



A Whole-Blood Functional Assay for Identifying Glucocorticoid Resistance in Dogs

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Introduction

Cortisol is the primary glucocorticoid hormone secreted in response to many adverse conditions, such as chemical, physical, and emotional stress. It is well-known for its anti-inflammatory and immunosuppressive actions at high levels. Glucocorticoid resistance, a decrease in the sensitivity of immune cells to glucocorticoid that normally suppresses the inflammatory response, can lead to poor health outcomes; however, identifying glucocorticoid-resistant patients remains a challenge due to a great deal of variability in serum cortisol levels across individuals. Thus, it is more valuable to measure the functional responses to cortisol level. This study proposes an *in vitro* model for measuring glucocorticoid sensitivity of immune cells in whole blood under conditions that mimic acute stress.

Hypothesis

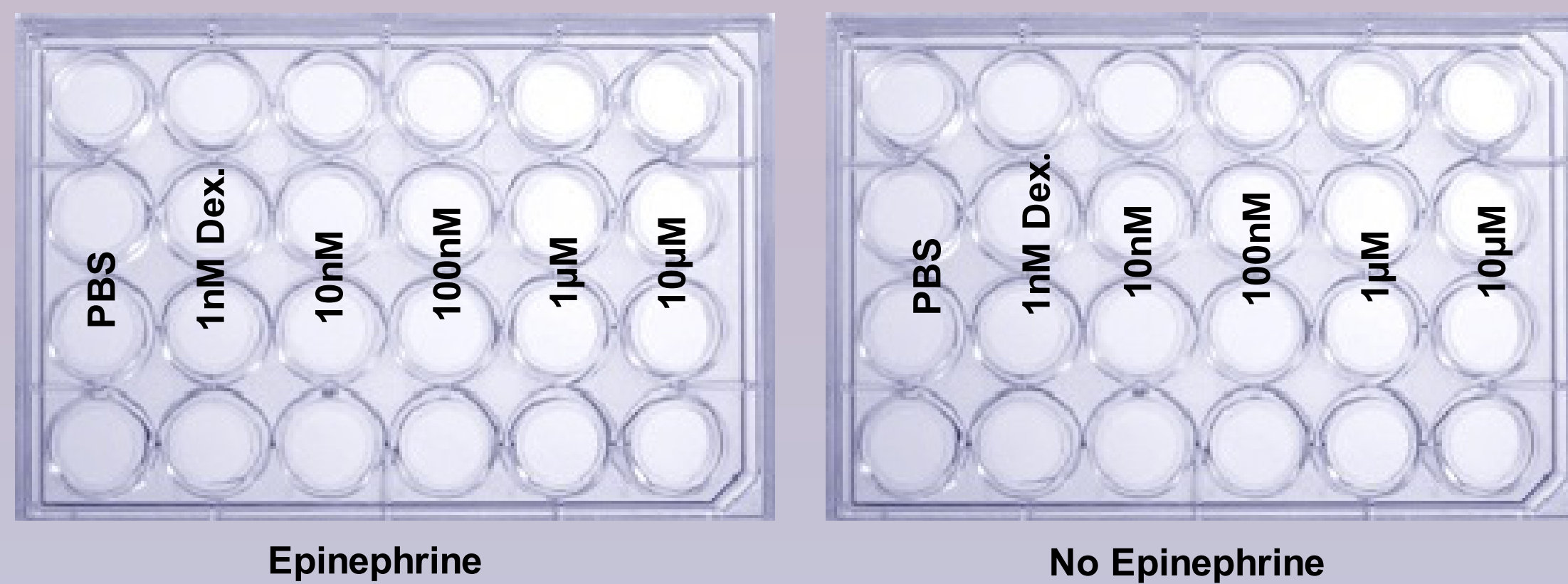
- Epinephrine will play a role in sensitization of immune cells to dexamethasone in blood from healthy dogs
- The concentration of dexamethasone that inhibits 50% of cytokine response (IC50) will be measured. Shifts in dexamethasone sensitivity (IC50) will predict patient outcomes.

Objective

To develop a whole-blood based assay that enables a functional quantification of glucocorticoid sensitivity.

Methods

- Whole blood culture



Each well contained the following:

- Whole blood diluted 1:2 with RPMI medium
- Lipopolysaccharide (100 ng/ml) or PBS
- 5 µM of Epinephrine or PBS
- Variable [Dexamethasone]
- Control (Phosphate buffered saline or PBS)

- ELISA and MFI for cytokines

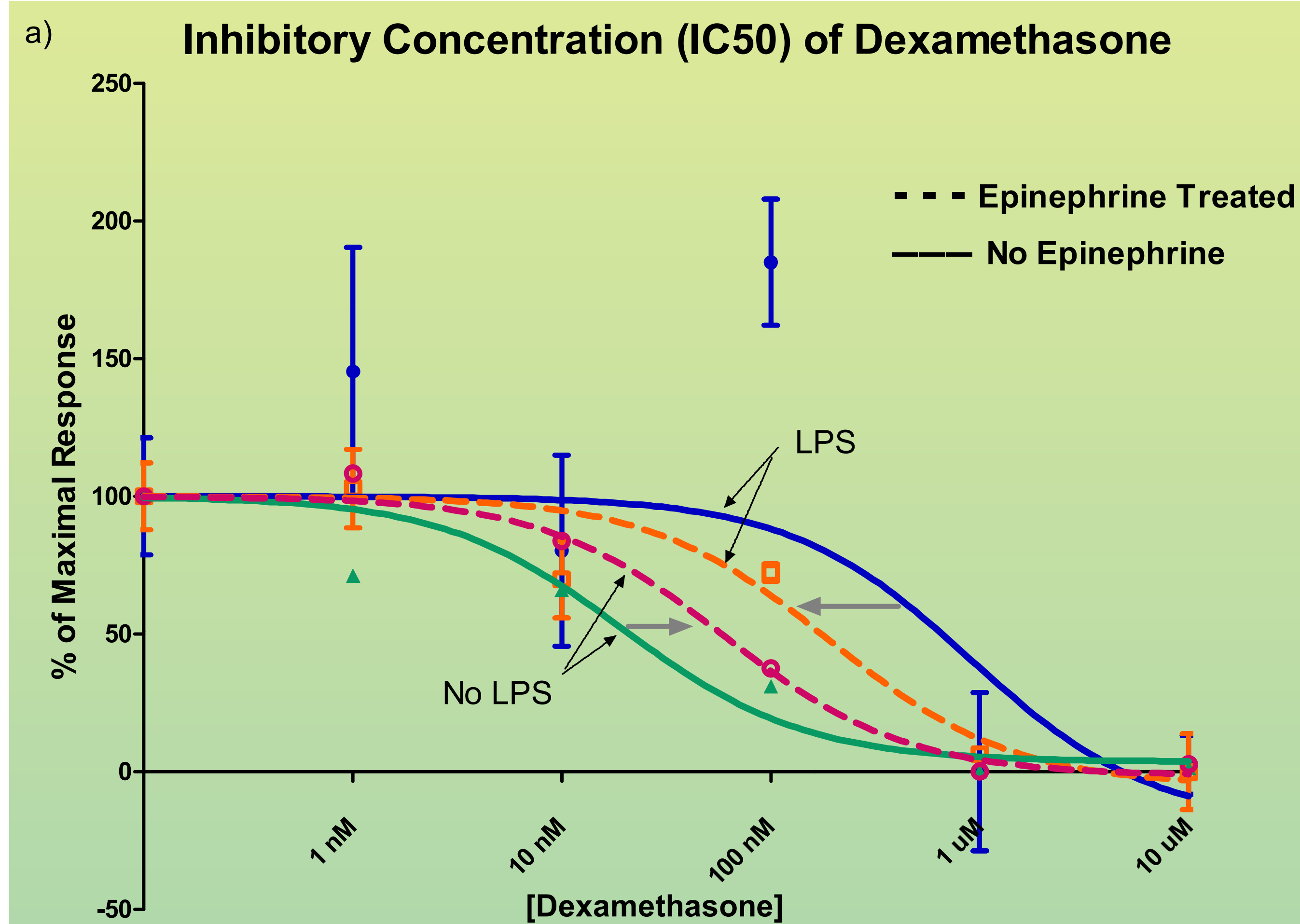


Figure a) IC50, the concentration of dexamethasone that inhibits 50% of cytokine (IL-6) response was measured in the presence and absence of epinephrine. In a clinically healthy dog, *in vitro* "stressor," epinephrine, shifts IC50 of LPS stimulated cells to the left, which indicates that epinephrine sensitized the immune cells to dexamethasone. Conversely, IC50 of non-stimulated cells shifts to the right in the presence of epinephrine. ** Data is in log scale.

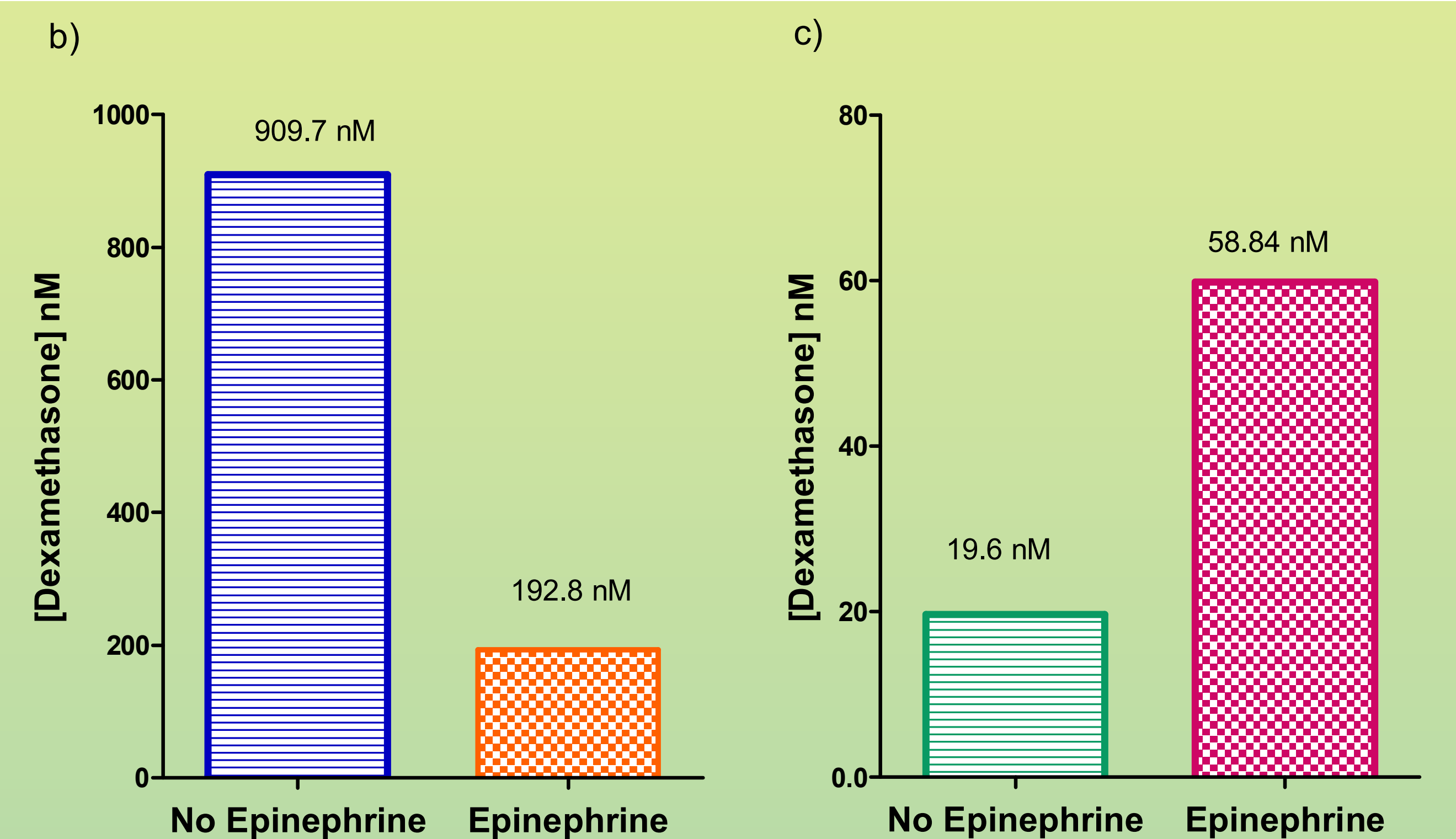


Figure b) Exposure to epinephrine increases the sensitivity to dexamethasone to suppress LPS-induced IL-6 cytokine secretion. **Data is from a single animal, thus there are no standard error to report.

Figure c) Epinephrine decreases the sensitivity to dexamethasone to suppress non-stimulated (basal) IL-6 cytokine secretion. ** Data is from a single animal, thus there are no standard error to report.

d) IL-6 Production with LPS Stimulation

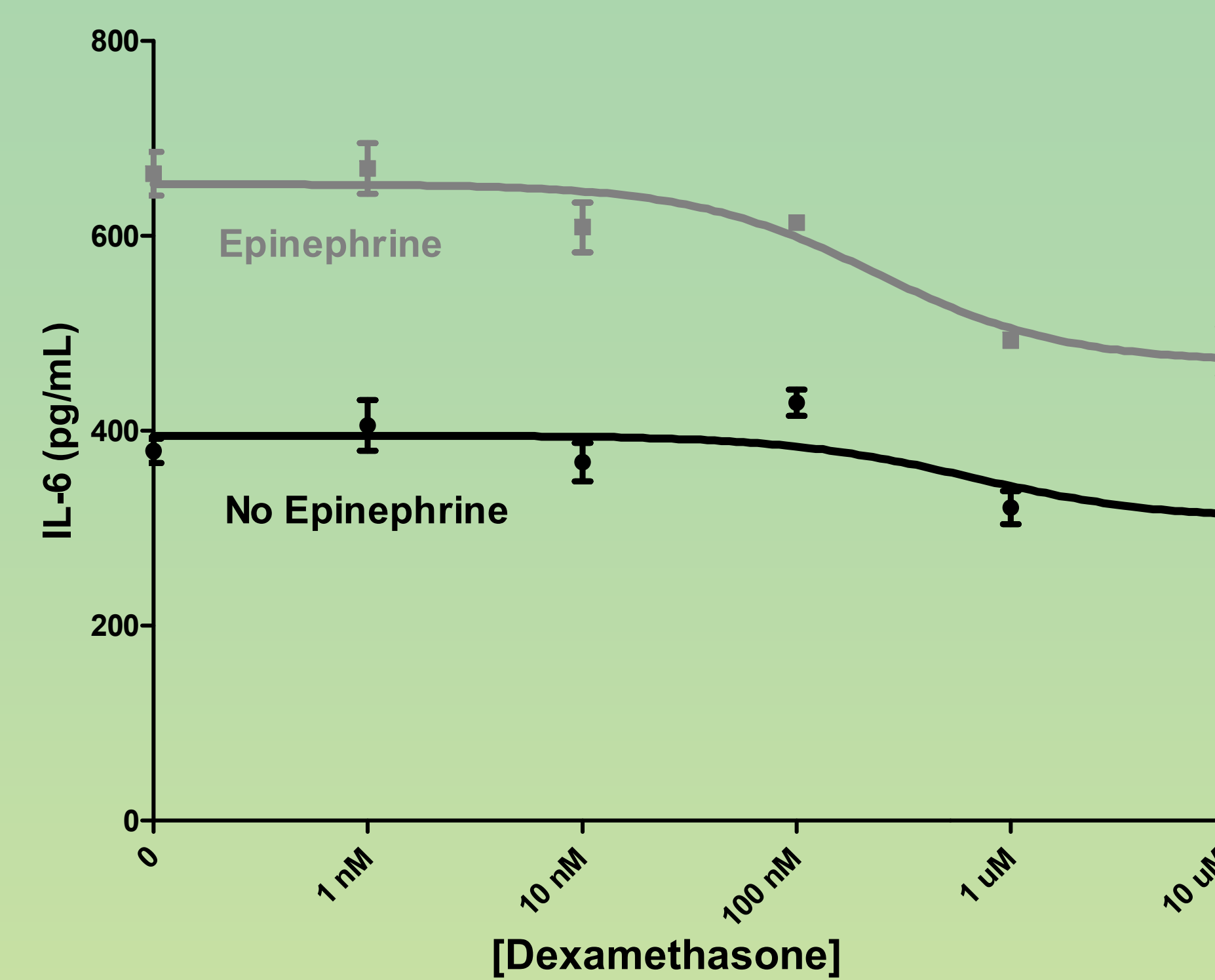
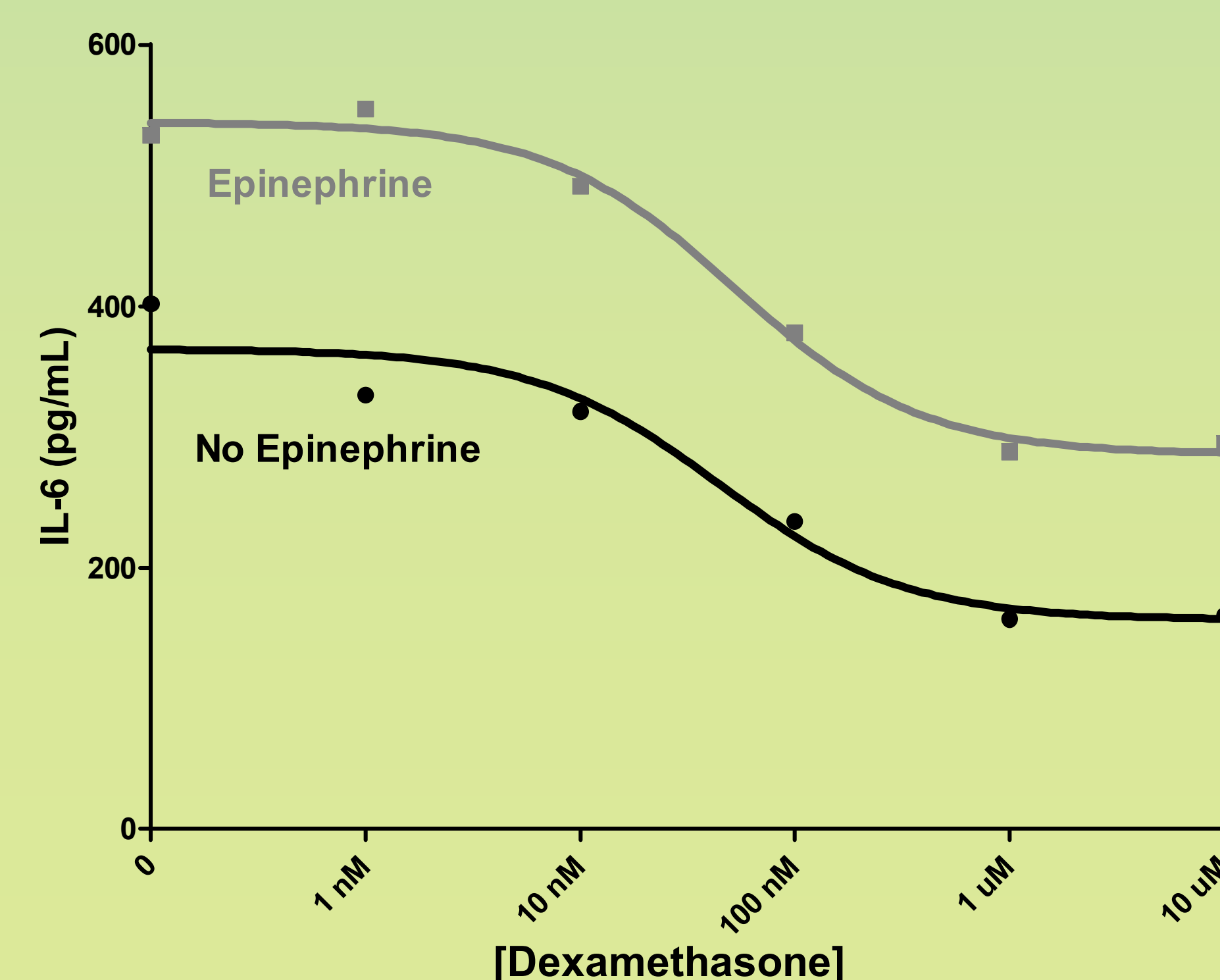


Figure d) and e) Epinephrine enhances the production of IL-6 cytokine in both the presence and the absence of LPS stimulation. This result suggests that epinephrine mimicked an acute stress condition with respect to immune cell responses. The increase in IL-6 secretion is higher in the cells stimulated by LPS (**Figure d**), which is expected with an active immune stimulus.

e) IL-6 Production without LPS Stimulation



Conclusion

Can the effect of stress on glucocorticoid sensitivity of immune cells in blood be modeled *in vitro*?

- Epinephrine recapitulates the effects of acute stress *in vitro*.
- Epinephrine plays a role in sensitizing the cells in whole blood of a healthy dog to dexamethasone.
- In a healthy dog, it takes less [dexamethasone] for the cells in the whole blood to suppress IL-6 secretion in the presence of stressor.
- The cell response to epinephrine is more prominent in the presence of an active immunological stimulant, LPS.

Ongoing & Further Studies

Can we inform prognoses of critical patients with this assay by measuring their glucocorticoid sensitivity?

- Whole blood culture of a variety of sick dogs will be compared with cultures from healthy dogs to determine whether results from this assay correlate with the prognoses of dogs with critical illness.
- Patients with poorer prognoses are expected to have an increased IC50 or become insensitive to dexamethasone.
- Interestingly, we observed a decrease in cell sensitivity to dexamethasone with respect to suppression of basal IL-6 secretion in the presence of epinephrine. We will explore whether this result is repeatable in additional healthy animals. This suggests a possible mechanism by which the stress of being in the hospital could affect patient outcomes.

Acknowledgments

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