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CORTICAL AND PONTINE VARIATIONS OCCURRING IN THE VOLTAMMETRIC NO SIGNAL THROUGHOUT THE SLEEP-WAKE CYCLE IN THE RAT

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INTRODUCTION

Studies related to the endothelium-derived relaxing factor (10) initiated researches that led to the discovery of a biological paracrine messenger identified as nitric oxide (NO) (15). It is now also currently reported that the synthesis of this gaseous messenger is achieved, from L-arginine, by NO-synthases (NOS: neuronal, endothelial and inducible isoforms) and that it lasts in an equimolar production of NO and L-citrulline (2, 17, 27). To date, the implication of NO has been documented for three main functional aspects, i.e. anti-microbial and anti-tumoral activities in immune responses, vasodilatation and neurotransmission (2, 26, 29). Regarding neurotransmission, it appears that NOS is colocalised in the brain systems involved in the sleep-wake states genesis and/or regulation (11, 20, 21), at least, with neurotransmitters like serotonin, acetylcholine or somatostatin (1, 23, 28). Moreover, on the basis of pharmacological approaches, it is now also reported that NO contained in neurons of the pontine tegmentum facilitates mainly PS (3, 4, 9,14). This is particularly true for the nucleus raphe dorsalis (nRD) where local injections of either NOS inhibitors or NO donors inhibit or facilitate PS respectively (7).

RESULTS AND DISCUSSION

In order to further specify the results as yet reported on the basis of local micropharmacology, in the present approach we investigated the modalities through which the spontaneous release of NO occurs within the nRD using a voltammetric NO sensor. The frontal cortex (Cx), where the axon processes coming from the nRD serotoninergic perikarya arise, was also analysed in the same manner.

For this study, OFA male rats (250 g; IFFA CREDO, France) were used in compliance with the relevant decree of the French Agriculture Ministry (N°: 03-505). For polygraphic measures, animals were chronically implanted (chloral hydrate anaesthesia, 400 mg/kg, i.p., Merck), with cortical electroencephalographic (EEG) and neck muscle (EMG) electrodes as previously described (4). They were also equipped with reference (Ag/AgCI wire) and auxiliary (Tungsten wire) electrodes

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necessary for voltammetric measures, as described (6). In order to precisely adjust the NO sensor in the brain areas defined, a micro-manipulator was also stereotaxically implanted into the frontal cortex (0.2 mm caudal to bregma; 1.5 mm lateral; first 500 micrometers in depth) or the nRD (Angulation of 28°/sagittal plane; 7.8 mm caudal to bregma; 0.5 mm lateral; vertical, 2 mm above the nRD, i.e. 4.5 mm in depth/ brain surface, the NO sensor penetrating 2 additive mm when inserted in the canula) according to Paxinos and Watson's atlas (18). Afterwards, all the electrodes were soldered to a pair of sub-miniature 5-pin connectors (Sei 3D, Lyon, France) and the entire assembly cemented to the skull using dental acrylic cement (Sun Medical & Co., Shiga, Japan). The rats were then placed in individual home-cages at 24 ± 1 °C and maintained under a 12:12 h light-dark cycle with food and water *ad libitum*. After a two-week recovery period, combined voltammetric and sleep polygraphic recordings began. Polygraphic recordings were automatically interrupted only for differential normal pulse voltammetry (DNPV) measurements (1 every 5 min). Variations in the NO peak height occurring during either SWS or PS were expressed in percent versus the preceding waking state (W, 100%). The NO sensor was prepared on the basis of the carbon fiber sensor previously described (6). Briefly, its active part, constituted by a carbon fiber (diameter, 30 micrometers; length, 500 micrometers), was first pre-treated with a triangular current (80 Hz, 2.9 V/20 s, 1.3 V/4 s) in phosphate buffer saline (PBS) 10 mM and then successively coated with porphyrin-nickel (Interchim, France) and nafion (Sigma-Aldrich, France). DNPV voltammetric (DNPV) measurements (linear potential sweep, 400-1350 mV; scan rate, 10 mW/s; measuring pulse, amplitude 40 mV, duration 60 ms) were performed *in vitro* (calibration of the NO sensor) and *in vivo* (anaesthetised animals) in using a "Biopulse-Unit" (Radiometter-Tacussel, Villeurbanne, France) displaying a three-electrode potentiostat (NO sensor, reference and auxiliary electrodes). At the end of each experiment the position of the active part of the NO sensor was checked by applying a 2 mA anodic current. Afterwards, the brains were removed, serially cut-off (20 micrometers thick slices) and stained with cresyl violet. Statistical significance of the variations occurring in the height of the voltammetric signal were determined by a Wilcoxon signed rank test.

In the frontal cortex, a NO signal was obtained at 650 mV as previously described (3, 4, 6). Throughout light and dark periods, the highest values of its height were always measured during the waking state (W, referenced at 100%, Fig. 1A), i.e. when the animals are more active. During SWS and PS, it decreased progressively (-4% during SWS/W; -8% during PS/W, Fig. 1A). When considering light and dark periods separately, the same tendency was confirmed, but the decrease occurring during PS versus W was more marked during the light period (-9%). These results fully confirm previous ones obtained in the same conditions (3). They illustrate again that the electrochemical sensor used is well adapted for approaches combining electrochemical NO measurements and behavioural studies in freely moving animals. Here, it can also be argued that the active NO release taking place in the cortex during waking is analogous to that described for serotonin (5, 19). Such a release might thus result from the serotonin nerve endings coming from the nRD and

impinging the frontal cortex (22). As suggested above, serotoninergic neurons of the nRD are, indeed, capable to synthesise NO (12, 28) and are mainly active during the waking state (16). However, within the cortex several other sources for NO exist, i.e. local GABA-ergic interneurons (25) or axon nerve endings coming from the basal hypothalamus (24). It is not thus excluded that the NO release observed within the frontal cortex might represent the average variations of various sources, each one releasing NO with different relationships towards the sleep-waking cycle.

In the nRD, a NO signal peaking at 650 mV was also recorded. Throughout the lightdark periods, its height tended towards an increase during SWS which was more important during PS (+4%/W, Fig. 1A). It is also to be noticed that during PS the increase observed in the nRD was significantly different from that observed in the Cx (Fig. 1A, $p < 0.05$). However, when light-dark periods were considered separately, the increase observed during PS versus W was more marked during the dark period (+ 13%). We emphasise here the fact that data obtained in the nRD could be preliminary since obtained in a limited sample of animals. They nevertheless suggest that an active release of NO may occur within the nRD, particularly during PS. This result is in keeping with the data obtained by local nRD injections of either NOS inhibitors or NO donors, capable to decrease or increase PS respectively (7). Beside, as discussed for the Cx, the NO release occurring within the nRD may also result, at least, from two different sources, i.e. local serotoninergic neurons (12) and nerve endings coming from the laterodorsal pontine tegmentum (LDT) (13). In this respect, it can be argued that local serotoninergic neurons may contribute to the PS-related NO release by way of their somatodendritic processes as suggested for serotonin (5). Such a paracrine release could contribute, through a feedback mechanism, to the PS-related silencing of the serotoninergic neurons. Regarding the NO-ergic-cholinergic nerve endings coming from the LDT (13), an area where the perikarya are more active during PS (20), it is likely that their synaptic release taking place within the nRD might reinforce the local paracrine influence exerted by NO.

Finally, recordings performed either in the Cx or in the nRD exhibited opposite nycthemeral changes throughout the light (12-h) and dark (12-h) periods, i.e. the signal height was higher in the Cx and lower in the nRD during the dark period (animals more active) and conversely for the light one (Fig. 1B). In this respect, variations reported for the Cx are in keeping with those previously described (8) while those described for the nRD are original. Such changes indicate that, beside the ultradian variations occurring in relation with the sleep-wake states alternance, a circadian component also regulates the NO release throughout the light-dark cycle. Mechanisms involved remain to be further investigated.

CONCLUSION

In conclusion, reported results emphasise the fact that NO released within the nRD and synthesised either locally or within the lateral pontine tegmentum, is actively involved in PS triggering and maintenance. Paracrine and synaptic influ-

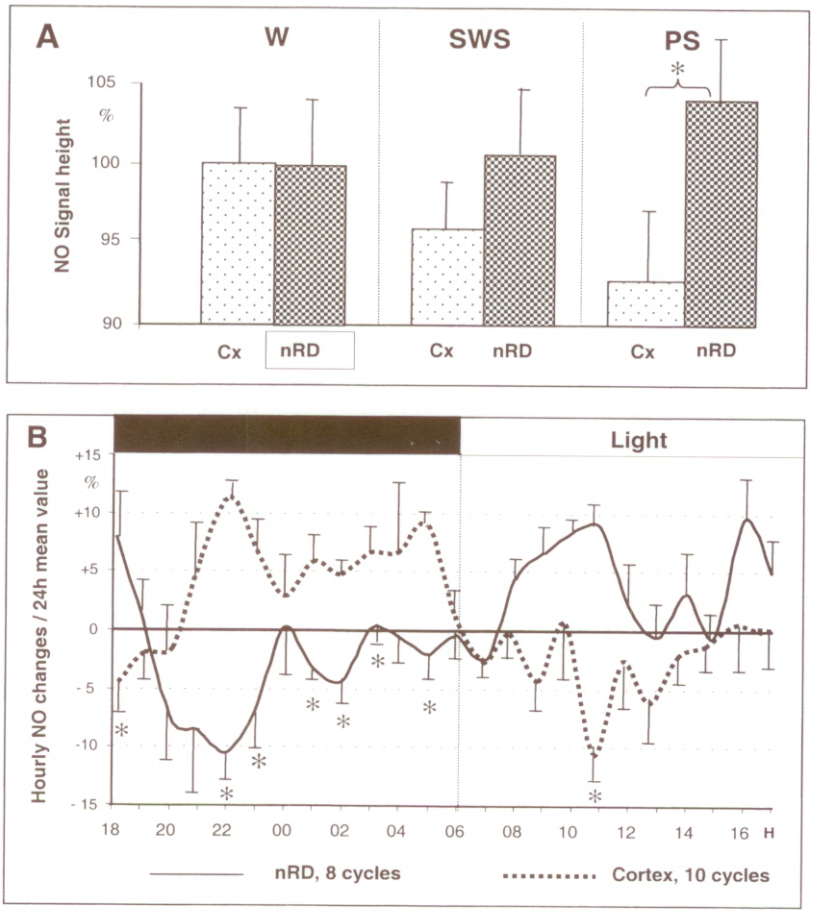

Fig. 1. *Spontaneous and nycthemeral changes occurring in the height of the voltammetric NO signal in relation wlth the sleep-wake states.*

A. - Mean values of the NO signal height measured either in the frontal cortex (Cx) or in the nucleus raphe dorsalis (nRD). Measurements were taken during spontaneous episodes of waking (W), slow wave sleep (SWS) and paradoxical sleep (PS) occurring either during the dark (12h) or the light (12h) periods. It is to be noticed that versus W (referenced at 100%) the changes occurring in the Cx during either SWS or PS are opposite to those of the nRD. For the Cx, the histograms were determined as follows: - W, mean ± SEM of 253 measurements; - SWS, mean ± SEM of 243 measurements; - PS, mean ± SEM of 82 measurements. For the nRD: - W, mean ± SEM of 154 measurements; - SWS, mean ± SEM of 157 measurements; -PS, mean ± SEM of 31 measurements; - Statistics: changes occurring in the cortex versus nRD are significantly different during PS (Wilcoxon signed rank test, $^{*}p < O.05$).

B. - Throughout a nycthemeron, the whole changes occurring in the NO signal height either in the Cx or in the nRD evolve also in an opposite manner: - in the Cx, the NO signal is higher during the dark period (when the animals are more active) and lower during the light one (when the animals are more sleepy); - in the nRD, the NO signal is lower during the dark period and higher during the light one. In the Cx, 10 cycles of 24h were determined from 7 rats while in the nRD 8 cycles of 24h were determined from 2 rats. For representation, the mean of the whole values obtained either in the Cx or in the nRD, over the 24h cycles taken into account, were calculated and seated at zero (horizontal line); afterwards, the mean ± SEM of the hourly changes occurring in each structure investigated were plotted in percent versus the mean. Statistics: significance of the differences between Cx and nRD were checked with a Wilcoxon signed rank test, $^{*}p < 0.05$.

ences taking place within the pons are, at least partly, also reflected in the Cx, a structure receiving axon nerve endings from the nRD.

SUMMARY

Voltammetric measurements of nitric oxide (NO) were performed either in the frontal cortex (Cx) or in the nucleus raphe dorsalis (nRD) of rats equipped for polygraphic recordings. In the frontal cortex, the 650 mV signal related to NO exhibited its highest height during the waking state (W) and decreased slightly during slow-wave sleep (SWS) and even more during paradoxical sleep (PS). In the nRD, opposite variations were observed, i.e. the signal tended towards an increase during SWS and raised more consistently during PS versus W. Recordings performed either in the Cx or the nRD, throughout the light (12-h) and dark (12-h) periods, exhibited opposite nycthemeral changes, i.e. the signal height was higher in the Cx and lower in the nRD during the dark period and conversely for the light one. Paracrine and synaptic mechanisms taking place within the pons and, at least partly, also reflected in the Cx need to be further investigated.

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