SHORT COMMUNICATIONS

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Influence of constant light and darkness on arginyl aminopeptidase activity

Arginyl-aminopeptidase activity (ArgAP) hydrolyzes basic N-terminal residues from peptides and arylamide derivatives (4), and has been implicated in the metabolism of Met-enkephalin (2), angiotensin III (1) and neurotensin (3). These substrates are also hydrolyzed by Leu-aminopeptidase activity (LeuAP), which exhibits similar substrate specificity and patterns of behavior (6, 7). A heterogeneous distribution, significant changes during the day, and an asymmetrical distribution of ArgAP and LeuAP at selected time points during 12 h light and 12 h dark periods, have been reported in some structures of the photoneuroendocrine circuit related to the melatonin rhythm-generating system (5-7). Because the structures of such circuit are functionally connected, it might be that environmental light influences ArgAP activity. Therefore, to evaluate the effects of light and dark conditions on ArgAP activity and its left-right distribution, we have compared the results obtained under two altered environmental light conditions (constant light and constant darkness) at the same time-point, and demonstrate a highly significant difference between both experimental groups.

Twenty-one male Sprague-Dawley rats weighing 200-250 g were used. The animals were given food and water *ad libitum* and were housed under controlled temperature (25 °C). They were divided into two experimental groups: 1) animals housed under constant light; 2) animals housed under constant darkness, during at least two weeks before they were killed; the sacrifice was always done at 10.00 h.

The selected areas (table I) were collected and processed as previously described (6). The experiments were done at the same season as the previous circadian studies (6, 7) (winter, northern hemisphere). ArgAP activity was measured in a fluorometric assay with Arginyl-β–naphthylamide (ArgNNap) as the substrate (7). Specific ArgAP activity was expressed as nmol of ArgNNap hydrolyzed/min/mg of protein. Paired Student's *t* tests were used to analyze differences between left and right structures. To assess differences between areas, the data were analyzed with an ANOVA test. Differences between light and darkness conditions were determined with unpaired Student's *t* tests; p values below 0.05 were considered significant.

Table I shows the mean values of soluble ArgAP activity obtained at 10.00 h in the different areas, under constant light and darkness conditions. The pattern of regional distribution described in the present work under constant light and darkness proportionally reproduced the pattern obtained previously under standard conditions (12:12 h light:dark cycle) (7). However, whereas ArgAP exhibited an uneven distribution, with a 4-fold difference between the region with the highest activity (occipital cortices) and the region with the lowest one (superior cervical ganglia) at 10.00 h of the light period of a 12:12 h light:dark cycle (7), such a difference remained unaltered under constant light conditions, but increased to 6-fold under constant darkness. The asymmetrical distribution of aminopeptidase activity, with left predominance in the

Table I. *Arginyl aminopeptidase activity under constant light and dark conditions.*
Values represent mean ± SEM of ArgAP activity, at 10.00 h, in the left and right retina (Re), anterior hypothalamus (AHt), posterior hypothalamus (PHt), superior cervical ganglia (SCG) and occipital cortices (OC), anterior (Ap) and intermediate-posterior pituitary (IPP), pineal gland (Pi) and serum (S). p values indicate the difference between constants light and dark conditions, and (a) left vs right difference of $p < 0.05$.

Source	Light (n = 11)		Darkness (n = 11)		Significance level
	Left	Right	Left	Right	(p)
Re	21.6 ± 2.7	15.5 ± 1.4[a]	24.6 ± 2.5	22.3 ± 2.9	0.045
AHt	28.0 ± 2.1	29.1 ± 1.3	55.7 ± 6.6	52.4 ± 6.3	0.000
PHt	28.8 ± 1.9	30.4 ± 2.1	51.0 ± 6.2	59.5 ± 9.3	0.000
SCG	9.4 ± 1.0	9.2 ± 0.8	13.0 ± 2.0	12.5 ± 2.5	0.045
OC	39.7 ± 1.9	41.0 ± 2.4	76.8 ± 9.5	70.9 ± 10.8	0.000
AP	24.5 ± 2.2		37.9 ± 4.3		0.008
IPP	17.8 ± 1.3		30.0 ± 5.5		0.030
Pi	31.7 ± 2.7		51.6 ± 9.2		0.034
S	0.11 ± 0.008		0.35 ± 0.02		0.000

retina at 10.00 h of the light period in a 12:12 h light:dark cycle (80 % of the animals with left predominance) (6, 7) also appeared at the same time-point under constant light conditions (80 % of the animals with left predominance). However, no left vs right differences were observed in the retina under constant darkness. Previous exposure to constant light and darkness conditions led to highly significant changes in all structures: activity increased under constant darkness, the highest levels of significance appearing in the hypothalamus, occipital cortex, anterior pituitary and serum. These results demonstrate that constant light and dark conditions influence ArgAP activity levels and also show the occurrence of left-right differences in the retina under constant light conditions. The detection of neuropeptide-degrading activity under artificially modified standard light-dark conditions is of importance not only in elucidating the physiological mechanisms behind these variations, but also because changes in this activity may induce (or reflect) an alteration in the normal physiological functions of the substrates involved.

Key words: Aminopeptidase, Light, Darkness, Brain, Asymmetry.

Palabras clave: Aminopeptidasa, Luz, Obscuridad, Cerebro, Asimetría.

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