

Physiological Research Pre-Press Article

Short Term Methylphenidate Treatment Does Not Increase Myocardial Injury in the Ischemic Rat Heart

Short title: Impact of methylphenidate on the ischemic heart

Sarah L. Seeley¹,BS; Manoranjan S. D'Souza¹, PhD; Thorne S. Stoops¹, Boyd R. Rorabaugh PhD²

¹Ohio Northern University School of Pharmacy, 525 South Main Street, Ada, Ohio 45810 USA;

²Marshall University School of Pharmacy, 1 John Marshall Drive, Huntington, WV 25755 USA

Corresponding Author:

Boyd R. Rorabaugh, Ph.D.

Marshall University School of Pharmacy

1 John Marshall Drive

Huntington, WV 25755

Email: rorabaughb@marshall.edu

Telephone: 304-696-7289

Abstract

Methylphenidate is commonly used for the treatment of attention deficit hyperactivity disorder. The cardiovascular safety of methylphenidate has been a subject of debate with some studies indicating that methylphenidate increases the likelihood of experiencing a myocardial infarction. However, it is unknown whether methylphenidate worsens the extent of injury during an ischemic insult. The purpose of this study was to determine whether short term exposure to methylphenidate increases the extent of myocardial injury during an ischemic insult. Male and female rats received methylphenidate (5 mg/kg/day) or saline for 10 days by oral gavage. Hearts were subjected to 20 min of ischemia and 2 hours of reperfusion on a Langendorff isolated heart apparatus on day 11. Cardiac contractile function was monitored via an intraventricular balloon and myocardial injury was assessed by triphenyltetrazolium chloride staining. Methylphenidate significantly increased locomotor activity in male and female rats, confirming absorption of this psychostimulant into the central nervous system. Male hearts had significantly larger infarcts than female hearts, but methylphenidate had no impact on infarct size or postischemic recovery of contractile function in hearts of either sex. These data indicate that methylphenidate does not increase the extent of injury induced by an ischemic insult.

Key Words

Myocardial ischemia; methylphenidate; heart attack; attention deficit hyperactivity disorder

Introduction

Methylphenidate is commonly used for the treatment of attention deficit hyperactivity disorder and narcolepsy. Prescriptions for 18.6 million tons of methylphenidate were filled in the United States in 2016 (Piper et al. 2018). The cardiovascular safety of methylphenidate has been a topic of debate. Some reports suggest that methylphenidate increases the likelihood of experiencing a heart attack (Thompson and Thompson 2010; Munk et al. 2015; Shin et al. 2016), while other studies indicate that methylphenidate does not significantly increase this risk (Schultz et al. 1998; Antel et al. 2015; Liu et al. 2019). Package inserts for methylphenidate include warnings that patients have experienced myocardial infarctions while using this drug at prescribed dosages. Previous clinical studies regarding the impact of methylphenidate on the heart have assessed the cardiac risk of this drug in terms of whether methylphenidate increases the likelihood of experiencing a heart attack (Antel et al. 2015; Shin et al. 2016). In contrast, we are not aware of any studies that have assessed whether or not methylphenidate alters the extent of myocardial injury when an ischemic insult occurs.

Previous work in our laboratory demonstrated that female rats (but not their male siblings) that are repeatedly exposed to methamphetamine over a 10 day period develop myocardial hypersensitivity to ischemic injury (Rorabaugh et al. 2017c). This is evidenced by increased infarct sizes and attenuation of postischemic recovery of contractile function in hearts of methamphetamine-treated rats. Importantly, this effect persists following at least a month of subsequent abstinence from methamphetamine, indicating that this psychostimulant induces long lasting cardiac effects that persist after use of the drug has been discontinued (Rorabaugh et al. 2017c). We have also reported that female rats (but not their male siblings) that were prenatally exposed to methamphetamine become hypersensitive to ischemic injury as adults (Rorabaugh et

al. 2016). This is consistent with previous work demonstrating that prenatal exposure to methamphetamine causes behavioral and neurological deficits in adult offspring (Hrebickova et al. 2016; Holubova et al. 2017; Dong et al. 2018; Slamberova 2019). Methylphenidate and methamphetamine both increase dopaminergic and adrenergic signaling, but they differ with respect to their mechanisms of action. Methamphetamine actively promotes the release of these neurotransmitters from intracellular storage vesicles by reversing the direction of their transport through NET and DAT (Han and Gu 2006). Methylphenidate does not induce the release of norepinephrine and dopamine (Luethi et al. 2018). Rather, methylphenidate act as a competitive antagonist of NET and DAT and prevents the reuptake of norepinephrine and dopamine after they have been released. In contrast to methamphetamine, it is unknown whether methylphenidate treatment increases the extent of myocardial injury during an ischemic insult. The purpose of the present study was to determine whether methylphenidate hypersensitizes the heart to ischemic injury. We hypothesized that methylphenidate would worsen the extent of myocardial injury in the ischemic heart in a manner similar to that of methamphetamine.

Methods

Animals

Sprague Dawley rats used for this study were obtained from a breeding colony maintained at Ohio Northern University. The colony originated from rats purchased from Charles River Laboratories (Boston, MA) Strain Code 001. Rats were pair housed in standard Plexiglas cages with free access to food and water and were maintained on a reversed 12 hour / 12 hour light / dark schedule (lights off at 07:00 to 19:00). Administration of methylphenidate for the treatment of attention deficit hyperactivity disorder in humans is typically limited to the waking hours because this psychostimulant interferes with sleep when administered at night.

Thus, rats were maintained on a reversed light cycle in this study to facilitate the administration of methylphenidate during the “active” (dark) phase of the rodent diurnal cycle to better mimic clinical use of the drug. All procedures were approved by the Institutional Animal Care and Use Committee of Ohio Northern University and were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Subchronic Treatment of Rats with Methylphenidate

A total of 47 male and female rats (8 weeks of age) were randomly assigned to methylphenidate (11 male; 13 female) or saline (11 male; 12 female) treatment groups. The animals received a single daily dose of a racemic mixture of d-threo- and l-threo-methylphenidate (5 mg/ kg/day) or saline by oral gavage at 10:00 am for 10 consecutive days. Previous work indicating that oral administration of this dose produces plasma methylphenidate concentrations within the clinically therapeutic range (Gerasimov et al. 2000; Montagnini et al. 2014). Hearts were isolated on day 11 (24 hours after the last dose), mounted on a Langendorff isolated heart apparatus, and subjected to an ischemic insult as described below. Methylphenidate was purchased from Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ).

Spontaneous Locomotor Activity

Locomotor activity was measured using a three-channel Opto-M4 Auto-Track System (Columbus Instruments, Columbus, OH) located in a dark room illuminated by a red 60 W light bulb as previously described (Rorabaugh et al. 2016; Rorabaugh et al. 2017a). Each rat was individually placed in the open field immediately after administration of methylphenidate on day 1 and day 10. Locomotor activity (determined by the total number of photo beam breaks) was

monitored for 1 hour. The total number of photobeam breaks was automatically converted into distance travelled (cm) by the Opto-M4 software that controls the apparatus.

Langendorff isolated heart experiments

The Langendorff isolated heart model was used to assess the impact of subchronic methylphenidate treatment on myocardial sensitivity to ischemic injury. Rats were anesthetized with sodium pentobarbital (100 mg/kg ip), and their hearts were rapidly removed and mounted on a Langendorff isolated heart apparatus. Krebs solution (in mM: 118 NaCl, 4.7 KCl, 1.2 MgSO₄, 25 NaHCO₃, 1.2 KH₂PO₄, 0.5 Na₂EDTA, 11 glucose, and 2.5 CaCl₂, pH 7.4) was perfused through an aortic cannula at a constant pressure of 80 mmHg. Contractile function of the left ventricle was measured using an intraventricular balloon connected to a pressure transducer and Powerlab 4SP data acquisition system (AD Instruments, Colorado Springs, CO) as previously described (Rorabaugh et al. 2016; Rorabaugh et al. 2017c). Hearts were equilibrated for 25 min prior to the onset of 20 min global ischemia (induced by terminating the flow of Krebs solution) and 2 hours of reperfusion. Preischemic contractile function was measured immediately prior to ischemia, and postischemic recovery of contractile function was measured following 10 min, 20 min, 40 min, and 1 hour of reperfusion. Coronary flow rate was measured by quantifying the volume of perfusate collected over a 30 seconds time period immediately prior to the onset of ischemia and during a 30 second time period following 1 hour of reperfusion. Hearts were perfused for an additional hour (2 hours of total reperfusion) prior to triphenyltetrazolium chloride (TTC) staining. Infarct sizes of TTC stained hearts were measured by an investigator who was blinded to the drug treatment and sex of each animal using NIH Image J software as previously described (Rorabaugh et al. 2016; Rorabaugh et al. 2017c).

Statistical analysis

Infarct sizes were compared by two-way ANOVA and Bonferoni's posthoc analysis using sex (male vs female) and drug treatment (methylphenidate vs saline) as factors. Parameters of cardiac function (developed pressure, +dP/dT, -dp/dT, heart rate, and end diastolic pressure, and coronary flow rate) were analyzed by three-way ANOVA and Bonferonni's posthoc analysis with sex, drug treatment, and time as factors. Time (preischemic contractile function and 10 min, 20 min, 40 min, and 60 min postischemic recovery of contractile function) was analyzed as a repeated measure. Locomotor activity data were analyzed by two-way ANOVA with time (day 1 and day 10 = repeated measure) and drug treatment as factors. All data analyses were performed using Graphpad Prism software (Graphpad Inc., San Diego, CA). Hearts from 2 methylphenidate-treated rats (one male and one female) were omitted from infarct size analyses because of a technical error that occurred during the TTC staining procedure. However, all other data from these animals (locomotor activity and parameters of contractile function) were retained.

Results

Body Weight and Heart Weight

Starting body weight, weight gain over the 10 day treatment period, and heart weight were greater in male rats than in female rats (Table 1). However, methylphenidate had no impact on these parameters in animals of either sex. Heart weight / body weight ratio was unaffected by methylphenidate (Table 1).

Methylphenidate Increases Locomotor Activity in Male and Female Rats

Locomotor activity was measured to verify that the orally administered methylphenidate was being absorbed and producing a centrally mediated response. Two-way ANOVA indicated significant overall effects of methylphenidate treatment in both male [$F = 21 (1,20)$, $p < 0.0005$] and female [$F = 15 (1, 23)$, $p < 0.001$] rats (Fig. 1). Bonferonni's posthoc analysis indicated that methylphenidate significantly increased locomotor activity in animals of both sexes and that this effect was not diminished with repeated exposure to the drug over the course of the 10-day treatment.

Preischemic contractile function

Hearts were isolated and mounted on a Langendorff isolated heart apparatus 24 hours after the final methylphenidate or saline gavage (day 11). Ten days of methylphenidate treatment had no significant impact on preischemic parameters of contractile function (Fig. 2A-E) or coronary flow rate (Fig. 2F) in hearts isolated from either male or female rats.

Postischemic recovery of contractile function and infarct size

Two-way ANOVA indicated that female hearts had significantly [$F = 5.4 (1, 40)$, $p < 0.05$] smaller infarcts than hearts from male rats (Fig. 3) following exposure to a 20 min ischemic insult. This is consistent with previous work from our laboratory (Rorabaugh et al. 2016; Rorabaugh et al. 2017c) and the work of others (Brown et al. 2005; Johnson et al. 2006). Methylphenidate had no effect on infarct size [$F = 1.9 (1, 40)$, $p = 0.18$] in hearts of either sex (Fig. 3).

Three way ANOVA indicated significant effects of time (before ischemia vs postischemic recovery) on developed pressure [$F = 78 (3,64)$, $p < 0.0001$], $+dP/dT$ [$F = 152 (3, 98)$, $p < 0.0001$], $-dP/dT$ [$F = 153 (3, 121)$, $p < 0.0001$], heart rate [$F = 152 (3,98)$, $p < 0.001$],

end diastolic pressure [$F = 203 (1,43)$, $p < 0.0001$], and flow rate [$F = 138 (1,43)$, $p < 0.0001$]. This reflects the fact that cardiac contraction stopped during the 20 min period of ischemia and did not return to the preischemic level of contractility during the recovery period. There was also a significant interaction between time and drug treatment with respect to $+dP/dT$ [$F = 5.6 (6, 239)$, $p < 0.01$], $-dP/dT$ [$F = 3.0 (6, 249)$, $p < 0.01$], and heart rate [$F = 2.3 (6, 253)$, $p < 0.05$]. However, methylphenidate had no effect on parameters of preischemic contractile function or postischemic recovery of contractile function in either male or female hearts at any time point before or after ischemia (Fig. 2). Heart rate was nominally (but not significantly) depressed in male rats at the end of the reperfusion period (Fig. 1B). This reflects the fact that 5 male hearts (2 from saline treated rats and 3 methylphenidate treated rats) did not recover a stable level of contractile function during the recovery period. These 5 hearts either contracted intermittently or did not contract at all and were assigned values of “0” for heart rate and other postischemic contractile parameters. Only 1 female heart (from a saline-treated animal) failed to recovery any postischemic contractile function.

Discussion

Prior studies assessing the cardiac risk associated with using methylphenidate for the treatment of ADHD have focused on determining whether or not methylphenidate increases the likelihood of experiencing a myocardial infarction. Some clinical studies have concluded that methylphenidate produces little or no increased cardiovascular risk (Antel et al. 2015; Liu et al. 2019) while other reports indicate that methylphenidate may increase the risk of experiencing a heart attack (Thompson and Thompson 2010; Munk et al. 2015; Shin et al. 2016). The present study differs from previous work because we did not assess the impact of methylphenidate on the likelihood of experiencing a myocardial infarction. Rather we assessed whether or not

methylphenidate alters the extent of myocardial injury that develops when an ischemic insult occurs. Our finding that methylphenidate does not worsen the extent of myocardial injury in hearts subjected to ischemia may provide an additional level of confidence in the cardiac safety of this commonly prescribed psychostimulant.

The observation that infarct sizes in female hearts were significantly smaller than those of male hearts (Fig. 2) is consistent with prior work from our own lab and the work of others (Johnson et al. 2006; Rorabaugh et al. 2016; Rorabaugh et al. 2017b). Resistance of the female heart to ischemic injury has been attributed to sex differences in cardioprotective signaling pathways involving ATP-dependent potassium channels (Johnson et al. 2006), nitric oxide (Shao et al. 2016), the mitochondrial permeability transition pore (Milerova et al. 2016), and to the cardioprotective effect of estrogen (Ma et al. 2009; Wang et al. 2010). Our previous work demonstrated that methamphetamine exposure during either the prenatal period (Rorabaugh et al. 2016) or during early adulthood (Rorabaugh et al. 2017c) negates the cardioprotective benefit of being female. In contrast, the current data indicate that methylphenidate does not interfere with the endogenous cardioprotective mechanisms of the female heart.

Methylphenidate and methamphetamine both increase adrenergic and dopaminergic signaling, but only methamphetamine causes the heart to become hypersensitive to ischemic injury (Rorabaugh et al. 2017c). Our previous study (Rorabaugh et al. 2017c) demonstrated that subcutaneous injection of methamphetamine (5 mg/kg for 10 days) caused hearts of female rats (but not their male littermates) to become hypersensitive to ischemic injury as evidenced by increased infarct size and attenuated postischemic recovery of contractile function (Rorabaugh et al. 2017c). Methylphenidate (5 mg/kg for 10 days) was administered orally in the present study to mimic the clinical route of administration of this drug. Repeating these experiments with

subcutaneously administered methylphenidate (5 mg/kg for 10 days) produced similar results (data not shown), indicating that the route of administration does not account for the observed differences in the impact of these psychostimulants on the ischemic heart. Methamphetamine and methylphenidate both produce pharmacological effects that are mediated through the central nervous system (CNS). However, studies with isolated cardiomyocytes provide evidence that methamphetamine also has direct effects on cardiomyocytes that are independent of the CNS. Methamphetamine has been reported to stimulate apoptosis (Leung et al. 2014), hypertrophy (Maeno et al. 2000), reorganization of myofibrils (Maeno et al. 2000) and enhances the activity of L type calcium channels (Sugimoto et al. 2009) in isolated cardiomyocytes that are separated from autonomic influence. We do not know whether the previously observed effects of methamphetamine on the ischemic heart (Rorabaugh et al. 2017c) is mediated through the CNS or if it resulted from a direct action of methamphetamine on the heart independent of the CNS. It is also unknown whether methylphenidate can act directly (independent of the CNS) on cardiomyocytes to induce the same changes that have been reported for methamphetamine. These gaps in our knowledge of the cardiac effects of methylphenidate make it difficult to determine why methylphenidate does not have the same detrimental impact on the ischemic heart that occurs following exposure to methamphetamine (Rorabaugh et al. 2017c).

The half-life of methylphenidate is less than 3 hours in humans (Wong et al. 1998). Consequently, it is often administered twice / day or administered as a long acting formulation to achieve more prolonged therapeutic efficacy in the treatment of ADHD. In addition, methylphenidate use typically follows a chronic (years) time course. The 10 day treatment protocol used in this study was based on prior work demonstrating that 10 days of methamphetamine treatment hypersensitizes the heart (Rorabaugh et al. 2017c) and brain

(Zuloaga et al. 2016) to injury caused by a subsequent ischemic insult. We cannot rule out the possibility that methylphenidate may worsen ischemic injury if administered multiple times / day, as a long acting formulation, or for a more chronic duration. However, our data provide evidence that short term exposure (10 days) does not hypersensitize the heart to ischemic injury.

Although methylphenidate is most commonly associated with the treatment of children with ADHD, there is growing evidence that this psychostimulant may be useful for the treatment of geriatric depression (Lavretsky et al. 2003; Lavretsky et al. 2015; Patel et al. 2017) and cognitive function in elderly patients with Alzheimer's disease (Rosenberg et al. 2013; Padala et al. 2018). A recent study of the French General Health Insurance Database indicated that 34 % of new methylphenidate users in France are adults (Pauly et al. 2018). Ischemic heart disease primarily impacts the elderly population, but the animals used in this study were young adults (8 weeks). Further work is needed to determine whether long term methylphenidate use alters the cardiac response to an ischemic insult or increases the likelihood of an adverse cardiovascular event in the geriatric population.

The hearts in the present study exhibited relatively low levels of postischemic recovery of contractile function relative to their small infarct sizes, suggesting that the postischemic recovery may have been attenuated by stunning rather than by extensive tissue death. Our data indicate that methylphenidate had no impact on postischemic recovery of contractile function during the first hour of reperfusion. However, we do not know how methylphenidate may have impacted postischemic recovery over a longer time course. The inability of the Langendorff isolated heart model to measure long-term recovery of myocardial contractile function following an ischemic insult is a limitation of this experimental model. Another limitation of this work is the use of a relatively short period of ischemia (20 min). We cannot exclude the possibility that

methylphenidate may have negatively impacted infarct size if a longer period of ischemia (resulting in more severe myocardial injury) had been utilized. Finally, methylphenidate is typically prescribed for a chronic time period. Our data provide evidence that short-term (10 days) methylphenidate treatment does not worsen myocardial ischemic injury, but we do not know if there is a detrimental cardiac effect on the ischemic heart following more chronic exposure.

In summary, prior studies investigating the impact of methylphenidate on the ischemic heart have focused on assessing whether or not methylphenidate increases the likelihood of experiencing a myocardial infarction. These studies have produced mixed results. Some investigators have reported that methylphenidate increases the likelihood of experiencing a heart attack (Thompson and Thompson 2010; Munk et al. 2015; Shin et al. 2016), while others have concluded that methylphenidate does not significantly increase this risk (Schultz et al. 1998; Antel et al. 2015; Liu et al. 2019). This is the only study that we are aware of to measure the extent of myocardial injury in the ischemic heart following a period of methylphenidate exposure. Caution is warranted regarding the use of this psychostimulant in patients who already have risk factors for cardiovascular disease. However, our data suggest that short term use of methylphenidate does not worsen the extent of myocardial injury that occurs during an ischemic insult.

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Table 1. Body weight, weight gain, and heart weight following saline or methylphenidate treatment

	Male Saline	Male Methylphenidate	Female Saline	Female Methylphenidate
Starting Body Weight (g)	387 ± 13	393 ± 14	226 ± 6 ^a	232 ± 5 ^b
Weight Gain (g)	55 ± 3	58 ± 7	5 ± 2 ^a	8 ± 3 ^b
Heart Weight (mg)	1,085 ± 52	1198 ± 56	709 ± 20 ^a	759 ± 21 ^b
Heart Weight / Body Weight Ratio (mg/g)	2.8 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.2 ± 0.1

Two-way ANOVA indicated significant effects of sex on starting body weight [F = 252 (1, 42), $p < 0.0001$], weight gain [F = 157 (1, 41), $p < 0.0001$], heart weight [F = 252 (1, 42), $p < 0.001$], and heart weight / body weight ratio [F = 7.7 (1, 41), $p < 0.01$]. However, methylphenidate had no significant effect on these parameters. ^a indicates a significant difference compared to saline-treated male rats. ^b indicates a significant difference ($p < 0.0001$) compared to methylphenidate-treated male rats.

Fig. 1. Methylphenidate increases locomotor activity in male and female rats after both acute and repeated administration. Male (A) and female (B) rats were treated with saline or methylphenidate (5 mg/kg) by oral gavage for 10 consecutive days. Locomotor activity was measured for 1 hour immediately after the first (day 1) and last (day 10) drug treatment. Methylphenidate significantly increased locomotor activity of both male and female rats on day 1 and day 10. Data represent the mean \pm SEM of 11 – 13 rats. ^a indicates $p < 0.001$. ^b indicates $p < 0.05$.

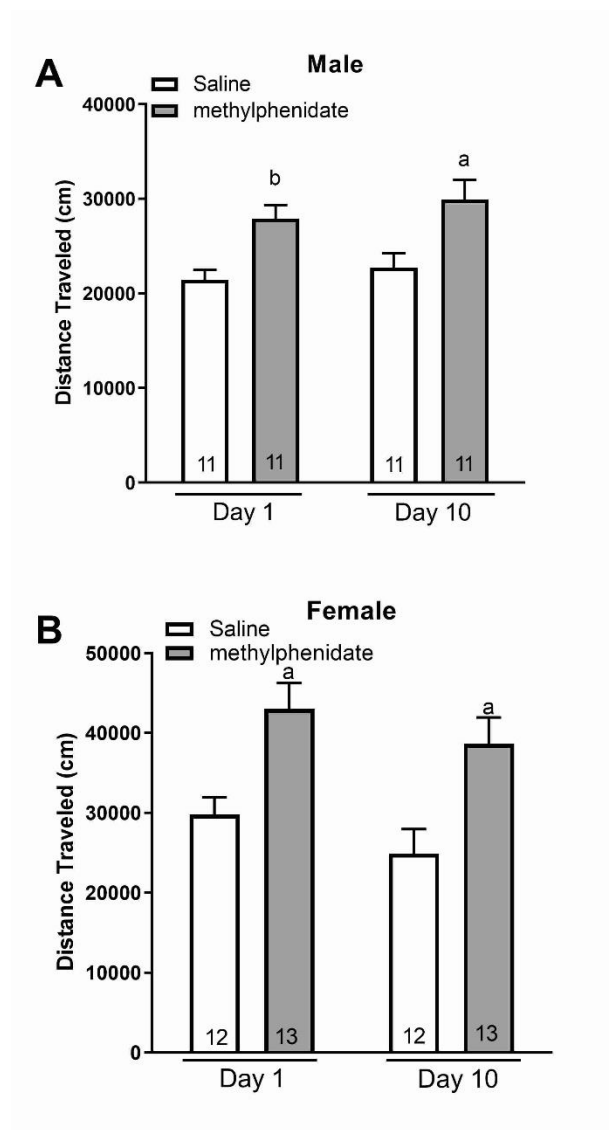


Fig. 2 Repeated methylphenidate administration for 10 days has no effect on preischemic contractile function or postischemic recovery of contractile function. Three way ANOVA indicated significant effects of time (before ischemia vs postischemic recovery) on developed pressure [F = 78 (3,64), $p < 0.0001$] (**A**), heart rate [F = 152 (3,98), $p < 0.001$] (**B**), +dP/dT [F = 152 (3, 98), $p < 0.0001$] (**C**), -dP/dT [F = 153 (3, 121), $p < 0.0001$] (**D**), end diastolic pressure [F = 203 (1,43), $p < 0.0001$] (**E**), and flow rate [F = 138 (1,43), $p < 0.0001$] (**F**). There was also a significant interaction between time and drug treatment with respect to heart rate [F = 2.3 (6, 253), $p < 0.05$] (**B**), +dP/dT [F = 5.6 (6, 239), $p < 0.01$] (**C**), and -dP/dT [F = 3.0 (6, 249), $p < 0.01$] (**D**). However, methylphenidate had no effect on parameters of preischemic contractile function or postischemic recovery of contractile function in either male or female hearts.

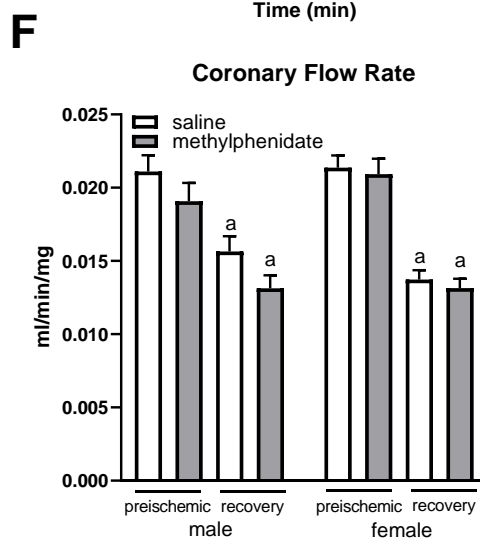
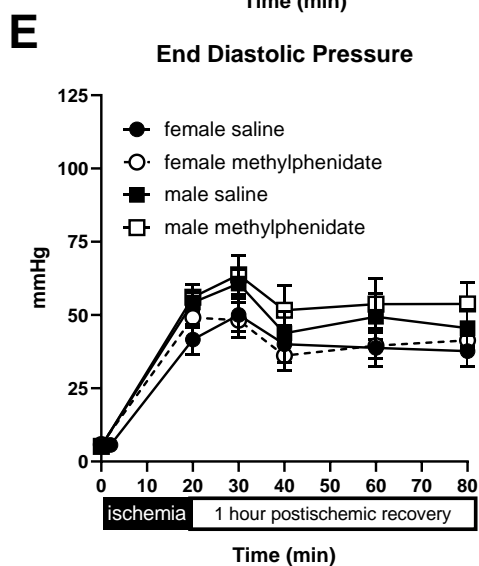
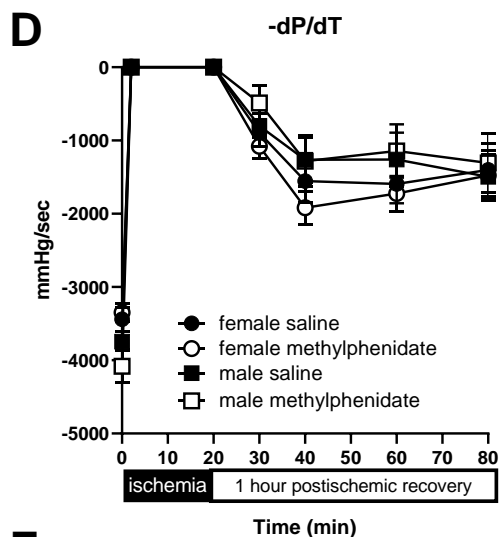
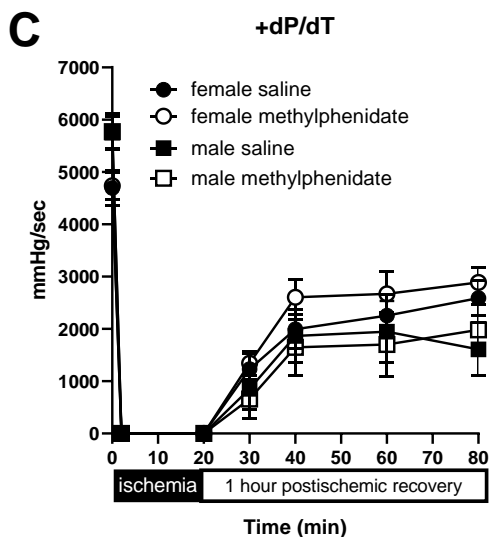
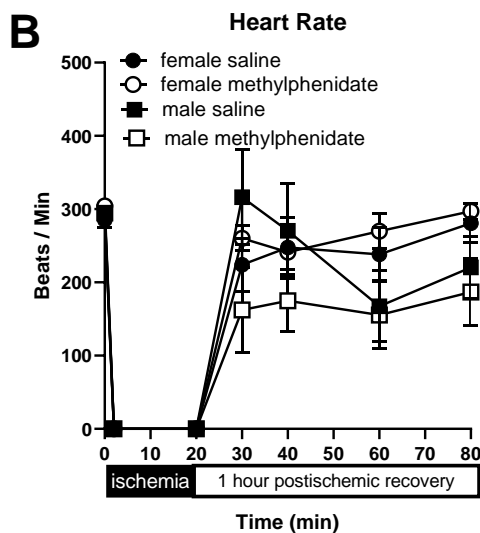
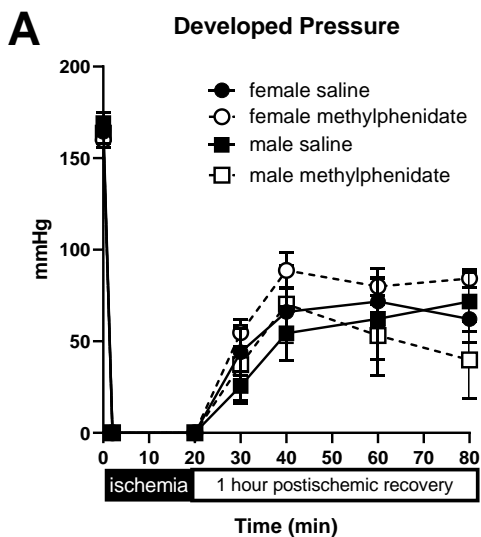


Fig. 3. Repeated administration of methylphenidate for 10 days does not hypersensitize the heart to ischemic injury. Male and female rats were treated with saline or methylphenidate (5 mg/kg) by oral gavage for 10 consecutive days. Isolated hearts were subjected to a 20 min ischemic insult on day 11. Infarct sizes were measured by triphenyltetrazolium chloride staining. Infarcts were significantly larger in male hearts compared to female hearts [$F = 5.4 (1, 40)$, $p < 0.05$]. However, methylphenidate had no effect on infarct size. Data in panel **A** represent the mean \pm standard deviation of hearts from 10 – 12 rats. Photographs of representative tissue slices from stained hearts are shown in panel **B**.

