EFFECTS OF SURGICAL STRESS ON THE SECRETION OF LUTEINIZING HORMONE, TESTOSTERONE AND CORTISOL IN THE DOMESTIC CAT (*Felis catus*)

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RESUMO

EFEITOS DO ESTRESSE CIRÚRGICO SOBRE A SECREÇÃO DE HORMÔNIOS: LUTEINIZANTE, TESTOSTERONA E CORTISOL NO GATO DOMÉSTICO (*Felis catus*)

A influência do estresse sobre as diversas funções fisiológicas tem sido por longo tempo estudada. Observações de que situações de estresse são geralmente acompanhadas de alterações nas atividades reprodutivas são também conhecidas. Assim, utilizaram-se 21 gatos (machos, adultos) em procedimento cirúrgico (24, 48 e 72 h, após canulação do vaso jugular direito), coletando-se sangue para dosagem, via radioimunoensaio, dos hormônios cortisol, testosterona e hormônio luteinizante (LH). Os resultados obtidos demonstraram que, para o gato doméstico, os níveis séricos dos hormônios estudados apresentaram uma elevação acentuada em suas concentrações, após o ato cirúrgico, e que diminuía gradativamente, voltando aos valores basais ao final de 48 h (2 ng/ml para o cortisol, 1 ng/ml para testosteona, e 3,5 ng/ml para LH), aproximadamente, regularizando-se a seguir. Esses resultados demonstram que, após procedimento cirúrgico, os felinos têm suas concentrações especialmente de cortisol aumentadas significativamente. O intervalo de pelo menos três dias deve ser aguardado antes que se proceda a quaisquer outros processos de manipulação que envolvam o sistema endócrino dessa espécie animal, já que durante esse período as concentrações de cortisol, bem como de outros hormônios, estão alteradas.

PALAVRAS-CHAVE: Cortisol, estresse, gato, LH, testosterona.

SUMMARY

EFFECTS OF SURGICAL STRESS ON HORMONE SECRETION: LUTEINIZING TESTOSTERONE AND CORTISON IN DOMESTIC CAT (*FELIS CATUS*)

The influence of stress interference in diverse physiological functions has long been studied. Observations that a stressful situation is generally accompanied by alterations in reproductive activity are also known. In the present study, blood was collected from 21 male adult cats 24, 48 and 72 h after surgery for determination of cortisol, testosterone and LH levels by radioimmunoassay. Hormonal plasma concentrations were found to be increased after surgery, followed by a gradual decrease, with plasma levels returning to basal values after approximately 48 h and remaining unchanged thereafter. These results demonstrate that hormone levels, particularly cortisol, are significantly increased in cats after surgery, a procedure routinely carried out in veterinary practice. An interval of at least three days is required before adopting any other process of manipulation involving the hormonal system, since the concentrations of cortisol as well as other hormones are altered during this period.

KEY WORDS: Cat, cortisol, LH, stress, testosterone.

INTRODUCTION

An increase in the plasma concentration of glucocorticoids originating from the adrenal is one of the most significant factors classifying a stressful situation, although other physiological events may occur simultaneously (BROOM & JOHNSON, 1993). The stress response involves a broad integration of the organism due to alterations in the hypothalamus-pituitary-adrenal (HPA) axis, as well as alterations in the hypothalamus-pituitary-gonadal (HPG) axis (WELSH & JOHNSON, 1981), immune response, and behavior (CARLSTEAD & BROWN, 1993) etc.

Among the responses of the organism to stress, i.e., to physical, psychological or infectious aggressions capable of influencing homeostasis, various situations such as social, food, or thermal stress etc. exist that may culminate in an increase in glucocorticoids (AGUILERA et al., 1983; PLOTSKY & VALE, 1984) and other hormones. The aim of the present study was to determine post-surgical variations in luteinizing hormone (LH), testosterone, and cortisol concentrations in the domestic cat, which represent the hormonal response to stress situations routinely encountered in veterinary practice.

MATERIAL AND METHODS

Twenty one adult male crossbred cats housed at the Central Animal House of the Faculty of Medicine of Ribeirão Preto/USP were submitted to the experimental procedures according to the guidelines of the National Institutes of Health (NIH). The animals were kept in the Laboratory of Physiology for at least 15 days before and during the experiments on a 12/12-h light/dark schedule at controlled temperature (23°C), with free access to water and commercial ration (Purina Nutriments Ltda.).

Operative technic: The right jugular vein was cannulated by the modified technique of HARMS & OJEDA (1974). The cannulae were introduced 24 h before the experiments and cleaned at least twice daily by infusion of heparinized saline (1/40, 100 IU/ml).

After fasting (12 h), the animals were anesthetized with a combination of ketamine HCl (20 mg/kg, *im* - intramuscular, Francotar – Virbac do Brasil Ind. Com. Ltd) and xylazine (1 mg/kg, *im*, Coopazine – Coopers do Brasil LTD).

The experiments were carried out during the same period of the day (morning) to prevent possible cyclic flutuations in hormone secretion and variation due to the nutritional status of the animals.

The cats were kept freely moving in individual cages in an isolated room. Blood was collected through P^{60} polyethylene cannulae attached to long extensions to which they were connected about 2 h before the beginning of the experiments. After each blood collection, the volume removed was replaced with 0.9% NaCl kept at 38.8°C.

Blood was centrifuged at 2500 rpm for 20 min at 4°C and the resulting plasma was stored at – 20°C for the determination of plasma LH, testoserone and cortisol concentrations by RIA (NISWENDER et al., 1969; GENARO, 1993; GENARO & ROSA e SILVA, 1997; LACERDA NETO, 1996).

Cortisol was measured as described by VIEIRA (1977), with nonspecific binding ranging from 1.0 to 6.9 %. Plasma testosterone was determined by RIA using the double antibody method according to the technique standardized by ROSA e SILVA (1997). Nonspecific binding was 2.6 %, the minimum dose detected was 0.1 pg/tube and the intra-assay error was 9.5 %. For LH (GENARO & ROSA e SILVA, 1997), the minimum dose detected was 149.57 ± 23.48 pg/tube and the intra-assay error was 4.5 % (for 500 pg/tube). All samples were analyzed in a single RIA.

RESULTS

As shown in Figure 1, plasma cortisol concentration was increased 24 h after surgery and progressively declined over time until reaching its minimum concentration, approximately 2 ng/ml plasma, at 72 h. This result demonstrates that cortisol requires a relatively longer period to return to normal levels than the other two hormones studied.

Testosterone concentrations showed a gradual decline immediately after surgery similar to that observed for cortisol. Similarly to cortisol and testosterone, LH concentration decreased soon after

the first collection (24 h after surgery) and remained constant after 48 h.

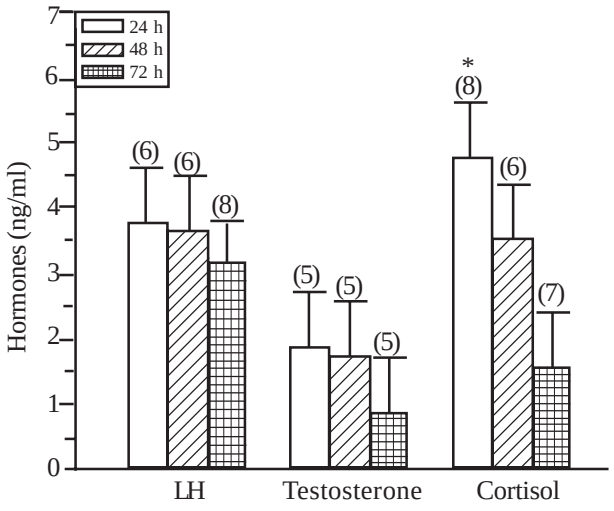

FIGURE 1. Plasma cortisol, testosterone and LH concentrations in male adult cats 24, 48 and 72 h after surgery. Data are reported as means ± SEM. The number of animals studied at each time point is given above the columns in parentheses. (* to $p<0.05$ between 24 and 72 h to plasma cortisol).

DISCUSSION

The capacity of stress to interfere with reproductive functions has long been recognized (SELYE, 1939; RIVIER & RIVEST, 1991; GENARO, 1993; LACERDA NETO, 1996). The original observations by SELYE (1939) in which stress is accompanied by an increase in HPA axis activity and a reduction in reproductive functions have suggested a relationship between HPA and HPG axis hormones. CRH, ACTH and glucocorticoids play an important role in the modulation of the effect of stress on reproductive functions. However, the exact mechanism of action of CRH, ACTH and glucocorticoids on the reproductive process remains to be established, with a wide variety of models having been proposed to explain these effects (RIVIER & RIVEST, 1991; GENARO, 1993; LACERDA NETO, 1996).

Stress-related hormones can influence reproductive functions basically at three sites of the HPG axis: in the central nervous system, by inhibiting LHRH secretion, in the pituitary by interfering with the LHRH-induced release of LH, or in the gonads, by altering the stimulating effect of gonadotrophins on the secretion of sex steroids (MOBERG, 1991; RIVIER & RIVEST, 1991).

Cortisol, the most important glucocorticoid in the cat (JOHNSTON & MATHER, 1979; FELDMAN, 1983), is an essential hormone, with total cortisol suppression leading to rapid death. This hormone is involved in several metabolic processes in the organism, thus explaining its great physiological importance (WILSON & FOSTER, 1985).

Among other metabolic effects, glucocorticoids play a special role in the inhibition of inflammatory responses. Since cortisol levels are increased immediately after surgery, the use of corticotherapy immediately after surgery or some time later (three days after surgery) should be carefully evaluated, since the profile of this hormone shows a drastic change immediately after surgery. The biphasic release of cortisol requires a dynamic approach to its utilization to help recovery after surgical processes.

CASTRO et al. (1995) demonstrated a marked increase in plasma corticosterone concentration in sham-adrenalectomized rats 3 h after surgery, followed by a gradual decrease, with plasma levels returning to basal values after 48 h. The authors attributed this time course of corticosterone to surgery-induced stress.

A similar behavior was observed in the present study for the domestic cat, i.e., hormones showing elevated levels decreased within the same interval of time, with a considerable decrease being observed 3 days after surgery and levels returning to basal values thereafter.

CONCLUSIONS

In conclusion, the present results demonstrate that animals submitted to surgery, a process known to be stressful for the animal, show a significant increase in cortisol concentration. Thus, an interval of at least three days is required before adopting any other process of manipulation involving the hormonal system, since the concentration of cortisol and other hormones is altered during this period, thus leading to an altered outcome of the performed manipulation due to the metabolic effects of cortisol and other involved hormones present at high concentrations.

ACKNOWLEDGMENTS

The authors thank Rogério R. Azevedo for technical assistance and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo – Process No. 90/1534-5) for financial support.

REFERENCES

AGUILERA, G.; HARWOOD, J.P.; WILSON, J.X.; MOREL, J.; BROWN, J.H.; CATT, K.J. Mechanism of action of CRH and other regulators of corticotropin release in rat pituitary cells. **Journal of Biological Chemistry**, v. 258, p. 80-93, 1983.

BROOM, D.M.; JOHNSON, K.G. **Stress and animal welfare**. London: Chapman & Hall, Animal Behaviour Series, 1993. 211 p.

CARLSTEAD, K.; BROWN, J.L. Behavioral and physiological correlates of stress in laboratory cats. **Aplied Animal Behaviour Science**, v.38, p. 143-158, 1993.

CASTRO, M.; FIGUEIREDO, F.; MOREIRA, A.C. Time course of hypothalamic CRH and pituitary ACTH contents and pituitary responsiveness to CRH stimulation after bilateral adrenalectomy. **Hormone Metabolism Research**, v. 27, p. 10-15, 1995.

FELDMAN, E.C. The adrenal cortex. In: ETTINGER, S.J. **Text book of veterinary internal medicine.** 2. ed.Philadelphia: Saunders, 1983. 1650 p.

GENARO, G. **Hormônio luteinizante no gato**. **Efeitos agudos e crônicos do análogo do LHRH [LHRH-A]. Efeitos do ACTH e CRH *in vivo* e *in vitro***. Ribeirão Preto, SP, 1993, 190f. Thesis (MS) – USP, Ribeirão Preto, SP, 1993.

GENARO, G.; ROSA E SILVA, A.A.M. Setting up and validation of the radioimmunoassay method for determination of the LH concentrations in felines. **Ars Veterinária**, v. 13, n. 1, p. 44-51, 1997.

HARMS P.G.; OJEDA, S.R. A rapid and simple procedures for chronic cannulation of the rat jugular vein. **Journal of Applied Physiology**, v. 36, p. 391-392, 1974.

JOHNSTON, S.D.; MATHER, E.C. Feline plasma cortisol (hydrocortisone) measured by RIA. **American Journal of Veteterinary Research**, v. 40, p. 190-192, 1979.

LACERDA NETO, J.C. **Estudos dos efeitos de agonista sintético do hormônio liberador de hormônio luteinizante (LH-RH-A) nas gônadas e adrenais de gatos (*Felis catus*)**. Ribeirão Preto, 1996, 140f. Thesis (PhD) – Ribeirão Preto, SP, 1996.

MOBERG, G.P. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. **Journal of Dairy Science**, v. 74, p. 304-311, 1991.

NISWENDER, G.D.; REICHERT JR., L.E.; MIDGLEEY, A.R.; NALBANDOV, A.V. Radioimmunoassay for bovine and ovine LH. **Endocrinology**, v. 84, p. 1166-1173, 1969.

PLOTSKY, P.; VALE, W. Hemorrhage-induced secretion of CRH-like immunoreactivity in to the rat portal circulation and its inhibition by glucocorticoids. **Endocrinology**, v.114, p. 164-169, 1984.

RIVIER, C.; RIVEST, S. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: Peripheral and central mechanism. **Biological Reproduction**, v.45, p. 523-532, 1991.

ROSA E SILVA, A.A.M. Padronização e validação do método de radioimunoensaio para dosagem da testosterona no plasma, no testículo e no fluido seminal intersticial. **Ars Veterinária**, v. 15, p. 74-78, 1997.

SELYE, H. Effect of adaptation to various damaging agents on the female sex organs in the rat. **Endocrinology**, v. 25, p. 615-624, 1939.

VIEIRA, J.G.H. **Radioimunoensaio do cortisol sérico**. São Paulo, 1977, 68f. Thesis (MS) – Escola Paulista de Medicina, São Paulo, 1977.

WELSH, T.H.; JOHNSON, B.H. Stress-induced alterations in secretion of corticosteroids, progesterone, LH and testosterone in bulls. **Endocrinology**, v. 109, p. 185-190, 1981.

WILSON, J.D.; FOSTER, D.W. **Textbook of endocrinology**. 7th ed., Philadelphia: Saunders, 1985. 1413 p.

Protocolado em: set. 2003. Aceito em: dez. 2003.