortic and pulmonary valves for homograft valve implantation. These hearts were stored at 0-4 °C in a sterile organ protecting solution (UW, EuroCollins, or HTK-Brettschneider, see Ploeg et al., 1992) immediately after circulatory arrest. After excision, tissue samples were placed in ice-chilled oxygenated Kreb's buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, $KHPO_4$ 1.2 and glucose 8.3) and trabeculae (<1 mm thickness) were carefully dissected free. The trabeculae were mounted in organ baths (15 ml Kreb's buffer, 37°C, gassed with 95% O_2 and 5% CO_2), and attached to Harvard transducers for isometric tension measurement. Resting tension was set to 750 mg for atrial, and to 1950 mg for ventricular tissue in order to provide optimal loading conditions. Tissues were paced at 1.5 Hz for porcine and 1 Hz for human tissue, using electrical field stimulation

(3 ms, voltage 20% above threshold), according to the different heart rates *in vivo* of the two species.

4.3.2 Experimental protocol

After stabilization, resting tension and baseline contractile force were measured. A concentration-response curve to noradrenaline was obtained to check inotropic responsiveness of the tissues. Since the tissues were found irreversibly damaged after exposure to 10^{-4} M (the baseline contractile force decreased after washout and did not recover in reasonable time), the maximum concentration of noradrenaline was restricted to 10^{-5} M. Since no maximum was reached for the concentration-response curve for noradrenaline, pD_2 values were not calculated. Tissues with less than 25 mg response were excluded from further analysis. Responses to histamine were expressed as percentage of the response to 10^{-5} M noradrenaline.

After washing (six times) and stabilization (at least 10 min), the porcine atrial and ventricular tissues were divided into four groups. In group 1, serving as control, a concentration response curve for histamine (10^{-6} M to 10^{-3} M) was obtained without antagonists. In the three other groups, a histamine concentration-response curve was obtained after 30 min incubation with either 10^{-5} M cimetidine to block H_2 receptors (English et al., 1986), 10^{-7} M mepyramine to block H_1 receptors (Black et al., 1982) or 10^{-6} M propranolol to investigate a possible indirect ß-adrenoceptor-mediated effect via endogenous noradrenaline release (Ginsburg et al 1980a). In order to avoid possible desensitization to histamine, only one histamine curve per preparation was obtained. After washing and restabilization, another noradrenaline concentration-response curve was obtained in each preparation to check viability of the tissue.

Since the effects of histamine on the human cardiac tissue are known, only two groups of experiments were performed; control and after incubation of 10^{-5} M cimetidine.

4.3.3 Data presentation and analysis

Data are presented as mean± S.E.M. Baseline values for atrial and ventricular tissue were compared using an unpaired Student's t-test. The effects of noradrenaline and histamine were analyzed using an ANOVA for repeated measurements. Differences were regarded statistically significant, if $p<0.05$. In the case of a complete concentration response curve (for control histamine the response to 10^{-3} M was taken as the maximum response), curves were fitted to a four parameter logistic function (Lean et al., 1987) which provided the PD_2 values.

4.3.4 Drugs used

The compounds used in the present study were: noradrenaline bitartrate and histamine dihydrochloride (Sigma, St. Louis, MO, U.S.A.), cimetidine base (Smith Kline Beecham, Rijswijk, The Netherlands), propranolol hydrochloride (Imperial Chemical Industries Limited, Macclesfield, U.K.), mepyramine maleate (Rhóne-poulenc, Paris, France).

4.4 Results

4.4.1 Porcine myocardium

4.4.1.1 Effects of noradrenaline and histamine. An example of the recording obtained from atrial and ventricular trabeculae is shown in figure 4.1. In both tissues noradrenaline and histamine increased contractile force in a concentration-dependent manner. In all ventricular tissues, at each histamine concentration the positive inotropic effect was preceded by a transient negative inotropic effect.

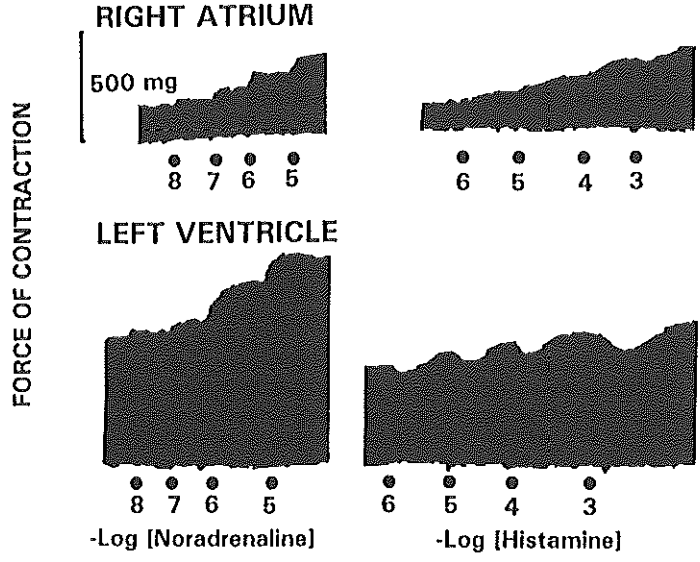


Figure 4.1. Recording of the effects of cumulative concentrations of noradrenaline (10^{-8}-10^{-5} M) and histamine (10^{-6}-10^{-3} M) on porcine atrial and ventricular trabeculae. Note that in ventricular tissue the positive inotropic effect of histamine is preceded by a transient negative inotropic effect.

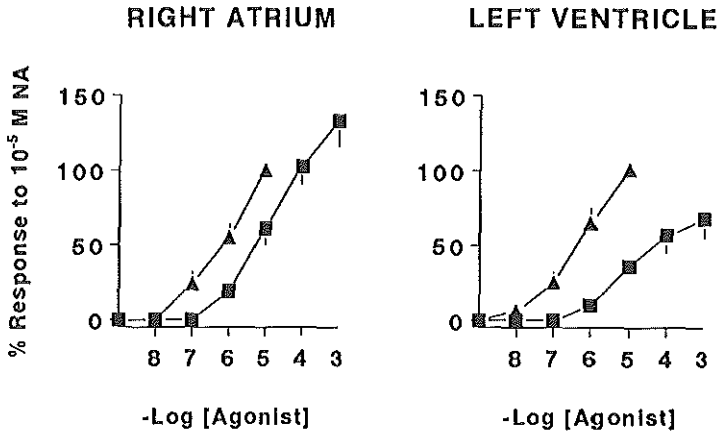


Figure 4.2. Cumulative concentration response curves for histamine (■) in porcine atrial (n=7) and ventricular (n=6) tissues, as compared to the contractile effects of noradrenaline (▲).

The mean concentration-response curves are shown in figure 4.2. After stabilization, the baseline contractile force was 134±36 mg (n=29) and 320±56 mg (n=25) in atrial and ventricular tissues, respectively. At 10^{-5} M noradrenaline, force of contraction increased to 290±43 mg (n=29) in atrial and to 588±91 mg (n=25) in ventricular tissues. Over the concentration range of 10^{-6} M to 10^{-3} M, histamine also caused concentration-dependent increases in force of contraction. Atrial tissue generated a relatively greater response to histamine (133±17% of response to 10^{-5} M noradrenaline, at 10^{-3} M, n=6) than ventricular tissue (68±13% of response to 10^{-5} M noradrenaline at 10^{-3} M, n=6). The pD_2 for histamine was 4.9±0.2 in atrial and 5.1±0.2 in ventricular tissue. After histamine had been washed out, the contractile force in atrial, but not in ventricular tissues, remained significantly elevated compared to that before histamine (table 4.1). This was not observed with noradrenaline (differences between before and after noradrenaline: 29±34 and 50±30 mg, in atrial and ventricular tissue, respectively). At the end of the protocol, noradrenaline could increase force of contraction in all tissues up to comparable values as at the beginning of the protocol (at 10^{-5} M: 372±64 and 545±74 mg in atrial and ventricular tissue, respectively).

4.4.1.2 Effects of antagonists. Three antagonists were used to block either H_1 (mepyramine), H_2 (cimetidine) or ß (propranolol) receptors. None of them affected baseline contractile force in either atrial or ventricular tissues (cimetidine: 101±18 and

103±16 mg (atria), 264±48 and 267±49 mg (ventricles); mepyramine: 112±48 and 112±48 mg (atria), 124±29 and 116±27 mg (ventricles); propranolol: 293±137 and 266±124 mg (atria), 240±72 and 260±67 mg (ventricles), before and after 30 min incubation, respectively).

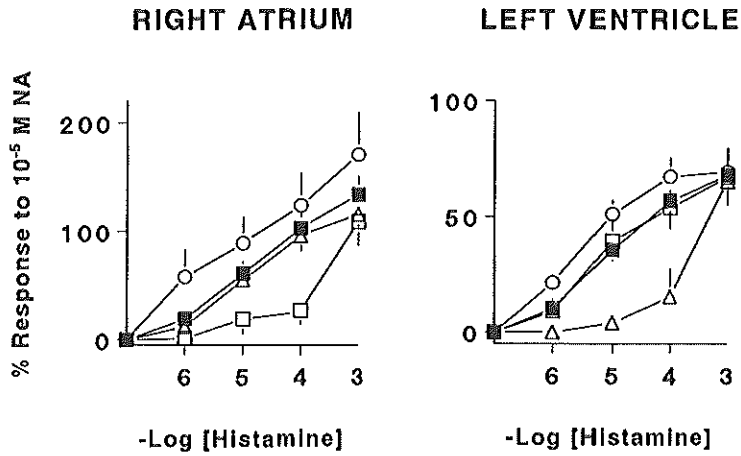


Figure 4.3. Concentration response curves of histamine in porcine atrial and ventricular tissues without (■, control, n=6) or after incubation with either 10^{-7} M mepyramine (△, n=6), 10^{-5} M cimetidine (□, n=6) or 10^{-6} M propranolol (○, n=7).

Table 4.1. Contractile force (mg) in porcine atrial and ventricular trabeculae (n=6 each) before exposure to histamine and after washout of histamine.

Preincubation	Atrium		Ventricle	
	Before	After	Before	After
None (control)	124±56	236±69*	385±101	468±106
Mepyramine (10^{-7} M)	98±42	323±148*	110±26	162±50
Cimetidine (10^{-6} M)	134±42	246±80*	343±85	397±90

* Significantly different from values before histamine.

Cimetidine (10^{-5} M) antagonized the contractile effects of histamine in the atrial but not in ventricular tissue. In contrast, mepyramine (10^{-7} M) antagonized positive inotropic effects of histamine in ventricular but not in atrial tissues (figure 4.3). However, the concentration response curve for histamine in the presence of cimetidine and mepyramine suggest a biphasic course rather than a parallel shift to the right. Propranolol (10^{-6} M) did not block the increase in contractile force produced by histamine in either atrial or ventricular tissue (figure 4.3). Additionally, mepyramine but not cimetidine or propranolol eliminated the transient negative inotropic effects of histamine in the ventricle (data not shown). The increase in baseline contractile force following exposure to histamine was not attenuated by previous treatment with either cimetidine or mepyramine (table 4.1).

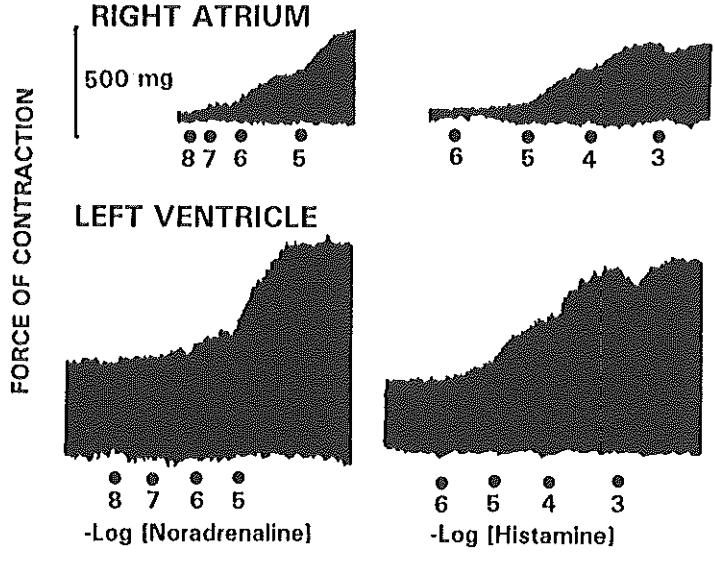


Figure 4.4. Recording of the effects of cumulative concentrations of noradrenaline (10^{-8}-10^{-5} M) and histamine (10^{-6}-10^{-3} M) on human atrial and ventricular trabeculae.

4.4.2 Human myocardium

An example of the recording obtained from atrial and ventricular trabeculae is shown in figure 4.4. Noradrenaline and histamine increased contractile force in a concentration dependent manner in both tissues. The negative inotropic effect preceding the positive inotropic effect, which was seen in all porcine ventricular tissues at each histamine concentration, occurred in human tissue only at 10^{-3} M in about 75% of the preparations.

The mean results are presented in figure 4.5. Baseline contractile force in left ventricular tissues was significantly higher (242±42 mg, n=12) than in right atrial tissues (47±9 mg, n=13). At 10^{-5} M noradrenaline, the force of contraction went up to 298±50 mg (n=12) in atrial and 636±112 mg (n=13) in ventricular tissues. Histamine (10^{-6} M to 10^{-3} M) caused concentration-dependent increases in force of contraction in atrial (at 10^{-3} M: 85±12% of response to 10^{-5} M noradrenaline) and in ventricular (at 10^{-3} M: 122±61% of response to 10^{-5} M noradrenaline) tissues (figure 4.5). The pD_2 for histamine was 4.9±0.2 in atrial and 5.0±0.1 in ventricular tissues.

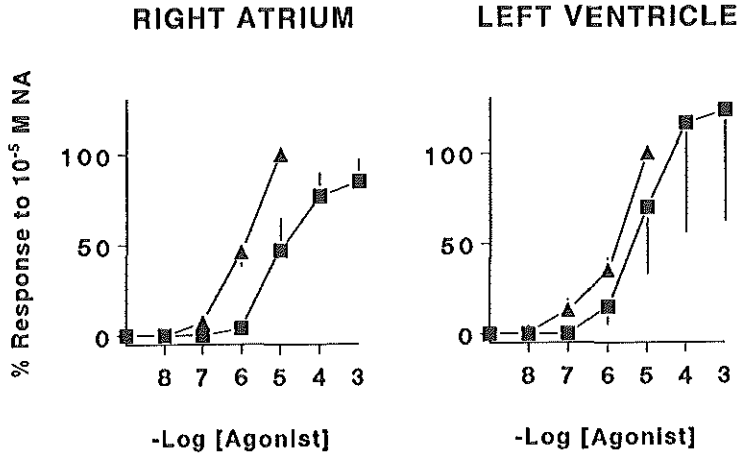


Figure 4.5. Cumulative concentration response curves for histamine (■) in human atrial (n=6) and ventricular (n=6) tissue, compared to contractile effects of noradrenaline (▲).

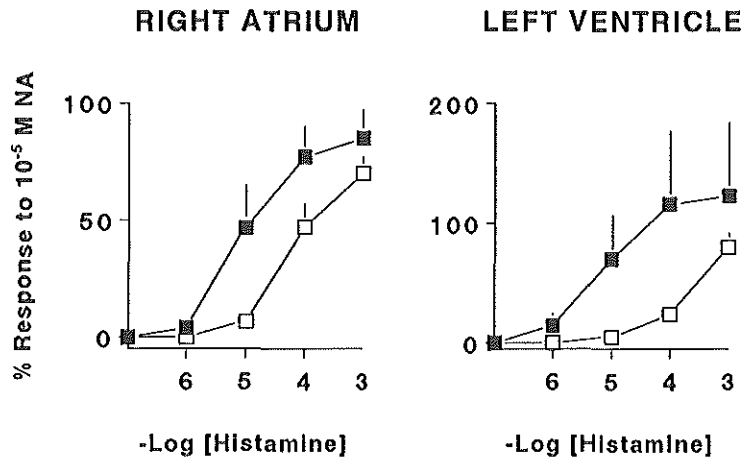


Figure 4.6. Concentration response curves of histamine in human atrial and ventricular tissues in the absence (■, control, n=6) or presence of 10^{-5} M cimetidine (□, n=7).

Table 4.2. Contractile force (mg) in human cardiac tissues (n=6 each) before exposure to histamine and after washout of histamine.

Preincubation	Atrium		Ventricle	
	Before	After	Before	After
None (control)	52±12	225±51*	350±86	590±185*
Cimetidine (10^{-6} M)	72±27	218±69*	240±55	330±81*

* Significantly different from values before histamine.

Cimetidine shifted the concentration-response curve for histamine to the right (figure 4.6); for atrial tissue with 1.1 and for ventricular tissue with 2.1 log units. Cimetidine did not block the transient negative inotropic effect observed at 10^{-3} M histamine (results not shown). After histamine had been washed out, both atrial and

ventricular tissues restabilized at a higher baseline contractile force (table 4.2), which was not observed with noradrenaline (differences between before and after noradrenaline: 15±16 and 29±52 mg, in atrial and ventricular tissue, respectively). Cimetidine did not abolish the increase in baseline contractile force in atrial tissue, and only partly reversed it in ventricular tissue (table 4.2). Noradrenaline stimulation at the end of the protocol increased contractile force to comparable values at 10^{-5} M noradrenaline as at the beginning of the protocol (275±74 and 633±111 mg, for atrial and ventricular tissues, respectively).

4.5 Discussion

In the present study, we investigated the contractile response of histamine in porcine myocardium as a potential model for human myocardium. In the latter, histamine increases contractile force using the adenylyl cyclase/cAMP pathway (Bristow et al., 1982). Therefore, to begin with we checked contractile responsiveness of the tissues though stimulation of the adenylyl cyclase/cAMP pathway using noradrenaline (Brodde, 1991). Baseline contractile force as well as the response to 10^{-5} M noradrenaline appeared higher in ventricular than in atrial trabeculae, which may be related to the different *in vivo* functions of the tissues. The data are comparable to those previously reported by our group (Schoemaker et al., 1992, 1993).

In the present study, the increase in force of contraction in human right atrial and left ventricular trabeculae induced by histamine as well as the ability of cimetidine to antagonize the responses to histamine are in agreement with previous studies (Gristwood et al., 1980, 1981; Bristow et al., 1982), in which the positive inotropic effect of histamine was completely mediated by H_2 receptors. The transient negative inotropic effect, observed at 10^{-3} M histamine, was not blocked by cimetidine and therefore appeared not to be mediated by H_2 receptors. An H_1-mediated mechanism could be responsible since these receptors are present in the heart (Ginsburg et al 1980a; Gristwood et al., 1980) and are described to cause negative inotropic effects (Guo et al., 1984). Interestingly, after histamine had been washed out thoroughly (six times), a sustained increased in contractile force was noticed. This increase was also found in tissues pretreated with cimetidine. Although we cannot exclude a tight binding of histamine to the H_2 receptor, the poor antagonist effects of cimetidine may suggest that activation of intracellular mechanisms rather than a sustained receptor occupation is responsible for this observation. However, adenylyl cyclase activation is probably not involved, since noradrenaline and 5-HT (Schoemaker et al., 1992, 1993) did not have any sustained effect on the baseline contractile force.

Similar to human atrial tissue, porcine atrial tissue was stimulated by histamine, and cimetidine, but not mepyramine, antagonized the histamine-induced inotropic effects, suggesting the involvement of H_2 receptors. However, the shape of the histamine concentration response curve in the presence of cimetidine did not show a completely parallel rightward shift, indicating a lack of a simple, one receptor-mediated, competitive antagonism. Comparable to human tissue, in porcine atrial tissue histamine caused a sustained elevation of the baseline contractile force after washing and stabilization.

In contrast to the human ventricular tissue, cimetidine failed to block the histamine-induced increase in the force of contraction in porcine ventricular tissue. Instead, the H_1 receptor antagonist mepyramine effectively antagonized the contractile effect of histamine. However, comparable to the results with cimetidine in the porcine atrium, the shape of the histamine concentration response curve in the presence of mepyramine suggested a biphasic course rather than a parallel shift to the right, indicating that the antagonism was not simply competitive in nature. In any case, the effect of histamine could not be attributed to an indirect release of noradrenaline from sympathetic nerve terminals, as has been found in the rat and cat (Laher and McNeill, 1979, 1980), since propranolol failed to block the histamine-induced increase in force of contraction in porcine ventricular tissue. Thus, the data indicate that the responses to histamine in porcine ventricular tissues were mainly mediated by H_1 receptors.

The mechanism of action for the H_1 receptor mediated inotropic response is still not clear. The activation of H_1 receptors can lead to an increased level of cellular cyclic GMP (Johnson, 1982), or phosphoinositide hydrolysis (Hattori et al., 1991), but any relationship with H_1 receptor-induced inotropic effect is yet to be elucidated. Moreover, H_1 receptor stimulation has been associated with both positive (Ginsburg et al., 1980a) and negative (Guo et al., 1984) inotropic effects. The observation that in porcine ventricular tissues, the positive inotropic effect of histamine (sensitive to mepyramine) was preceded at each concentration by a negative inotropic effect (also sensitive to mepyramine) suggests the involvement of H_2 receptors in both positive and negative inotropic effects. The shape of the histamine concentration-response curve in the presence of mepyramine indicates a complex concentration-effect relation. The lack of change in baseline contractile force in this tissue, in contrast to the other investigated tissues, further indicates the involvement of different receptors and/or mechanisms in the contractile effects of histamine in the porcine ventricle.

A non-homogeneous distribution of histamine receptors in the heart has been found in other species as well. For example, in the guinea-pig heart, H_1 receptors are present in the left atrium, H_2 receptors in the right atrium and both H_1 and H_2 receptors in the right ventricle (Verma and McNeill, 1977). The positive inotropic effect of histamine in rabbit

atrium is mediated predominantly by H_2 receptors, whereas in papillary muscle it is mainly mediated by H_1 receptors (Hattori et al., 1988). Our present study provides a new example of atrium/ventricle difference among mammalian species. These observations imply that in the heart, atrial and ventricular contractility can be regulated separately.

In conclusion, the results of our study demonstrate that histamine can significantly increase the force of contraction in porcine right atrial and left ventricular trabeculae. Similar to human myocardial trabeculae, the inotropic effect of histamine on porcine right atrial trabeculae is mediated by H_2 receptors. However, in contrast to that in humans, the inotropic effect of histamine on porcine left ventricular trabeculae appears to be mediated by H_1 receptors. Although pig hearts are often used as a model for human hearts, the results from the present study indicate that this model too has its limitations.

Chapter 5 Human calcitonin gene-related peptide and porcine isolated myocardium

5.1 Summary

Calcitonin gene-related peptide (CGRP) is considered to be a neuropeptide, potentially involved in the regulation of cardiac contractility. Previous investigations have demonstrated that in most species, including guinea-pig, rat and human, CGRP exerts positive inotropic effect in atrial but not in ventricular tissue. In the present study, we investigated the effects of human α-CGRP in porcine atrial and ventricular trabeculae. In contrast to human cardiac tissue, human α-CGRP (10^{-9} to 10^{-7} M) induced a concentration-dependent positive inotropic effect in porcine ventricular, but not in atrial trabeculae. Whether this discrepancy is due to the use of human CGRP or to differences in the distribution of CGRP receptors in different parts of the heart remains unknown. These results show that a biogenic substance can induce a different response in different species, which may probably be mediated by the same receptor. Although porcine and human heart show similar responses to several biogenic substances, the present results indicate that the porcine heart model also has its limitation.

5.2 Introduction

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide released by sensory nerve fibres. CGRP is found in almost all tissues and is considered to be a neurotransmitter or neuromodulator, also within the cardiovascular system (Gennari et al., 1985). Its effects on the cardiovascular system, including positive chronotropic and inotropic actions (Ishikawa et al., 1988; Anand et al., 1991), suggest that CGRP may be involved in the regulation of cardiac contractility. Previous investigations have shown that in most species, such as rat (Mulderry et al., 1985) and guinea-pig (Ishikawa et al., 1988), as well as in man (Franco-Cereceda et al., 1987a,b; Du et al., 1994), rat or human α-CGRP exerted positive chronotropic and inotropic effect in atrial but not in ventricular tissue. However, in contrast to these species, a positive inotropic effect of human CGRP was reported in porcine ventricular false tendons (Miyauchi et al., 1988). Meanwhile, CGRP is species-specific and using the CGRP from one species in another may afflict its effects. For instance, rat CGRP was about 10 times more potent than human CGRP in increasing the rate of contraction in guinea-pig atria, and both human and rat CGRP failed to altered the rate of contraction in rabbit isolated perfused heart (Marshall et al., 1986).

Porcine heart is used as a model for the human heart, as it responds similarly to several biogenic substances, including noradrenaline (Du et al., 1993; Schoemaker et al.,

1993), histamine (Du et al., 1993) and 5-HT (Schoemaker et al., 1993). These substances act by stimulation of the same receptor subtypes in porcine and human hearts, except for histamine where the H_1 receptor instead of H_2 was involved in the response of porcine ventricular tissue. Because of this similarity of human and porcine heart, and the fact that porcine CGRP is not yet available, we studied the effects of human α-CGRP in porcine atrial and ventricular tissue, and compared the results to the effects in the human myocardium (Du et al., 1994).

5.3 Material and methods

5.3.1 Preparations

Right atrial and left ventricular tissues were obtained from 3 months old pigs, used as saline-controls in *in vivo* experiments (under pentobarbital aneasthesia, 12 to 20 mg/kg.h). The tissues were placed in ice-chilled oxygenated Kreb's buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 8.3), and trabeculae (<1 mm thickness) were carefully dissected free. The trabeculae were mounted in organ baths (37°C, gassed with 95% O_2 and 5% CO_2), and attached to Harvard isometric transducers. Resting tension was set to approximately 750 mg for atrial and 1950 mg for ventricular tissue, to provide optimal loading conditions. Tissue was paced at 1.5 Hz, using field stimulation (3 ms, voltage 20% above threshold).

5.3.2 Experimental protocol

Similar to our previous studies (Schoemaker et al., 1993; Du et al., 1993), after a stabilization period of at least 40 min, baseline contractile force was recorded and a concentration-response curve for noradrenaline was obtained to check the viability of the tissue. The maximal concentration of noradrenaline was limited to 10^{-5} M, and all responses were expressed as percentage of the response to this concentration. Tissues with less than 25 mg response to 10^{-5} M noradrenaline were excluded from further analysis.

After washing and restabilization a concentration-response curve for CGRP (10^{-9} to 10^{-7} M) was obtained in both atrial and ventricular tissue. Following another washing and stabilization period, the concentration-response curve for noradrenaline was repeated to testify if the tissue was still viable.

5.3.3 Data presentation and analysis

Data are presented as mean ± S.E.M. Baseline values for atrial and ventricular tissue were compared using an unpaired Student's t-test. Effects of noradrenaline and CGRP

were analyzed using an ANOVA for repeated measurements. Differences were regarded statistically significant, if $p<0.05$.

5.3.4 Compounds used

The compounds used in the present investigation were: norepinephrine bitartrate (Sigma, St. Louis, MO, U.S.A.) and human CGRP (Saxon, Hannover, F.R.G.).

5.4 Results

An example of the recording obtained from atrial and ventricular trabeculae is shown in figure 5.1. In both tissues noradrenaline increased the force of contraction in a concentration-dependent manner. CGRP clearly increased the ventricular contractile force, but, in contrast, was virtually inactive in the atrial preparation.

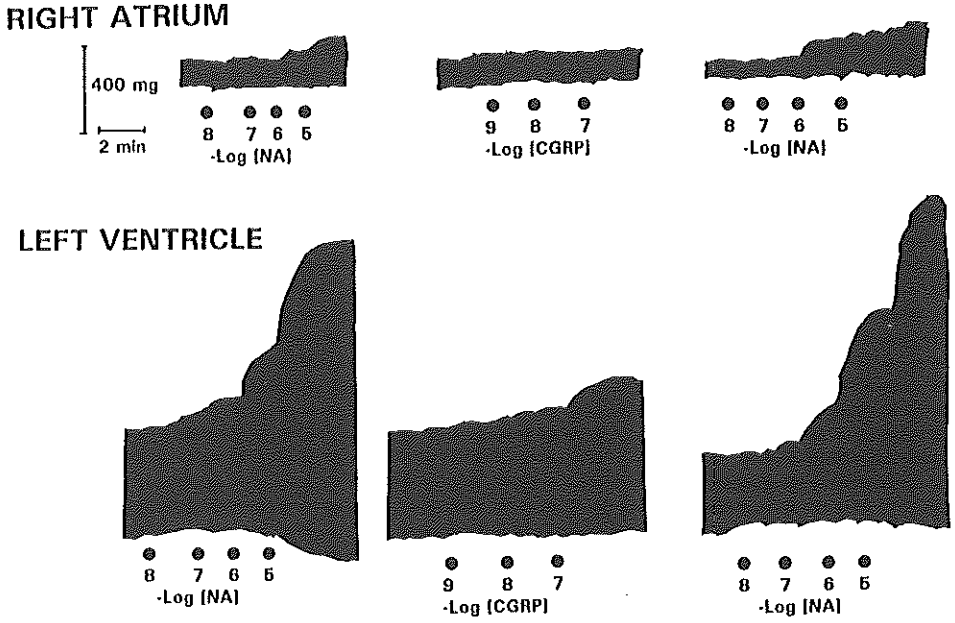


Figure 5.1. Recording of force of contraction in atrial and ventricular trabeculae showing the effects of noradrenaline (before and after CGRP) and human CGRP in increasing concentrations.

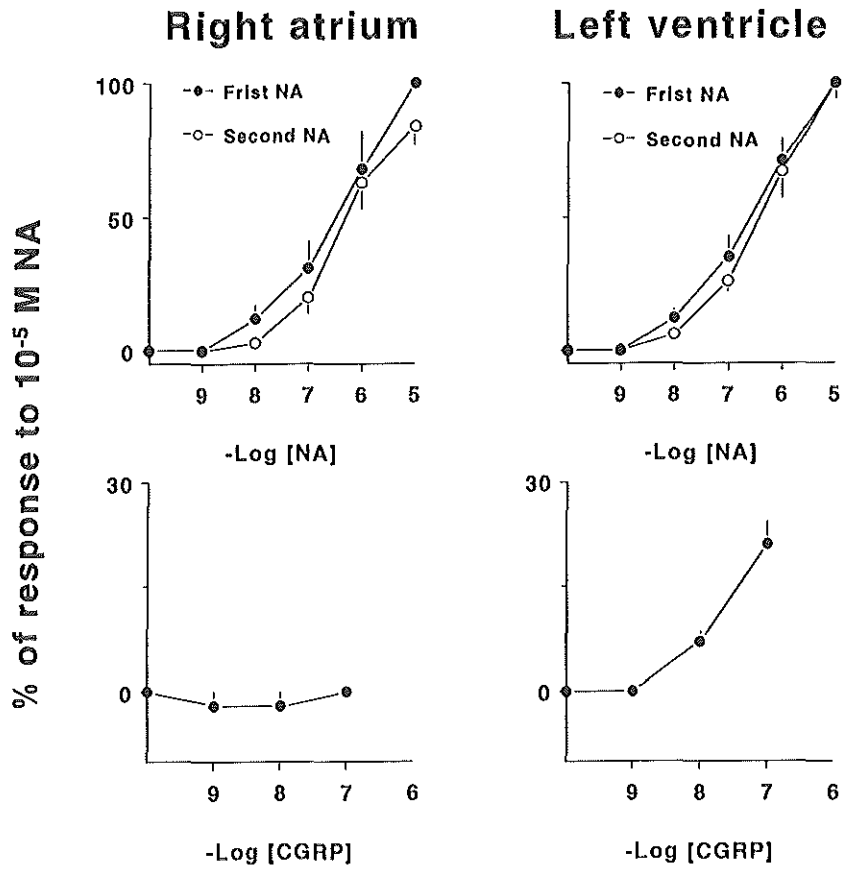


Figure 5.2. Cumulative concentration-response curves of noradrenaline (NA, before and after CGRP, upper panels) and CGRP (lower panels), obtained in atrial (n=6) and ventricular (n=10) trabeculae, respectively.

Mean results (figure 5.2) from all experiments showed that baseline contractile force was significantly lower in atrial (50±16 mg, n=6) than in ventricular tissue (595±138 mg, n=10). Noradrenaline (before CGRP concentration-response curve) increase in force of contraction up to 213±59 mg in atrial (n=6) and 1151±540 (n=10) mg in ventricular trabeculae at the concentration of 10^{-5} M. CGRP (10^{-9} to 10^{-7} M) did not change the atrial

contractility (baseline: 107±26 mg, 10^{-7} M CGRP: 103±25 mg, n=6). However, in ventricular trabeculae, CGRP induced a concentration-dependent increase in force of contraction from 545±110 mg at baseline up to 663±119 mg, or 21±3 % of the response to 10^{-5} M noradrenaline (n=10, $P<0.05$). After the CGRP concentration-response curve, noradrenaline again showed a concentration-dependent increase in contractile force in both atrial and ventricular trabeculae (at 10^{-5} M: 84±7%, n=6 and 100±6%, n=10 of the response to this concentration before CGRP, in atrial and ventricular trabeculae, respectively).

5.5 Discussion

In the present study, we observed that atrial baseline contractile force was much lower than that in ventricular preparations and that responses to noradrenaline in both atrial and ventricular preparations were similar before and after CGRP. These results confirm our previous observations (Schoemaker et al., 1993; Du et al., 1993) and rule out a major influence of variation or deterioration of the tissue in the present experiments.

We demonstrated that human CGRP induced a positive inotropic effect in porcine ventricular tissue but not in atrial tissue. This is in contrast with our previous findings using human CGRP in human cardiac tissues; human CGRP increased human atrial but not ventricular contractility (Du et al., 1994). The present results are, however, in accordance with previous observation by Miyauchi et al. (1988), where human CGRP induced a positive inotropic response in porcine ventricular tissue. Moreover, our observations are also compatible with observation that human α-CGRP increases cardiac output in aneasthesized pig (Van Gelderen et al., unpublished).

In literature, evidence has been presented that the positive inotropic and chronotropic effects in the atrium of several species, including humans, are mediated via specific CGRP receptors coupled to adenylyl cyclase (Franco-Cereceda et al., 1987; Miyauchi et al., 1987; Ono et al., 1989; Anand et al., 1991). In the heart, specific binding sites for CGRP linked to stimulation of adenylyl cyclase activity have been identified. Whereas these binding sites reach high concentrations in the rat atrium (Gennari et al., 1985; Sigrist et al., 1986), such sites in the ventricles from guinea pig are scarce (Ishikawa et al., 1988). This may explain the lack of response in the ventricular muscle of most species, including humans. However, the results from the present study in porcine heart indicate the opposite: a substantial response in ventricular tissue, but not in atrial tissue. At least two possibilities for the discrepancy can be discussed. Firstly, the specific binding sites for CGRP may be absent in the porcine atrium, but present in the ventricle. Although no data are available about specific binding sites for CGRP in porcine atrium, these sites have been

demonstrated in porcine ventricular tissue (Miyauchi et al., 1988). Secondly, CGRP may be species-specific. CGRPs from different species have a slightly different amino acid sequence. For instance, the human CGRP, where the amino acid sequence differs from that of the rat CGRP at four positions, showed different potency in increasing the heart rate of guinea-pig atrium (Marshall et al., 1986). In this regard, we cannot exclude that if porcine CGRP (not available yet) had been used in the porcine tissue, this would mimic the effects in human tissue. If the latter possibility would be true, it is interesting to investigate the effect of porcine CGRP in human myocardium.

In the present study, porcine isolated myocardium showed a different response to human CGRP, compared to most other species, including human. In combination with our previous investigations in porcine isolated myocardium (Schoemaker et al,1993, Du et al., 1993), we conclude that although the basic response and mechanisms in cardiac contractility derived from investigation in porcine heart may be applicable to the human heart, results from the present study suggest that this model, too, has its limitations.

Chapter 6 Different pharmacological responses of atrium and ventricle: studies with human cardiac tissue

6.1 Summary

It has been recently reported that 5-hydroxytryptamine (5-HT) increases force of contraction in atrial tissue but not in ventricular tissue. In the present study with trabeculae obtained from non-diseased human hearts, we investigated whether this difference in the contractile responses is specific for 5-HT or is also observed for other substances: calcitonin gene-related peptide (CGRP), angiotensin II, adenosine, somatostatin and acetylcholine. CGRP (10^{-9}-10^{-7} M) and angiotensin II (10^{-9}-10^{-5} M) caused concentration-dependent increases in force of contraction in atrial trabeculae (up to 36±8% and 42±8% of the response to 10^{-5} M noradrenaline, respectively). Similar to 5-HT, no effects were observed with CGRP and angiotensin II in ventricular trabeculae. Adenosine (10^{-8} to 10^{-5} M) and somatostatin (10^{-8} to 10^{-6} M) caused concentration-dependent negative inotropic effects on baseline atrial contractility (-54±17% and -51±25%, respectively), but no response was found on baseline ventricular contractility. Adenosine, but not somatostatin, reduced force of contraction after pre-stimulation with 10^{-5} M noradrenaline in atrial tissue and, to a lesser extent, in ventricular tissue. Acetylcholine exhibited a biphasic concentration-response curve in the atrial tissue, consisting of an initial negative inotropic response (10^{-9}-10^{-7} M, from 120±41 mg at baseline to 48±16 mg at 10^{-7} M), followed by a positive inotropic response (10^{-6}-10^{-3} M, from 48±16 mg at 10^{-7} M to 77±15 mg). On the baseline ventricular force of contraction, acetylcholine (10^{-9}-10^{-4} M) induced only a positive inotropic effect, starting at 10^{-9} M (from 252±65 mg at baseline to 353±71 mg at 10^{-4} M). After pre-stimulation with 10^{-5} M noradrenaline, acetylcholine reduced force of contraction in both tissues at 10^{-3} M (atrium: -14±4%, ventricle: -61±5%). The data indicate that, in atrial tissue, force of contraction can be affected by either positive or negative inotropic agents. However, in ventricular tissue only positive inotropic effects could be detected. Since atrial and ventricular tissues display different responses to the above biogenic substances, a different mechanism of regulation of contractility seems feasible.

6.2 Introduction

In humans the ß-adrenoceptor-G_s-protein-adenylyl-cyclase pathway is the most powerful mechanism for the regulation of cardiac contractility. By using either the same or alternative pathways, several other biogenic substances can also affect cardiac contractility, and hence, they may also be involved in the regulation of cardiac function. Substances that evoke positive inotropic effects include 5-hydroxytryptamine (5-HT) (mediated by 5-HT_4 receptor; Kaumann et al., 1991; Schoemaker et al., 1993; Zerkowski et al., 1993), histamine (mediated

mainly by H_2 receptor; Bristow et al., 1982; Du et al., 1993; Zerkowski et al., 1993), vasoactive intestinal peptide (VIP) (mediated by VIP receptor; Hershberger et al., 1989), angiotensin II (mediated by angiotensin AT_1 receptor; Urata et al., 1989; Zerkowski et al., 1993) and endothelin (mediated by ET_A receptor; Brodde et al., 1992). Substances that evoke negative inotropic effects include adenosine (mediated by adenosine A_1 receptor; Hershberger et al., 1987), somatostatin (mediated via somatostatin receptors; Hershberger et al., 1988), and acetylcholine (mediated by M_2 receptor; Brodde et al., 1992).

It has been recently reported that 5-HT increases the atrial force of contraction without affecting the ventricular contraction in the pig (Saxena et al., 1992; Schoemaker et al., 1992) as well as in humans (Jahnel et al., 1992; Schoemaker et al., 1993), thus suggesting that an agent can elicit a positive inotropic response in the atrium without having a corresponding effect in the ventricles. In this investigation with human heart, we report similar characteristics of several other substances — human calcitonin gene-related peptide (CGRP), angiotensin II, adenosine, somatostatin and acetylcholine. We focused on the comparison of the responses of atrial and ventricular tissues obtained, in most cases, from the same non-diseased human hearts. To our knowledge, this is the first study to investigate the contractile responses of atrial and ventricular tissues from the same heart in parallel.

6.3 Material and methods

6.3.1 General preparations

Right atrial and left ventricular trabeculae were obtained from 42 'beating heart' organ donors (27 males, 15 females; age 1-54 years) who died of non-cardiac disorders (26 cerebrovascular accident, 14 polytrauma, 1 encephalopathy, 1 hypoxia) less than 24 h before the tissue was taken to the laboratory. The hearts were kindly provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation/Eurotransplant Foundation) after removal of the aortic and pulmonary valves for homograft valve implantation. The hearts were stored at 0-4 °C in a sterile organ protecting solution (UW, Eurocollins, or HTK-Brettschneider; see Ploeg et al., 1992) immediately after circulatory arrest.

After excision, pieces of atrial and ventricular myocardium were placed in ice-chilled oxygenated Krebs buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, $KHPO_4$ 1.2 and glucose 8.3) and atrial and ventricular trabeculae (<1 mm thickness) were carefully dissected free. The trabeculae were mounted in organ baths (gassed with 95% O_2 and 5% CO_2; 37°C) and then prepared for isometric tension recording with a Harvard transducer. Resting tension was set to 750 and 1950 mg for atrial and ventricular

tissues, respectively, in order to provide optimal loading conditions. Tissues were paced at 1 Hz, using electrical field stimulation (3 ms, voltage 20% above threshold).

6.3.2 Experimental protocol

After stabilization, the baseline force of contraction was measured. A concentration-response curve for noradrenaline was obtained to check the inotropic responsiveness of the tissues. Since the tissues were irreversibly damaged after a concentration of 10^{-4} M (the baseline force of contraction decreased after washout and did not recover in a reasonable time), the maximum concentration of noradrenaline was restricted to 10^{-5} M. The responses to all other substances are expressed as percentages of either the response to 10^{-5} M noradrenaline or the baseline force of contraction. Tissues with a response to 10^{-5} M noradrenaline smaller than 25 mg were excluded from further analysis.

After the tissues had been washed (6 times) and had stabilized, a concentration-response curve for CGRP (10^{-9}-10^{-7} M), angiotensin II (10^{-9}-10^{-5} M), adenosine (10^{-8}-10^{-5} M), somatostatin (10^{-8}-10^{-6} M), or acetylcholine (10^{-9}-10^{-3} M, in the presence of 10^{-5} M physostigmine to prevent rapid degradation of acetylcholine by cholinesterase; see Chatonnet and Lockridge, 1989) was obtained in parallel in *both* atrial and ventricular tissues. Only one substance was studied per tissue. After another wash and stabilization period, the concentration-response curve for adenosine and somatostatin was constructed again following pre-stimulation with 10^{-5} M noradrenaline. Because of desensitization occurring after repeated exposure to acetylcholine, the above-described experiments with this substance after pre-stimulation with 10^{-5} M noradrenaline were carried out in separate tissues. At the end of each experiment without pre-stimulation with noradrenaline (except in a few cases where the tissue was frozen for further investigations which are not reported here), another noradrenaline concentration-response curve was obtained after washing and re-stabilization (at least 10 min) to check the viability of the tissue. In the case of investigations with adenosine and acetylcholine in pre-stimulated (10^{-5} M noradrenaline) myocardium, we checked the stability of the noradrenaline response by administering 10^{-5} M noradrenaline at the end of protocol.

6.3.3 Data presentation and analysis

All data are presented as means±S.E.M. Baseline values for atrial and ventricular tissues were compared by using an unpaired *t*-test. The effects of noradrenaline, CGRP, angiotensin II, adenosine, somatostatin and acetylcholine were analyzed by using an analysis of variance (ANOVA) for repeated measurements. Differences were regarded statistically significant, if $P<0.05$.

6.3.4 Compounds used

The compounds used in the present study were: noradrenaline bitartrate (Sigma, St. Louis, MO, U.S.A.), adenosine (Janssen, Geel, Belgium), angiotensin II, human CGRP and somatostatin-14 (Saxon, Hannover, F.R.G.), acetylcholine chloride (Ciba, Breda, The Netherlands), atropine sulphate (Centrafarm, Etten-Leur, The Netherlands), physostigmine salicylate (Sandoz, Basel, Switzerland) and propranolol hydrochloride (Imperial Chemical Industries Limited, Macclesfield, U.K.).

6.4 Results

6.4.1 Viability of the tissues

On average, the baseline force of contraction was significantly lower in atrial tissue (126±25 mg, n=37) than in ventricular tissue (221±34 mg, n=34). In both tissues noradrenaline (10^{-8}-10^{-5} M) increased force of contraction in a concentration-dependent manner. After exposure to 10^{-5} M noradrenaline, the force of contraction went up to 548±55 mg (n=37) and 599±78 mg (n=34) in the atrial and ventricular trabeculae, respectively. When the response to noradrenaline (10^{-5} M) was elicited again at the end of the protocol, similar (in atrial trabeculae: 107±10% of the initial noradrenaline response, n=33) or even greater (in ventricular trabeculae: 177±29% of the initial noradrenaline response, n=31) increases in force of contraction were observed.

6.4.2 Positive inotropic effects

6.4.2.1 CGRP. An example of the recordings obtained from the atrial and ventricular trabeculae is shown in figure 6.1. Unlike noradrenaline, CGRP increased force of contraction in atrial but not in ventricular tissue. The mean results are shown in figure 6.2 (left panel). CGRP (10^{-9}-10^{-7} M) exerted a positive inotropic effect in a concentration-dependent manner in the atrial tissue. At 10^{-7} M CGRP, force of contraction increased significantly from 374±128 mg at baseline up to 545±151 mg, or 36±8% of the response to 10^{-5} M noradrenaline (n=8). In contrast, no significant change was noticed in the ventricular force of contraction (baseline: 356±73 mg, 10^{-7} M CGRP: 374±79 mg, n=8).

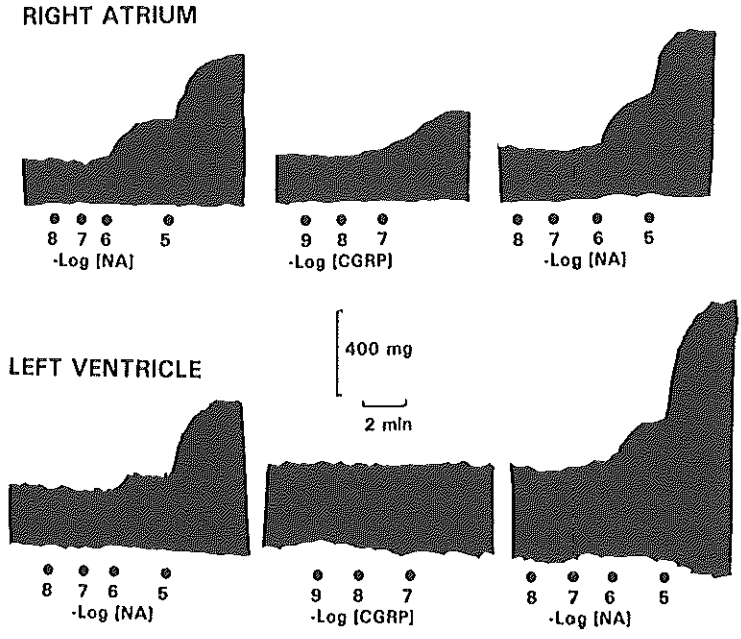


Figure 6.1. Recordings of force of contraction of a right atrial (top) and a left ventricular (bottom) trabecula showing the effects of cumulative concentrations of CGRP as compared to those of noradrenaline (NA), before and after CGRP.

6.4.2.2 Angiotensin II. On average angiotensin II (10^{-9} M to 10^{-5} M) caused a concentration-dependent increase in the atrial force of contraction, but 2 out of 6 atrial trabeculae (33%) did not respond to angiotensin II. Data from the other 4 trabeculae (67%) showed an enhanced force of contraction, which increased from 70±50 mg at baseline to 398±117 mg with 10^{-5} M angiotensin II (see figure 6.2, right panel). No response was seen in any of the 5 ventricular trabeculae.

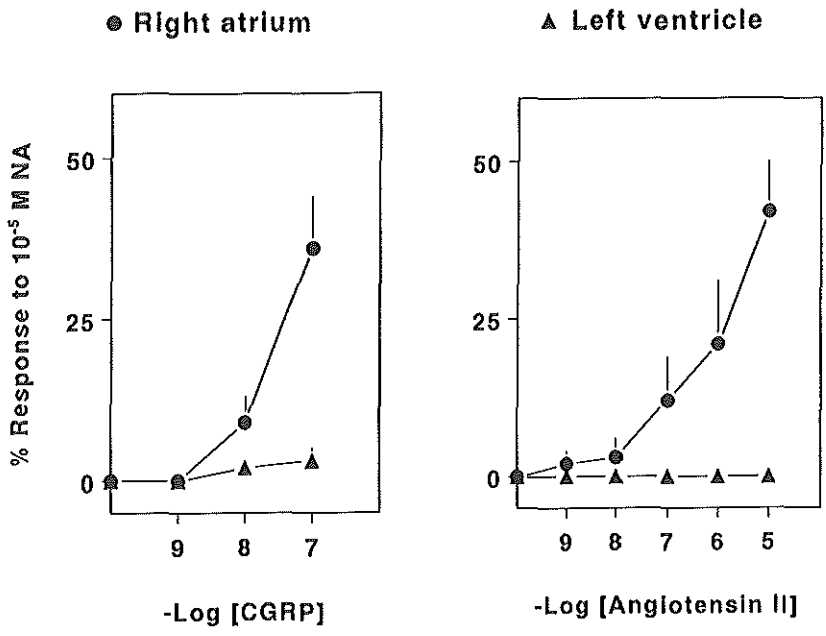
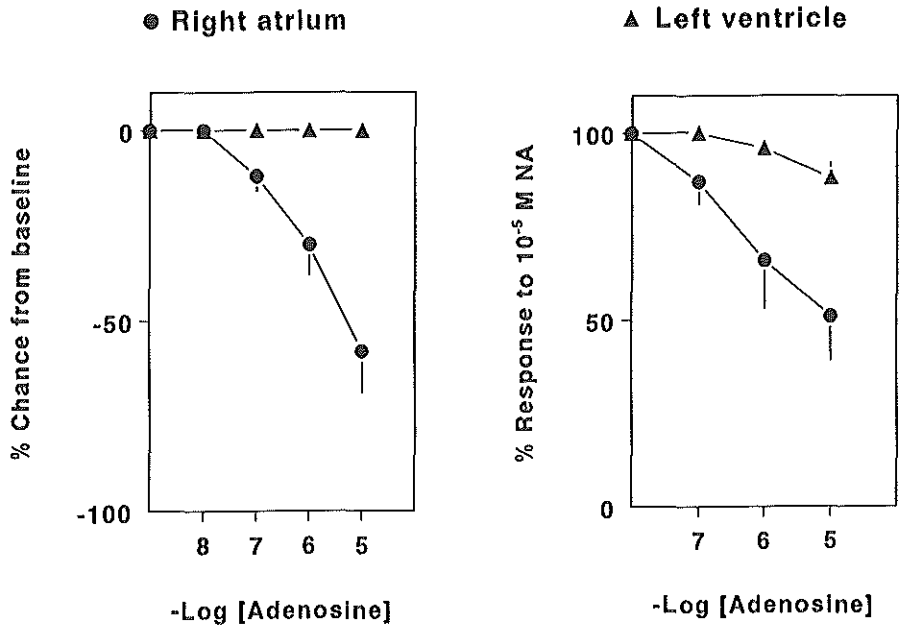


Figure 6.2. Cumulative concentration-response curves of CGRP and angiotensin II in atrial and ventricular trabeculae (CGRP: n=8 each; angiotensin II: n=4 and 5 for atrium and ventricles, respectively). Responses to both compounds are expressed as percentages of the response to 10^{-5} M noradrenaline (NA).

6.4.3 Negative inotropic effects

6.4.3.1 Adenosine. The effects of adenosine (10^{-8}-10^{-5} M) on the atrial and ventricular force of contraction at baseline or in the presence of 10^{-5} M noradrenaline are presented in figure 6.3. In the atrial tissue, adenosine significantly reduced baseline force of contraction (from 116±57 mg to 58±40 mg at 10^{-5} M; n=5), as well as force of contraction in the presence of 10^{-5} M noradrenaline (503±134 mg to 317±114 mg at 10^{-5} M; n=6). In the ventricular tissue, adenosine did not affect baseline contractility (baseline: 86±30 mg, 10^{-5} M adenosine: 93±30 mg; n=5) and only slightly (but significantly) decreased force of contraction after pre-stimulation with 10^{-5} M noradrenaline (from 657±140 mg down to 597±137 mg, at 10^{-5} M; n=6).



Figrue 6.3. Concentration-response curves of adenosine on the baseline force of contraction (n=6) and on the force of contraction after pre-stimulation with 10^{-5} M noradrenaline (NA; n=5), obtained with atrial and ventricular tissues.

6.4.3.2 Somatostatin. The mean results from 5 pairs of atrial and ventricular trabeculae are shown in figure 6.4. Over a concentration range of 10^{-8}-10^{-6} M, somatostatin decreased the atrial baseline force of contraction from 464±184 mg to 180±124 mg (-51±25%) at 10^{-6} M, but it did not affect the atrial force of contraction after pre-stimulation with 10^{-5} M noradrenaline (928±83 mg before and 936±95 mg after 10^{-6} M somatostatin). Neither at baseline nor after pre-stimulation with noradrenaline did somatostatin affect ventricular contractility.

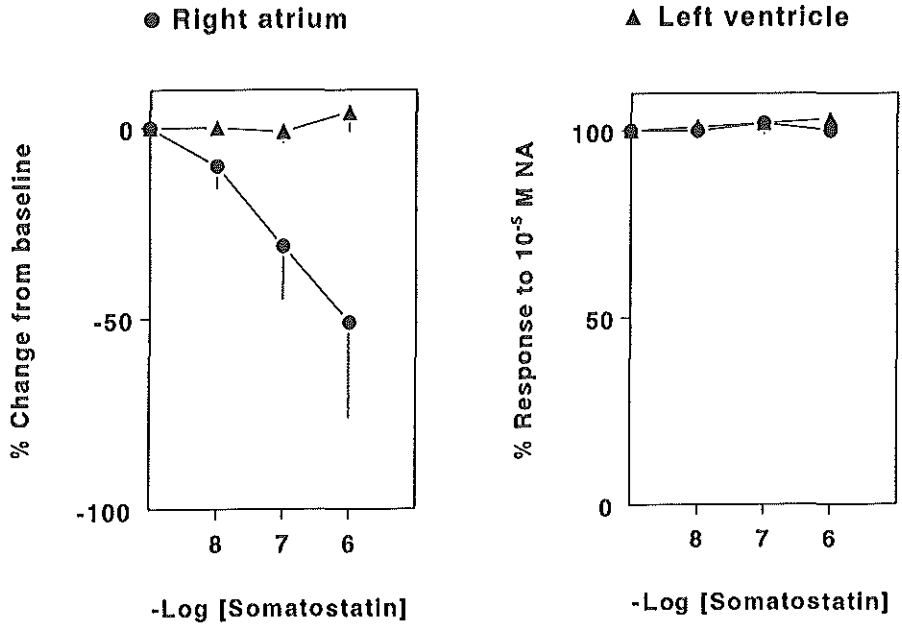


Figure 6.4. Concentration-response curves of somatostatin on the baseline force of contraction (n=5) and on the force of contraction after pre-stimulation with 10^{-5} M noradrenaline (NA; n=5), obtained with atrial and ventricular tissues.

6.4.4 Mixed inotropic effects of acetylcholine

An example of recordings showing the effects of acetylcholine on the right atrial and left ventricular trabeculae is presented in figure 6.5. In the atrial trabecula, acetylcholine elicited a biphasic effect, consisting of an initial negative inotropic response (from 10^{-9}-10^{-7} M) followed by a positive inotropic response (from 10^{-6}-10^{-4} M). It should be noted that even after the highest concentration of acetylcholine (10^{-3} M) the force of contraction did not exceed the baseline value. In the ventricular trabecula, acetylcholine caused a clear concentration-dependent positive inotropic effect. After administration of 10^{-5} M noradrenaline, which resulted in a stable increase in the atrial and ventricular force of contraction, acetylcholine produced only negative inotropic effects in both tissues.

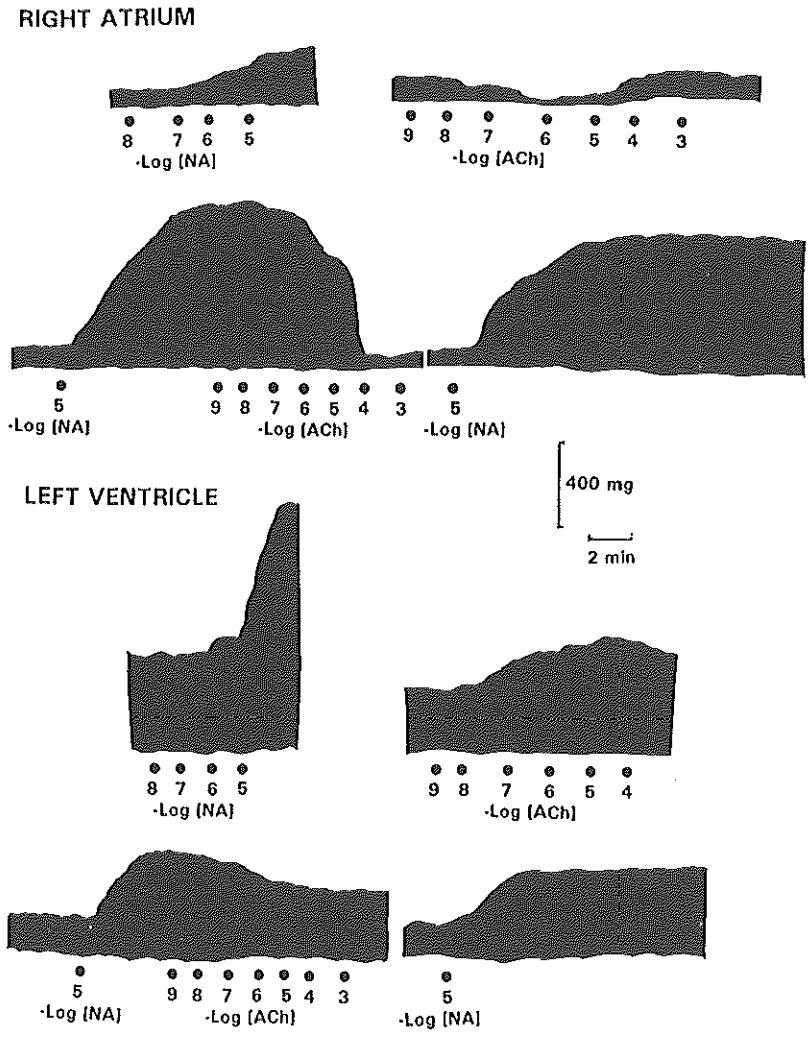


Figure 6.5. Recordings of force of contraction of two right atrial (top 2 panels) and two left ventricular (bottom 2 panels) trabeculae obtained from the same hearts. The recordings show the effects of cumulative concentrations of acetylcholine (in the presence of 10^{-5} M physostigmine) on the baseline force of contraction (first and third panels) and after pre-stimulation with 10^{-5} M noradrenaline (NA; second and fourth panels). For comparison, the sustained effect of stimulation with 10^{-5} M noradrenaline is also presented.

The mean results of the effects of acetylcholine on the atrial and ventricular baseline force of contraction are shown in figure 6.6 (left panel). In the atrial tissue, at low concentrations acetylcholine decreased baseline force of contraction from 120±41 mg to 48±16 mg (-50±11%) at 10^{-7} M (n=8), whereas in concentrations above 10^{-6} M, acetylcholine increased force of contraction (up to 77±15 mg at 10^{-3} M; n=8) back towards baseline values. In the ventricular tissue, acetylcholine increased baseline force of contraction in a concentration-dependent manner (maximal response 358±70 mg or 56±16% increase from baseline at 10^{-5} M; n=6). Figure 6.6 (right panel) presents the mean results of the effects of acetylcholine on force of contraction after pre-stimulation with 10^{-5} M noradrenaline. At 10^{-3} M, acetylcholine reduced the atrial (n=7) and ventricular (n=6) force of contraction from 504±141 mg and 463±93 mg, respectively, to 43±6 mg (14±4%) and 288±59 mg (61±5%), respectively.

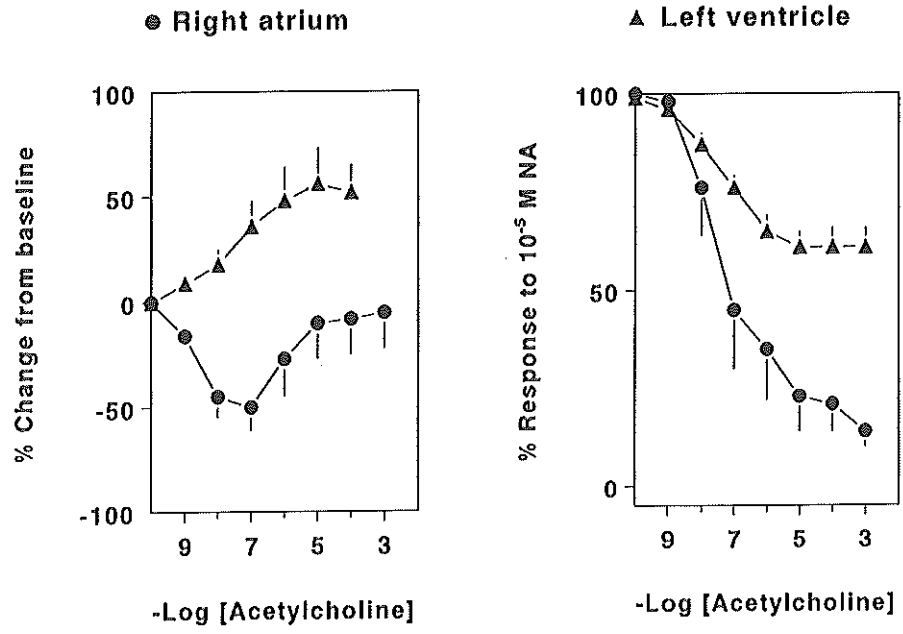


Figure 6.6. Concentration-response curves of acetylcholine on the baseline force of contraction and on the force of contraction after pre-stimulation with 10^{-5} M noradrenaline (NA) in atrial and ventricular tissues. The number of atrial and ventricular trabeculae used were: 8 and 6, respectively, in the left panel; and 7 and 6, respectively, in the right panel.

6.5 Discussion

Recent investigations have established that 5-HT, which increases heart rate and atrial contractility in both porcine (Saxena, 1986; Bom et al., 1988; Kaumann, 1990; Villalón et al., 1990) and human (Kaumann et al., 1990, 1991) heart by acting at 5-HT_4 receptors, does not have a direct effect on ventricular contractility (Jahnel et al., 1992; Saxena et al., 1992; Schoemaker et al., 1992, 1993). Therefore, the main purpose of the present investigation was to establish whether or not such a differential effect is also noticed with other biogenic substances in atrial and ventricular trabeculae obtained from the same non-diseased human heart. Since the adenylyl cyclase/cAMP pathway is the major system regulating force of cardiac contraction (Brodde et al., 1992), noradrenaline was used to check the inotropic responsiveness and viability of the tissues, both at the beginning and at the end of the experiments. As in our previous studies (Du et al., 1993; Schoemaker et al., 1993), tissues not responding to noradrenaline at the beginning of the experiment were excluded. All tissues responded to noradrenaline at the end of experiment, confirming that these tissues remained viable during the course of the experiment.

6.5.1 Positive inotropic effects

CGRP, a neuropeptide released by the endings of sensory nerve fibres, exerts a positive inotropic effect on atrial but not on ventricular myocardium of the rat (Sigrist et al., 1986; Ishikawa et al., 1987) and guinea-pig (Saito et al., 1987; Ishikawa et al., 1988; Giuliani et al., 1992). In both species the atrial effect of CGRP is associated with an increase in cAMP (Sigrist et al., 1986; Ishikawa et al., 1988). In the dog, CGRP (up to 1 μM) has no effect on either the atrial or ventricular trabeculae (Rigel et al., 1989). In the human myocardium, a positive inotropic effect has been reported on the atrial tissue (Franco-Cereda et al., 1987), but no data are available about the effect of CGRP on the ventricular contractility. Our results show that, similar to the rat and guinea-pig, a positive inotropic effect of CGRP was observed in human atrial trabeculae. However, CGRP had no effect on ventricular tissue obtained from the same heart. As was the case with 5-HT (Schoemaker et al., 1993), the lack of effect of CGRP on the ventricular myocardium was not due to deterioration of the tissue during the experiment. Animal studies have shown that specific binding sites for CGRP can be identified in high concentration in the atrium (Sigrist et al., 1986), but such sites are scarce in the ventricles (Ishikawa et al., 1988). Therefore, a low receptor density may explain the lack of response in the ventricular muscle.

As has been reported earlier (Urata et al., 1989; Moravec et al., 1990; Zerkowski et al., 1993), angiotensin II did increase atrial contractility in our experiments, though not all atrial preparations responded to this peptide. In addition, our experiments show that angiotensin

did not affect human ventricular contractility. Though Moravec et al. (1990) reported that angiotensin II has a positive inotropic effect on human ventricular trabeculae, the responses in the atrium were more pronounced. The reason for this difference is not clear, but it may be related to the heterogeneous receptor distribution in the human myocardium (Urata et al., 1989). The effects of angiotensin II on atrial tissue are mediated by angiotensin AT_1 receptors (Pieske et al., 1993; Zerkowski et al., 1993), which are coupled to the production of phospholipase C/diacylglycerol from inositol-1,4,5-trisphosphate (Brodde et al., 1992; Zerkowski et al., 1993). Thus, the discrepancy between atrial and ventricular contractile responsiveness is not restricted to adenylyl cyclase/cAMP mediated mechanisms.

6.5.2 Negative inotropic effects

Adenosine elicited a negative inotropic effect on the baseline as well as noradrenaline-stimulated atrial contractility, but it had no (baseline) or little (noradrenaline-stimulated) effect on ventricular contractility. Adenosine as well as some of its analogues have been shown to inhibit isoprenaline-stimulated force of contraction in ventricular myocardium at concentrations above 10^{-5} M (Böhm et al., 1985; Jakob et al., 1989). The negative inotropic effect of adenosine seems to be mediated by A_1 receptors, which are negatively coupled to adenylyl cyclase and have been identified in both human atrial and ventricular myocardium (Böhm et al., 1985; Hershberger et al., 1987; Schmitz et al., 1987). However, in human atrial tissue, the density of the A_1 receptors is about twice as high as in ventricular tissue (Böhm et al., 1989), which could offer an explanation for the difference in the pharmacological responses of the atrium and ventricle.

Somatostatin exerted a concentration-dependent negative inotropic action on the baseline atrial force of contraction. No effect was noticed on the noradrenaline-stimulated atrial trabeculae or on the ventricular trabeculae, with or without stimulation with noradrenaline, despite a report that somatostatin may inhibit adenylyl cyclase activity in the human ventricular myocardium (Hershberger et al., 1988). Somatostatin is suggested to act via a receptor that is negatively coupled to the adenylyl cyclase pathway via G_i-proteins (Brodde et al., 1992). In atrial fibres, the inhibitory effect of somatostatin on contractility was associated with a reduction of intracellular calcium (Hou et al., 1987). The concentration of somatostatin in the atrioventricular node and right atria was found to be significantly higher than in other heart regions (Day et al., 1985), suggesting that somatostatin has a role in the cardiac conduction system rather than in the direct regulation of ventricle contractility. The lack of a negative inotropic effect on ventricular contractility is apparently an optimistic sign from the point of the clinical use of somatostatin. Thus, for example, long-term (3-6 months) treatment with this drug has been reported to reverse left ventricular hypertrophy and improve exercise performance in acromegalic patients (Tokgözoğlu et al., 1993).

6.5.3 Mixed inotropic effects

Acetylcholine caused a mixed positive and negative inotropic response in the present study. Both negative (at concentrations $<10^{-6}$ M) and positive (at concentrations $\geq 10^{-6}$ M) inotropic effects of acetylcholine and other cholinergic agonists have been found in isolated atrial tissue from several animal species, including rabbit (Endoh and Blinks, 1984) and chick (Tajima et al., 1987), as well as rat and guinea-pig (Eglen et al., 1988). In ventricular tissue, only a positive inotropic effect on baseline contractility (started at 10^{-9} M) and a negative inotropic effect (though weaker than in the atrial tissue) after pre-stimulation with noradrenaline were seen. The negative inotropic effect is consistent with findings reported in the literature (see Jakob et al., 1989); the effect depends on the presence of drugs that increase force of contraction by augmenting cellular cAMP (Korth and Kühlkamp, 1987; Löffelholz and Pappano, 1987). In most species, the positive inotropic effect of muscarinic agonists in ventricular tissues is only seen at concentrations above 10^{-5} M (Korth and Kühlkamp, 1987; Tsuji et al., 1987). However, our results are consistent with a report by Gilmour and Zipes (1985) that acetylcholine elicits a positive inotropic response at concentrations ranging from 10^{-9}-10^{-4} M in canine cardiac Purkinje fibres. Both the positive and the negative inotropic effects could be antagonized by atropine (Du et al., unpublished observations), indicating the involvement of muscarinic receptors. In the human heart, M_2-receptors are predominantly present (Brodde et al., 1992) and these seem to exist in two different states (Brown and Brown, 1984): a high-affinity state associated with inhibition of adenylyl cyclase causing negative inotropy (Korth and Kühlkamp, 1987; Eglen et al., 1988; Brodde et al., 1992), and a low affinity state, coupled to a pertussis toxin insensitive G protein to stimulate inositol-1,4,5-trisphosphate hydrolysis (Poggioli et al., 1986; Tsuji et al., 1987; Eglen et al., 1988), causing positive inotropy. This hypothesis provides a good explanation for the biphasic response in atrial tissue. In ventricular tissue, these high- and low-affinity states might also exist, but in most species only the latter is expressed, so that acetylcholine has predominantly a positive inotropic effect.

In conclusion, data from the present study show that several endogenous substances (CGRP, angiotensin II, adenosine, somatostatin and acetylcholine) exhibit different effects on atrial and ventricular contractility. In general, atrial tissue shows more marked responses than ventricular tissue. Although the effects and the mechanisms of the above substances are different, in the end these substances affect Ca^{2+} influx. Fabiato (1982) has suggested that, in many mammalian species, the Ca^{2+}-induced release of Ca^{2+} is more developed in the atrium than in the ventricle. This implies that the atrium is more sensitive than the ventricle to substances that modify Ca^{2+} influx. Therefore, a low sensitivity to Ca^{2+}-induced release of Ca^{2+}, as well as a lower density or absence of the involved receptors, may be responsible for the poor ventricular responses to these agents. The results of this study may help provide a

better understanding of the physiology of atrial and ventricular contractility, as well as an important direction for drug development.

Chapter 7 Characterization of the positive and negative inotropic effects of acetylcholine in human isolated atrial and ventricular trabeculae

7.1 Summary

In the human isolated myocardium, acetylcholine (10^{-9} to 10^{-3} M) elicited a biphasic inotropic effect (a decrease in the lower and an increase in higher concentration range) in atrial and a positive inotropic effect in ventricular trabeculae. However, under conditions of raised contractility achieved by exposure to noradrenaline (10^{-5} M), negative inotropic effects were observed in both atria and ventricles. Atropine (10^{-6} M), but not propranolol (10^{-6} M), antagonized both the positive and negative inotropic effects of acetylcholine, thus showing that the responses were mediated by muscarinic acetylcholine receptors. The use of subtype selective muscarinic receptor antagonists (10^{-7} to 10^{-5} M), pirenzepine (M_1>M_3>M_2), AF-DX 116 (M_2>M_1>M_3) and HHSiD (M_3≥M_1>>M_2), revealed that the negative inotropic effect of acetylcholine in atrial as well as the positive inotropic effect in ventricular trabeculae were best antagonized by AF-DX 116 and not by pirenzepine, suggesting the involvement of the muscarinic M_2 receptor subtype, possibly linked to different second messenger systems. On the other hand, the positive inotropic effect of acetylcholine (10^{-6} to 10^{-3} M) in the atrial tissue, observed only in preparation with depressed contractility, was not effectively antagonized by either AF-DX 116 or HHSiD, but was significantly reduced by pirenzepine. Furthermore, the muscarinic M_1 subtype selective agonist McN-A-343 (10^{-9} to 10^{-3} M), which failed to significantly change the baseline contractility in either atrial or ventricular trabeculae, produced a positive inotropic effect in atrial preparations when contractility had been depressed by prior treatment with acetylcholine (10^{-9} to 10^{-7} M). This effect of McN-A-343 was effectively antagonized by pirenzepine (10^{-5} M). These data show that, besides the muscarinic M_2 receptor mediating both negative (atria) and positive (ventricle) inotropic effects, muscarinic M_1 receptors, capable of reversing depressed atrial contractility, are also present in the human heart.

7.2 Introduction

It is well known that acetylcholine causes negative inotropic and chronotropic effects in the mammalian heart, including humans (Brodde et al., 1992; Caulfield, 1993; Böhm et al., 1994; Landzberg et al., 1994). In addition, studies have shown that acetylcholine can elicit a positive inotropic response in isolated cardiac tissue of some species (Endoh and Blinks, 1984; Tajima et al., 1987; Eglen et al., 1988). In our previous study in isolated human myocardium, we reported that acetylcholine elicited a biphasic response (an initial decrease followed by an increase in contractility back to baseline values) in the atrial trabeculae, whereas only a positive inotropic effect was noticed in the ventricular trabeculae (Du et al., 1994). This latter effect was not observed earlier (Jakob et al., 1989; Deighton et al., 1990; Böhm et al., 1994).

Five muscarinic acetylcholine receptor subtypes genes have been cloned and expressed, but only muscarinic M_2 receptors have been detected in the human heart (Maeda et al., 1988; Ford et al., 1992; Caulfield, 1993). Correspondingly, the responses to muscarinic receptor agonists in the cardiac muscle appear to be mediated by the activation of a well-characterized muscarinic M_2 receptor, which are coupled to different signal transduction pathways (Schimerlik, 1989). However, the cardiac effects of muscarinic receptor stimulation are not yet fully understood and evidence is emerging that, besides the muscarinic M_2 receptor, other muscarinic receptor are also expressed in the heart. For example, muscarinic M_1 receptors seem to mediate positive inotropic effects in cells isolated from adult guinea-pig ventricles (Gallo et al., 1993) as well as the increase in automaticity in canine Purkinje fibres (Rosen et al., 1990). Thus, it is suggested that although the predominant population of muscarinic receptors at postsynaptic sites in the heart is of the muscarinic M_2 subtype, a small population of muscarinic M_1 subtype is also present (Watson et al., 1983; Evans et al., 1985).

The purpose of the present study was to characterize the receptors involved in the observed inotropic effects of acetylcholine in the human cardiac tissue (Du et al., 1994) by using relatively selective antagonists at the muscarinic M_1, M_2 and M_3 receptors (Doods et al., 1987) as well as a selective muscarinic agonist at the muscarinic M_1 receptor.

7.3 Material and methods

7.3.1. Preparations

Right atrial and left ventricular trabeculae were obtained from 61 heart beating organ donors (43 males, 18 females aged 2-55 years), who died of non-cardiac disorders (36 cerebrovascular accident, 20 polytrauma, 5 hypoxia) less than 24 h before the tissue was brought to the laboratory. The hearts were kindly provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation/Eurotransplant Foundation) after removal of the aortic and pulmonary valves for homograft valve implantation. The hearts were stored at 0-4°C in a sterile organ protecting solution (UW, Eurocollins, or HTK-Brettschneider, see Ploeg et al. 1992) immediately following circulatory arrest. After excision, tissue samples were placed in ice-chilled oxygenated Kreb's buffer (composition in Mm: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, $KHPO_4$ 1.2 and glucose 8.3) and atrial and ventricular trabeculae (<1 mm thickness) were carefully dissected free. The trabeculae were mounted in organ baths containing Kreb's buffer (37°C, gassed with 95% O_2 and 5% CO_2) and paced at 1 Hz using electrical field stimulation (3 ms, voltage 20% above threshold). The developed tension was recorded using isometric Harvard transducers and

Goerz flatbed recorders (Schoemaker et al., 1993; Du et al., 1994). Based on preliminary experiments, the chosen optimal resting load, yielding the highest developed tension, was 750 mg and to 1950 mg for atrial and ventricular tissue, respectively.

7.3.2 Experimental protocol

After stabilization, resting tension and baseline contractile force were measured. A concentration-response curve for noradrenaline was obtained to check the viability of the tissues (Schoemaker et al., 1993; Du et al., 1994). Tissues with less than 25 mg response to 10^{-5} M noradrenaline were excluded from further analysis.

After washing (6 times) and stabilization, 10^{-6} M physostigmine was added to the Kreb's buffer in experiments involving acetylcholine to prevent its rapid degradation by cholinesterase (see Chatonnet and Lockridge, 1989). One cumulative concentration response curve for acetylcholine (10^{-9} to 10^{-3} M) was constructed in each atrial and ventricular preparation, in the absence (control) or after 30 min incubation with one of the following antagonists: propranolol (10^{-6} M, non-selective ß-adrenoceptor antagonist), atropine (10^{-6} M, non-selective muscarinic receptor antagonist), pirenzepine (10^{-7}, 10^{-6} or 10^{-5} M, muscarinic M_1 receptor antagonist, Hammer et al., 1980), AF-DX 116 (10^{-7}, 10^{-6} or 10^{-5} M, muscarinic M_2 receptor antagonist, Giachetti et al., 1986) and HHSiD (10^{-6} or 10^{-5} M, muscarinic M_3 receptor antagonist, Lambrecht et al., 1989). In addition, the effects of acetylcholine on trabeculae pre-stimulated with noradrenaline (10^{-5} M) were investigated either in the absence or presence of 10^{-6} M atropine.

Since not all effects of acetylcholine could be fully explained after the use of antagonists, we also investigated the effects of a muscarinic M_1 receptor agonist, McN-A-343. One cumulative concentration response curve for McN-A-343 (10^{-9} to 10^{-3} M) was obtained in each atrial and ventricular preparation. In addition, in the atrial tissue, cumulative concentration response curves for McN-A-343 (10^{-9} to 10^{-3} M) were also constructed following a maximal negative inotropic effect induced by acetylcholine (10^{-9} to $\geq 10^{-6}$ M), in the absence or presence of pirenzepine (10^{-5} M).

There was no apparent difference in the responses to noradrenaline or acetylcholine observed in trabeculae obtained from subjects of different age or dying from different causes. This is also true for a number of other substances, including 5-hydroxytryptamine and histamine (Schoemaker et al., 1992; Du et al., 1993, 1994).

7.3.3 Data presentation and analysis

Data are presented as means±S.E.M. Baseline values for atrial and ventricular tissue were compared using an unpaired t-test. The effects of acetylcholine and McN-A-343 in the absence or presence of various antagonists were analyzed using an analysis of variance

for repeated measurements. Differences were regarded statistically significant, if $p \leq 0.05$. Since the negative inotropic response in atrial and the positive inotropic response in ventricular tissues reached their maximum, the curves were fitted to a four parameter logistic function (Lean et al., 1978) to calculate, where applicable, apparent pD_2 values (negative logarithm of the molar concentration eliciting half maximum effect). Apparent pK_B values were derived from pD_2 values, using the following equation:
$pK_B = -Log[B] + Log\{([A_2]/[A_1])-1\}$, where [B] is the molar concentration of antagonists and $[A_1]$ and $[A_2]$ represent molar concentrations of agonists eliciting half maximal effect in the absence and presence of antagonists, respectively.

7.3.4 Chemicals used

The chemicals used in the present study were: acetylcholine chloride (Ciba, Breda, The Netherlands), AF-DX 116 (11-({2-[(diethylamino)-methyl]-1-piperidyl}acetyl)-5,11-dihydro-6H-pyridol[2,3-b][1,4] benzodiazepine-6-one base; Thomae, Biberach/Riss, Germany), atropine sulphate (Centrafarm, Etten-Leur, The Netherlands), HHSiD (*p*-fluorohexahydro-siladifenidol hydrochloride; Research Biochemicals Incorporated, Natick, USA), McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride; McNiel, Fort Washington, PA, USA), noradrenaline bitartrate (Sigma, St. Louis, MO, U.S.A.), physostigmine salicylate (Sandoz, Basel, Switzerland), pirenzepine base (Thomae, Biberach/Riss, Germany), propranolol hydrochloride (Imperial Chemical Industries Limited, Macclesfield, U.K.). Drugs were dissolved in distilled water.

7.4 Results

7.4.1 Viability of the tissues

Baseline contractile force was significantly lower in the atrial (97±13 mg, n=57) than in ventricular (236±30 mg, n=54) tissue. In both tissues, noradrenaline (10^{-8} M to 10^{-5} M) increased contractile force in a concentration-dependent manner. After exposure to 10^{-5} M noradrenaline, the force of contraction went up to 381±35 mg (n=57) and 627±65 mg (n=54) in the atrial and ventricular trabeculae, respectively.

7.4.2 Inotropic responses to acetylcholine in atrial and ventricular trabeculae

7.4.2.1 Effect on baseline contractility. The effect of acetylcholine on the baseline atrial and ventricular contractility as well as the influence of atropine and propranolol are shown in figure 7.1. In right atrial trabeculae, acetylcholine exhibited a biphasic

contractile response consisting of an initial negative inotropic response followed by a positive inotropic response. At low concentrations (10^{-9} to 10^{-7} M), acetylcholine clearly decreased the baseline contractile force (maximum decrease from baseline: 67±13% at 10^{-7} M), but with higher concentrations the contractile force started to increase towards baseline values (0±23% change from baseline at 10^{-3} M). If after exposure to 10^{-7} M acetylcholine higher concentrations of acetylcholine were not used, the contractile force remained depressed without coming back towards baseline values. However, when 10^{-4} M acetylcholine was administrated without the preceding part of the concentration response curve, no positive inotropic effect was observed (data not shown). In contrast to atria, acetylcholine caused only a positive inotropic effect in left ventricular trabeculae. The maximum increase in contractile force (53±17% from baseline) was observed at 10^{-3} M.

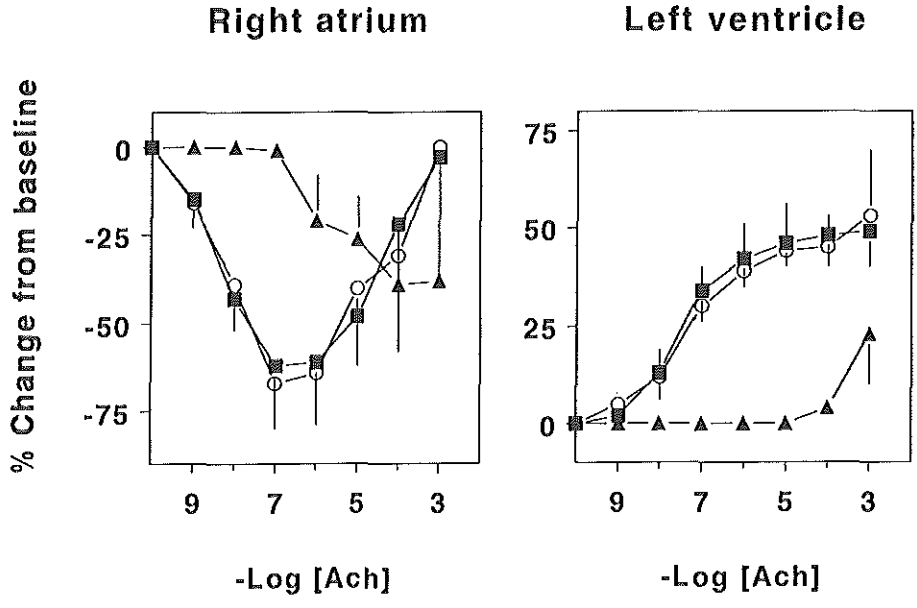


Figure 7.1. Cumulative concentration-response curves of acetylcholine (Ach) on the baseline force of contraction, obtained in the atrial (left panel) and ventricular (right panel) trabeculae in the absence (○, n=5 and 6, respectively) or presence of 10^{-6} M atropine (▲, n=6 each) or propranolol (■, n=7 and 6, respectively).

Atropine (10^{-6} M) effectively antagonized both the negative and positive inotropic effects of acetylcholine in atrial as well as the positive inotropic effect in ventricular trabeculae. Since in the presence of atropine a complete concentration-response curve to acetylcholine could not be constructed, pD_2 values of acetylcholine were not calculated. However, from figure 7.1 it can be seen that atropine shifted the concentration-response curve to the right by more than 3 and 4 log units in atrial and ventricular trabeculae, respectively. Propranolol (10^{-6} M) did not modify the responses to acetylcholine.

7.4.2.2 Effect on noradrenaline-stimulated contractility. In the presence of noradrenaline (10^{-5} M), acetylcholine produced only a negative inotropic effect in both atrial and ventricular trabeculae (figure 7.2). The reduction of the contractile force by 10^{-3} M acetylcholine was from 504±141 to 43±6 mg (86±4%) in atrial trabeculae (n=7) and from 463±93 to 288±59 mg (39±5%) in ventricular trabeculae (n=6). The pD_2 value for acetylcholine was 7.0±0.6 (n=5) and 6.8±0.9 (n=6) in the atrial and ventricular tissues, respectively.

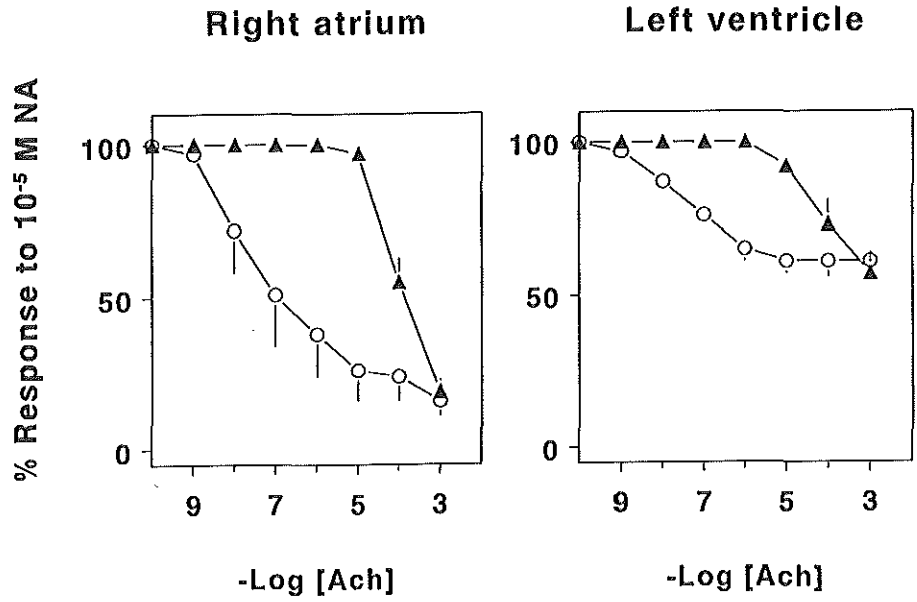


Figure 7.2. Cumulative concentration-response curves of acetylcholine (Ach) on force of contraction after pre-stimulation with noradrenaline (NA; 10^{-5} M), obtained in the atrial (left panel) and ventricular (right panel) trabeculae in the absence (○, n=7 and 6, respectively) or presence of atropine (10^{-6} M; ▲, n=7 and 5, respectively).

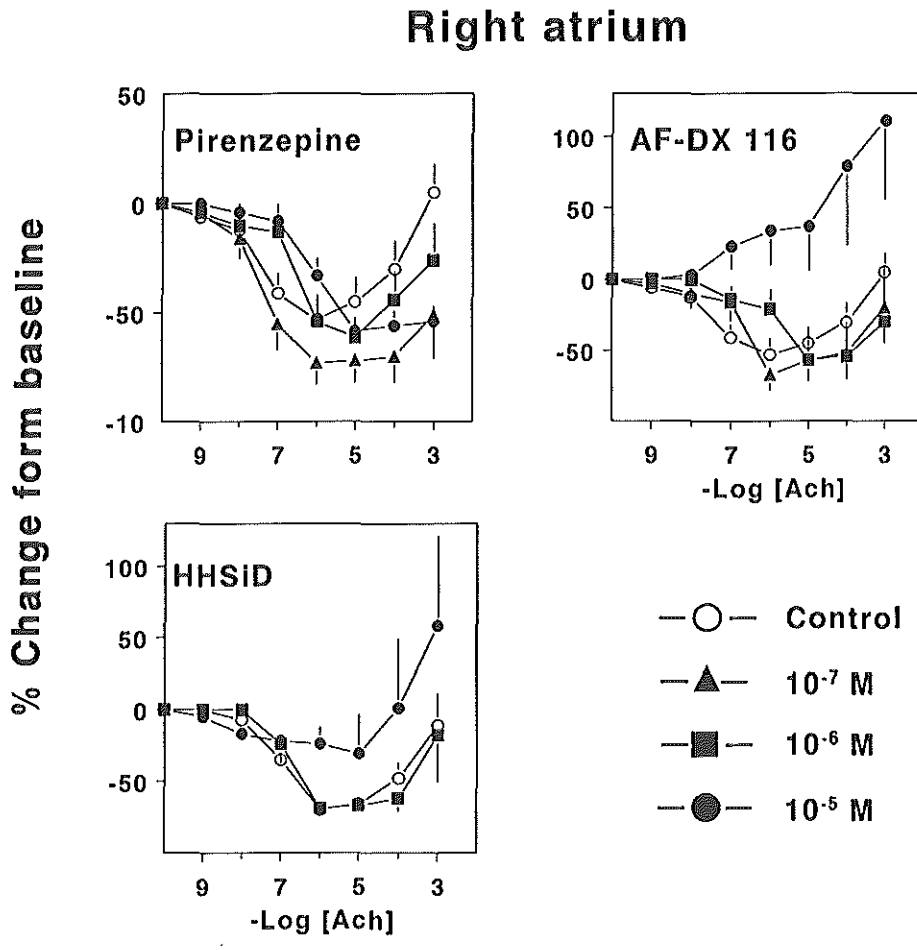


Figure 7.3. Cumulative concentration-response curves of acetylcholine (Ach) on baseline force of contraction in the atrial trabeculae in the absence or presence of antagonists. Upper left panel: acetylcholine alone (n=16) or in the presence of pirenzepine (10^{-7}, 10^{-6} and 10^{-5} M; n=6, 8 and 6, respectively). Upper right panel: acetylcholine alone (n=16) or in the presence of AF-DX 116 10^{-7}, 10^{-6} and 10^{-5} M; n=6, 8 and 6, respectively). Lower left panel: acetylcholine alone (n=10) or in the presence of HHSiD (10^{-6} and 10^{-5} M; n=6 each).

Atropine shifted the concentration-response curve for acetylcholine to the right in both atrial and ventricular tissue following pre-stimulation with 10^{-5} M noradrenaline (figure 7.2). The pD_2 values for acetylcholine were decreased in both atrial (4.5±0.1; n=7) and ventricular (4.8±0.3; n=5) trabeculae.

Table 7.1. Maximum negative (with apparent pD_2) and positive inotropic effects in atrial and maximum positive inotropic effect (with apparent pD_2) in ventricular trabeculae induced by acetylcholine.

			Atrium				Ventricle	
Antagonist	μM	n	Negative[a]	pD_2	Positive[b]	n	Positive[c]	pD_2
Control		16	47±12	6.8±0.2	78±11	14	33±6	5.6±0.4
Pirenzepine	0.1	6	73±10	7.1±0.2	29±15*	5	37±5	6.3±0.2
Pirenzepine	1	8	61±7	6.4±0.2	37±11*	4	56±16	5.1±0.6
Pirenzepine	10	6	58±6	6.3±0.2	4±3*	7	50±20	4.7±1.8
Control		16	66±5	6.8±0.2	68±12	16	46±13	5.8±0.2
AF-DX 116	0.1	6	67±11	6.6±0.1	50±23	6	38±9	6.1±0.1
AF-DX 116	1	8	57±20	5.9±0.2*	47±37	6	26±11	4.8±0.6
AF-DX 116	10	6	#*	#*	111±51	7	29±12	3.9±0.1*
Control		10	70±7	7.1±0.2	75±30	10	36±6	6.2±0.3
HHSiD	1	6	69±6	6.8±O.1	51±31	6	44±12	6.0±0.2
HHSiD	10	6	31±28*	6.4±0.4	106±57	5	32±10	5.3±0.4

[a], Change from baseline values by acetylcholine (10^{-6} M or 10^{-5} M in presence of the two highest concentrations of pirenzepine); [b], Change by 10^{-3} M acetylcholine, calculated as the difference from the maximal negative effect (pD_2 not calculable); [c], Change from baseline values by acetylcholine (10^{-4} M or 10^{-3} M). *, Significantly different from values in control experiments run in parallel. #, Only positive inotropic response.

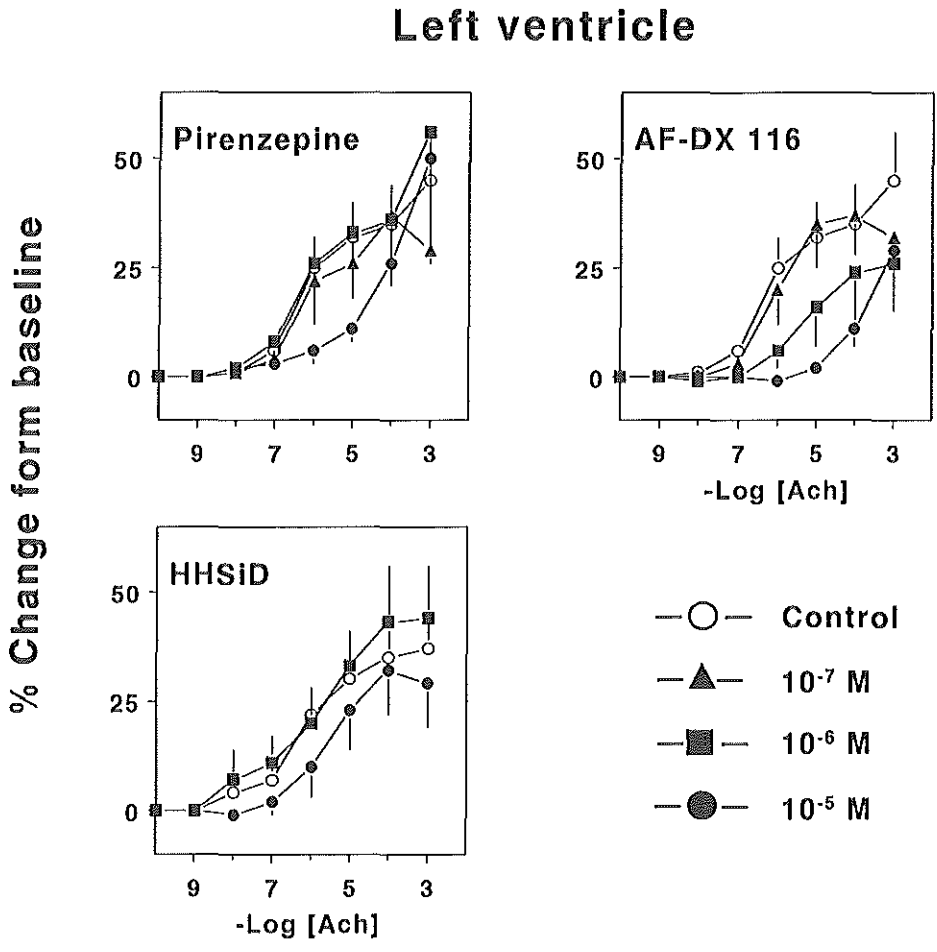


Figure 7.4. Cumulative concentration-response curves of acetylcholine (Ach) on baseline force of contraction in the ventricular trabeculae in the absence or presence of antagonists. Upper left panel: acetylcholine alone (n=14) or in the presence of pirenzepine (10^{-7}, 10^{-6} and 10^{-5} M; n=5, 4 and 7, respectively). Upper right panel: acetylcholine alone (n=16) or in the presence of AF-DX 116 10^{-7}, 10^{-6} and 10^{-5} M; n=6, 6 and 7, respectively). Lower left panel: acetylcholine alone (n=10) or in the presence of HHSiD (10^{-6} and 10^{-5} M; n=6 and 5, respectively).

7.4.3 Effect of muscarinic receptor antagonists on acetylcholine-induced inotropic responses

7.4.3.1 Right atrium. Figure 7.3 presents concentration-response curves to acetylcholine in atrial trabeculae in the absence (control) or presence of the three relatively selective muscarinic receptor antagonists, whereas the relevant parameters of these curves are summarized in table 7.1. The muscarinic M_1 receptor antagonist pirenzepine (10^{-7}, 10^{-6} or 10^{-5} M) did not significantly change the negative inotropic effect of acetylcholine (10^{-9} to 10^{-6} M), whereas the positive inotropic effect of acetylcholine (10^{-6} to 10^{-3} M) was significantly reduced by pirenzepine at the highest concentration (10^{-5} M; pK_B could not be calculated).

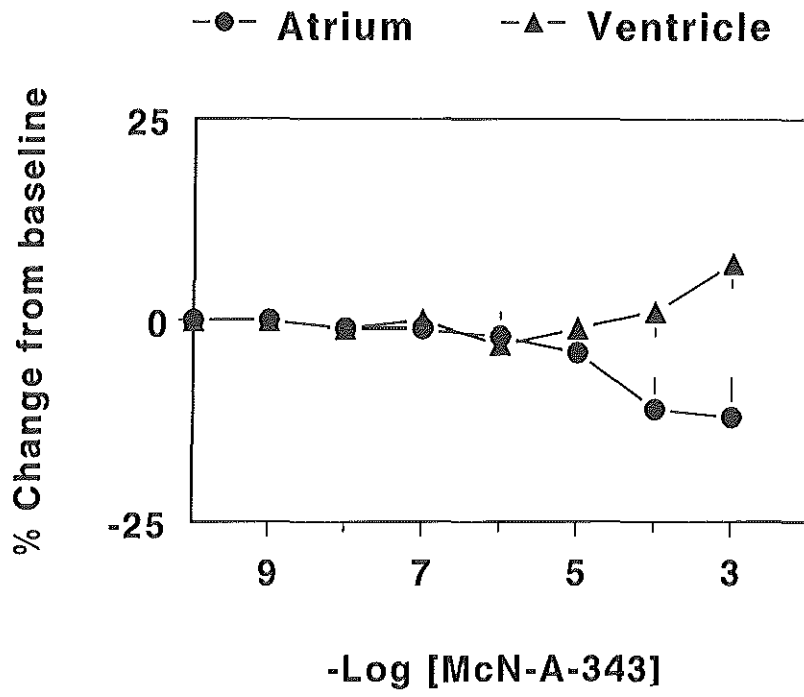


Figure 7.5. Cumulative concentration-response curves of McN-A-343 on baseline force of contraction, obtained in atrial (●) and ventricular (▲) trabeculae (n=6 each).

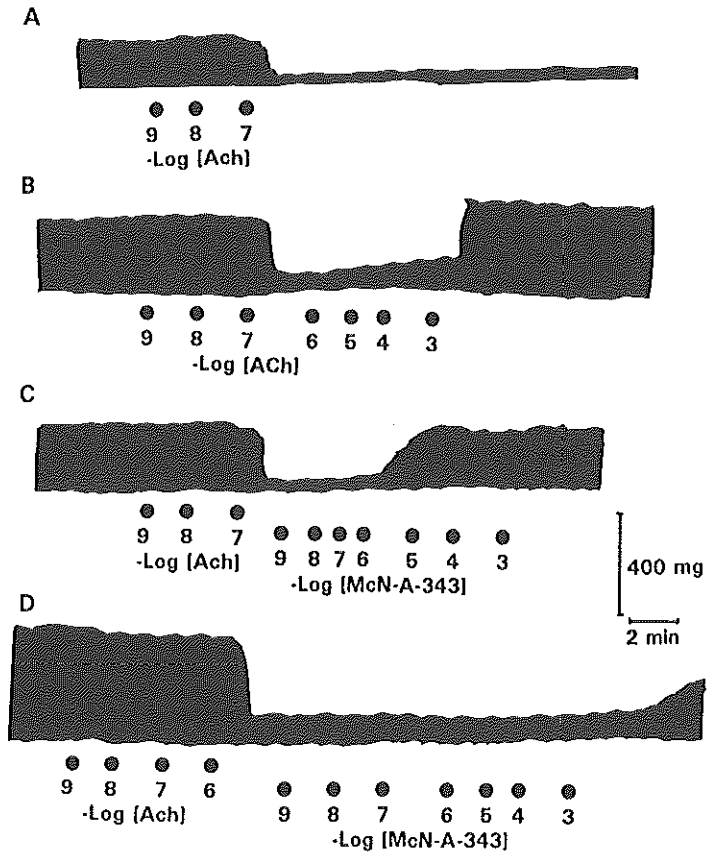


Figure 7.6. Recordings of force of contraction in four right atrial trabeculae showing the effects of acetylcholine (Ach) in different concentrations. Note that the negative inotropic effect of acetylcholine (10^{-7} M), which was maintained for several min (panel A), was reversed by higher concentrations of acetylcholine (panel B) and McN-A-343 (panel C). The reversal of the negative inotropic effect of acetylcholine by McN-A-343 was not observed in atrial trabeculae pre-treated with pirenzepine (10^{-5} M) (panel D).

The muscarinic M_2 receptor antagonist AF-DX 116 did not significantly modify the negative inotropic effect of acetylcholine at 10^{-7} M, but 10^{-6} M AF-DX 116 produced a

parallel shift to the right of the acetylcholine curve (apparent pK_B: 6.7±0.4; n=8) and 10^{-5} M AF-DX 116 completely abolished the negative inotropic effect. AF-DX 116 either did not affect (10^{-7} and 10^{-6} M) or even seemed to increase (10^{-5} M) the positive inotropic component of the effects of acetylcholine. The preferential muscarinic M_3 receptor antagonist HHSiD (10^{-6} M) failed to modify the effects of acetylcholine, but its higher concentration (10^{-5} M) reduced the negative inotropic component (apparent pK_B: 6.0±0.6; n=6), without affecting the positive inotropic component.

7.4.3.2 Left ventricle. The effects of the muscarinic receptor antagonist on acetylcholine-induced positive inotropic effects in ventricular trabeculae are shown in figure 7.4 and table I. Pirenzepine did not efficiently antagonize the responses to acetylcholine; only at 10^{-5} M pirenzepine, the curve for the acetylcholine seemed to be slightly shifted to the right (apparent pK_B: 5.7±0.5; n=6). On the other hand, AF-DX 116 (10^{-6} and 10^{-5} M) caused a concentration-dependent antagonism of the responses to acetylcholine (apparent pK_B: 6.7±0.6 and 6.2±0.3, respectively; n=6 each). HHSiD failed to modify the responses to acetylcholine.

7.4.4 Effects of McN-A-343

7.4.4.1 Baseline contractility. The effects of McN-A-343 (10^{-9} to 10^{-3} M), a relatively selective muscarinic M_1 receptor agonist, on baseline contractility in the atrial and ventricular trabeculae are presented in figure 7.5. McN-A-343 failed to significantly alter the force of contraction in either tissue; the values before and after McN-A-343 (10^{-3} M) were 230±51 and 208±56 mg in atrial trabeculae and 348±139 and 358±130 mg in ventricular trabeculae.

7.4.4.2 Acetylcholine-induced depressed contractility in atrial trabeculae. Figure 7.6 presents examples of original tracings showing the effects of acetylcholine and McN-A-343 in atrial trabeculae. Acetylcholine (10^{-7} M) produced a sustained negative inotropic effect (panel A), which was reversed by higher concentrations of acetylcholine ($>10^{-6}$ M; panel B) as well as by McN-A-343 ($\geq 10^{-8}$ M; panel C). Pre-treatment of the atrial trabeculae with pirenzepine (10^{-5} M) antagonized the positive inotropic effect of McN-A-343 (panel D).The mean data obtained in atrial trabeculae with McN-A-343 in the absence or presence of 10^{-5} M pirenzepine (after maximum negative inotropic effect was reached by low concentrations of acetylcholine) are presented in figure 7.7. Acetylcholine decreased the baseline contractile force from 868±169 mg at baseline down to 140±63 mg (-84±5%) at 10^{-7} M (n=5). McN-A-343 (10^{-9} to 10^{-3} M) increased the contractile force back to baseline values (902±170 mg at 10^{-3} M; n=5) in a concentration-dependent way (pD_2: 6.75±0.42).

Although pirenzepine (10^{-5} M) slightly shifted the acetylcholine curve to the right, it strongly antagonized the McN-A-343-induced positive inotropic effect. In the presence of pirenzepine, the force of contraction was not changed by McN-A-343 (10^{-9} to 10^{-4} M). Only at 10^{-3} M, McN-A-343 increased the contractility from 67±17 to 285±42 mg, close to the baseline value (380±110 mg) (n=4); the pD_2 value of McN-A-343 based on the last response was 3.56±0.06. The apparent pK_B value of pirenzepine against McN-A-343 was found to be 8.57±0.33 (n=4).

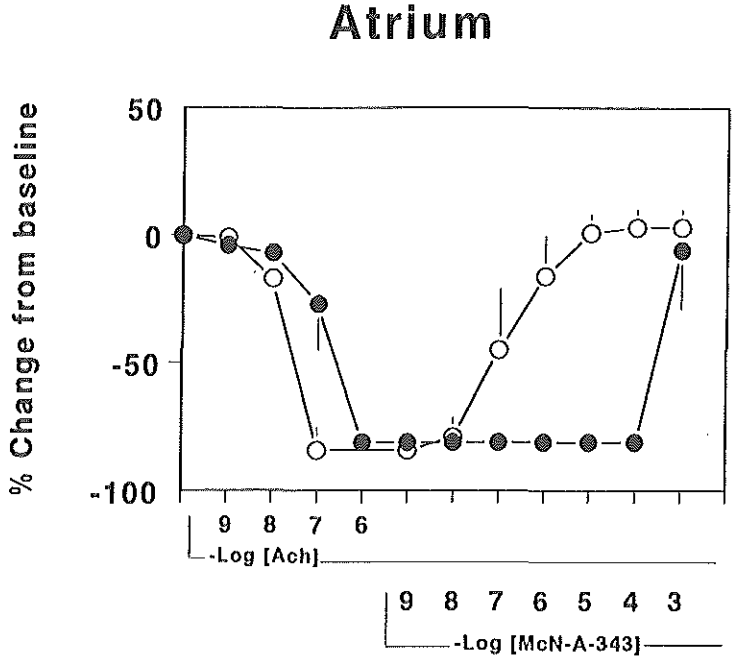


Figure 7.7. Cumulative concentration-response curves of McN-A-343 on the force of contraction in atrial trabeculae after depression of contractility by acetylcholine (Ach; 10^{-9} to 10^{-7} or 10^{-6} M), obtained in the absence (○; n=5) or presence (●; n=4) of pirenzepine (10^{-5} M).

7.5 Discussion

7.5.1 Involvement of muscarinic receptor in acetylcholine responses

The results from the present and previous (Du et al., 1994) studies show that in the human isolated myocardium acetylcholine elicits complex inotropic effects, consisting of a biphasic response (decrease followed by increase at high concentrations) in atrial baseline contractility and only an increase in ventricular baseline contractility. However, in conformity with previous observations (Jakob et al., 1989; Deighton et al., 1990; Böhm et al., 1994) in isoprenaline-augmented human isolated myocardium, acetylcholine decreased the contractility in both atrial and ventricular preparations after prior exposure to noradrenaline. The negative inotropic effect of muscarinic cholinergic agents (antagonized by atropine) is well known, but their positive inotropic action, though reported in some animal species (rat atria, Imai and Ohta, 1982; guinea-pig papillary muscles, Korth and Kühlkamp, 1987; guinea-pig atria, Eglen et al., 1988; chick ventricles, Tsuji et al., 1987), has not been reported in humans (see Jakob et al., 1989; Deighton et al., 1990; Böhm et al., 1994). The reasons for this discrepancy are not entirely clear. However, in contrast to our investigations performed in non-diseased hearts and with acetylcholine in ventricular trabeculae, the previous authors (Jakob et al., 1989; Deighton et al., 1990; Böhm et al., 1994) studied the effects of carbachol on papillary muscles obtained from hearts removed for transplantation surgery.

The precise receptor mechanisms involved in the positive inotropic effects of muscarinic cholinergic agents is not well understood. In the rat atria (Imai and Ohta, 1982), guinea-pig papillary muscle (Korth and Kühlkamp, 1987) and chick ventricles (Tsuji et al., 1987), the positive inotropic effect was antagonized by atropine and, in the last two tissues, it was also shown that the effect, being unaffected by prior reserpinization (Korth and Kühlkamp, 1987) or propranolol (Tsuji et al., 1987), was independent of endogenous catecholamines. In accordance with these observations, we also found that atropine not only blocked the negative inotropic effect in the atria, but also the positive inotropic responses in both atria and ventricles, which were unaffected by propranolol. Thus, the results show that both the negative and positive inotropic effects of acetylcholine in the human isolated myocardium are mediated by muscarinic receptors.

7.5.2 Subtypes of muscarinic receptors involved in acetylcholine responses

Although, at present, highly selective antagonists of muscarinic receptor subtypes are not available, a combination of relatively selective antagonists can be employed to characterize the muscarinic receptor subtypes. In the present investigation, we used three such antagonists having different selectivity profiles, namely pirenzepine (M_1>M_3>M_2),

AF-DX 116 (M_2>M_1>M_3) and HHSiD (M_3≥M_1>>M_2) (Doods et al., 1994). However, it should be pointed out that the affinity values of these relatively selective antagonists obtained in the present experiments in the human myocardium are further prejudiced by the complex nature of acetylcholine response.

7.5.2.1 Involvement of M_2 receptor subtype. The present study showed that AF-DX 116, followed by HHSiD and pirenzepine) most effectively antagonized the atrial negative inotropic (figure 7.3) and ventricular positive inotropic (figure 7.4) effects of acetylcholine; the concentration-response curves were shifted to the right. The calculated apparent pK_B values of AF-DX 116 in the atria (6.7±0.4) and ventricles (6.7±0.6) match with pK_i values observed in the two tissues (7.14±0.06 and 7.18±0.06, respectively) for the displacement of [^{3}H]-N-methyl scopolamine in guinea-pig membranes (Michel and Whiting, 1987). Thus, it appears that the muscarinic M_2 receptor subtype mediates both the negative (in atria) and positive (in ventricles) inotropic responses. This conclusion is in agreement with the demonstration of muscarinic M_2 receptor mRNA and protein in human heart (Maeda et al., 1988; Caulfield, 1993). Moreover, it is now well established that the muscarinic M_2 receptor is coupled to three signal transduction pathways (Schimerlik, 1989): (i) inhibition of adenylyl cyclase to reduce cAMP (Fleming et al., 1987), (ii) opening of potassium channels (Ray et al., 1993) and (iii) hydrolysis of phosphatidylinositol. The first two may contribute to the negative inotropic response of muscarinic receptor stimulation (Hanf et al., 1993), while the third may induce positive inotropic responses (Mizushima et al., 1987; Kohl et al., 1990). Although the exact molecular processes are unclear, it is believed that the two opposite functional responses are mediated via muscarinic M_2 receptor coupled to different G-proteins and occurring in different states: a high affinity state associated with inhibition of adenylate cyclase and a low affinity state associated with the phosphatidylinositol breakdown (Brown and Brown, 1984).

A recent study on comparison of muscarinic K^+ channels between human atrium and ventricle (Koumi et al., 1994) showed that whole cell acetylcholine-induced K^+ current in human atria and ventricles exhibit substantially similar characteristics, with the exception of the channel density. Acetylcholine-induced K^+ current in atria was approximately two to three times higher than that in ventricles. Another study on the comparison of coupling of muscarinic receptors in guinea-pig atrial and ventricular myocardium showed that atrial and ventricular receptors were similar, but receptor coupling to cyclase inhibition or phosphatidylinositol hydrolysis was distinguishable (Woodcock et al., 1987). Thus, it is possible that all three pathways operate to mediate the observed tissue responses, but their importance may vary in different tissues and/or conditions. Based on the effect of acetylcholine on the baseline contractility (without prior stimulation with, for example,

noradrenaline), it would appear that the coupling of muscarinic M_2 receptors to adenylyl cyclase (negatively) and K^+ channels may be more important in the atrial tissue, whereas in the ventricular tissue muscarinic M_2 receptors couple preferentially to phosphatidylinositol hydrolysis. However, it is possible that the coupling can alter in different conditions. Indeed, it is interesting to recall that in the human ventricular myocardium a concentration-dependent negative inotropic effect was seen after pre-stimulation with sympathomimetic agents (Jakob et al., 1989; Landzberg et al., 1994; present results).

7.5.2.2 Involvement of muscarinic M_1 receptor subtype. Experiments with AF-DX 116 and HHSiD in atrial tissue showed that the positive inotropic effect of acetylcholine, observed with high concentrations and only when the atrial contractility had been depressed, was not attenuated and seemed to be potentiated by these compounds. On the other hand, pirenzepine seemed to attenuate this response, thereby accentuating the preceding negative inotropic effect (figure 7.3). It therefore appears that the acetylcholine-induced increase in depressed contractility is mediated by the muscarinic M_1 receptor subtype rather than the M_2 or M_3 subtype. Although HHSiD has been reported to be a more potent antagonist against M_2 or M_3 receptors (see Doods et al., 1994), this apparently dose not seem to hold true for the human heart, where this compound blocked M_2 receptor mediated responses (negative inotropism in atria and positive inotropism in ventricles) more effectively.

The conclusion that the muscarinic M_1 receptor subtype mediates acetylcholine-induced increases in atrial contractility is further substantiated by the results obtained with McN-A-343, which in the radioligand binding assays has similar affinities for the M_1 and M_2 receptor subtypes, but in functional assays, probably due to differences in intrinsic efficacy and/or tissue receptor reserve, shows selectivity for the muscarinic M_1 receptor subtype (Eglen et al., 1987). Indeed, McN-A-343 failed to decrease atrial or increase ventricular contractility (figure 7.5), thus ruling out activation of the muscarinic M_2 receptor subtype in the human heart. This compound, however, mimicked acetylcholine (higher concentrations) in increasing atrial contractility, once this had been depressed by prior administration of low concentrations of acetylcholine (figure 7.6). The reversal of the negative inotropic effect of acetylcholine by McN-A-343 cannot be due to a blockade of muscarinic M_2 receptors, since the effect of McN-A-343 was antagonized by pirenzepine. The apparent pK_B value of pirenzepine against McN-A-343 (8.57±0.33) was similar to the binding affinity of drug at the human cloned muscarinic M_1 receptor (pK_i: 8.20±0.13; Dorje et al., 1991), thus confirming the involvement of muscarinic M_1 receptor subtype. Additional evidence for functional atrial muscarinic M_1 receptors comes from studies of Pitsschner and Wellstein (1988), who observed that low doses (<3mg) of pirenzepine decrease heart rate in humans subjects. Similarly, the bradycardia caused by low doses of

atropine in humans may also be related to blockade of muscarinic M_1 receptors (Wellstein and Pitschner, 1988; present results), rather than central vagal stimulation (Weiner, 1990).

Kellar et al. (1985) examined the binding of [^{3}H]acetylcholine and suggested that most of the muscarinic M_2 sites had a high affinity for acetylcholine, whereas the majority of muscarinic M_1 sites had a low affinity for acetylcholine. This is in agreement with our observations that the positive inotropic response in the atrial trabeculae was obtained with high concentrations of acetylcholine (10^{-4} or 10^{-3} M). It is possible that the muscarinic M_1 receptor mediating increases in atrial contractility is coupled to phosphatidylinositol hydrolysis (Gallo et al., 1993; Wess et al., 1993).

In conclusion, the present investigation in the human isolated myocardium shows that: (i) in addition to the generally accepted negative inotropic effect, acetylcholine also causes positive inotropic effects at similar concentration in human ventricular trabeculae and both these responses are mediated by the muscarinic M_2 receptor subtype, possibly having preferential coupling to adenylyl cyclase (negatively) and K^+ channels in atria and to phosphoinositol breakdown in ventricles, (ii) in trabeculae pre-stimulated with noradrenaline, acetylcholine elicited negative inotropic responses in both atrial and ventricular trabeculae, suggesting that the preferential coupling in the ventricles can be altered, and (iii) when atrial contractility has already been depressed, high concentrations of acetylcholine as well as McN-A-343 can increase the atrial contractility back towards baseline values via the muscarinic M_1 receptor subtype. The physiological relevance of the atrial muscarinic M_1 receptor is not yet clear.

Chapter 8 General discussion

The studies presented in this thesis are composed of *in vitro* experiments studying pharmacological responses of isolated cardiac tissue. Three aspects make this thesis rather unique.

i) The studies were performed on human cardiac tissue and, therefore, the results can be directly translated to the human situation without extrapolation from animal data. Nevertheless, some studies were performed in porcine cardiac tissue in parallel to evaluate this species as a model for human tissue.

ii) The studies were performed in human healthy (without cardiac diseases) atrial and ventricular tissue, which, especially the latter, is difficult to obtain on a regular basis. The results provide information about the regulation of contractility in human cardiac muscle in non-diseased states.

iii) The studies were performed on right atrial and left ventricular tissues obtained from the same heart. The results may represent the different contribution of the atrium and ventricle to overall function of the heart. The use of the atrial and ventricular tissue in parallel enhances the value of comparison of the responses of the two tissues with regard to their function in vivo.

The present chapter discusses the results with respect to comparison of human and porcine heart, atrial versus ventricular tissue responses, and receptor subtypes mediating the responses.

8.1 Species differences

The use of laboratory animals as models for man provides an important and convenient method for pharmacological research. However, for such an approach to succeed, it is imperative that chosen animal species does not show species-related differences with human pharmacology. For evaluation of porcine cardiac tissue as a model, in some of our studies the differences and similarities of the porcine and human tissue in contractile responses are discussed.

8.1.2 Different response

In chapter 5, we showed that human α-CGRP exerted different effects, presumably through the same receptor, on porcine and human hearts. α-CGRP induced a positive inotropic response in porcine ventricular, but not in atrial trabeculae. In contrast, α-CGRP

increased the contractility in human atrial, but not in ventricular trabeculae (chapter 6). It is known that positive inotropic effect of human CGRP in most species is mediated by specific CGRP receptors coupled to adenylyl cyclase (Franco-Cereceda et al., 1987; Miyauchi et al., 1987; Ono et al., 1989; Anand et al., 1991). We do not know whether the different responsiveness of human and porcine myocardium is due to the use of human CGRP in both human and porcine heart or to a different distribution or type of functional CGRP receptors in the two species. Specific binding sites for CGRP have been demonstrated in porcine ventricular tissue (Miyauchi et al., 1988) and rat atrium (Sigrist et al., 1986), whereas for porcine atrial and human cardiac tissue this is not known.

The above observation showed, probably for the first time, that a biogenic substance induces a positive inotropic response in a mammalian ventricular tissue without a corresponding effect in atrial tissue. The opposite has been shown with several substances in the heart from several species (Ziogas et al., 1985; Schoemaker et al, 1992, 1993; Feolde et al., 1993; Du et al., 1994; Ishihata et al., 1995). The results of this study suggest that the response to an endogenous substance could be species-specific as it induces different responses in different species.

8.1.3 Similar response but different receptors

In chapter 4, we showed that histamine induced a similar response in different species mediated by different receptor subtypes. Histamine produced a positive inotropic effect in human atrial and ventricular trabeculae as well as porcine atrial trabeculae via histamine H_2 receptors. In porcine ventricular trabeculae, histamine induced a similar response, however, via histamine H_1 receptors. This receptor subtype difference is not unique for the porcine heart and it occurs in other species as well. For instance, the positive inotropic effect of histamine in dog atrium (Chiba et al., 1977) as well as in guinea pig left atrium (Verma and McNeill 1977) is due to stimulation of H_1 receptors. On the other hand, in rabbit ventricular muscle, the effect is mediated by both H_1 and H_2 receptors (Elizalde, 1986). Similarly, as reviewed by Saxena and Villalón (1991), cardiac stimulation by 5-HT is mediated by 5-HT_1-like, 5-HT_2, 5-HT_3 and 5-HT_4 receptors in, respectively, the cat, rat, rabbit and pig; however, in this case, porcine and human heart resemble each other. Thus, according to the results described in this thesis (see chapter 4 and 5), we conclude that porcine cardiac tissue, although pharmacologically similar in several respects, is still distinguishable from that of humans, indicating that the porcine heart model too has its limitation. Although animal models may provide a useful tool for answering specific questions, an evaluation of interference of species-related differences with regard to pharmacologic regulation of cardiac contractility is necessary. Therefore, we restricted further experiments to only human tissue.

8.2 Different response in human atrial and ventricular tissue

Focusing on human atrial and ventricular tissue, we found that biogenic substances could induce similar or different responses in atrial and ventricular tissue from the same heart. A parallel experiment in atrial and ventricular tissue is necessary for studies on cardiac contractility.

8.2.1 Different response but similar receptor

Chapter 7 showed that a biogenic substance can induce different responses in atrial and ventricular tissue mediated by the same receptors. Acetylcholine ($\leq 10^{-6}$ M) produced a negative inotropic effect in human atrial trabeculae, but a positive inotropic effect in ventricular trabeculae; both effects were mediated by M_2 muscarinic receptors. It is well known that M_2 receptors are coupled to multiple second messenger systems (Schimerlik, 1989): 1. Inhibition of adenylyl cyclase to reduce cAMP and opening of potassium channels, which may contribute to the negative inotropic response of muscarinic receptor stimulation (Hanf et al., 1993); 2. Stimulation of phosphoinositide hydrolysis, which may be responsible for the positive inotropic effect of choline esters (Korth et al., 1987). The difference in atrial and ventricular response may be explained by coupling of the M_2 muscarinic receptor to different second messenger systems: in atrial tissue, the M_2 receptor may couple predominantly negatively to the cAMP pathway, whereas in ventricular tissue, the M_2 receptor may couple mainly to the IP_3 pathway. The above observations provide a clear example that, although both tissues were taken from the same heart, the results from atrial tissue are not representative for the rest of the heart, including ventricular tissue. The difference in atrial and ventricular tissue is further discussed below.

8.2.2 Lack of ventricular response

At the beginning of the present studies (chapters 2 and 3), we observed that atrial and ventricular trabeculae from the same human heart showed different response to 5-HT: 5-HT induced a positive inotropic effect in atrial but not in ventricular tissue. Similar results were obtained in porcine heart. The effect of 5-HT in atrial tissue is mediated by 5-HT_4 receptors coupled to the adenylyl cyclase /cAMP second messenger system (Kaumann et al., 1991a,b). The results have led to two questions: 1. Is this phenomenon unique for 5-HT or could it be observed for other substances as well? 2. Why does ventricular tissue fail to respond to this and other substances?

With regard to the first question, our results (chapter 6) showed that other substances, including human α-CGRP and angiotensin II, increased atrial, but not ventricular

contractility. Moreover, substances that decrease cardiac contractility (adenosine and somatostatin) affected atrial tissue, but they were virtually inactive in ventricular tissue. We, therefore, conclude that the effect of 5-HT with regard to the different atrial and ventricular responses was not limited to 5-HT, but could also be observed with other substances. Atrial tissue may be more sensitive to endogenous inotropic substances than ventricular tissue.

Based on our studies, a clear explanation for the different responses of atrial and ventricular trabeculae (question 2) is difficult to give. Obviously, the force of contraction of ventricular tissue can be increased by positive inotropic agents, since this tissue showed a substantial response to noradrenaline and histamine (chapter 4). Because both noradrenaline and histamine exert their positive inotropic effect by increasing cAMP levels through adenylyl cyclase stimulation, we conclude that this pathway was intact in our preparations. However, in contrast to atrial tissue, ventricular tissue did not respond to angiotensin II, human α-CGRP, adenosine and somatostatin. Since 5-HT, α-CGRP, adenosine and somatostatin all act via their receptors coupled to the adenylyl cyclase pathway, which, as argued above, seems to function normally, the lack of response in ventricular tissue may be caused by either a too low receptor number or by receptors that are not coupled to their signal transduction pathway. Moreover, this lack of ventricular response is not restricted to substances acting via adenylyl cyclase/cAMP pathway, since it was also observed for angiotensin II, that acts via the PLC/IP_3/DG pathway.

For at least some substances, there is evidence for reduced receptor density or different receptor subtype in ventricular tissue. In a recent review, Kaumann (1994) suggested that functional 5-HT_4 receptors may be absent in human ventricular myocytes. Nozawa et al. (1994) suggest that, in contrast to human atrial myocardium with mainly AT_1 receptors, the predominant angiotensin II receptor subtype in human ventricular myocardium is AT_2, which does not modulate contractility. Thus, in addition to a difference in receptor density between atrial and ventricular tissue, the different subtypes of angiotensin II receptors may also contribute to the lack of ventricular response. Specific binding sites for CGRP were identified in high concentration in rat atrium, but very few were found in guinea pig ventricles (Sigrist et al., 1986; Ishikawa et al., 1988). Since the response in rat and guinea pig cardiac tissue is similar to that in human tissue, this explanation may also be true for the human heart. In human atrial tissue, the density of adenosine A_1 receptors is about twice as high as in the ventricle (Böhm et al., 1989). These data may explain the lack of response with adenosine in human ventricular tissue.

The above results show that atrial contractility is more sensitive to several cardiovascular relevant endogenous substances than ventricular contractility. This observation may have important implications for drug development. Usually, development

of therapeutic agents that influence cardiac contractility is directed to increase ventricular contractile force, as it is a major determinant of left ventricular function. In this regard, the use of atrial tissue as representing "cardiac tissue" is questionable and can be misleading. On the other hand, since atrial tissue seems more sensitive to most of the substances investigated so far, the contribution of atrial contraction to the overall cardiac function may have been generally underestimated.

8.2.3 Functional importance

Data from this thesis showed that ventricular tissue from both man and pig did not respond to several positive inotropic substances. Also some negative inotropic substances, acetylcholine, adenosine and somatostatin, decreased force of contraction only in atrial, but not in ventricular tissue. In vivo, under normal conditions, the atrial contraction causes an additional 30% of filling of the ventricle, which is normally not really needed because of the 300-400% overcapacity of heart (Guyton, 1986). However, under pathological conditions such as myocardial infarction, when cardiac reserve has decreased, the contribution of atrial contraction to ventricular filling increases to 42% (Matsuda et al., 1983). The atrial contraction contributes by about 35% to the stroke volume after myocardial infarction, compared to 22% under normal conditions. Thus, although atrial contraction under healthy conditions may not be important for cardiac function, its contribution becomes more significant in pathological condition.

It is interesting to notice that a direct negative inotropic response on baseline ventricular contractility was not detected with any of the biogenic substances studied here. The present results and those from other laboratories have shown that in mammalian ventricular tissue, a negative inotropic response can only be observed after pre-stimulation with catecholamines (Böhm et al., 1985; 1988; Endoh et al., 1985), though a direct negative inotropic effect of carbachol (a muscarinic agonist) has been reported in chick ventricular muscle strips (Sorota et al., 1986). These results suggest that left ventricular tissue, at rest, can only be stimulated, but not depressed by biogenic substances. If these results from *in vitro* studies can be extrapolated to the *in vivo* situation, it would suggest that a lowering in left ventricular contraction can only be obtained indirectly by reduction of atrial contractility and, hence, ventricular filling. Enhanced left ventricular contraction could be obtained either directly via ß-adrenergic stimulation, histamine and, surprisingly, acetylcholine or indirectly by increasing atrial contraction via substances, such as α-CGRP, angiotensin II and 5-HT.

On the basis of the results, we can only speculate about the physiological meaning of the atrial-ventricular differences. It is possible that atrial contractile force is regulated

more subtly than that of ventricles, and relatively small changes in left ventricular filling affect left ventricular function via the Frank Starling mechanism. Only in case of pronounced extra need for cardiac output, for instance, during sympathetic stimulation, the ventricle itself is stimulated to enhance contractile force, with concomitant extra energy costs. Since all experiments were performed in non-diseased cardiac tissue, the outcome may be different in pathological conditions where direct ventricular stimulation is needed for sufficient cardiac output.

Further investigation may provide a better understanding of the physiology of atrial and ventricular contractility, from which drug development can benefit.

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Summary

The first purpose of the present thesis was to investigate the effects and the possible mechanisms of several biogenic substances (5-HT, histamine, human α-CGRP, angiotensin II, adenosine, somatostatin and acetylcholine) in human isolated myocardium, although some studies were performed on porcine heart to evaluate this as a model for human heart. Myocardial contractility, as the major parameter of the function of the heart, has been studied in different situations. In vivo, the changes in force of contraction in the heart can be markedly influenced by reflex mechanisms as well as hemodynamic changes. Therefore, isolated, electrically driven cardiac preparations provide a useful model to examine the direct effects of substances on cardiac contractility. Since endogenous substances are associated with the physiologic and pathophysiological changes in human body, pharmacologic investigations on cardiac contractility in isolated myocardium can provide useful information for developing better drugs. From our studies on the inotropic effects of several biogenic substances, it is interesting to notice that different pharmacologic responses were observed not only in different species, but also in atrial and ventricular tissue from the same heart.

The second aim of our study was to explore the differences in the pharmacology of human isolated atrial and ventricular trabeculae. Evidence shows that atrium and ventricle may play different roles in the regulation of cardiac contractility. To distinguish between atrial and ventricular responses, these tissues should be studied separately, but in parallel, implying *in vitro* methods.

Species-related pharmacologic differences were observed in functional responses as well as receptor subtypes. The difference in the functional response is exemplified by the effects of human α-CGRP in human and porcine isolated myocardium (chapter 5 and 6). Human α-CGRP induced a concentration-dependent positive inotropic response in human atrial but not in ventricular trabeculae. In contrast, in porcine heart, the human α-CGRP induced a positive inotropic effect in ventricular but not in atrial trabeculae. Furthermore, the effects of histamine provide a good example for the species-related receptor subtype dissimilarity. Histamine (chapter 4) increased the contractility in both human and porcine myocardium. However, the effects of histamine in human atrial and ventricular trabeculae as well as in porcine atrial trabeculae were mediated by H_2 histamine receptor. In contrast, the effect in porcine ventricular trabeculae was mediated by H_1 histamine receptor. This data indicated that a similar response in atrial and ventricular tissues could be mediated by different receptors. Our results obtained from porcine isolated myocardium showed similar

responses and mechanisms in most cases when compared to human myocardium. However, also dissimilar, substantiated by responses to human α-CGRP as well as to histamine with human tissues were observed, suggesting that this model, too, has its limitations.

An important aspect of this thesis is that atrial and ventricular tissues obtained from the same heart showed differences. Firstly, baseline contractile force was significantly lower in the atrial than in ventricular tissue in both human and porcine myocardium. This difference is compatible with the different *in vivo* function of these two parts of the heart. Secondly, the pharmacological differences of these two tissues concerned both functional responses, receptor subtypes and coupling. As shown in chapter 2 and 3, it was found that 5-HT increased the force of contraction in atrial trabeculae from human and pig in concentration-dependent manner, but, it failed to affect the force of contraction in ventricular trabeculae from both species. Similar to 5-HT, several other substances including human α-CGRP and angiotensin II also caused concentration-dependent increases in force of contraction in human atrial (chapter 6), but not in human ventricular trabeculae. Moreover, not only positive inotropic substances, also some negative inotropic substances were virtually inactive in modifying the baseline ventricular contractility. Adenosine and somatostatin (chapter 6) induced a concentration-dependent negative inotropic effect on baseline human atrial contractility, but no response was found on baseline human ventricular contractility. Adenosine, but not somatostatin, reduced force of contraction after pre-stimulation with 10^{-5} M noradrenaline in atrial tissue and, to a lesser extent, in ventricular tissue.

Different responses of atrial and ventricular tissue can also be observed with acetylcholine, which elicited a biphasic inotropic effect (an initial decrease followed by an increase in contractility back to baseline values) in human atrial trabeculae, whereas, only a positive inotropic effect was observed in the ventricular trabeculae (chapter 7). It was interesting to discover that the negative inotropic effect of acetylcholine ($\leq 10^{-6}$ M) in atrial trabeculae and the positive inotropic effect of acetylcholine in ventricular trabeculae were mediated by the same M_2 muscarinic receptor. Under conditions of raised contractility achieved by exposure to noradrenaline (10^{-5} M), negative inotropic effects, mediated by M_2 muscarinic receptor too, were observed in both atria and ventricles. However, our results suggest that the positive inotropic effect of acetylcholine (10^{-6} to 10^{-3} M) in the atrial tissue, observed only in preparations with depressed contractility, was associated with M_1 receptor stimulation. From this study, we can conclude that a different response in atrial and ventricular tissue could even be mediated by the same receptors.

The data from the present thesis indicate that force of contraction in atrial tissue can be affected by either positive or negative inotropic substances, whereas in ventricular

tissue, only positive inotropic effects could be detected on baseline contractility. Atrial tissue appeared to be more sensitive to endogenous inotropic substances than ventricular tissue. Since atrial and ventricular tissues display different responses to above biogenic substances, a different mechanism of regulation of contractility seems feasible. If these results from *in vitro* studies can be extrapolated to the *in vivo* situation, the contribution of atrial contractility to overall cardiac function may be generally underestimated. Our results may provide a better understanding of the physiology of atrial and ventricular contractility, from which drug development can benefit.

Samenvatting

Het hart is een van de vitale organen in het menselijk lichaam en hart en vaatziekten zijn al gauw levensbedreigend. Onderzoek naar de effecten van geneesmiddelen zijn dan ook erg belangrijk. Het gebruik van menselijk weefsel daarbij is uiterst beperkt. Soms kan tijdens operaties (bv. by-pass) een stukje atrium verkregen worden dat gebruikt kan worden voor onderzoek. Resultaten verkregen m.b.v. dit stukje weefsel worden vaak geëxtrapoleerd naar het hele hart, inclusief het voor de functie zo belangrijke linker ventrikel.

Het eerste doel van dit proefschrift was de effecten van verscheidene endogene stoffen te bestuderen op humaan geïsoleerd hartspierweefsel. Tevens zijn enkele van de studies ook uitgevoerd op hartspierweefsel van varkens, om dit als model voor humaan hartweefsel te evalueren. Geïsoleerde, elektrisch gestimuleerde trabeculea vormen een praktische opstelling voor studies naar effecten op contractiekracht zonder de invloed van reflex mechanismen en wisselende hemodynamische en neurohumorale invloeden.

Species gerelateerde verschillen werden gevonden in de response zelf en in de receptoren betrokken bij de response. Een species-verschil in response is gevonden bij humaan α-CGRP (hoofdstuk 5 en 6), hetgeen in menselijk materiaal een positief inotrope response gaf in atriaal, maar niet in ventriculair weefsel. Voor de varkensharten werd het omgekeerde gevonden. Histamine daarentegen (hoofdstuk 4) gaf in atrium en ventrikel weefsel van zowel mens als varken een vergelijkbare response. Echter de receptoren betrokken in deze response waren in menselijk atrium en ventrikel, en in varkens atrium de H_2, en in varkensventrikel de H_1 receptor. Dus ondanks overeenkomsten in reacties zijn er ook verschillen aan te tonen in varkensharten t.o.v. mensenharten, hetgeen aangeeft dat ook dit model zo zijn beperkingen kent.

Uit de studies naar inotrope effecten van endogene stoffen kwamen niet alleen species-verschillen naar voren maar ook verschillen in reacties van atriale en ventriculaire trabeculae uit het zelfde hart.

Het tweede doel van dit proefschrift was deze atrium/ventrikel verschillen verder te onderzoeken. Beide delen van het hart hebben een verschillende bijdrage aan de totale hartfunctie en daarom mogelijk ook een verschillende regulatie van de contractiliteit. Om atrium en ventrikel weefsel apart te onderzoeken is een in vitro benadering noodzakelijk.
Het verschil tussen atriale en ventriculaire reacties vormt een belangrijk aspect van dit proefschrift. Allereerst was de basale contractiekracht van atriale trabeculae lager dan die van ventriculaire trabeculae. Dit is compatibel met de verschillen in in vivo functies van

de beide delen. In hoofdstuk 2 en 3 wordt aangetoond dat serotonine wel een positief inotroop effect op atriaal weefsel heeft, maar niet op ventriculair weefsel. Deze observatie is niet beperkt tot serotonine; ook CGRP en angiotensine II laten dit zien (hoofdstuk 6). Niet alleen positief inotrope stoffen maar ook negatief inotrope stoffen, zoals adenosine en somatostatine (hoofdstuk 6) gaven wel response in atriaal, maar niet in ventriculair weefsel.

Naast een afwezigheid van reactie in ventriculair weefsel is ook een verschillende reacties van ventriculair t.o.v. atriaal weefsel gevonden voor acetylcholine; in atrium een bi-fasisch effect (eerst negatief inotroop en bij hogere concentraties terug naar de uitgangswaarde) en in het ventrikel alleen een positief inotroop effect (hoofdstuk 7). Verrassend was dat en het negatief inotrope effect in atriale trabeculae en het positief inotrope effect in ventriculaire trabeculae door dezelfde M_2 receptoren gemedieerd worden, doch gekoppeld aan verschillende second messenger systemen. Het positief inotrope effect bij hoge concentraties in atriaal weefsel bleek gemedieerd door M_1 receptoren. Uit deze experimenten hebben we geconcludeerd dat een verschillende response in atriaal en ventriculair weefsel terug te voeren kan zijn op verschillen in receptordichtheid, receptorsubtype en zelfs gemedieerd kan worden door hetzelfde receptorsubtype, maar verschillend gekoppeld.

De data in dit proefschrift suggereren dat de contractiekracht in atrieel weefsel beïnvloed kan worden door zowel positief als negatief inotrope stoffen, terwijl ventriculair weefsel alleen reageert op positief inotrope invloeden. Het atrium lijkt meer gevoelig voor endogene inotrope invloeden dan het ventrikel. Indien de resultaten van deze in vitro studies kunnen worden geëxtrapoleerd naar de in vivo situatie, dan kunnen ze een belangrijke bijdrage leveren aan het inzicht in de regulatie van contractiliteit en hartfunctie. Een inzicht waar de ontwikkeling van geneesmiddelen zijn voordeel mee kan doen.

摘 要

本文的目的之一是研究一些生物活性物质，包括5-羟色胺、组织胺、降钙素基因相关肽、血管紧张素、腺甙、生长激素释放抑制激素以及乙酰胆碱对心肌收缩力的作用。这些活性物质和人体的一些生理和病理变化密切相关，所以这一研究将对发展新药提供帮助。另外，为评估猪心肌作为人心的研究模型的可行性，一些实验在猪心肌上进行。

心肌的收缩力做为心脏功能的主要参数之一，得到从不同条件下的广泛研究。在整体情况下，心肌收缩力受到反射机制、血液动力学变化及激素等因素的影响。而离体心肌实验则可在一定条件下直接观察其变化，为药理学研究提供有用的方法。

结果表明，药理学差异不仅表现在不同的生物种属上，还表现在同一心脏的不同部位(心房肌和心室肌)上。本文的另一目的就是对心房肌和心室肌在同一条件下进行比较研究。

与种属相关的药理学差异可表现在受缩力的变化及受体的类型上。前者以降钙素基因相关肽的作用为例(第5章及第6章)。降钙素基因相关肽可增加人心房肌的收缩力而不影响其心室肌的收缩力。另外，与种属相关的受体类型的差异可以从对组织胺的反应观察到(第4章)。虽然人及猪的心肌收缩力在组织胺的作用下都得到增加，但在人心肌上是通过H_2受体，而猪只有心房肌是通过H_2受体，心室肌是通过H_1受体起作用的。本文结果表明，虽然猪心肌在某种程度上和人心肌相似，但也有种属的差异，使其研究结果有局限性。

本文的一个重要结论是来至同一心脏的心房肌和心室肌对某些生物活性物有不同的反应，在调节控制心脏收缩力上有不同机制。这种区别表现在几个方面：其一，在基础收缩力上心房肌要显著低于心室肌。其二，对这些生物活性物质的反应，心房肌在大多数情况下比心室肌更敏感。有些物质包括5-羟色胺、降钙素基因相关物质、血管紧张素、腺甙、生长激素释放抑制激素只对人心房肌有作用，而对心室

肌基础收缩力无影响。其三，同一种物质对心房肌和心室肌产生不同甚至相反的作用(以乙酰胆碱对人心肌作用为例，见第7章)。 所以对心肌收缩力的研究不仅要考虑外部条件、种属等因素，而且要注意同一心脏的房/室肌的差异。

List of Publication

Full papers

Du XY, Schoemaker GR, Bax WA, Bos E, Saxena PR (1993) Effects of histamine on porcine isolated myocardium: differentiation from effects of on human tissue. J Cardiovasc Pharmacol 22:468-473

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Curriculum vitae

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