Author’s response to reviews

Title: Effects of dexmedetomidine on porcine pulmonary artery vascular smooth muscle

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Author’s response to reviews:

Response letter
Professor Martin Kaczocha, Ph.D.
Editor-in-Chief
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Effects of dexmedetomidine on porcine pulmonary artery vascular smooth muscle
Kenichi Sato; Mami Chikuda

7, July 2019

Dear Martin Kaczocha

Thank you very much for your consideration of our manuscript. We fundamentally agree with all of these comments and have incorporated them to the revised version of our manuscript.

Red indicates the parts that I changed according to Rewerer #1.

Blue indicates the parts that I changed according to Rewerer #2.
We hope that you are able to positively evaluate our revisions.

Sincerely yours,

Kenichi Sato

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Department of Dental Anesthesiology

Responses to Reviewers’ Comments

James P Dilger, PhD (Reviewer 1):

The authors note that Dex is often administered with local anesthetics, so it is important to investigate whether Dex may have side effects, such as on the circulatory system. They chose to study its effect on pulmonary arteries. Following the lead of a similar study on gastroepiploic arteries (Ref 7), they perform a logical series of experiments and come to similar conclusions.

Note: The changes you requested appear in red font in the revised manuscript.

General Comments.

1. Manuscript organization.

The Methods section should contain a general description of the experimental approach rather than a figure-by-figure description. Moving some of the text in the Methods to the appropriate place in the Results section, would make the manuscript easier to read. In addition, in the Results section, the authors could add a sentence explaining the rationale for each experiment. This is, indeed, found in the Discussion, but the paper would flow more clearly with some commentary in the Results. The Discussion could then be shortened somewhat.

Response: Thank you for your Comment. We are not sure how to perform a simple revision of the sections that you requested. It would require a large amount of work. Perhaps with the further editing that the manuscript has undergone, it is now more easily understood without making those changes. We respectfully ask you to take a second look at it to see if you still think such changes are necessary.

2. Dex concentrations.
I could not find any mention of clinical concentrations that are used with Dex. This should be provided to allow the reader to assess the clinical importance of the findings.

Response: Thank you. We added the following sentences in the Discussion (Page 15 Lines 12-23 and Page 16, lines 1-9).

“The selection of Dex at a concentration of 10-6 M was based on the following data. We considered that the inhibitory concentration of 50% of Dex was 2.083 µM according to the dose-dependent curve (Fig. 4). We therefore needed to have 60–70% maximum inhibition. Figure 2 shows that Dex increased the 60 mM KCl-induced contraction tension, with the observed increases reaching significance at a Dex concentration of ≥5×10-6 M. Hence, we decided to use 5 µM. Dex at high doses activates the α2β-receptors distributed in vascular smooth muscle, causing hypertension resulting from contraction of vascular smooth muscle. At low doses, Dex causes hypotension resulting from vasodilation and bradycardia due to parasympathetic dominance [19]. The blood concentration of Dex required to maintain a sedative effect in humans is reported to be similar, at 1.0 × 10-9 g/mL—i.e., 4.0 × 10-9 mol/L (23, 24). The present results show that Dex had no effect on vasoconstrictor responses in porcine pulmonary arteries when applied in clinically effective concentrations. The previous reported systemic effects of Dex observed in the clinical setting, including decreased blood pressure, may not be the result of direct actions on vascular smooth muscle but possibly due to decreased central and peripheral sympathetic nervous system activity [11]. One clinical report also suggested that, at large doses (>10-8 mol/L), Dex increases peripheral vascular resistance leading to increased blood pressure [13]. Although the mechanism of the blood pressure increase is unclear, it cannot be ruled out that the vasoconstrictive effects of Dex shown in this study (i.e., those possibly mediated by VDCC activation or in case of accidental intravenous administration) may be relevant in such cases.”

Dentists usually use 2% lidocaine with 1:80,000 adrenaline in Japan, which comes in a 1.8-ml cartridge for use as a local anesthetic. Adrenaline contained in lidocaine is widely used in neurosurgery, otorhinolaryngological procedures, dental treatment, and oral and maxillofacial surgery to decrease surgical bleeding, lessen mucosal congestion, and maintain a clear field of view. The 1:80,000 adrenaline is 22.5 µg/1.8 ml in a cartridge (12.5 µg/ml = 40 µM). We occasionally use 4–6 cartridges (90–135 µg) for minor surgery or extraction. During general anesthesia for oral maxillofacial surgery, 1% lidocaine containing 1:100,000 adrenaline is often used (10–30 ml = 100–300 µg) during an operation. If Dex were to be added to local anesthetics in the dental field, higher concentrations of Dex would be used rather than higher adrenaline doses.

The authors do not provide any motivation for this choice. When Dex is given with a local anesthetic, is there a possibility of sufficiently high Dex concentrations in the lung?

Response: Thank you for your comment. We added the following sentence in the Background section (Page 5, lines 18-23 and Page 6 lines 1-2).

“Although several in vivo studies have shown the effects of Dex on aortic or coronary arteries in various animals, there are few reports regarding its effect on other peripheral vessels. The pulmonary artery has been relatively unexplored, even though it is a prominent artery that nourishes the lungs. Although pulmonary vasoconstriction, with its resultant progressive elevation of pulmonary arterial resistance and pressure, plays a central role in pulmonary arterial hypertension, which could be fatal (7, 8), there are only a few reports of the effects of Dex on the pulmonary artery. The diversity of effects of Dex on smooth muscle precludes guessing its effects on any individual smooth muscle.”

We think it is possible that when Dex is given with a local anesthetic the Dex concentration in the lung is insufficient.


Please provide the statistical test used in each figure legend. For example, in Fig 2D, this should be analyzed with ANOVA and then a post-hoc test. Was the post-hoc test performed for each concentration vs control?

Response: Thank you for your comment. We analyzed the data using ANOVA and the post-hoc test with Scheffe’s multiple comparison procedure. When we reexamined Fig. 2D, however, we found a mistake and so deleted the asterisk.

We provided the statistical tests used in figure legends for Figs. 1-4.

“Statistical analysis was performed with repeated-measures analysis of variance followed by Scheffe’s multiple comparison procedure.”

We added the following sentence to the legends for Figs. 5 and 6.

“Statistical analysis was performed with Student’s t test.”

<Abstract>

1. p 3, line 33. Please include Dex concentrations in the abstract.
Response: Thank you. We added the following sentence in the Abstract, Background (Page 3, Lines 15-17).

“The concentration–response relation was determined at Dex concentrations of 10–10, 10–9, 10–8, 10–7, 10–6, 5×10–6, and 10–5 M and for other experiments at 5×10–6 M.”

2. p. 5, line 12. This would be a good place to include the concentration of Dex used in local anesthesia.

Response: Thank you. We added the following sentence in the Background (Page 5, Lines 6-8).

“For example, administration of Dex 0.5 µg/kg with 0.5% lidocaine to the brachial plexus for brachial nerve block significantly extends the duration of the local anesthetic’s effect [2] ....”

3-1. p. 6, line 56. What is the "incubator"?

Response: Thank you for your comment. We changed it to “perfusion chamber,” which we generally say (Page 7, line 13).

We are sorry for the confusion.

“Specimens were placed in a perfusion chamber (with 3 ml of perfusate),...”

3-2. At what temperature were the performed?

Response: Thank you. We added the following sentence in the Methods (Page 7, Lines 14-15):

“The perfusate, adjusted to 37°C, flowed at a rate of 1.6 ml/min controlled by a peristaltic pump (SMP-23; Tokyo Rikakikai Co., Fujisawa, Japan).


Response: Thank you. We deleted the following sentence: “Effects of various α receptor *ligands* on the vascular smooth muscle of pulmonary arteries.”

5. p. 10, line 33. Are the "untreated samples" the control muscles?
Response: Yes, they are, but we decided to delete “untreated samples” altogether.

6. p. 10, line 30-35. It is contradictory to say "no significant changes" in one sentence then "slight increases" in the next sentence. It looks as if the changes seen at high [Dex] in Fig 1 are significant. Please clarify.

Response: Thank you for your comment. We do not think that the changes seen at high Dex are significant. Therefore, we deleted the following sentence “However, slight increases in contraction tension were observed with high concentrations of Dex and imidazoline.”

7. p. 10, line 56. I don't normally think of having only 1 or 2 points different from control as evidence for a "concentration dependent" effect.

Response: Thank you for your comment. Having only 1 or 2 points different from the control is not evidence for a "concentration-dependent" effect. We deleted “…in a concentration-dependent manner” and have rewritten it as follows rewritten it in the Methods section (Page 9, Lines 13-14).

“Dex enhanced the contraction induced by high KCl stimulation, with the increases reaching significance at Dex concentrations of ≥5×10^{-6} M (Fig. 2).”

8. p. 11, line 51. It would be better to write "a transient increase" rather than "transient increases".

Response: Thank you. We changed “transient increases” to “a transient increase” (P10, Line 13).

9. p. 13, lines 7-12. This is not a complete sentence.

Response: I am sorry. You are correct. This sentence (p13, line 7-12) is not a complete sentence. We therefore deleted it.

10. p. 14, line 51. You are not measuring effects on "cell membrane depolarization".

Response: Thank you. I am sorry. We did not measure effects on “cell membrane depolarization”. We therefore deleted the following sentence: “Imidazoline had weaker effects than Dex on cell membrane depolarization.”
Response: Thank you for your comment. Ebert et al. [19] discussed “The effects of increasing plasma concentrations of dexmedetomidine in humans.” (Anesthesiology. 2000;93:382–94). They stated that “the pump infused the study drug according to pharmacokinetic models to achieve the following seven, ascending, targeted, plasma concentrations: 0.5, 0.8, 1.25, 2.0, 3.2, 5.0, and 8.0 ng/ml. Specifically, targeted mean achieved dexmedetomidine levels were as follows (ng/ml, ± SD): 0.5:0.7 ± 0.22; 0.8:1.2 ± 0.22; 1.2:1.9 ± 0.23; 2.0:3.2 ± 0.48; 3.2:5.1 ± 0.64; 5.0:8.4 ± 0.82; and 8.0:14.7 ± 0.78 ng/ml. The first two infusions of dexmedetomidine significantly lowered the MAP by 13%, whereas higher concentrations resulted in progressive increases in MAP, with the average peak individual increases averaging 12% more than the baseline value. The first two infusion steps did not significantly change CVP, PCWP, mean PAP, or the calculated pulmonary or systemic vascular resistances. The third infusion step, however, which increased plasma levels of dexmedetomidine to 1.9 ng/ml, was associated with significant increases in these variables that were sustained throughout subsequent infusion steps. We therefore define “high doses” as within the range of 1.2–8.0 ng/ml.

12. p. 17, line 20. Here we have a definition of "high doses" as (>10−8 mol/L). (P16, Line 4: We changed “high doses” to “large doses” ) That would suggest that most of your significant results are with high doses. Please comment.

Response: Thank you for your comment. You are correct, most of our significant results were with high doses. Dentists have usually used local anesthetics that contain a high concentration of adrenaline, with the result that various adverse events have occurred. In previous reports and in our experience, high concentrations of dexmedetomidine have caused various effects. Hence, it is necessary to avoid the addition of high concentrations of dexmedetomidine to local anesthetics to avoid such adverse events.

13. p. 17, line 40. You used multiple concentrations of Dex, so this sentence does not make sense.

Response: Thank you. Yes, we did use multiple concentrations of Dex. We deleted the following sentence: “It is unclear whether the 5 µM Dex concentration used in the present study is the optimal dose to add to local anesthetics. Despite previous reports and our experience, the optimal concentration should continue to be explored in the future.

14. p. 24, lines 20-25. Are these changes significant? If not, do not give the numbers.
Response: Thank you. The changes in Fig. 1 are not significant, so we deleted the following sentences: “In the pulmonary artery samples, addition of Dex at concentrations of 10−6, 5 × 10−6, and 10−5 M caused contraction tension to increase by 0.4±0.1%, 2.1±0.8%, and 5.2±1%, respectively. The addition of imidazoline at concentrations of 10−6, 5 × 10−6, and 10−5 M caused contraction tension to increase in the pulmonary artery samples by 0.2±0.1%, 1.6±0.6%, and 3.6±0.8%, respectively.”

15. Fig 1. Are the tensions measured relative to the adjacent baseline (before adding KCl + drug) or the KCl baseline? This also applies to other figures.

Response: Thank you for your comment. The tensions were measured relative to the KCl baseline (before adding KCl only). We added the following sentence in the legends for Figures 1, 2, 4, 5, 6: “The tensions were measured relative to the KCl baseline.”

16. Fig 2 B, D. The y-axis label should be ”Relative tension (%)” only.

Response: Thank you. We changed it to “Relative tension (%).”

17. Fig 3. Here, I refer to panels such that the upper part has panels 1, 2, and 3 and the lower has 4, 5, and 6. The effect of Dex shown in panel 4 is much smaller than that reported in Fig 2. Please comment.

Response: Thank you. We apologize. When we created the lower panels (4, 5, 6) with Excel, we mistakenly included incorrect numbers for the 10-6 M data for the column. We appreciate your pointing it out. We remade the figure, as follows.

18. Fig 3. Panels 5 and 6 contradict panels 2 and 3. Or, are the bars mislabeled such that R should be D and P should be D?

Response: Thank you. As you pointed out, the bars are mislabeled in panels 5 and 6. We changed R to D and P to D.

19. Fig 4. IC50 values should be calculated for the various ligands.

Response: Thank you. We added the followed IC50 values of the various ligands in the Results section (Page10, Lines 6-8).
“The inhibitory concentrations of 50% were 2.083 µM for Dex, 0.8996 µM for imidazoline, 0.376 µM for yohimbine, and 0.5702 µM for rauwolscine.”

20. Fig 5. My question about baselines (question 15) is relevant here.

Response: Thank you. Baselines are the KCl baselines (before adding KCl only).

Jean-Pierre Estèbe, MD, PhD (Reviewer 2)

The authors attempted to evaluate the effect of Dexmedetomidine (Dex) on smooth muscle (ex vivo endothelium-denuded porcine pulmonary arteries model).

Note: The changes you requested appear in blue font in the revised manuscript

1) Unfortunately, a controlled evaluation at the end of each experiment (i.e. as at the beginning) does not allow to estimate the effect of exhaustion and/or of depletion effect which could modify the interpretation of the results (or discuss). To make it clear, specifies that each new experiment was carried out with new fresh preparations.

Response: Thank you for your comments. We are not quite clear of your meaning regarding the exhaustion/depletion effect that might modify the interpretation of the results. Could we respectfully ask you to provide an example to clarify?

As for the second suggestion regarding each new experiment, we added the following sentences in the Results (Page 8, Lines 16-18).

“For all experiments, each new experiment was carried out with fresh preparations because it has been suggested that down-regulation of α2-adenoreceptors often occurs after prolonged α2-agonist treatment (10, 11).”

We also remade all the graphics.

2) For each step, give the number of manipulations performed.

Response: Thank you. We are sorry, but we do not understand what you mean. Do you mean how many steps did it take to complete each experiment? Should we have presented each experiment in numbered form, as in a list?
3) It must be discussed (see proofs) that the method of euthanasia does not introduce bias in the results (i.e. a major stress of the animal can produce the depletion of Ca 2+; discuss). A control with some pigs with an experimentally euthanized experiment would be useful for the discussion.

Response: Thank you. The pigs are slaughtered using a routine procedure in a slaughterhouse named Iwate Chikusan Ryutsu Center Co. Ltd., which processes and sells food. We obtained porcine lung tissue from this corporation. We added the following in the Discussion section (Page 15, Lines 8-11).

“When the 6-month-old pig whose tissues were used in the present experiments was euthanized, the major stress of the animal could have produced depletion of the Ca2+ reservoir. All pigs at that location are euthanized routinely with an electrical method. Hence, the euthanasia protocol did not introduce bias in the results.

4) Throughout the manuscript, the authors refer to the possible use of Dex as a possible adjunct to regional anesthesia. Their intentions may be laudable, but it is a systemic effect that has been evaluated especially at blood level concentrations that cannot be observed during a local administration. It would be better to speak only about the systemic effect of Dex (maybe in case of accidental IV administration; the pharmacokinetics of resorption do not allow to observe these blood levels).

Response: Thank you for your comments. We added the following sentences in the Discussion (Page 15, Lines 20-23, Page 16, Lines 1-9).

“The blood concentrations of Dex required to maintain a sedative effect in humans are reported to be similar, at 1.0 ×10-9 g/mL (i.e., 4.0 × 10-9 mol/L [23, 24]. The present results show a lack of effect of Dex on vasoconstrictor responses in porcine pulmonary arteries when applied in clinically effective concentrations. The previous reported systemic effects of Dex observed in the clinical setting, including decreases in blood pressure, may not be the result of direct actions on vascular smooth muscle but possibly due to decreases in central and peripheral sympathetic nervous system activity [11]. One clinical report also suggested that, at large doses (>10-8 mol/L), Dex increases peripheral vascular resistance, leading to increased blood pressure [13]. Although the mechanism of blood pressure increase is unclear, it cannot be ruled out that the vasoconstrictor effects of Dex shown in this study (i.e., those that maybe mediated by VDCC activation or in case of accidental intravenous administration) may be relevant in such cases.”
5) The rationale of the experimental plan must be clarified in the introduction of the Material and Methods chapter (i.e. alpha-2 + Vs. alpha-2 - etc. not at the end of this chapter or during the discussion).

The reader will be more comfortable to follow the strategy of the experimental plan.

Response: Thank you. We added the following sentences in the Discussion (Page 6, Lines 6-9).

“To evaluate Dex’s possible mechanisms of action, we investigated components of the two most important pathways involved in intracellular Ca2+ fluctuations during vascular smooth muscle contraction—i.e., intracellular influx of extracellular Ca2+ and release of stored intracellular Ca2+ within the cell.”

6) The quality of graphics with bar graphs needs to be improved.

Response: Thank you. We remade all the graphics.

Reviewer: In conclusion, it is after a clarification of these different elements (as baseline) that the results can be interpreted.

To the Reviewers:

Thank you for your reviews and helpful suggestions. We hope that our responses have addressed your concerns. Please let us know if you require further clarification.