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The effect of Substance P on hemorrhage and secondary injury after spinal cord injury

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Introduction

Spinal cord injury (SCI) is a life-altering event that can have numerous lasting effects. Previous work has shown that pain input after SCI engages nociceptive fibers, resulting in an adverse effect on locomotor recovery alongside an increase in tissue loss (secondary injury) at the injury site (Grau et al, 2004). These effects can be attributed in part to an increase in hemorrhage at the injury site.

Substance P (SP) is a neuropeptide that is released from sensory neurons to act as a messenger of injury and pain via the neurokinin-1 receptor (NK-1). Past studies have found that administration of a SP antagonist after a traumatic brain injury (TBI) had a neuroprotective effect in injured rats, showing that SP plays a role in the formation of secondary injury and the decrease in locomotor function (Corrigan et al, 2015).

In an SCI model, SP has been shown to have both beneficial and detrimental effects. SP improved recovery of locomotor function after SCI while also promoting an anti-inflammatory environment by increasing levels of anti-inflammatory cytokines and decreasing levels of inflammatory cytokines and markers of cell death (Jiang et al, 2012, Jiang et al, 2013). On the other hand, rats treated with a SP antagonist post SCI displayed decreased spinal cord blood flow, which may result in further damage by restricting blood and oxygen supply (Freedman et al, 1998).

There is also evidence that substance P may have an adverse effect. SP has been shown to induce vasodilation, increase vascular permeability, and create an inflammatory environment via regulation of macrophages, lymphocytes, and mast cells (Bartold et al, 1994, Lundblad et al, 1983). Additionally, SP is a key element in the production of prolonged states of overexcitation, thus potentially leading to the development of central sensitization (De Koninck & Henry, 1991).

The present study sought to determine the effects of SP after SCI and whether the administration of SP is linked to the induction of hemorrhage.

Methods

Treatment:

Male Sprague Dawley rats (n = 6) received a contusion at T12. Twenty-four hours later, subjects were given 40uL of 30nmol substance P, 60nmol substance P or saline vehicle administered intrathecally.

Behavioral and Physiological Testing:

Twenty-four hours after given a contusion, rats were analyzed using the Basso, Beattie, and Bresnahan (BBB) scale. BBB scores and blood pressures were measured every hour for the three hours following substance P administration.

Spectrophotometry:

Three hours after drug administration, rats were sacrificed and a one-centimeter section of the spinal cord around the site of injury was collected and processed for protein extraction. 1.5uL of the purified protein extract was analyzed under a spectrophotometer to observe the extent of hemorrhage using the Drabkin assay and Nanodrop.

References

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Does substance P affect locomotor recovery?

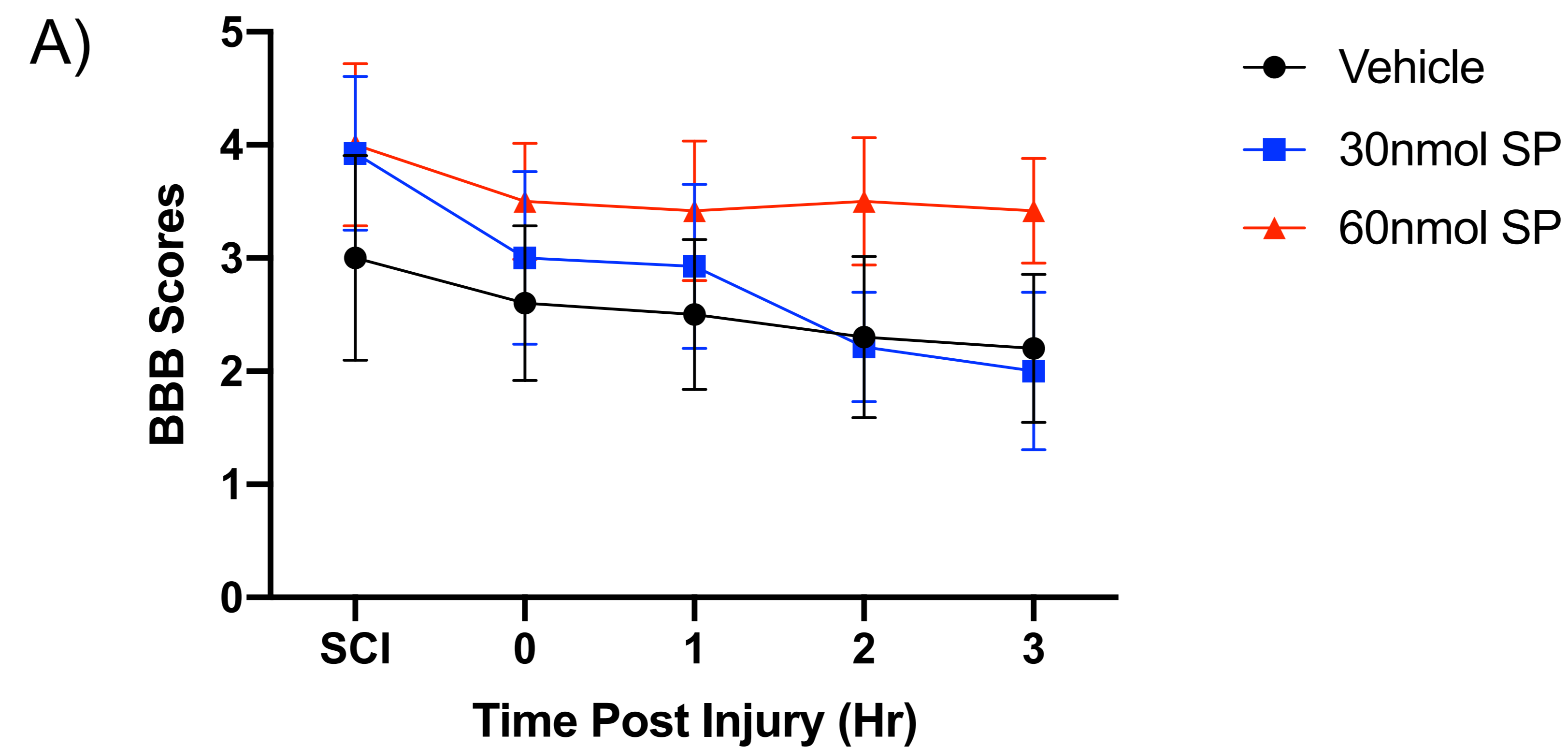


Figure 1. (A) shows the mean BBB locomotor scores for the three hours following treatment. An analysis of covariance, using the pretreatment (SCI) as the covariate, revealed that Substance P did not have a significant effect on locomotor recovery ($p > 0.05$).

How does substance P affect blood pressure?

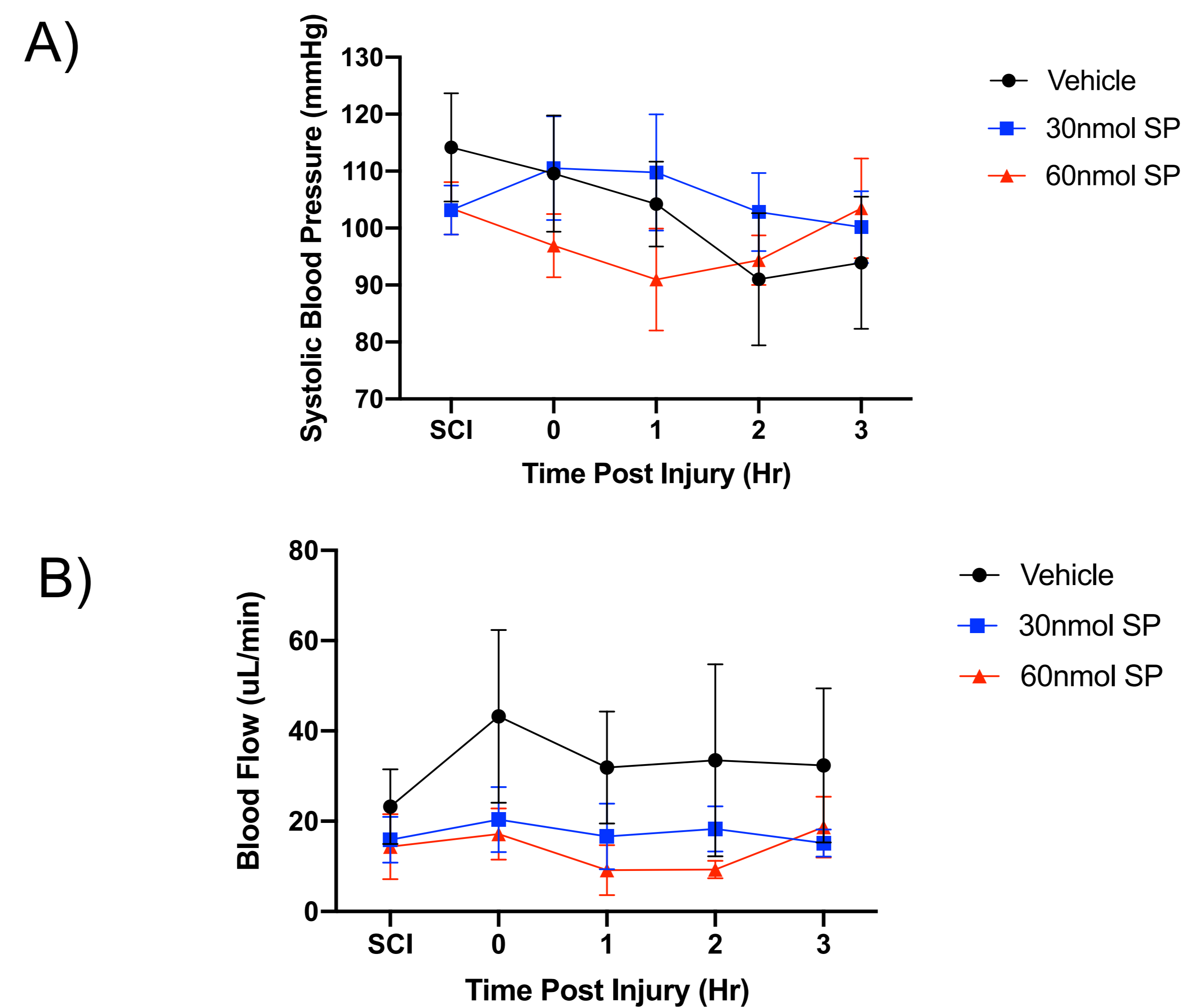


Figure 2. (A) shows the mean systolic blood pressures for the three hours following treatment. (B) shows the mean tail volume for three hours following treatment. Substance P did not have a significant effect on the mean systolic blood pressure or the mean tail volume ($p > 0.05$).

Does substance P reduce hemorrhage?

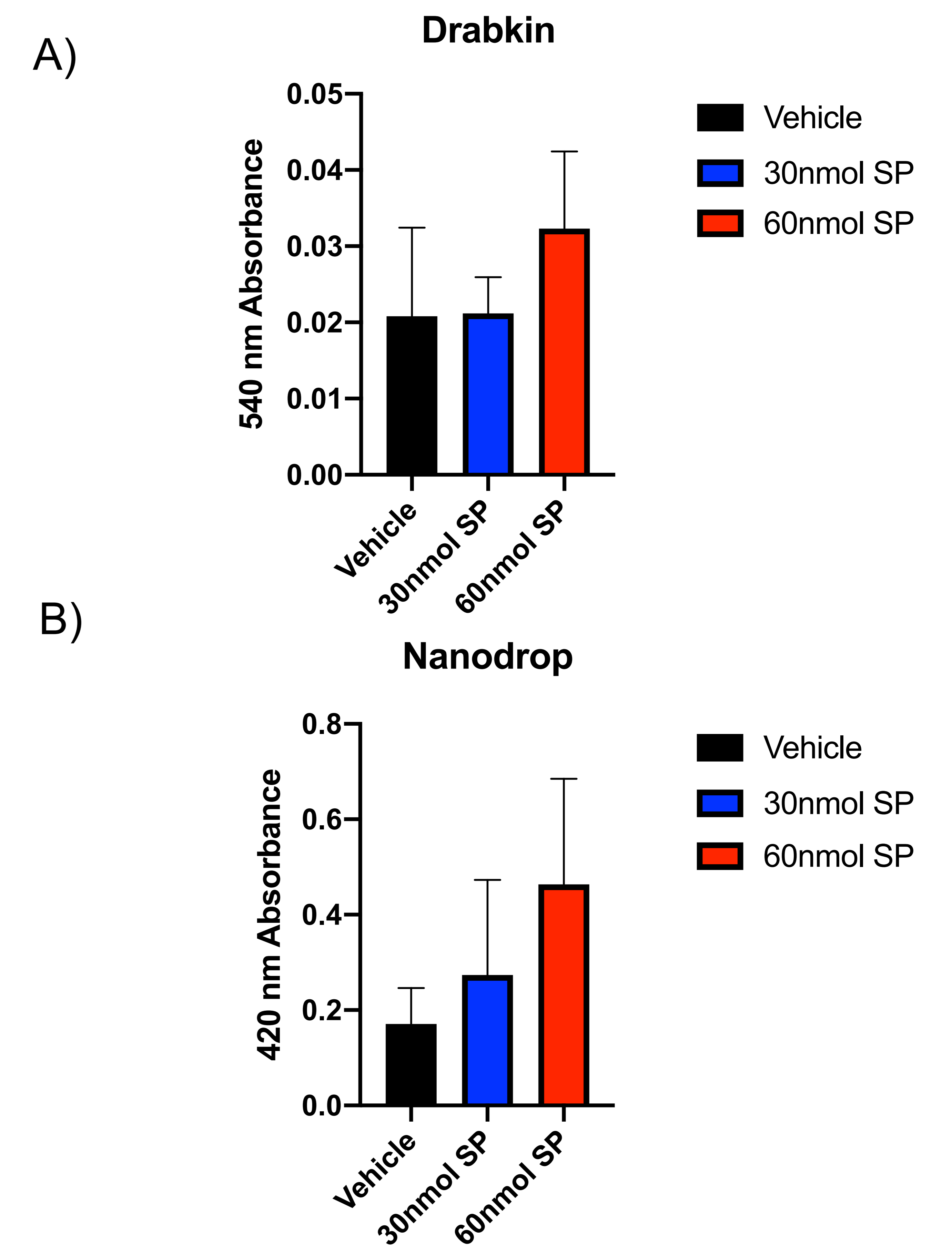


Figure 3. (A) shows the level of absorbance at 540nm (hemoglobin) using the Drabkin assay. (B) shows the level of absorbance at 420nm (hemoglobin) for each group using Nanodrop. Substance P did not have a significant effect on hemorrhage ($p > 0.05$).

Conclusions

- Substance P did not have a significant effect on locomotor function, hemorrhage, or blood pressure.

Future work includes:

- Further elucidating the role of substance P after SCI
- Use of an NK-1 receptor antagonist

