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Margarida Maria Velez Laranjeira Tapadas

Vascular Effects of 3,4-methylenedioxymethamphetamine (MDMA) and the Impact of Interindividual Variability

Dissertação no âmbito do Mestrado em Farmacologia Aplicada orientada pelo Professor Doutor Diogo André Afonso da Fonseca e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

Marie Curie

Agradecimentos

A ver chegar o final deste meu percurso na Faculdade de Farmácia da Universidade de Coimbra, não poderia deixar de agradecer a todas as pessoas que tornaram tudo isto possível. Desde a licenciatura em Farmácia Biomédica até ao presente Mestrado, os ensinamentos foram muitos assim como toda a magnifica experiência que é estudar nesta Faculdade.

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A Coimbra, por me ter acolhido, visto crescer e evoluir nos seus braços!

Nada disto teria sido possível sem nenhum destes intervenientes.

Para sempre grata.

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ABBREVIATIONS

5-HT	5-hydroxytryptamine
ANOVA	Analysis of variance
AUC	Area Under the Curve
BBB	Blood-Brain Barrier
COMT	Catecol-*O*-methyltransferase
CYP	Cytochrome P450
DAT	Dopamine transporter
E_{max}	Maximum contraction
g	Gram
HHA	3,4-dihydroxyampthetamine
HHMA	3,4-dihydroxymethamphetamine
ITA	Internal thoracic artery
KCL	Potassium chloride
MAO	Monoamine oxidase
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
mg	milliGram
mN	milliNewton
NET	Noradrenaline transporter
pEC_{50}	Negative logarithm of the effective concentration of agonist able to induce half of the maximum concentration
SEM	Standard error of the mean
SERT	Serotonin transporter
VIF	Variance inflation factor
VMAT	Monoamine vesicular transporter

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RESUMO

A 3,4-metilenodioximetanfetamina (MDMA), mais conhecida como "ecstasy", é uma droga recreativa usada em todo o mundo pelos seus efeitos psicotrópicos. Apesar do seu reconhecido impacto a nível neuronal, os seus efeitos cardiovasculares a nível da pressão arterial e da frequência cardíaca são também relevantes, tendo os seus efeitos vasculares e dos seus metabolitos sido o foco de estudos recentes, bem como a sua correlação com a hipertermia, o efeito secundário mais frequente do consumo desta droga de abuso. O presente trabalho teve como objetivo geral o estudo dos efeitos vasculares da MDMA e o impacto da variabilidade interindividual nestes efeitos, através de uma análise retrospetiva de resultados, previamente obtidos no nosso laboratório, relativos à resposta contrátil à MDMA em artérias torácicas internas humanas (também designadas de artérias mamárias internas). Através de uma análise retrospetiva conjunta (28 doentes), demonstrou-se que a resposta contrátil à MDMA apresenta um perfil bifásico, sugerindo o envolvimento de mais do que um alvo farmacológico, tal como já proposto na literatura. Acresce referir que as diferenças observadas entre condições térmicas dos ensaios (ou seja, 37°C vs 40°C) não se apresentaram como significativas estatisticamente, o que foi confirmado através da análise de uma amostra de 4 doentes emparelhados (cujos segmentos foram analisados a ambas as temperaturas). Contudo, foram detetadas associações independentes significativas através de análise multivariada entre a temperatura do ensaio e a resposta farmacológica. Alguns fatores clínicos, nomeadamente história de hipertensão arterial, doença vascular periférica e área de superfície corporal podem estar associados a pequenas diferenças nos parâmetros farmacológicos da resposta contrátil ao MDMA. Em conclusão, não foi observada uma relação marcada entre as características individuais dos doentes incluídos no estudo e os parâmetros de resposta contrátil vascular à MDMA, sugerindo que a variabilidade interindividual desempenha um papel limitado na resposta vascular a esta droga de abuso.

Palavras-chave: 3,4-metilenodioximetanfetamina, efeitos vasculares, hipertermia, pressão arterial, frequência cardíaca, variabilidade interindividual.

ABSTRACT

3,4-methylenedioxymethamphetamine (MDMA), better known as "ecstasy", is a recreational drug used around the world for its psychotropic effects. Despite its recognized neuronal impact, its cardiovascular effects on blood pressure and heart rate are also relevant, and its vascular effects and its metabolites have been the focus of recent studies, as well as its correlation with hyperthermia, the most frequent side effect of the use of this drug of abuse. The present work aimed to study the vascular effects of MDMA and the impact of interindividual variability on these effects, through a retrospective analysis of results previously obtained in our laboratory, related to the contractile response to MDMA in human internal thoracic arteries (also known as internal mammary arteries). Through a pooled retrospective analysis (28 patients), it was shown that the contractile response to MDMA displays a biphasic profile, suggesting the involvement of more than one pharmacological target, as already suggested in the literature. It should also be noted that the differences observed between the thermal conditions of the experiments (i.e. 37°C vs 40°C) were not statistically significant, which was confirmed by the analysis of a sample of 4 paired patients (whose segments were analyzed at both temperatures). However, significant independent associations were detected through multivariable analysis between the temperature of the assay and the pharmacological response. Some clinical factors, such as a history of hypertension, peripheral vascular disease and body surface area, may be associated with small differences in the pharmacological parameters of the contractile response to MDMA. In conclusion, a marked relationship was not observed between individual characteristics of patients included in the study and the parameters of vascular contractile response to MDMA, suggesting that interindividual variability plays a limited role in the vascular response to this drug of abuse.

Keywords: 3,4-methylenedioxymethamphetamine, vascular effects, hyperthermia, blood pressure, heart rate, interindividual variability.

CHAPTER I.

General Introduction

1. GENERAL INTRODUCTION

1.1. Historical context and chemical characteristics of MDMA

The 3,4-methylenedioxymethamphetamine (MDMA), more recognized as "ecstasy", is a recreational drug used worldwide for its psychotropic effects. MDMA causes effects such as a feeling of pleasure and well-being as well as a feeling of empathy. It has a great impact on the level of the central nervous system (CNS), the cardiovascular system, and the vascular system of the human being, which can make this substance quite dangerous.

It is known that MDMA was first synthesized in 1912 by chemists Carl Mannich and Willy Jacobson in Germany, having been patented in 1914 as an appetite suppressant (Hegadoren, Baker e Bourin, 1999).

From the 1970s, MDMA started to gain great popularity in the psychiatric community as an adjunct to some treatments. In the 1980s, MDMA started to be used in psychotherapy and was said to increase patient self-esteem and facilitate therapeutic communication. In such settings, it was administered orally (75-175 mg) and it was noted that it produced acute sympathomimetic effects, such as increased heart rate and blood pressure, and transient anxiety (Greer, G; Strassman, 1985; Grinspoon e Bakalar, 1986).

The structural similarity between MDA and MDMA led the latter, in 1985, to be considered as a "substance of great abuse potential, without acceptable safety even with medical supervision" (schedule 1) by the "Drug Enforcement Administration" (DEA). A major controversy surrounding its therapeutic potential led to a reconsideration of MDMA for use under medical supervision, having been added to schedule 3 (scale of least risk and severity). This decision was only temporary, and in 1988, MDMA was once again added to schedule 1 until today (Carvalho, 2007).

As can be seen, MDMA was quickly considered and used as a drug of abuse due to its psychotropic effects, making its illegal consumption very common all around the world. At this point, the first studies that attempt to report the dangers of the consumption of MDMA appear: 3,4-methylenedioxyamphetamine (MDA), a similar compound of MDMA and metabolite resulting from its degradation. This compound has been shown to induce degeneration of serotonergic nerve terminals in studies carried out in the rat brain (Ricaurte *et al.*, 1985).

Illegally, MDMA has been consumed under various forms and such as a crystal or powder which can be injected, subcutaneously and intravenously, smoked or ingested orally (Ropero-

Miller e Goldberger, 1998). Nevertheless, oral consumption in the form of "ecstasy" pills has been the most common route (Cole e Sumnall, 2003).

There is a wide variety in these pills, with the degree of purity and amount of MDMA varying from one to the other (Cole *et al.*, 2002). However, several pills do not contain MDMA as an active substance, but rather another type of substances such as other amphetamines or methamphetamines or caffeine (Baggott *et al.*, 2000). This point is extremely important in determining whether the clinical effects are due to MDMA or not, and it is also important to bear in mind that these substances are normally consumed together with depressant substances, such as heroin or benzodiazepines. There are also reports of MDMA being consumed together with alcohol, cannabis, or other hallucinogenics or stimulants (Scholey *et al.*, 2004). In fact, some of the dangerous and deadly effects can be a consequence of the simultaneous consumption of various substances.

According to the report by the European Monitoring Center for Drugs and Drug Addiction, studies suggest that about 13 million Europeans have tried amphetamines and that approximately 2 million consumed MDMA in 2012 (OEDT, 2012). In 2016/17, ecstasy emerged as the third drug preferentially consumed in Portugal, both generally in the population aged 15-74, as in the population aged 15-34, with a prevalence of consumption lower than cannabis and cocaine (SICAD, 2018).

Despite its recreational and clandestine use, MDMA contains properties that are still the subject of scientific study today. Chemically, MDMA is a synthetic compound derived from amphetamine (a potent CNS stimulant) and mescaline (a hallucinogenic compound), thus sharing several properties of these compounds (Figure 1). It is considered a methamphetamine derivative with the chemical formula $C_{11}H_{15}NO_2$, a molecular weight of 193.24 g/mol, and two enantiomeric forms *S* and *R*. The enantiomeric forms of MDMA have distinct pharmacological properties (Pitts *et al.*, 2018). The *S*-enantiomer has more affinity with the dopamine (DAT), serotonin (SERT) and noradrenaline (NET) transporters reversing the action of these transporters (Verrico, Miller e Madras, 2007) while the *R*-enantiomer has 3 to 5 times more affinity for 5-HT_{2A} serotonergic receptors (Lyon, Glennon e Titeler, 1986). Studies have shown that the S-(+)-MDMA enantiomer has a more stimulating action on the central nervous system (Nichols *et al.*, 1982), but is also responsible for a greater serotonergic degeneration associated with the use of MDMA (Schmidt, Levin e Lovenberg, 1987).

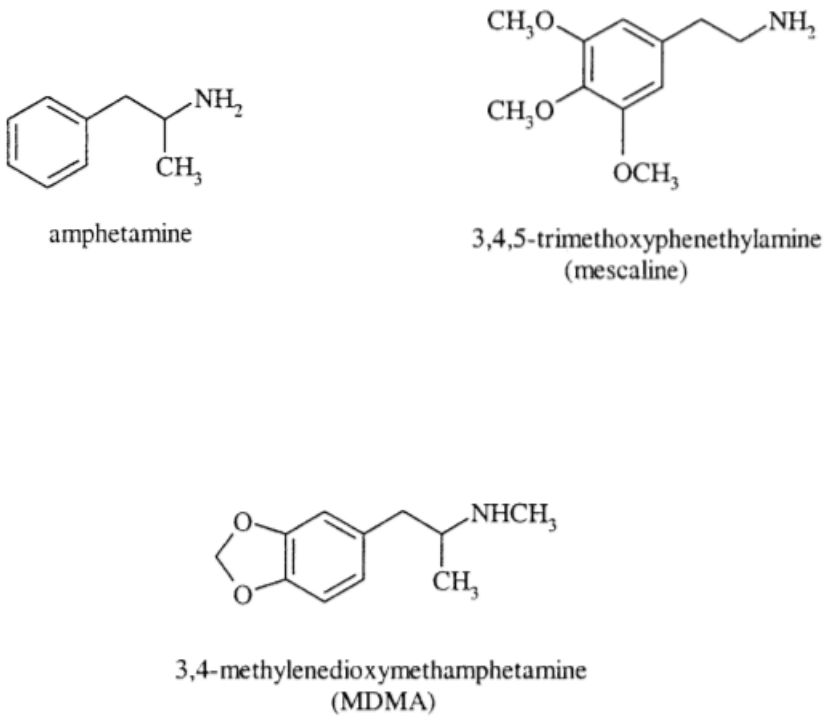

Figure 1 - Structural similarity between MDMA, amphetamine and mescaline. Retrieved from (Green *et al.* 2003).

1.2. Pharmacokinetics of MDMA

It has already been described in the literature that MDMA has an efficient gastrointestinal absorption, with plasma concentration peaks occurring between 1 hour and 3 hours after oral ingestion of the substance. It displays a plasmatic half-life of approximately 8 hours, most of which is excreted in the urine (Mas *et al.*, 1999).

It is known that for small increases in dose there are high plasma concentrations, and there may even be an increased risk of overdose, which suggests that MDMA follows non-linear kinetics (La Torre, De *et al.*, 2000).

The metabolism of MDMA, both in humans and in rats, occurs mainly at the hepatic level, through cytochrome P450 (CYP), specifically the set of isoenzymes CYP1A2 and CYP2D6. The existence of genetic polymorphisms of these isoenzymes can generate changes in metabolism which are dose-independent, as a slow metabolism increases the risk of acute toxicity (Carmo *et al.*, 2006).

The metabolism of MDMA initially involves the *N*-demethylation to form MDA, by the action of CYP1A2. Both MDMA and MDA can undergo *O*-demethylation via CYP2D6, leading to the metabolites 3,4-dihydroxymethamphetamine (*N*-Me-α-MeDA or HHMA) and 3,4-dihydroxyamphetamine (α-MeDA or HHA), respectively. In humans, HHMA is the main

plasma metabolite, whereas in rats the most abundant metabolite is HHA (La Torre, De e Farré, 2004).

1.3. Pharmacodynamics of MDMA

The pharmacodynamics of MDMA is mainly associated with monoamine transporters, namely the serotonin (5-hydroxytryptamine, 5-HT) transporter (SERT), the dopamine transporter (DAT), and the noradrenaline transporter (NET). In addition, MDMA is responsible for the inhibition of monoaminoxidase (MAO-B), an enzyme responsible for the metabolism of serotonin and dopamine, thus leading to the accumulation of these neurotransmitters. Overall, there is an increase in the concentration of these neurotransmitters in the synaptic cleft, mainly of 5-HT, which is followed by a period of decrease of this neurotransmitter.

Taking this into account, it is clear that there is a potential for interactions with some drugs, namely 5-HT reuptake inhibitors, such as fluoxetine, among other compounds which interact with the same targets, e.g. cocaine and amphetamine derivatives. In fact, these substances also inhibit CYP2D6, thus increasing plasma concentrations of MDMA (Ramamoorthy *et al.*, 2002).

At the vascular level, there is already evidence of hallucinogenic effects that may be mediated by 5-HT_2 and the interaction of MDMA with additional targets, namely with serotonergic receptors (Silva *et al.*, 2016).

Among the young population of consumers, MDMA is generally regarded as a harmless drug. However, MDMA can prove to be a major toxicity-causing substance in the human body. This toxicity can be revealed in terms of neuronal degeneration, liver toxicity, cardiovascular toxicity, renal toxicity, psychiatric events and hyponatremia. The phenomenon of hyponatremia occurs because normally individuals who consume MDMA drink large amounts of water, which can lead to the formation of cerebral edema (Caballero *et al.*, 2002).

At the CNS level, this substance is a powerful stimulant, even though not as much as amphetamine. The main symptoms that are observed are an increase in euphoria and alertness, an increase in feelings of well-being and confidence, hyperactivity, hyperthermia, bruxism, appetite suppression, and even a decrease in auditory and visual capacity (Hegadoren, Baker e Bourin, 1999). Regular use can lead to chronic psychosis, and this psychosis can lead to an even more dangerous effect, the act of suicide (McGuire e Fahy, 1991).

In addition, other side effects include abdominal pain, tremors, cold/heat sensation, intensification of reflexes, blurred vision, muscular tension, dry mouth, anxiety and insomnia (Mccann, State e Ricaurte, 1996).

At the cardiovascular level, increased blood pressure and heart rate, hypotension, palpitations, arrhythmias, cardiomyopathies, circulatory collapse, cardiovascular hypertrophy, interstitial fibrosis, and microvascular diseases or even myocardial infarction have been reported, which can lead to death from cardiac insufficiency or stroke (Henry, Jeffreys e Dawling, 1992; Milroy e Parai, 2011).

1.3.1. Blood Pressure and Heart Rate

The two major cardiovascular effects of MDMA consumption are increased blood pressure and heart rate. This is supported by consistent findings from several clinical trials in controlled settings (Fonseca *et al.*, 2021) but also in recreational use settings (Halpern *et al.*, 2011; Irvine *et al.*, 2006) or intoxications in children (Eifinger *et al.*, 2008; Melian *et al.*, 2004).

In pre-clinical studies, the findings have proven to be less consistent with those found in clinical reports. Several studies have shown increases in systolic and diastolic blood pressure and/or mean arterial pressure (Badon *et al.*, 2002; Bexis e Docherty, 2006; McDaid e Docherty, 2001; O'Cain *et al.*, 2000; Schindler *et al.*, 2014), despite one study reported no significant changes in blood pressure (Rusyniak *et al.*, 2007). Moreover, it was found that this increase occurs dose-dependently (O'Cain *et al.*, 2000; Schindler *et al.*, 2014).

Interestingly, it was demonstrated that the response of the diastolic blood pressure was biphasic, as an initial pressor response (mediated by the action of α_1- and α_2-adrenoceptors) was followed by a depressor response (mediated by α_2-adrenoceptors) (McDaid e Docherty, 2001). This type of pressor response appears to be dose-dependent, that is, while a dose of 5 mg/kg produces a biphasic response, a dose of 20 mg/kg produces only a pressor response (Vandeputte e Docherty, 2002).

Regarding heart rate, one study has also shown a biphasic response (Badon *et al.*, 2002). Conversely, other studies have shown either tachycardia (Alsufyani e Docherty, 2015; McDaid e Docherty, 2001; Rusyniak *et al.*, 2007; Schindler *et al.*, 2014) or bradycardia (O'Cain *et al.*, 2000; Zhuo *et al.*, 2013). Studies have already been carried out where the involvement of β-adrenoreceptors in the tachycardia process has been verified and where propranolol has also managed to block this effect (Alsufyani e Docherty, 2015; Schindler *et al.*, 2014).

Compared to other amphetamines, MDMA display an effect similar to amphetamine (O'Cain *et al.*, 2000) and 3,4-methylenedioxyamphetamine or MDA (Bexis e Docherty, 2006). Regarding its metabolites, the dihydroxyl metabolites (HHA and HHMA) cause a large increase in heart rate, as HHMA showed a higher efficacy and potency compared to MDMA and HHA. Other metabolites (HHMA and HMA) do not appear to exhibit such impact on heart rate and blood pressure (Schindler *et al.*, 2014).

Several underlying mechanisms, which are represented in Figure 2, have also been identified, such as the interaction with α-adrenoceptors and increased renal sympathetic nerve activity (O'Cain *et al.*, 2000), interaction with 5-HT receptors (McDaid e Docherty, 2001), but also the interaction with β-adrenoceptors (Alsufyani e Docherty, 2015; Schindler *et al.*, 2014).

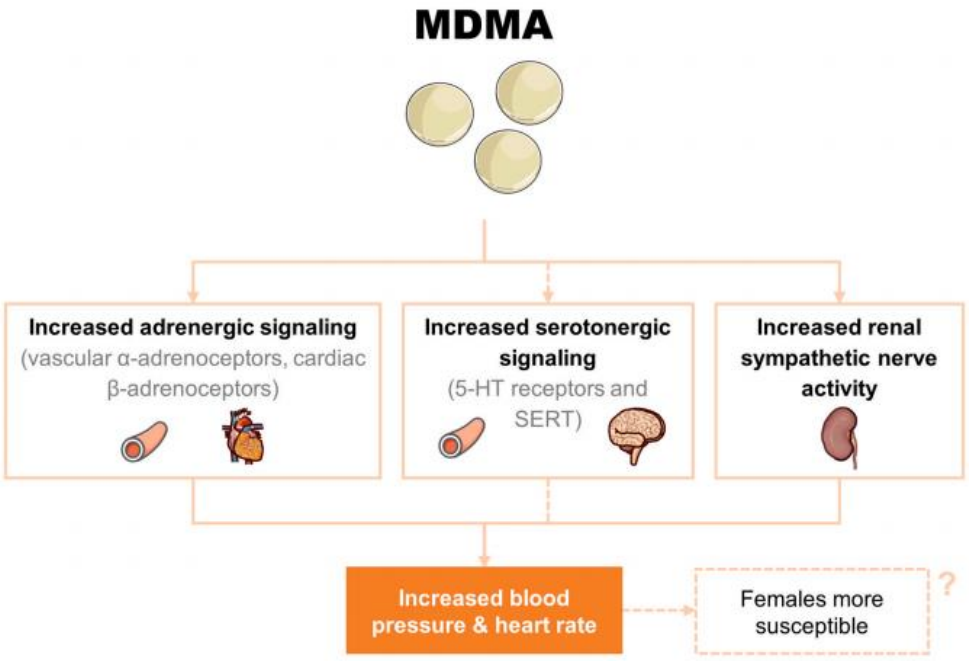

Figure 2 - Summary of the mechanisms involved in the MDMA-induced increases in blood pressure and heart rate (Fonseca *et al.*, 2021).

As mentioned above, several clinical trials have been carried out that showed consistently an increase in blood pressure and heart rate with the oral administration of MDMA (for more details see our recent review: Fonseca *et al.*, 2021). Notably, the individuals included in such clinical trials are typically young male individuals with no underlying cardiovascular disease.

In this context, a few studies have explored the influence of specific factors on the blood pressure and heart rate response to MDMA, namely: gender (male vs female) and interindividual genetic differences (i.e. genetic polymorphisms) in the serotonergic, adrenergic and dopaminergic systems and in the CYP-mediated hepatic metabolism.

As to gender, the evidence has not been consistent and thus whether this is a factor to take into account when analyzing the cardiovascular response to MDMA remains to be clarified. In one study, equal doses of MDMA provoked a greater response in women compared to men, however men initially showed higher levels of blood pressure (Liechti, Gamma e Vollenweider, 2001). In contrast, a recent study showed that, taking into account body weight, males displayed a higher increase in systolic blood pressure compared to females (Vizeli e Liechti, 2017).

Regarding genotypes of CYP-mediated hepatic metabolism, studies have demonstrated that the CYP2C19 genotype can influence this response, unlike CYP1A2 and CYP2B6 (Vizeli *et al.*, 2017).

Moreover, one study has explored the social context of MDMA consumption relating to its recreational use as a drug of abuse. In this study, a greater increase in heart rate was observed when MDMA was taken in a group compared to when taken alone or accompanied by a study assistant (Kirkpatrick e Wit, De, 2015).

Regarding the underlying mechanisms, clinical trials have mainly focused on testing the involvement of monoaminergic targets, such as monoamine transporters and receptors (Fonseca *et al.*, 2021). In the adrenergic system, reboxetine (NET blocker) has been shown to decrease the rise in blood pressure and heart rate caused by MDMA (Hysek *et al.*, 2011), as well as the NET and SERT blocker duloxetine (Hysek *et al.*, 2012). These studies suggest that NET can play a role in the MDMA-induced cardio-stimulation. There here is also evidence that beta (β) and alpha (α) adrenoceptors are involved in these cardiovascular effects. Carvedilol, a α_1- and β-adrenoceptor antagonist, can inhibit the cardiovascular response to MDMA (Hysek *et al.*, 2012), just as the β-blocker pindolol can prevent the increase in heart rate (Hysek, Vollenweider e Liechti, 2010). On the contrary, clonidine, α_2-adrenoceptor agonist, has failed to show any results in inhibiting/decreasing such cardio-stimulating effects (Hysek *et al.*, 2012).

Focusing on the serotonergic system, high-activity genotypes of SERT and COMT were associated with increased systolic blood pressure and heart rate (Pardo-Lozano *et al.*, 2012). Serotonin reuptake inhibitors citalopram (Liechti e Vollenweider, 2000) and paroxetine (Abanades *et al.*, 2007) have been shown to decrease the heart rate response and increased blood pressure caused by MDMA, while a study says fluoxetine (Tancer e Johanson, 2007) only blocked the increase in heart rate.

Nowadays, taking into account all the preclinical studies and clinical trials already carried out in this area, it is also important to take into account that there is a probability that the genetic and endogenous factors of each human being can also influence the response of the human body. when exposed to MDMA.

1.3.2. Cardiac effects

In addition to increasing heart rate and blood pressure, MDMA displays many other effects on the human body. Many of these at the level of the heart, where it can cause disorders such as arrhythmias, and contractile dysfunction, or even heart valve disease.

In regard to heart valve disease, there is a marked scarcity of studies. However, it is thought that the appearance of valvular heart disease may also be related to the consumption of MDMA. In an in vitro assay using interstitial valve cells, MDMA promoted fenfluramine-like proliferation of these cells, as both MDMA and MDA (*N*-demethylated metabolite of MDMA), can bind to 5-HT_{2B} receptors, which may also be evidence that these receptors are involved in the onset of MDMA-associated valvular disease (Setola *et al.*, 2003). Another study has been carried out in individuals who were current or former users of MDMA, which showed that the use of this drug may be associated with mitral and tricuspid valve regurgitation (Droogmans *et al.*, 2007).

In addition to this cardiac complication, it is also thought that MDMA consumption leads to a greater predisposition to develop cardiac pathologies, namely cardiomyocyte necrosis, tissue inflammation, and even myocardial infarction (Fonseca *et al.*, 2021). In a preclinical trial, a single administration of MDMA was shown to promote myocardial contraction band necrosis, inflammatory infiltrate and calcium deposits within ventricular cardiomyocytes. In addition to these findings, it also decreased the antioxidant activity of some enzymes and decreased levels of ascorbic acid and total glutathione (Cerretani *et al.*, 2008).

In another study on adolescent and aged male rats, aged rats were found to be more susceptible to heart damage, higher increases in body temperature and to serotonergic neurotoxicity, which can be an indication that age might be relevant in the toxicity of MDMA. In addition, this study also showed that aged rats were more susceptible to kidney damage (Feio-Azevedo *et al.*, 2018).

Over the years and in all the studies carried out, it has become increasingly obvious that there is a relationship between the excessive consumption of MDMA and the emergence of contractile dysfunction and arrhythmias (Eigsti, Firchau e Nashelsky, 2018; Greene *et al.*, 2003;

Voizeux *et al.*, 2019). Several preclinical and clinical studies also support this relationship, and heart failure has also been observed even in acute consumption settings (Kafle *et al.*, 2019).

Both in vitro and in vivo studies have suggested that the consumption of MDMA can interfere with the function of cardiomyocytes, involving several mechanisms. Of note, concentrations of MDMA equivalent to those used in recreational settings have been shown to impact the function of human iPSC-derived cardiomyocytes, causing: a decreased spike amplitude at 100 μM and beat rate in electrophysiology field potential recordings, which could indicate negative inotropic and chronotropic effects; arrhythmia-like events at concentrations equal to or higher than 300 μM and a small decrease in metabolic activity at 1000 μM (Zwartsen *et al.*, 2019).

In fact, MDMA consumption has been associated with left ventricle (LV) systolic dysfunction in rats, as the autophagy-lysosomal pathway has been suggested as the underlying mechanism (Shintani-Ishida *et al.*, 2014).

As it is known, MDMA is frequently co-consumed with alcohol in recreational settings. In this context, a study has shown that this combination can trigger the activation of cardiac sympathetic pathways in LVs of adolescent mice as well as increased expression and phosphorylation of heat shock protein 27 (Navarro-Zaragoza *et al.*, 2019). More recently, this research group has also shown that concomitant exposure of MDMA and binge ethanol increased the expression and phosphorylation of TH in right ventricles of adolescent mice (Navarro-Zaragoza *et al.*, 2019).

At the moment, there is very limited clinical evidence on the relationship between MDMA consumption and cardiac contractile dysfunction, as the existing clinical studies (Kanneganti *et al.*, 2008; Lester, 1983) do not seem to confirm this relationship.

1.3.3. Vascular effects

In addition to the previously explored cardiovascular effects, MDMA has also been shown to elicit direct effects on the vasculature and cause several phenomena, such as: vasoconstriction, thrombosis and disruption of vascular integrity.

As explored above, there is strong evidence that MDMA increases blood pressure. Considering that blood pressure is derived from cardiac and vascular factors, such effects on the blood pressure could result from either an increase in cardiac output, an increase in peripheral vascular resistance or both (Fonseca *et al.*, 2021). MDMA and its metabolite MDA

have already been the subject of several studies, as an increase in the contraction to the α_1-agonist phenylephrine was observed in rat aortic rings, which can be an indication of role for the α_1-adrenoceptors (Bexis e Docherty, 2006). In the porcine coronary artery, MDMA was mainly shown to cause vasoconstriction, however, it was also observed that the presence of cocaine or prazosin and the removal of the endothelium did not affect the observed vasoconstriction, thus there is a hypothesis for the involvement of mechanisms other than α_1-adrenoceptors and sympathomimetic activity (Baker, Herbert e Broadley, 2007).

In a study also performed using rat aortic artery rings, it was found that MDMA does not significantly interfere with the efficacy and potency of 5-HT-induced vasoconstriction (Murphy *et al.*, 2002).

Our group has explored the effects of MDMA using a human arterial model, the human internal thoracic artery (ITA) also known as internal mammary artery. In these studies, we have shown that: (a) MDMA and its metabolites cause changes in serotonergic activity both under normothermic or hyperthermic conditions, (b) MDMA and two metabolites (HHMA and HHA) elicit concentration-dependent contraction which is potentiated in hyperthermic conditions (Fonseca *et al.*, 2017, 2019). Based on these findings, it is possible to formulate the hypothesis that the rise in blood pressure may be due to a direct vasoconstrictor effect involving 5-HT receptors, namely 5-HT_1 and 5-HT_{2A}. Despite this, other mechanisms may also be involved, namely the SERT-mediated release of 5-HT (Silva *et al.*, 2016).

Several studies have also been conducted where it was concluded that the consumption of MDMA can cause the disruption of the blood-brain barrier (BBB). This has been associated with several mechanisms, such as: increased expression of pro-inflammatory cytokines, increased BBB permeability, production of reactive oxygen species, and also upregulation of heat-shock protein 72 (Kousik, Napier e Carvey, 2012; Sharma e Ali, 2008). In fact, it has already been observed that the increase in BBB permeability associated with MDMA is mediated by a mechanism involving $P2X_7$ receptors, which in turn promotes the activity of matrix metalloproteinases (MMPs), specifically MMP-9 and MMP-3, and subsequently extracellular matrix degradation (Rubio *et al.*, 2014).

In addition to BBB disruption, MDMA may also promote other forms of disruption of vascular integrity such as aortic dissection (Kanahara *et al.*, 2016), similar to methamphetamine.

In general, amphetamines are associated with acute vascular problems, many of those being thrombosis or even myocardial infarction. In regard to MDMA, it has been associated with venous renal thrombosis (Eldehni *et al.*, 2010) and acute myocardial infarction (Israelit,

Strizevsky e Raviv, 2012; Lai *et al.*, 2003; Möller *et al.*, 2010; Okunoye e Dutton, 2013; Papachristidis *et al.*, 2016; Qasim, Townend e Davies, 2001), as well as several forms of hemorrhage (Fonseca *et al.*, 2021).

Despite the large body of evidence, it is notorious how much this substance still has to be studied, at different levels. Even though it is mainly considered a drug of abuse for recreational settings, MDMA has attracted some interest for its potential in psychotherapy. However, the multiple activities displayed by this substance warrant a need for further study of its systemic effects, particularly at the cardiovascular level where there are still many unknowns.

1.4. Human Internal Thoracic Artery

The human ITA, or internal mammary artery, originates from the subclavian artery and is located inside the anterior chest wall (Miyamoto e Enomoto, 1990) (Figure 3). This vessel allows blood to reach the region of the anterior thoracic wall, branching into other vessels of a smaller caliber that supply the diaphragm and abdominal wall.

This artery has been of great interest in the scientific community as it is the most successful arterial graft in coronary artery bypass grafting (CABG). Its use instead of other vessels has led to better long-term clinical results, improving the survival rate and quality of life of patients (Sabik *et al.*, 2013).

The ITA has mostly histomorphology characteristics of an elastic artery, having in its constitution three main layers: the tunica intima, the tunica media, and the adventitia. Although the ITA was initially classified as an artery with elastic characteristics, it was confirmed that there are histological differences along its section from the subclavian artery to the epigastric bifurcation (Fonseca, Antunes e Cotrim, 2017; Kraler *et al.*, 2021). The histomorphology of this artery becomes a better choice compared to other arteries due to some important factors. Comparing ITA with the saphenous vein, Motwani and Topol showed that the ITA had few endothelial fenestrations, a superior intercellular communication capacity, permeability of the inferior intercellular junction, little dependence on vasa vasorum and greater resistance to harvest trauma (Motwani e Topol, 2018).

Furthermore, this artery has a low incidence of developing atherosclerosis (Sims, 1983). According to the literature already described, ITA has been considered as a vessel free of atherosclerosis after several studies have demonstrated the absence of atherosclerotic lesions (Fonseca, Antunes e Cotrim, 2017). Currently, there is already a better understanding of the

particular and beneficial characteristics that this vessel has, such as shear forces, set of endothelial cells with rigid endothelial junctions and formation of important molecules such as nitric oxide, as well as antithrombotic factors and resistance to the formation of selectins and other adhesion molecules, can help understand why ITA is resistant to the development of atherosclerosis (Otsuka *et al.*, 2013). Thus, all these characteristics make the ITA an artery of great importance and interest at the level of clinical investigation.

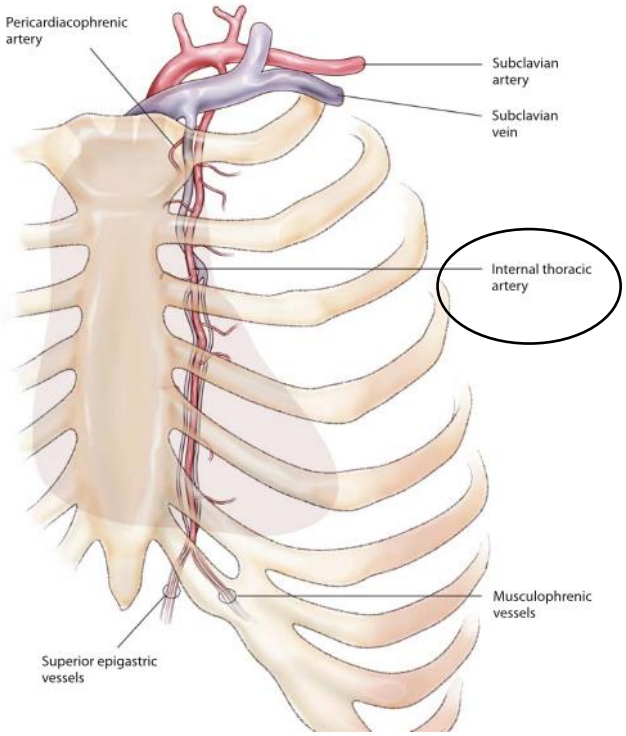

Figure 3 - Representation of the anatomical site of the human mammary artery. Adapted from (He 2006).

This vessel is generally less susceptible to vasospasm, but the distal portion can be the a part of the this vessel more susceptible to this phenomenon, nevertheless the underlying mechanisms that can lead to spasms are not yet fully understood and may be provoked by some type of physical stimulus (Fonseca, Antunes e Cotrim, 2014). Furthermore, a relevant feature of this artery is that the distal portion is pharmacologically active and can easily respond both to vasoconstrictor agents, such as α-adrenoreceptors, 5-HT agonists, and to vasodilators, such as calcium channel antagonists (He *et al.*, 1989).

According to the literature, the artery displays serotoninergic receptors and α and β-adrenoceptors in the constitution of its endothelium and smooth muscle. Interestingly, there are 5-HT receptor subtypes, the 5-HT_{2A} e 5-HT_{1B}, which have vasoconstrictor activity for

example found in this artery. Studies carried out to clarify which subtypes of 5-HT receptors are responsible for the contractile activity of ITA have also shown that 5-HT_{1B} and 5-HT_{2A} receptors are the mediators of the 5-HT-induced contractile response in this artery (Tanaka *et al.*, 2008). In another study carried out with a 5-HT_{2A} receptor antagonist, ketanserin, a considerable reduction in the response to serotonin was observed, but this reduction did not happen completely (Conti *et al.*, 1990), what can shows that there may be a possible involvement of 5-HT_1 receptors in this contractile process. Also according to the already published literature, the vasoconstrictor response of ITA can be mediated by 5-HT_{1B} receptors (Conti *et al.*, 1990). When its contractile capacity is tested with a 5-HT_{1B} receptor agonist, sumatriptan, it appears that there is a contractile response by the artery, although there is some reduction in contraction (Yildiz *et al.*, 1996). Therefore, some indicators can prove that in ITA, the contractile response that exists is explained by the activity of 5-HT_{1B} and 5-HT_{2A} receptors.

Other types of receptors that exist in the human thoracic artery are adrenergic receptors, which have also been studied to try to understand the role they play in this artery. These receptors can be divided into two types, α-adrenergic receptors and β-adrenergic receptors. According to some study results, there is evidence that this type of receptor causes vasoconstriction in the artery. A study already described in the literature shows that stimulation of α-receptors with norepinephrine elicited a contractile response mediated mainly by type 1 and type 2 α-adrenergic receptors (Giessler *et al.*, 2002).

Contrary to adrenergic receptors, concerning β-adrenergic receptors, these have a completely different function than the receptors discussed above. Based on the literature review, it is known that the process of vasodilation in ITA also occurs, and these effects are mediated predominantly by β_2-adrenergic agents and a small part of this relaxation is also due to β_1-adrenergic agents (Ferro, Kaumann e Brown, 1993). Another study was also able to show that isoprenaline, a non-selective β-adrenergic agonist, could cause relaxation effects in β_2-adrenergic receptors located in this artery (Molenaar *et al.*, 1988). There are, therefore, data that evidence and confirm that these receptors, when stimulated, cause the artery to relax.

CHAPTER II.

Rationale and Aims

2. RATIONALE AND AIMS

Ecstasy has become one of the most widely consumed drugs in the world, establishing itself as one of the most popular recreational drugs. As detailed in the previous chapter, there is a large body of evidence on the effects of MDMA on the human body, particularly concerning its effects on the brain and liver. As this substance has attracted the interest of the scientific community due to its potential in psychotherapy, its consumption may start to extend beyond the classical recreational use by young individuals. However, it is notorious how much is unknown regarding the cardiovascular effects of this substance, particularly at the vascular level. In fact, there is very limited evidence on the implications of its consumption by individuals with preexisting cardiovascular conditions as well as to whether there are individuals with higher risk of developing vascular complications.

The present work focused on the vascular effects of MDMA in individuals with preexisting cardiovascular conditions and the impact of interindividual variability on the vascular response to this drug of abuse. Methodologically, we carried out a pooled retrospective analysis on previously obtained results from our lab regarding the functional vascular response to MDMA using ITA samples. Regarding the impact interindividual variability we first analyzed the influence of age, taking into account previous studies on the comparison of adolescent and aged rats. Other baseline patient characteristics were also considered, such as: obesity, history of hypertension, diabetes, peripheral vascular disease, myocardial infarction, among others.

CHAPTER III.

Methods and Materials

3. METHODS AND MATERIALS

The present study consisted of a retrospective analysis of data that were previously collected for a pooled analysis of pharmacological parameters of vascular contractile response to MDMA. Furthermore, we also evaluated the impact of interindividual variability on the vascular response to this drug of abuse.

This study was carried out with the approval from the research ethics committee of the Faculty of Medicine of University of Coimbra (CE-068/2021). Written informed consent was obtained from each patient for the harvesting of the arterial samples with the approval of the local research ethics committees of the Faculty of Medicine of University of Coimbra and Coimbra University Hospitals (CE-084/2020 and PC-388/08, respectively), as the access to anonymized clinical data was granted by ethics committee approval (CE-084/2020 and CE-068/2021).

3.1. Vascular contractile response to MDMA

3.1.1. Reagents

MDMA (hydrochloride salt) was extracted and purified from high-purity MDMA tablets that were kindly provided by the Portuguese Criminal Police Department through Prof. Dr. Félix Carvalho (Faculty of Pharmacy, University of Porto). The obtained hydrochloride salt was characterized by nuclear magnetic resonance and mass spectrometry, as previously detailed and reported (Capela *et al.*, 2007). All other chemicals were purchased from Sigma-Aldrich® (St. Louis, Missouri) and correspond to the highest grade commercially available.

3.1.2. Harvesting and preparation of human arterial samples

Arterial segments were harvested from 28 patients, aged between 47 and 82 years. The arteries were dissected as a pedicle from the anterior internal surface of the chest after sternal incision, externally irrigated with papaverine, a vasodilator used to prevent perioperative vasospasm, and cut distally. The distal segments remaining from surgery were placed in cold (4°C) Krebs-Henseleit bicarbonate buffer (in mM: NaCl 118.7, KCl 5.4, $CaCl_2.H_2O$ 1.9, KH_2PO_4 0.9, $MgSO_4.7H_2O$ 0.6, $NaHCO_3$ 25 and glucose 11.1), previously adjusted to pH 7.4

and aerated with carbogen (95% O2 / 5% CO2), and transferred to the Laboratory of Pharmacology and Pharmaceutical Care, Faculty of Pharmacy of the University of Coimbra.

To fully isolate the vessel and avoid the potential influence on tissue reactivity, the perivascular tissue - fat, muscle, and vasa vasorum - was carefully removed with surgical tweezers and scissors to maintain tissue viability. Afterwards, the distal segments of ITA were isolated by removing the perivascular tissue, cut into small rings (about 3 mm in length) and mounted in 10-ml tissue organ baths filled with KHBB. Throughout the experiments, arterial rings were maintained either at physiological temperature (37°C) or at hyperthermic conditions (40°C), with a thermostat (PanLab®). The isometric tension of the rings was recorded with force transducers (AD Instruments®). After mounting, each ring was adjusted to an optimal resting tension, which referred to the equilibrium state reached after the application of a 2-g passive force (19.6 mN). Rings were allowed to stabilize and equilibrate for 2 hours, with regular washes (each 30 min).

3.1.3. Vasoreactivity protocol and pharmacological analysis

At the beginning and at the end of each experiment, arterial rings were stimulated with potassium chloride (KCl, 60 mM) to check for tissue viability throughout the duration of the experiments.

Vascular contractile response to MDMA was assessed by cumulative concentration-response curves (10-1360 μM), as each concentration was added when the response to the previous concentration reached a plateau, i.e. a stabilization of the ring tone.

A concentration-response curve is typically fitted with nonlinear regression models (Figure 4), such as the Hill model with the following logistic equation in semi-logarithmic scale (Figure 4A), where E_{max} is the maximum effect produced by an agonist, E_{min} the baseline, *[A]* the concentration of the agonist, EC_{50} the half-maximum concentration and *HS* the Hill slope:

$$y = E_{min} + \frac{E_{max} - E_{min}}{1 + 10^{(logEC_{50} - [A]) \times HS}}$$

In a three-parameter logistic equation, the Hill slope parameter is equal to 1, whereas it can vary in a four-parameter equation.

However, in some cases, a biphasic model may better explain a concentration-response relationship, as visible in Figure 4B. In these cases, the following equation is used, where F_{max} is the proportion of maximal response due to the more potent phase:

$$y = E_{min} + \frac{(E_{max} - E_{min}) \times F_{max}}{1 + 10^{(logEC_{50(1)} - [A]) \times HS_{(1)}}} + \frac{(E_{max} - E_{min}) \times (1 - F_{max})}{1 + 10^{(logEC_{50(2)} - [A]) \times HS_{(2)}}}$$

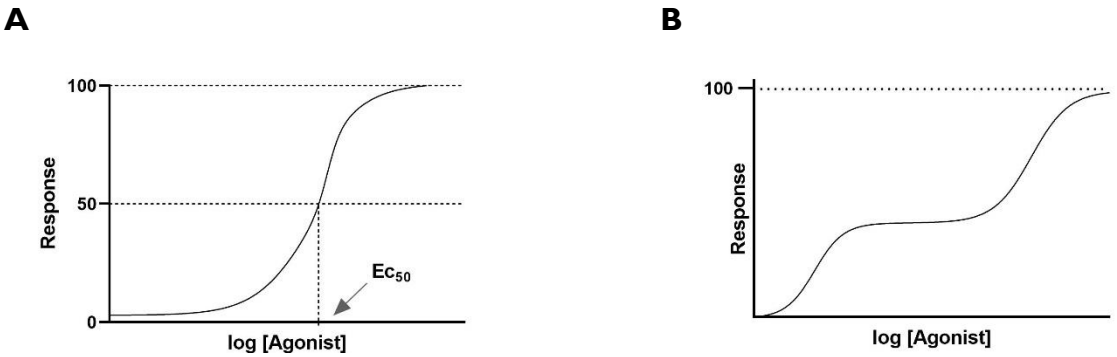

Figure 4 - Representative curves for the Hill model (a) and the biphasic model (b).

The maximal contraction (E_{max}), either as absolute (in mN) or relative contraction (in % of maximal response to KCl or % KCl), was considered as a parameter of intrinsic activity of MDMA. Potency is usually expressed as the negative logarithm of the effective concentration (in mol/L or M) of agonist able to induce half of the maximum contraction (pEC_{50}, -log[M]), by interpolation in a half-logarithmic scale (% E_{max} vs. -log [M]). In biphasic concentration-response curves, this can be more difficult. Instead, we considered a combined measure of efficacy and potency, the area under the concentration-response curve to MDMA (AUC), i.e. area from the curve to baseline of 0 mN or %.

3.2. Clinical characteristics definition

Several clinical characteristics were considered which can be classified into demographic and anthropometric factors, risk factors for coronary artery disease and others. In regard to demographic and anthropometric factors, (a) age was defined as the age of the patient in years, at the time of surgery, (b) gender was defined as gender of the patient at birth, as male or female, (c) body surface area was expressed as m^2 at the time of surgery and was calculated according to the DuBois & DuBois formula. Definitions for risk factors are presented in Table 1.

Table 1 - Definitions of risk factors for coronary artery disease, analyzed in the presented study (ordered alphabetically).

Factor	*Definition*
Diabetes mellitus	History of diabetes mellitus, regardless of duration of disease, including patients currently medicated with oral antidiabetics and/or insulin.
Dyslipidemia	History of dyslipidemia diagnosed and/or treated by a physician. Documentation of one or more of the following: total cholesterol >200 mg/dL, LDL≥130 mg/dL, HDL<30 mg/dL, admission cholesterol >200 mg/dL, triglycerides >150 mg/dL, treatment with cholesterol-lowering drugs initiated due to LDL > 100 mg/dL (2.59 mM) in patients with known coronary artery disease (CAD) and any treatment with cholesterol-lowering drugs.
Family history of CAD	Presence of absence of any direct blood relative who have had any of the following diagnosis (at an age lower than 55 or 65 years for male and female relatives, respectively): angina, acute myocardial infarction, sudden cardiac death without obvious cause, coronary bypass surgery, percutaneous coronary intervention.
Hypertension	Diagnosis of hypertension documented by ate least one of the following criteria: documented history of arterial hypertension diagnosed and treated with medication, diet and/or exercise, documented history of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg for patients without diabetes mellitus or chronic kidney disease, documented history of systolic blood pressure >130 mmHg or diastolic blood pressure >80 mmHg on at least two occasions for patients with diabetes mellitus or chronic kidney disease and any pharmacologic treatment to control arterial hypertension.
Smoking	Current (≤30 days), past (>30 days) or no history of consumption of any form of tobacco (e.g. cigarettes, cigars, smoking pipe and others).

Other clinical factors were also considered, namely:

a) Angina pectoris class – graded according to the Canadian Cardiovascular Society into one of four categories: (I) angina only with strenuous exertion, (II) angina with moderate exertion, (III) angina with mild exertion and (IV) angina at rest;
b) Unstable angina, defined as angina at rest requiring nitrates intravenous therapy within 48h prior to surgery and/or until the entrance in the operating room;
c) History of peripheral vascular disease (PVD), including upper and lower extremities, renal, mesenteric and abdominal aortic systems, documented according to the following criteria: claudication, either with exertion or at rest, amputation due to arterial insufficiency, vascular reconstruction, CABG or PCI to the extremities (excluding dialysis fistulas and vein stripping), documented aortic aneurysm (with or without repair), positive non-invasive test (e.g. ankle brachial index ≤0.9, ultrasound, magnetic resonance or computed tomography imaging with diameter stenosis >50% in any peripheral artery i.e. renal, subclavian, femoral, iliac);

d) History of cerebrovascular disease (CBVD), documented by one of the following criteria: (a) cerebrovascular accident with symptoms more than 24h after onset and presumed vascular etiology, (b) transient ischemic accident with recovery within 24h, (c) non-invasive carotid test with diameter occlusion higher than 79% and (d) prior carotid surgery (neurological disease processes such as metabolic and/or anoxic ischemic encephalopathy were excluded).

3.3. Analysis of results

The data was initially collected in units of gram (g) and converted to milliNewton (mN). In addition, we expressed the results as a percentage of the maximum contraction to KCl (% KCl) to control for differences in the contractility of the tissue.

Overall, data was expressed as mean ± standard error of mean (SEM) unless specified otherwise and *n* indicates the number of patients. *P* values lower than 0.05 ($P<0.05$) were considered to indicate statistically significant differences. Statistical analysis was performed in GraphPad Prism 9® (GraphPad Inc., La Jolla, CA, USA) and IBM SPSS Statistics® version 19.0.0 (IBM Corp., Armonk, NY, USA).

Curves were first analyzed by Mann-Whitney U-test for curve means and multiple Mann-Whitney U-tests to identify differences in specific concentrations between different thermic conditions (37°C vs 40°C). Curve parameters (E_{max} and AUC) were analyzed by Mann-Whitney U-tests. Additionally, we compared paired samples ($n= 4$) in both thermic conditions with the paired samples Wilcoxon test.

Multivariable linear regression analysis was carried out to identify independent predictors of the pharmacological parameters of MDMA-induced contractile response. This method corresponds to linear regression modelling of a continuous outcome variable with multiple continuous and/or categorical explanatory variables (Peters, 2008). This was achieved by first screening the explanatory variables by univariate analysis (using the Spearman's correlation for continuous variables, the Mann-Whitney *U*-test for variables for two categories and the Kruskal-Wallis H-test for variables with 3 categories), as variables were retained for multivariable regression by the backward stepwise method if the *P* value was lower than 0.20 in the first stage of the analysis. Due to a small effective sample size, a *P* value lower than 0.10 was considered for variable retention in the final regression models. The R^2 is reported as a measure of the percentage of explanation of the regression model, i.e. an R^2 of 0.50 indicates that 50% of the variation may be explained by the variables included in the model. Statistical

validation of these models was carried out by ANOVA (constructed model vs no model) and a *P* value lower than 0.05 indicated that the constructed model displayed higher predictive power compared to no model. Considering that some explanatory variables may be related to each other, collinearity was assessed by the value of the variance inflation factor (VIF), as a VIF lower than 2 indicated the absence of multicollinearity in the variables that entered the regression models.

CHAPTER IV.

Results and Discussion

4. RESULTS AND DISCUSSION

4.1. Baseline patient characteristics

As detailed above, we aimed to carry out a pooled retrospective analysis on previously obtained results from our lab regarding the functional vascular response to MDMA using ITA samples. This study included a total of 28 patients (26 males and 2 females), with a mean age of 62.8 years (Table 2). A considerable number of patients had medical history of hypertension (85.7%) and dyslipidemia (92.9%). Of note, 12 patients had history of smoking (3 current smokers and 9 past smokers, who did not smoke for at least 30 days prior to surgery). Also, 9 patients had history of myocardial infarction (7 in the last 30 days before surgery).

Table 2 - Patients' baseline clinical characteristics. Continuous variables were expressed as mean ± SEM and categorical variables as counts (percentages).

Characteristics	***N= 28***
Age (years)	62.8 ± 2.0
Male gender	26 (92.9%)
Body surface area (cm^2)	183.9 ± 2.6
Family history CAD	6 (21.4%)
Current smoking	3 (10.7%)
Past smoking	9 (32.1%)
Hypertension	24 (85.7%)
Diabetes	9 (32.1%)
Dyslipidemia	26 (92.9%)
Peripheral vascular disease	4 (14.3%)
Cerebrovascular disease	3 (10.7%)
Recent myocardial infarction	7 (25.0%)
Past myocardial infarction	2 (7.1%)

This group of patients corresponds to a population undergoing coronary artery bypass grafting. Overall, the characteristics are consistent with previous reports by our group on the histomorphological aspects of the ITA (Fonseca *et al.*, 2019) and on vasomotion in the ITA (Fonseca *et al.*, 2018).

4.2. Characterization of the vascular contractile response to MDMA

The pooled analysis of previous results on the vascular response to MDMA revealed a biphasic contractile response to MDMA at both normothermic and hyperthermic conditions (Figure 5). As can be seen, the response was consistent when comparing the results expressed as absolute contraction (mN, Figure 5A) with the results expressed as relative contraction (% KCl, Figure 5B).

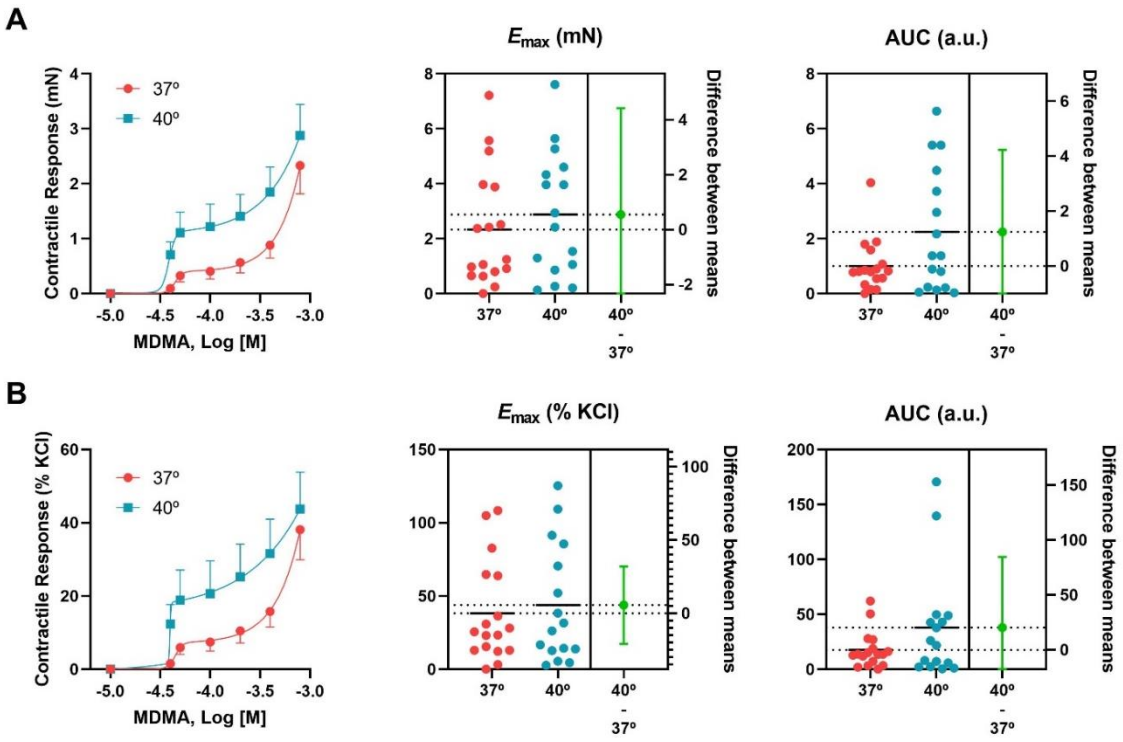

Figure 5 - Contractile response of MDMA in mN (A) and % KCl (B) and evaluation of the means of pharmacological parameters: Emax and AUC. Data is presented in mean ± SEM in plots of concentration-response curves (left). In green, the mean difference is presented with 95% confidence intervals.

This type of response hints towards the intervention of more than one target. In fact, this has been suggested in the literature, with previous reports proposing a role for monoamine receptors and transporters, among other targets. In this context, our group has shown that both 5-HT_2 (Silva *et al.*, 2016) and 5-HT_1 (Fonseca *et al.*, 2019) may be involved in the vascular contractile response to MDMA in ITAs, as well as SERT (Ribeiro, 2020). Other targets have been proposed, such as α_1-adrenoceptors (Bexis e Docherty, 2006) and NET (Al-Sahli *et al.*, 2001) However, contradictory evidence has also emerged as there have been reports showing: no changes in 5-HT efficacy and potency in rat aortic rings (Murphy *et al.*, 2002) and no influence of indirect sympathomimetic activity and α_1-adrenoceptors in porcine coronary arteries (Baker, Herbert and Broadley, 2007). Supporting this notion, clinical studies focused

on the role of genetic polymorphisms in the cardiostimulant effects of MDMA have suggested no major role for the serotonergic (Vizeli, Meyer Zu Schwabedissen e Liechti, 2019) and dopaminergic (Vizeli e Liechti, 2019) systems and NET (Vizeli, Meyer zu Schwabedissen e Liechti, 2018). Therefore, the precise mechanisms involved in this vascular contractile response remain to be fully elucidated.

As previously mentioned, hyperthermia is one of the major effects of MDMA consumption. Our group has studied the influence of hyperthermia on the vascular contractile response both to MDMA and to its metabolites and we have suggested an increased contractile response to MDMA, HHA and HHMA at hyperthermic conditions (Fonseca *et al.*, 2017). In this pooled analysis, the comparison of the two thermal conditions (37°C vs 40°C) suggested an increased contractile response at 40°C (Figure 5 and Table 3). However, the analysis of the pharmacological parameters (E_{max} and AUC) revealed no statistically significant differences between thermal conditions.

Table 3 - Pharmacological parameters (E_{max} and AUC) at both experimental conditions: 37°C and 40°C. Data presented as mean ± SEM.

T (°C)	*mN*		*% KCl*	
	E_{max}	*AUC*	E_{max}	*AUC*
37	2.331 ± 0.515	1.003 ± 0.231	38.13 ± 8.28	17.55 ± 4.04
40	2.880 ± 0.565	2.242 ± 0.558	43.76 ± 10.08	37.89 ± 12.39

To further understand this relationship between temperature and contractile response, we compared paired ITA samples from 4 patients (Figure 6) and observed no significant differences between the two experimental conditions.

This pooled analysis included data from (Fonseca *et al.*, 2017) and (Ribeiro, 2020). In our previous publication on the subject (Fonseca *et al.*, 2017) we showed a 4-fold higher contractile response to MDMA at 40°C compared to 37°C (E_{max}=1.98±0.77 mN at 37°C vs E_{max}=8.03±0.49 mN at 40°C, $p< 0.001$). However, recent work by our lab (Ribeiro, 2020) showed no significant differences (E_{max}=2.79±1.03 mN at 37°C vs E_{max}=7.23±4.54 mN at 40°C). Further studies are needed to clarify the influence of hyperthermia on the vascular contractile response to MDMA.

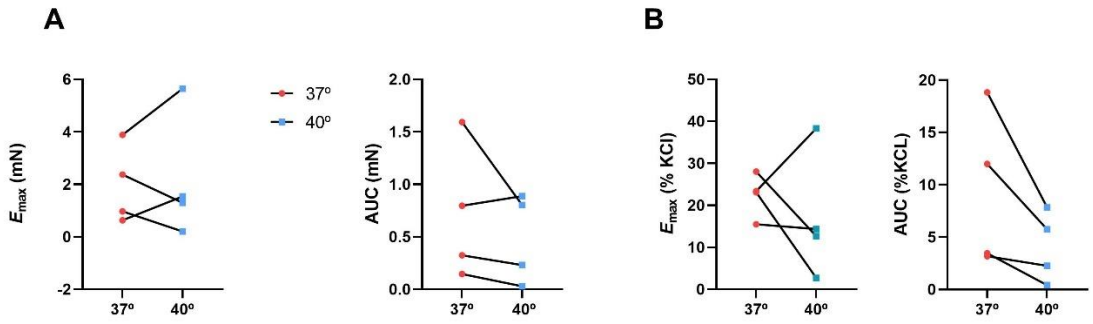

Figure 6 - Comparison of the contractile response to MDMA in mN (A) and % KCl (B) in paired segments of ITA, based on temperature (37°C vs 40°C).

4.3. Impact of clinical factors

The main goal of this work was to assess the impact of interindividual variability on the vascular response to this drug of abuse. To this purpose, we conducted a statistical analysis of the pharmacological parameters described above (E_{max} and AUC) taking into consideration the baseline clinical characteristics of the patients and also the experimental temperature that was used (37°C or 40°C). This analysis combined univariate and multivariable approaches to account for the multiple variables and the interaction between them, as detailed in the Methods section. To avoid over- and underestimation due to the overlap of clinical and pharmacological information from the 4 patients with paired data at both thermal conditions, we excluded these patients from this analysis. Table 4 displays the characterization of the 24 patients included in this analysis.

A first univariate statistical screening revealed a significant difference in the AUC (results expressed in mN) between the temperature of 37°C and 40°C (1.09±0.28 vs 3.07±0.67 respectively, p=0.022). A borderline non-significant difference was detected in E_{max} (expressed as % KCl) between hypertensive and non-hypertensive patients (41.92± 7.99% vs 86.61±20.31% respectively, p=0.053).

Table 4 - Baseline clinical characteristics of patients included in the analysis of the impact of interindividual variability on the vascular response to MDMA.

Characteristics	*N= 24*
Age (years)	61.3 ± 2.0
Male gender	23 (95.8%)
Body surface area (cm^2)	184.4 ± 2.8
Family history CAD	6 (25.0%)
Current smoking	3 (12.5%)
Past smoking	8 (33.3%)
Hypertension	20 (83.3%)
Diabetes	8 (33.3%)
Dyslipidemia	22 (91.7%)
Peripheral vascular disease	4 (16.7%)
Cerebrovascular disease	3 (12.5%)
Recent myocardial infarction	7 (29.2%)
Past myocardial infarction	2 (8.3%)

As previously mentioned, a criterion of $p<0.10$ was used for variable retention for the regression models. In addition to assay temperature and history of hypertension, a medical history of peripheral vascular disease also followed this criterion regarding the E_{max} expressed as mN ($p=0.075$). Multivariable linear regression models were constructed for several parameters (Table 5), except for E_{max} in mN, for which no model explained the observed variability.

Table 5 - Multivariable linear regression analysis of the association between selected clinical variables and pharmacological parameters.

Model	*Predictors*	*β*	*P*	*VIF*
AUC (mN) [a]	Assay temperature	0.524	0.009	1.000
E_{max} (% KCl) [b]	Hypertension	0.430	0.036	1.000
AUC (% KCl) [c]	Assay temperature	0.415	0.044	1.000

[a] Adjusted R^2= 0.241; [b] Adjusted R^2= 0.148; [c] Adjusted R^2= 0.134.

As can be seen, assay temperature was an independent predictor of the parameter AUC (results expressed both in mN and in % KCl). Also, history of hypertension was an independent predictor of the parameter E_{max} (expressed as % KCl). Considering the results detailed above, this suggests that an assay temperature of 40°C is independently associated with a higher AUC (compared to 37°C) and history of hypertension with a lower contractile response to MDMA.

Despite these models allowed the prediction of the mentioned pharmacological parameters, the use of a criterion for variable entry (p<0.10 in the univariate analysis) could theoretically lead to a selection bias of the variables and an overestimation of the effect of these variables. Therefore, we then conducted multivariable regression analysis with the inclusion of all clinical variables by a backward stepwise method (Table 6), with the exception of variables with N<3 in at least 1 of the groups

Table 6 - Multivariable linear regression analysis of the association between all clinical variables and pharmacological parameters.

Model	*Predictors*	*β*	*P*	*VIF*
E_{max} (mN) [a]	Diabetic	-0.404	0.058	1.222
	Hypertensive	0.416	0.054	1.248
	Cerebrovascular disease	0.701	0.054	3.524
	Peripheral vascular disease	-1.113	0.005	3.703
AUC (mN) [b]	Cerebrovascular disease	-0.265	0.087	1.024
	Family history CAD	-0.303	0.055	1.040
	Assay temperature	0.645	<0.001	1.039
E_{max} (% KCl) [c]	Hypertension	0.433	0.044	1.000
AUC (% KCl) [d]	Cerebrovascular disease	-0.352	0.052	1.026
	Body surface area (cm^2)	-0.368	0.041	1.004
	Assay temperature	0.418	0.024	1.030

[a] Adjusted R^2= 0.319; [b] Adjusted R = 0.561; [c] Adjusted R^2= 0.147; [d] Adjusted R^2= 0.414.

Regarding E_{max} (expressed as mN), this second multivariable analysis allowed the construction of a model composed of 4 variables, of which only history of PVD was an independent predictor (p=0.005). Although this factor may be related to the contractile response to MDMA, no significant difference was observed between patients with and without history of PVD (4.67±1.07 mN vs 2.51±0.48 mN respectively, p=0.075) in the univariate analysis. Moreover, an issue of multicollinearity must be noted between the history of PVD and CBVD (VIF >2 for both), thus the observed independent association could be an overestimation. When expressing E_{max} in % KCl, the second model confirmed an independent association with hypertension (Table 6), even though a low R^2 was observed (14.7%).

Consistently with the initial model (Table 5), assay temperature remained as an independent predictor of AUC expressed in mN (p<0.001, Table 6). No collinearity issues were found. Of note, the R^2 (measure of percentage of explanation) of the second model for AUC expressed in mN was markedly higher than the first (56.1% vs 24.1%, respectively)

suggesting a better predictive power, even though the association with other variables (i.e. CBVD and history of CAD) was not significant.

The relationship between assay temperature was also observed regarding AUC from results expressed in % KCl (Table 6). Interestingly, two clinical variables were also included in the model: history of CBVD and body surface area. As the association with the history of CBVD was not significant, an independent association was observed with body surface area (AUC mildly decreased with increasing body surface area, but not significantly). No collinearity issues were observed. From the initial model to the second model (including all variables), the value of R^2 increased markedly from 13.4% to 41.4%.

Overall, a limited relationship was observed between the vascular contractile response to MDMA and the clinical characteristics of the patients, suggesting that interindividual variability could play a minor role on the variability of this vascular response. Previous studies were also carried out with the aim of observing whether factors such as age or gender interfere in the response to MDMA. For example, a study that, despite not focusing on the contractile response to MDMA, was able to observe that this drug of abuse causes more intense psychoactive effects in women than in men, but in contrast, men showed higher increases in blood pressure than woman (Liechti, Gamma e Vollenweider, 2001). On the other hand, a recent pooled analysis of placebo-controlled studies concluded that the acute physiological and psychological response to MDMA depends mainly on MDMA plasma concentration rather than on non-pharmacological factors such as gender or age, even though an older age was significantly associated with small changes in heart rate (Studerus *et al.*, 2021). These distinct observations may also indicate that the role of these two factors should be better understood in the future. Furthermore, the influence of hyperthermia remains to be fully understood, as the results from the pooled analysis and paired data were not in accordance with the sample of patients selected for the multivariable analysis.

The study of all these factors becomes extremely important due to the current discussion on the therapeutic use of MDMA in psychotherapy. For this reason, it is important to know more about its effects and its possible relationship with cardiovascular risk factors. The fact that there is no strong association with clinical factors may suggest that the risk of vasoconstriction may depend on factors other than those analyzed.

CHAPTER V.

Conclusion

5. CONCLUSION

Ecstasy has become one of the most illegally used drugs in the world. There are several studies that have been done in recent years on the effects this drug produces in the human body. It is known that its consumption is considered a risk factor for the development of some cardiovascular diseases, like valvular heart disease (Cosyns *et al.*, 2013), but it is also responsible for changes such as the appearance of hypertension and other disorders at the vascular level, which have already been supported by several studies carried out over the years. Because it can cause all these disorders that have been described in this study and in the past, the consumption of MDMA can be considered a risk factor for developing complications at the cardiovascular and vascular level.

With this purpose, in my 2nd year in the Master of Applied Pharmacology at the Faculty of Pharmacy of the University of Coimbra, a pooled retrospective study was carried out on the vascular effects of MDMA and the impact of inter-individual variability. This retrospective analysis used results from previous vascular studies with ITA specimens retrieved from patients undergoing coronary revascularization. Through this study, we aimed to provide scientifically relevant knowledge on this subject and were able to conclude that:

- MDMA produces a vascular contractile response in human arteries, which may result in vasoconstriction and blood pressure increases;
- The vascular contractile response displayed a biphasic profile, suggesting the interaction with more than one target;
- The profile of the contractile response was independent of the way the results were expressed, either as absolute response (mN) or as relative response (% KCl);
- The pooled analysis suggested no significant relationship between hyperthermic conditions and pharmacological parameters of contractile response, which was confirmed by paired data from 4 patients;
- Multivariable analysis suggested, however, a relationship between experimental temperature (37°C vs 40°C) and AUC parameter;
- Clinical factors, such as history of hypertension and peripheral vascular disease and body surface area may be associated with minor differences in pharmacological parameters of contractile response to MDMA.

Some limitations should be highlighted in this work. First, a low number of patients was included and a low homogeneity was observed in terms of clinical characteristics (particularly gender). Second, the fact that arteries were harvested from patients with coronary

atherosclerosis that required coronary artery bypass grafting may influence our findings as potential functional and structural impairment may have been present. Nevertheless, previous studies have suggested a better integrity of this vessel in comparison with others and a low incidence of atherosclerotic lesions at the time of surgery (Otsuka *et al.*, 2013). Moreover, the use of human tissue provides a better translation of the results to the human.

For future studies, it will be important to fully characterize the mechanisms involved in the vascular effects of MDMA. Specifically, the role of the adrenergic system must be addressed, as well as the potential role of the vascular endothelium. It will also become important to characterize the vascular effects of specific MDMA enantiomers. Furthermore, the development of safe analogs similar to MDMA could provide useful treatments for post-traumatic stress disorders.

In conclusion, the limited relationship between clinical characteristics of the patients and the vascular contractile response to MDMA suggests that interindividual variability plays a minor role on the variability of this vascular response.

CHAPTER VI.

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