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Contractile effects of 3,4-methylenedioxymethamphetamine on the human internal mammary artery

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ABSTRACT

Since the late 1980s numerous reports have detailed adverse reactions to the use of 3,4-methylenedioxymethamphetamine (MDMA) associated with cardiovascular collapse and sudden death, following ventricular tachycardia and hypertension. For a better understanding of the effects of MDMA on the cardiovascular system, it is critical to determine their effects at the vasculature level, including the transporter or neurotransmitter systems that are most affected at the whole range of drug doses. With this purpose in mind, the aim of our study was to evaluate the contractile effect of MDMA in the human internal mammary artery, the contribution of SERT for this effect and the responsiveness of this artery to 5-HT in the presence of MDMA. We have also studied the possible involvement of 5-HT_2 receptors on the MDMA contractile effect in this human blood vessel using ketanserin. Our results showed that MDMA contracted the studied human's internal mammary artery in a SERT-independent form, through activation of 5-HT_{2A} receptors. Considering the high plasma concentrations achieved in heavy users or in situations of acute exposure to drugs, this effect is probably involved in the cardiovascular risk profile of this psychostimulant, especially in subjects with pre-existing cardiovascular disease.

1. Introduction

The recreational use of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) has long been associated with potential toxic effects to several organs and organ systems (Campbell and Rosner, 2008; Capela et al., 2009; Carvalho et al., 2010; Shenouda et al., 2010). The generalized toxicity of MDMA is a consequence of multiple factors, both at molecular and physiological levels, namely the formation of reactive MDMA metabolites, the sustained stimulation of serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) receptors, the enzymatic oxidation of 5-HT and DA and the non-enzymatic oxidation of DA, hyperthermia, hyponatremia, oxidative stress, inflammation, and disturbance of the cardiovascular system. From this host of adverse events, the mechanisms underlying the cardiovascular effects of MDMA are among the less understood.

In humans, MDMA produces dose-dependent increases in heart rate and blood pressure, in controlled experimental settings (Vollenweider et al., 1998; Mas et al., 1999; de la Torre et al., 2000; Lester et al., 2000; Liechti et al., 2000; Farré et al., 2004, 2007; Tancer and Johanson, 2007; Kolbrich et al., 2008; Hysek et al., 2010), during recreational use (Irvine et al., 2006, Halpern et al., 2011) and even in children intoxications (Melian et al., 2004; Eifinger et al., 2008). In line with these effects, MDMA abuse has been shown to induce significant cardiovascular toxicity, including hypertension, cardiac arrhythmias, myocardial infarction, aortic dissection, short gastric artery perforation, cardiovascular collapse, cardiomyocyte necrosis (Dowling et al., 1987; Suarez and Riemersma, 1988; Milroy et al., 1996; Williams et al., 1998; Lai et al., 2003; Sano et al., 2009; Shenouda et al., 2010; Turillazzi et al., 2010) or even subarachnoid and intracranial haemorrhage and cerebral infarction (Gledhill et al., 1993; McEvoy et al., 2000; Auer et al., 2002; Lee et al., 2003; Kahn et al., 2012), which are known causes of permanent cardiac and/or neurologic impairments, or even sudden death.

MDMA affects the nervous system by activating monoaminergic neurons, through the stimulation of 5-HT, noradrenaline (NA) and DA release from nerve vesicles into the synaptic cleft. MDMA also contributes to a steady concentration of these neurotransmitters in the synaptic cleft by partially inhibiting its oxidation through monoamine oxidase (MAO) and by competitively blocking their reuptake into nerve terminals through the respective transporters (Capela et al., 2009). Besides its well known psychotropic effects, MDMA has also been shown to stimulate the release of 5-HT, NA and adrenaline, and to block its cellular uptake in peripheral tissues (Badon et al., 2002; Shenouda et al.,

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2010). Thus, the activation of the autonomic nervous system by MDMA has probably a determinant influence on its cardiovascular effects.

The human cardiovascular system is continuously exposed to plasma 5-HT, usually released from platelets (which contain close to a millimolar level of 5-HT) (Ni et al., 2008). When 5-HT is sustainably released in high amounts, it can produce harmful acute and chronic effects. Acute vascular constriction mediated by 5-HT is usually shared by 5-HT_{1B} and 5-HT_{2A} receptors, except in intracranial arteries, which constrict mainly through 5-HT_{1B} receptors as reported by several authors (Razzaque et al., 2002; van den Broek et al., 2002; Silva et al., 2007) and reviewed by Kaumann and Levy (2006), making their activation the pharmacological basis of potential ischemic cardiovascular events induced by drugs that are agonists at these receptor subtypes. Although concentration of free circulating 5-HT, not stored in platelets (in the nanomolar range) is low in peripheral arteries, a local 5-hydroxytryptaminergic system (with tryptophan hydroxylase isoform TPH1 for 5-HT synthesis, MAO A for metabolism, and serotonin transporter SERT for uptake and release, on smooth muscle cells) with physiological function exists as proven by Ni et al. (2004, 2006, 2008) on rat aorta and superior mesenteric artery. In peripheral tissues SERT is important in that it plays a critical role in regulating the level of activation of 5-HT receptors via modulation of extracellular and intracellular 5-HT concentrations. This endogenous 5-HT release from peripheral arteries can potentiate noradrenaline-induced arterial contraction with hazardous consequences especially in subjects with pre-existing cardiovascular disease.

After the demonstration by Hekmatpanah and Peroutka (1990), showing that 5-HT uptake blockers dose dependently block MDMA induced [^{3}H]-5-HT release, meaning that MDMA interferes particularly with SERT, many others followed, showing that MDMA evokes transporter-mediated release of 5-HT, both in the brain and in the blood, where MDMA mainly targets platelet SERT to increase plasma 5-HT (Yubero-Lahoz et al., 2012).

Experimental data has been shedding some light on our understanding of the MDMA effects on the cardiovascular system in humans. Ketanserin, a 5-$HT_{2A/2C}$ antagonist, prevented the increase of diastolic blood pressure elicited by 1.5 mg/kg MDMA (Liechti et al., 2000). On the other hand, the same study showed that MDMA-elicited increases in systolic blood pressure and heart rate were not affected by ketanserin. Treatment with fluoxetine, a selective 5-HT reuptake inhibitor (SSRI), for at least 5 days attenuated the effects of 1.5 mg/kg MDMA on heart rate, but blood pressure was not affected (Tancer and Johanson, 2007). More recently, it was demonstrated that pindolol, a β- and 5-HT_1 antagonist, prevented MDMA-induced tachycardia but not hypertension or other adverse effects associated with MDMA (Hysek et al., 2010). MDMA and its metabolite 3,4-methylenedioxyamphetamine (MDA) were also shown to be associated with proliferative disease and thickening of cardiac valves, mediated through 5-HT_{2B} receptors (Kaumann and Levy, 2006). In spite of this interesting data, obtained in humans, much needs to be investigated, especially in human vessels at the *in vitro* level, for a better understanding and prevention of the cardiovascular adverse effects of MDMA.

The internal mammary artery has been used for coronary artery bypass grafting because of its convenient diameter, flow capacity, and freedom from atherosclerosis (Koike et al., 1990). It is a model of a resistance artery sensitive to 5-HT (Conti et al., 1990) and also a model of a blood vessel to study the so called chest symptoms, adverse effects attributed to vasoconstrictor drugs, like triptans (Wackenfors et al., 2005). According to molecular and functional studies of Tanaka et al. (2008), 5-HT_{2A} and 5-HT_{1B} receptors are present in smooth muscle cells of this vessel.

Considering the above racionale, the aim of the present study was to evaluate the *in vitro* contractile effects of MDMA in the human internal mammary artery, as well as the real contribution of 5-$HT_{2A/2C}$ receptors (using ketanserin as a selective inhibitor) and of SERT (using fluoxetine as a specific inhibitor) for this activity. In addition, the responsiveness of this blood vessel to 5-HT in the presence of MDMA was also studied.

2. Materials and methods

2.1. Tissue collection

Human internal mammary arteries were collected from patients undergoing coronary artery bypass graft surgery. Mammary arteries were dissected from the internal surface of the anterior chest after sternal incision. Short length samples remaining after the surgery were immediately immersed in a cold, oxygenated Krebs–Henseleit solution (mmol/L: NaCl 118.67; KCl 5.36; $MgSO_4 \cdot 7H_2O$ 0.57; $CaCl_2 \cdot 2H_2O$ 1.90; KH_2PO_4 0.90; $NaHCO_3$ 25.0; glucose 11.1), pH 7.4, and taken immediately to the laboratory. The tissue was provided by the Cardiothoracic Surgery Service of the University Hospital of Coimbra [Serviço de Cirurgia Cardiotoráxica dos Hospitais da Universidade de Coimbra], Portugal, and the local research ethics committee approval was obtained and numbered as: PC-388/08.

2.2. Experimental protocols

Upon arrival at the laboratory, excess fat and connective tissue were trimmed off and the arteries were cut into 3 mm-long rings. Then they were suspended on stainless steel hooks under a passive force of 19.6 mN in 10 mL organ baths (Panlab, Barcelona, Spain) filled with a Krebs–Henseleit solution aerated with 5% CO_2–95% O_2 and maintained at 37 °C. The basal tension in our experiments was that occurring spontaneously after an equilibrium state had been reached in a tissue submitted to the referred 19.6 mN. After an equilibration period of 2 h, with periodic washings of the preparations, isometric contractions of two successive cumulative concentration-response (CR) curves for MDMA (10–1600 μM) and/or 5-HT (0.1–90 μM) were recorded using Panlab isometric transducers (Barcelona, Spain) connected to a PowerLab data acquisition package (ADInstruments, Australia) (Fig. 1). The two CR curves were performed with an interval of time of one hour to avoid tachyphylaxis. Thirty minutes before the second MDMA CR curve, single applications of 1 μM ketanserin (a 5-$HT_{2A/2C}$ antagonist) or 0.1 μM fluoxetine (a SERT specific inhibitor), were made in volumes of 100 μL to the static organ bath and left in contact with the tissue for that period of time (pre-incubation) to guarantee a homogeneous distribution in the biophase prior the performance of the second MDMA CR curve, for receptor and transport characterization. To test the MDMA effect on the 5-HT CR curve, another set of experiments was performed with 400, 800 or 1600 μM MDMA, also added to the organ bath

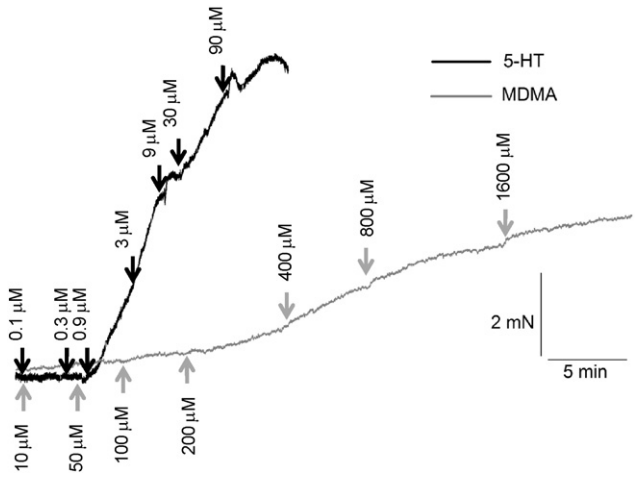

Fig. 1. Representative recording of concentration-response curves for MDMA and 5-HT on the human internal mammary artery.