Check for updates

Methamphetamine increases force of contraction in isolated human atrial preparations through the release of noradrenaline

Joachim Neumann [a], Wilhelm Hußler [a], Karyna Azatsian [a], Britt Hofmann [b], Ulrich Gergs [a,*]

[a] Institute for Pharmacology and Toxicology, Medical Faculty, Martin Luther University Halle-Wittenberg, D-06097 Halle, Germany
[b] Department of Cardiac Surgery, Mid-German Heart Center, University Hospital Halle, D-06097 Halle, Germany

ARTICLE INFO

ABSTRACT

We measured the cardiac contractile effects of the sympathomimetic amphetamine-like drug methamphetamine alone and in the presence of cocaine or propranolol in human atrial preparations. For a more comprehensive analysis, we also examined the effects of methamphetamine in preparations from the left and right atria of mice and, for comparison, analyzed the cardiac effects of amphetamine itself. In human atrial preparations, methamphetamine and amphetamine increased the contractile force, the relaxation rate, and the rate of tension development, and shortened the time to maximum tension and the time to relaxation. Likewise, in mice preparations, methamphetamine and amphetamine increased the contractile force in the left atrium and increased the beating rate in the right atrium. The effect in human atrial preparations started at 1 µM, therefore methamphetamine was less effective and potent than isoproterenol in increasing contractile force. These positive inotropic effects of methamphetamine were greatly attenuated by 10 µM cocaine and abolished by 10 µM propranolol. The inotropic effects of methamphetamine in human atrial preparations were associated with, and are believed to be mediated at least in part by, an increase in the phosphorylation state of the inhibitory subunit of troponin. In conclusion, the sympathomimetic central stimulant drug methamphetamine (as well as amphetamine) increased contractile force and protein phosphorylation, presumably through a release of noradrenaline in isolated human atrial preparations. Thus, methamphetamine acts as an indirect sympathomimetic in the human atrium.

1. Introduction

The organic molecules methamphetamine (N-methylamphetamine, desoxy-ephedrine, N-methyl-1-phenylpropane-2-amine, N,α-dimethyl-phenethylamine) or amphetamine (1-phenylpropane-2- amine, α-methylphenethylamine) can be regarded as phenylpropane amines, but also as phenylethane derivatives (Fig. 1). They show structural similarities to 2-amino-1-(3,4-dihydroxyphenyl)ethanol (norepinephrine or noradrenaline, Fig. 1). Unlike noradrenaline, methamphetamine and amphetamine are often considered to be indirect sympathomimetics: they seem to act primarily through the release of noradrenaline from tissues or cells. Released noradrenaline activates both α- and β-adrenoceptors. This probably explains the central and peripheral effects of methamphetamine. Because methamphetamine has no hydroxyl groups on its benzene ring, methamphetamine is, in contrast to noradrenaline, poorly metabolized in the gastrointestinal tract.

Therefore, methamphetamine can be administered orally. Nevertheless, methamphetamine can be degraded to active metabolites such as amphetamine (Carvalho et al., 2012; Brunton et al., 2018).

In principle, drugs can be applied in many galenic forms in humans. For example, in the case of methamphetamine, the medical literature reports oral route, nasal route, intravenous route, rectal route, through human skin, smoking, and inhalation of the free base of methamphetamine (Chiadmi and Schlatter, 2009; Salocks et al., 2012; Lowder and Caplan, 2020). Even accidental absorption from the vagina with fatal outcome has been described (Jones et al., 2014). Methamphetamine was initially produced by the reduction of ephedrine by Nagai in Japan (Nagai, 1893). A simpler synthesis of methamphetamine from cheaper starting chemicals (from phenylacetone = 1-phenyl-2-propanon and methyl-amine) was described later (German patent 1937): this cheap synthesis by pharmaceutical companies (and illegal laboratories) led to widespread (mis)use of methamphetamine: methamphetamine became
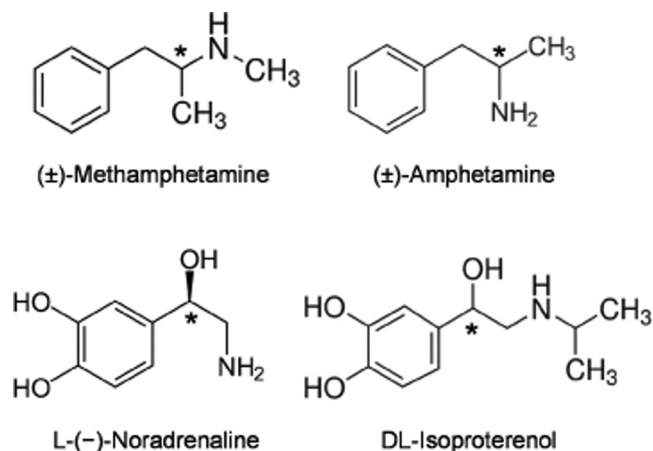

Fig. 1. Structural formulae of methamphetamine, amphetamine, noradrenaline, and isoproterenol. Asterisks indicate chiral carbon atoms.

popular among the public over the following decades, for example, to increase wakefulness, enhance aggressiveness, improve physical endurance and lose weight. Methamphetamine was found to be useful to treat narcolepsy (Mitler et al., 1993). However, there is currently no accepted medical indication for methamphetamine. Interestingly, methamphetamine is a regular metabolite of selegiline, a drug used to treat Parkinson's disease and some forms of depression (Scheinin et al., 1998). 10 mg of peroral selegiline led to serum concentrations of methamphetamine of about 0.1 μM in these volunteers (Scheinin et al., 1998). The illicit use of methamphetamine presents a major health problem: methamphetamine remains one of the most often used illicit central stimulants (Hoffmann et al., 2018; Substance Abuse and Mental Health Services Administration, 2021). Methamphetamine use should be discouraged because it has various detrimental health effects. These detrimental effects occur in the central nervous system but also in peripheral tissues. On the one hand, central deleterious side effects of acute or chronic use of methamphetamine include psychosis, schizophrenia, depression and dependence (Yang et al., 2018). On the other hand, methamphetamine also induces peripheral detrimental effects: methamphetamine may lead to deadly cardiac arrhythmias in drug users (Derlet and Horowitz, 1995; Kaye et al., 2007). Methamphetamine can also induce hypertension and this can result in fibrosis, left ventricular or right ventricular cardiac hypertrophy, cardiac failure or stroke, but also to myocardial infarction and thereby to sudden cardiac death (Wijetunga et al., 2003; Ho et al., 2009; Huang et al., 2016; Lappin et al., 2017; Zamanian et al., 2018; Zhao et al., 2018).

Yet, nearly 425 clinical studies are currently found at www.clinicaltrials.gov (last accessed March 18, 2023), where clinical studies are archived: these past or ongoing clinical studies try, for instance, to clarify the pharmacokinetics of methamphetamine or to test drugs to treat the symptoms of methamphetamine dependence or methamphetamine withdrawal in humans. Hence, we see legitimate reasons to study methamphetamine actions in human cardiac preparations.

To the best of our knowledge, the effects of methamphetamine on contractility in the isolated human atrial cardiac muscle have never been reported. Therefore, we started this project to fill this gap. Moreover, the action of methamphetamine varies between species. Depending on the species studied, methamphetamine may increase force of contraction as a direct or indirect sympathomimetic drug or both, decrease force of contraction or may be without measurable inotropic effect. Hence, we investigated for comparison with the human heart also the mouse heart to get hold on hypothetical species differences. Another reason why we also used mouse cardiac preparations resides in the fact, that in mouse right atrial preparations, we could examine the putative chronotropic effects of methamphetamine independent of any influences of the sympathetic nervous system. In humans, the main active metabolite of ingested methamphetamine (formed by the cytochrome enzyme CYP2D6) is amphetamine. For instance, in a typical study in human volunteers, after oral administration of 10 mg of methamphetamine, approximately 20% of the total molar concentration of methamphetamine amounted to amphetamine, suggesting a relevant metabolism of methamphetamine to amphetamine in healthy humans (Schepers et al., 2003). Hence, we thought it potentially useful to study in the present work methamphetamine and amphetamine in close comparison.

To summarize: in this study, we tested the hypotheses that, firstly, methamphetamine alters cardiac contractility in human atrial preparations and, secondly, that methamphetamine alters force of contraction or beating rate also in mouse atrial preparations and, thirdly, that methamphetamine increases the phosphorylation state of troponin inhibitor in the mammalian heart.

2. Materials and methods

2.1. Contractile studies in mice

In brief, right and left atrial preparations from mice were isolated and mounted in organ baths similar as previously described (Boknik et al., 2019; Gergs et al., 2021; Neumann et al., 2021a; Neumann et al., 2021b). The bathing solution of the organ baths contained 119.8 mM NaCI, 5.4 mM KCI, 1.8 mM $CaCl_2$, 1.05 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 22.6 mM $NaHCO_3$, 0.05 mM Na_2EDTA, 0.28 mM ascorbic acid, and 5.05 mM glucose. The solution was continuously gassed with 95% O_2 and 5% CO_2 and maintained at 37 °C and pH 7.4. Spontaneously beating right atrial preparations from mice were used to study any chronotropic effects.

The drug application was as follows. After equilibration was reached, 1–10 μM methamphetamine was cumulatively added to the mouse atrial preparations. After complete washout of the inotropic or chronotropic effects, rolipram (0.1 μM) was added for 10 min and then, a cumulative concentration-response curve with 1–10 μM methamphetamine was performed again. Finally, 10 μM propranolol was added. In some experiments, in the presence of 10 μM methamphetamine or after addition of 10 μM propranolol we also added without any washout, 10 μM isoproterenol to test whether the efficacy of 10 μM methamphetamine was different from the efficacy of 10 μM isoproterenol. The same procedure was followed using amphetamine instead of methamphetamine.

The investigation conformed to the Guide for the Care and Use of Laboratory Animals as published by the National Research Council (2011). The animals were handled and maintained according to the approved protocols of the Animal Welfare Committee of the University of Halle-Wittenberg, Halle, Germany.

2.2. Contractile studies on human preparations

The contractile studies on human preparations were performed with the same setup and buffer as used for the mouse preparations described in the preceding paragraph (Gergs et al., 2009; Boknik et al., 2019; Gergs et al., 2021). The samples were obtained from ten patients (six male, four female) suffering from three vessel coronary heart disease, aged 49–79 years (mean ± SD: 65 ± 10.8 years) undergoing bypass surgery. Our methods used for atrial contraction studies in human atrial samples have been previously published and were not altered in this study (Gergs et al., 2009). Here, the drug application was as follows: After equilibration was reached, 1–10 μM methamphetamine (or amphetamine) was cumulatively added to the human atrial preparations followed by addition of 10 μM propranolol. In another setup of experiments, a single concentration of 10 μM methamphetamine was applied followed by the following variations. A) Snap-frozen in liquid nitrogen at maximum inotropic effect, b) additional 10 μM propranolol was added before snap-freezing, c) after complete washout of all drugs, cocaine (10 μM) was added for 10 min and then 10 μM methamphetamine, followed by snap-freezing. The frozen preparations were stored at − 80 °C until

biochemical analysis.

This study complies with the Declaration of Helsinki and has been approved by the local ethics committee (hm-bü 04.08.2005). Informed consent was obtained from all patients included in the study.

2.3. Western blotting

The homogenization of the samples, protein measurements, electrophoresis, primary and secondary antibody incubation and quantification were performed following our previously established protocols (Boknik et al., 2018; Boknik et al., 2019; Gergs et al., 2021; Neumann et al., 2021a; Neumann et al., 2021b). Here, we used following primary antibodies: anti phosphorylated troponin inhibitor (P-TnI; #4004, Cell Signaling Technology, Leiden, Netherlands) and as cardiac myocytes-specific loading control anti calsequestrin (CSQ; #ab3516, abcam, Cambridge, UK).

2.4. Data analysis

Data shown are means ± standard error of the mean. Statistical significance was estimated using the analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered to be significant. Following software applications were used to record and analyze the data and to prepare the graphics: LabChart 8
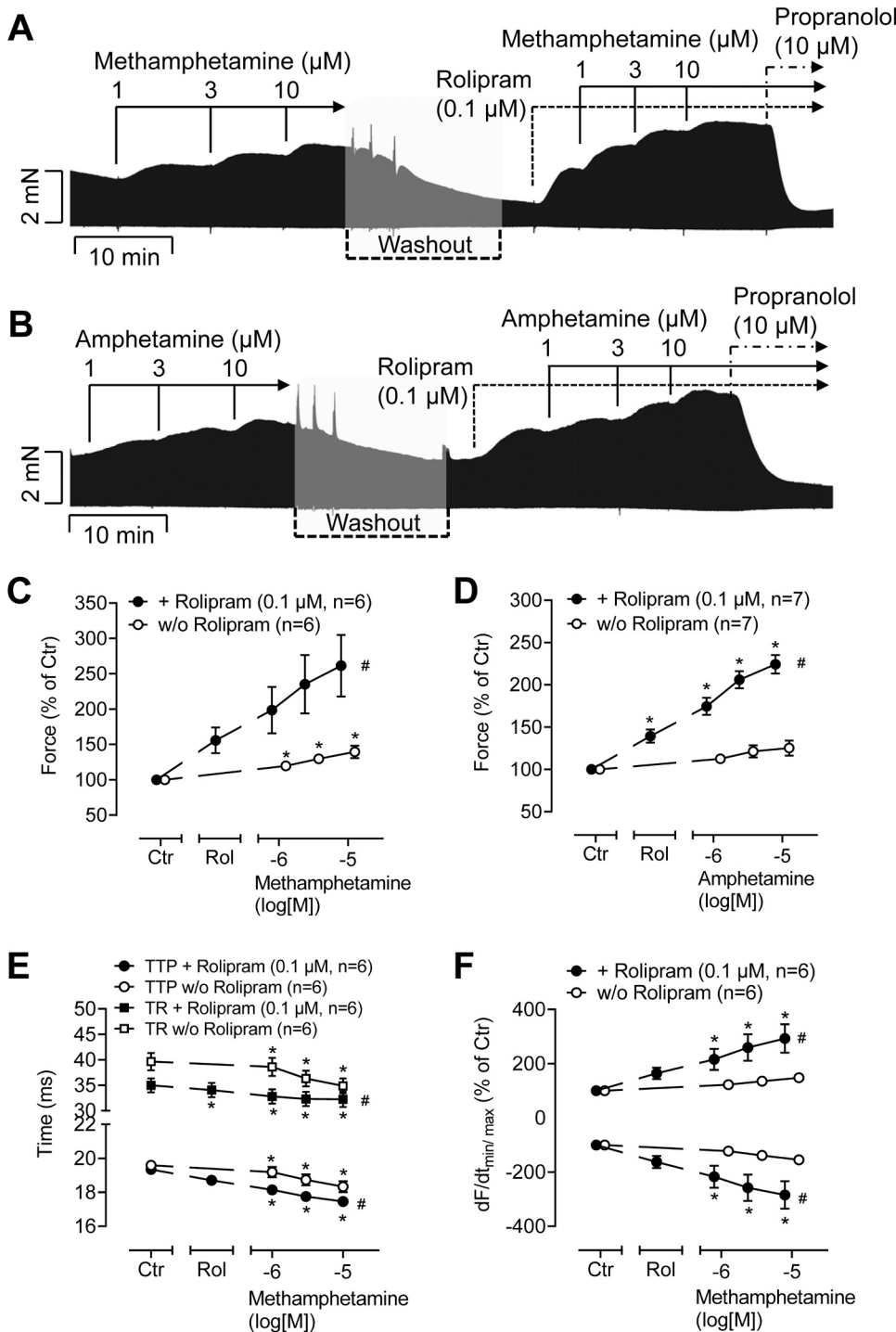

Fig. 2. Effects of methamphetamine and amphetamine on force of contraction in the mouse left atrium. Original recordings of methamphetamine (A) and amphetamine (B) show concentration- and time-dependent positive inotropic effects in isolated electrically driven (1 Hz) left atrial preparations of mice. Pre-stimulation by phosphodiesterase inhibition with rolipram (Rol, 0.1 μM) enhanced the positive inotropic effects of methamphetamine and amphetamine, whereas propranolol (10 μM) antagonized the effects. Horizontal bars: time axis. Vertical bars: developed tension in milli Newton (mN). (C-F) Effects of methamphetamine/amphetamine alone (open circles) or in the presence of 0.1 μM rolipram (closed circles) on left atrial force of contraction (C, methamphetamine; D, amphetamine), on time to peak tension (TTP) or time of relaxation (TR) (E), and on maximum rate of tension development or tension relaxation (dF/dt max and min) (F). *p < 0.05 vs. control (Ctr = pre-drug value), #p < 0.05 vs. w/o rolipram.

(ADInstruments, Spechbach, Germany), ImageQuant 10 (Cytiva, Freiburg, Germany), Prism 9.0 (Graphad Software, San Diego, California, USA).

2.5. Drugs and materials

The drugs isoproterenol-bitartrate salt, propranolol and cocaine were purchased from Sigma-Aldrich (Steinheim, Germany). Racemic methamphetamine hydrochloride and racemic amphetamine were from Logical (Luckenwalde, Germany). All other chemicals were of the highest purity grade commercially available. Deionized water was used throughout the experiments. Stock solutions were prepared fresh daily.

3. Results

3.1. Studies on isolated left atrial preparations from mice

We found that methamphetamine or amphetamine alone just slightly raised the contractile parameters in the mouse left atrial preparations (Fig. 2). They increased the force of contraction, the rate of tension development, and the rate of relaxation and they shortened the time to peak tension as well as the time of relaxation in a concentration- and time-dependent manner (Fig. 2C-F). The time course of the increase in force of contraction was slower after addition of methamphetamine or amphetamine than after addition of isoproterenol (data not shown), suggesting that methamphetamine and amphetamine use a signal transduction system divergent from that used by isoproterenol. The

positive inotropic effects of methamphetamine were eliminated by additionally applied propranolol (Fig. 2), a β-adrenoceptor antagonist. The efficacy of methamphetamine and amphetamine to increase force of contraction could be increased by pre-treatment of the mouse atrial preparations with the phosphodiesterase 4 inhibitor rolipram (0.1 μM). We used rolipram here because phosphodiesterase 4 is the major isoform of the phosphodiesterases in the mouse atrium (Neumann et al., 2021c).

3.2. Studies on isolated right atrial preparations from mice

Cumulatively applied methamphetamine (1–10 μM) increased, like amphetamine, the beating rate in isolated spontaneously beating mouse right atrial preparations in a concentration- and time- dependent manner: this is discernible in the original recordings (Fig. 3A-B) and summarized in Fig. 3C-D. The positive chronotropic effects of methamphetamine and amphetamine were reversed by additionally applied propranolol, suggesting the effects were due to stimulation of β-adrenoceptors (Fig. 3). These negative chronotropic effects of propranolol have been overcome by isoproterenol (data not shown), further supporting the involvement of β-adrenoceptors in the positive chronotropic effects of methamphetamine and amphetamine. Rolipram (0.1 μM) slightly increased the beating rate on its own, consistent with our previous report where we had compared several concentrations of rolipram (Neumann et al., 2021). However, rolipram did not affect the efficacy of methamphetamine and amphetamine to increase the beating rate, suggesting that the β-adrenoceptors in the right atrium were maximal stimulated after addition of 10 μM methamphetamine or amphetamine
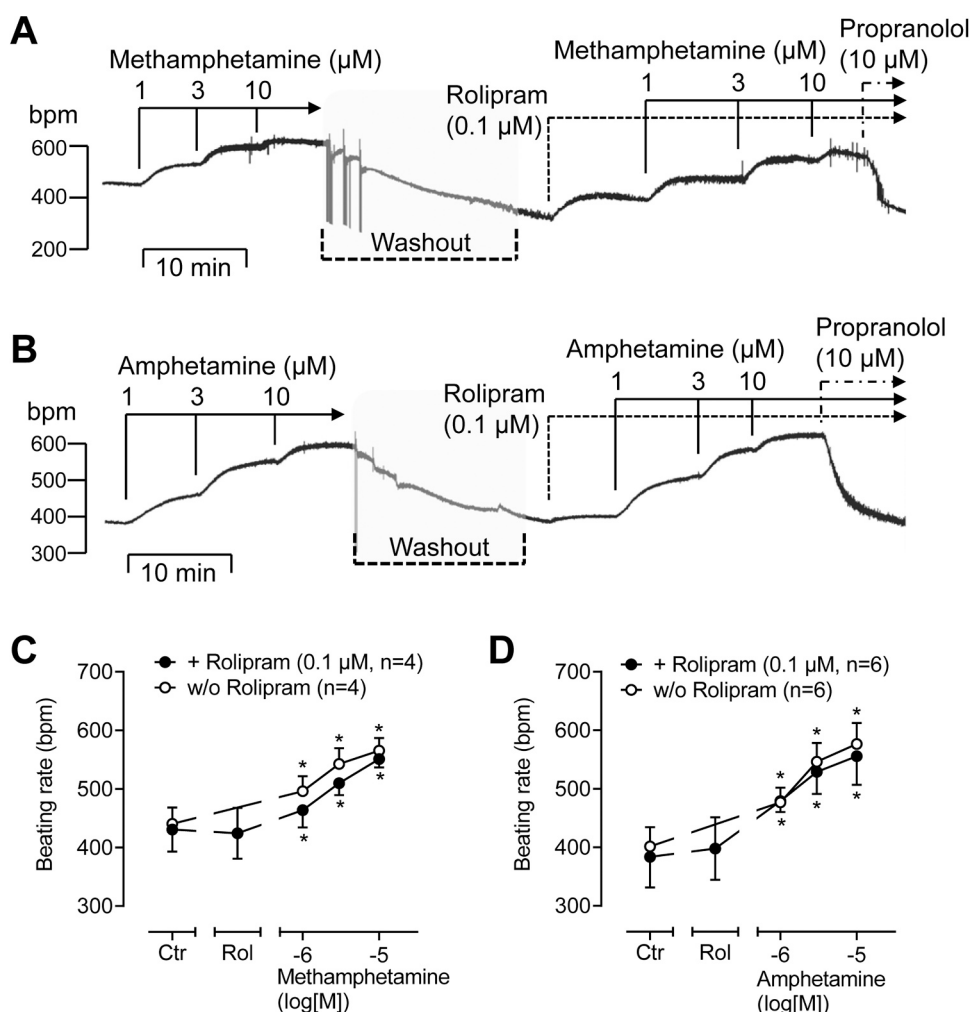

Fig. 3. Effects of methamphetamine and amphetamine on beating rate in the mouse right atrium. Original recordings of methamphetamine (A) and amphetamine (B) show concentration- and time-dependent positive chronotropic effects in isolated spontaneously beating right atrial preparations of mice. Pre-stimulation by phosphodiesterase inhibition with rolipram (Rol, 0.1 μM) had no effect on the positive chronotropic effects of methamphetamine and amphetamine, whereas propranolol (10 μM) antagonized the effects. Horizontal bars: time axis. Vertical bars: beating rate in beats per minute (bpm). Effects of methamphetamine (C) or amphetamine (D) alone (open circles) or in the presence of 0.1 μM rolipram (closed circles) on the beating rate in spontaneously beating right atrial preparations of mice. *p < 0.05 vs. control (Ctr = pre-drug value).

alone under our experimental conditions (Fig. 3).

3.3. Contractile studies in human atrial preparations

Methamphetamine, cumulatively applied (1–10 μM), increased force of contraction in isolated electrically stimulated human right atrial preparations (Fig. 4). The data were summarized in Fig. 4C-F. For comparison, we also studied amphetamine, which increased the force of contraction with similar potency and efficacy as methamphetamine (Fig. 4G-I). In the human preparations, it was not necessary to use a phosphodiesterase inhibitor to further enhance the inotropic effects of methamphetamine or amphetamine.

Like for amphetamine, the inotropic effect of methamphetamine took more time (10–20 min) to reach the maximum than the positive inotropic effect of isoproterenol (which plateaued within two minutes) on the very same human muscle preparation, suggesting different signal transduction mechanisms of methamphetamine and isoproterenol in the human atrium (data not shown). Furthermore, the positive inotropic effects of 10 μM methamphetamine could be blocked by 10 μM propranolol (Fig. 4A and F). These positive inotropic effects were accompanied by increased rates of tension development and rates of relaxation (Fig. 4D) and shortened time to peak tension (Fig. 4E). Finally, we wanted to know whether cocaine, an inhibitor of mono amine transporters, can block inotropic responses to the drugs of interest. Indeed, any inotropic effects were blocked by previously given cocaine (10 μM) (Fig. 4B and F).

3.4. Studies on protein phosphorylation in human atrial preparations

Fittingly, in contracting human atrial preparations, methamphetamine and amphetamine increased the TnI phosphorylation (Fig. 5 and supplementary Figure 1). These effects, like the contractile effects, were reduced by additionally applied propranolol (Fig. 5 and supplementary Figure 1).

4. Discussion

Firstly, methamphetamine alone slightly stimulated force of contraction in isolated mouse left atrial preparations, but after pre-stimulation by phosphodiesterase inhibition, the positive inotropic effect of methamphetamine was much pronounced. A pronounced positive chronotropic effect of methamphetamine alone was noted in spontaneously beating mouse right atrial preparations. Secondly, methamphetamine alone exerted strong positive inotropic effects in human atrial preparations. Thirdly, methamphetamine raised the phosphorylation state of TnI in isolated human atrial preparations. All these effects were also noted for amphetamine.

Increased phosphorylation of TnI (Kentish et al., 2001) as well as increased phosphorylation of, for example, phospholamban (Tada et al., 1976) accelerate myofibrillar relaxation and can explain why methamphetamine reduces the time of relaxation and increases the rate of relaxation in human atrial preparations: phosphorylated phospholamban increases the rate at which Ca^{2+} is pumped from the cytosol into the sarcoplasmic reticulum and phosphorylated TnI decreases myofibrillar Ca^{2+} sensitivity. As a result, Ca^{2+} dissociates faster from troponin C and Ca^{2+} concentrations decline more rapidly near the myofilaments and thus myofilaments relax more rapidly (Tada et al., 1976; Kentish et al., 2001; Hamstra et al., 2020).

Under our experimental conditions, methamphetamine probably did not directly stimulate β-adrenoceptors in the human atrium and thus increased force of contraction: this conclusion is based on the observation that the contractile effects of methamphetamine were absent in the presence of cocaine in the human atrium. Cocaine inhibits the entrance of methamphetamine (as well as of amphetamine) into cells from which methamphetamine can release noradrenaline.

We explain the inotropic and biochemical effects of

methamphetamine in the human atrium as follows. Methamphetamine releases noradrenaline from cardiac stores. Released noradrenaline stimulates β-adrenoceptors on the contracting human atrial myocardium (as well as on murine left atrial preparations). This conclusion is based on the finding that the positive inotropic effect of methamphetamine was reversed by propranolol, a β-adrenoceptor antagonist in the human atrium.

In neonatal rat cardiomyocytes, 100 μM and higher concentrations of methamphetamine reduced Ca^{2+} transients and increased the beating rate (Sugimoto et al., 2009). The authors argued that their choice of the high methamphetamine concentration was relevant because methamphetamine users inject about 10 g methamphetamine daily, which translated to a plasma concentration of about 1 mM methamphetamine (Cohen, 1975). The increase in beating rate by 500 μM methamphetamine of neonatal rat cardiomyocytes was blocked by 10 nM nifedipine (a L-type calcium channel (LTCC) antagonist) but not by 10 μM ruthenium red (a ryanodine receptor antagonist) or 10 μM cyclopiazonic acid (a sarcoplasmic calcium ATPase inhibitor) or propranolol or prazosin suggesting to the authors that methamphetamine directly stimulated the LTCC (Sugimoto et al., 2009). This contradicts apparently our findings but can be explained by different methods employed: our samples are from adult mice and not neonatal rats. Hence, species and developmental stage differ.

Although our data in atrial mouse preparations are novel, they seem to be contradictory compared to findings by others in isolated perfused mouse hearts or isolated ventricular mouse cardiomyocytes. For instance, in isolated retrogradely perfused spontaneously beating adult wild type mouse hearts, methamphetamine from 10 to 1000 μM did not alter the beating rate but diminished the first derivative of developed left ventricular pressure at 100 μM and 1000 μM, reaching a plateau after 15 min. And the effects were reversible after washout (Turdi et al., 2009). Methamphetamine blunted the inotropic response to isoproterenol, suggesting a functional antagonism between methamphetamine and isoproterenol on the β-adrenoceptor (Turdi et al., 2009). Interestingly, 100 μM and 1000 μM acutely applied methamphetamine reduced maximal lengthening of isolated electrically driven mouse ventricular cardiomyocytes, arguing for a direct impairment of cardiac function (Turdi et al., 2009). Their functional data (unchanged relaxation rates) would argue against an action of methamphetamine on phospholamban or TnI phosphorylation. The expression of phospholamban as protein was unaltered whereas methamphetamine (30 min, 1000 μM, adult mouse cardiomyocytes, not contracting) elevated the expression of sodium calcium exchanger in these cells (Turdi et al., 2009). The authors argued that this might lead to a loss of Ca^{2+} and thus would explain why they noted a negative inotropic effect of methamphetamine (Turdi et al., 2009). In addition, the authors noted altered protein carbonylation and speculated that methamphetamine might have interfered with the function of the mitochondria which could also have had contributed to the negative inotropic effect in adult mouse preparations (Turdi et al., 2009).

Although our data are contrary to those of Turdi et al. 2009, they extend the findings to isolated isometrically contracting left atria and isolated spontaneously beating right atria; in contrast to whole mouse hearts (where auxotonic contractions prevail). They reported on an antiadrenergic effect at high concentrations of methamphetamine that we did not find in atrial preparations of mice and humans. However, our data agree with findings of others in frogs: in the bullfrog isolated atrium or ventricle, methamphetamine initially increased force of contraction. This positive inotropic effect of methamphetamine in the bullfrog heart was blocked by pre-treatment with a β-adrenoceptor antagonist or cocaine (Urabe, 1982). Moreover, we argue that in human cardiac preparations, which are the relevant tissue samples from a clinical standpoint, methamphetamine clearly increases the force of contraction under our experimental conditions.

As concerns drug targets for methamphetamine, a vast number of studies are found in the literature. We would like to mention just a few
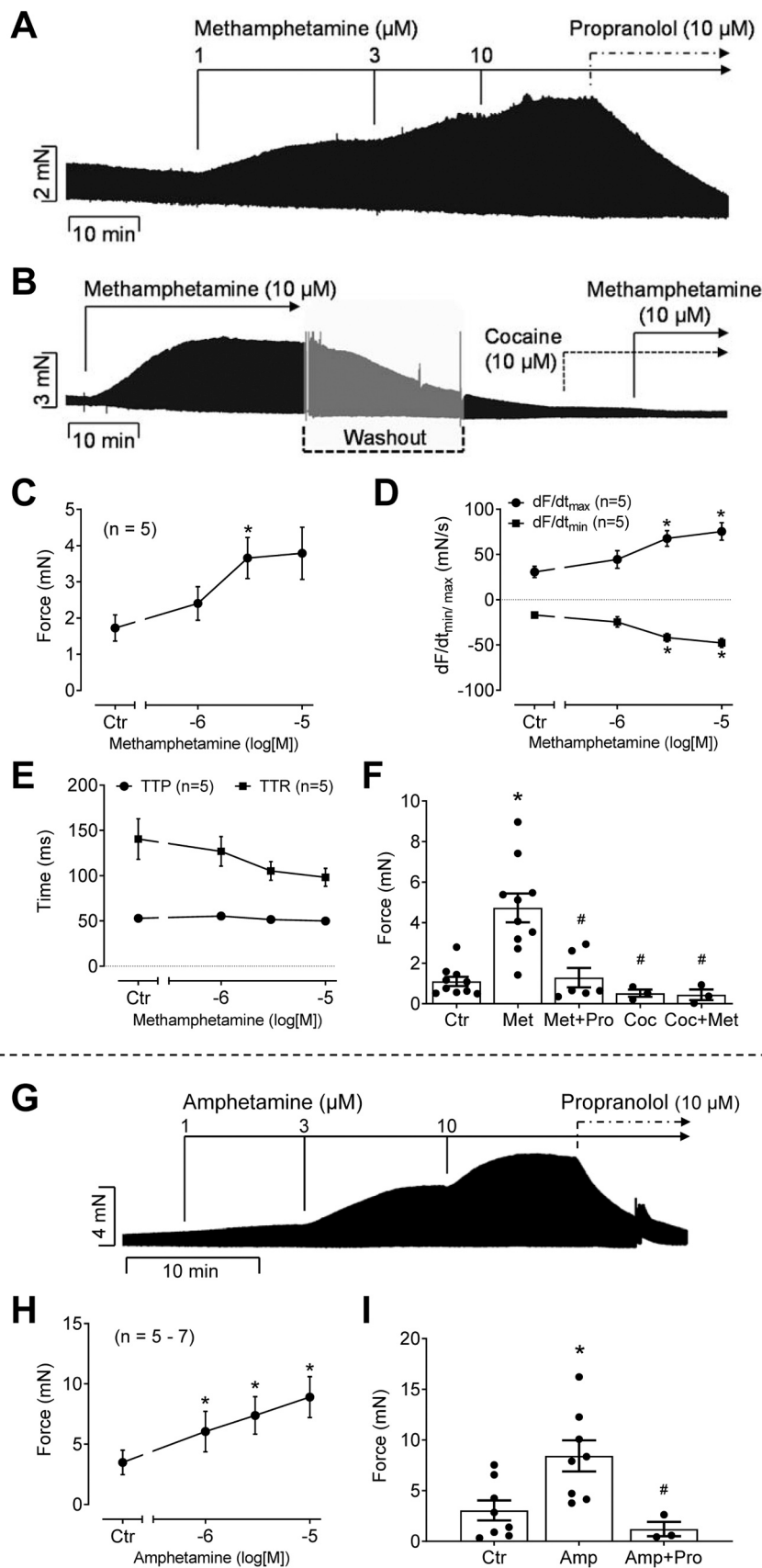
Fig. 4. Effect of methamphetamine on force of contraction in the human atrium. Original recordings of methamphetamine (A-B) or amphetamine (G) show concentration- and time-dependent positive inotropic effects in isolated electrically driven (1 Hz) right atrial preparations from human hearts. Propranolol (10 μM) as well as cocaine (10 μM) antagonized the positive inotropic effects. Horizontal bars: time axis. Vertical bars: developed tension in milli Newton (mN). Concentration-dependent effects of methamphetamine on (C) force of contraction (H, amphetamine for comparison), on (D) rate of tension development (dF/dtmax) or rate of tension relaxation (dF/dtmin) and on (E) time to peak tension (TTP) and time of relaxation (TR) in isolated electrically driven (1 Hz) right atrial strips from human hearts. *p < 0.05 vs. control (Ctr = pre-drug value). (F) Propranolol (Pro, 10 μM) as well as cocaine (Coc, 10 μM) antagonized the positive inotropic effect of 10 μM methamphetamine (Met). (I) For comparison, the antagonistic effect of 10 μM propranolol on the positive inotropic effect of 10 μM amphetamine (Amp) is shown. Numbers of experiments are given in the graphs. *p < 0.05 vs. Ctr, #p < 0.05 vs. Met or Amp.

A

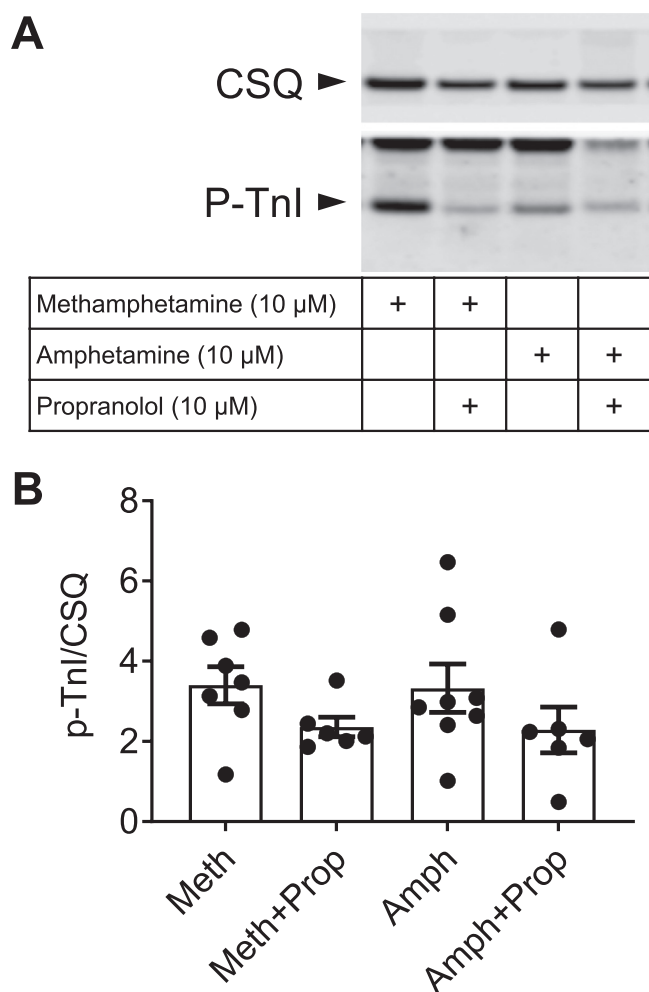| Methamphetamine (10 µM)	+	+		
Amphetamine (10 µM)			+	+
Propranolol (10 µM)		+		+

B

Fig. 5. Methamphetamine and amphetamine increase phosphorylation in isolated human atrial preparations. Effect of 10 µM methamphetamine (Meth) or 10 µM amphetamine (Amph) on the phosphorylation state of the inhibitory subunit of troponin (P-TnI) in isolated electrically stimulated right atrial preparations from human hearts in the absence and presence of 10 µM propranolol (Prop). An exemplary Western blot is shown in (A) and the scatter plot in (B) summarizes the data. As loading control, we assessed the protein expression of cardiac calsequestrin (CSQ) by cutting the lanes of the blots and incubating the lower and upper halves with different primary antibodies. The P-TnI signal was normalized to CSQ (B). The complete Western blots are presented in the supplementary Figure 1.

examples here that are relevant to the present work. Hence, inhibitors of the VMAT2 function (Meyer et al., 2013) may interact with the methamphetamine-mediated monoamine release. Methamphetamine can inhibit the action of MAO-A and MAO-B (Sulzer et al., 2005). Methamphetamine can also slow down its own metabolism by impeding the activity of cytochrome CYP2D6 (Wu et al., 1997). Down-stream targets of methamphetamine, at least in the brain, include CREB-phosphorylation (Krasnova et al., 2016). This may result from β-adrenoceptor stimulation followed by cAMP increase and subsequent PKA activation (Krasnova et al., 2016; Shaerzadeh et al., 2018). Methamphetamine may also inhibit noradrenaline transporters in cell surface membranes (Fleckenstein et al., 2007).

One can ask why the effects of methamphetamine are much larger in the human heart than in the mouse heart. Part of the explanation may lie in the observation that the noradrenaline content in the human heart is about 300 ng/g tissue (Neubauer and Christensen, 1976), while about 0.6 ng/g has been reported in the heart of mice (Sharman et al., 1962). We assume that methamphetamine releases about 10% of the

noradrenaline in the hearts of mice and humans. Then, about 500 times more noradrenaline would be released in the human heart than in the mouse heart, and this could easily lead to a more effective increase in the force of contraction in the human compared to the mouse.

Our data in the human atrium have limitations: we cannot know on which cell type in the atrium methamphetamine acts. Methamphetamine should be studied in future work in freshly isolated human atrial myocytes. Moreover, due to lack of availability, we were not able to study human ventricular samples. The effects we report here in atrial preparations may be due to direct effects of methamphetamine on cardiomyocytes or to neuronal release of noradrenaline from ganglia, which is probably the main mechanism, but this is currently speculative. Perhaps we should emphasize that our data comparing the force of contraction in the human and mouse atrium are another example of species differences in cardiac function. For example, previously we reported: functional H_2 histamine or $5-HT_4$ serotonin receptors are absent in the mouse heart but are present in the human atrium. Here, we extend this concept by presenting evidence for interspecies differences not only with regard to receptor function, but probably also to storage and/or release of noradrenaline in the mouse and human heart. Our data underscore the importance of using human cardiac tissue in order to understand human cardiac pharmacology.

Clinical relevance: "recreational drugs" such as methamphetamine, can lead to intoxication and death. One reason for a fatal course of intoxication with methamphetamine could be cardiac arrhythmias. One manifestation of cardiac arrhythmias is tachycardia. In vivo, coronary constriction by released noradrenaline acting on alpha-adrenergic receptors in the vessel wall could contribute to cardiac arrhythmias. Furthermore, our data suggest that methamphetamine intoxications can be treated with propranolol, at least in terms of effects on the atrium and contractility. If a typical concentration of 10 mg methamphetamine is administered orally, increases in systolic and diastolic blood pressure and heart rate have been measured in human volunteers compared to placebo (Schepers et al., 2003). Increased cardiac contractility in healthy volunteers has also been observed with inhaled methamphetamine using non-invasive methods (Perez-Reyes et al., 1991).

One can ask: what are the highest concentrations of methamphetamine reached in the human body? Some information might be gained from intoxications, which should represent the upper concentration limits in humans. Values up to 1 mM have been reported there (Cohen, 1975). Thus, our concentrations are clinically relevant. A caveat is in order, as we tested only acute effects of methamphetamine. Deaths have been reported at methamphetamine blood levels of approximately 10 µM (Jones et al., 2014). Hence, it is difficult to define a "therapeutic window" for methamphetamine because "therapeutic" and deathly plasma concentrations overlap.

However, lower methamphetamine concentrations were measured at lower doses. For instance, when a single retarded oral form of 10 mg methamphetamine was administered, the peak plasma concentrations varied between 0.1 and 0.22 µM methamphetamine (Schepers et al., 2003). Assuming linear kinetics, one would expect that oral administration of 100 mg methamphetamine would result in 1–2.2 µM methamphetamine with a time to peak of about 3 h, depending on the galenic form, and a plasma half-life of about 10 h (discussed in table 1 from (Schepers et al., 2003)). The highest methamphetamine concentrations that patients survived were in one case reported about 5 µM, 7 µM, or 19 µM, which suggests to us that the highest methamphetamine concentration, 10 µM, that we used in the present study, is clinically relevant (Nakashima et al., 2003; Irvine et al., 2006; Uekusa et al., 2013).

When methamphetamine is administered chronically, other effects may predominate. There is a wealth of clinical data, mostly case reports, indicating that prolonged use of methamphetamine not only leads to cardiac arrhythmias, as discussed above, but also to heart failure manifesting e.g. by pulmonary edema. In such chronic methamphetamine intoxications, other mechanisms have been convincingly described in animal studies.

Extrapolating to the human heart in patients, one might be tempted to speculate that direct contractile effects of methamphetamine may occur, albeit at the upper limit of the therapeutic window, where intoxication already occurs in some individuals. On the other hand, it could be argued that tachycardia in methamphetamine users could also be due to direct effects on the heart and not solely to indirect effects, initiated in the brain via the sympathetic nervous system, and that such tachycardia should be treatable by application of β-adrenoceptor antagonists. In this case, β-adrenoceptor blockers would be useful due to their direct action on cardiac β-adrenoceptors and not alone due to their action on the central nervous system. As mentioned in the Introduction, methamphetamine is metabolized by CYP2D6 and to a minor extent by CYP3A4. This cytochrome is well known to be subject to polymorphisms. There are poor and rapid metabolizers in connection with CYP2D6. Indeed, there are clinical data that in poor metabolizers, the area under the curve is higher for methamphetamine than in rapid metabolizers (Scheinin et al., 1998). Thus, one could predict that in poor metabolizers, cardiac side effects of methamphetamine should be more frequent and last longer. Moreover, one may suggest that users of methamphetamine who also take inhibitors of CYP2D6 (for instance fluoxetine) are prone to cardiac side effects of methamphetamine. For instance, CYP2D6 inhibitors like ritonavir or bupropion, elevate in patients plasma levels of methamphetamine (Hales et al., 2000; Newton et al., 2005).

In summary, we have presented evidence that methamphetamine can increase force of contraction in the isolated human (and to a lesser extent mouse) atrium. We therefore hypothesize that drugs blocking β-adrenoceptors (e.g. propranolol) should be tried for methamphetamine intoxication.

CRediT authorship contribution statement

Conceptualization, **J.N., U.G.**; Conduction of experiments. **W.H., K.A.**; Materials. **B.H.**; data analysis. **W.H., K.A., U.G.**; visualization. **U.G.**; original draft preparation, **J.N.**; review and editing. **J.N., W.H., K.A., B.H., U.G**.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

Consent for publication

All authors approved the final manuscript for publication.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxlet.2023.06.012.

References

Boknik, P., Drzewiecki, K., Eskandar, J., Gergs, U., Grote-Wessels, S., Fabritz, L., Kirchhof, P., Müller, F.U., Stümpel, F., Schmitz, W., Zimmermann, N., Kirchhefer, U., Neumann, J., 2018. Phenotyping of mice with heart specific overexpression of A2A-

adenosine receptors: evidence for cardioprotective effects of A2A-adenosine receptors. Front. Pharmacol. 9, 13. https://doi.org/10.3389/fphar.2018.00013.

Boknik, P., Drzewiecki, K., Eskandar, J., Gergs, U., Hofmann, B., Treede, H., Grote-Wessels, S., Fabritz, L., Kirchhof, P., Fortmüller, L., Müller, F.U., Schmitz, W., Zimmermann, N., Kirchhefer, U., Neumann, J., 2019. Evidence for arrhythmogenic effects of A2A-adenosine receptors. Front. Pharmacol. 10, 1051. https://doi.org/10.3389/fphar.2019.01051.

Brunton, L.L., Gilman, A., Goodman, L.S., 2018. Goodman & Gilman's the pharmacological basis of therapeutics: The Pharmacological Basis of Therapeutics, thirteenth ed. McGraw Hill Medical,, New York, p. 1419.

Carvalho, M., Carmo, H., Costa, V.M., Capela, J.P., Pontes, H., Remião, F., Carvalho, F., Bastos, M.D.L., 2012. Toxicity of amphetamines: an update. Arch. Toxicol. 86 (8), 1167–1231. https://doi.org/10.1007/s00204-012-0815-5.

Chiadmi, F., Schlatter, J., 2009. Crystal meth: une forme de méthamphétamine (Paris, France: 1983). Presse Med. 38 (1), 63–67. https://doi.org/10.1016/j.lpm.2008.07.017.

Cohen, S., 1975. Amphetamine abuse. JAMA 231 (4), 414–415.

Derlet, R.W., Horowitz, B.Z., 1995. Cardiotoxic drugs. Emerg. Med. Clin. North Am. 13 (4), 771–791.

Fleckenstein, A.E., Volz, T.J., Riddle, E.L., Gibb, J.W., Hanson, G.R., 2007. New insights into the mechanism of action of amphetamines. Annu. Rev. Pharmacol. Toxicol. 47, 681–698. https://doi.org/10.1146/annurev.pharmtox.47.120505.105140.

Gergs, U., Boknik, P., Schmitz, W., Simm, A., Silber, R.-E., Neumann, J., 2009. A positive inotropic effect of adenosine in cardiac preparations of right atria from diseased human hearts. Naunyn-Schmiede 'S. Arch. Pharmacol. 379 (5), 533–540. https://doi.org/10.1007/s00210-008-0374-8.

Gergs, U., Büxel, M.L., Bresinsky, M., Kirchhefer, U., Fehse, C., Höring, C., Hofmann, B., Marušáková, M., Čináková, A., Schwarz, R., Pockes, S., Neumann, J., 2021. Cardiac effects of novel histamine H2 receptor agonists. J. Pharmacol. Exp. Ther. 379 (3), 223–234. https://doi.org/10.1124/jpet.121.000822.

Hales, G., Roth, N., Smith, D., 2000. Possible fatal interaction between protease inhibitors and methamphetamine. Antivir. Ther. 5 (1), 19.

Hamstra, S.I., Whitley, K.C., Baranowski, R.W., Kurgan, N., Braun, J.L., Messner, H.N., Fajardo, V.A., 2020. The role of phospholamban and GSK3 in regulating rodent cardiac SERCA function. American journal of physiology. Cell Physiol. 319 (4), C694–C699. https://doi.org/10.1152/ajpcell.00318.2020.

Ho, E.L., Josephson, S.A., Lee, H.S., Smith, W.S., 2009. Cerebrovascular complications of methamphetamine abuse. Neurocritical care 10 (3), 295–305. https://doi.org/10.1007/s12028-008-9177-5.

Hoffmann, L., Schumann, N., Richter, M., 2018. Methamphetaminkonsum in mitteldeutschland: eine qualitative studie zu konsumentengruppen und -motiven aus expertensicht. Psychother., Psychosom., Med. Psychol. 68 (8), 329–336. https://doi.org/10.1055/s-0043-118655.

Huang, M.-C., Yang, S.-Y., Lin, S.-K., Chen, K.-Y., Chen, Y.-Y., Kuo, C.-J., Hung, Y.-N., 2016. Risk of cardiovascular diseases and stroke events in methamphetamine users: a 10-year follow-up study. J. Clin. Psychiatry 77 (10), 1396–1403. https://doi.org/10.4088/JCP.15m09872.

Irvine, R.J., Keane, M., Felgate, P., McCann, U.D., Callaghan, P.D., White, J.M., 2006. Plasma drug concentrations and physiological measures in 'dance party' participants. Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol. 31 (2), 424–430. https://doi.org/10.1038/sj.npp.1300896.

Jones, P., Mutsvunguma, R., Prahlow, J.A., 2014. Accidental death via intravaginal absorption of methamphetamine. Forensic Sci., Med., Pathol. 10 (2), 234–238. https://doi.org/10.1007/s12024-014-9538-7.

Kaye, S., McKetin, R., Duflou, J., Darke, S., 2007. Methamphetamine and cardiovascular pathology: a review of the evidence. Addict. (Abingdon, Engl.) 102 (8), 1204–1211. https://doi.org/10.1111/j.1360-0443.2007.01874.x.

Kentish, J.C., McCloskey, D.T., Layland, J., Palmer, S., Leiden, J.M., Martin, A.F., Solaro, R.J., 2001. Phosphorylation of troponin I by protein kinase A accelerates relaxation and crossbridge cycle kinetics in mouse ventricular muscle. Circ. Res. 88 (10), 1059–1065. https://doi.org/10.1161/hh1001.091640.

Krasnova, I.N., Justinova, Z., Cadet, J.L., 2016. Methamphetamine addiction: involvement of CREB and neuroinflammatory signaling pathways. Psychopharmacology 233 (10), 1945–1962. https://doi.org/10.1007/s00213-016-4235-8.

Lappin, J.M., Darke, S., Farrell, M., 2017. Stroke and methamphetamine use in young adults: a review. J. Neurol., Neurosurg., Psychiatry 88 (12), 1079–1091. https://doi.org/10.1136/jnnp-2017-316071.

Lowder, K.N., Caplan, J.P., 2020. Waterlining of methamphetamine. Psychosomatics 61 (1), 103–104. https://doi.org/10.1016/j.psym.2019.09.006.

Meyer, A.C., Neugebauer, N.M., Zheng, G., Crooks, P.A., Dwoskin, L.P., Bardo, M.T., 2013. Effects of VMAT2 inhibitors lobeline and GZ-793A on methamphetamine-induced changes in dopamine release, metabolism and synthesis in vivo. J. Neurochem. 127 (2), 187–198. https://doi.org/10.1111/jnc.12373.

Mitler, M.M., Hajdukovic, R., Erman, M.K., 1993. Treatment of narcolepsy with methamphetamine. Sleep 16 (4), 306–317.

Nagai, N., 1893. Kanyaku Maou Seibun Kenkyu Seiseki (Results of the Research of the Contents of Chinese Medicine Maou). Yakugaku Zasshi. J. Pharm. Soc. Jpn. 139, 901–933.

Nakashima, K., Kaddoumi, A., Ishida, Y., Itoh, T., Taki, K., 2003. Determination of methamphetamine and amphetamine in abusers' plasma and hair samples with HPLC-FL. Biomed. Chromatogr.: BMC 17 (7), 471–476. https://doi.org/10.1002/bmc.278.

Neubauer, B., Christensen, N.J., 1976. Norepinephrine, epinephrine, and dopamine contents of the cardiovascular system in long-term diabetics. Diabetes 25 (1), 6, 10. 10.2337/diab.25.1.6.

Neumann, J., Binter, M.B., Fehse, C., Marušáková, M., Büxel, M.L., Kirchhefer, U., Hofmann, B., Gergs, U., 2021a. Amitriptyline functionally antagonizes cardiac H2 histamine receptors in transgenic mice and human atria. Naunyn-Schmiede 'S. Arch. Pharmacol. 394 (6), 1251–1262. https://doi.org/10.1007/s00210-021-02065-7.

Neumann, J., Grobe, J.M., Weisgut, J., Schwelberger, H.G., Fogel, W.A., Marušáková, M., Wache, H., Bähre, H., Buchwalow, I.B., Dhein, S., Hofmann, B., Kirchhefer, U., Gergs, U., 2021b. Histamine can be formed and degraded in the human and mouse heart. Front. Pharmacol. 12, 582916 https://doi.org/10.3389/fphar.2021.582916.

Neumann, J., Voss, R., Laufs, U., Werner, C., Gergs, U., 2021c. Phosphodiesterases 2, 3 and 4 can decrease cardiac effects of H2-histamine-receptor activation in isolated atria of transgenic mice. Naunyn-Schmiede 'S. Arch. Pharmacol. 394 (6), 1215–1229. https://doi.org/10.1007/s00210-021-02052-y.

Newton, T.F., Roache, J.D., La Garza, R., de, Fong, T., Wallace, C.L., Li, S.-H., Elkashef, A., Chiang, N., Kahn, R., 2005. Safety of intravenous methamphetamine administration during treatment with bupropion. Psychopharmacology 182 (3), 426–435. https://doi.org/10.1007/s00213-005-0102-8.

Perez-Reyes, M., White, W.R., McDonald, S.A., Hill, J.M., Jeffcoat, A.R., Cook, C.E., 1991. Clinical effects of methamphetamine vapor inhalation. Life Sci. 49 (13), 953–959. https://doi.org/10.1016/0024-3205(91)90078-p.

Salocks, C.B., Hui, X., Lamel, S., Qiao, P., Sanborn, J.R., Maibach, H.I., 2012. Dermal exposure to methamphetamine hydrochloride contaminated residential surfaces: surface pH values, volatility, and in vitro human skin. Food Chem. Toxicol.: Int. J. Publ. Br. Ind. Biol. Res. Assoc. 50 (12), 4436–4440. https://doi.org/10.1016/j.fct.2012.08.002.

Scheinin, H., Anttila, M., Dahl, M.-L., Karnani, H., Nyman, L., Taavitsainen, P., Pelkonen, O., Bertilsson, L., 1998. CYP2D6 polymorphism is not crucial for the disposition of selegiline*. Clin. Pharm. Ther. 64 (4), 402–411. https://doi.org/10.1016/S0009-9236(98)90071-6.

Schepers, R.J.F., Oyler, J.M., Joseph, R.E., Cone, E.J., Moolchan, E.T., Huestis, M.A., 2003. Methamphetamine and amphetamine pharmacokinetics in oral fluid and plasma after controlled oral methamphetamine administration to human volunteers. Clin. Chem. 49 (1), 121–132. https://doi.org/10.1373/49.1.121.

Shaerzadeh, F., Streit, W.J., Heysieattalab, S., Khoshbouei, H., 2018. Methamphetamine neurotoxicity, microglia, and neuroinflammation. J. Neuroinflamm. 15 (1), 341. https://doi.org/10.1186/s12974-018-1385-0.

SHARMAN, D.F., VANOV, S., VOGT, M., 1962. Noradrenaline content in the heart and spleen of the mouse under normal conditions and after administration of some drugs. Br. J. Pharmacol. Chemother. 19, 527–533. https://doi.org/10.1111/j.1476-5381.1962.tb01458.x.

Substance Abuse and Mental Health Services Administration, 2021. Key substance use and mental health indicators in the United States: Results from the 2020 National Survey on Drug Use and Health (HHS Publication No. PEP21–07-01–003, NSDUH Series H-56). Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration. ⟨https://www.samhsa.gov/data/⟩. Accessed 12 December 2022.

Sugimoto, K., Okamura, K., Tanaka, H., Takashima, S., Ochi, H., Yamamoto, T., Matoba, R., 2009. Methamphetamine directly accelerates beating rate in cardiomyocytes by increasing Ca(2+) entry via L-type Ca(2+) channel. Biochem. Biophys. Res. Commun. 390 (4), 1214–1220. https://doi.org/10.1016/j.bbrc.2009.10.124.

Sulzer, D., Sonders, M.S., Poulsen, N.W., Galli, A., 2005. Mechanisms of neurotransmitter release by amphetamines: a review. Prog. Neurobiol. 75 (6), 406–433. https://doi.org/10.1016/j.pneurobio.2005.04.003.

Tada, M., Kirchberger, M.A., Katz, A.M., 1976. Regulation of calcium transport in cardiac sarcoplasmic reticulum by cyclic AMP-dependent protein kinase. Recent Adv. Stud. Card. Struct. Metab. 9, 225–239.

Turdi, S., Schamber, R.M., Roe, N.D., Chew, H.G., Culver, B., Ren, J., 2009. Acute methamphetamine exposure inhibits cardiac contractile function. Toxicol. Lett. 189 (2), 152–158. https://doi.org/10.1016/j.toxlet.2009.05.015.

Uekusa, K., Hayashida, M., Saito, N., Mashiko, K., Hara, K., Waters, B., Ohno, Y., 2013. Methamphetamine and amphetamine concentrations in survivors of body-packer syndrome in Japan. Forensic Sci. Int. 227 (1–3), 45–47. https://doi.org/10.1016/j.forsciint.2012.10.005.

Urabe, M., 1982. Inhibitory mechanism of methamphetamine in the isolated myocardium of bullfrog. Arch. Int. De. Pharmacodyn. Et. De. Ther. 257 (2), 239–254.

Wijetunga, M., Seto, T., Lindsay, J., Schatz, I., 2003. Crystal methamphetamine-associated cardiomyopathy: tip of the iceberg? J. Toxicol. Clin. Toxicol. 41 (7), 981–986. https://doi.org/10.1081/clt-120026521.

Wu, D., Otton, S.V., Inaba, T., Kalow, W., Sellers, E.M., 1997. Interactions of amphetamine analogs with human liver CYP2D6. Biochem. Pharmacol. 53 (11), 1605–1612. https://doi.org/10.1016/s0006-2952(97)00014-2.

Yang, X., Wang, Y., Li, Q., Zhong, Y., Chen, L., Du, Y., He, J., Liao, L., Xiong, K., Yi, C.-X., Yan, J., 2018. The main molecular mechanisms underlying methamphetamine-induced neurotoxicity and implications for pharmacological treatment. Front. Mol. Neurosci. 11, 186. https://doi.org/10.3389/fnmol.2018.00186.

Zamanian, R.T., Hedlin, H., Greuenwald, P., Wilson, D.M., Segal, J.I., Jorden, M., Kudelko, K., Liu, J., Hsi, A., Rupp, A., Sweatt, A.J., Tuder, R., Berry, G.J., Rabinovitch, M., Doyle, R.L., Jesus Perez, V., de, Kawut, S.M., 2018. Features and outcomes of methamphetamine-associated pulmonary arterial hypertension. Am. J. Respir. Crit. Care Med. 197 (6), 788–800. https://doi.org/10.1164/rccm.201705-0943OC.

Zhao, S.X., Kwong, C., Swaminathan, A., Gohil, A., Crawford, M.H., 2018. Clinical characteristics and outcome of methamphetamine-associated pulmonary arterial hypertension and dilated cardiomyopathy. JACC Heart Fail. 6 (3), 209–218. https://doi.org/10.1016/j.jchf.2017.10.006.