



Influence of transportation, subclinical *Salmonella* infection and slaughter on plasma histamine level of pigs

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Abstract

Pigs are regularly subjected to stress around slaughter. Stress hormones released during such periods of sustained environmental challenges could be important to assess the procedural impact on the well-being of the animal, as well as the impact on meat quality. Besides classical stress hormones, the present study mainly focussed on histamine because histamine has been suggested to be a suitable stress indicator in rats. In Trial 1, histamine, catecholamines and cortisol were measured in plasma samples obtained by venipuncture before stunning and slaughter or during exsanguination. Median plasma concentrations in venipuncture samples amounted to 1.13 nM (adrenaline), 2.33 nM (noradrenaline), 18.3 nM (cortisol) and 79.3 nM (histamine). The concentrations of adrenaline, noradrenaline and cortisol increased from venipuncture to slaughter ($P < 0.01$) 142-, 40- and 3-fold, respectively. Plasma histamine concentration tended to double in parallel ($P = 0.09$). In exsanguination samples, plasma histamine concentration tended to correlate to plasma adrenaline ($P = 0.13$) and plasma noradrenaline ($P = 0.07$) concentrations. In Trial 2, pigs were infected with *Salmonella typhimurium* DT 104. After the infection had become clinically inapparent, pigs were either rested overnight or transported for 8 hours prior to slaughter. Uninfected control pigs were also either rested or transported before slaughter. Plasma histamine levels were measured in two exsanguination samples taken within 10-30 s of bleeding and 1 min thereafter. The plasma concentration of histamine decreased by transportation ($P < 0.05$). However, subclinical *Salmonella* infection did not affect histamine concentration in plasma. The plasma concentration of histamine increased from early to late exsanguination samples ($P < 0.05$). Agonal stress during stunning and slaughter triggers an exorbitant release of classical stress hormones in pigs. Histamine is released, too. In view of the physiological impact of histamine on vascular and intestinal permeability, the histamine release could be relevant for the quality of meat. However, plasma histamine level is not a suitable stress indicator in pigs because of a high interindividual variability and because of paradoxical decreases after prolonged stress.

Key words: Adrenaline, catecholamines, cortisol, histamine, noradrenaline, pig, *Salmonella*, slaughter, stress response.

Introduction

Pigs in the current production systems are subjected to a variety of stressors like confinement, loading/unloading and transportation¹⁻³. The stress of slaughter pigs has three major dimensions: an animal welfare dimension with ethical, political and aesthetic aspects³, an economical dimension due to transportation losses and impaired meat quality⁴ and a public health dimension due to facilitation of food-borne diseases, e.g. salmonellosis⁵. Accordingly, there are three major reasons to minimize stress and/or the consequences thereof. To do this, objective criteria are needed to evaluate the responses to stressing challenges and to compare the responses between different systems.

A variety of neuronal and hormonal mediators is released into the systemic circulation during stress. Some of these mediators appear suitable to assess the impact of a certain stressor on the individual animal, e.g. adrenaline, noradrenaline and cortisol⁶⁻⁸. Plasma histamine level could be another indicator for the impact of stress^{9,10}. However, there appear to be differences between species. Whereas rats show marked increases in plasma histamine

level during even moderate stress^{9,10}, dogs do not respond to any stressor with increased plasma histamine levels^{11,12}. In a previous trial, it was suggested that plasma histamine level could be a stress indicator in pigs¹³. This would not only be important from a diagnostic and animal welfare point of view. The permeability-enhancing properties of histamine^{14,15} could be important for meat quality. The objective of the present study was to test inasmuch the plasma histamine level of pigs is affected by different stressors, i.e. transportation, subclinical *Salmonella* infection and agonal stress during stunning and slaughter. For comparison, the plasma levels of established stress mediators, i.e. catecholamines and cortisol, were also monitored in one trial.

Experimental

Experimental procedures were in accordance with the German legislation on animal welfare and were approved by the appropriate authority, Regierungspräsidium Leipzig (TVV 24/00 and AZ 74-9162.11-01-T47/01).

Trial 1: Histamine, catecholamines and cortisol: Eight growing-finishing pigs (German Landrace x German Large White, female, 90–110 kg) were transported approximately 8 km from the university farm to the Faculty of Veterinary Medicine in Leipzig and held in individual pens. Pigs were slaughtered on separate days at 7:30 a.m. On the day of slaughter, the pig was led into a transport cage. It was restrained by a snare but care was taken to keep the restraint as little and as short as possible (< 1 min). A first blood sample was taken into 10 ml Plasma Lithium Heparin tubes (Sarstedt, Nümbrecht, Germany) by venipuncture of the jugular vein. The pig was then transported to the faculty's slaughterhouse by a hand-pulled cart (approximately 200 m). Within 15 min of arrival, pigs were stunned by bolt pistol and exsanguinated. A second blood sample was taken from the exsanguination blood immediately after placing the bleeding incision.

Trial 2: Transportation and Salmonella infection: Forty one pigs (fattening hybrids, castrated male, 30–35 kg) were obtained from a herd without previous history of salmonellosis. Pigs were tested negative for Salmonella, using serology and bacteriology of faeces at the beginning of the experiment. Nineteen pigs served as an uninfected control group. The other 22 pigs were infected intragastrically with a highly virulent, penta-resistant field isolate of *Salmonella typhimurium* DT 104 according to the protocol described in Marg *et al.*⁵. All infected animals developed clinical salmonellosis within 24 h. After 1 wk post infection, pigs were essentially free of clinical signs (body temperature, diarrhoea, appetite, general demeanour) except for some sporadic inconsistency of faeces occurring still after 2 wk. Three weeks post infection, eight pigs of the control group and eleven pigs of the infected group were transported separately at night time for 8 h on small local roads in Saxony. On arrival (8:00–10:00 a.m.), pigs were stunned by bolt pistol and slaughtered. Blood samples for histamine analyses were obtained in Plasma Lithium Heparin tubes immediately after placing the bleeding incision (10–30 s after stunning) and 1 min thereafter. Non-transported animals were killed at the same time of the day and sampled equivalently. The subclinical Salmonella carriage of pigs in the infected groups was verified by positive Salmonella recovery from at least one of the following organs: tonsils, jejunum, ileum, caecum, colon or from jejunal, iliocaecal or colonic lymph nodes as described in Marg *et al.*⁵. The Salmonella-free status of non-infected animals was confirmed by negative testing of all sampled organs.

Sample processing, storage and analyses: All blood samples were immediately placed on ice after taking and centrifuged within 1 h (1500 g, 10 min, 4°C). Sample aliquots for analyses of catecholamines and cortisol in Trial 1 were stored at -80°C until analyses by radioimmunoassay (RIA). The RIA for catecholamines was performed according to the manufacturer's procedure (KatCombi RIA, IBL, Hamburg, Germany). Plasma cortisol concentrations were measured in duplicate as described by Abraham *et al.*¹⁶, using an own RIA system and commercially available ³H-cortisol (Amersham Biosciences, Freiburg, Germany). Radioactivity of the KatCombi RIA was measured by gamma counting (Wallac 1470 Automatic Gamma Counter, Wallac, Turku, Finland). Radioactivity of the cortisol RIA was measured by liquid scintillation counting (Wallac 1409 Liquid Scintillation Counter) using the liquid scintillation fluid Rotiszint[®] Mini (Roth, Karlsruhe,

Germany).

In Trial 1, a second sample aliquot was stored at -80°C and thawed in cold water shortly before histamine extraction and analysis. Histamine extraction was performed by the two-step procedure described previously by Aschenbach *et al.*¹⁷ (ion-pair extraction using a 50 mM bis(2-ethylhexyl) phosphate solution in n-heptane followed by acid extraction into 0.1 N HCl). The histamine concentration in the extract was determined by HPLC as previously described¹⁸. Despite using recommended low-temperature storage at -80°C, it was noted that the plasma content of histamine was always decreased when plasma samples were refrozen and thawed again for repeated analyses. Therefore, histamine was extracted before storage in Trial 2. The acid extract was then stored at -80 °C until HPLC analysis.

Chemicals: Methanol, water, acetonitrile, n-heptane (all HPLC-grade) and ortho-phthalaldehyde were supplied by Fluka (Buchs, Switzerland). Bis(2-ethylhexyl) phosphate and cortisol were supplied by Serva (Heidelberg, Germany). All other chemicals that are not explicitly mentioned in the text were obtained either from Merck (Darmstadt, Germany) or from Sigma (Taufkirchen, Germany).

Presentation of results and statistical analysis: Statistical comparisons of different groups started with testing for normality (Kolmogorov-Smirnov's test with Lilliefors' correction) and equal variance (Levene's Median test). If both criteria were fulfilled, data are presented as means and SEM and were compared by Student's paired t-test. If one criterion was biased, data are presented as medians and percentiles, and tested with Wilcoxon's signed rank test. For the complex data set of Trial 2, three-way analysis of variance (ANOVA) was used. Data were tested for effects of Salmonella infection, transportation and time of blood sampling after stunning, as well as for interactions between each two or three of these factors. All tests were performed using the software SigmaStat[®] 2.0 (Systat, Erkrath, Germany). The same software was used to calculate correlation coefficients and to estimate regression parameters. Differences between regression parameters were assessed by Student's t-test.

Results

The aim of the first trial was to assess the stress response of pigs at slaughter in general and to compare histamine as a potential stress indicator with the established indicators adrenaline, noradrenaline and cortisol. Stunning and exsanguination caused 142-fold and 40-fold increases in the median concentrations of plasma adrenaline and noradrenaline, respectively, compared with samples obtained by venipuncture before stunning ($P < 0.01$; Fig. 1). Plasma cortisol level also increased albeit to a lower extent (threefold; $P < 0.01$; Fig. 1). The plasma concentration of histamine tended to be greater ($P = 0.09$; Fig. 1) in samples obtained at exsanguination compared with samples obtained by venipuncture. Plasma concentrations of adrenaline and noradrenaline were correlated to each other ($P < 0.01$) in samples obtained from both venipuncture and exsanguination (Fig. 2). However, the slope of the regression line was decreased ($P < 0.01$) at exsanguination (Fig. 2), concomitant with a decrease in the noradrenaline:adrenaline ratio (from 2.42 ± 0.39 to 0.71 ± 0.12 ; $P < 0.01$; means \pm SEM). Cortisol plasma values were correlated to both

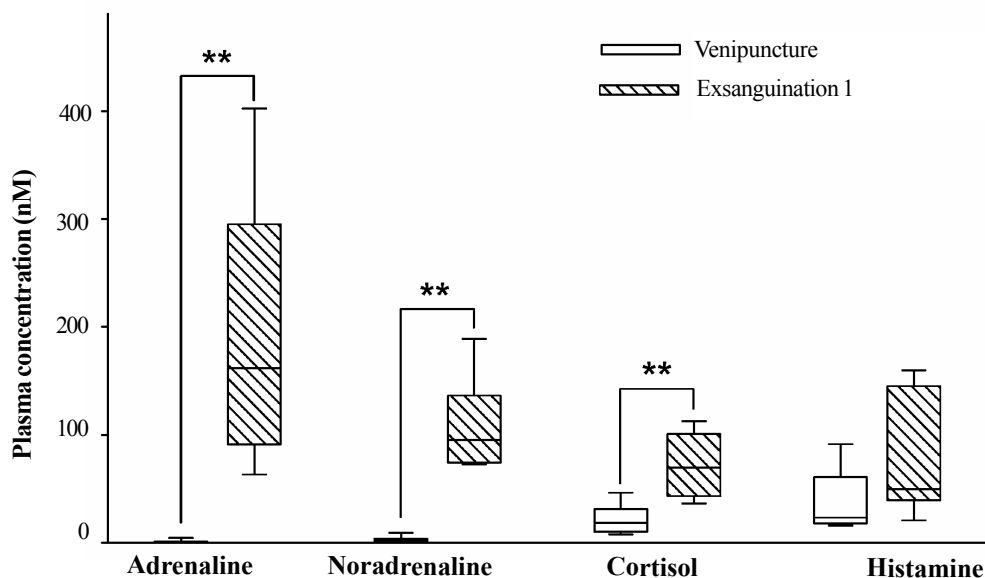


Figure 1. Plasma concentrations of established stress hormones and histamine in pigs of Trial 1. Blood samples were obtained by venipuncture before stunning and slaughter or during early exsanguination at slaughter. The early exsanguination samples are termed Exsanguination 1 in conformity with the nomenclature used in legend to Trial 2 (Fig. 3). Values of eight animals are presented as box and whisker plots. The boxes show the 25th, 50th (median), and 75th percentiles. The whiskers at the top and bottom of the boxes extend from the 5th and 95th percentile, respectively. **P<0.01.

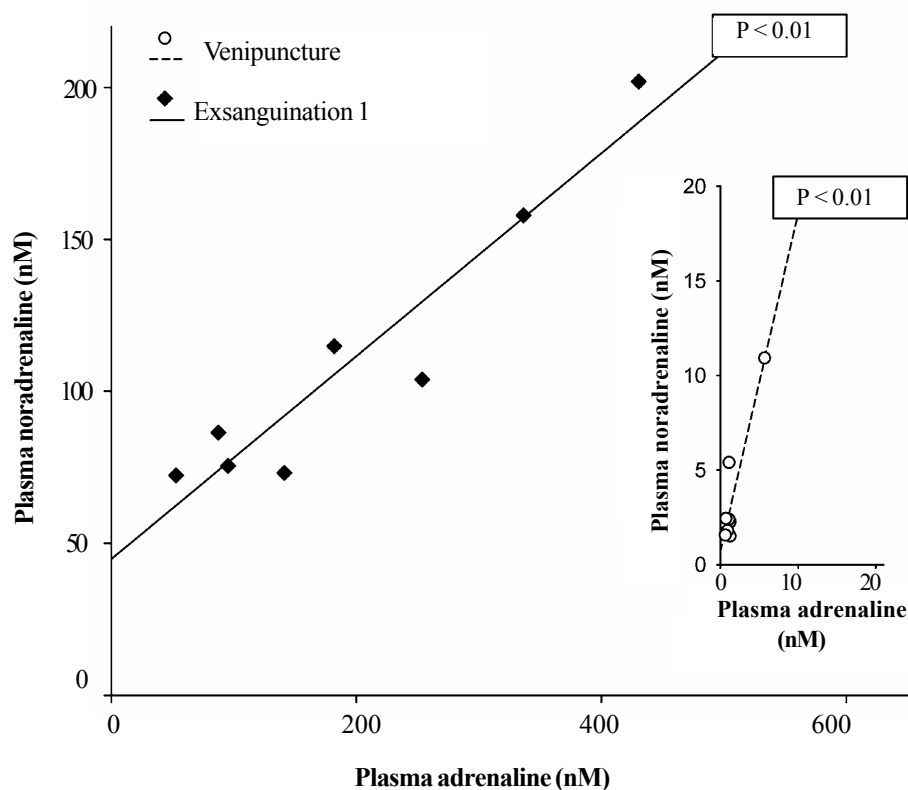


Figure 2. Relationship between plasma noradrenaline and adrenaline concentrations during venipuncture and early exsanguination in Trial 1. Plasma noradrenaline concentrations of eight individual animals are plotted against the corresponding adrenaline concentration during either venipuncture or early exsanguination. The significance level of correlation is indicated at the end of the regression lines. The slopes of the regression lines are different from zero and different from each other. The intercept of the regression line of the exsanguination data is different from zero (P < 0.05). Regression estimates are:

- : noradrenaline = $(0.67 \pm 0.66) \text{ nM} + (1.79 \pm 0.30) \cdot \text{adrenaline}$; $r = 0.93$
- ◆: noradrenaline = $(44.8 \pm 10.6) \text{ nM} + (0.33 \pm 0.05) \cdot \text{adrenaline}$; $r = 0.95$.

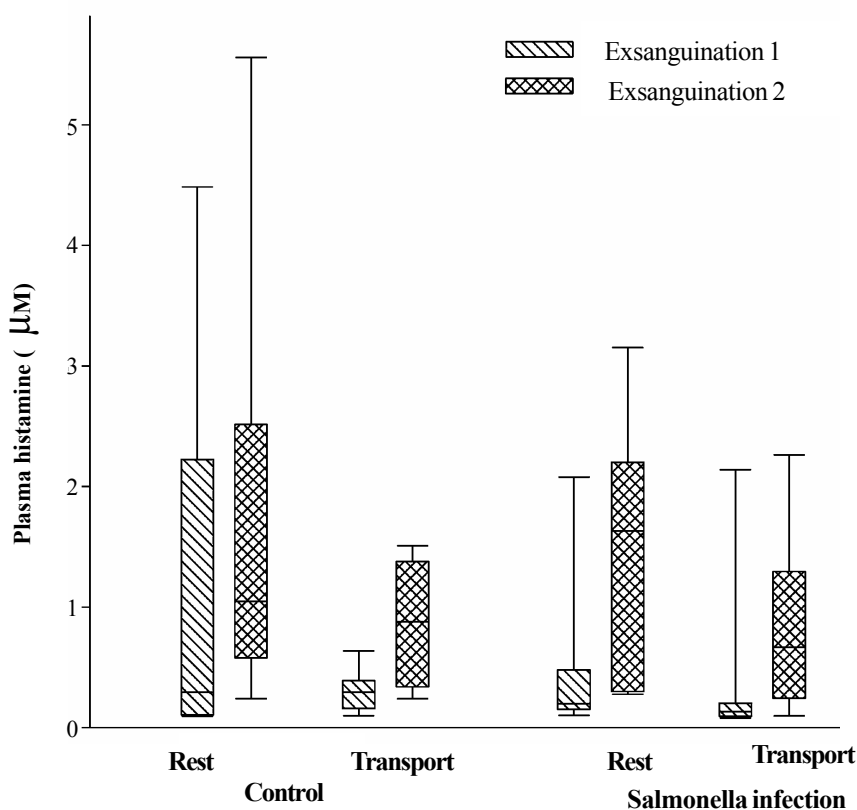


Figure 3. Influence of an 8-h transportation and subclinical Salmonella infection on the plasma concentration of histamine during early or late exsanguination in Trial 2. Values of 8–11 animals are presented as box and whisker plots. The early exsanguination sample (Exsanguination 1) was obtained 10–30 s after stunning. The late sample (Exsanguination 2) was taken 1 min thereafter. The plasma histamine concentration was higher in the late exsanguination sample compared with early samples ($P < 0.05$) and histamine concentration decreased in transported animals ($P < 0.05$). Subclinical Salmonella infection did not affect histamine levels at slaughter.

adrenaline ($r = 0.77$; $P < 0.05$) and noradrenaline ($r = 0.70$; $P = 0.05$) in the venipuncture samples but not in the exsanguination samples. By contrast, plasma histamine concentration tended to correlate to plasma adrenaline ($r = 0.58$; $P = 0.13$) and plasma noradrenaline ($r = 0.67$; $P = 0.07$) only in the exsanguination samples but not in the venipuncture samples (data not shown).

In a second trial, the pre-slaughter stressors, transportation and subclinical infection, were tested in combination as to their impact on plasma histamine level. The results are shown in Fig. 3. Transportation decreased ($P < 0.05$) the plasma concentration of histamine, whereas subclinical Salmonella infection was without effect. The third factor tested in this trial was sampling time after stunning and initiation of bleeding. The histamine concentration in a later exsanguination sample was generally higher ($P < 0.05$) than in an early exsanguination sample.

Discussion

The close association between mast cells and nerves¹⁹, as well as enterochromaffine-like cells and nerves²⁰, make histamine a neurally releasable mediator. In rats, both neurally releasable histamine pools are activated sequentially during immersion stress²¹. Mast cells contribute to a marked but transient increase of plasma histamine concentration in the acute phase of stress, whereas enterochromaffine-like cells cause a more subtle but sustained increase of plasma histamine concentration during

prolonged stress²¹. The practically important question whether histamine is likewise a stress-inducible plasma constituent in pigs has two aspects. From a diagnostic aspect, plasma histamine could be a valuable parameter to assess and classify stress responses in scientific investigations to improve the management and handling of commercial pigs. From a meat hygiene aspect, the multiple physiological functions of histamine would have to be considered for slaughter pigs within the phenomenon stress. Especially in slaughter pigs, the permeability changes induced by histamine in the intestine¹⁴ and the vasculature¹⁵ could be important for the microbiological and organoleptic quality, as well as the storage life of the end-product meat.

The stressors in the present study were especially chosen to reflect potential stressors of slaughter pigs, i.e. transportation, possible re-activation of subclinical infection and, most importantly, the slaughter procedure itself. It has been shown in previous studies that stunning and slaughter induce increases in the plasma concentrations of so-called stress hormones that are superior to any other stress situation^{22,23}. To set the suspected histamine release during stunning and slaughter in relation to the release of those classical stress hormones, catecholamines and cortisol were measured, too. The absolute values of adrenaline concentrations in the exsanguination plasma are comparable to the values described previously by D'Souza *et al.* (~400 nM)²² and Hartung *et al.* (~715 nM)²³. Cortisol values are also in line

with literature data from slaughtered pigs (~130 nM)²⁴. Only the noradrenaline concentrations measured in the present study are much lower than those reported in literature. In the previous studies using CO₂ stunning^{22,23}, noradrenaline concentrations in the exsanguination plasma were about 10 times higher than those in the present study using stunning by bolt pistol. Accordingly, the noradrenaline:adrenaline ratio in the exsanguination plasma remained > 2 in those previous studies, whereas this ratio fell from 2.42 to 0.71 in the present study. Such decrease in the noradrenaline:adrenaline ratio due to preferential adrenomedullary stimulation is usually interpreted as specific excitation of centres for emotional arousal and fear⁷. Consequently, the excitation of these brain centres and/or their subordinate centres appears to be a main and dominating effect of stunning by bolt pistol. By contrast, CO₂ stunning seems to induce an additional sympathoneural activity, most likely by local or local reflex effects on noradrenaline release from sympathetic nerve endings^{25,26}. The differential increases of catecholamines in differently stunned pigs resemble earlier observations in decapitated vs. CO₂-stunned mice²⁷ and could be significant from animal welfare and meat quality points of view.

In comparison to catecholamines, histamine release by stunning and slaughter was relatively small. Nevertheless, stress can be considered to release some histamine into the systemic circulation

of pigs. This can be deduced, firstly, from the trend towards an increase in plasma histamine concentration from the venipuncture samples to the exsanguination samples in Trial 1. Secondly, the plasma histamine concentration in Trial 1 rose most prominently in those animals which developed the highest plasma concentrations of catecholamines during stunning and slaughter, i.e., a correlation developed between plasma histamine and plasma catecholamine levels during exsanguination. Thus, it can be assumed that the degree of agonal stress is reflected by the relative increase of both plasma catecholamine and plasma histamine levels. Thirdly, a significant increase in plasma histamine concentration occurred between the early exsanguination sample and the later exsanguination sample in Trial 2. The latter was statistically significant and finally showed that histamine release occurs during agonal stress but its peak is not yet reached at the onset of exsanguination. As mentioned above, agonal histamine release appears to be rather moderate in comparison to the release of catecholamines. However, it cannot be inferred that it is of minor importance for meat quality. Histamine-producing cells are highly concentrated below the contact surfaces to the environment (e.g. lung and gastrointestinal tract)²⁸. Thus, the topical release of histamine in these organs can be quite high despite an only moderate increase of plasma concentration.

Trial 2 was not only designed to check for agonal histamine release but also to assess the impact of an acute stressor (stunning and slaughter) placed on top of an enduring stressor. The enduring stressors were subclinical infection with *Salmonella typhimurium* and long-distance transportation. Subclinical *Salmonella* infection was chosen since feeding of *Salmonella* endotoxin to pigs over 14 days had induced a significant increase in plasma histamine level at slaughter in a previous study¹⁷. In the present trial, however, a latent infection with *Salmonella* was without an effect on plasma histamine concentration at slaughter. This was unexpected given the dual role of histamine as a stress-induced mediator^{9,10,21} and an inflammatory mediator²⁹. The missing effect of *Salmonella* infection on plasma histamine level may point to the fact that the subclinical carriage of *Salmonella* was rather well tolerated by the animals and did neither act as a marked stressor nor as a potent inflammatory stimulus. In contrast to *Salmonella* infection, long-distance transportation induced a marked depression of plasma histamine level at slaughter. It seems reasonable to assume that this decreased histamine concentration at slaughter likely reflects ongoing histamine release during transportation stress. As the histamine released during transportation would be removed very quickly from the circulation³⁰, the decreased plasma histamine concentration in transported pigs at slaughter could indicate exhaustion of their neurally releasable histamine pools.

Both trials of our study confirmed that the plasma histamine level of pigs is very high compared to most other species. However, published plasma histamine levels of pigs differ between studies (~35 nM¹⁷, ~300-550 nM²⁸, ~400 nM³⁰, ~300 nM³¹, ~300 nM³², ~150-250 nM³³, ~300 nM³⁴, ~50 nM³⁵, ~200 nM³⁶). Two reports even claim the physiological plasma concentration of pigs to be only ~2-5 nM^{13,37}. We experienced in the first trial, that even strict temperature control during plasma freezing and thawing in combination with low temperature storage might not completely prevent some histamine loss in porcine plasma samples. This might partly explain differences in resting plasma histamine values in our two trials, although sex and age differences of the animals

could have contributed to these differences, too. We finally found that extraction of histamine before storage (as done in Trial 2) gives the most reliable results and should be the method of choice in future experiments.

Conclusions

The results of this study show that a huge stress response occurs during stunning and slaughter. The released cocktail of stress mediators seems to differ with different stunning methods, which should be evaluated in further trials from a meat hygiene point of view. Apart from classical stress hormones, histamine can also be released in stressed pigs. However, one may find paradoxically decreased plasma histamine levels after prolonged stress situation (e.g. transportation), which may point to a limitation of the neurally releasable histamine pool. Together with high interindividual variations in plasma histamine levels and difficulties of accurate sample handling, plasma histamine level is not an easy-to-use stress indicator in pigs. Further studies should address the question whether the excretion of histamine metabolites in urine would be a better measure to describe the histamine burden of stressed pigs³⁸.

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References

- Swanson, J.C. 1995. Farm animal well-being and intensive production systems. *J. Anim. Sci.* **73**:2744-2751.
- Perremans, S., Randall, J.M., Rombouts, G., Decuyper, E. and Geers, R. 2001. Effect of whole-body vibration in the vertical axis on cortisol and adrenocorticotrophic hormone levels in piglets. *J. Anim. Sci.* **79**: 975-981.
- Brown, S.N., Knowles, T.G., Wilkins, L.J., Chadd, S.A. and Warriss, P.D. 2005. The response of pigs to being loaded or unloaded onto commercial animal transporters using three systems. *Vet. J.* **170**:91-100.
- Warriss, P.D. and Brown, S.N. 1994. A survey of mortality in slaughter pigs during transport and lairage. *Vet. Rec.* **134**:513-515.
- Marg, H., Scholz, H.C., Arnold, T., Rösler, U. and Hensel, A. 2001. Influence of long-time transportation stress on re-activation of *Salmonella typhimurium* DT104 in experimentally infected pigs. *Berl. Münch. Tierärztl. Wochenschr.* **114**:385-388.
- Neubert, E., Gürtler, H. and Vallentin, G. 1996. Einfluss einer Fixation von Mastschweinen mittels Oberkieferschlinge auf das Verhalten der Plasmakonzentrationen an Catecholaminen, Cortisol, Insulin und Stoffwechselfparametern. *Berl. Münch. Tierärztl. Wochenschr.* **109**:409-413.
- Otten, W., Puppe, B., Kanitz, E., Schon, P.C. and Stabenow, B. 2002. Physiological and behavioral effects of different success during social confrontation in pigs with prior dominance experience. *Physiol. Behav.* **75**:127-133.
- Koopmans, S.J., Ruis, M., Dekker, R., van Diepen, H., Korte, M. and Mroz, Z. 2005. Surplus dietary tryptophan reduces plasma cortisol and noradrenaline concentrations and enhances recovery after social stress in pigs. *Physiol. Behav.* **85**:469-478.
- Huang, Z.L., Mochizuki, T., Watanabe, H. and Maeyama, K. 1999. Activation of sensory nerves participates in stress-induced histamine release from mast cells in rats. *Neurosci. Lett.* **270**:181-184.

- ¹⁰Huang, Z.L., Mochizuki, T., Watanabe, H. and Maeyama, K. 1999. Histamine release induced by immobilization, gentle handling and decapitation from mast cells and its inhibition by nedocromil in rats. *Jpn. J. Pharmacol.* **80**:255-262.
- ¹¹Ahrens, F., Knies, K., Schneider, M., Köhler, F. and Erhard, M.H. 2005. Influence of different training and outdoor conditions on plasma histamine and cortisol concentrations in search-and-rescue dogs. *Inflamm. Res.* **54**(Suppl.1):S34-S35.
- ¹²Knies, K., Erhard, M.H. and Ahrens, F. 2005. Effect of moderate stress on plasma histamine concentration in laboratory dogs. *Inflamm. Res.* **54**(Suppl. 1):S32-S33.
- ¹³Schlumbohm, C., Menzler, M. and Harmeyer, J. 1999. Gastrin und Histamin im Plasma beim Schwein: Einfluss von Stress und Ca-Gehalt der Ration. *Proc. Soc. Nutr. Physiol* **8**:93 (Abstract).
- ¹⁴Groot, J.A. 1998. Correlation between electrophysiological phenomena and transport of macromolecules in intestinal epithelium. *Vet. Q.* **20**(Suppl. 3):S45-S49.
- ¹⁵Tirupathi, C., Minshall, R.D., Paria, B.C., Vogel, S.M. and Malik, A.B. 2002. Role of Ca²⁺ signaling in the regulation of endothelial permeability. *Vascul. Pharmacol.* **39**:173-185.
- ¹⁶Abraham, G., Gottschalk, J. and Ungemach, F.R. 2005. Evidence for ototopical glucocorticoid-induced decrease in hypothalamic-pituitary-adrenal axis response and liver function. *Endocrinology* **146**:3163-3171.
- ¹⁷Aschenbach, J.R., Seidler, T., Ahrens, F., Schrödl, W., Buchholz, I., Garz, B., Krüger, M. and Gäbel, G. 2003. Luminal *Salmonella* endotoxin affects epithelial and mast cell function in the proximal colon of pigs. *Scand. J. Gastroenterol.* **38**:719-726.
- ¹⁸Aschenbach, J.R., Oswald, R. and Gäbel, G. 2000. Transport, catabolism, and release of histamine in the ruminal epithelium of sheep. *Pflügers Arch.* **440**:171-178.
- ¹⁹Peters, E.M., Kuhlmei, A., Tobin, D.J., Müller-Röver, S., Klapp, B.F. and Arck, P.C. 2005. Stress exposure modulates peptidergic innervation and degranulates mast cells in murine skin. *Brain Behav. Immun.* **19**:252-262.
- ²⁰Norlen, P., Ericsson, P., Kitano, M., Ekelund, M. and Hakanson, R. 2005. The vagus regulates histamine mobilization from rat stomach ECL cells by controlling their sensitivity to gastrin. *J. Physiol.* **564**:895-905.
- ²¹Huang, Z.L., Mochizuki, T., Watanabe, H., Kagoshima, M. and Maeyama, K. 1998. Biphasic elevation of plasma histamine induced by water immersion stress, and their sources in rats. *Eur. J. Pharmacol.* **360**:139-146.
- ²²D'Souza, D.N., Warner, R.D., Leury, B.J. and Dunshea, F.R. 1998. The effect of dietary magnesium aspartate supplementation on pork quality. *J. Anim. Sci.* **76**:104-109.
- ²³Hartung, J., Nowak, B., Waldmann, K.H. and Ellerbrock, S. 2002. CO₂-Betäubung von Schlachtschweinen: Einfluss auf EEG, Katecholaminausschüttung und klinische Reflexe. *Dtsch. Tierärztl. Wochenschr.* **109**:135-139.
- ²⁴Forslid, A. and Augustinsson, O. 1988. Acidosis, hypoxia and stress hormone release in response to one-minute inhalation of 80% CO₂ in swine. *Acta Physiol. Scand.* **132**:223-231.
- ²⁵Borovsky, V., Herman, M., Dunphy, G., Caplea, A. and Ely, D. 1998. CO₂ asphyxia increases plasma norepinephrine in rats via sympathetic nerves. *Am. J. Physiol.* **274**:R19-R22.
- ²⁶Myre, K., Rostrup, M., Eriksen, M., Buanes, T., Raeder, J. and Stokland, O. 2004. Increased spillover of norepinephrine to the portal vein during CO₂-pneumoperitoneum in pigs. *Acta Anaesthesiol. Scand.* **48**:443-450.
- ²⁷Lucot, J.B., Jackson, N., Bernatova, I. and Morris, M. 2005. Measurement of plasma catecholamines in small samples from mice. *J. Pharmacol. Toxicol. Methods* **52**:274-277.
- ²⁸Lorenz, W., Barth, H., Kusche, J., Reimann, H.J., Schmal, A. and Metejka, E. 1971. Histamine in the pigs: determination, distribution, release and pharmacological actions. *Eur. J. Pharmacol.* **14**:155-175.
- ²⁹Malaviya, R., Ikeda, T., Abraham, S.N. and Malaviya, R. 2004. Contribution of mast cells to bacterial clearance and their proliferation during experimental cystitis induced by type 1 fimbriated *E. coli*. *Immunol. Lett.* **91**:103-111.
- ³⁰Lorenz, W., Hell, E., Boeckl, O., Reimann, H.J., Zimmermann, G., Seidel, W., Laszcz, M. and Uhlig, R. 1973. Histamine release during orthotopic homologous liver transplantation in pigs. *Eur. Surg. Res.* **5**:11-20.
- ³¹Kusche, J., Stahlknecht, C.D., Lorenz, W., Reichert, G. and Dietz, W. 1979. Comparison of alterations in the histamine-diamine oxidase system during acute intestinal ischaemia in pigs, dogs and rabbits; evidence for a uniform pathophysiological mechanism? *Agents Actions* **9**:49-52.
- ³²Almeida, A.P., Flye, W., Deveraux, D., Horakova, Z. and Beaven, M.A. 1980. Distribution of histamine and histaminase (diamine oxidase) in blood of various species. *Comp. Biochem. Physiol. C.* **67C**:187-190.
- ³³Man, W.K., Rago, D., Manolas, K., Welbourn, R.B. and Spencer, J. 1984. Plasma histamine in pig: effect of food and pentagastrin. *Agents Actions* **14**:529-533.
- ³⁴Sattler, J., Lorenz, W., Kubo, K., Schmal, A., Sauer, S. and Luben, L. 1989. Food-induced histaminosis under diamine oxidase (DAO) blockade in pigs: further evidence of the key role of elevated plasma histamine levels as demonstrated by successful prophylaxis with antihistamines. *Agents Actions* **27**:212-214.
- ³⁵Stinner, B., Kunneke, M., Thiel, T., Hasse, C., Kapp, B. and Lorenz, W. 1995. Modification of cardiovascular response and histamine release by prophylactic antibiotic drugs in complicated surgery: a prospective randomized trial in a pig experimental model. *Inflamm. Res.* **44**(Suppl. 1):S78-S79.
- ³⁶Celik, I., Stinner, B., Thiel, T., Bauhofer, A., Rothmund, M. and Dietz, W. 2004. Antibiotic prophylaxis influences cardiovascular stability in complicated surgery. *Inflamm. Res.* **53**(Suppl. 2):S116-S121.
- ³⁷Kovacs, J., Kaszaki, J., Temesvari, P., Czesznak, A., Abraham, C.S. and Joo, F. 1995. The role of cerebral microvessels in the elimination of histamine released during postasphyxial reperfusion in newborn piglets. *Neurosci. Lett.* **195**:25-28.
- ³⁸Fornhem, C., Dahlback, M., Kumlin, M., Lundberg, J.M. and Alving, K. 1996. Effects of local and systemic budesonide on allergen-induced airway reactions in the pig. *Br. J. Pharmacol.* **118**:989-997.