Different catecholamines induce different patterns of takotsubo-like cardiac dysfunction in an apparently afterload dependent manner.

Masters Thesis in medicine

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Gothenburg, Sweden 2015

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Abstract

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Background: Stress induced cardiomyopathy(SIC) is an acute cardiac syndrome with symptoms reminiscent of an acute coronary syndrome but with different pathology and prognosis. As in the case of myocardial infarction part of the ventricle stops contracting and becomes akinetic, but unlike MI the akinetic part does not undergo necrosis and over time normal function is regained if the patient survives. The akinetic area does not confirm to the area of ventricle wall supplied by any one coronary artery and when angiography is performed no occlusions are found in the coronary arteries. The precise mechanism behind this is unknown, but high concentrations of catecholamines are known to play a central role. The condition is often brought on by somatic and or emotional stress. As of now there exists no evidence based treatment guidelines.

Aims: To study the role of the different adrenergic receptors in the development of SIC using a rat model and catecholamines with different receptor affinities. And to study how intervening to alter afterload affects size and location of the SIC-like dysfunction.

Methods: Ten week old male Sprauge-Dawley rats weighting 250-350 g where used for all experiments. Rats where anesthetized using a mixture of ketamine and Midazolam. Catecholamines where administered as a bolus intraperotineally. The catecholamines used where adrenaline, noradrenaline, phenylephrine, dopamine and isoprenaline all of whom display different, well established, affinities for the various adrenergic receptors. Additional drugs where administered as a continuous infusion into the right jugular vein. Left ventricular

function was studied by echocardiography and both evaluated for akinesia/no akinesia, percentage affected and fractional area change was calculated.

First maximum tolerated dose for each catecholamine was titrated, using 6 rats for each dose and 5e doses per catecholamine. Then we took the dose most likely to produce SIC-like dysfunction for each catecholamine and gave it to 7 rats while monitoring hemodynamics invasively. Then we gave adrenaline, noradrenaline and isoprenaline at the same dose while giving Hydralazine or nitroprusside to lower blood pressure in the adrenaline and noradrenaline rats and phenylephrine to increase blood pressure in the isoprenaline rats in order to alter afterload.

Results: All included catecholamines caused tachycardia, hair erection and increase in body temperature. All catecholamines were able to cause SIC like dysfunction in the rat heart. The catecolamines with affinity for the α-adrenergic receptor were most likely to cause akinesia in the basal part of the ventricle whereas isoprenaline which only stimulates the β-receptors caused primarily apical akinesia. The catecholamines stimulating α-receptors also caused hypertension in the rats while isoprenaline which only stimulates β-receptors caused hypotension.

When intervention was performed to lower blood pressure in rats receiving adrenaline or noradrenaline with Hydralazine or nitroprusside all basal akinesia disappeared and only apical akinesia was seen. When the opposite intervention was performed in hypotonic isoprenaline rats all akensia disappeared.

Conclusion: The ability of both α and β-agonists to cause SIC-like dysfunction makes it unlikely that any one adrenergic receptor is the culprit in SIC. The importance of afterload as determined by vascular tonus for the morphology of the akinesia in the heart points to a cardiovascular pathogenesis rather than simply a cardiac one.

Ethics: We received permission in advance for all experiments from the ethical committee for animal experiments at Sahlgrenska academy.

Abbreviations and terms:

SIC: Stress induced cardiomyopathy, one of several names for the syndrome, Takotsubo and broken heart syndrome being the other common ones. I have opted to use SIC in my paper but at present no consensus on naming exists.

MI: Myocardial infarction

STEMI: ST-segment elevation myocardial infarction, severe form of MI

Akinesia: Lack of contraction in an area of the myocardium, when echocardiography or angiography is performed the affected myocardium only moves secondary to contraction in unaffected myocardium. Akinesia can develop anywhere in the left ventricle and even include parts of the right ventricle.

Hyperkinesia: In SIC the unaffected part of the ventricle tends to contract more forcefully than normal to compensate for the loss of the akinetic part.

Apical akinesia: Akinesia in the apex of the ventricle, i.e. the area of the apex furthest from the mitral/aortic valves. Most common form of SIC.

Basal akinesia: Instead of the apex the area most proximal to the valves becomes akinetic and instead the apex becomes hyperkinetic. In some papers there is also mention of mid-vetricular akinesia, we don’t use this classification in this paper as rat hearts are too small for us to distinguish between this and basal if it even exists in rats.

ECG: Electrocardiogram

Introduction

“You would rather help this brute, Achilles, whose mind is warped, his will of adamant. The man’s heart is like a lion’s, wild and powerful is that creature’s in its urge to slaughter the shepherds’ flocks for meat. Achilles is as devoid of pity, and of the shame that benefits men, urging restraint.’”

Illiad Bok XXIV

In most languages an ancient conception survives in various figures of speech. It is the notion that the heart is not, as a modern physiology claims, merely a complex pump but also a source of strong emotions, subconscious drives and frequently the very essence of a man’s character. When Homer in the quote above has Phoebus Apollo describing the makeup of Achilles character locates these traits in his heart. Another good example is the prophet Jeremiah who in the Old Testament refers to the heart as “deceitful above all things”, making it the source of something akin to Freud’s id.

In English we have sayings like “follow your heart”, a bad boxer is said to “lack heart” and at the time of the American civil war soldiers suffering from what we call PTSD were said to suffer from “soldier’s heart. This presumably is because changes in cardiac activity are one of the more dramatic physical manifestations of stress, anger or sorrow. Resulting in the impression that the heart is a driving force in our emotions, rather than simply reacting to them.

Showing how strongly ingrained these ideas are is the fact that many, when heart transplants where first performed, believed that the recipient would also get the donors personality along with the heart. In a study 6% of Austrian heart transplant receivers still believe that their personality has changed as a direct consequence of the transplant the received[1].

Enter takotsubo

Having people die from strong emotions, especially sorrow has always been common in classical fiction, especially of the tragic and romantic varieties. A typical example can be seen in Antony and Cleopatra where Shakespeare has Domitus Enobarbus die of a broken heart brought on by the shame of his betrayal of Marc Anthony. In Tristan and Isolde Wagner has Isolde presumably die of grief at the sight of her dead lover Tristian's corpse.

But as the 20 Th. century progressed despite the occasional case report of a patient dying of grief or some other stressful event cardiologists preferred more substantial problems and with few exceptions little interest was shown. Walter B. Cannon a Harvard physiologist who coined the term "fight or flight" studied deaths brought on by emotional stress with a particular interest in deaths brought on by curses in primitive societies, he termed these "psychosomatic" deaths as "Voodoo death" [33], a term that sadly didn't stick. One reason for this was that the patients dying or falling ill suddenly following strong emotional shock displayed symptoms typical of myocardial infarction leading to the quite reasonable conclusion that perhaps stress was a cause of heart attacks rather than causing a separate syndrome with symptoms mimicking a myocardial infarction.

Among biologists on the other hand the problem was well known and studied. As capture for the purpose of breeding of rare and endangered species became common many took note of the high mortality associated with the particularly stressful captures of wild animals. This was known as Capture Myopathy and was known to correlate to the total amount of stress the animal experienced during capture [2]. Harvard psychiatrist Curt Richter also noticed that if you cut the whiskers of wild adult rats they would become highly stressed and many would suffer spontaneous death [31]. He believed stress to be the cause and considered it an animal example of Cannon's "Voodoo death".

The term stress cardiomyopathy (SIC) was first coined by two American pathologists in 1980[3]. They studied patients who died following physical assault but where no damage to the internal organs to explain the mortality was found. They found myofibrillar degeneration in their hearts not found in the hearts of matched controls. They even found two patients who had survived assault for a while in hospital but died after suffering frequent arrhythmias neither displaying any damage to the internal organs during autopsy. They even suggested catecholamines as a possible cause of this but the article seems to have had limited impact in the cardiological community.

It was first in 1990 that Japanese doctors would show all the features of “broken heart” secondary to emotional stress, especially the complete recovery of heart function after several weeks. Takotsubo became accepted as a diagnosis in Japan and studies on it where published in Japanese journals throughout the 90’s [4]. It was first in 2001 when Japanese cardiologists published about it in international journals [5] that takotsubo cardiomyopathy as a diagnosis became widely accepted internationally. What they showed was that these women suffered from an akinesia in the left ventricle but unlike myocardial infarctions the akinesia did not match the wall area supplied by any one coronary artery. Rather it was the entire apex that was akinetic while the base was hyperkinetic to compensate for the loss of the apex. But the most remarkable part was that almost all patients regained normal cardiac motility within weeks. The shape of the left ventricle with apical akinesia reminded the doctors of the shape of a traditional Japanese octopus trap, a takotsubo.

Besides the fact that the hypokinesia did not conform to the area of the myocardium supplied by any single coronary artery in most cases and the remarkable spontaneous return to normal function there was the fact that patients would frequently survive with large percentages of the left ventricle akinetic where patients with a myocardial infarction of the same size usually died [6]. All this pointed to the existence of a subgroup among what previously had been

thought of as myocardial infarction patients who had similar symptoms and clinical findings but another pathology and prognosis. A variety of definitions of the disease, diagnostic criteria and names for the disease have come in to being since then(See Gothenburg criteria below for an example of a recently developed). Stress induced Cardiomyopathy(SIC), broken heart syndrome and takotsubo cardiomyopathy are the most common names in use today. As there still isn't any reliable noninvasive way of distinguishing between SIC and MI most diagnostic criteria use the motion and shape of the left ventricle in angiography together with absence of occlusions of the coronary arteries as a foundation [7]. Other common criteria are that, as previously mentioned, akinesia that does not conform to the area supplied by any one coronary artery. Also fairly common is the criteria that the patient must regain normal function of the heart.

New Gothenburg criteria[9] from 2014:

• Transient hypokinesis, akinesis, or dyskinesis in the left ventricular segments and frequently, but not always, a stressful trigger (psychical or physical)

• The absence of other pathological conditions (e.g. ischemia, myocarditis, toxic damage, tachycardia, etc.) that more credibly explain the regional dysfunction

• No elevation or modest elevation in cardiac troponin (i.e. disparity between the troponin level and the amount of dysfunctional myocardium)

• Normal, or near normal left ventricular filling pressure*

• Low, or near normal peripheral vascular resistance and normal or near-normal cardiac output*

*Optional diagnostic criteria that are not mandatory, but when positive they increase the likelihood of takotsubo syndrome diagnosis

These criteria are not entirely unproblematic. The first criteria, open coronary arteries, makes sense as MI is a more severe diagnosis that needs to be ruled out or treated first but this

assumes that patients with MI do not suffer from simultaneous SIC. When in fact MI causes massive both emotional and somatic stress which we today know to cause SIC, making highly probable that patients with MI may suffer simultaneous SIC.

In most studies there has been consistently low mortality, usually around 1-2%[8], which led some to conclude that this was a fairly benign condition, but this may to some degree be due to diagnostic criteria excluding groups of patients with higher mortality. The requirement that the akinesia be transient obviously rules out patients who die in the acute phase. By having angiography as a diagnostic requirement patients where there is no indication for angiography or patients who are in such bad shape that it's not considered meaningful are excluded. Some criteria also exclude patients with intracranial bleeding [9] which excludes patients with subarachnoid hemorrhage whom frequently display SIC-like dysfunction of the heart and have a considerably worse prognosis.

SIC today

The clinical aspects of SIC remain largely unchanged today. No reliable way has been found to differentiate between SIC and MI non-invasively and therefore the diagnosis is still dependent on angiography excluding MI for a diagnosis[7]. Echocardiography may give a strong suspicion of SIC when the typical akinesia is seen but does not rule out MI as a cause of the akinesia it can be very helpful in measuring heart function and visualizing the location and extent of the akinesia providing valuable prognostic data [28]. ECG and troponin are also unreliable for differentiating between the two, ECG in SIC is typically abnormal and although ST-segment elevation and negative T-waves are the most common abnormalities degree and percentage of SIC patients exhibiting vary widely in published material [29]. Biomarkers for MI like troponin are typically elevated but although significantly lower than in MI and or STEMI they are still not low enough to rule out an MI [30].

One point where progress has been made is in understanding the role of catecholamines. Clinical experience and preclinical experiments both point to high levels of catecholamines in the blood causing SIC although the actual mechanism is still unknown[11]. The role of catecholamines was further cemented when it was shown that catecholamines in large doses could produce dysfunction closely resembling SIC in rats and that histology from these areas was similar from histology taken from akinetic areas in the heart of SIC patients[16]. As a result of this beta blockers are frequently employed and catecholamine inotropes are avoided as far as possible [12]. Angiotensin-converting enzyme inhibitors, diuretics and anticoagulants to prevent thrombosis formation are also often used [31].

Another thing that further complicates our understanding of this condition is the discovery of basal and mid-ventricular forms of SIC. They are rarer than the apical form and therefore often referred to as atypical[12]. The suggestive name takotsubo and the very characteristic shape of the left ventricle when studied with ultrasound and angiography together with the frankly odd cause of a “broken heart” is intuitive to understand and easy to remember, with some odd cause of stress it makes for great case reports. But slightly more obscure, and perhaps not as obvious to PCI operators and cardiologists performing echocardiography, and therefore less likely to be included in studies or publicized as case reports are the more recently discovered basal and mid ventricular variants. These ad further questions to this already mysterious condition. What causes the akinesia to be localized at the apex in one patient, at the base in one and somewhere else in another?

Animal models

One problem with studying SIC in humans is that when patients with SIC are diagnosed the SIC appears to be fulminant and there doesn't seem to be any significant increase of the size of the akinesia after diagnosis[27]. This indicates that the pathological process takes place in

the timespan from when symptoms first present until angiography is performed, or it might be slightly longer if the pathological process begins some time before symptoms manifest. Either way there exists a need to find out what happens in this timespan and as it is somewhat hard to get ethical permission to stress postmenopausal women until they develop SIC there has been demand for animal models.

There exists several animal models. One Japanese model restrains rats for one hour which causes enough stress for them to develop SIC-like dysfunction in the left ventricle[13]. In the west ethical concerns has prevented this model form becoming widely used. One group has published a model that uses what is referred to as an intruder rat protocol[14]. This form of experiment is well established in behavioral studies and is known to introduce copious amounts of stress in a male rat as his fight or flight response is activated. Essentially the protocol consists of housing a male of an aggressive strain (Sprauge-Dawley rats are to nice) with a sterilized female for one week without changing the bedding, essentially allowing him to make the cage his territory. The female is then removed and another male is inserted in her place ten minutes later, causing the male to feel that his territory is being invaded and triggering a fight or flight response as he has to decide whether to fight the intruder or submit/escape. This is a very interesting model in several ways, but in the published article no echocardiography was performed and the existence of SIC in the rats was inferred from increased stress hormone and troponin I levels in the blood, ECG changes and post sacrifice heart weight/body weight ratio and left ventricle/body weight ratio . The strength of models like these are that the cause of the SIC is endogenous and therefore can be studied. Another plus is that studying the effects of treatments affecting catecholamine pathways becomes more reliable than in models where exogenous catecholamines are administered in large doses as these models makes it harder to say whether one is merely preventing the insult that simulates the disease or actually treating the disease when one administers for example beta blockers.

But the most common method has been to administer various catecholamines and then wait and study the heart with ultrasound to evaluate the effect. Paur et al. have published a study where they administered adrenaline to the rats to get SIC-like dysfunction [15]. In this paper they also tried using noradrenaline but where not able to induce SIC-like dysfunction with it. Various other groups have published similar models and as of now models using catecholamines are widely accepted but there is no wide consensus on which catecholamine or what dosage to use.

The animal model our group uses was developed and validated by Björn Redfors during his time as a PhD student [16]. It uses young male rats weighting 250-350 g who are given 50 mg/kg isoprenaline, a β_1/β_2 agonist but not a α_1 agonist. The lack of alpha stimulation coupled with strong beta stimulation gives a low afterload, tachycardia and increased inotropy simultaneously.

One possible objection to this model is the use of males in modeling a predominantly female disease. We use males as females have a wider variation in their normal physiological data owning to their menstrual cycles which translates into having to use more animals to achieve statistical power[17]. The decision to use young males is both practical and economical, as it is impossible to buy old rats from the breeder we would have to house the rats we intend to use for one to one and a half years for them to become “old”, this would both significantly increase the cost per animal for us and a demand a great deal of advance planning of experiments. The downside of this is of course that we study a disease primarily affecting post-menopausal women in the rat equivalent of a young and healthy man.

Context of the study

The mechanism that translates circulating catecholamines into akinesia is still unknown. There are several theories. One was that SIC was secondary to an abnormal LAD, this one

was ruled out by showing that carriers of the proposed abnormality was were not overrepresented among SIC patients[18].

Another theory is that the catecholamines cause a temporary vasospasm on some level and that the resulting temporary ischemia causes the akinesia [19]. It is well established that a temporary ischemia in myocardium can cause ischemic stunning [34], an akinesia in the ischemic area that also regains normal function over time. Another argument in favor of this is that isoprenaline given repeatedly in high doses in rats causes nerocis of the myocardium and is used in some MI models [20]. The lack of correlation between akinetic myocardium and myocardium supplied by any single artery is hard to explain with this theory but disturbances of the microcirculation in akinetic areas is a possible explanation.

There also is what is sometimes referred to as the direct effect theory. In this theory the akinesia was an effect of the stimulation, or perhaps overstimulation, of adrenergic receptors on cardiac myocytes of the ventricle [22]. This theory focuses on the effect of stimulating the receptors and the intracellular signaling cascade that followed rather than the physiological effects of chronotropy, inotropy and blood pressure. Especially the β_2 receptor was seen as the one necessary to stimulate to cause SIC and some have suggested a gradient in the ventricle with density of β_2-receptors increasing distally as a cause of apical ballooning [35].

A fourth theory that is dominant in our group is that the physiological effects of the catecholamine is most important and that secondary to the increased work an increased chonotropy and inotropy a supply-demand mismatch in the myocardium causes the akinesia.

So we decided to perform a study to further the understanding of the role of different adrenergic receptors and impact of physiological variables in the development of SIC. There are a number of substances that stimulate the adrenergic receptors, there is a well-established

difference among these in affinity for the various adrenergic receptors. So we decided to use five of them to study the role of the α, β_1 and β_2 receptors in SIC development.

Aims

* Establishing the roles of the various catecholamine receptors using five different catecholamines with well-known receptor affinities at different doses.

* Studying the hemodynamics accompanying SIC-like dysfunction by running seven rats at the catecholamine dosage that most reliably produces SIC for each catecholamine with invasive hemodynamic monitoring.

* Studying the role of afterload in shaping the location of the akinesia by altering it by administering vasoconstrictors and dilators in a continuous intravenous infusion parallel to catecholamines.

Material and methods

The animals

Ten week old male Sprauge-Dawely rats in the range of 250-350g where used. This strain is an albino strain popular in labs throughout the world in part for their calmness and ease of handling. They were housed in a temperature controlled environment at 25 C° with a 12 hour light/dark cycle with free access to food and water.

The rats where first anesthetized using a mixture of Ketamine (50mg/kg) and Midazolam (5mg/kg). A trimmer was used to shorten the hair on the chest of the rat and remaining hair was removed using hair removal cream to enable echocardiography. The rats where placed on a heating pad, the temperature of the pad was adjusted to keep the body temperature of the rats at about 38 degrees. The right carotid was then dissected free and canulated in order to monitor blood pressure invasively. This was done by inserting a pressure catheter connected to a display (Radianalyzer, St Jude medical) through the canula and down the aorta into the left ventricle and then backing it up 3 mm. In the experiments where an infusion of vasoconstrictors or vasodilators was used the jugular vein was also canulated for access.

The experiment included 255 rats in total. For titrating the maximum tolerated dose of each catecholamine 48 rats where used. 150 rats where used for testing the effect of the various catecholamines on the heart, 30 for each catecholamine and these where then subdivided into groups of 6 for each different dose. Finally 57 rats where used to study the effect of hemodynamic intervention.

Protocol

The rats where prepared as described. They were then allowed to stabilize during 30 minutes before the chosen catecholamine was administered intraperitionealy. Cardiac function was

then evaluated after 90 minutes using echocardiography. A subset of rats given each catecholamine showing SIC on echocardiography(n=3) where then put back in their cages and echocardiography was performed again after seven days, the rest where sacrificed post echo using a lethal dose of Penorbital administered intraperitonealy.

Table 1

Receptor specifity for different catecholamines.			
Catecholamine:	β_1	β_2	α
Isoprenaline	+	+	-
Adrenaline	+	+	+
Noradrenaline	+	-	+
Dopamine	+	+	+
Phenylephrine	-	-	+

The chosen catecholamines (see table 1) where all administered intraperitonealy as a bolus dose. We used isoprenaline(β_1 and β_2 agonist), adrenaline(β_1, β_2 and α agonist), noradrenaline(β_1 and α agonist), dopamine(non-selective β and α agonist but also a agonist to the dopamine receptors) and phenylephrine(selective α agonist).

Table 2

Rat Parameters

Catecholamine:	Weight:	Heart rate(at 90 min):	Peak body temperature(C):
Isoprenaline(N=7)	324 ± 18	548 ± 48	41.2 ± 0.6
Adrenaline(N=7)	321 ± 17	385 ± 59	39.9 ± 0.8
Noradrenaline(N=7)	311 ± 14	449 ± 32	40.0 ± 0.2
Dopamine(N=7)	298 ± 41	459 ± 101	40.2 ± 0.7
Phenylephrine(N=7)	330 ± 24	367 ± 48	39.8 ± 0.4

We defined maximum tolerated dose as the dose where mortality was >50% and increased to 100% if doubled and <50% if halved. For isoprenaline the highest tolerated dose had already been titrated [22]. For Adrenaline and Noradrenaline we started from previously published doses [23], dosage was reduced 50% for each decrease in dose. For phenylephrine we started out with maximum tolerated dose for noradrenaline and then increased it 100% each step. With dopamine we also started out with the maximum tolerated dose for noradrenaline and

titrated using a stepwise reduction of 50%. Experiments where then performed (n=7) with the dose that most reliably produced SIC (see table 3) with invasive hemodynamic measuring.

To reduce hypertension in the rats who received adrenaline and noradrenaline we infused Hydralazine or nitroprusside, saline infusion was given as control. The infusion was continuous and adjusted to keep blood pressure below 120 mm hg. In rats who receive isoprenaline there is hypotension instead so we induced hypertension by giving phenylephrine, or saline as control, as a continuous infusion keeping blood pressure above 120 mg hg. Infusions where always started one minute before the catecholamines where administered.

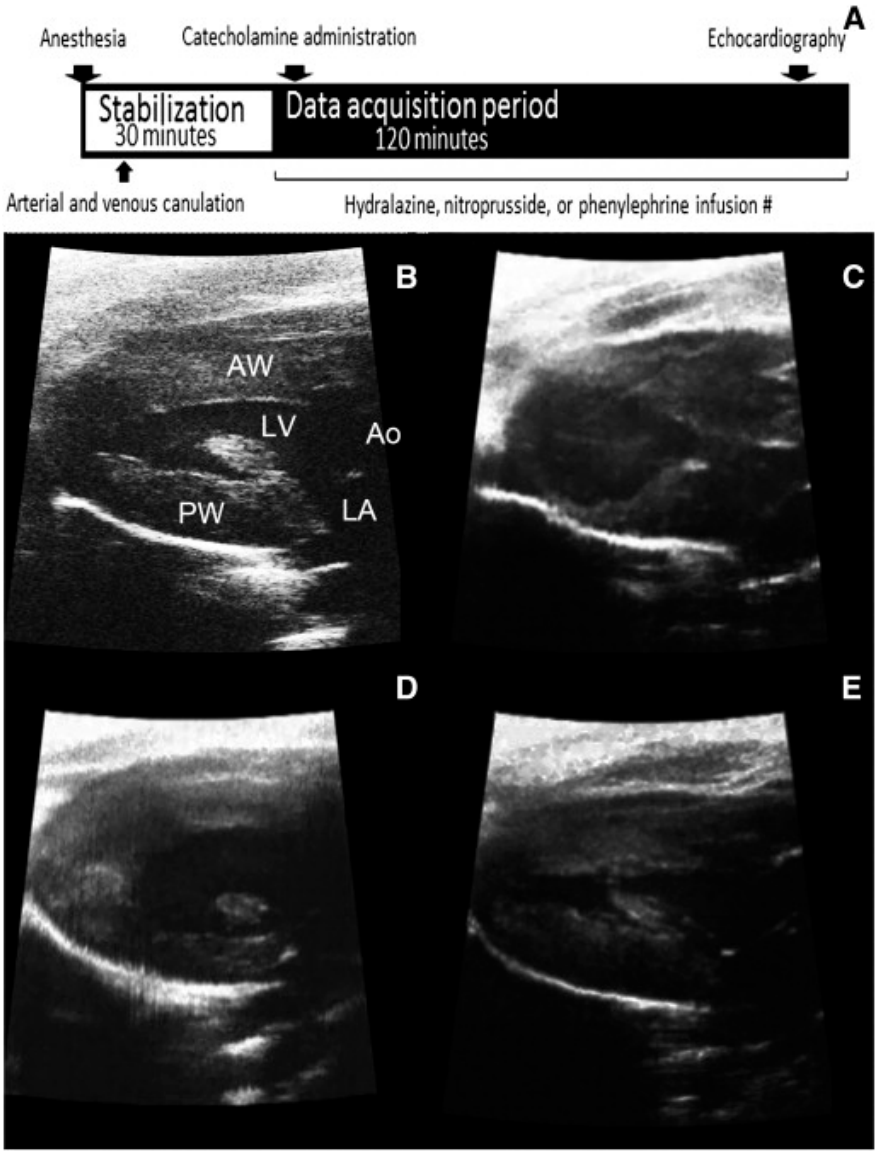

Fig 1: A)Our protocol . B-E) Showing end systolic echocardiographic images. B) Normal rat heart contracting uniformly. C) Apical akinesia in a rat heart. D) Basal akinesia with a normal apex. E) Normal contraction seven days later. AW: Anterior wall. LV: Left ventricle. Ao: aorta. LA: left atrium. PW: posterior wall

Measuring outcomes

Echocardiography was performed using a Visualsonics 770 Vevo imaging station with an integrated rail system for positioning the ultrasound probe and maintaining position throughout. An optimal parasternal view of the heart was acquired by visualizing the atrial and mitral valves and keeping them in view while trying to find a view showing maximal distance between them and the apex(See Fig 1). When we felt that we had found an optimal long axis view the probe was fixated and a high resolution recording of the cardiac cycle was recorded using ECG-gated kilohertz visualization. Using the high resolution slow motion recording of the heart the length of the akinesia was traced and expressed as a percentage of the total endocardial length of the left ventricle. For a measure of cardiac contractile function we used Fractional area change which is calculated as (End diastolic area-end systolic area)/End diastolic area=FS[36]. Ejection fraction and other measurements that are common in clinical use and would be preferable are hard to perform reliably due to the small size of the heart, fraction of area change is a common measure of ventricular contractile function we chose as a substitute.

Blood pressure was measured using a Radi analyzer from St Jude Medical as previously described.

Statistical methods

For all statiscal analysis we used STATA v13.1. Mann-Whitney or Anova(Analysis of variance) tests were performed independently with Turkey test performed post-hoc to compare groups.

These tests were only performed in the intervention study, comparing data from intervention for each catecholamine with data from the same catecholamine without intervention.

Ethics

The use of animals in research has always been contentious. The relatives of the early modern philosopher and pioneer of live vivisections Descartes started the very first animal protection society in response to the cruelty of his experiments.

Our research has been approved by the ethics committee at Gothenburg University. In designing our studies we also try to abide by what is commonly referred to as the three R:s of animal studies: reduce, replace and refine. Replacing animals with something else nonliving is not a realistic option in our field of research at present. Reducing the number of animals used in experiments is done by having clear goals and questions with each experiment and performing proper planning to reduce wasted animals. Refinement centers on reducing animal suffering, in our experiments this takes the form of ensuring adequate anesthesia trough out the experiment.

As to the philosophical question as to whether performing experiments on animals is right or wrong I think any conclusive answer is unobtainable and everyone will have to make up his own mind. Personally I consider the life of a human being as more valuable than that of a rat or mouse without being able to provide some form of airtight philosophical argument from first principles. And therefore I’m comfortable performing experiments with a clear benefit and a minimum of suffering for the animals used.

Results

Table 3

Dose titration

Catecholamine:	Dose:	Number of rats:	Apical pattern (n)	Atypical patterns (n)	Dead <90 min (n)
Isoprenaline	25 mg	6	0	0	0
	50 mg	6	4	0	0
	100 mg	6	3	0	0
	300 mg	6	1	0	2
	450 mg	6	1	1	2
Adrenaline	0.25 mg	6	0	0	0
	0.5 mg	6	0	3	0
	1 mg	6	0	6	0
	2 mg	6	0	1	4
	3 mg	6	0	1	5
Noradrenaline	0.25 mg	6	0	0	0
	0.5 mg	6	0	3	1
	1 mg	6	0	6	0
	2 mg	6	0	2	2
	3 mg	6	2	1	1
Dopamine	10 mg	6	1	0	0
	25 mg	6	0	2	1
	50 mg	6	1	1	1
	100 mg	6	0	2	3
	300 mg	6	0	0	6
Phenylephrine	0.5 mg	6	0	0	0
	1 mg	6	1	3	0
	2 mg	6	0	2	1
	5 mg	6	0	2	3
	10 mg	6	0	0	6

All catecholamines induced some form of SIC like regional cardiac dysfunction. Isoprenaline in the 25-450 mg/kg range typically induced apical akinesia(30%) and basal akinesia in only 3% of rats. Adrenaline(0,25-3 mg/kg) only induced basal akinesia(37%) whereas noradrenaline(0,25-3 mg/kg), phenylephrine(0,5-10 mg/kg) and dopamine where more likely to cause basal akinesia(40%, 23% and 17% respectively) but also could cause

apical akinesia(7%, 3% and 7%). Isoprenaline only induced apical akinesia at the dose most likely for inducing SIC-like dysfynction. Isoprenaline also caused the highest fraction of area change in relation to dose of all catecholamines (see Fig 2). When echocardiography was performed after seven days on the rats we saved all rats showed complete recovery of normal cardiac function.

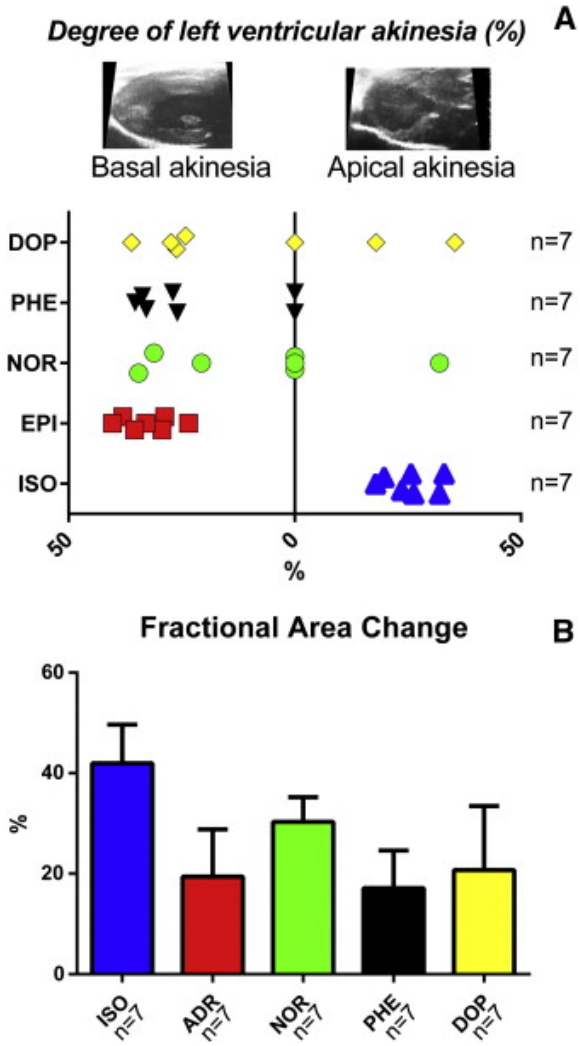

Fig 2: A) Percentage of the left ventricle affected by and location of akinesia at dosage most likely to cause akinesia for each catecholamine. B)Fractional area change for each catecholamine at dosage most likely to cause akinesia.

Each catecholamine produced a different hemodynamic response(see fig 3). Isoprenaline was the only one that caused mean arterial pressure to drop, all the others caused it to rise initially

to varying degrees before gradually decreasing throughout. Changes in diastolic blood pressure mirrored those of systolic pressure.

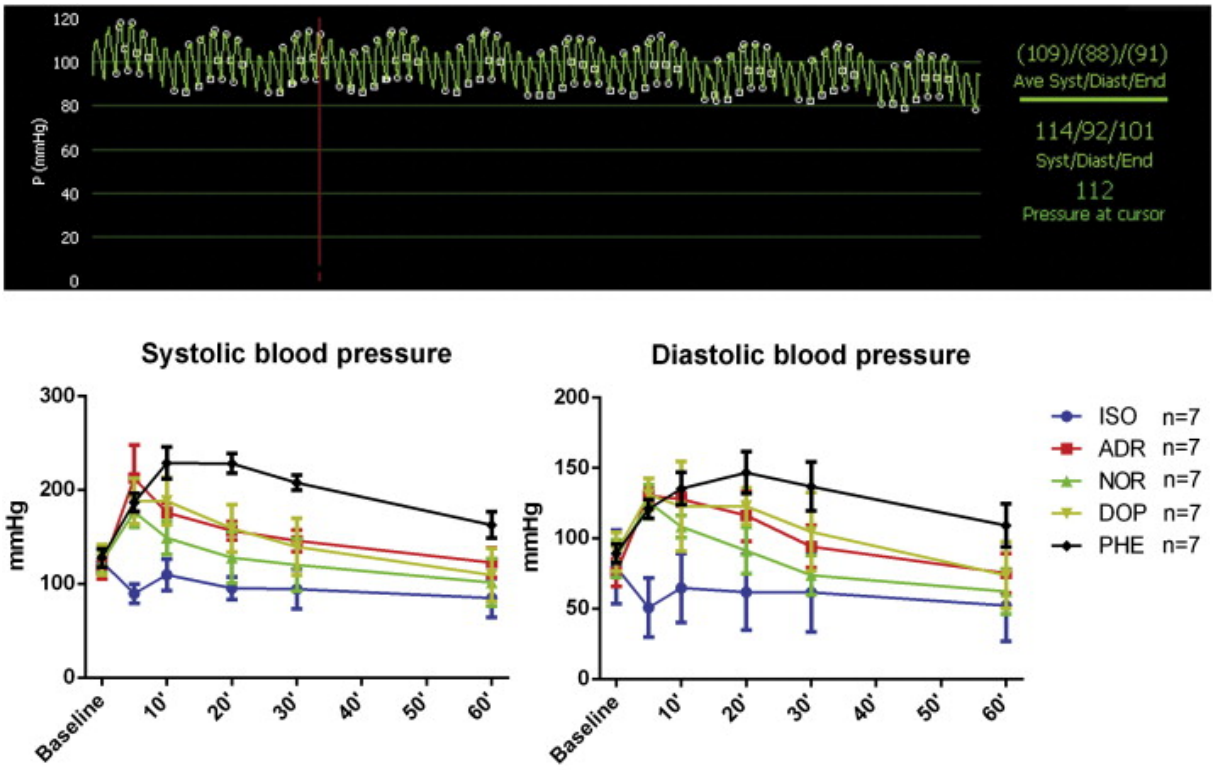

Fig 3: Above: Blood pressure as displayed on our measuring display. Below: Systolic and diastolic blood pressure throughout the experiment for each catecholamine at dose most likely to cause akinesia.

Intervening (See fig 4) with Hydralazine or nitroprusside to lower the mean arterial pressure below 120 mm hg and then keep it in the 100-120 mm hg range removed basal dysfunction entirely ($P < 0,05$) and instead increased the incidence of apical akinesia($P < 0,05$).

Table 4

Hemodynamic intervention

Catecholamine:	Dose	Number of rats		Apical pattern (n)	Atypical pattern (n)	Dead <90 min(n)
Adrenaline	1 mg	8		0	7	1
Noradrenaline	1 mg	9		1	3	2
Isoprenaline	50 mg	7		7	0	0
Adrenaline + nitroprusside	1 mg	9		1	0	2
Adrenaline + Hydralazine	1 mg	9		3	0	2
Noradrenaline + Hydralazine	1 mg	8		7	0	1
Isoprenaline + phenylephrine	50 mg	7		0	0	0

Intervening to raise the blood pressure in rats who received isoprenaline above 120 mm hg eliminated all cardiac dysfunction($P < 0,05$).

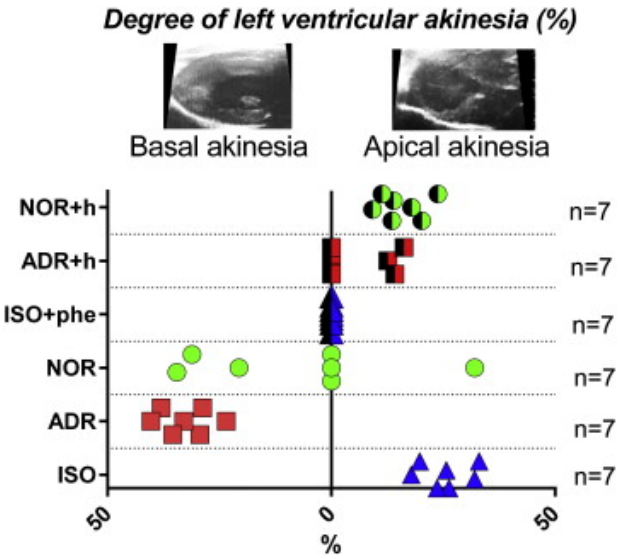

Fig 4: Results of hemodynamic intervention on akinesia. NOR+h: Noradrenaline + Hydralazine. ADR+h: Adrenaline + Hydralazine. ISO+phe: isoprenaline + phenylephrine

Discussion

Firstly this study showed that all included catecholamines are capable of causing SIC-like cardiac dysfunction in rats. The second important finding was that when a catecholamine activated the α-adrenergic receptor the dysfunction became most likely to take the shape of a basal akinesia. Stimulation of the α-adrenergic receptor was also associated with a higher blood pressure than pure β-receptor stimulation which instead produced hypertonia and only apical akinesia.

Paur et al. [24] and others have, based on their experiments, maintain that the β_2-receptor is the adrenergic receptor responsible for the development of SIC in the presence of catecholamines. In the case of Paur this was because they only managed to produce apical akinesia using adrenaline but not with noradrenaline which has a lower affinity for the β_2-receptor[24]. But unlike us they only tested noradrenaline at a single dose.

In our model all catecholamines cause some form of SIC-like dysfunction at some dose indicating that the important mechanisms still to be found are not specific for one adrenergic receptor subtype. Although all catecholamines except adrenaline where able to produce apical akinesia they were more likely to produce basal akinesia. This seems to be a product of the increase in afterload brought on by α-receptor stimulation when one takes the result of our intervention experiments into consideration.

This and the fact that when phenylephrine was added to isoprenaline to raise blood pressure above 120 mm Hg all dysfunction disappeared while moving the akinesia to the apex from the base when we intervened with Hydralazine in rats who received adrenaline and noradrenaline points to factors beyond the stimulation of some specific adrenergic receptor as the key to understanding the pathology of SIC.

The results of intervention to modulate the blood pressure and thereby left ventricular afterload show the close connection between function of the heart and the vasculature. This interaction between the arterial system and left ventricle is referred to as arterial ventricular coupling, the relationship between arterial elastance and left ventricular end systolic elastance. In a healthy individual this relationship is maintained by feedback from the baroreceptors in the aorta and carotids changing cardiac rate and force of contraction and vascular tone. Aging and diseases like heart failure and hypotension affect this coupling by changing the structure and function of the arteries and left ventricle[25].

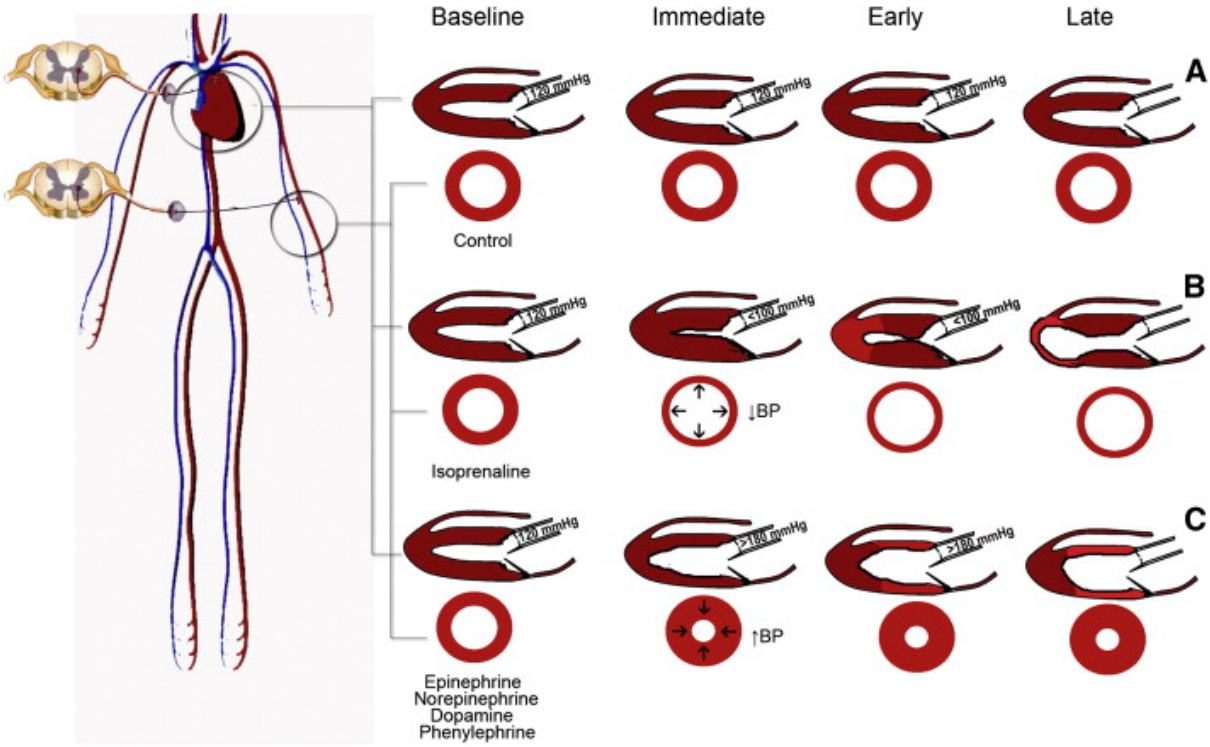

Fig 5: Representation of hypothetical effects of varying afterload on the left ventricle. A) Normal heart. B) Strong chronothropic and inotropic stimulation with a low afterload causes outflow obstruction and maximum wall stress in apical region. C) Strong chronothropic and inotropic stimulation but now with a high afterload causes maximum wall tension in the basal region.

We propose that in in the presence of a strong chronotropic and/or inotropic drive the addition of a strong vasopressor or dilator stimuli may cause “uncoupling” off the reflexes that maintain normal ventriculo-arterial coupling. When there is strong chronotropic and inotropic stimulation and hypotension, wall tension may be redistributed towards the apex of the ventricle while simultaneously the low afterload allows the lumen of the ventricle to occlude totally or partially. This would result in outflow obstruction further increasing load in the apex of the ventricle (see fig 5). Given the same chronotropic/inotropic stimulation but a higher afterload we might see a shift of the point of maximum wall stress towards the basal area of the ventricle, this would be in line with our results but needs further study.

The role of afterload in shaping SIC morfology points to a broader pathology for SIC than previously suggested. Previously SIC has been conceived primarily as a form of cardiomyopathy [26]. Patients presenting typical SIC with apical left ventricle ballooning are usually hypotensive but despite having normal left ventricle filling pressure show decreased peripheral resistance [26]. This finding is in line with the findings in our model. Data on hemodynamics of patients with atypical SIC is scarce so far. But based on these findings we believe SIC to be a cardio circulatory syndrome rather than a cardiomyopathy.

Extrapolating findings from the rat heart to the human heart is not necessarily unproblematic. But rat hearts have been used a long time as models for the human heart, among other things for testing therapies for heart failure[37] and elucidating the mechanism behind the effects of various drugs[38] and studying MI[20]. One specific difference between human and rat hearts that is significant in this context is that rats exhibit more α-receptors in the ventricle than humans do[39]. Another weakness I’ve touched on previously, and why we chose to do it this way, is that we are using young healthy male rats to study a disease that primarily manifests in post-menopausal women. We are as of now planning further studies in older post-menopausal and/or ovarectomised female rats to overcome this.

A relatively low number of rats where used to titrate the maximum tolerated dosage for each catecholamine, making it possible that this dosage is perhaps a little off. But we instead tested many different dosages. Myocardial perfusion of the akinetic area, compared to akinesia in a ischemic are, is only studied in isoprenaline rats in a previous study. This is simply due to lack of access to an MRI machine, time and resources.

We show that all five studied catecholamines are capable of inducing SIC-like dysfunction in rat hearts. The location of the dysfunction is to some degree dependent on the afterload the left ventricle is working against. We also show that SIC-like dysfunction can develop in the absence of β_2-receptor stimulation.

Populärvetenskaplig sammanfattning

Att ett brustet hjärta till följd av sorg kan döda var länge något som kanske främst författare av litteratur med tvivelaktig kvalitet hävdade. Men på 1990 talet kom nya fynd som visade att några procent av patienterna som man tidigare trott drabbats av hjärtinfarkter istället led av just ett ”brustet hjärta” till följd av sorg eller stress.

Att dessa patienter länge gömt sig bland hjärtinfarktpatienterna berodde på att de hade liknande symtom, provsvar och undersökningsfynd. Vi har fortfarande inget pålitligt sätt att skilja ett brustet hjärta från en hjärtinfarkt utan att genomföra en kärlröntgen för att utesluta proppar i kärlen som förser hjärtmuskeln med blod. Det som hände på 90 talet var att japanska läkare kunde visa att bland de äldre kvinnor som kom till sjukhuset med symtom som vid hjärtinfarkt fanns det en liten grupp med en helt annan sjukdom. Dessa kvinnor hade hjärtan där spetsen av vänster hjärthalva stod stilla medan resten arbetade mer än vanligt för att kompensera bortfallet av den stillastående delen. Området som stod stilla motsvarade inte försörjningsområdet för någon av de artärer som försörjer hjärtmuskeln med blod vilket är fallet vid infarkter där en av dessa artärer täppts igen. Till skillnad från vid hjärtinfarkter där den drabbade vävnaden dör så återfick dessa patienter sin normala hjärtfunktion efter en tid, efter några veckor slog hjärtat oftast som vanligt igen. Mest uppseendeväckande var att alla patienterna hade en annan sak gemensamt, de var alla nyblivna änkor där makens död hade utlöst symtomen. Formen på vänster kammare, den del av hjärtat som pumpar ut blodet i kroppspulsådern, när man undersökte den med ultraljud eller kärlröntgen påminde de japanska läkarna om en sorts kruka fiskare i japan använder för att fånga bläckfisk, denna kruka heter Takotsubo vilket också blev ett av namnen sjukdomen fick.

Idag vet vi mer om tillståndet men inte så mycket som man hade kunnat hoppas. Det största framsteget är kunskapen om den centrala roll höga halter cirkulerande katekolaminer i blodet

spelar. Katekolaminer är ett samlingsnamn för adrenalin, noradrenalin och liknande substanser som kroppen frisätter i blodet vid stress. Men varför hjärtat reagerar som det gör och hur det går till inne i cellerna är fortfarande okänt. Det finns heller inte mycket kunskap om hur sjukdomen skall behandlas. Betablockerare, en medicin som blockerar katekolaminers effekt genom att hindra dem från att binda till sina receptorer på cellerna, och blodtryckssänkande ACE-hämmare brukar användas men bra bevis för effektiviteten hos någon viss behandling saknas fortfarande. Prognosen sågs initialt som god med en dödlighet på bara 1-2% men även om nyare studier pekar på en relativt låg dödlighet för dessa patienter på lång sikt så verkar det som att dödligheten i samband med insjuknande kan vara betydlig högre än vad man först trodde.

Detta arbete är utfört för att studera rollen de olika katekolaminreceptorerna i kroppen spelar genom att använda fem olika katekolaminer som binder olika starkt till de olika receptorerna. Utöver att det som tidigare nämnts finns flera olika katekolaminer så finns det ett flertal receptorer som binder olika starkt till de olika katekolaminerna och spelar olika roller i regleringen av blodtryck, puls och kraften i hjärtats slag. Vi ville också studera hur variationer i blodtryck, dvs trycket hjärtats vänsterkammare måste arbeta mot för att pressa ut blodet från lungorna i kroppspulsådern, påverkar vilken form sjukdomen tar i hjärtat. Vi gjorde detta I en råttmodell som utvecklats av min handledare Björn Redfors. Den bygger på att råttorna, som är sövda, får en katekolamin med spruta i buken, den tas därifrån upp i blodet och sedan utvärderas hjärtats funktion efter nittio minuter med ultraljud som ger oss en bild av hur hjärtat rör sig när det pumpar ut blodet. När vi ville se effekten av förändringar i blodtryck gjorde vi likadant men gav även blodtryckssänkande/höjande medicin samtidigt.

Det vi fann var att alla katekolaminer i studien kunde ge sjukdomen. De som stimulerade receptorerna av alfa typ gav dock oftast en form där hjärtats bas, delen närmast utgången till kroppspulsådern, slutade röra sig istället för spetsen medan de som endast stimulerade beta

receptorer istället främst gav formen där spetsen av hjärtat slutar röra sig. Stimulering av alfareceptorer leder också till ökat blodtryck då de får artärerna att dra sig samman. När vi gav katekolaminer som stimulerade alfareceptorerna men samtidigt gav blodtryckssänkande medicin så såg vi åter att spetsen slutade röra sig. Detta pekar på en viktig roll för blodtrycket då det verkar avgöra vart i hjärtat tillståndet visar sig.

Slutsatsen vi drog är att alla receptorer för katekolaminer kan orska sjukdomen men också att istället för att bara se sjukdomen som en hjärtsjukdom så är det, då blodtrycket tycks spela en avgörande roll för vilken form sjukdomen tar, troligen mer korrekt att se den som kardiovaskulärt syndrom, dvs ett sjukdomstillstånd som innefattar både hjärta och blodkärl.

Acknowledgments

Bjorn Redfors

Elmir Omerovic:

Anwar Ali

Jessica Khuory

Dennis Mesinovic

Jonatan Oras

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