

● PERSPECTIVE

Sympathetic stimulation with norepinephrine may come at a cost

Norepinephrine (NE; also known as noradrenaline) is the body's primary adrenergic neurotransmitter which belongs to the catecholamine family. Norepinephrine has pharmacologic effects on the α_1 (Suita et al., 2015), α_2 (Schwartz, 1997), β_1 , β_2 and β_3 (Tsukada et al., 2003) adrenoceptors. In the brain, norepinephrine increases arousal and alertness, promotes vigilance, enhances formation and retrieval of memory, and focuses attention. It also increases restlessness and anxiety. In the remainder of the body, norepinephrine increases heart rate and blood pressure, triggers the release of glucose from energy stores, increases blood flow to skeletal muscle and increases muscle contraction, reduces blood flow to the gastrointestinal system and its motility and lastly, inhibits voiding of the bladder (Goldstein, 2010). This last point is particularly interesting in the context of this perspective piece.

An abundant number of current studies in the literature administer NE to small animal models. In these studies, NE is often given intraperitoneally (Im et al., 1998; Tsukada et al., 2003; Suita et al., 2015; Zhang et al., 2018) or *via* intravenous infusion (Chi et al., 1997; Deten et al., 2004). Exogenous NE plays a key role in stimulating heart and circulatory functions (Chi et al., 1997; Deten et al., 2004). Furthermore, NE reduces the rate of gastric emptying (Tsukada et al., 2003) and attenuates the efficacy of anti-angiogenic drugs in cancer mouse models (Deng et al., 2014). However, to date, none of these studies

have reported major adverse effects of NE in animals, although administration routes varied significantly between the investigations. As summarized in **Figure 1**, these studies have considerable variations in i) species or strain of mice used; ii) route of administration; iii) dose of NE; iv) length of NE administration and v) purpose of NE administration. It is entirely possible that the adverse outcomes of NE could be dependent on factors such as species of rodent, strain of mice, age and sex of animals, dietary modifications, animal facility status (PC1 or PC2) and the pathogen status of animals. Our mice were specific pathogen free male C57BL6/J mice.

Intravenous infusion is the most common form of NE administration to laboratory rodents. It may be speculated that intravenous administration of NE may promote systemic effects which could be detrimental to the animals if dose and time course of administration is not carefully considered. In addition, infusion rates and total infusion volume must be carefully determined as large volumes can result in immediate distress, pulmonary and cardiac abnormalities and death. On the other hand, substances delivered intraperitoneally are typically absorbed at a much slower rate which may minimise adverse events (Turner et al., 2011).

Given the wide use of NE in animal studies, it is important to understand the functions of NE, both beneficial and detrimental. Intraperitoneal administration of NE has shown to reduce endogenous NE suggesting a counter regulatory effect in rabbits and rats. However, intravenous administration considerably increased endogenous NE (Zavodskaya and Moreva, 1980). Hence it can be suggested that the differences seen in the level of endogenous NE could be due to different rates of absorption determined by the route of administration.

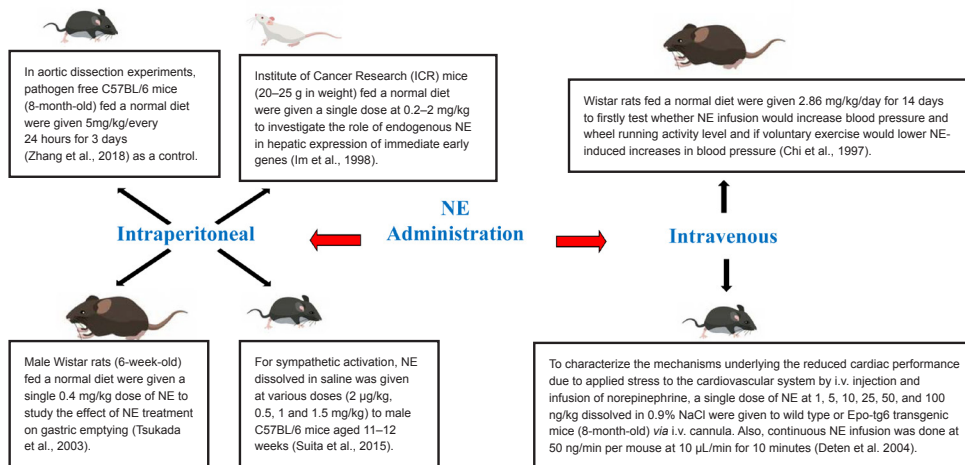


Figure 1 The variation in rodent studies where norepinephrine is administered. NE: Norepinephrine.

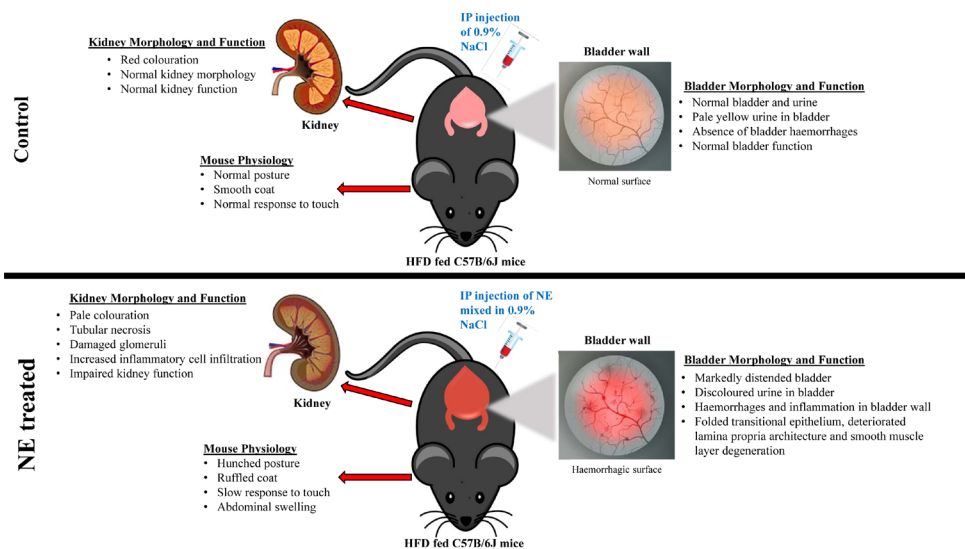


Figure 2 Possible adverse events due to NE treatment in mice. Results were obtained from the study of Matthews et al., 2018. HFD: High-fat diet.

In our recent publication in *Biochemistry and Biophysics Reports*, we highlight a number of adverse outcomes of exogenous NE administration to mice (Matthews et al., 2018). Our goal was to administer NE to a subset of high fat diet fed mice (fed the high fat diet for 10 weeks) to mimic the sympathetic hyperactivity observed in human obesity and type 2 diabetes. In our studies, we intraperitoneally injected mice with 0.2 or 2 mg/kg/day of NE dissolved in 0.9% NaCl. A subset of the NE treated mice showed an unexpected rapid decline in their health and wellbeing (Figure 2). Of 55 mice, 11 mice either died or were euthanised early to avoid further adverse events. We also ceased NE administration as soon as adverse events were observed.

In short, affected NE treated mice showed a hunched posture, ruffled coat, slow response to touch and abdominal swelling. Upon post-mortem examination of these animals, the urinary bladder was noted to be markedly distended with discoloured urine in the bladder. One mouse out of the 11 effected mice showed the capacity to expel urine from the distended bladder suggesting that a partial or non-obstructive urinary bladder may have contributed to the extended bladder phenotype. However, the majority of the NE affected mice ($n = 10$) could not expel urine even upon application of forceful pressure. It is entirely likely that the normal urinary flow was prevented due to the obstruction of the urethra. Furthermore, bladder haemorrhages represented damage, irritation and/or inflammation of the bladder wall. Histological analysis of the bladder revealed severe structural abnormalities such as folded transitional epithelium, deteriorated lamina propria architecture and degeneration of the smooth muscle layer reflecting bladder dysfunction. It is shown that $\alpha 1$ adrenoceptors promote contraction of the bladder neck and the urethra to enhance bladder outlet resistance. Whereas, the $\alpha 2$ adrenoceptors may promote weak contraction of the urethra in species other than humans (Michel and Vrydag, 2006). When agonists for the $\alpha 1$ (e.g., Phenylephrine), $\alpha 2$ (e.g., Clonidine) and $\beta 1$ (e.g., Dobutamine) adrenoceptors are used, it is not known whether these compounds may also have similar effects as seen in our recent study. However, these compounds are believed to have lower affinity for the receptor than NE.

In NE treated mice, both kidneys appeared pale in colour. The kidney morphology of these mice were significantly affected and showed tubular necrosis, damaged glomeruli and increased infiltration of inflammatory cells (Figure 2). The morphological damage observed in NE treated mouse kidney is likely a consequence of NE induced abnormalities in urethral contraction and voiding of the bladder. The extensive distention of the bladder would not only lead to sustained mechanical pressure on the bladder wall but would also result in increased pressure in the upper urinary tract, particularly the ureter and renal pelvis with functional consequences resulting in renal impairment (Mustonen et al., 1999).

Although many studies administer exogenous NE particularly to rodents, none to date have reported adverse events similar to what we noted in our cohort of mice. A recent study administered NE intraperitoneally at a much higher dose (5 mg/kg/every 24 hours for 3 days) but does not report any adverse outcomes (Zhang et al., 2018). Our cohort of mice given NE were fed a high fat diet and we and others have previously shown hyperactivity of the sympathetic nervous system in these mice. It is likely that treatment with NE further aggravated the already hyperactivated sympathetic nervous system leading to a detrimental outcome in a subset of mice. The damaging effect of NE was more pronounced in the 2 mg/kg/day treatment group of mice in comparison to the 0.2 mg/kg/day group. The phenotype observed in our study could be a combination of several factors such as dose, route of administration and modified diet.

Hence, researchers considering administering NE to animals must carefully evaluate the dose, route and frequency of administration, preparation of NE, animal species used and modifications to diet prior to commencing experiments. Furthermore, it is vital that more studies involving administration of exogenous NE are completed to fully understand any pathogenic mechanisms involved with NE *in vivo*.

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