Original paper

EFFECT OF HIGH LEVELS OF AMMONIA IN AIR ON ADRENAL RESPONSE TO ADRENOCORTICOTROPIN AND FORCED RUNNING IN RABBITS

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Abstract

The aim of the present study was to assess adrenal response to high air ammonia levels in rabbits. Twenty male rabbits of the New Zealand White breed at the age of 4 months were randomly allocated into two groups: control - reared under low air ammonia levels (1.4-14.6 ppm) and experimental – reared under higher ammonia levels (28-57 ppm). The rabbits of both groups were subjected to forced running for 15 min on day 37 of the trial and two weeks later they received i.m. injection of 0.1 mg synthetic adrenocorticotropin ($ACTH_{1-24}$) per rabbit. The animals were sacrificed two days after termination of the trial and some internal organs and glands were excised and weighed. Plasma cortisol levels in both groups were not significantly altered at 20 and 60 min following the end of forced running relative to basal levels. Plasma corticosterone level in the control rabbits declined at 20 min ($P<0.01$) and 60 min ($P<0.05$) following exposure to forced running and remained unchanged in the experimental group. Plasma cortisol and corticosterone concentrations in both groups declined significantly at 60 and 120 min following ACTH injection. The rabbits under high ammonia levels had heavier adrenal glands than control rabbits ($P<0.01$). The results are interpreted to suggest that ammonia-induced higher adrenal weight was due to hypertrophy of adrenal zona glomerulosa.

Key words: *adrenal gland, corticosterone, cortisol, rabbit, tetracosactide*

Introduction

Most of the ammonia related investigations are focused on the deleterious effect of ammonia on defense mechanisms of the respiratory tract against pathogens (Drummond et al., 1981; Al-Mashhadani, 1984; Gustin et al., 1994).

It is worth to note that exposure of weaned pigs to 35 and 50 ppm of ammonia for 19 days was found to increase serum cortisol level on day 19, but the magnitude of the increase was of moderate value (von Borell et al., 2007). On the contrary, exposure of pigs to 0, 25, 50 and 100 ppm of ammonia for 6 days did not modify plasma cortisol level (Gustin et al., 1994). Slight but significant increase of plasma cortisol levels have been reported in nursery pigs following chronic (20 days) or acute (96 h) exposure to 30 and 50 ppm of ammonia (Mitloehner, 2004). Olanrewaju et al. (2008) did not find significant

change of plasma cortisol level in broiler chickens exposed to 0, 25 and 50 ppm of ammonia for 14 days. Thus, the aim of the present study was to investigate the effect of enhanced level of air ammonia on adrenal response to stress.

Materials and methods

Twenty New Zealand male rabbits (*Oryctolagus cuniculus*) at the age of 4 months and average weight of 3 kg were randomly allocated into 2 groups (control and experimental) - 10 rabbits in each. Rabbits were reared individually in wire-floor cages, provided with feeders and automated drinkers - feed and drinking water were supplied ad libitum. Air temperature, relative humidity and CO_2 levels were within the following limits - 16-24 °C; 40-70% and 480-1260 ppm. The rabbits of both groups were reared together from birth until the start of the experiment in a room with natural ventilation and relatively high air ammonia levels (15-21 ppm). During the 51 days long experiment the rabbits of the control group were reared under low air ammonia levels (1.4 – 14.6 ppm), whereas the experimental rabbits were kept under higher levels of naturally occurring ammonia (28-57 ppm) via window closing. Thirty seven days from the start of the experiment all rabbits were exposed to 15 min forced running. Blood samples were collected by ear venepuncture before (basal level) and after the forced running session (at 20 and 60 min). Two weeks later the rabbits of both groups were injected with 0.1 mg tetracosactide (Adrenocorticotropin, 1-24). Blood was taken before and following adrenocorticotropin (ACTH) injection (at 1 and 2 h). The acute effect of air ammonia on adrenal function was assessed by spreading liquid ammonia on the floor, immediately after the end of forced running session and ACTH injection, which led to increased air ammonia level as high as 158 ppm. All rabbits were sacrificed two days after termination of the trial and the following internal organs and glands were excised and weighed – liver, lung, spleen, testes and adrenal glands. Plasma cortisol and corticosterone were measured by Rabbit cortisol and corticosterone ELISA kits, manufactured by Cusabio Biotech. CO., LTD. Air ammonia was recorded via AeroQual S200 Monitor, equipped with ammonia sensor head (0-100±0.1 ppm). The results of one factor statistical analysis are expressed as means ± S.E.M. and were analyzed by ANOVA.

Results and discussion

Chronic exposure to ammonia had no effect on basal plasma levels of cortisol (Figure 1). Ammonia prevented cortisol decline, observed in the control rabbits at 20 min following the end of forced running session (Figure 1). Our data, concerning rabbit exposure to forced running, are not consistent with those reported in rats subjected to acute exercise session (Fortunato et al., 2008). The authors found an increase of corticosterone level at 30 min after the termination of 20 min long treadmill exercise followed by sharp decline. The observed discrepancy of cortisol dynamics between our and their experiment could be due to species specific difference in the rate of cortisol clearance.

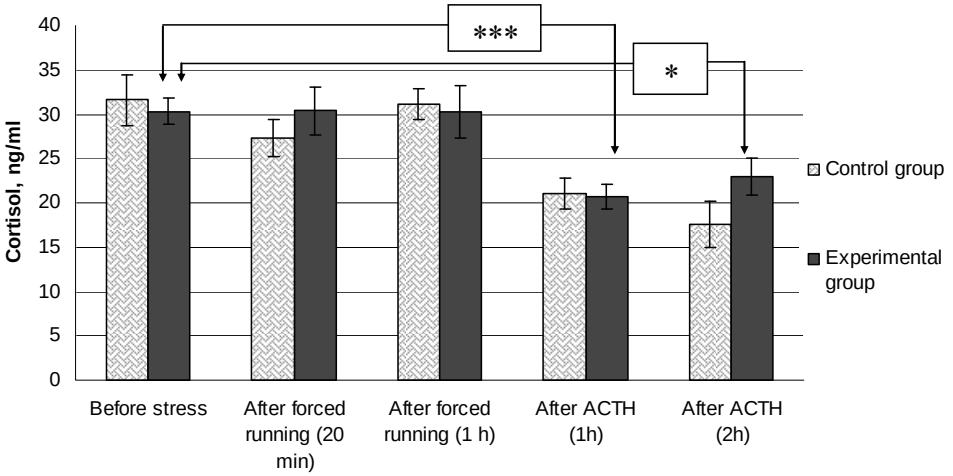

Figure 1. *Effect of forced running and ACTH injection on plasma levels of cortisol in rabbits, reared under low and high ammonia levels*
* - P<0.05 *** - P<0.001

Table 1. *Relative weight of liver, lung, spleen, testes and adrenal glands in rabbits, reared under low and high ammonia levels*

Organs and glands	n	Control group		Experimental group	
		x	Sx	x	Sx
Liver	9	0.0272	0.0012	0.0249	0.0017
Lung	9	0.0039	0.00014	0.0042	0.00026
Spleen	9	0.00049	0.00006	0.00037	0.00004
Testes	9	0.00126	0.00020	0.00182	0.00036
Adrenal gland	9	0.000073	0.0000036	0.000101**	0.0000081

** - P<0.01 significantly different versus control group

Surprisingly, plasma cortisol levels declined significantly in both groups at 1 and 2 h following adrenocorticotropin ($ACTH_{1-24}$) injection. Our data are not consistent with the reported 7-fold increase of corticosterone within 30 min after ACTH injection in neonatal mother-deprived rabbits (Brecchia et al., 2009). Earlier studies involving wild rabbits have shown completely different stress response to psychological stress which did not involve the adrenal cortex (Kracht and Kracht, 1952; Kracht, 1954.) The authors found symptoms of thyrotoxicosis and suggested that the observed stress response was completely mediated by the pituitary-thyroid system. On the contrary, Brown-Grant et al. (1954) reported that rabbits subjected to restraint or electrical current had reduced rate of release of Iodine-131 from the thyroid gland, which was not due to stress-induced renal clearance of iodine, since the inhibitory effect of stress on thyroid function was also observed in adrenalectomized rabbits. It seems that the lack of adrenal response to forced

running and ACTH injection in our study was influenced by NH_3 despite the similar response in the control and experimental rabbits. This view stems from the fact that many generations of rabbits in the rabbit farm have been raised under high ammonia levels, especially in winter when all doors and windows were kept closed because of the low ambient temperature. Furthermore, the rabbits of the control group have been reared under high ammonia level for 4 months before being moved to a clean, well ventilated room at the start of the experiment. Numerous experiments on animal and man indicated that exposure to stress during pregnancy and neonatal period alters hypothalamic-pituitary adrenal activity of the offspring (Sternberg and Ridgway, 2003; Davis et al., 2004; Huang, 2011). Consequently, we assume that adrenal response to stress in the control rabbits might have been modified by the elevated air ammonia level during pregnancy, neonatal or early postnatal period.

Alleviated adrenal response to ACTH in hyperammonemic rats was reported by Llansola et al. (2013**)**. Ammonia is known to increase NO synthesis (Swamy et al., 2005) and NO was found to modulate cortisol and corticosterone secretion (Adams et al., 1992).

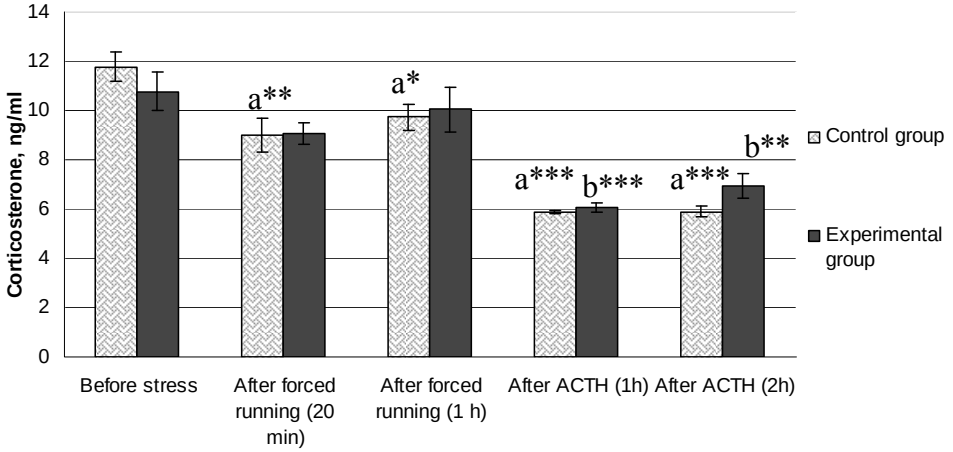

Figure 2. *Effect of forced running and ACTH injection on plasma levels of corticosterone in rabbits, reared under low and high ammonia levels*

* - P<0.05 ** - P<0.01 *** - P<0.001

a – significantly different versus basal level in the control group

b – significantly different versus basal level in the experimental group

Plasma corticosterone levels declined in the control group (P<0.01) at 20 and 60 min following exposure to 15 min forced running (Figure 2). It is worth to note that plasma corticosterone level in the experimental group did not decline significantly at 20 and 60 min. following the termination of forced running session. The observed difference in plasma corticosterone levels between the two groups could be due to ammonia-induced increase of plasma aldosterone and the use of corticosterone as a precursor of aldosterone (Müller, 1965, Perez et al., 1977; Yamauchi et al., 1997). The experimental rabbits had heavier adrenal glands (P<0.01) relative to the control rabbits (Table 1). Adrenal

hypertrophy in the hyperammonemic rabbits was obviously not due to increased secretion of glucocorticoids as seen in Figure 1 and 2. Ammonium chloride has been used in many animal studies to induce metabolic acidosis since around 98% of endogenous ammonia exists as NH_4^+ (Wright, 2010**)**. Ammonium chloride ingestion was reported to decrease serum cortisol level (Müller, 1965; Yamauchi et al., 1997; Llansola et al., 2013). Also, hyperammonemia-induced metabolic changes are only partially mediated by adrenaline (Wiechetek et al., 1989). Consequently, the higher adrenal weight in the experimental rabbits was most probably due to hypertrophy of adrenal zona glomerulosa. This view is in agreement with the reported zona glomerulosa hypertrophy in ammonium chloride treated rats (Lina and Kuijpers, 2004). Furthermore, hepatic encephalopathy is accompanied by hyponatremia (Bhatia et al., 2006) and it is well known that hyponatremia stimulates aldosterone secretion.

Adrenocorticotropin administration caused significant decline of plasma corticosterone levels in both groups of rabbits at 1 and 2 h following the injection (Figure 2). However, the rate of corticosterone decline was more pronounced than that of cortisol level and constituted almost half of the basal corticosterone level.

Conclusion

Rabbits did not respond to forced running and ACTH administration by increase of cortisol and corticosterone levels. Exposure to high air ammonia caused adrenal hypertrophy.

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