Validation of urinary catecholamines as a stress indicator following electrical stunning

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Background. The collection of voided urine samples for the measurement of stress hormones, such as catecholamines and metabolite is a useful non-invasive method for assessing animal stress and welfare (Hay and Mormede, 1998). If collected from the bladder, after slaughter, urine catecholamines and their metabolites may provide a "stress record" of the recent events preceding slaughter. However validation of the method is still required for a number of animal species and different experimental situations.

Plasma catecholamine levels can rise rapidly in response to stress, and they fall rapidly because they have a half life in the blood of on seconds, making surges in levels difficult to measure. The sensitivity of animals to different blood sampling techniques further complicates attempts to measure baseline catecholamine levels, or the effects of specific stressors. These characteristics of catecholamine release and metabolism have lead to the use of measurement of urine catecholamine levels to indicate the effects of cumulative stress.

The pathway for the breakdown of catecholamines is important in the interpretation of physiological sympathetic activity. Reuptake and metabolism of norepinephrine in peripheral sympathetic nerve endings results in little non-metabolised NE being released into the circulation. However, circulating catecholamines (released from the adrenal gland) and infused catecholamines can result in more non metabolised catecholamines being excreted due to less efficient metabolism (Maas and Landis 1971). The main metabolic product of both NE and epinephrine (E) is vanillomandelic acid (VMA). This leads to the suggestion that the ratio of non-metabolised to metabolised catecholamines may provide a more sensitive index of sympathoadrenal activity, than VMA alone.

Electrical stunning, which occurs during routine commercial slaughter, significantly elevates blood catecholamines in sheep (Pearson, 1979), but there are no published studies, detailing the chronological relationship between increases in plasma catecholamines and corresponding changes in the urine catecholamine concentration. In sheep, electrical stunning has been shown to produce a 40-50 fold increase in plasma catecholamines 20 s after a stun, levels then decrease rapidly to baseline within 2 min (Pearson 1979).

Objectives.

- To confirm the time course and magnitude of the release of catecholamines into the blood following electrical head-only stunning.
- To measure the time delay between catecholamines being released into the blood and the subsequent detection of catecholamines and VMA in the urine.
- To determine if electrical stunning at slaughter confounds the measurement of catecholamines and their metabolites in urine as measure of stress occuring immediately preslaughter in a commercial slaughterhouse.
- To assess the use of non-metabolised urinary catecholamine ratios as an index of sympathoadrenal activity.

Methods. This experiment was conducted with the approval of the Ruakura Animal Ethics Committee, Hamilton, New Zealand. Twelve sheep (ewes, 50-55kg) were electrically head-only stunned (Cook et al. 1995), six with short stuns (0.2 s) and six with long stuns (8 s). Each animal was fitted with a jugular cannula for blood collection, a urethral catheter for urine collection, and placed in a restraining cradle for stunning. They were not stuck, but instead, they were allowed to recover from the stun. Blood samples (5 mls) were taken 10 min and 1 min before stunning, and 0.5, 1, 2, 5, 15, and 30 min after stunning. Samples were placed on ice and centrifuged within 5 min of collection. The plasma was frozen in liquid nitrogen and stored at –80°C until analysis. Urine samples were taken 10 minutes and 1 minute before stunning, and 5, 15, and 30 min after stunning. Urine samples were acidified using HCL immediately after collection and stored at -80 °C until analysis. Plasma and urinary NE, E and VMA, were determined using HPLC and electrochemical detection. Catecholamines were extracted from 0.5 mL of plasma or 0.1 mL urine using the method described by He et al. (1997). The coefficient of variation for external standards were 4.66% and 5.65% for NE and E respectively. The extraction efficiency was 63.65 ± 3.46, 64.71 ± 3.62 and 66.83 ± 3.54 for NE, E and epinine, respectively. To measure VMA, urine was diluted 10-100 fold in 0.08M acetic acid and centrifuged at 24000g for 5 minutes, the supernatant injected directly onto the column. The column was a Prodigy™ 150×4.6mm 5µ ODS-3 (Phenomenex), and the mobile phase was 0.1M phosphate, 0.2 g L^{-1} tetra butyl ammonium hydrogen sulphate and 1%methanol adjusted to a pH of 6.9. Urine creatinine was determined by a colourmetric method. Urinary values for NE, E and VMA are all adjusted for glomeruler filtration rate, and expressed as g mg $creatinine^{-1}$.

T-tests were used to test for differences between the peak values of plasma NE, E, urinary NE, E, and VMA, between the 0.2 and 8s stuns. Repeat measures ANOVA was used to determine differences in catecholamines and metabolites with time in each treatment.

Results and discussion. Electrical head-only stunning rapidly increased plasma levels of both NE and E in sheep stunned for 8 and 0.2 s. NE increased 34 fold above basal levels, while E increased 100 fold (figure 1). Levels of both NE and E peaked between 30 s and one min and had returned to baseline 5 min after the stun. These are similar findings to those of Pearson (1979). Peak levels of plasma NE were higher in sheep stunned for 8 s duration than those stunned for 0.2 s ($p<0.05$), whereas there was no difference in peak plasma E levels. This trend followed for peak levels in the urine where peak NE was significantly higher in the 8s stun group, but E was not. These results suggest that urinary catecholamines can be used to assess a stressor in a quantitative manner, but to simplify the presentation of results all figures presented are pooled results from both 8 and 0.2s stuns. There was a significant time delay of between 5-15 min before urinary NE, E or VMA increased after the stun (figure 2). Clearly the release of catecholamines during stunning is both rapid and of large magnitude, and this was reflected soon afterwards by changes in urinary catecholamines and metabolites.

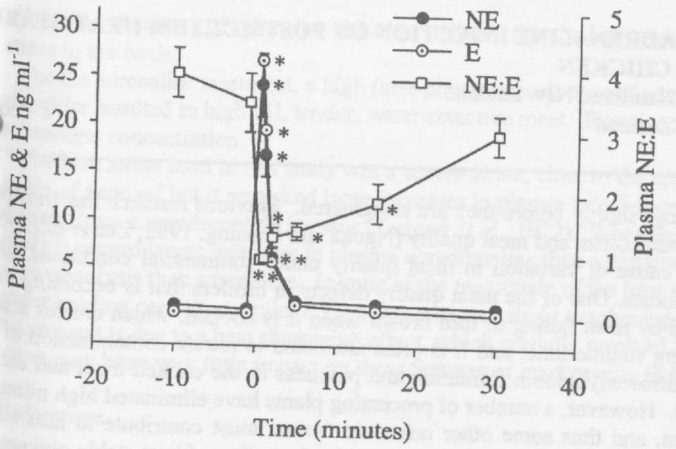

Figure 1. Changes in plasma NE and E concentrations, and NE:E ratios over time following a head-only stun. *(p<0.05) - significantly different from baseline.

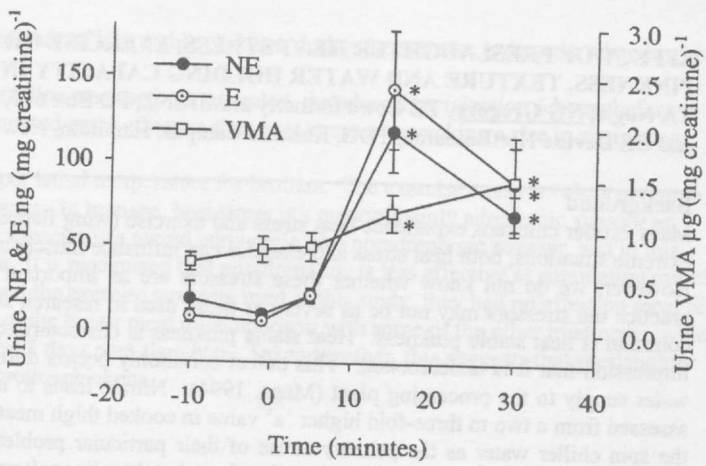

Figure 2. Changes in urinary NE, E and VMA concentrations over time following a head-only stun. *(p<0.05) - significantly different from baseline.

In a commercial slaughter house animals are usually bled out within 20 s of being stunned. Once blood flow to the kidneys ceases, urinary production is prevented, thus the physiological stress associated with stunning and slaughter should not impact upon urinary concentrations of NE, E or VMA.

The relative rise in concentration of urinary NE and E, is much greater than for VMA. This effect is shown in figure 3 where the ratios of NE, E, and VMA are presented. The percentage of unmetabolised NE, E in the urine increased from approximately 2% to 14% following stunning. This reflects increased secretion of catecholamines directly into the circulation by the adrenal medulla, and less efficient metabolism of plasma catecholamines. Another aspect of the release of catecholamines from the adrenal medulla is the relative ratio of NE:E. At peak levels in the plasma following stunning NE:E are in a ratio of 1:1, whereas, prestun levels are in a ratio of 3.75:1 (figure 1). These ratios are reflected in the urinary levels (figure 3). Perhaps the most interesting feature of this result is that the ratio of NE:E in urine appears to decrease in advance of significant changes in the concentrations of these compounds (figure 3).

The stress response induced in this study may not typify responses to other stressors. Thus, further study involving different stressors, including physical, and psychological, will allow further understanding of the sensitivity of ratios of urinary NE:E as a stress measure.

Conclusions

- The massive release of catecholamines into the blood circulation following electrical stunning, is unlikely to have a significant effect on levels of urinary catecholamines in samples collected from the bladder postmortem.
- Levels of non-metabolised catecholamines in the urine, and their relative concentrations have potential as sensitive measures for evaluating sympathoadrenal activity.

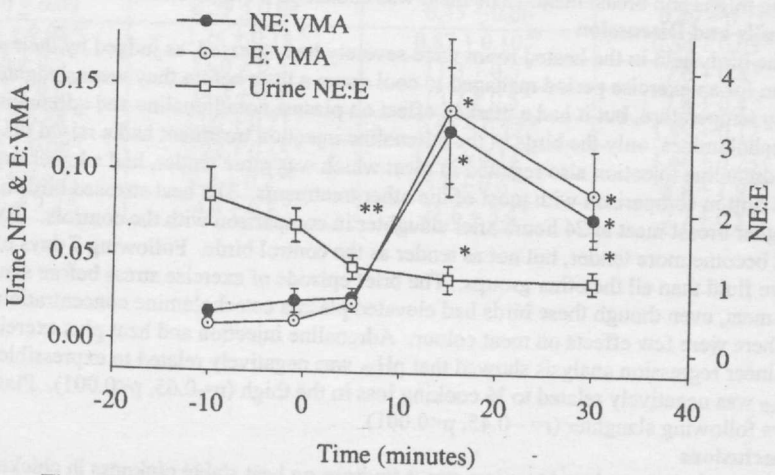

Figure 3. Changes in ratios of urinary NE, E and VMA over time following a head-only stun. *(p<0.05), **(p<0.10) - significantly different from baseline.

References

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