# LACK OF CIRCADIAN CHANGE ON CONCENTRATION OF C-TYPE NATRIURETIC PEPTIDE IN RABBITS AQUEOUS HUMOR

## *AUSÊNCIA DE MUDANÇA NO RITMO CIRCADIANO DA CONCENTRAÇÃO DE PEPTIDEO NATRIURETICO TIPO C NO HUMOR AQUOSO DE COELHOS*

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**ABSTRACT: Purpose**: To determinate whether there is a circadian change of the concentration of C-Type Natriuretic peptide (CNP) in rabbit aqueous humor.

**Methods**: Forty-one male white New Zealand rabbits were enrolled to a 12 hr light: 12hr dark lighting schedule; lights on was at 0 hr, lights off at 12 hr. CNP was assayed at 3 light (2,6 and 10 hr) and 3 dark (14,18 and 22 hr) times. All groups had four animals but two that had bigger sample sizes to increase the power of tests (6 hr, n=12; 22 hrs, n=13). Dependency between the two eyes was tested by Pearson correlation. The mean of two eyes was considered for analysis. Differences in concentration in pg/200ml were evaluated by one way ANOVA, T test and COSINOR analysis.

**Results**: There were no significant differences for the 6 groups analyzed (one way ANOVA p=0,157). Pooled into two groups (light and dark), the CNP concentration of the light group was lower, 2.626 ± 0.92 pg/200ml compared with the dark group 3.02 ± 1.16 pg/200ml but did not reach a significant difference (t-test p= 0.23). COSINOR analysis was not statistically significant (R%= 7.72 P= .209)

**Conclusion**: These data demonstrate that the concentration of CNP in aqueous humor did not show a circadian change in rabbits entrained to a 12 hr light: 12hr dark lighting schedule.

## INTRODUCTION

Type A, B and C Natriuretic peptides (ANP, BNP, CNP) have been identified in aqueous humor (COCA-PRADOS, M., et al., 1999, p 403-429; FERNANDEZ-DURANGO, R., et al., 1999, p 107-113) and there is evidence that all can influence intraocular pressure (IOP) (COCA-PRADOS, M., et al., 1999, p 403-429; FERNANDEZ-DURANGO, R., et al., 1999, p 107-113; TAKASHIMA, Y., et al., 1998, p 89-96;TAKASHIMA, Y., et al., 1996, p 2671-2677; PALM, D.E., et al., 1995, p 193-203). Fernandez-Durango showed that all three are produced in the eye and their concentrations are not dependent on the systemic plasma concentration (DE JUAN, J.A., et al., 1995, p 25-33). The concentration, receptor affinity and hypotensive effects of the 3 peptides differ. Stimulating two of the natriuretic peptide receptors in the eye, (NPRA and NPRB) increases cGMP; the third, NPRC, functions as a clearance receptor (DE JUAN, J.A., et al., 1995, p 25-33). ANP, BNP and CNP increase cGMP; CNP was the most potent. As far as the receptor response to increase the cGMP concentration, NPRB subtype-selective agonist receptor was most important (PANG, I.H., et al., 1996, p 1724-1731) but NPRC occurs at higher density in the trabecular meshwork (CHANG, A. T., et al., 1996, p 137-143) and ciliary body (MOYA, F. J., et al., 1998, p 3833-3841).

Rowland at al described a model for the circadian rhythm of IOP in New Zealand White rabbits; maximum IOP was during the dark and minimum during the light phase (ROWLAND, J.M., et al., 1981, p 169-173). This was confirmed by Gregory at al who also showed that superior cervical ganglionectomy decreased IOP in the dark and therefore that sympathetic tone is important at night (GREGORY, D.S., et al., 1985, p 1273-1279). Aqueous humor production, like the rhythm of IOP, is higher during the dark (SMITH, S.D., et al., 1989, p 775-778).

The purpose of this study was to determine whether there is a circadian change of CNP in rabbit aqueous humor as a first step toward determining its relation to the circadian rhythms of IOP and aqueous humor production.

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## MATERIALS AND METHODS

All experimental procedures employing animals adhered to the ARVO Resolution on the Use of Animals in Research. Forty one male New Zealand white rabbits weighing 2 –3 kg were enrolled to a 12 hr light: 12hr dark lighting schedule. Lights on was at 0 hr and lights off at 12 hr.

Animals were sacrificed as described elsewhere SMITH, S. D., et al., 1989, p 775-778 every 4 hours (light phase: 2h, 6h, 10h and dark phase: 14h, 18h, 22h). Temporal paracentesis was performed in both eyes with a 28 g needle and aqueous humor was collected, added to 0.5 ml polypropylene microtubes containing anti-protease solutions (1 ml of 2mM EDTA, 1 ml of 2 mM PMSF and 5 ml of 0,2 mM pepstatin A) and immediately frozen and stored at -84ºC. CNP was measured by radioimmunoassay using kits from Peninsula Laboratories, INC, Belmont, CA-USA.

Correlation between the two eyes was tested by Pearson correlation. The mean of two eyes was considered for analysis. Differences in concentration in pg/200ml were evaluated by one way ANOVA, T test and COSINOR analysis (COSINA 3.1, 1996 by Ana Amelia Benedito Silva). Differences were considered significant if $p<0.05$.

## RESULTS

There was a significant correlation of CNP concentration between the two eyes ($r=0.46$, $p=0.00223$) so we used a mean of the two for analysis. There were no statistical differences among the six tested periods ($F=1.72$; $P=0.157$). When the data were combined into two groups, (1) light (mean of 2h,6h,10h) and (2) dark (mean of 14h,18h,22h), the concentration of CNP appeared less in the light than in the dark (2.626 ±0.917 compared with 3.02 ±1.16 picograms/200milliliters). However, the two groups were not statistically significantly different ($P= 0.23$) (table 1).

The COSINOR analysis showed no circadian rhythmus during 24h analysis: mesor: -0.13; gama=0.44; beta=0.55; amplitude =0.47; acro=2.51; R%=7.72 p=0.209.

**Table 1.** Evaluation of the difference on cnp concentration (picograms/200milliliters) during 6 periods.

| Time | Light | Mean | SD | Minimum | Maximum | n | P |
|---|---|---|---|---|---|---|---|
| 2:00 | On | 2.40 | 0.66 | 1.50 | 3.10 | 4 | |
| 6:00 | On | 2.84 | 1.03 | 1.71 | 5.10 | 12 | |
| 10:00 | On | 2.20 | 0.72 | 1.50 | 3.20 | 4 | |
| 14:00 | Off | 2.20 | 0.58 | 1.50 | 2.90 | 4 | |
| 18:00 | Off | 2.55 | 0.72 | 2.00 | 3.60 | 4 | |
| 22:00 | Off | 3.42 | 1.24 | 1.76 | 6.52 | 13 | 0.157 |
| From 2:00 to 10:00 | On | 2.626 | 0.917 | 1.50 | 5.10 | 20 | |
| From 14:00 to 22:00 | Off | 3.02 | 1.16 | 1.50 | 6.52 | 21 | 0.230 |

## DISCUSSION

This study failed to demonstrate a daily change of the concentration of CNP in rabbit aqueous humor, maybe due to the sample size as the difference should be more then 2 picograms/200milliliters to reach statistical significance. Nevertheless, there is strong evidence that natriuretic peptides, especially CNP (FERNANDEZ-DURANGO, R., et al., 1999, p 107-113), can modulate intraocular pressure (FERNANDEZ-DURANGO, R., et al., 1999, p 107-113; TAKASHIMA, Y., et al., 1998, p 89-96; TAKASHIMA, Y., et al., 1996, p 2671-2677; PALM, D.E., et al., 1995, p 193-203; WOLFENSBERGER, T.J., et al., 1994, p 446-448; KORENFELD, M.S., et al., 1989, p 2385-2392; TSUKAHARA, S., et al., 1988, p 104-109; MITTAG, T. W., et al., 1987, p 1189-1196). Fernandez – Durango et al reported that the effect of CNP on intraocular pressure reduction was 9-fold and 20-fold higher than the effect of BNP and ANP, respectively (FERNANDEZ-DURANGO, R., et al., 1999, p 107-113). CNP reduces IOP by increasing outflow facility and does not affect aqueous humor flow or uveoscleral outflow when injected intravitrially. (TAKASHIMA, Y., et al., 1998, p 89-96) All natriuretic pepetides can increase ocular cGMP concentrations but CNP was the most potent (FERNANDEZ-DURANGO, R., et al., 1999, p 107-113 ; PANG, I.H., et al., 1996, p 1724-1731) cGMP can decrease $Ca^{+2}$ by stimulating cGMP dependent protein kinase (HASSID, A., 1986, p C681-686),

increasing $Na^{+}/Ca^{+2}$ exchange (FURUKAWA, K., et al., 1991, p 12337-12341) or increasing $Ca^{+2}$ sequestration from the sarcolema by activating the $Ca^{+2}$ bomb or both (POPESCU, L.M., et al., 1985, p 393-394). Although the mechanism it is not known, these are possible ways that natriuretic peptides can act as "relaxants" of trabecular meshwork. (PANG, I.H., et al., 1996, p 1724-1731)

The lack of substantial variation on the CNP concentration does not reflect a continuous amount of production. Because aqueous flow changes during the 24 hour light-dark cycle (SMITH, S.D., et al., 1989, p 775-778) CNP production would also have to change in order for its aqueous concentration to remain constant.

The function of CNP in normal regulation of IOP is still not clear. Studies in pathological situations such as glaucoma are very important to better understand the roles of these peptides in the eye and could lead to new pharmacological therapies.

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**RESUMO: Objetivo**: verificar se ha mudança na concentração circadiana de peptídeo natriuretico tipo C (CNP) no humor aquoso de coelhos.

**Métodos**: Quarenta e um coelhos machos da raça New Zealand foram admitidos numa rotina de 12 horas de luz e 12 de escuridão; luzes eram acesas na hora zero e apagadas na hora 12. O CNP era medido em três períodos com luz acesa (2,6 e 10h) e 3 com luz apagada (14,18 e 22 h). Todos os grupos tinham 4 animais com exceção de dois períodos em que o n foi incrementado para aumentar o poder dos testes (6h, n=12; 22h, n=13). A dependência entre os olhos foi testada por meio da correlação de Pearson. A media dos dois olhos foi usada para a analise. Diferença quanto a concentração media de CNP em pg/200ml foi avaliada por meio do teste de one-way ANOVA, teste T e analise de COSINOR.

**Resultados**: Não foi encontrada diferença estatisticamente significante nos 6 grupos analisados (one way ANOVA p=0,157). Reunidos em dois grupos (luz e escuridão), a concentração de CNP com a luz acesa foi mais baixa (2.626 ± 0.92 pg/200m) quando comparada ao grupo com luz apagada (3.02 ± 1.16 pg/200m) porem sem alcançar significância estatística (t-test p= 0.23). Analise de COSINOR não foi estatisticamente significante (R%= 7.72 P= .209)

**Conclusão**: Nossos dados demonstraram que a concentração do CNP no humor aquoso de coelhos não variou circadianamente quando analisadas num ritmo de luz/escuro 12/12 h.

**UNITERMOS:** Peptídeo natriuretico, CNP, Glaucoma, Pressão intra-ocular.

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