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ORIGINAL RESEARCH

High-Dose Glucagon Has Hemodynamic Effects Regardless of Cardiac Beta-Adrenoceptor Blockade: A Randomized Clinical Trial

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BACKGROUND: Intravenous high-dose glucagon is a recommended antidote against beta-blocker poisonings, but clinical effects are unclear. We therefore investigated hemodynamic effects and safety of high-dose glucagon with and without concomitant beta-blockade.

METHODS AND RESULTS: In a randomized crossover study, 10 healthy men received combinations of esmolol (1.25 mg/kg bolus+0.75 mg/kg/min infusion), glucagon (50 µg/kg), and identical volumes of saline placebo on 5 separate days in random order (saline+saline; esmolol+saline; esmolol+glucagon bolus; saline+glucagon infusion; saline+glucagon bolus). On individual days, esmolol/saline was infused from –15 to 30 minutes. Glucagon/saline was administered from 0 minutes as a 2-minute intravenous bolus or as a 30-minute infusion (same total glucagon dose). End points were hemodynamic and adverse effects of glucagon compared with saline. Compared with saline, glucagon bolus increased mean heart rate by 13.0 beats per minute (95% CI, 8.0–18.0; $P<0.001$), systolic blood pressure by 15.6 mm Hg (95% CI, 8.0–23.2; $P=0.002$), diastolic blood pressure by 9.4 mm Hg (95% CI, 6.3–12.6; $P<0.001$), and cardiac output by 18.0 % (95% CI, 9.7–26.9; $P=0.003$) at the 5-minute time point on days without beta-blockade. Similar effects of glucagon bolus occurred on days with beta-blockade and between 15 and 30 minutes during infusion. Hemodynamic effects of glucagon thus reflected pharmacologic glucagon plasma concentrations. Glucagon-induced nausea occurred in 80% of participants despite ondansetron pretreatment.

CONCLUSIONS: High-dose glucagon boluses had significant hemodynamic effects regardless of beta-blockade. A glucagon infusion had comparable and apparently longer-lasting effects compared with bolus, indicating that infusion may be preferable to bolus injections.

REGISTRATION INFORMATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT03533179.

Key Words: beta blocker ■ glucagon ■ hemodynamics ■ toxicology

Glucagon is a peptide hormone secreted by the alpha cells in the pancreas. Glucagon is best known for its ability to increase glucose production in the liver, thereby controlling fasting blood glucose levels in balance with insulin.[1] Because of the glucoregulatory effect, pharmacological glucagon preparations are used to counteract acute insulin-induced hypoglycemia.[2] Effects of glucagon, however, exceed glucoregulation:[3,4] Data obtained from preclinical and uncontrolled clinical studies and case

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CLINICAL PERSPECTIVE

What Is New?

- This is the first randomized, placebo-controlled, clinical trial investigating the effects of glucagon in the high doses (50 μg/kg) recommended for the treatment of beta-blocker poisonings.
- In a randomized crossover trial, 10 healthy male participants received combinations of the beta-blocker esmolol, glucagon, or placebos on 5 separate trial days.
- Our results show that a 2-minute, high-dose glucagon bolus injection rapidly and significantly increased heart rate, blood pressure, and measures of cardiac contractility regardless of concomitant beta-blockade, and without causing serious adverse effects. Comparable maximal and longer-lasting hemodynamic effects were obtained with a 30-minute infusion of an identical dose of glucagon.

What Are the Clinical Implications?

- Intravenous high-dose glucagon is a recommended antidote against beta-blocker poisonings, but clinical effects are currently unclear because of a lack of controlled clinical trials.
- Procurement of enough glucagon in the emergency department for sustained hemodynamic support is a concern with glucagon therapy.
- Our results indicate that administration of high-dose glucagon infusion instead of repeated bolus injections might be preferable for hemodynamic support in beta-blocker–poisoned patients; the results have potential clinical implications because glucagon administered as an infusion instead of bolus requires less glucagon for the same hemodynamic effects to occur.

Nonstandard Abbreviations and Acronyms

bpm	beats per minute
GLP-1	glucagon-like peptide 1
iAUC	incremental area under the curve

reports have shown that intravenous administrations of glucagon in high doses under most circumstances lead to increases in heart rate, blood pressure (BP), and cardiac contractility.[3–8] This is the rationale behind the clinical use of glucagon as a treatment for hypotension and bradycardia caused by severe overdoses with beta-adrenoceptors (beta-blockers) and calcium channel blockers.[6,9] No randomized controlled trial has, however, investigated the glucagon doses used for toxicological emergencies[9,10] and our knowledge about the effects of high-dose glucagon alone and during beta-blockade in humans is thus limited. There are, in addition, only limited data on adverse effects of high-dose glucagon, and this topic is unlikely to be explored further because of a lack of current commercial interest. To provide human data on the clinical use of high-dose glucagon treatment during beta-blocker poisoning, we therefore conducted a randomized, placebo-controlled, clinical crossover trial. Ten healthy male trial participants received in random order combinations of the beta-blocker esmolol (used because of a favorable pharmacokinetic profile and vasodilatory properties in addition to beta-blocking effects), glucagon (administered intravenously as a 2-minute bolus or as a 30-minute infusion) and identical volumes of saline placebos on 5 separate trial days (day A: saline+saline; day B: esmolol+saline; day C: esmolol+glucagon bolus; day D: saline+glucagon infusion; day E: saline+glucagon bolus).

METHODS

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

Study Design and Inclusion Criteria

This randomized, single-blinded, 5-armed, placebo-controlled crossover trial was conducted at Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark, between September 2018 and September 2019. The study was conducted according to the Declaration of Helsinki,[11] and written and oral informed consent was received from participants before inclusion. Ten healthy male participants, 18 to 40 years of age, with normal body mass indices (≥18.5–≤25 kg/m^2) deemed healthy by investigator, with no regular use of medication, each completed the 5 trial days (A-E) in random order (Figure 1). Potential risk of carry-over effects was sought, minimized by a minimum 7-day washout period (deemed ample compared with the rapid elimination of glucagon and esmolol).[4,12] Upon withdrawal or exclusion, an enrolled participant was replaced with another, who was assigned an identical intervention sequence. Data from withdrawn or excluded participants were not analyzed. An interim evaluation of trial design and procedures was conducted after the 2 first enrolled participants had completed all 5 trial days. After the interim evaluation, the trial was continued without changes.

Procedures

Participants fasted from midnight the day before each trial day. A trial day started at the same time of day

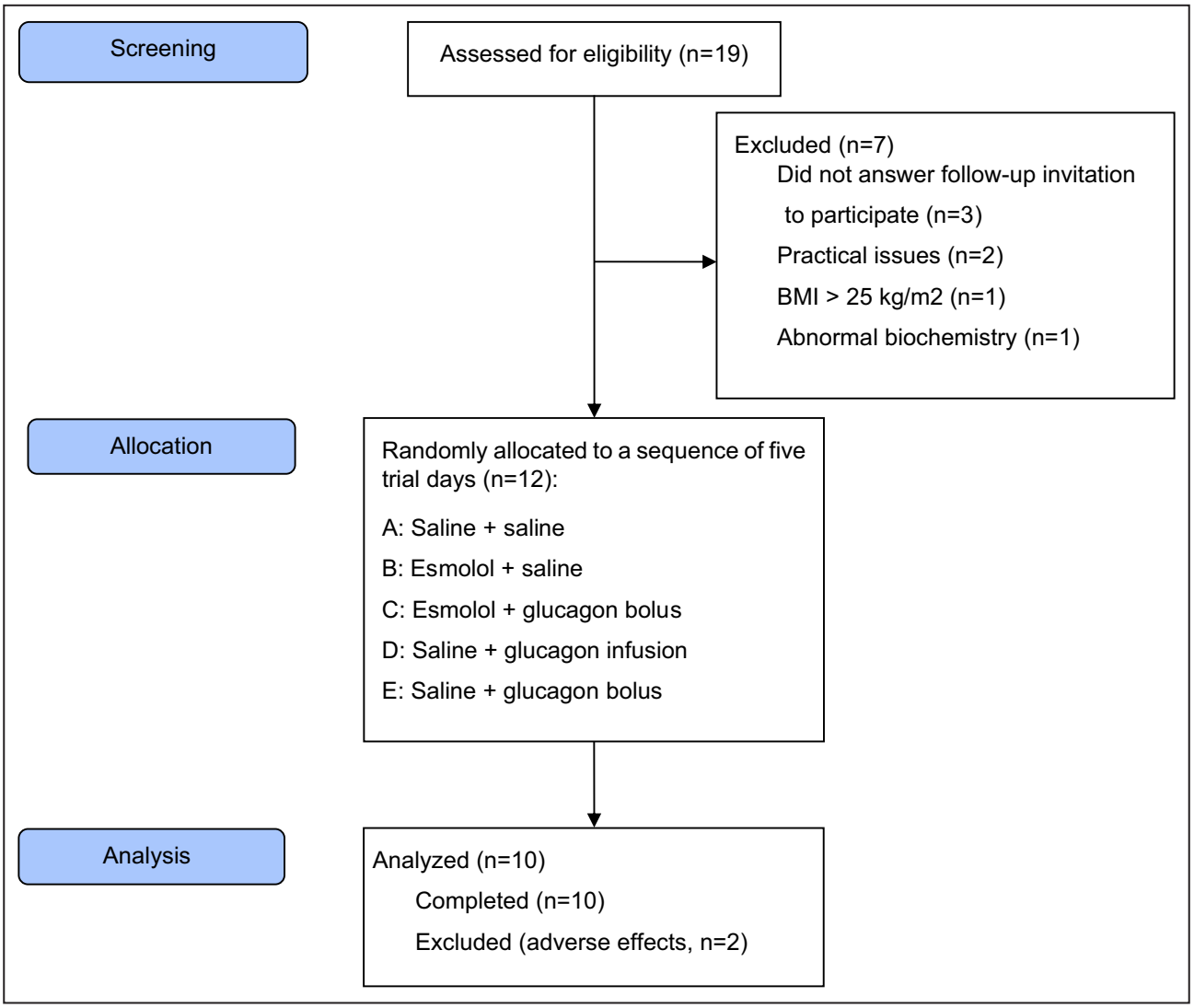

Figure 1. Flowchart of the trial and analyses of trial participants.
Nineteen potential participants were assessed, and 12 were allocated to a random sequence of interventions (**A-E**). In case of withdrawal or exclusion, an enrolled participant was replaced with another who was assigned to an identical intervention sequence. Ten participants completed 5 trial days and were included in analyses. Data from withdrawn or excluded participants were excluded from analyses.

for each participant, at 8 AM or at 11 AM. Participants were administered an 8-mg tablet of the serotonin 3 (5-HT_3) receptor antagonist ondansetron (Fresenius Kabi AG, Bad Homburg, Germany) on all trial days 60 minutes before glucagon/saline administration. A 5-lead ECG was recorded from –20 to 60 minutes as a safety measure. Participants were administered the following combinations of the beta-blocker esmolol, glucagon, and identical volumes of saline: trial day A: saline+saline; day B: esmolol+saline; day C: esmolol+glucagon(-bolus); day D: saline+glucagon(-infusion); day E: saline+glucagon(-bolus). Esmolol, 10 mg/mL (Brevibloc, Baxter, Deerfield, IL) was administered as a 1-minute, 1.25 mg/kg intravenous bolus at –15 minutes followed immediately by infusion of 0.75 mg/kg per minute to 30 minutes (Figure S1). Glucagon, 50 µg/kg (GlucaGen lyophilized glucagon, Novo Nordisk A/S, Bagsværd, Denmark) was (immediately before administration) reconstituted with 50 mL of isotonic saline with 1% human albumin (CSL Bering, Lyngby, Denmark) to reduce adhesion of glucagon to the inside of infusion sets. Glucagon was administered intravenously at baseline (0 minute) either as a 2-minute bolus or as a 30-minute infusion (day D) (Figure S1). For control infusions, identical volumes of saline were prepared with 1% human albumin. Infusions were administered using an Infusomat Space infusion pump (B. Braun Melsungen AG, Hessen, Germany). Hemodynamics were recorded every 5 seconds from timepoint –20 to 60 minutes

by a transducer connected to an arterial catheter inserted into the radial artery in the wrist. The transducer was connected to a S/3 Datex Ohmeda anesthesia monitor (GE Datex Ohmeda, Helsinki, Finland)[13] and the transducer was routinely zeroed before measurement start. Arterial pulse pressure waves were recorded every 10 milliseconds from the analog port in the monitor using in-house developed hardware and software. If the arterial line malfunctioned, heart rate data were obtained from the ECG recording. Ten participants were used in all analyses of heart rate except for day D, where data from one individual were discarded because of vomiting and premature discontinuation of infusions. On 5 of the 50 trial days, the arterial line could either not be placed or malfunctioned. Thus, BP, stroke volume, cardiac output, and systemic vascular resistance from 8 participants from day A; 9 from days B, D, and E, respectively (missing data from participants attributable to problems with the arterial line); and 10 from day C were analyzed. Blood was drawn for measurements of plasma glucagon at baseline and at 2, 4, 6, 10, 15, 20, 30, 40, 50, and 60 minutes (on day D at baseline and at 6, 10, 20, 30, 32, 34, 36, 40, 45, 50, and 60 minutes). Blood for measurements of plasma norepinephrine was drawn at baseline and at 4, 30, and 60 minutes. Vials were kept on ice before blood sampling, and samples were immediately pipetted, centrifuged, and stored at −80°C until analysis after trial conclusion (last participant's last visit).

End Points

The primary end point was the mean change in heart rate from baseline to the 5-minute time point on days with glucagon bolus compared with matching saline days. Secondary hemodynamic end points were heart rate, BP, cardiac output, stroke volume, and (post hoc) systemic vascular resistance at 3, 10, 15, 20, 30, 40, 50, and 60 minutes compared between corresponding glucagon and saline days. The relative stroke volume was calculated with the Liljestrand-Zander pulse pressure wave equation: $\text{Stroke volume} = k \times \left(\frac{\text{pulse pressure}}{\text{systolic BP} + \text{diastolic BP}} \right)$.[14,15] Systemic vascular resistance was calculated with the formula: $\frac{\text{Mean arterial pressure}}{\text{Cardiac output}}$. Two-minute averages of hemodynamic end points were used to adjust for fluctuations (eg, time point 3 minutes = time point 1–3 minutes, time point 5 minutes = time point 3–5 minutes). Plasma glucagon and norepinephrine were measured at the above-specified times of blood sampling. Nausea was verbally scored by participants at time 6, 10, 30, and 60 minutes, using a scoring scale used to verbally rate nausea from 0 (no nausea) to 6 (the worst nausea imaginable). Participants were also encouraged to immediately report any sensation of discomfort during the trial. To compare the hemodynamic effects of glucagon bolus with infusion of an identical dose of glucagon, the integrated responses (expressed as incremental [baseline subtracted] area under the curves [iAUCs]) of heart rate and BP parameters were compared between days D and E. Likewise, to compare the acute hemodynamic effects of glucagon bolus with and without esmolol (day C versus day E), the iAUCs of heart rate and BP from baseline to the 10-minute time point were calculated and compared.

Power

A heart rate change of approximately 10 beats per minute (bpm) after intravenous administration of 50 μg of glucagon has been observed previously.[4] Population size (N) was calculated using the formula: $\text{Power} = pt\left(qt(0.025, n-1, 0), n-1, -\left(\frac{\mu 1-\mu 2}{\Sigma}\right) \times \sqrt{N}\right)$,[16] where μ1–μ2 is the expected 10-bpm difference between glucagon and saline, and N is the number of participants. Based on the expected 10-bpm difference, a 2-sided significance (α) of 0.05, a power (1 – β) of 0.08, and an estimated SD of the difference (Σ) between 2 experimental days for the same participant of 7 bpm, the formula results in an 87% probability of detecting a difference of 10 bpm when enrolling 8 participants. We included 10 participants to increase power in terms of exploring end points.

Randomization and Blinding

Before recruitment began, a list allocating participant numbers to a random intervention sequence (1–10 in random order)[17] was generated by personnel not otherwise involved in the trial. After inclusion by the investigator, participants were consecutively assigned a number corresponding to a random intervention sequence on the list. Personnel checked the list before each trial day to ensure that participant and visit numbers matched the list. Participants and the outcome assessor (for measures of plasma norepinephrine) were blinded. The investigator was not blinded; this was not possible because of the nature of effects and particularly adverse effects of the infusions. Study drugs and saline were visually identical but were nonetheless handled and administered behind a curtain to ensure participant blinding.

Statistical Analysis

Mean baseline subtracted changes in hemodynamic values after 5 minutes on days with glucagon compared with matching saline (day A versus day E and day B versus day C) (the primary end point) were compared with the paired t-test using Prism

version 8.0.0 (GraphPad Software, San Diego, CA). Differences in secondary hemodynamic end points compared between days with glucagon and corresponding saline (absolute values) were analyzed by a mixed model (PROC MIXED) with fixed (intervention, time points) and random (ID) variables, using LSMEANS to estimate least square means in SAS Enterprise 7.1 (SAS Institute Inc, Cary, NC). Multiple comparisons were adjusted using Tukey's test, and missing values (completely at random) were handled by maximum likelihood estimation. Differences in plasma norepinephrine were compared between corresponding days using a mixed model fitted in GraphPad Prism with Sidak correction of multiple comparisons. iAUCs (day E versus day D and day C versus day E) from baseline were calculated for heart rate and BP parameters using the trapezoidal rule (ie, the area under the curve approximated as a trapezoid of units per minute) and compared using the paired t-test. Stroke volume, cardiac output, and systemic vascular resistance obtained from the arterial pulse pressure wave were unitless. Therefore, the relative change from baseline (expressed as a percentage) were compared between days with glucagon and corresponding saline. Values relative to baseline were base-*e* log-transformed for analyses and back-transformed for reporting geometric means and 95% CIs. For all comparisons, two-sided *P* values < 0.05 were considered statistically significant.

Study Approval

The trial was approved by the Research Ethics Committee of the Capital Region of Denmark (journal number: H-17019944), the Danish Medicines Agency (journal number: 2017064670), and the Danish Data Protection Agency (journal number: 2012-58-0004/BFH-2017-093).

RESULTS

Participant Flow

Nineteen potential participants were screened between September 2018 and July 2019; 12 were included (1 later withdrew because of nausea and 1 was excluded at the discretion of the investigator because of vomiting), and 10 completed the trial (Figure 1, Table 1). There was an average 17-day washout period between trial days (total range, 7–96 days).

Table 1. Baseline (Screening) Demographics

	Mean	SD
Age, y	23.6	1.7
Weight, kg	71.7	6.5
Height, cm	181.7	6.7
Body mass index, kg/m^2	21.7	1.3
Systolic blood pressure, mm Hg	124.4	9.4
Diastolic blood pressure, mm Hg	70.4	6.5
Heart rate (beats per minute)	60.8	9.1

Plasma Glucagon Concentrations

On the 2 days with glucagon bolus, mean plasma glucagon concentrations increased to 81.2 nmol/L (95% CI, 53.3–109.1) and 106.6 nmol/L (95% CI, 75.4–137.8), respectively, after 2 minutes, from 7 pmol/L on average at baseline. Concentrations at the 4-minute time point were 107.9 (95% CI, 79.9–135.8) and 110.2 (95% CI, 84.2–136.2) nmol/L, respectively (Figure 2A). Mean glucagon concentrations stayed in elevated ranges of 28.3 and 24.5 nmol/L (range, 16.0–40.7 nmol/L) at the 10-minute time point, and decreased from> 9 nmol/L on average at 15 minutes to a supraphysiological range of 2–300 pmol/L at 60 minutes. Glucagon infusion increased mean glucagon concentrations to 28.2–26.6 nmol/L (95% CI, 19.8–35.5) 20–30 minutes after infusion start (Figure 2A). Mean glucagon concentration had decreased to 17.7 nmol/L (95% CI, 14.8–20.6) 2 minutes after stop of infusion and to 361 pmol/L (95% CI, 276–447) at 60 minutes (Figure 2A). The plasma glucagon iAUC was significantly –110.6 nmol/L × min lower on the day with infusion compared with bolus (95% CI, –217.0 to –4.2; *P* = 0.04, Figure 2B). On days without glucagon administrations, mean plasma glucagon concentrations remained unchanged at basal levels (8 pmol/L on average; total range, 1–37) (data not shown).

Heart Rate

Days without esmolol (days A, D, and E): Compared with saline, the glucagon bolus increased mean heart rate 13.0 bpm (95% CI, 8.0–18.0 bpm; *P* < 0.001) after 5 minutes from 55.5 bpm (95% CI, 51.2–59.9 bpm) at baseline (0 min) (Table S1, Figure 3A and 3B). Differences in mean heart rate between glucagon bolus and saline were statistically significant at 3 and 5 minutes after infusion start (Figure 3, Table S2). Glucagon infusion significantly increased mean heart rate by 13.2 to 15.6 bpm 15 to 30 minutes after start of infusion (*P* < 0.01) (Figure 3A and 3B) and the heart rate incremental iAUC was significantly larger with glucagon infusion compared with glucagon bolus (mean difference, 237.3 bpm × min; 95% CI, 33.0–441.7 bpm × min; *P* = 0.03). On days with esmolol (days B and C), the average heart rate was 58.2 bpm at baseline (Table S1). Compared with saline, the glucagon bolus increased mean heart rate 9.2 bpm (95% CI, 3.3–15.2 bpm; *P* = 0.006) after 5 minutes (Table S1, Figure 3C and 3D). Differences in heart rates between days with glucagon and saline were statistically significant from 3 to 10 minutes after infusions (9.9 bpm; *P* < 0.001 and 6.7 bpm; *P* = 0.04, respectively) (Table S2, Figure 3C and 3D). Relative percentage changes

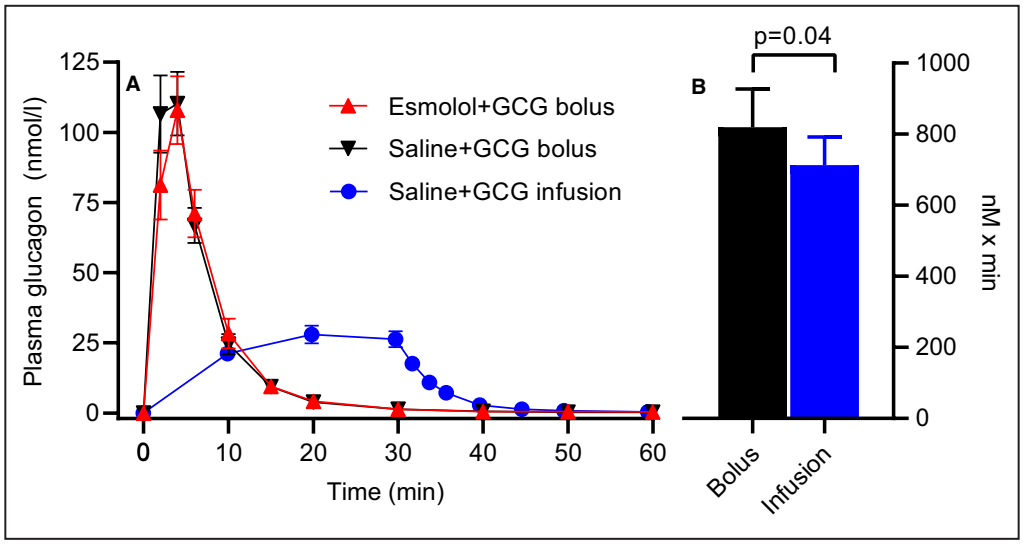

Figure 2. Intravenous glucagon bolus and infusion results in glucagon concentration–time curves coinciding with hemodynamic effects.
(**A**) Plasma glucagon concentrations (nmol/L) presented as means ± SEM (red, esmolol + glucagon bolus; black, saline + glucagon bolus; blue, saline + glucagon infusion). Days without glucagon administrations (and glucagon concentrations at basal levels of 8 pmol/L on average) are not shown. Measures before baseline are moved forward to baseline (the 0-minute time point). (**B**) Plasma glucagon incremental area under the curve (iAUC) on the day with glucagon infusion (blue) was significantly lower compared with the AUC on the saline + glucagon bolus-day (black). iAUCs were compared with the paired t-test.

in heart rate from baseline on all trial days are shown in Figure S2.

Systolic Blood Pressure

On days without esmolol, the glucagon bolus increased mean systolic BP 15.6 mm Hg (95% CI, 8.0–23.2 mm Hg; P = 0.002) compared with saline after 5 minutes, from 128.1 mm Hg on average at baseline (Table S1, Figure 4A and 4B). The increase in systolic BP after glucagon remained significant (17.2 mm Hg, P<0.001) compared with saline until the 10-minute time point. Glucagon infusion increased the systolic BP significantly 15.9 to 21.5 mm Hg between 10 and 30 minutes after start of infusion (P < 0.01) (Figure 4A and 4B). The systolic BP iAUC was insignificantly larger with glucagon infusion compared with bolus (mean difference, 226.2 mm Hg × min; 95% CI, –165.4–617.9 mm Hg × min; P = 0.21). On the 2 days with esmolol infusion, mean systolic BP decreased from 127.5 mm Hg at the –15-minute time point to 110.8 mm Hg at baseline. Compared with saline, the glucagon bolus increased mean systolic BP 16.2 mm Hg (95% CI, 10.7–21.6 mm Hg; P < 0.001) after 5 minutes (Table S1, Figure 4C and 4D). The 18.3 mm Hg difference between glucagon and corresponding saline observed 10 minutes from baseline was statistically significant (P = 0.001) (Table S2, Figure 4C and 4D). Relative percentage changes in systolic BP from baseline shown in Figure S2.

Diastolic Blood Pressure

On days without esmolol infusion, the glucagon bolus increased mean diastolic BP 9.4 mm Hg (95% CI, 6.3–12.6 mm Hg; P < 0.001) after 5 minutes, from 66.7 mm Hg (95% CI, 61.9–72.0 mm Hg) at baseline (Table S1, Figure 5A and 5B). Differences between glucagon and saline were statistically significant also after 3 minutes (5.8 mm Hg, P = 0.02) and 10 minutes (11.8 mm Hg, P < 0.001) (Table S2, Figure 5A and 5B). Glucagon infusion increased average diastolic BP by 8.6 to 9.2 mm Hg 10 to 30 minutes after start of infusion (P < 0.01) (Figure 5A and 5B). On days with esmolol, the glucagon bolus increased diastolic BP by 12.9 mm Hg (95% CI, 7.0–18.2 mm Hg; P < 0.001) after 5 minutes compared with saline (Table S1, Figure 5C and 5D). The approximate 12 mm Hg differences in mean heart rate between glucagon and saline 3 and 10 minutes after start of injections were statistically significant (P < 0.001) (Table S2, Figure 5C and 5D). Relativepercentage changes in diastolic BP from baseline are shown in Figure S2.
Changes in mean arterial pressure are included in Table S3 and S4 and Figure S3.

Acute Hemodynamic Effects of Glucagon Bolus With and Without Esmolol Pretreatment

The iAUCs of acute effects elicited by glucagon on heart rate and BP parameters up to the 10-minute time

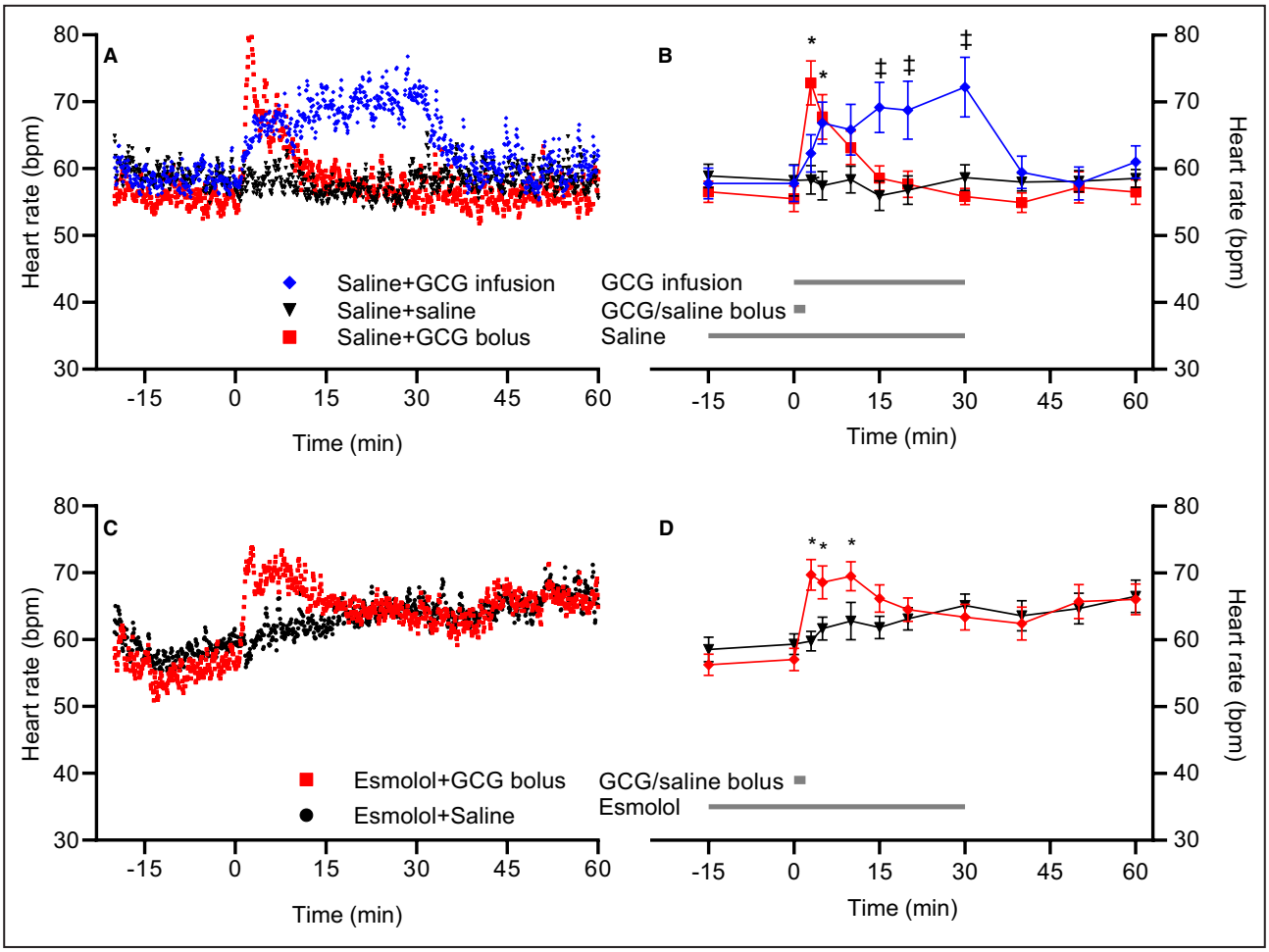

Figure 3. Glucagon injections has positive chronotropic effects regardless of beta-blockade.
(**A**) Scatter plot of 5-second heart rate (beats per minute) means on trial days without esmolol (blue, glucagon infusion; black, saline; red, glucagon bolus). (**B**) Two-minute heart rate means ± SEM on days without esmolol. (**C**) Scatter plot of 5-second heart rate means on trial days with esmolol (black, saline; red, glucagon bolus). (**D**) Two-minute heart rate means ± SEM on days with esmolol. Horizontal gray lines mark durations of infusions and boluses. *Statistically significant difference between glucagon bolus and corresponding saline. ‡Statistically significant difference between glucagon infusion and saline. Differences were analyzed by a mixed model with Tukey correction of multiple comparisons (baseline subtracted changes to the 5-minute time point were compared using the paired t-test).

point were not significantly different when comparing days with and without esmolol (day C versus day E; *P*=0.16–0.98) (Figure S4).

Stroke Volume

On days without esmolol, the glucagon bolus insignificantly decreased stroke volume after 3 and 5 minutes, followed by a significant 7.4% to 8.9% increase after 20 to 40 minutes compared with saline (*P* = 0.002–0.03) (Table S5, Figure 6A). Esmolol reduced mean stroke volume 13.7% and 25.8% from –15 minutes to baseline on days B and C, respectively (Figure 6B). Compared with saline, the glucagon bolus increased stroke volume insignificantly from baseline by 3.8% to 12.9% between 10 and 30 minutes (95% CI, –23.9 to 10.2) (Table S5, Figure 6B).

Cardiac Output

On days without esmolol, compared with saline, glucagon bolus increased cardiac output 18.0% (95% CI, 9.7–26.9; *P* = 0.003) after 5 minutes (Figure 6C). There was a higher cardiac output at all time points with glucagon compared with saline, which was statistically significant between 3 and 15 minutes after start of injections (27.5–14.7 %; *P* < 0.001–0.04) (Table S5, Figure 6C). The glucagon infusion significantly increased mean cardiac output by 27.9% to 23.7% 15 to 30 minutes after start of injections (*P* < 0.001) (Figure 6C). On days with esmolol, cardiac output was insignificantly 15.8% to 17.2% higher 10 to 20 minutes after start of injections on days with glucagon compared with saline (*P* = 0.7–0.9) (Table S5, Figure 6D).

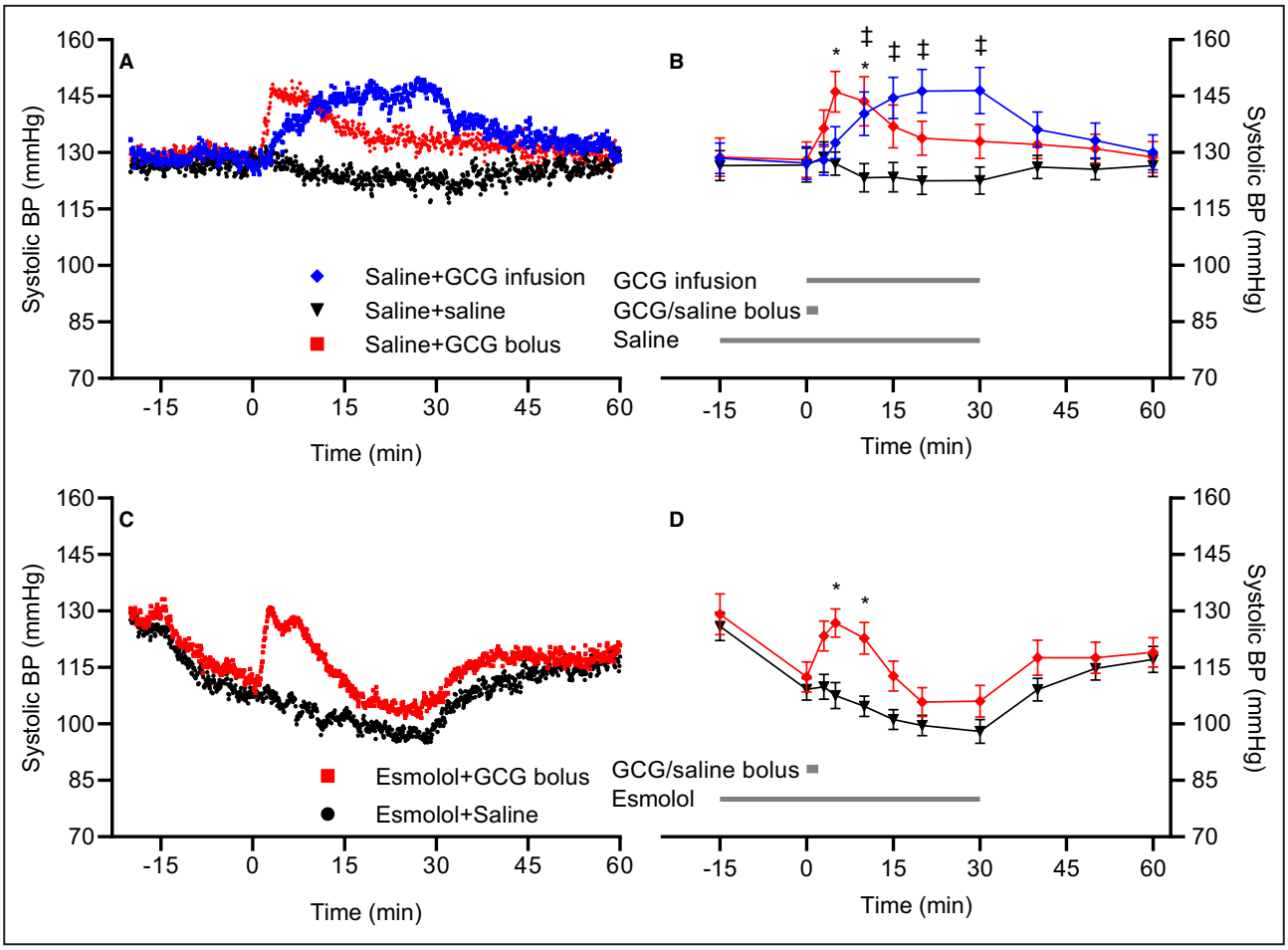

Figure 4. Glucagon injections increase systolic blood pressure (BP) regardless of beta-blockade.
(**A**) Scatter plot of 5-second systolic BP (mm Hg) means on trial days without esmolol (blue, glucagon infusion; black, saline; red, glucagon bolus). (**B**) Two-minute systolic BP means ± SEM on days without esmolol. (**C**) Scatter plot of 5-second systolic BP means on trial days with esmolol (black, saline; red, glucagon bolus). (**D**) Two-minute systolic BP means ± SEM on days with esmolol. Horizontal gray lines mark durations of infusions and boluses. *Statistically significant difference between glucagon bolus and corresponding saline. ‡Statistically significant difference between glucagon infusion and saline. Differences were analyzed by a mixed model with Tukey correction of multiple comparisons (baseline subtracted changes to the 5-minute time point were compared using the paired t-test).

Systemic Vascular Resistance

Compared with saline, the glucagon bolus decreased systemic vascular resistance after 3 minutes (14.4%; 95% CI, 8.3–20.0; P = 0.003) on days without esmolol. Systemic vascular resistance remained lower with glucagon bolus compared with saline, but between-day differences after 3 minutes were insignificant (Table S5, Figure 7A). Compared with saline, the glucagon infusion insignificantly reduced systemic vascular resistance like the bolus between the 5- and 60-minute time points. Likewise, on days with esmolol, mean systemic vascular resistance was insignificantly lower on days with glucagon compared with saline 10 to 60 minutes after start of injection (Table S5, Figure 7B).

Plasma Norepinephrine Concentrations

No differences in plasma norepinephrine concentrations between days with glucagon and corresponding saline infusions at any time point regardless of whether esmolol administration were observed (P = 0.56–0.99) (Figure S5). On all trial days, peak plasma norepinephrine concentrations were reached at the 30-minute time point: Without esmolol pretreatment, mean plasma norepinephrine increased 0.331 nmol/L to the 30-minute time point (from 0.615 nmol/L at baseline), compared with a 0.218 nmol/L increase on the saline day. The plasma norepinephrine versus time curves were similar in response to glucagon infusion and bolus (Figure S5A). On both esmolol days, mean norepinephrine levels increased 0.638 and 0.633 nmol/L, respectively, from –20 to 30 minutes (Figure S5B). Norepinephrine concentrations returned toward baseline values at the 60-minute time point.

Adverse Effects

Nausea was the most common adverse effect despite administration of 8 mg of ondansetron at the

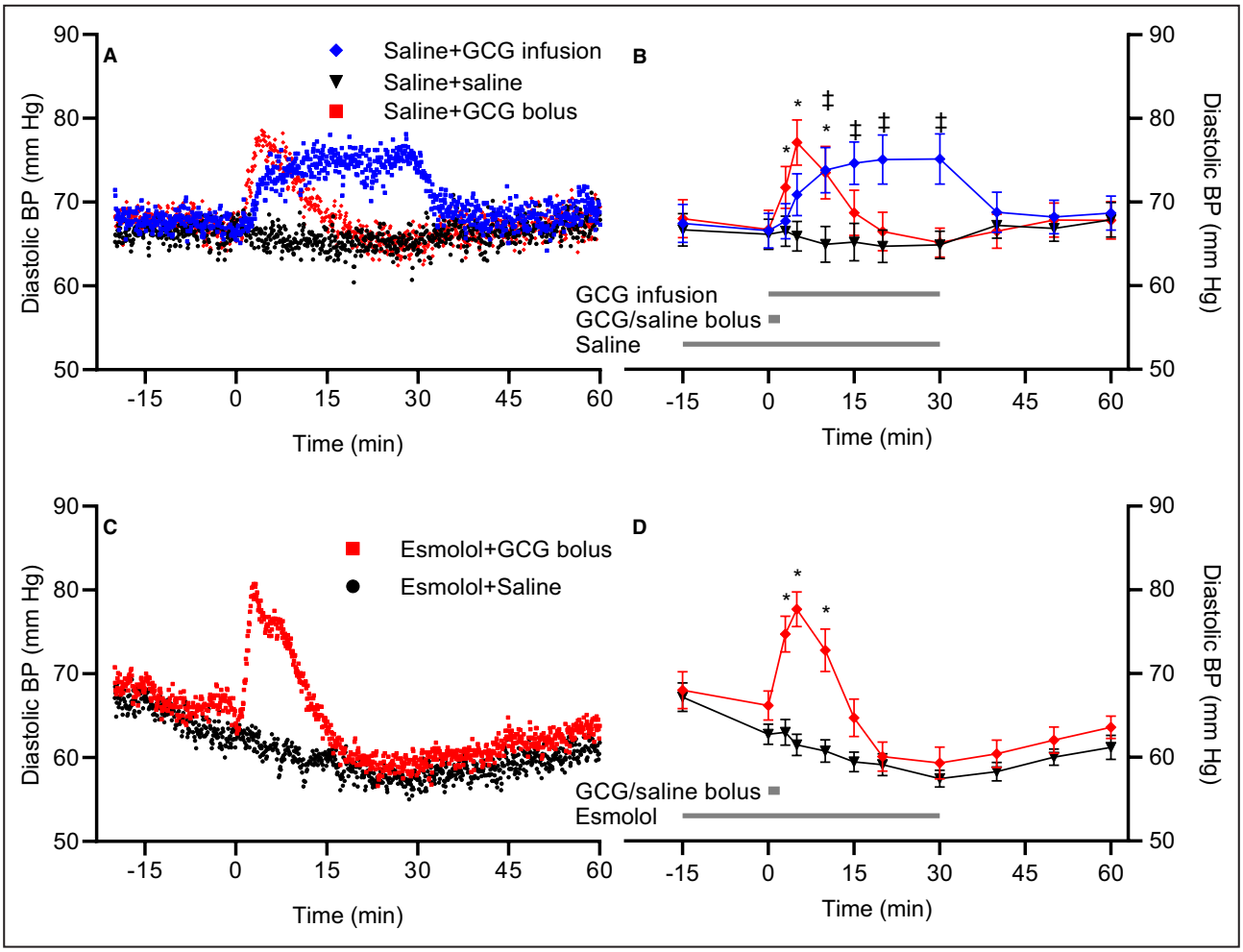

Figure 5. Glucagon injections increase diastolic blood pressure (BP) regardless of beta-blockade.
(**A**) Scatter plot of 5-second diastolic BP (mm Hg) means on trial days without esmolol (blue, glucagon infusion; black, saline; red, glucagon bolus). (**B**) Two-minute diastolic BP means ± SEM on days without esmolol. (**C**) Scatter plot of 5-second diastolic BP means on trial days with esmolol (black, saline; red, glucagon bolus). (**D**) Two-minute diastolic BP means ± SEM on days with esmolol. Horizontal gray lines mark durations of infusions and boluses. *Statistically significant difference between glucagon bolus and corresponding saline. ‡Statistically significant difference between glucagon infusion and saline. Differences were analyzed by a mixed model with Tukey correction of multiple comparisons (baseline subtracted changes to the 5-minute time point were compared using the paired t-test).

–60-minute time point on all trial days. Except for 1 incidenct after 6 minutes on a day with saline alone, nausea was reported only on glucagon days. On days with glucagon bolus, nausea was reported after 2 minutes by 4 of 10 and 5 of 10 participants, respectively. Eight of 10 and 7 of 10 participants reported nausea after 6 minutes (average score, 2.8 [range, 1–6] and 2.4 [range 1–4], respectively), and in 1 participant nausea was sustained to the 30-minute time point. In comparison, during glucagon infusion, nausea was reported by 5 of 10 participants at 6 minutes (average score 1.8; range, 1–3) and by 5 of 10 participants at 30 minutes (average score, 2.2; range, 1–4), 4 of whom had reported nausea at 6 minutes. Two participants experienced considerable nausea and vomiting, and their glucagon infusions were stopped at 8 and 17 minutes, respectively. Any nausea lasted <30 minutes. No other gastrointestinal side effects and no serious adverse effects occurred (Table S6).

DISCUSSION

An intravenous bolus of high-dose glucagon significantly increased mean heart rate and BP 3 to 5 minutes after start of injection compared with saline irrespective of concomitant infusion of a beta-blocker. Glucagon also increased cardiac output and stroke volume while lowering vascular resistance regardless of beta-blocker infusion.

Glucagon bolus increased BP within 3 to 5 minutes despite reducing systemic vascular resistance

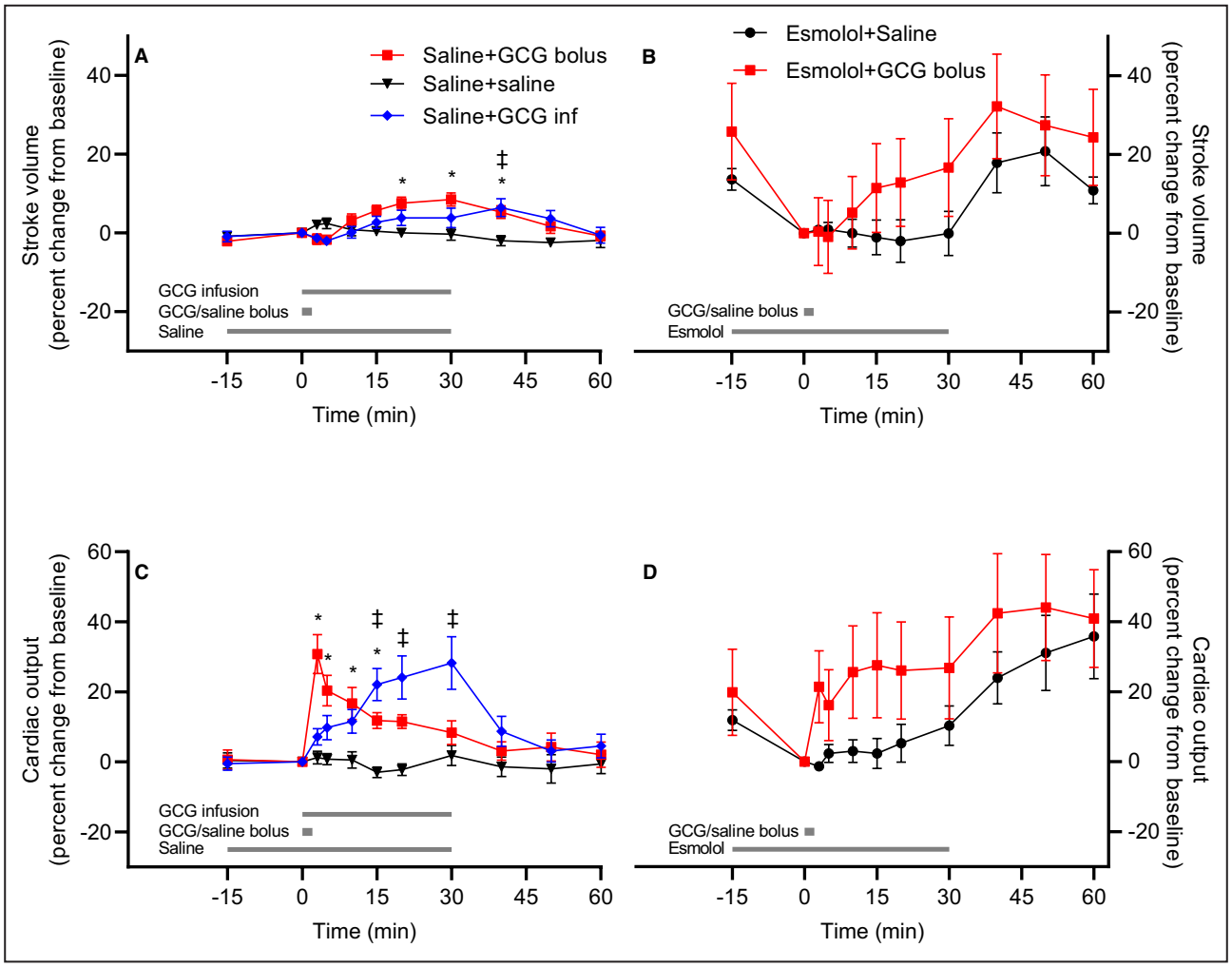

Figure 6. Glucagon injections increase stroke volume and cardiac output.
(**A**) Two-minute stroke volume means ± SEM (depicted as percentage change from baseline) on days without esmolol (blue, glucagon infusion; black, saline, red: glucagon bolus) (**B**) Two-minute means ± SEM of stroke volume percentage change from baseline on days with esmolol (red, glucagon bolus; black, saline). (**C**) Two-minute means ± SEM of cardiac output percentage change from baseline on days without esmolol (blue, glucagon infusion; black, saline; red, glucagon bolus) (**D**) Two-minute means ± SEM of cardiac output percentage change from baseline on days with esmolol (red, glucagon bolus; black, saline). Horizontal gray lines mark durations of infusions and bolus. *Statistically significant difference between glucagon bolus and corresponding saline. ‡Statistically significant difference between glucagon infusion and saline. Differences were analyzed by a mixed model with Tukey correction of multiple comparisons.

and not increasing stroke volume. This indicates that the BP increase was mainly driven by the approximate 20% increase in heart rate followed by a 20% increased cardiac output at the 5-minute time point. The hemodynamic effects reflected the plasma glucagon versus time curve, except for the more delayed increase in stroke volume. Effects of glucagon infusion were almost identical to those of the bolus but appeared after approximately 10 minutes. Integrated hemodynamic responses expressed as iAUCs were all numerically higher with glucagon infusion compared with bolus, and there was no indication of tachyphylaxis/desensitization during the infusion in contrast with previous observations.[18,19] This altogether signifies that the glucagon concentration necessary for maximal hemodynamic responses was reached by the infusion, and this was much lower than the approximate mean maximum plasma concentration of 100 nmol/L attained by a glucagon bolus. Nausea caused by glucagon occurred despite ondansetron administration in 70% to 80% of the participants on days with a glucagon bolus shortly after start of injections, and apparently more prolonged nausea occurred in 50% of the participants on the glucagon infusion day. This common prevalence of nausea agrees with most available literature.[4,6] The rapid onset and course apparently coinciding with the plasma glucagon concentration versus time

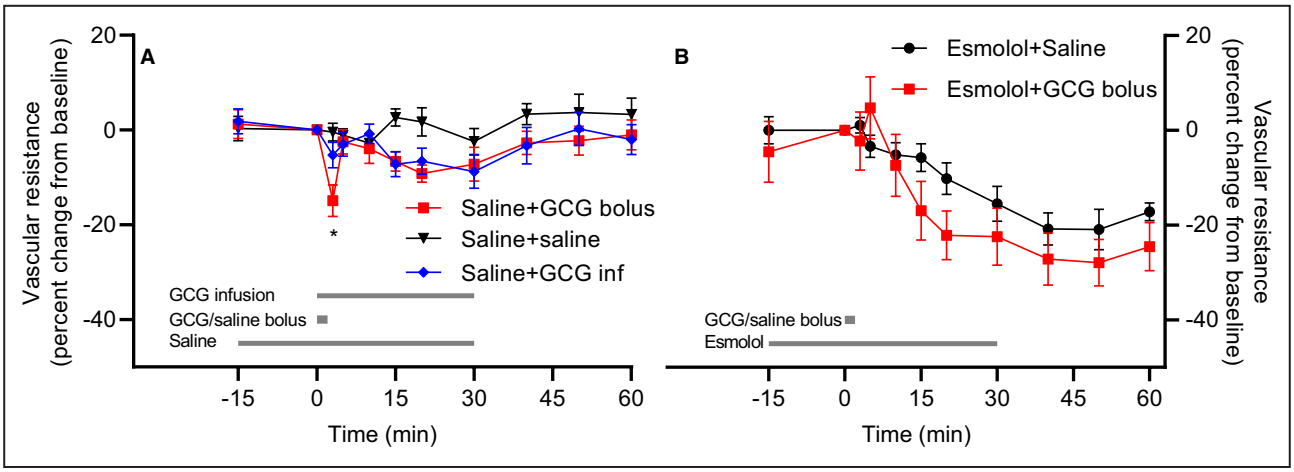

Figure 7. Glucagon injections reduce relative systemic vascular resistance.
(**A**) Relative systemic vascular resistance (percentage change from baseline) over time depicted as 2-minute means ± SEM on trial days without esmolol (blue, glucagon infusion; black, saline; red, glucagon bolus). (**B**) Two-minute means ± SEM of systemic vascular resistance percentage change from baseline on days with esmolol (red, glucagon bolus; black, saline). Horizontal gray lines mark durations of infusions and boluses. *Statistically significant difference between glucagon and corresponding saline. Differences were analyzed by a mixed model with Tukey correction of multiple comparisons.

curve is consistent with a direct nausea-inducing effect on the brain by glucagon, as opposed to nausea secondary to delayed gastric emptying or distention evoked by prolonged glucagon infusions.[20]

The glucagon bolus decreased mean stroke volume (insignificantly) and systemic vascular resistance for 3 to 5 minutes after the injection on days without esmolol. The reduced systemic vascular resistance by glucagon has been ascribed to a decreased sympathetic tone secondary to the improved cardiac performance in patients with heart failure.[21] In our healthy trial participants, however, the initial decrease in systemic vascular resistance coincided with a rapid increase in heart rate (and cardiac output). This resulted in a BP increase, which possibly led to a counterbalancing (reflex) reduction in vascular resistance to maintain hemodynamic homeostasis. In addition, a vasodilatory effect of glucagon caused by direct stimulation of vascular glucagon and glucagon-like peptide 1 (GLP-1) receptors has been documented in rat arterial tissues,[22] and our results might therefore reflect a direct effect of glucagon on vascular tone. On esmolol days, the systemic vascular resistance did not decrease abruptly because of glucagon, most likely because of the esmolol-induced decreased systemic vascular resistance (in turn decreasing the afterload) and BP.

Plasma norepinephrine maximum plasma concentration was observed at the 30-minute time point on all days. Changes in plasma levels of norepinephrine, the principal sympathetic neurotransmitter,[23,24] reflect changes in sympathetic activity attributable to spillover from synaptic clefts. Norepinephrine is central for cardiovascular tone via alpha agonism; however, it also activates to some degree beta receptors, which convey the positive inotropic and chronotropic effects of catecholamines. Glucagon bolus did not alter plasma norepinephrine concentrations significantly compared with saline irrespective of concomitant esmolol infusion. This, together with the similar effects on heart rate and BP regardless of cardiac beta 1 receptor blockade, contradict that hemodynamic effects of glucagon are conveyed through activation of the sympathetic nervous system.

Glucagon's cardiac action has classically been attributed to a direct effect on the heart through activation of myocardial glucagon receptors.[6] In a recent study, no expression of glucagon receptors could be detected in biopsies taken from any of the 4 chambers of the human heart,[25] but whether there is expression in the sinoatrial node remains unknown. Therefore, a direct effect on the human sinoatrial node by high-dose glucagon remains hypothetical but would be a mechanistically plausible main driver of the observed effects of glucagon bolus. Importantly, glucagon is a full, low-affinity agonist on the GLP-1 receptor with 50% receptor activation occurring at 4.9 nmol/L, which is well below the plasma glucagon concentration we obtained (~100 nmol/L after bolus administration and ~25 nmol/L after infusion).[26] The receptor for GLP-1 is expressed in human myocardium and perhaps also in the sinoatrial node,[25,27] and administration of GLP-1 (and GLP-1 analogs) increases heart rate and cardiac output.[3,28] Thus, activation of cardiac GLP-1 receptors by high-dose glucagon may be a major contributor to the observed stimulatory effects on the heart as observed in the present study.

Glucagon has been used as an antidote for systemic beta-blocker toxicity for nearly 50 years. It has rarely

been the only therapy for beta-blocker poisoning, reports of no response to glucagon therapy also exist, and there is no evidence supporting that glucagon has beneficial effects on survival after beta-blocker poisoning.[9,10] Several published papers, however, describe favorable outcomes in beta-blocker–poisoned patients after normalization of hemodynamics by glucagon injection(s).[8,10] The observed hemodynamic effects of glucagon, even when including the counteracting effects of a beta-blocker, might therefore have clinical relevance.

Limitations to this study include that participants were healthy, young male volunteers who were administered intravenous beta-blocker infusions. This limits translation of our results to incidents of life-threatening overdoses. For technical reasons, we did not measure plasma epinephrine, which—compared with norepinephrine—is more associated to the response of the hypothalamic-pituitary-adrenocortical axis than of the sympathetic nervous system.[29] The strengths of this study are the randomized, placebo-controlled crossover design and the invasive hemodynamic data recording. To our knowledge, there are no previous controlled studies documenting the hemodynamic effects and safety of the glucagon dose recommended for beta-blocker poisonings. Our study therefore provides novel information about the effects of high-dose glucagon alone and with a simultaneous beta-blocker infusion. To increase generalizability, esmolol was administered in a dose sufficient to block >85% of cardiac beta 1 receptors.[12] Esmolol was also chosen from a safety perspective because of its short half-life. In addition to beta-blocking characteristics, esmolol has vasodilatory and hypotensive effects in high doses,[30] which in our study resulted in a BP reduction and a reflex heart rate increase. This phenomenon is sometimes observed after toxicologic doses of other beta-blockers.[31]

In conclusion, intravenous high-dose glucagon administrations significantly increased heart rate, BP, and measures of cardiac contractility while reducing the vascular tone. Heart rate and BP were increased by glucagon in a plasma concentration–dependent fashion regardless of beta-blockade. Nausea caused by glucagon occurred in most of the participants despite pretreatment with ondansetron, but glucagon caused no serious adverse effects. The rapid onset of the increases in heart rate and BP by glucagon bolus injections might indicate a direct action of high-dose glucagon on the sinoatrial node, perhaps partly or fully mediated via the GLP-1 receptor. Hemodynamic effects like those achieved by bolus injections occurred 10 to 15 minutes after the start of the 30-minute glucagon infusion (same total dose) and lasted for the duration of the infusion. This has potential clinical relevance, as glucagon is usually in limited stock in the emergency department, while using an infusion (perhaps including an initial loading dose) will require less glucagon than repeated bolus administrations for comparable hemodynamic effects to occur.

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Disclosures

None.

Supplementary Material

Tables S1–S6
Figures S1–S5

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Supplemental Material

Table S1. Mean changes in hemodynamic parameters from baseline to the five-minute (min) timepoint.

Trial day:	A	E	B	C	D
			Heart rate (beats per min)		
T-15 min (mean±95% CI)	58.9 (54.9-62.9)	56.6 (52.9-60.1)	58.6 (54.4-62.7)	56.3 (52.6-59.9)	57.8 (52.6-63.0)
Baseline (T0) (mean±95% CI)	58.3 (53.5-63.6)	55.5 (51.2-59.9)	59.3 (55.8-62.9)	57.1 (53.2-60.8)	57.8 (51.7-63.9)
T5 min (mean±95% CI)	57.5 (52.6-62.3)	67.7 (60.2-75.3)	61.7 (57.8-65.5)	68.6 (63.1-74.2)	66.8 (59.8-73.9)
Change from baseline (mean±SEM)	-0.8 (1.0)	12.2 (2.6)	2.3 (1.6)	11.6 (1.5)	9.1 (1.8)
Mean difference ±95% CI (A versus E; B versus C)	13.0 (8.0-18.0) p<0.001		9.2 (3.3-15.2) p=0.006		
			Systolic blood pressure (BP) (mm Hg)		
T-15 min (mean±95% CI)	126.5 (116.9-136.0)	128.1 (117.1-140.5)	125.9 (117.3-134.6)	129.1 (116.9-141.3)	128.5 (119.3-137.7)
Baseline (T0) (mean±95% CI)	126.6 (115.9-137.3)	128.1 (117.3-138.9)	109.2 (102.6-115.8)	112.5 (103.4-121.5)	127.2 (117.3-137.1)
T5 min (mean±95% CI)	127.0 (119.6-134.4)	146.1 (133.6-158.7)	107.6 (99.6-115.5)	126.8 (118.4-135.3)	132.6 (122.9-142.4)
Change from baseline (means±SEM)	0.4 (2.1)	18.0 (1.9)	-1.6 (1.4)	14.4 (1.4)	5.5 (2.0)
Mean difference ±95% CI (A versus E; B versus C)	15.6 (8.0-23.2) p=0.002		16.2 (10.7-21.6) p<0.001		
			Diastolic BP (mm Hg)		
T-15 min (mean±95% CI)	66.7 (62.1-71.3)	68.0 (62.8-73.2)	67.2 (63.2-71.1)	68.0 (63.0-72.9)	67.5 (62.3-72.6)
Baseline (T0) (mean±95% CI)	66.1 (61.8-70.4)	66.7 (61.9-72.0)	62.8 (60.0-65.5)	66.2 (62.2-70.1)	66.6 (61.8-71.3)
T5 min (mean±95% CI)	65.9 (61.7-70.1)	77.1 (70.9-83.4)	61.4 (58.6-64.4)	77.7 (73.0-82.4)	70.1 (65.2-76.5)
Change from baseline (mean±SEM)	-0.2 (0.7)	10.4 (1.3)	-1.3 (0.7)	11.5 (2)	4.2 (1.2)
Mean difference ±95% CI (A versus E; B versus C)	9.4 (6.3-12.6) p<0.001		12.9 (7.0-18.2) p<0.001		
			Stroke volume (%)		
Change from baseline (mean±95% CI)	2.4 (-0.7-5.5)	-1.7 (0.5-3.9)	0.9 (-3.3-5.1)	-0.9 (-21.8-20.0)	-2.0 (-4.3-0.2)

Mean difference ±95% CI (A versus E; B versus C)	4.1 (-7.5-1.0) p=0.75	3.6 (-9.5-18.6) P=0.9

Changes to the five-min timepoint are compared between glucagon bolus and corresponding saline using the paired t-test. Data may not sum up due to rounding and missing values. T: timepoint.

Table S2. Differences in mean heart rates (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) compared between days with glucagon and corresponding saline±95% CIs.

	Differences in mean HR (beats per minute (min))			Differences in mean SBP (mm Hg)			Differences in mean DBP (mm Hg)		
	A vs E			A vs E			A vs E		
Timepoint (min)	Mean	95 % CI	p	Mean	95% CI	p	Mean	95% CI	p
-15	2.4	-5.9 - 10.6	1.00	0.0	-11.5 - 11.6	1.00	-1.9	-7.1 - 3.2	0.99
0	2.7	-5.5 - 10.9	0.99	0.9	-10.7 - 12.4	1.00	-1.2	-6.4 - 4.0	1.00
3	-14.5	-6.3- -22.8	<0.001	-5.3	-16.8- -6.2	0.97	-5.8	-0.7 - -11.0	0.02
10	-4.7	-12.9 - 3.5	0.89	-17.2	-28.7- -5.7	<.001	-11.8	-17.0 - -6.6	<0.001
15	-2.6	-10.9 - 5.6	0.99	-10.7	-22.3 - 0.8	0.09	-3.4	-8.6 - 1.7	0.67
20	-0.9	-9.1 - 7.4	1.00	-5.8	-17.7 - 6.1	0.96	-0.9	-6.1 - 4.3	0.68
30	2.9	-5.4 - 11.1	0.99	-4.9	-16.8 - 7.0	0.99	-0.1	-5.2 - 5.4	1.00
40	3.1	-5.2 - 11.3	0.99	-3.7	-15.2 - 7.8	0.99	0.1	-5.2 - 5.4	1.00
50	0.9	-7.3 - 9.2	1.00	-3.2	-14.7 - 8.3	1.00	0.1	-5.1 - 5.3	1.00
60	2.1	-6.2 - 10.3	1.00	3.3	-8.5 - 15.2	1.00	-1.6	-6.8 - 3.6	1.00
	B vs C			B vs C			B vs C		
Timepoint (min)	Mean	95 % CI	p	Mean	95% CI	p	Mean	95% CI	p
-15	2.3	-4.9 - 8.9	0.99	-3.4	-17.6 - 10.9	1.00	-0.7	-8.0 - 6.6	1.00
0	2.3	-4.3 - 8.9	0.99	-3.5	-17.7 - 10.8	1.00	-3.3	-10.6 - 4.1	0.99
3	-9.9	-3.3- -16.5	<0.001	-13.6	-27.9 - 0.6	0.07	-11.6	-18.9 - -4.3	<0.001
10	-6.7	-13.3 - -0.1	0.04	-18.3	-32.5 - -4.0	0.001	-11.8	-19.2 - -4.5	<0.001
15	-4.3	-10.9 - 2.3	0.71	-11.7	-25.9 - 2.6	0.27	-5.1	-12.4 - 2.2	0.60
20	-1.4	-8.0 - 5.2	1.00	-6.4	-20.6 - 7.9	0.98	-0.8	-8.1 - 6.5	1.00
30	1.7	-4.9 - 8.3	1.00	-8.3	-22.5 - 6.0	0.87	-1.7	-9.0 - 5.6	1.00
40	1.2	-5.4 - 7.8	1.00	-8.6	-22.9 - 5.6	0.82	-1.9	-9.2 - 5.4	1.00
50	-1.0	-7.6 - 5.6	1.00	-3.0	-17.3 - 11.2	1.00	-1.9	-9.2 - 5.4	1.00
60	0.5	-6.1 - 7.1	1.00	-2.1	-16.3 - 12.2	1.00	-2.2	-9.5 - 5.1	1.00

Differences between glucagon and corresponding saline are analyzed by a mixed model with Tukey correction of multiple comparisons.

Table S3. Average changes in mean arterial pressure from baseline to the five-min timepoint.

Trial day:	A	E	B	C	D
T-15 (mean±95% CI)	85 (79-91)	87 (80-93)	85 (81-90)	86 (80-93)	87 (80-94)
Baseline (T0) (mean±95% CI)	84 (79-90)	85 (79-92)	77 (74-80)	80 (76-85)	86 (79-93)
Heart rate T5 (means±95% CI)	85 (79-90)	100 (91-108)	76.0 (72-81)	94 (89-100)	90 (83-97)
Change from baseline (means±sem)	0.4 (0.7)	14.3 (1)	-1.2 (0.8)	13.9 (1.5)	5.1 (0.7)
Mean±95% CI of difference (A vs E; B vs C)	12.2 (8.9-15.5) p<0.001		15.2 (10.7-19.7) p<0.001		

Changes to the five-minute timepoint were compared between glucagon bolus and corresponding saline placebo using the paired t-test. Data may not sum up due to rounding/missing values. ‘T’: timepoint.

Table S4. Differences in average mean arterial pressure compared between days with glucagon and corresponding saline±95% CIs.

	A vs E		
Timepoint (minute)	difference	95 % CI	p
-15	-2.2	-8.7 - 4.2	0.999
0	-1.2	-7.6 - 5.3	1.000
3	-7.5	-13.9 - -1.0	0.007
10	-11.8	-18.2 - -5.3	<0.001
15	-5.6	-12.1 - 0.8	0.178
20	-3.3	-9.8 - 3.1	0.953
30	-1.4	-7.8 - 5.1	1.000
40	-0.6	-7.0 - 5.8	1.000
50	-1.3	-7.8 - 5.1	1.000
60	0.7	-5.7 - 7.2	1.000
	B vs C		
Timepoint (minute)	Mean	95 % CI	p
-15	-1.0	-9.6 - 7.7	1.000
0	-2.7	-11.4 - 5.9	0.999
3	-12.9	-21.6- -8.1	<0.001
10	-14.5	-23.1 - -5.8	<0.001
15	-7.2	-15.8 - 1.5	0.252
20	-2.3	-10.9 - 6.3	1.000
30	-2.9	-11.5 - 5.8	0.999
40	-3.4	-12.0 - 5.3	0.998
50	-1.8	-10.4 - 6.8	1.0000
60	-1.8	-10.4 - 6.8	1.0000

Differences between glucagon and corresponding saline are analyzed by a mixed model with Tukey correction of multiple comparisons.

Table S5. Differences in mean % changes from baseline in stroke volume, cardiac output and systemic vascular resistance (SVR) compared between days with glucagon and corresponding saline ±95% CI.

	Stroke volume			Cardiac output			SVR		
	A vs E			A vs E			A vs E		
Timepoint (minute)	Mean	95 % CI	p	Mean	95% CI	p	Mean	95% CI	p
-15	-1.3	-4.7 - 2.2	1.00	-0.7	-7.6 - 6.8	1.00	1.56	-5.2-8.8	1.00
0	-0.1	-3.5 - 3.5	1.00	0.4	-6.6 - 8.0	1.00	0.71	-6.0-7.8	1.00
3	-1.7	5.2 - 1.8	0.33	27.5	19.0 - 37.1	<0.001	-14.4	-20.0- -8.3	0.003
10	3.1	-0.5 - 6.8	0.98	14.7	6.6 - 23.3	0.04	-0.62	-7.4-6.6	1.00
15	5.3	1.5 - 9.2	0.41	17.5	9.3 - 26.4	0.005	-8.35	-14.6-1.7	0.67
20	7.6	3.7 - 11.6	0.02	13.6	5.4 - 22.4	0.12	-9.92	-16.0-3.3	0.32
30	8.9	4.9 - 12.9	0.002	5.7	-1.9 - 13-9	1.00	-4.39	-10.9-2.6	1.00
40	7.4	3.5 - 11.4	0.03	4.1	-3.4 - 12.1	1.00	-5.04	-11.5-1.9	1.00
50	4.4	0.8 - 8.2	0.69	5.8	-1.8 - 14.1	1.00	-5.54	-12.0-1.4	0.99
60	-2.4	-5.8 - 1.1	0.99	1.6	-5.5 - 9.3	1.00	-3.57	-10.0-3.3	1.00
	B vs C			B vs C			B vs C		
Timepoint (minute)	Mean	95 % CI	p	Mean	95% CI	p	Mean	95% CI	p
-15	-7.9	-19.6 - 5.4	0.99	-4.1	-18.3 - 12.5	1.00	4.6	-14.2 - 23.4	1.00
0	-0.9	13.4 - 13.5	1.00	-0.5	-15.2 - 16.8	1.00	-0.03	-18.8 - 18.8	1.00
3	-2.0	-14 - 11.8	0.76	-17.1	-29.3 - 2.7	0.76	3.4	-15.4 - 22.2	1.00
10	-3.8	-15.9 - 10.2	1.00	-15.8	-28.3 - 1.2	0.87	2.2	-16.6 - 21.0	1.00
15	-9.8	-21.2 - 3.2	0.99	-17.2	-29.5 - 2.9	0.75	11.2	-7.6-30.0	0.98
20	-12.2	-23 - 0.5	0.95	-14.6	-27.2 - 0.3	0.94	11.9	-6.8 - 30.8	0.96
30	-12.9	-23.9 - 0.3	0.91	-10.7	-23.9 - 4.9	1.00	6.9	-11.9 - 25.7	0.99
40	-9.9	-21.4 - 3.1	0.99	-10.7	-23.9 - 4.9	1.00	6.4	-12.4 - 25.2	0.99
50	-4.5	-16.6 - 9.3	0.99	-8.1	-21.7 - 7.9	1.00	7.0	-11.8 - 25.8	0.99
60	-1.6	-14.1 - 12.6	1.00	-3.3	-17.6 - 13.5	1.00	7.3	-11.5 - 26.1	1.00

Differences between glucagon and corresponding saline are analyzed by a mixed model with Tukey correction of multiple comparisons.

Table S6. Adverse effects during the trial.

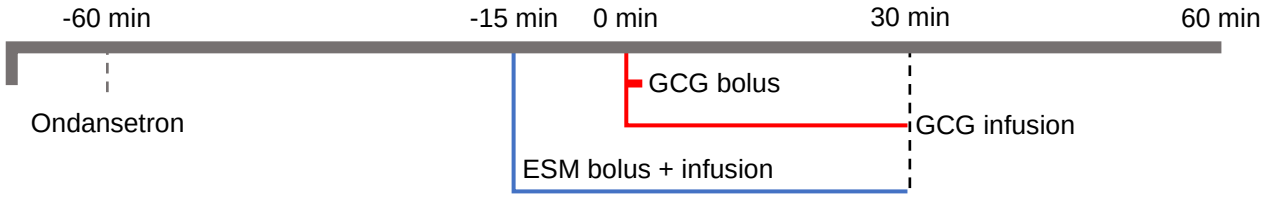
Participant ID	Nausea T6	Nausea T10	Nausea T30	Nausea T60	Other adverse effect (duration / intensity / consequences for trial day completion)
Day A: Saline+saline					
1	1	0	0	0	
2	0	0	0	0	
4	0	0	0	0	
5	0	0	0	0	
6	0	0	0	0	
7	0	0	0	0	

8	0	0	0	0	Pain at site of infusion (T23-T100/mild/no consequences)
9	0	0	0	0	
10	0	0	0	0	
12	0	0	0	0	
Day B: Esmolol+saline					
1	0	0	0	0	
2	0	0	0	0	
4	0	0	0	0	
5	0	0	0	0	
6	0	0	0	0	
7	0	0	0	0	Dizziness (T6-T10/mild/no consequences)
8	0	0	0	0	
9	0	0	0	0	
10	0	0	0	0	
12	0	0	0	0	
Day C: Esmolol+glucagon bolus					
1	6	6	5	0	Nausea (T2) severity: “6”
2	0	0	0	0	
4	0	0	0	0	
5	4	2	0	0	Nausea (T2) severity: “4”
6	1	1	0	0	Nausea (T2) severity: “4”
7	4	1	0	0	
8	3	0	0	0	
9	1	0	0	0	Dizziness (T2-T10/mild/no consequences)
10	1	1	0	0	Nausea (T2) severity: “2”
12	2	1	0	0	
Day D: Saline+glucagon infusion					
1	0	1	0	0	Dizziness (T40-T50/mild/no consequences)
2	1	1	2	0	Headache (T6-T9/mild/no consequences)
4	0	0	0	0	

5	3	5	4	1	
6	0	1	0	0	
7	0	0	0	0	
8	1	1	2	0	
9	0	1	1	0	
10	2	2	0	0	Nausea and vomiting (T6-T20/considerable/infusion stopped at T17)
12	2	2	2	0	
Day E: saline+ glucagon bolus					
1	4	1	0	0	
2	0	0	0	0	Nausea (T4) severity: “2”
4	2	0	0	0	Nausea (T4) severity: “3”
5	4	3	0	0	
6	0	0	0	0	
7	3	0	0	0	
8	1	0	0	0	Nausea (T2) severity: “3”
9	2	0	0	0	
10	1	0	0	0	Nausea (T2) severity: “3”
12	0	0	0	0	Heat sensation (T2-T3/mild/no consequences) Nausea (T2) severity: “1”

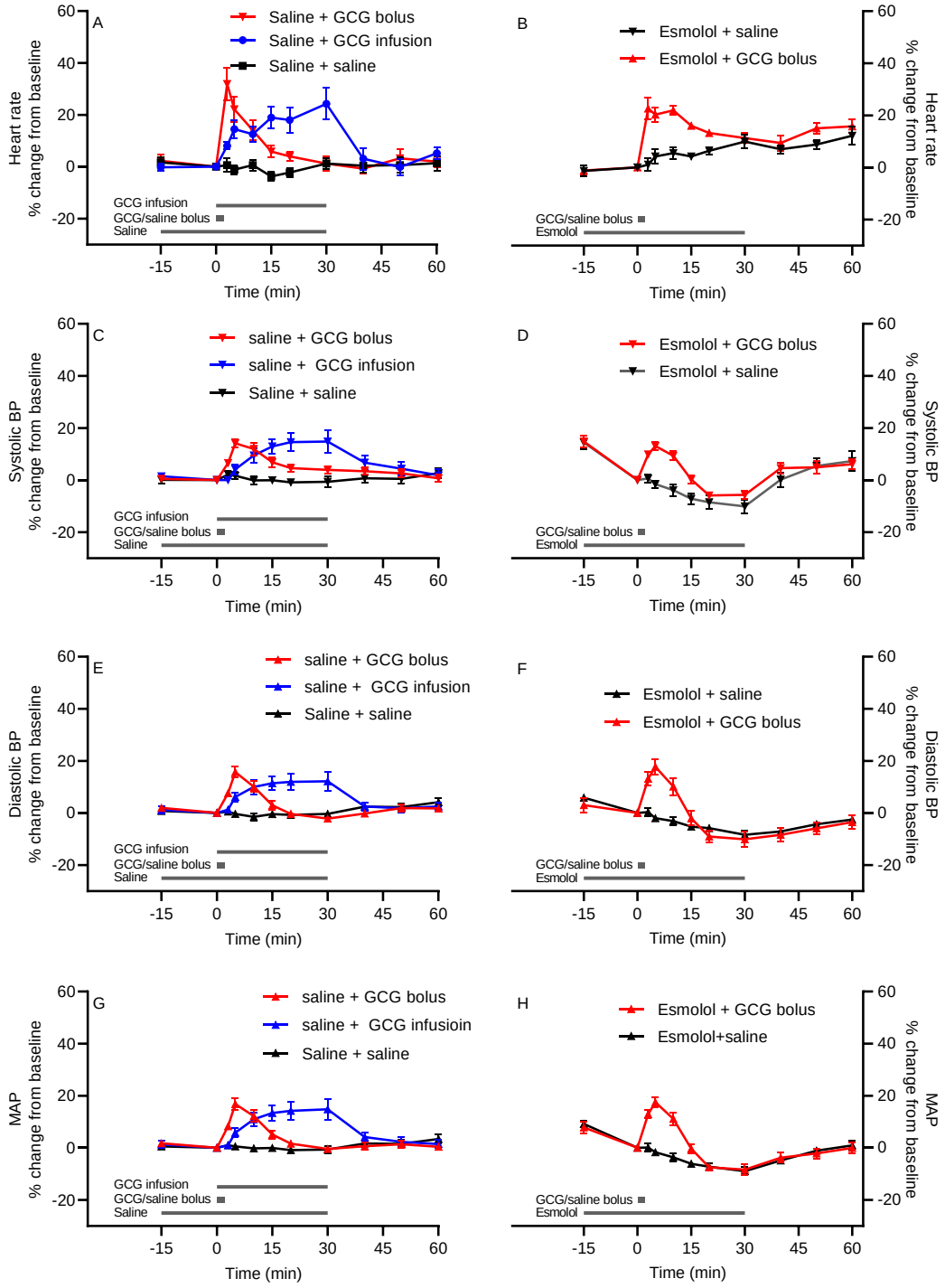
Nausea was systematically evaluated at prespecified timepoints by a verbal scoring scale ranging from 0 (no nausea) to 6 (extreme nausea). ‘T’ designates timepoint, i.e. ‘T6’ = the six-minute timepoint.

Figure S1. Procedures on individual trial days and timepoints of interventions.



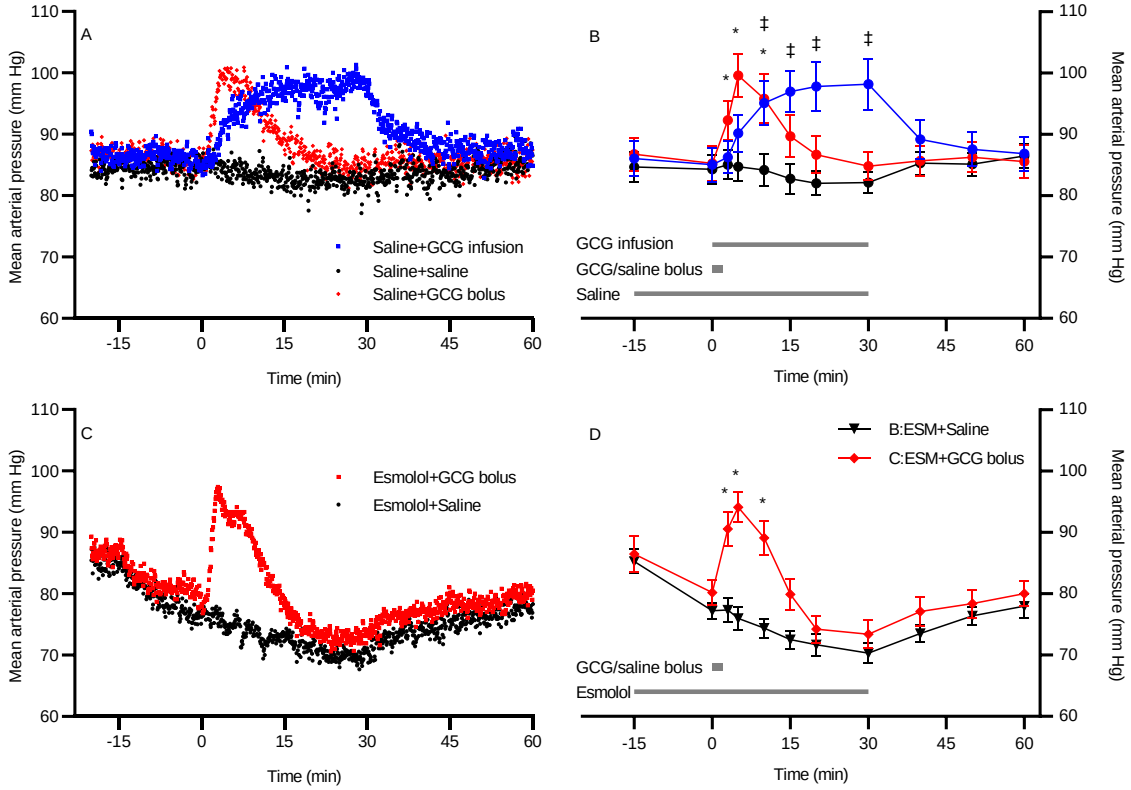
Combinations of esmolol (ESM), glucagon (GCG) and saline placebos (day A: saline+saline; B: esmolol+saline; C: esmolol + glucagon bolus; D: saline + glucagon infusion; E: saline + glucagon bolus) were administered in random order. Eight mg of ondansetron was administered 60 minutes (min) before baseline (0 min). Esmolol was administered from -15 to 30 min as a 1.25 mg/kg IV bolus for one min followed by a 0.75 mg/kg/min infusion (blue line). Glucagon (50 µg/kg) was administered at baseline as a two-min IV bolus or as a 30-min infusion (red lines). Matching volumes of saline were administered at identical timepoints.

Figure S2. Glucagon injections increase heart rate and blood pressure regardless of beta-blockade.



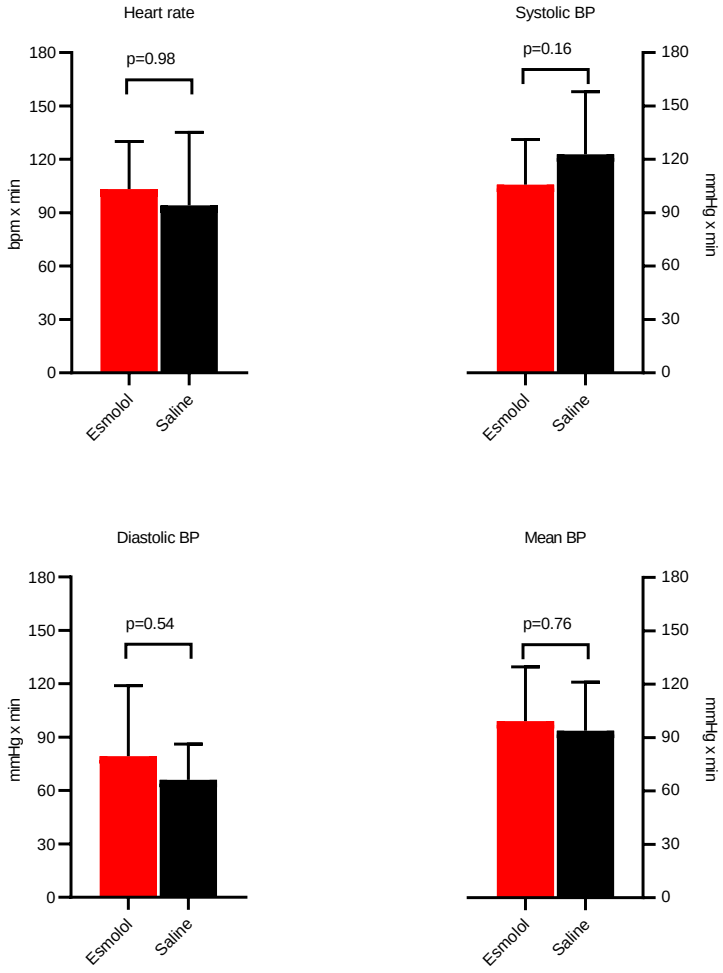
Hemodynamic effects of glucagon boluses expressed as relative % changes from baseline. (A) two-minute (min) heart rate means±SEM on days without esmolol. (B) two-min heart rate means±SEM on days with esmolol. (C) two-min systolic blood pressure (BP) means±SEM on days without esmolol. (D) two-min systolic BP means±SEM on days with esmolol. (E) two-min diastolic BP means±SEM on days without esmolol. (F) two-min diastolic BP means±SEM on days with esmolol. (G) two-min mean arterial BP (MAP) means±SEM on days without esmolol. (H) two-min MAP means±SEM on days with esmolol. GCG: glucagon.

Figure S3. Glucagon injections increase mean arterial pressure regardless of beta-blockade.



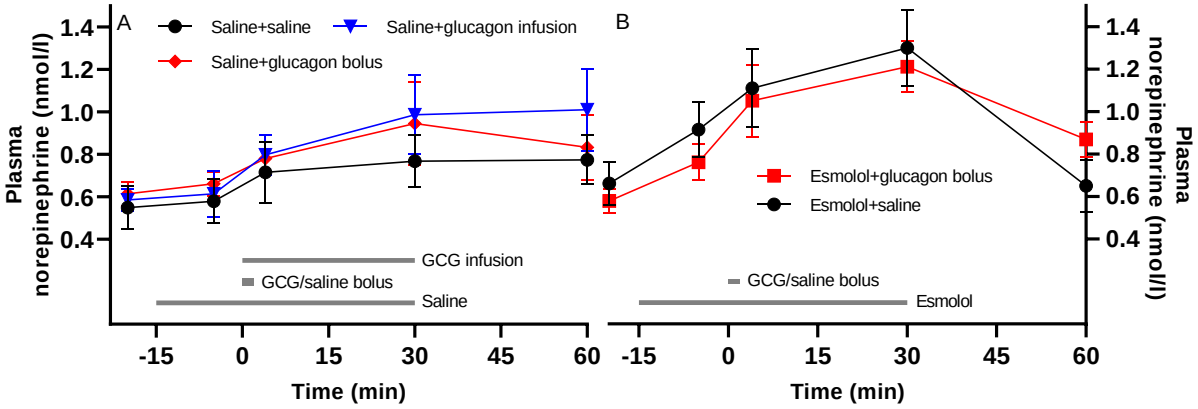
(A) Scatter plot of five-second (s) mean arterial pressure (MAP) (mm Hg) means on trial days without esmolol (blue: glucagon infusion; black: saline; red: glucagon bolus). (B) two-minute (min) MAP means±SEM on days without esmolol. (C) Scatter plot of five-s MAP means on trial days with esmolol (black: saline; red: glucagon bolus). (D) two-min MAP means±SEM on days with esmolol. Horizontal gray lines mark durations of infusions and boluses. *statistically significant difference between glucagon bolus and corresponding placebo; ‡Statistically significant difference between glucagon infusion and placebo, analyzed by a mixed model with Tukey correction of multiple comparisons. GCG: glucagon.

Figure S4. Glucagon bolus exerts similar acute hemodynamic effects irrespective of concomitant cardiac beta-blockade.



Hemodynamic effects of glucagon boluses expressed as incremental areas under the curves (mean±95% CI) from baseline to the 10-minute timepoint were calculated using the trapezoidal rule and compared using the paired t-test. Red: with concomitant esmolol infusion; black: with concomitant saline infusion. BP: blood pressure.

Figure S5. Glucagon has no significant effect on plasma norepinephrine levels compared to saline.



Plasma norepinephrine (nmol/l) is shown as means±SEM (A) days without esmolol (black: saline bolus; red: glucagon bolus; blue: glucagon infusion). (B) days with esmolol (black: saline bolus; red: glucagon bolus). Horizontal gray lines mark durations of infusions and boluses. Differences in norepinephrine concentrations compared between glucagon and corresponding saline were insignificant at all timepoints (p=0.45-0.99) (analyzed by a mixed model with Sidak correction of multiple comparisons). GCG: glucagon.