Reviewer's report

Title: Xylazine-induced Reduction of Tissue Sensitivity to Insulin Leads to Acute Hyperglycemia in Diabetic and Non-diabetic Monkeys

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Reviewer: Joseph J J. McArdle

Reviewer's report:

Xylazine is widely used in combination with ketamine for anesthesia of animals during veterinary care and laboratory research procedures. However, xylazine has a poorly understood hyperglycemic effect which contraindicates its use in animal experimental studies of diabetes. The goal of this work is to understand the hyperglycemic action of xylazine. Because xylazine is a drug of abuse, this work also has toxicologic significance to man. Therefore, the authors studied the hyperglycemic effect of xylazine in normoglycemic cynomolgus and rhesus monkeys. In addition, they studied a line of cynomolgus monkeys with congenital insulin-dependent diabetes to test the hypothesis that xylazine-induced hyperglycemia results from reduced insulin secretion. Overall, the manuscript is easy to read. Minor corrections are suggested at the end of this review. However, Revisions are needed to enhance the manuscript’s clarity.

Compulsory Revisions

1. Figure 1. Are these all data derived from the diabetic and non-diabetic cynomologus monkeys whose baseline data are presented in Table 1? Indicate the numbers of monkeys studied to produce the data of Figure 1. The error bars on the data points of B as well as the histogram bars of C and D should be made larger.

2. Figure 2 A. Are these data derived from the same monkeys presented in Table 1 and Figure 1? In lines 5-7 of paragraph 2 on page 9 the authors suggests that hyperglycemia reversed much more slowly for diabetic as opposed to normoglycemic monkeys; this conclusion is repeated in the discussion. However, the data do not support a statistically significant difference in time recovery time. Furthermore, the authors go on to state that “Only one out of 5 diabetic monkeys gradually returned to the pre-xylazine glucose level at 2 hr after xylazine administration.” The authors should discuss why this diabetic monkey recovered whereas the remaining 4 monkeys required yohimbine to recover. Does yohimbine increase the recovery rate from xylazine-induced hyperglycemia for normoglycemic cynomolgus monkeys?

3. The data of Figure 2B indicate that xylazine did decrease plasma insulin level for normoglycemic monkeys; this effect occurred at approximately the time when blood glucose concentration increased. These observations contradict the authors’ argument that xylazine-induced hyperglycemia does not involve
decreased secretion of insulin. Were tests of significance performed for the open circle data points of Figure 2B? The statements noted in paragraph 1 of page 10 need to be clarified. That is, the authors state that insulin levels of normoglycemic monkeys initially decreased after xylazine “…but remained unchanged at all other time points.”

4. On page 9 the authors assess the effects of xylazine on glucoregulatory hormones. The last sentence of paragraph 2 should be more clearly written.

5. Since the authors had serum samples, it is unclear why they did not measure the concentration of other hormones (e.g., epinephrine, corticosterone, somatostatin, growth hormone) which regulate blood glucose concentration. These measurements should be made to improve understanding of the hyperglycemic action of xylazine.

6. In line 3 from the bottom of page 10 the authors indicate that hyperinsulinemic-euglycemic clamp experiments were made in 8 normoglycemic rhesus monkeys. Representative records for 2 of these monkeys are presented in Figure 3. Because blood glucose concentration was not stable, the authors did not challenge with xylazine until approximately 200 min after beginning the clamp. At this time, xylazine produced a mild increase of blood glucose from 55 to 83 and 64 to 99 mg/dL for these two monkeys; Figure 2 indicates that xylazine increased glucose in normoglycemic cynomolgus monkeys from 58 ± 3 mg/dL to 108 ± 12 mg/dL. Thus, during the clamp experiment xylazine is approximately 45% as effective in increasing plasma glucose. In order to strongly support the authors’ conclusion presented in the last sentence of paragraph 1 on page 11 all of the euglycemic experimental data should be presented and analyzed for statistical significance of blood glucose changes.

7. In line 4 of paragraph 2 on page 11 the authors indicate that “…blood glucose in these animals (ie., those studied with euglycemic clamp) reached 40-min stabilization…”. However, in the representative records of Figure 3, blood glucose was apparently judged to be stable only after 200 min and then xylazine was given. Why was there a time difference for glucose stabilization in these 2 sets of experiments?

8. The hyperinsulinemic-euglycemic clamp was performed on 8 normoglycemic rhesus monkeys which had blood glucose of 86 ± 4.6 mg/dL as compared to 47.9 ± 5.4 mg/dL for the 5 cynomolgus monkeys of Table 1. Is this difference in blood glucose significant for these two populations of normoglycemic rhesus and cynomolgus monkeys?

9. The reduced glucose infusion rate of animals which were first clamped with exogenous insulin to minimize endogenous insulin glucoregulation prior to xylazine administration suggested to the authors that “…xylazine-induced hyperglycemia potentially resulted from a reduction of tissue sensitivity to insulin.” The authors should discuss alternative explanations and means to test the validity of their hypothesis.
Discretionary Revisions

Page 2 line 4: “… becomes clinically relevant…”
Page 8 line 8 from page bottom: “…insulin AUC was significantly ….”
Page 20: Figure 2 legend line 4 “…. B and C. ?????”.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.