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ORIGINAL ARTICLE

A humanized anti-cocaine mAb antagonizes the cardiovascular effects of cocaine in rats

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Abstract

The recombinant monoclonal anti-cocaine antibody, h2E2, sequesters cocaine in plasma increasing concentrations more than 10-fold. The increased levels of cocaine in the plasma could have detrimental peripheral effects, particularly on the cardiovascular system. We investigated the duration and magnitude of the effect of cocaine on the rat heart, and if h2E2 could antagonize that effect. Echocardiography was used to evaluate cardiac function under isoflurane anesthesia, while a tail-cuff was used to measure blood pressure. Cocaine was delivered intravenously and the rats were continuously monitored for a total of 45 min. Echocardiography measurements were recorded every 5 min and blood pressure measurements were recorded throughout the duration of the experiment using 30-s cycles. ECG recordings were taken simultaneously with the echocardiography measurements. An increase in ejection fraction was seen after the cocaine push with the maximum change occurring at 25 min. Treatment with h2E2 1 h before the cocaine push did not have any effect on cardiac parameters. Subsequent cocaine treatment had no effect on the ejection fraction, indicating that the antibody-bound cocaine does not affect the heart. This antagonism of cocaine's effects was greatly decreased after 1 week and entirely absent after 1 month. Cocaine in the presence of h2E2 is pharmacologically inert and h2E2 may have additional clinical utility for reversing cocaine effects on the cardiovascular system.

KEYWORDS
cocaine, humanized monoclonal antibody, cardiology, echocardiography, translational studies

1 | INTRODUCTION

Traditional pharmacotherapeutic approaches for cocaine addiction have targeted the dopaminergic neurotransmission in the brain, through which cocaine exerts its effects. There is no pharmacological treatment approved for cocaine abuse, despite the testing of agents that target nearly all aspects of the dopaminergic system.[1]

Immunotherapy provides a potential alternative to traditional small-molecule therapies. Anti-cocaine antibodies bind to cocaine and form a complex too large to cross the blood–brain barrier. Previously, antibodies have been raised through active immunization

Abbreviations: CO, cardiac output (ml/min); ECG, electrocardiogram; EF, ejection fraction (%); FS, fractional shortening; HR, heart rate (bpm); LV, left ventricular; mAb h2E2, humanized anti-cocaine monoclonal antibody; SV, stroke volume.

using a cocaine vaccine.[2–4] Importantly, recent clinical studies have demonstrated that a cocaine vaccine generating anti-cocaine antibodies decreased cocaine use in cocaine abusers.[5–7] Despite efforts to improve these vaccines, the response to the vaccine is slow to develop and unpredictable with regard to antibody titers, affinities, and specificities, causing efficacy to vary between individuals.[5,7–9]

Passive immunization, entailing the injection of an anti-cocaine monoclonal antibody (mAb), has a number of potential advantages.[2,10] With passive immunization, immediate immunity is conferred via injection of a well-characterized mAb with known high affinity and specificity for cocaine. Our group developed a unique anti-cocaine mAb, 2E2, which had a fully human sequence heavy (H) chain and Fc region but also had a murine lambda light (L) chain. This mAb passed key safety and efficacy milestones in animal studies that established the feasibility of this immunotherapeutic approach.[11,12] Subsequently, a reengineered version of 2E2, h2E2, was generated which has a human lamda light chain constant region replacing the murine constant region. This reengineered recombinant protein has met a series of preclinical milestones necessary for transitioning to clinical development.

In addition to the drug abuse-related risks, cocaine use has significant effects on cardiovascular function in humans and has been associated with the development of chest pain, myocardial ischemia and in rare cases cardiac death.[13,14] Interestingly, h2E2 in vivo produces a 10–20-fold increase in plasma cocaine[15] and cocaethylene[16] concentrations in mice and rats. The high affinity of h2E2 for cocaethylene and norcocaine has been established in previous studies.[15] As h2E2 produces a dramatic decrease in brain cocaine concentrations, it is assumed that the increased plasma cocaine levels[17,18] are due to h2E2-bound cocaine that is pharmacologically inert and a decreased free cocaine concentration. It is possible that the increased plasma cocaine concentration, increasing the circulation time of cocaine and the 92% decrease in cocaine excretion into the urine could have increased direct effects on the cardiovascular system.[19] There are concerns that the sequestration of cocaine by h2E2 may exacerbate the peripheral effects of cocaine. The objective of this study was to determine the dose-dependent duration and magnitude of effects of cocaine administration in the presence and absence of mAb h2E2 on echocardiographic variables in an in vivo rat model. We hypothesize that the cocaine-induced responses will be decreased in the presence of h2E2 as our models indicate that the cocaine is bound to h2E2 and is pharmacologically inert.

2 | MATERIALS AND METHODS

2.1 | Animals

All animal procedures were performed with the approval of the Institutional Animal Care and Use Committee of the University of Cincinnati and in accordance with the 8th Edition of the Guide for the Care and Use of Laboratory Animals.[20] The Sprague–Dawley (225–300 g) rats used in this study were purchased from Harlan Laboratories. Animals were maintained in the UC Laboratory Animal Medicine vivarium for a minimum of 1 week prior to surgery. The rats had indwelling catheters surgically implanted[19] and were allowed to recover for 7 days before echocardiography. After surgery, the rats were housed individually on a 14/10-h light/dark cycle with unrestricted access to food and water.

2.2 | Echocardiography

Echocardiography was performed as previously described using the Vevo 2100 with a MS250 transducer.[21] Briefly, the rats were anesthetized with 2.0% isoflurane and baseline echocardiography images were recorded. Images were also taken at the time of the cocaine push, as well as at 1, 3, and 5 min. Subsequent images were taken at 5 min intervals for a total of 45 min (Figure 1A,B). Parasternal long-axis (PSLAX) images were recorded and then analyzed on a separate workstation. From the M-mode images, left ventricular cavity size for systole and diastole (LV Vol;s and LV Vol;d) were measured. Heart rate (HR), stroke volume (SV), and the echocardiographic calculation for fractional shortening (FS), ejection fraction (EF), and cardiac output (CO) were obtained using the VevoStrain software (Version 3.1.1, VisualSonics). Each rat served as its own control and changes from baseline were calculated for the subsequent time points and the average was calculated for each experimental group.

2.3 | Experimental design

As described in Figure 1C, the experimental procedure was the same for each rat and consisted of catheter surgery (day −7), cocaine push (day 1), cocaine + vehicle (day 3), cocaine + h2E2 (day 5), 1 week post-pre-treatment with h2E2 (cocaine push), and 1-month post-pre-treatment with h2E2 (cocaine push). As previously determined, the equimolar h2E2 antibody dose of 360 mg/kg or vehicle (PBS, pH 7.0) was injected 1 h prior to cocaine push to allow for proper circulation throughout the animal.[19]

2.4 | h2E2 preparation and h2E2 concentration protocol

Norman et al.[15] describe the generation of h2E2. The h2E2 used in the study was manufactured by Catalent PharmaSolutions Inc. at a concentration of 20 mg/ml. To deliver the equimolar dose of 360 mg/kg, it was necessary to concentrate the antibody. The antibody was concentrated to 100 mg/ml using the Amicon® Pro Purification System (MilliporeSigma) according to the manufacturer's recommendations.

2.5 | Blood pressure measurements

Prior to echocardiography, the anesthetized rats were subjected to noninvasive tail-cuff (Kent Scientific, CODA Standard [Kent Scientific Corporation]) blood pressure (BP) measurements as indicated by manufacturer's guidelines. Measurements were taken

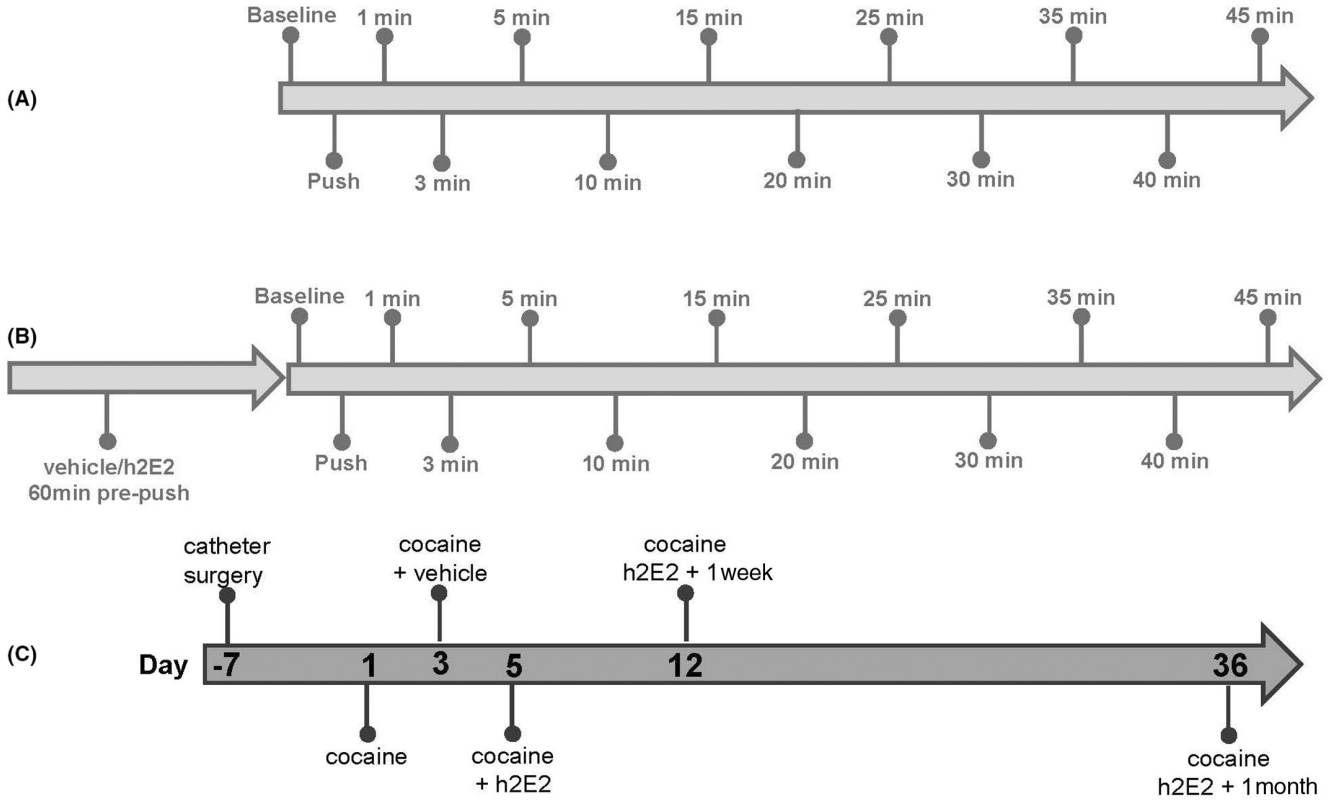

FIGURE 1 Schematics of the experimental design. (A) Echocardiography schematic for the saline and cocaine, 1 week post-h2E2, and 1-month post-h2E2 groups. Measurements were taken at baseline, cocaine push, 1, 3, 5 min, and at 5-min intervals for a total of 45 min. (B) Echocardiography schematic for the vehicle and h2E2 groups. Measurements were taken at baseline, cocaine push, 1, 3, 5 min, and at 5-min intervals for a total of 45 min. (C) Schematic showing the timeline of the rat procedures.

before baseline and then at 30-s cycles for the remainder of the experiment. Systolic and diastolic BP were determined.

2.6 | Statistical analysis

Data analysis was performed with SigmaPlot v.13 (Systat Software, Inc.). All data were tested for normality and equal variance. Statistical significance between multiple groups was assessed by analysis of variance using a two-factor mixed design with repeated measures as appropriate. Although significance was indicated, post hoc testing was performed using the Holm-Sidak method for comparing individual means and correcting for family-wise error (SigmaPlot v.13.0; Systat Software, Inc.). Data are presented as means ± SEM, and differences were regarded as significant at p-value of 0.05 or less.

3 | RESULTS

3.1 | Determination of cocaine HCl dose-response curve

Initial dose-ranging studies determined the effect of cocaine-HCl on cardiac parameters. The doses investigated were (in mg/kg): 0.56, 1.12, 1.68, 2.24, 3.36, and 4.48. Treatment with the higher cocaine doses of 3.36 and 4.48 mg/kg produced a reaction similar to overdose in the rats and was not pursued further. The echocardiography images for the remaining doses were analyzed and the measured changes in EF were 5.97% ± 2.59%, 7.64% ± 1.06%, 8.24% ± 0.83%, and 8.06% ± 0.95%, respectively (Figure 2).

3.2 | Cocaine-induced changes in echocardiography parameters that were blunted by h2E2 pretreatment

The echocardiography parameters of EF (Figure 3A), LV Vol;s (Figure 3B), HR (Figure 3C), and CO (Figure 3D) were evaluated for rats injected with saline, coc + veh, and coc + h2E2 over the course of the experiment. The saline injection did not result in a change in EF or systolic volume. However, there was a delayed decrease in HR and CO, likely due to the well-documented anesthetic effect of isoflurane on the rat heart over time.[22–24] Cocaine administration resulted in an initial drop in EF (−9.39% ± 2.71%, $p < .001$), followed by an increase in EF, with the peak value at 25 min after injection (9.48% ± 0.0.54%, $p < .001$). Inversely proportional changes in left ventricular systolic volume were also observed. Cocaine produced an immediate decrease in HR that did not fully return to baseline.

Similarly, cocaine caused a decrease in CO, which normalized over time. Administration of h2E2 had no detectable effect on cardiovascular measurements 1-h, 1-week, or 1-month post-injection (Table 1). Crucially, immediate pretreatment with the h2E2 antibody significantly blunted the effect of cocaine on EF, systolic volume, HR, and CO.

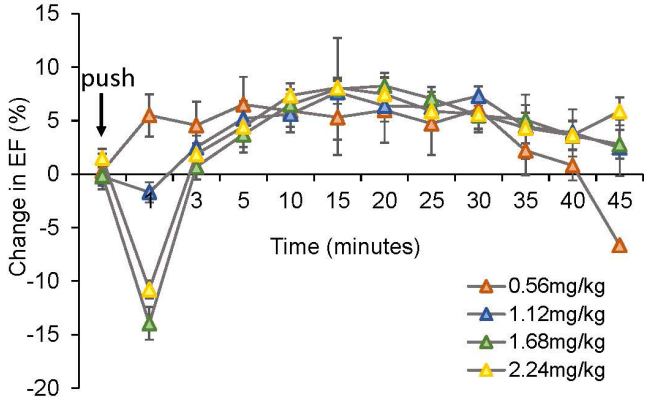

FIGURE 2 Determination of optimal cocaine dose based on echocardiographic parameters. Individual rats were injected with four different doses of cocaine-HCl, 0.56 mg/kg ($n = 6$), 1.12 mg/kg ($n = 8$), 1.68 mg/kg ($n = 17$), and 2.24 mg/kg ($n = 5$). The change in ejection fraction (EF) was measured for each dose at baseline, cocaine push, 1, 3, 5 min, and at 5-min intervals for a total of 45 min. Values are average ± SEM.

3.3 | Long-term effects of h2E2 pretreatment on cocaine-induced changes in cardiac function

A comparison of the immediate antibody effect (coc + h2E2) and that of 1-week pretreatment (coc + 1week) results in an increased inotropy from 10–35 min (Figure 4A) and subsequent decreased systolic volume (Figure 4B). However, this increase is significantly less than that observed with coc + veh from 10–30 min for the EF and at 20 and 25 min for systolic volume. Pretreatment with an antibody 1 month prior to cocaine did not significantly blunt the increased inotropy (Figure 4A) or inverse decrease in systolic (Figure 4B). The initial drop in HR was blocked by h2E2, even at 1-week post-injection (Figure 4C). h2E2 similarly antagonized the increase in CO, however, this effect was mitigated at 1 week and 1-month post-injection (Figure 4D).

3.4 | Cocaine-induced changes in the cardiac electrical parameters

ST-segment elevation was noted in the vehicle group consistent with the development of active ischemia, likely through macro and microvascular vasospasm secondary to the direct effect of cocaine on the vasculature (Figure 5, left). The effect was observed almost immediately after cocaine administration with resolution within a minute. There were no significant changes to the ST segments noted in the h2E2 groups (Figure 5, right).

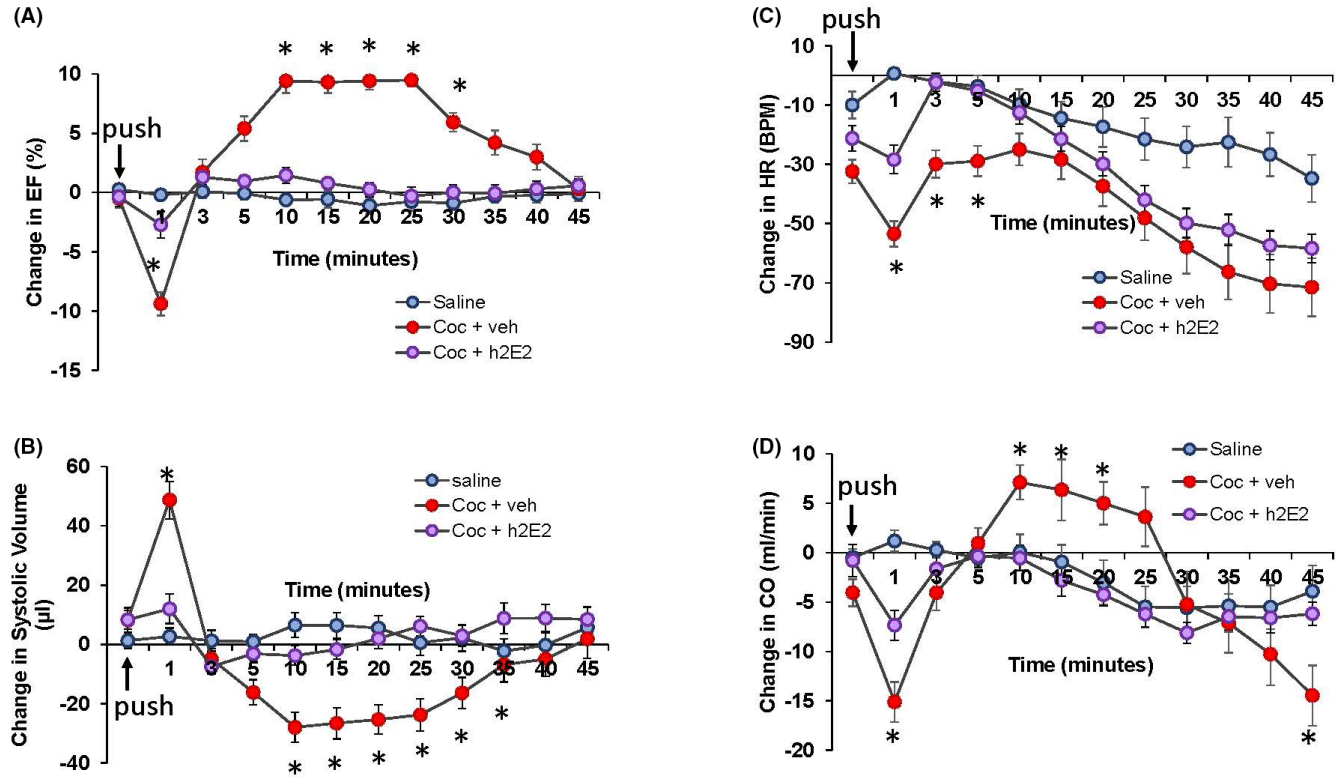

FIGURE 3 Echocardiography measurements for saline ($n = 8$), Coc + veh ($n = 13$) and Coc + h2E2 ($n = 13$). (A) Change in ejection fraction (% EF). (B) Change in systolic volume. (C) Change in heart rate (HR, bpm). (D) Change in cardiac output (CO, ml/min). Values are average ± SEM. *$p < .05$ for Coc + veh compared to saline and Coc + h2E2.

TABLE 1 The effect of h2E2 on the cardiovascular effects of cocaine in rats was measured by echocardiography

Baseline	Coc + veh $n = 13$	Coc + h2E2 $n = 13$	Coc + 1 wk $n = 11$	Coc + 1 mos $n = 12$
Heart rate (BPM)	342.30 ± 6.82	340.83 ± 5.24	332.90 ± 4.11	331.57 ± 5.21
Volume, s (μl)	128.42 ± 7.03	120.45 ± 6.08	125.56 ± 5.74	146.92 ± 7.07
Volume, d (μl)	337.14 ± 9.45	319.78 ± 12.38	332.93 ± 11.94	370.14 ± 17.64
Stroke volume (μl)	208.71 ± 6.54	199.33 ± 10.83	207.36 ± 9.34	223.23 ± 11.31
Ejection fraction (%)	62.04 ± 1.53	62.14 ± 1.70	62.22 ± 1.32	60.23 ± 0.67
Fractional shortening (%)	34.86 ± 1.15	34.88 ± 1.28	34.89 ± 0.99	33.49 ± 0.51
Cardiac output (ml/min)	71.45 ± 2.67	67.79 ± 3.56	68.97 ± 3.20	73.68 ± 3.47
Weight (g)	294.77 ± 5.17	295.85 ± 4.68	324.00 ± 4.07	366.75 ± 4.73

Abbreviations: Coc, cocaine; d, diastolic; mos, month; s, systolic; veh, the h2E2 vehicle; wk, week.

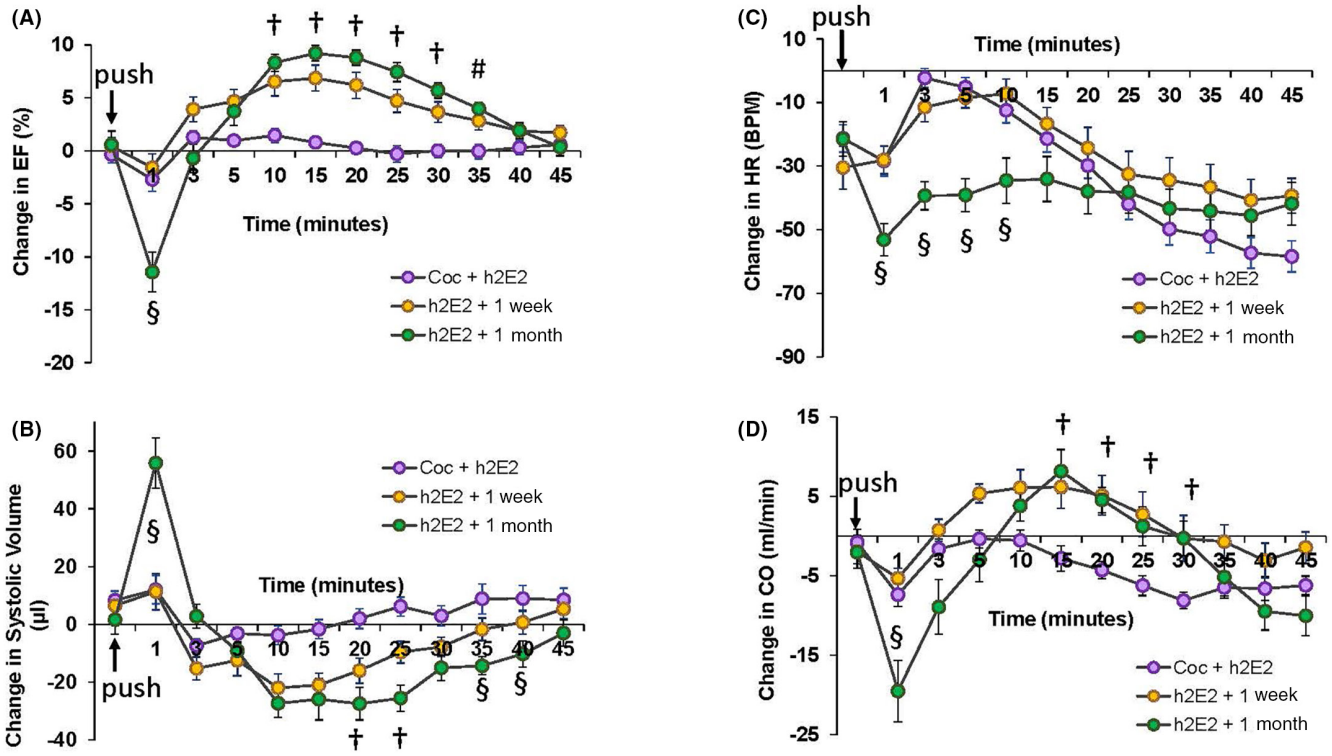

FIGURE 4 Echocardiography measurements for all experimental groups. The groups are coc + h2E2 ($n = 13$), h2E2 + 1 week ($n = 11$) and h2E2 + 1 month ($n = 12$). (A) Change in ejection fraction (% EF). (B) Change in systolic volume. (C) Change in heart rate (HR, bpm). (D) Change in cardiac output (CO, ml/min). Values are average ± SEM. $^{\S}p < .05$ for h2E2 + 1month compared to Coc + h2E2 and h2E2 + 1week. $^{\dagger}p < .05$ for Coc + h2E2 compared to h2E2 + 1 week and h2E2 + 1 month. $^{\#}p < .05$ for Coc + h2E2 compared to h2E2 + 1month.

3.5 | Cocaine-induced vasoconstriction was blocked by h2E2 pretreatment

We measured the BP at baseline and recovery (Figure 6A). Immediately following the cocaine push, the BP was no longer able to be determined by the tail cuff. Therefore, we counted the number of cycles that passed before readings returned (Figure 6B). The recovery values are an average of the first 10 cycles after the tail-cuff measurements resume.

4 | DISCUSSION

The goal of the current study was to address concerns that an increased concentration of cocaine in the plasma, facilitated by h2E2 binding will cause an adverse effect on the heart. We set out to determine what the effect of cocaine was on the rat heart, if h2E2 could antagonize that effect and if there was indeed a negative effect on cardiovascular function. Our preliminary studies included different doses of cocaine to investigate which, if any,

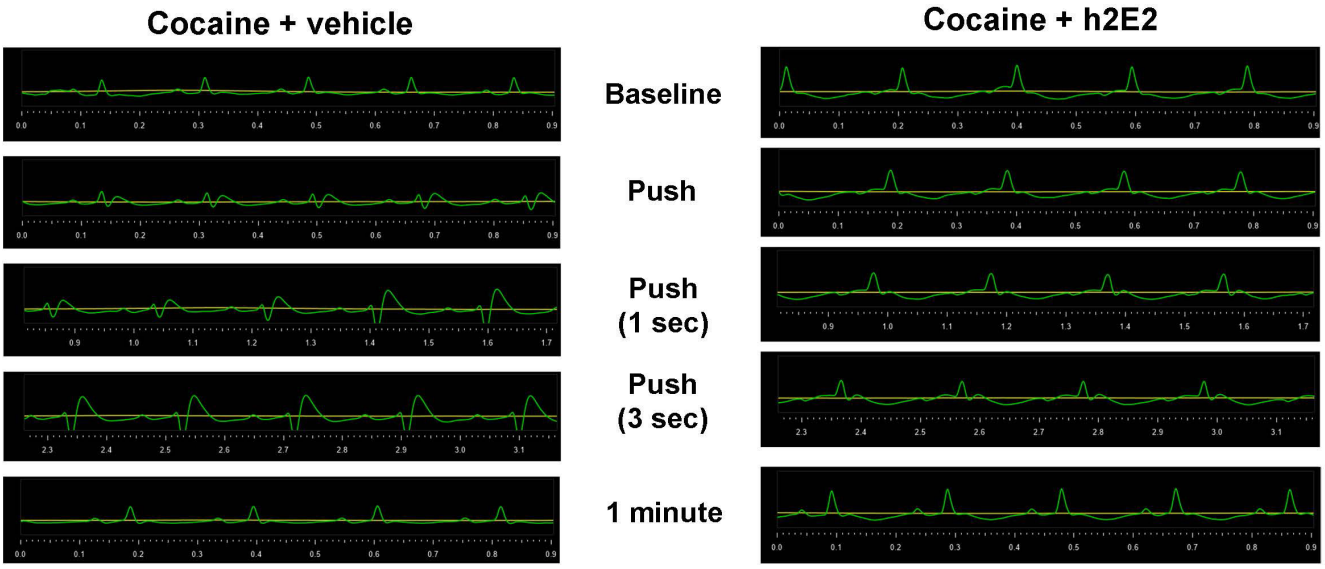

FIGURE 5 ECG recordings from Coc + veh and Coc + h2E2. ECG recordings are shown at baseline, push, push +1 s, push +3 s and 1 min after push.

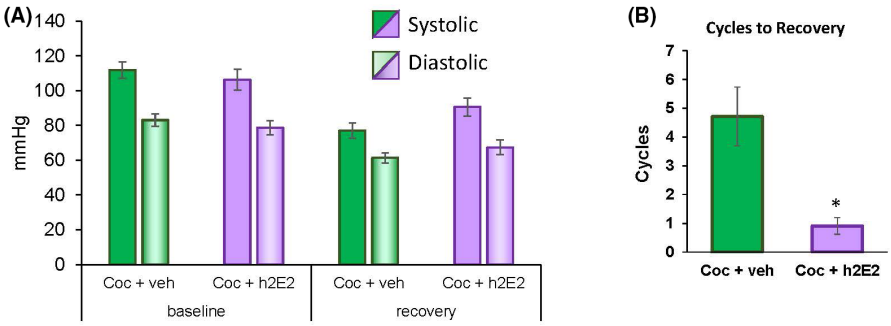

FIGURE 6 Blood pressure. Blood pressure measurements via tail-cuff at baseline and recovery from cocaine push for coc + veh ($n = 15$) and coc + h2E2 ($n = 12$). (A) Absolute BP measurements for baseline and recovery. (B) The number of cycles that passed before the BP measurements resumed. Values are average ± SEM. $p < .01$ for Coc + h2E2 compared to Coc + veh.

echocardiography parameters are influenced by cocaine. We began with the same dose used in our self-administration studies, 0.56 mg/kg cocaine HCl.[25] Our initial cocaine-HCl dose did not demonstrate a decreased EF, however, this dose did increase the EF, though the results were inconsistent with large deviations. Doubling the dose to 1.12 mg/kg had similar results, with a great deal of variability. The dose of 1.68 mg/kg gave the most consistent and reproducible results. To verify that higher doses would not be more effective, we also used 2.24, 3.36, and 4.48 mg/kg. The very high doses of 3.36 and 4.48 mg/kg resulted in shallow breathing and decreased EF, similar to overdose in humans.[26] Based on our previous studies, using the standard practice of equimolar concentrations of antibody and cocaine, and knowing that each antibody can bind two cocaine molecules,[19] we opted for the dose of 1.68 mg/kg.

The effect of cocaine on the cardiovascular system is via direct and indirect routes. It has been shown that at low doses, the effect is mostly indirect through the stimulation of sympathetic nerve activity by inhibiting the reuptake and stimulating the outflow of catecholamines, specifically norepinephrine and dopamine.[14] At higher doses, cocaine acts directly as an anesthetic and antiarrhythmic agent by blocking sodium, potassium, and calcium currents.[13,14] Finally, cocaine causes vasoconstriction by increasing the release of endothelin-I (vasoconstrictor) and inhibiting nitric oxide (vasodilator) production.[14]

We observed an initial severe drop in EF with cocaine administration, consistent with the standard physiological effect of cocaine as previously published.[14,27–30] However, this effect lasted for less than 3 min and by 5 min, we saw an increased EF with a peak around 25 min after the cocaine injection. The increase in EF is likely due to a secondary sympathetic stimulation by higher levels of norepinephrine.[31] Pretreatment with h2E2 blocked both the initial decrease and subsequent increase in EF. Furthermore, h2E2 treatment alone did not cause any adverse effects on the cardiovascular system, as evidenced by a comparison of the echocardiographic baseline measurements. Likewise, the T-wave inversion we observed that has been previously reported to occur with cocaine treatment[32] was mitigated by h2E2. Furthermore, pretreatment with h2E2 demonstrated no adverse effects on the ECG. Lastly, the vasoconstriction caused by cocaine was not evident in rats pretreated with h2E2, as there was no delay in BP readings. After 1 week, most of the inhibition by h2E2 was no longer measurable, though the initial decrease in EF did not occur and the EF increase was slightly lower than cocaine + vehicle. The clearing of h2E2 from the rat system is evident after 1 month since the cardiovascular parameters are similar to those of cocaine with only vehicle. The ability of h2E2 to antagonize the cardiovascular effects of cocaine in vivo predicts that h2E2 will be effective in the treatment of cocaine use disorder.

In summary, cocaine is pharmacologically inert in the presence of equimolar concentrations of h2E2 cocaine binding sites, and there is no evidence of an increased risk of adverse cardiovascular effects of cocaine in the presence of h2E2 despite elevated plasma cocaine concentrations. Indeed, the h2E2 antagonism of cocaine's cardiovascular effects may provide an additional clinical indication for h2E2 as a treatment for any cardiovascular toxicity of cocaine.

4.1 | Limitations

This study was limited to investigating the possible cardiovascular effects of treatment with h2E2 on rats. As the study was designed to be preclinical, overdosing and combined alcohol and cocaine consumption was not considered. The study investigated only short-term effects and as such the animals in the study were only observed via echocardiography until the heart function, as measured by EF, returned to baseline values. Future studies will investigate the long-term effects of h2E2 on the rat heart using self-administration to simulate chronic cocaine use, including post-treatment to prevent cocaine overdose. Due to the longitudinal nature of this study, it was not feasible to complete any terminal experiments. Therefore, additional planned studies will include invasive catheterization measurements and investigating levels of cardiac biomarkers.

AUTHOR CONTRIBUTIONS

All authors participated in the research design and contributed to drafting the manuscript. SEK and JAM conducted experiments and performed data analysis. SEK, JR, and ABN contributed to the writing of the manuscript.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

DISCLOURE

ABN is a coinventor on a series of patents on the matter and use of the h2E2 monoclonal antibody. All other authors declare they have no conflicts of interest.

ETHICS STATEMENT

These studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals under a protocol approved by the Institutional Animal Care and Use Committee at the College of Medicine, University of Cincinnati.

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