

EFFECTS OF XYLAZINE (ALPHA 2-ADRENERGIC AGONIST) ON THE STRESS RESPONSE TO IMMOBILIZATION AND HEAT IN RATS

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Summary

The effect of xylazine administration on plasma cortisol, prolactin, glucose and packed cell volume (PCV) responses to immobilization and heat stress was investigated. Immobilization of rats for 2 hours by ligation of the fore and hind legs strongly caused approximately two-fold increase in plasma cortisol and prolactin levels. Plasma glucose and PCV were not significantly changed. Pretreatment of immobilized rats with xylazine (20 mg/kg body weight i.m.) resulted in approximately 20% reduction in both plasma cortisol and prolactin concentrations. A marked hyperglycemia and increase in the PCV value was observed. On the other hand, rats exposed to acute heat stress (40°C and 60% relative humidity) for 2 hours, also developed two fold increase in both plasma cortisol and prolactin concentrations and the pretreatment with xylazine caused a 20% reduction in the levels of both hormones. Plasma glucose level was not significantly changed in heat stressed rats but it was markedly increased after pretreatment with xylazine. PCV was significantly increased under heat stress and pretreatment with xylazine induced a pronounced elevation in this value. It was suggested that stimulation of cortisol and prolactin secretion in response to immobilization or heat stress can be partially reduced by an alpha 2-adrenergic agonist.

(Key Words: Immobilization, Heat, Cortisol, Prolactin, Xylazine)

Introduction

Xylazine produces sedation, analgesia and muscular relaxation on the peripheral and central nervous system. Xylazine is an alpha 2-adrenergic receptor agonist and produces many effects through receptors either centrally or peripherally (Docherty and McGarth, 1980).

Previous attempts to attenuate the stressful effects of transport action by pentobarbitone anaesthesia were successful (Sanhoury et al., 1990, 1991a). Diazepam suppressed transport-induced hypercortisolaemia, hyperglycemia, tachypnea and tachycardia in goats (Sanhoury et al., 1991b). Pretreatment of goats with xylazine suppressed cortisol concentrations induced by transportation (Sanhoury et al., 1992).

An increase in adrenal corticosteroid secretion, as reflected by increased plasma corticosteroid concentrations, has been widely used as an index of stress in many species, including rats (Barlow

et al., 1979). Immobilization and heat stress are a well known stressor to increase plasma cortisol and prolactin levels in several animal species (Dantzer and Mormede, 1983; Marzourki and Tayeb, 1988; Mahmoud, 1990). An heat stress has been found to increase cortisol and prolactin levels and decrease T₃ and T₄ concentrations in several animal species (Marzouki and Tayeb, 1988). Several attempts have been done to alleviate the hormonal changes during heat stress by shades, cold water spray and other methods. However, no attempts has been done to use tranquilizers and sedative drugs to counteract the hormonal changes during heat stress.

Generally, stress conditions induced adverse effects on the reproductive performance of animals because it resulted in high serum levels of glucocorticoids, prolactin and endogenous opioid peptides which inhibit the hypothalamic - pituitary - gonadal axis (Mahmoud, 1990).

The present experiments were therefore undertaken to investigate the effect of immobilization and heat stress on plasma corticosteroid, prolactin, glucose and PCV values. The effect of pretreatment with xylazine (alpha 2-adrenergic agonist) on these parameters was investigated also.

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Materials and Methods

Thirty mature female albino rats weighing 175-200 g were housed in plastic cages (5 animals/cage) and were divided into 6 groups of five. Food and water were provided *ad libitum*.

Animals in group 1 were maintained at room temperature and injected with 0.25 ml saline i.m. and considered as control. Rats in group 2 were maintained at room temperature and administered with xylazine (Miles laboratories Inc., Shawnee, K. N.) at a dose of 20 mg/kg body weight i.m. Rats in the 3rd group were injected with 0.25 ml saline and immobilized on their back for 2 hours by ligation of the fore and hind legs strongly with a cotton thread. Animals in the 4th group were pretreated with xylazine at a dose of 20 mg/kg body weight, 10 minutes before immobilization for 2 hours. Rats in 5th group were exposed to heat stress (40°C and 60% relative humidity) for 2 hours. Animals in the 6th group were pretreated with xylazine at a dose of 20 mg/kg body weight i.m. 10 minutes before exposure to heat stress for 2 hours.

At the end of the experimental period, rats in

all groups were lightly anaesthetized by ether and blood samples were obtained by puncture of the orbital sinus plexus. Packed cell volume (PCV) was determined by a microhematocrit method. Plasma samples were separated and stored at -20°C until analyzed for cortisol, prolactin and glucose concentrations. Plasma cortisol and prolactin levels were determined by radio immuno assay (RIA) Kits (Diagnostic Product Corp., Los Angeles, California, USA). Glucose was measured by Glucofix Kits supplied from Menarini Diagnostics, Italy.

Data were statistically analyzed by analysis of variance (ANOVA) and least significant difference (LSD) method was used to detect differences between means (Snedecor and Cochran, 1980).

Results

Immobilization of the rats caused increase cortisol and prolactin concentrations while no change in plasma glucose and PCV (table 1). Administration of xylazine resulted in a slight (non significant) decrease in plasma cortisol and prolactin levels while a significant increase in glucose and PCV were observed (table 1).

TABLE 1. PROLACTIN, CORTISOL, GLUCOSE AND PCV VALUES OF IMMOBILIZED AND HEAT STRESSED RATS WITH OR WITHOUT XYLAZINE TREATMENT

Group	Prolactin (ng/ml)	Cortisol (ng/ml)	Glucose (mg/dl)	PCV (%)
Control	3.42 ± 0.11 ^a	4.86 ± 0.25 ^a	103.3 ± 2.3 ^a	38.0 ± 0.7 ^a
Xylazine	2.89 ± 0.07 ^a	3.98 ± 0.14 ^a	131.9 ± 2.3 ^b	43.2 ± 1.3 ^b
Immobilized	7.52 ± 0.12 ^b	7.49 ± 0.18 ^b	108.2 ± 3.4 ^a	38.8 ± 3.4 ^a
Immobilized + Xylazine	5.83 ± 0.21 ^c	6.08 ± 0.29 ^c	131.4 ± 4.0 ^b	43.6 ± 0.8 ^b
Heat	7.36 ± 0.11 ^b	7.59 ± 0.15 ^b	102.5 ± 5.2 ^a	40.4 ± 1.1 ^c
Heat + Xylazine	5.78 ± 0.17 ^c	6.11 ± 0.07 ^c	148.9 ± 13.7 ^c	46.8 ± 0.6 ^d

± Standard error of the mean (n = 5).

^{a,b,c,d} Means with different letters in the same column are significantly different (p < 0.05).

Pretreatment of immobilized rats with xylazine resulted in a reduction (approximately 20%) in plasma cortisol and prolactin concentrations compared to the immobilized rats alone. Glucose concentrations and PCV were also increased in immobilized rats pretreated with xylazine (table 1).

Acute heat stress resulted in approximately two fold increase in cortisol and prolactin concentrations. PCV value was also significantly increased

but no change in the glucose level (table 1). Pretreatment of rats with xylazine before heat stress, resulted in approximately 20% reduction in plasma cortisol and prolactin levels. Glucose concentration and PCV were also significantly increased (table 1).

Discussion

Immobilization and heat stress induced two-fold

increase in plasma cortisol and prolactin concentrations and peripheral administration of xylazine partially suppressed the increase in the levels of these hormones. In general, several kinds of stressor such as immobilization, heat, transportation, anaesthesia resulted in increase plasma corticosteroid, prolactin, epinephrine, norepinephrine and endogenous opioid peptides (Ajika et al., 1972; Alvarez and Johnson, 1973; Barlow et al., 1979; Valtorta, 1979; Marzouki and Tayeb, 1988; Fayed et al., 1989; Mahmoud, 1990; Sanhoury et al., 1991a). Several attempts have been conducted to counteract the stress-induced elevation in plasma hormone levels. Increased plasma prolactin level due to etherization stress could be blocked by nembutal (Ajika et al., 1972) and increased plasma cortisol level due to transportation and immobilization stress could be prevented by administration of pentobarbitone, xylazine and diazepam (Sanhoury et al., 1991a and b; Sanhoury et al., 1992).

In our study, stimulated cortisol and prolactin secretion due to immobilization or heat stress can be partially reduced by pretreatment with xylazine. This effect is similar to that after the administration of clonidine or medetomidine centrally in the dog and man (Ganong, 1980; Kallio et al., 1988; Slowinska - Szrednicka et al., 1988). Reduction of stress - induced cortisol or prolactin levels by xylazine could be mediated directly on the pituitary to decrease adrenocorticotrophin hormone (ACTH) release or more likely at the hypothalamus to decrease corticotrophin releasing factor (CRF) or prolactin releasing factor. However, it is possible that xylazine may have also reduced the afferent arm of the cortisol and prolactin response by reducing sensory input traffic.

Immobilization or heat stress had no significant effect on glucose level. However, administration of xylazine to non - stressed and stressed rats induced hyperglycemia which was pronounced in heat stressed rats. Xylazine has been shown to induce hyperglycemia in several animal species due to its α_2 -agonistic effect (Symonds, 1976; Hsu and Hummel, 1981; Thurmon et al., 1982). The pronounced hyperglycemic effect of xylazine in heat stressed animals is unclear but it might be due to certain hormonal and metabolic changes which potentiate the hyperglycemic effect of xylazine.

Packed cell volume (PCV) did not change in immobilized rats but increased in heat stressed rats and in rats treated with xylazine. PCV has been

found to increase in heat stress due to excessive loss of fluids which led to hemoconcentration (Swenson, 1982). The increased PCV in rats treated with xylazine could be attributed to the excessive loss of water in the urine as was reported that xylazine increases urine volume in mares (Thurmon et al., 1984). Moreover, the additive effect of heat stress and xylazine on excessive water loss from the body could explain the marked increase in the PCV value in rats treated with xylazine and exposed to heat stress.

It was concluded that xylazine partially reduced the hypercortisolemia and hyperprolactinemia resulted from the immobilization and heat stress in rats.

Literature Cited

- Ajika, K., S. P. Kalra, C. P. Fawcett, L. Krulich and S. M. McCann. 1972. The effect of stress and nembutal on plasma levels of gonadotrophins and prolactin in ovariectomized rats. *Endocrinology* 90:707-715.
- Alvarez, M. B. and H. D. Johnson. 1973. Environmental heat exposure on cattle plasma catecholamine and glucocorticoids. *J. Dairy Sci.* 56:189.
- Barlow, S. M., A. F. Knight and F. M. Sullivan. 1979. Plasma corticosterone response to stress following chronic oral administration of diazepam in the rat. *J. Pharm. Pharmacol.* 31:23-26.
- Dantzer, R. and P. Mormede. 1983. Stress in farm animals: A need for re-evaluation. *J. Anim. Sci.* 57: 6-18.
- Docherty, J. R. and J. C. McGarth. 1980. A comparison of pre- and post-junctional potencies of several α -adrenoreceptors agonists in the cardiovascular system and anococcygeus muscle of the rat. *Naunyn. Schmiedeberg's Arch. Pharmacol.* 312:107-116.
- Fayed, A. H., E. Abdalla, R. R. Anderson, K. Spencer and H. D. Johnson. 1989. The effect of xylazine in cattle under thermoneutral or heat stress conditions. *Am. J. Vet. Res.* 50:151-153.
- Ganong, W. F. 1980. Neurotransmitters and pituitary function: Regulation of ACTH secretion. *Fedn. Proc.* 39:2923-2930.
- Hsu, W. H. and S. K. Hummel. 1981. Xylazine-induced hyperglycemia in cattle: A possible involvement of α_2 -adrenergic receptors regulating insulin release. *J. Endocrinol.* 109:825-829.
- Kallio, A., M. Koulou, H. Scheinin, J. Viikari and M. Scheinin. 1988. Acute effects of medetomidine, a selective α_2 -adrenoreceptor agonist, on anterior pituitary hormone of cortisol secretion in man. *Acta Endocrinol. (Copenh.)* 119:11-15.
- Mahmoud, A. M. 1990. Effect of some stress factors on plasma testosterone and cortisol levels in male rats. *Assiut Medical Journal* 4:27-32.

- Marzouki, Z. M. H. and O. S. Tayeh. 1988. Diagnostic significance of selected serum hormones in heat stroke. *J. Medical Research Institute* 9:111-124.
- Sanhouri, A. A., R. S. Jones and H. Dobson. 1990. Plasma concentrations of cortisol, testosterone, glucose and blood gases in male goats during anaesthesia with pentobarbitone sodium. *Br. Vet. J.* 146:36-42.
- Sanhouri, A. A., R. S. Jones and H. Dobson. 1991a. Pentobarbitone inhibits the stress response to transport in male goats. *Br. Vet. J.* 147:42-48.
- Sanhouri, A. A., R. S. Jones and H. Dobson. 1991b. Preliminary results on the effects of diazepam on physiological responses to transport in male goats. *Br. Vet. J.* 147:388-389.
- Sanhouri, A. A., R. S. Jones and H. Dobson. 1992. Effects of xylazine on the stress response to transport in male goats. *Br. Vet. J.* 148:119-128.
- Slowinska-Szrednicka, J., S. Zgiczyński, P. Sobzynski, J. Pucilowska, H. Wierzbicki and W. Ijeske. 1988. Effect of clonidine on beta endorphin, ACTH and cortisol secretion in essential hypertension and obesity. *Eur. J. Pharmacol.* 15:115-121.
- Snedecor, G. W. F. and W. G. Cochran. 1980. *Statistical Methods*. 7th Edn. Iowa state University Press, Ames, Iowa USA.
- Swenson, M. J. 1962. Physiological properties and chemical constituents of blood. In: *Dukes' Physiology of domestic Animals*. 9th edition. Chapter 2, pp. 20 (ed. Swenson, M. J.) Cornell University Press, New York.
- Symonds, H. W. 1976. The effect of xylazine upon hepatic glucose production and blood flow rate in the lactating dairy cow. *Vet. Rec.* 99:234-236.
- Thurmon, J. C., C. Neff-Davis, L. E. Davis, R. A. Staker, G. J. Benson and T. F. Lock. 1982. Xylazine hydrochloride induced hyperglycemia and hypoinsulinemia in thoroughbred horses. *J. Vet. Pharmacol. Ther.* 5:241-245.
- Thurmon, J. C., E. P. Steffey, J. G. Zinkl, M. Walner and D. Howland. 1984. Xylazine causes transient dose related hyperglycemia and increased urine volume in mares. *Am. J. Vet. Res.* 45:224-227.
- Valtorta, S. 1979. Hormonal responses to heat in sheep and lactating dairy cow. M. S. Thesis, University of Missouri, Columbia, USA.