Electronic Supplementary Material:

Population Modelling of Dexmedetomidine Pharmacokinetics and Hemodynamic Effects after Intravenous and Subcutaneous Administration

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1. Study design

1.1. Subjects and Ethical considerations

Ten healthy male human subjects of median age 22y (19–27) and median weight 80kg (71–90) were recruited for our previously published phase 4 clinical trial that studied the bioavailability and pharmacodynamic profile of subcutaneous dexmedetomidine [1], and on which our modelling study is predicated. The study protocol ((EudraCT 2015-004698-34, ClinicalTrials.gov identifier NCT02724098) was put in practice after an approval from the Ethics committee of the Hospital District of Southwest Finland and by the Finnish Medicines Agency (FIMEA). The participants in the study consented via written forms, and it was ascertained that they were not using any medications at the time of study; did not have a severe illness, medicine allergies, alcohol dependence, psychological or emotional problems that may have caused problems in the execution of study protocol.

1.2. Study design

A two-phase cross over study design was followed with a three week wash out period between the IV and SC arms of the study. Subjects maintained a fasted state from midnight until 5 hours after the commencement of the study, while water consumption was allowed. All study participants stayed in a half supine position through the course of the study, with occasional breaks for toilet visits or eating meals. Standard meals were served at 5h and 9h after dexmedetomidine administration.

Dexmedetomidine was administered to the study participants at a dose of 1 μg/kg using the IV or SC route depending on the phase of the study. Dexmedetomidine (Dexdor®, 100 μg/mL, Orion Pharma, Espoo, Finland) was diluted using 0.9% saline (Natriumklorid B. Braun®, 9 mg/ml, B. Braun, Melsungen, Germany) and administered at a concentration of 8 μg/mL during a 10 mins infusion, using an infusion pump (Perfusor®, Space Infusion Pump, B. Braun). Dexmedetomidine was diluted to a final concentration of 50 μg/mL for 10 mins subcutaneous infusions, using the same pump.

1.3. Blood sampling and analysis

The start of the study was preceded by venous catheterisation in the large forearm vein, and arterial catheterisation in the radial artery. For the measurement of dexmedetomidine, norepinephrine (NE) and epinephrine (E) concentrations, 5 mL samples from radial artery were taken in EDTA tubes, immediately prior to and at 5, 10, 15, 20, 30, 45 mins and 1, 1.5, 2, and 3h after the start of the 10 mins dexmedetomidine infusions. In addition, blood samples were taken at 4, 5, 6, 8, and 10 h for dexmedetomidine concentration analysis. Plasma was obtained via refrigerated centrifugation, and stored at a temperature of −70 °C.

Reversed-phase high-performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS; Shimadzu Prominence HPLC connected to an AB Sciex API4000 mass spectrometer) was used for the analysis of dexmedetomidine in plasma samples, with slight modifications over previously reported method [2]. Sep-Pak® tC18 cartridges (Waters Corporation, Milford, MA, USA), and deuterium-labelled dexmedetomidine (from Toronto Research Chemicals, Toronto, ON, Canada) was used as the internal standard. The mobile phase was

0.1 % formic acid in a mixture of 1:1:1 (v/v/v) methanol/acetonitrile/water. The lower limit of quantification was 0.05 ng/mL and between-run precision of the assay (coefficient of variation) was within 5 % in the relevant concentration range.

In addition, HPLC and coulometric electrochemical detection methods were used for the determination of NE and E concentrations in the plasma samples [3], but now adapted to a dedicated HPLC system provided by Thermo Fisher Scientific (Waltham, MA, USA). The analytical column was a reversed-phase C18 column (HR-80 C1), the detector was a Dionex Ultimate ECD-3000RS instrument coupled to a model 6011RS Ultra dual electrode, and the system was operated with Chromeleon v. 7 software (all from Thermo Fisher Scientific). The LLOQ for both catecholamines was 0.1 nM. The within- and between-run precision of the assay (coefficient of variation) was within 10 % in the relevant concentration range.

1.4. Pharmacodynamic measurements

Hemodynamic PD variables of heart rate, systolic and diastolic blood pressure were recorded in conjunction with the blood sampling times, i.e. at baseline and then at 5, 10, 15, 30, and 45 mins and 1, 1.5, 2, 3, 5, 8, and 10 h after dexmedetomidine challenge for both IV and SC arms of the study. Subjective drug effects on vigilance (alert to drowsy) and performance (very good to very poor performance) were recorded at the same time points using 100-mm visual analogue scale (VAS) lines.

2. Model development

2.1. Pharmacokinetic model

Concentration-time data from intravenous administration of dexmedetomidine was used to test disposition models. At first an empirical one compartment model was tried, with subsequent testing of two and three compartment mammillary models. Difference amongst the performance of these models was evaluated using an objective function value (OFV) drop of greater than or equal to 3.84 points at a probability level of 0.05 for each degree of freedom amongst nested models. In addition, numerical results from final models, output of covariance matrices, and standard goodness of fit plots using model output were generated to evaluate the correlation between observed data and model predicted results.

Modelling the absorption of dexmedetomidine from the local SC injection site was challenging. A first order absorption process was initially anticipated to account for the relatively fast build-up of drug concentrations in the plasma, however, visual predictive checks did not produce consistent outcome and more complex absorption functions had to be tested. Following attempts included a first order absorption, single Weibull absorption function, and biphasic absorption with slow absorption ($k_{A,slow}$) modelled as first order or Michaelis Menten process, transit compartment model, fractional absorption, and a bioavailability model. All of these models converged to successful minimization, but the model predicted output did not coincide well with the observed trends in the data. Next, following *a priori* knowledge of dexmedetomidine logP/log k_{OW}(partition coefficient) value of 2.9 [4], we hypothesized that a fraction of the drug would permeate the subcutaneous fat layer.

A biphasic absorption model was next built up such that a fast absorption process ($k_{a,FAST}$) was responsible for the influx of drug from the subcutaneous depot into the systemic circulation (V_1) and a fat layer permeation constant (k_{FAT}) was added to describe secondary drug reservoir. A slow absorption process ($k_{a,SLOW}$) was coded in the model to describe zero order drug release from the subcutaneous fat layer. Although the scenario represented a biphasic absorption process, no strict criterion was coded into the model to describe the temporal shift between the two processes, and both were estimated simultaneously.

The addition of a bioavailability model was deemed important for the subcutaneous phase of the data. It has been documented in the original study that dexmedetomidine bioavailability is 81% (49 – 97) after SC dosing. Therefore, a bioavailability model was implemented in our modelling study, however due to the complexity of the final system, few adjustments and assumptions had to be made about this phenomenon. The permeation of the SC fat layer was described by a parameter (F_{FAT}) to describe the relative proportion of drug amount in the depot that gets permeated into the fat layer. Additionally, a bioavailability parameter was added in the model to work in conjunction with the fast absorption process to describe the extent to which the amount of drug remaining in the depot after fat permeation enters the systemic circulation (i.e. F_{DEPOT}). Finally, we also attempted to add a parameter in the model to describe the fraction of fat layer dexmedetomidine amount which gets redistributed into the central circulation, but it could not be estimated with precision and therefore, was assumed to be equal to 1. Such an arrangement allowed for the calculation of overall bioavailability as follows:

$$F = (1 - F_{FAT}) * F_{DEPOT} + F_{FAT}$$

$$F = (1 - 0.55) * 0.78 + 0.55$$

$$F = 0.89$$

The assumption that all the drug amount that has permeated the fat layer becomes bioavailable in the systemic circulation led to a slightly higher estimate for the overall bioavailability as compared to Uusalo et al. 2018 [1], but this assumption had to be made to achieve a numerically stable model.

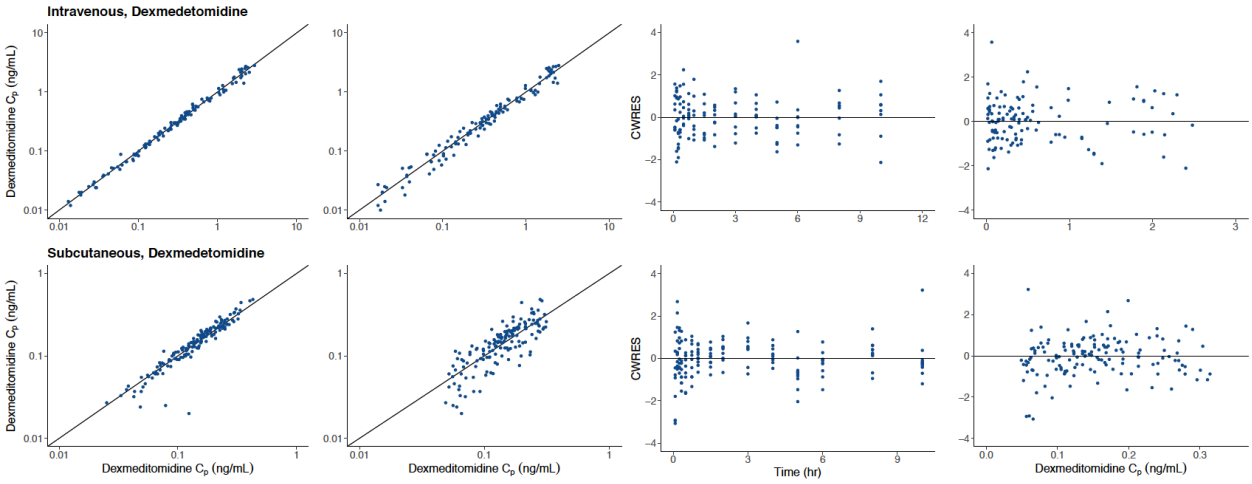
The differential equations for the pharmacokinetic model are as follows,

$$\frac{dA_{DEPOT}}{dT} = -k_{a,FAST} \cdot A_{DEPOT} \cdot F_{DEPOT} - k_{FAT} \cdot A_{DEPOT}$$

$$\frac{dA_{FAT}}{dt} = k_{FAT} \cdot A_{DEPOT} \cdot F_{FAT} - k_{a,SLOW} \cdot A_{FAT}$$

$$\frac{dA_1}{dt} = k_{a,FAST} \cdot A_{DEPOT} + k_{a,SLOW} \cdot A_{FAT} - k_{10} \cdot A_1 - k_{12} \cdot A_1 + k_{21} \cdot A_2$$

$$\frac{dA_2}{dt} = k_{12} \cdot A_1 - k_{21} \cdot A_2$$



Supplementary Figure 1. Goodness-of-fit plots for dexmedetomidine PK model. Observations vs. individual and population predictions; conditionally weighted residuals (CWRES) vs. time after dexmedetomidine dose and population predictions of dexmedetomidine after intravenous (*upper panel)* or subcutaneous (*lower panel*) 1 µg/kg dose administered during 10 min continuous infusion in 10 healthy volunteers (8 in intravenous phase).

2.2. Norepinephrine model

The next step was the development of a pharmacodynamic model for norepinephrine concentrations. The inhibitory effect of dexmedetomidine on the *in vivo* build-up of norepinephrine was modelled using an indirect response model connected with a biophase for mimicking catecholamine synthesis and release from adrenergic sympathetic nerve fibres in the human body.

Since the first observation in the data was taken before a dexmedetomidine challenge, a baseline level of norepinephrine in the plasma (${C_{NE}}^{t=0}$) could be specified for each individual as,

$$C_{NE,P} = {C_{NE}}^{t=0}$$

Plasma concentration of norepinephrine is known to represent a 15% spill over fraction (f_{SPILL}) of the concentrations at adrenergic nerves [5], hence the norepinephrine model was coded as a composite of two separate compartments, each representing sympathetic nerves and plasma respectively, and linked by rate constants for determining mass flow between these compartments. The baseline level at the release (nerve) compartment could be estimated in the model as,

$$C_{NE,P} = C_{NE,R} \cdot f_{SPILL}$$

Thereafter the initial conditions for the norepinephrine synthesis compartment were specified as,

$$k_{IN,R} = C_{NE,R} \cdot k_{OUT,R}$$

where $k_{IN,R}$ is the rate constant for the endogenous synthesis of norepinephrine, while $k_{OUT,R}$ is the rate constant for the release of norepinephrine from the synthesis compartment.

The spillage of norepinephrine from the release compartment into the parenteral circulation was added to the model as follows,

$$k_{IN,P} = k_{OUT,R} \cdot f_{SPILL}$$

where $k_{IN,P}$ is the rate of norepinephrine spillage from the nerve to plasma compartment and f_{SPILL} is a constant describing the relationship between $k_{IN,P}$ and $k_{OUT,S}$ (i.e. the former is 15% of the latter), and was fixed to a numerical value of 0.15. Since dexmedetomidine is known to reduce the rate and extent of catecholamine release at adrenergic nerves *in vivo*, an inhibitory effect on the release rate constant was coded into the model using a sigmoidal (E_{max}) model as follows,

$$k_{IN,R} = {k_{IN,R}}^{t=0} \cdot \left(1 - \frac{C_1}{C_{DEX,50} + C_1}\right)$$

where $k_{IN,S}$ defines the rate of norepinephrine release at adrenergic nerves at $time = t$, ${k_{IN,S}}^{t=0}$ defines the *de novo* rate of release of norepinephrine at adrenergic nerves at $time = 0$, C_1 is the concentration of dexmedetomidine at

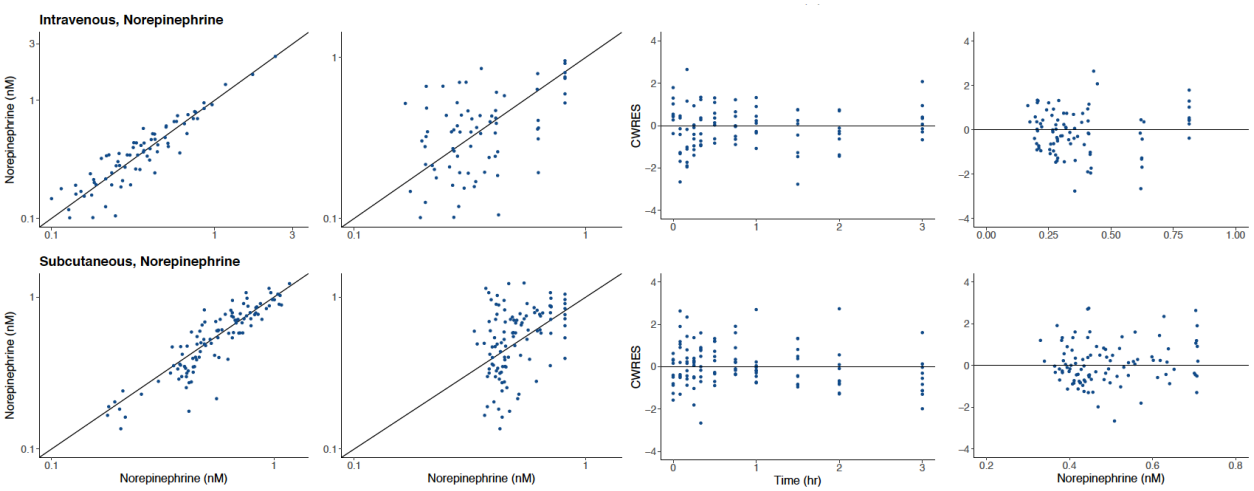
the central compartment, and $C_{DEX,50}$is the concentration of dexmedetomidine that causes 50% of maximal effect on NE release *in vivo*.

The differential equation describing mass balance of norepinephrine in the release and plasma compartments are as follows,

$$\frac{dA_5}{dt} = k_{IN,R} - k_{OUT,R} \cdot A_s$$

$$\frac{dA_6}{dt} = k_{IN,P} \cdot A_s - k_{OUT,P} \cdot A_P$$

where A_5 is the amount of norepinephrine in the release compartment, $k_{IN,R}$ is the *de novo* rate of release of norepinephrine in the human body, $k_{OUT,R}$ is the biological rate of norepinephrine degradation from the release compartment, A_6 is the amount of norepinephrine in the plasma compartment, $k_{IN,P}$ is the rate of norepinephrine spillage into the plasma from the release compartment and was numerically set to $k_{OUT,R} \cdot f_{SPILL}$, and $k_{OUT,P}$ is the rate of degradation of norepinephrine from the plasma compartment.



Supplementary Figure 2. Goodness-of-fit plots for norepinephrine model. Observations vs. individual and population predictions; conditionally weighted residuals (CWRES) vs. time after dexmedetomidine dose and population predictions of dexmedetomidine after intravenous (*upper panel)* or subcutaneous (*lower panel*) 1 µg/kg dose administered during 10 min continuous infusion in 10 healthy volunteers (8 in intravenous phase).

2.3. Epinephrine model

Development of epinephrine model was limited by concentration-time data, which contained multiple below limit of quantification (BLQ) levels for all individuals. Dexmedetomidine leads to a significant decrease in the endogenous production of epinephrine in the human body, as suggested previously shown by several authors [5,6]. The high degree of sparsity in the data hindered the modelling process and all attempts to estimate model parameter using modelling strategies previously tried for norepinephrine failed to produce a numerically stable model. High relative standard errors in the final model parameter estimates were noticed pointing to high uncertainty in model

parameters, which was also confirmed from the results of bootstrap analysis of the final epinephrine model. Finally, the epinephrine model was discarded and it was concluded that the sparsity challenged model fitting.

Considering the physiological role of epinephrine in hemodynamic homeostasis and the lack of a validated epinephrine model, subsequent pharmacodynamic variables of HR, SAP/DAP and subjective effects on vigilance and performance were assumed to be functions of norepinephrine concentrations. This assumption might have affected the model performance for the remaining PD endpoints due to missing information on epinephrine effect.

2.4. Systolic/Diastolic arterial blood pressure models

Norepinephrine concentrations at the synthesis compartment were used as the key determinant of the systolic/diastolic blood pressure effect. The choice of pharmacodynamic models for both SAP and DAP were effect compartment models with k_{E0} that defined norepinephrine mass transfer from the release to effect compartment. In conjunction, a sigmoidal E_{MAX} model was used for the estimation of the individual SAP or DAP values for the individuals. However, this approach resulted in clear misspecifications in the model outputs.

Previous literature demonstrates that dexmedetomidine exerts a biphasic effect on blood pressure in the human body [6–9]. At high initial concentrations, a hypertensive effect predominates due to an action on the α_{2B}- adrenergic receptors, while the role of α_{2A}- adrenergic receptors surfaces more as the concentrations of dexmedetomidine get lower, ultimately leading to a significant hypotensive effect. The hypertensive effect leads to a significant rise in blood pressure immediately following dose administration, but is short-lived and does not evoke marked clinical implications. However, in order for the model to accurately predict the time course of systolic and diastolic blood pressure development, a combination of both hypertensive and hypotensive effects had to be implemented.

We hypothesized that a combination of two indirect response models with separate k_{E0} values for norepinephrine and dexmedetomidine would suffice for describing the hypotensive and hypertensive roles of these mediators respectively. The concentrations of dexmedetomidine in central compartment and norepinephrine at the SAP or DAP effect compartments were used to drive the sigmoidal E_{max} models for the NE mediated hypotensive ($E_{BP,HPN}$) and dexmedetomidine mediated hypertensive ($E_{BP,HTN}$) effect components respectively, as follows:

$$E_{BP,HPN} = 1 + \left(\frac{E_{MAX} \cdot {C_{E,NE}}^{\gamma}}{{EC_{50,NE}}^{\gamma} + {C_{E,NE}}^{\gamma}} \right)$$

$$E_{BP,HTN} = 1 + \left(\frac{E_{MAX} \cdot {C_{E,DEX}}^{\gamma}}{{EC_{50,DEX}}^{\gamma} + {C_{E,DEX}}^{\gamma}} \right)$$

where $E_{MAX,BP}$ is the maximum effect for SAP or DAP and was fixed to 1, C_E is NE or dexmedetomidine concentration at the effect compartment, and EC_{50} is the concentration of norepinephrine or dexmedetomidine resulting in half maximum effect for hypotension or hypertension respectively.

The combined hypotensive and hypertensive effect of norepinephrine and dexmedetomidine on blood pressures respectively was modelled as,

$$E_{BP} = E_{MIN,BP} \cdot (E_{BP,NE} + E_{BP,DEX})$$

where $E_{MIN,BP}$ is the minimum for blood pressure estimated as a model parameter.

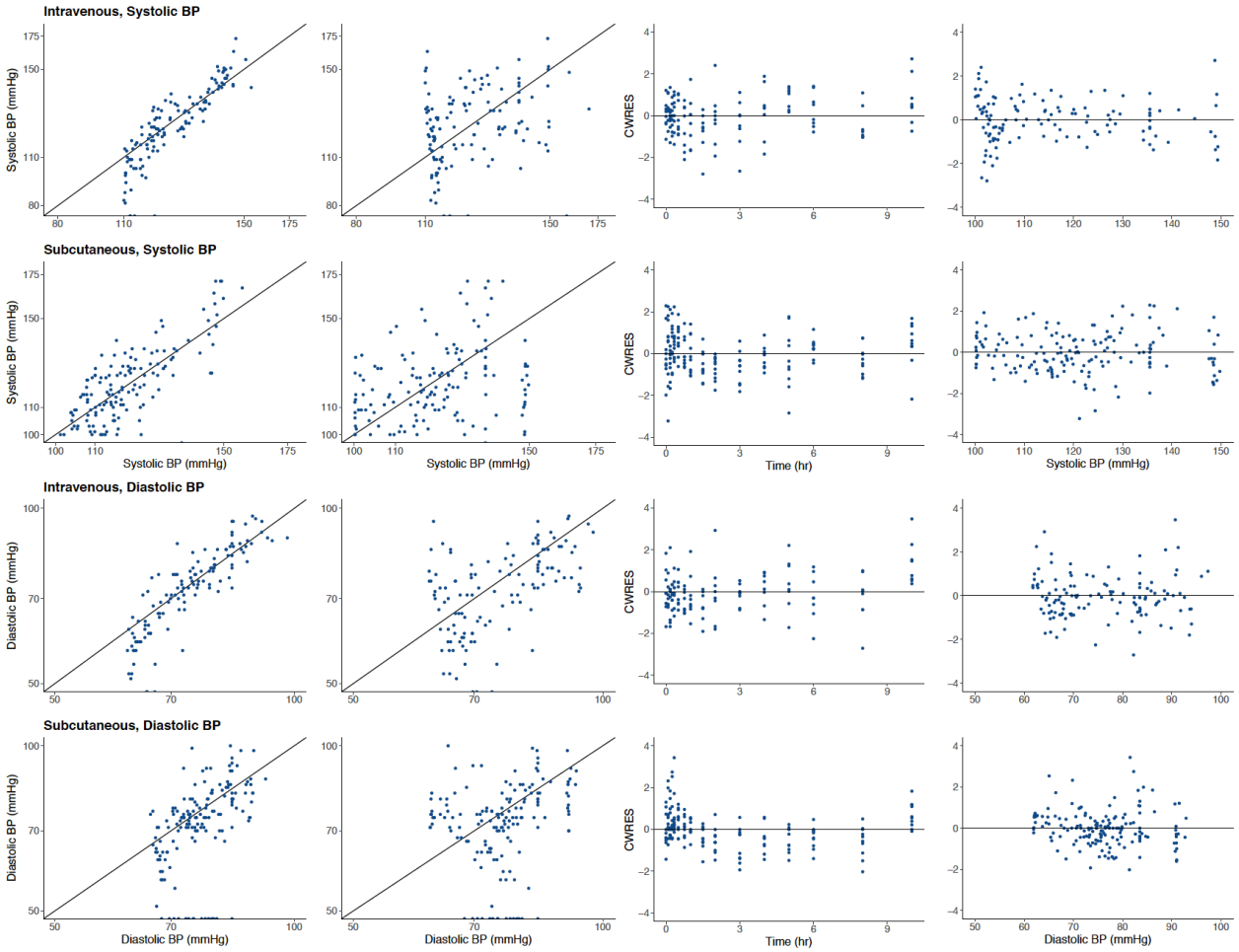
The differential equations for the compartments constituting SAP or DAP effect were added to the models as:

$$\frac{dA_{E,HPN}}{dt} = k_{e0,HPN} \cdot A_{R,NE} - k_{e0,HPN} \cdot A_{HPN,NE}$$

$$\frac{dA_{E,HTN}}{dt} = k_{e0,HTN} \cdot A_{C,DEX} - k_{e0,HTN} \cdot A_{HTN,DEX}$$

where EC_{HPN} represents the effect compartment for the hypotensive component of SAP or DAP, k_{E0_HPN} is the mass transfer rate constant for norepinephrine from the synthesis to the hypotensive effect compartment and vice versa, $A_{R,NE}$ is the amount of norepinephrine at the release compartment, $A_{HPN,NE}$ is the amount of norepinephrine at the hypotensive effect compartment, k_{E0_HTN} is the mass transfer rate constant for dexmedetomidine from the central to the hypertensive effect compartment, $A_{C,DEX}$ is the amount of dexmedetomidine in the central compartment scaled to V_C, and $A_{HTN,DEX}$ is the amount of dexmedetomidine at the hypertensive effect compartment. Model parameters were specified in both the SAP and DAP models with the aim of describing the baseline systolic or diastolic arterial pressure for each individual, i.e. $E_{BASE,SAP}$ or $E_{BASE,DAP}$ respectively and at $t = 0$, E_{BP} for the systolic or diastolic blood pressures were assumed to be equal to $E_{BASE,SAP}$ or $E_{BASE,DAP}$ accordingly. This allowed us to evaluate population trends in the data at the very early time points after dexmedetomidine dosing. Additionally, inter-individual variability was added in a sequential manner on the model parameters and observed for a betterment in model fit as suggested by a significant drop in OFV values, and low shrinkage in the added parameters. It was observed that only the addition of BSV parameters on $EC_{50,NE}$ and $EC_{50,DEX}$ resulted in a significant improvement in the model fit with <30% ETA-shrinkage, suggesting adequate information about these parameters in the data at hand.

As suggested by the output from final model, a combination of hypertensive and hypotensive predictive strategy accurately described the trends in the data and was able to predict SAP time course as observed for the study population. Standard GOF plots, low percent relative standard errors and narrow confidence intervals indicated model suitability and adequate predictive power for describing the SAP and DAP trends in the data.



Supplementary Figure 4. Goodness-of-fit plots for blood pressure models. Observations vs. individual and population predictions; conditionally weighted residuals (CWRES) vs. time after dexmedetomidine dose and CWRES vs population predictions of systolic (*upper panel)* or diastolic blood pressure (*lower panel*) after intravenous or subcutaneous 1 µg/kg dose administered during 10 min continuous infusion in 10 healthy volunteers (8 in intravenous phase).

2.5. Heart rate model

We assumed that the concentrations of norepinephrine at the release compartment is responsible for the development of the heart rate effect. Since a number of mediatory pathways contribute to the manifestation of heart rate, an effect compartment model with k_{E0} was implemented, where k_{E0} represents the transfer rate constant determining the mass balance of norepinephrine between the release and effect compartment.

The maximum effect ($E_{MAX,HR}$) for heart rate was fixed to a value of one and the minimum value of heart rate effect for the study population ($E_{min,HR}$) was included as a model parameter for estimation. The heart rate effect was modelled using a sigmoidal E_{max} function with the shape factor γ (Hill coefficient).

$$E_{HR,NE} = E_{MIN,HR} \cdot \left(1 + \left(\frac{E_{MAX} \cdot {C_{E,NE}}^{\gamma}}{{EC_{50,NE}}^{\gamma} + {C_{E,NE}}^{\gamma}}\right)\right)$$

where $E_{HR,NE}$ is the heart rate effect due to norepinephrine concentrations at the effect compartment, $C_{E,NE}$is the norepinephrine concentration at the effect compartment, and $C_{50,NE}$ is the norepinephrine concentration causing 50% maximum effect.

Thereby, at any time point, the individual specific concentrations of norepinephrine at the synthesis compartment were used to calculate the rise from E_{MIN} to determine the exact heart rate for each observation in the dataset. The above-mentioned equation was used in this form for $t > 0$, while at $time = 0$, E_{MIN} had to be replaced with $E_{BASE,HR}$ which was added to the model as a parameter. Using E_{MIN} at $t = 0$ caused a model misspecification that delayed the decrease in heart rate effect, not in line with the trend in the data at hand, which showed a sharp decline in HR after dexmedetomidine. Replacing E_{MIN} with $E_{BASE,HR}$ corrected the misfit in the model, and accurately described the steep initial decline in the heart rate, coinciding with norepinephrine profiles. Norepinephrine mass transfer to the heart rate effect compartment from the release compartment was coded into the model with differential equation as follows,

$$\frac{dA_{EC,NE}}{dt} = k_{e0,HR} \cdot A_{R,NE} - k_{e0,HR} \cdot A_{E,NE}$$

where k_{e0_HR} is the mass transfer rate constant for norepinephrine from the synthesis compartment to HR effect compartment ($EC_{HR,NE}$) and vice versa, $A_{R,NE}$ is the amount of norepinephrine in the release compartment, and $A_{E,NE}$ is the amount of norepinephrine at the EC_{HR}. Both $A_{R,NE}$ and $A_{E,NE}$ effectively represent the norepinephrine concentrations, assuming the negligible volumes at the NE release and heart rate effect compartment respectively.

Although the model using norepinephrine as the only mediator of heart rate was successful in predicting the response time course, there was a clear misspecification in the final model that was evident in the visual predictive check (VPC) diagnostics, especially during the SC phase. The population trend in the data was being correctly accounted for by the NE dependent bradycardia, but the final model under-predicted the overall effect, pointing towards an inhibitory mechanism independent of norepinephrine. It has been documented in recent literature that dexmedetomidine can cause a dose dependent vasoconstriction [10]. We hypothesized that the under-prediction of heart rate effect was due to missing information about changes in neural activity in the heart rate modulating reflex from the central nervous system (CNS) which potentiated bradycardia, especially during the vasoconstrictive phase of dexmedetomidine action.

The model predicted SAP levels ($IPRED_{SAP}$) from the validated blood pressure model were used as input into a turnover model for the reflex in CNS. We assumed that at baseline level of SAP, the baroreflex input to CNS is minimal and therefore reflex modulation of HR from CNS was fixed to a small initial value. We attempted to estimate baseline neural output as a model parameter, but due to the problem of over-parametrization it has to be fixed. The initial conditions for the 'build-up' compartment in CNS were specified in the model as,

$$k_{IN,CNS}{}^{t=0} = k_{OUT,CNS} \cdot C_{BASE,CNS}$$

And effect of $IPRED_{SAP}$ on the development of central reflex was defined using sigmoidal E_{max} model as:

$$k_{IN,CNS} = k_{IN,CNS}{}^{t=0} \cdot \left(1 - \left(\frac{E_{MAX} \cdot IPRED_{SAP}{}^{\gamma}}{EC_{50,SAP}{}^{\gamma} + IPRED_{SAP}{}^{\gamma}}\right)\right)$$

where $k_{IN,CNS}{}^{t=0}$ represents the baseline rate of neural reflex development in the CNS, k_{OUTCNS} describes its dissociation, and $C_{BASE,CNS}$ defines its level at time = 0 and $EC_{50,SAP}$ is the level of blood pressure required to cause 50% inhibition of central neural output.

The differential equations describing the development of neural reflex for HR modulation in CNS and its mass transfer to heart rate effect compartment is as under:

$$\frac{dA_{CNS}}{dt} = k_{IN,CNS} - k_{OUT,CNS} \cdot A_{E,CNS}$$

$$\frac{dA_{E,CNS}}{dt} = k_{e0,CNS} \cdot A_{CNS} - k_{e0,CNS} \cdot A_{E,CNS}$$

where A_{CNS} is the level of neural reflex in the build-up compartment, $k_{e0,CNS}$ represents its mass transfer rate constant into the HR effect compartment, and $A_{E,CNS}$ is its level in the HR effect compartment.

The component of heart rate effect attributable to neural output in CNS was calculated in the model as,

$$C_{E,CNS} = A_{E,CNS}$$

$$E_{HR,CNS} = E_{MIN} \cdot \left(1 - \left(\frac{E_{MAX} \cdot C_{E,CNS}{}^{\gamma}}{EC_{50,CNS}{}^{\gamma} + C_{E,CNS}{}^{\gamma}}\right)\right)$$

where C_{CNS} is the level of NA causing in the heart rate effect compartment and responsible for a component of the overall effect attributable to this activity i.e. $E_{HR,CNS}$, and $EC_{50,CNS}$ is the level of change in central NA required to cause a 50% inhibition of heart rate effect.

The net heart rate after norepinephrine effect and inhibitory action of lowered reflex in CNS was calculated as follows:

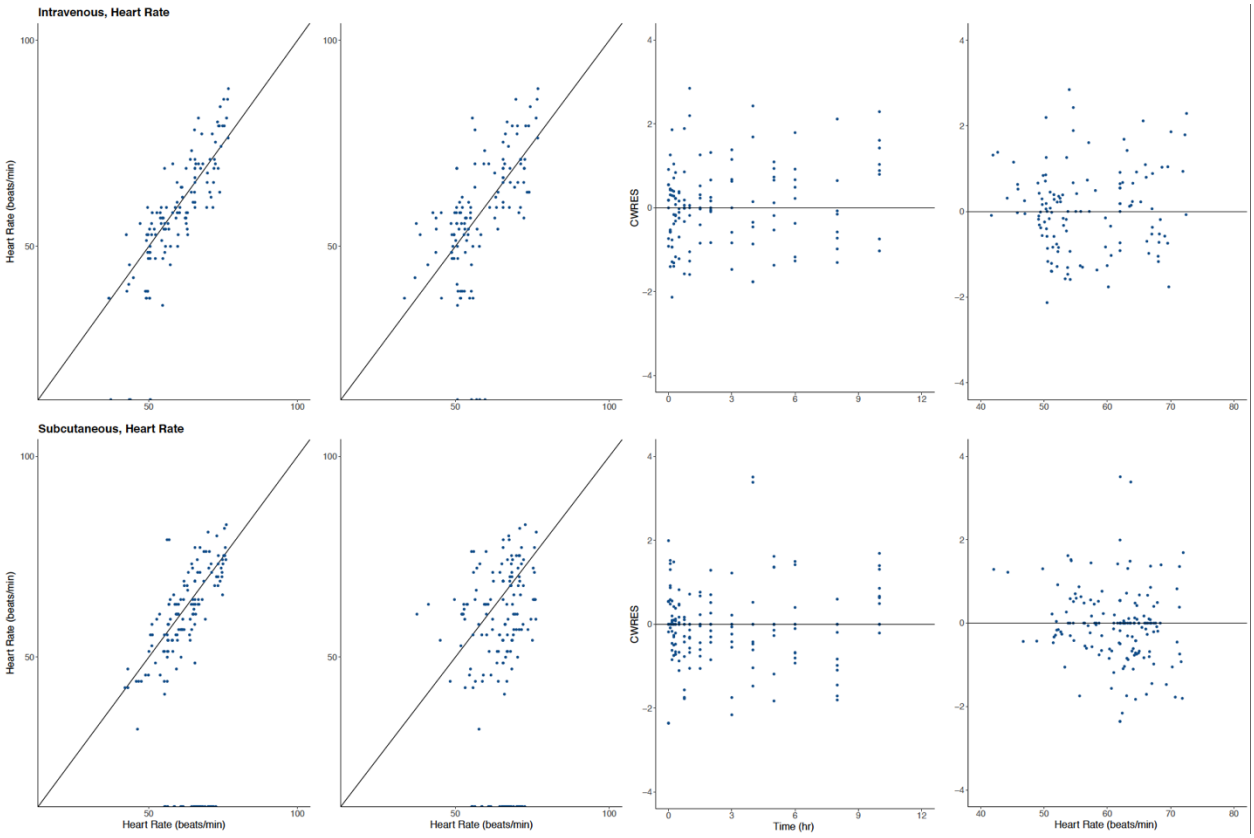
$$E_{HR} = E_{HR,NE} + E_{HR,CNS}$$

We postulated that neural reflex in CNS is minimal at resting state and was therefore fixed at a near zero starting value. In addition, the level of systolic blood pressure required to cause a significant change (indirectly linked to baroreflex), as well as the level of $EC_{50,CNS}$ for HR were fixed to near zero levels, considering their physiological role in the body. We noticed that an addition of this component to the HR model considerably improved the HR model fit without causing numerical instability due to over-parametrization in the final model. Simulations from the

HR model suggested a dose dependent drop in HR effect, and E_{MIN} as the lower bound and accounted for subtle differences in the intravenous and subcutaneous phases of dexmedetomidine administration.

Epinephrine model could not provide adequate results due to high level of sparsity in the data, and therefore, the role of epinephrine in the development of heart rate effect could not be modelled in conjunction with norepinephrine. In order to test for mechanisms other than norepinephrine in the development of heart rate effect, an effect compartment model was implemented with k_{E0} describing the residual effect of dexmedetomidine on heart rate. Such a combined model could not further improve the model fit, also the added model parameters were estimated with very high %RSE values. Thus, we discarded this model assumed that norepinephrine is the only physiological mediator of heart rate effect of dexmedetomidine.

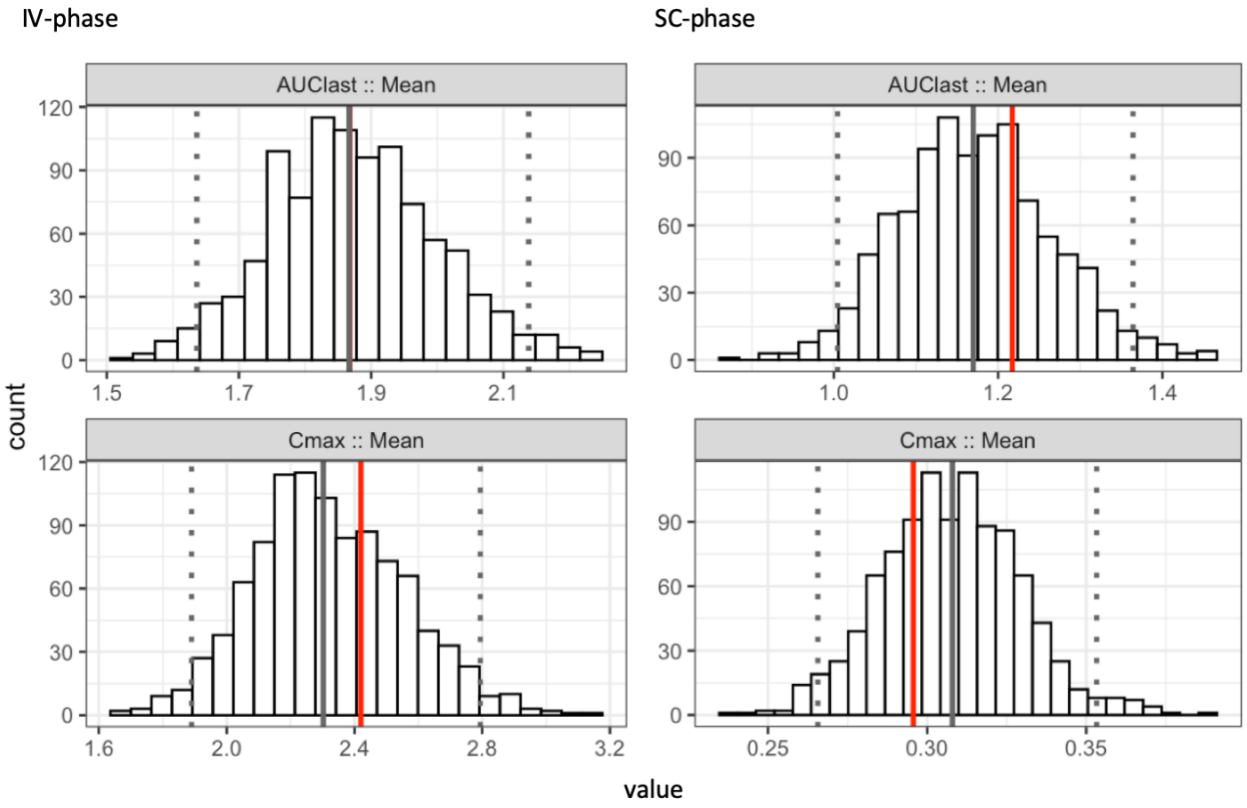
In addition, an inter-individual variability parameter was also tried on the baseline heart rate effect, however, was discarded due to high shrinkage and no further improvement in the objective function value (OFV). The final model included BSV on the $C_{50,NE}$ only, and accurately described the heart rate response time profile for the study individuals.



Supplementary Figure 4. Goodness-of-fit plots for heart rate model. Observations vs. individual and population predictions; conditionally weighted residuals (CWRES) vs. time after dexmedetomidine dose and CWRES vs population predictions of heart rate after intravenous (*upper panel)* or subcutaneous (*lower panel*) 1 µg/kg dose administered during 10 min continuous infusion in 10 healthy volunteers (8 in intravenous phase).

3. *Results from the NCA-simulations*

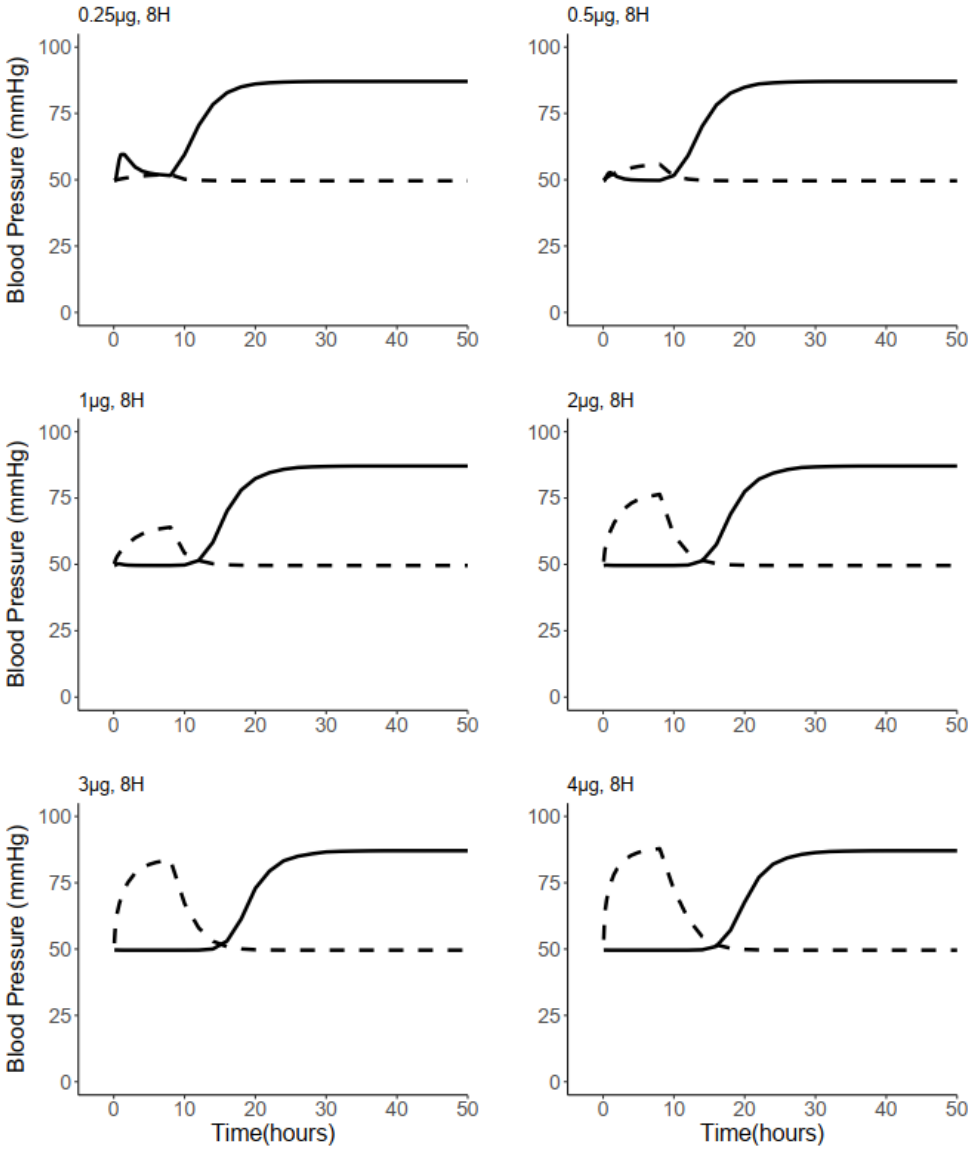
We used *ncappc* package of PsN [12] to perform a non-compartmental analysis (NCA) based comparison between the observed values in the dataset to model derived predictions for the PK and PD parameters as described previously [13]. Our results indicate that the model produces reasonably



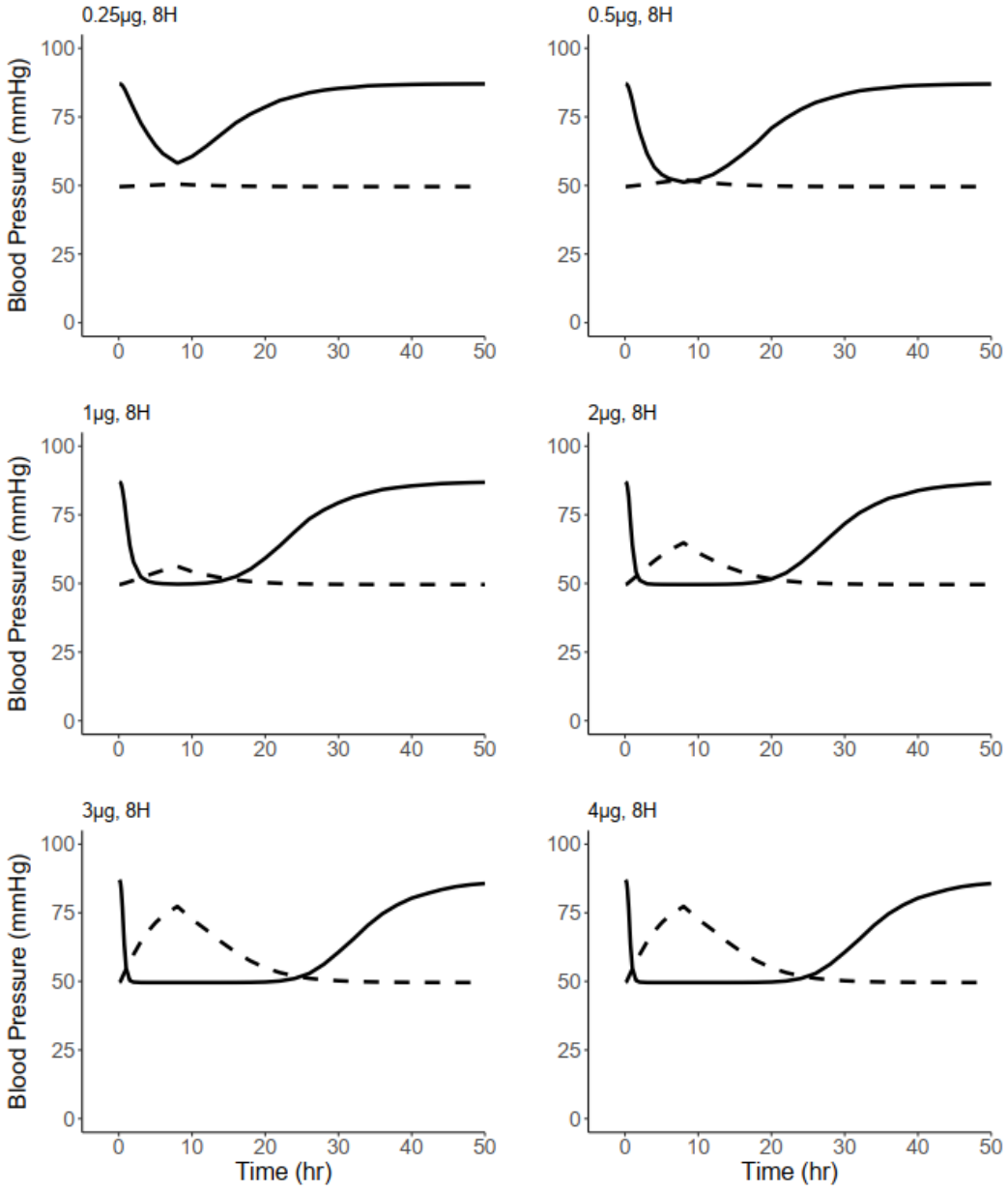
Supplementary Figure 1. Histogram of the population mean of the area under the concentration-time curve (AUC) and the peak concentration (Cmax) metrics. The observed parameter values are compared to percentiles of the simulated values computed from 1000 simulations. Red line shows the mean of the original observation, and median and 95% CI of the simulated values are shown in solid and dashed gray lines, respectively.

4. Results from the blood pressure model simulations

Final blood pressure models and different dexmedetomidine dosing schemes and the resulting NE profiles were used to evaluate the effects dexmedetomidine on hemodynamic parameters. Results from the simulations show a dynamic interplay between the hypotensive and hypertensive effects, depending upon the concentrations of NE and dexmedetomidine. At higher dexmedetomidine concentrations following intravenous dosing, the hypertensive effect can be seen to cause a spike in blood pressure which is swiftly followed by a hypotensive effect due to reduced NE release.



Supplementary figure 2. Time dependency of the hypotensive and hypertensive components of systolic blood pressure after 0.25 µg – 4 µg/h/kg constant 8 hour infusion. The solid and dashed lines show the time course of the hypo- and hypertensive effects, respectively.



Supplementary fig. 3. Time dependency of the hypotensive and hypertensive components of diastolic blood pressure after 0.25 µg – 4 µg/h/kg constant 8 hour infusion The solid and dashed lines show the time course of the hypo- and hypertensive effects, respectively.

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